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PLANT ANIMAL INTERACTIONS

Pollinator diversity affects plant reproduction and recruitment: the tradeoffs of generalization

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Abstract One outstanding and unsolved challenge in ecology and conservation biology is to understand how pollinator diversity affects plant performance. Here, we provide evidence of the functional role of pollination diversity in a plant species, *Erysimum mediohispanicum* (Brassicaceae). Pollinator abundance, richness and diversity as well as plant reproduction and recruitment were determined in eight plant populations. We found that *E. mediohispanicum* was generalized both at the regional and local (population) scale, since its flowers were visited by more than 100 species of insects with very different morphology, size and behaviour. However, populations differed in the degree of generalization. Generalization correlated with pollinator abundance and plant population size, but not with habitat, ungulate damage intensity, altitude or spatial location. More importantly, the degree of generalization had significant consequences for plant reproduction and recruitment. Plants from populations with intermediate generalization produced more seeds than plants from populations with low or high degrees of generalization. These differences were not the result of differences in number of flowers produced per plant. In addition, seedling emergence in a common garden was highest in plants from populations with intermediate degree of generalization. This outcome suggests the existence of an optimal level of generalizations even for generalized plant species.

Keywords *Erysimum* · Pollination generalization · Pollinator diversity · Spatial variation

Introduction

The evolution of plant–pollinator relationships has traditionally been viewed as a tight co-adaptive process in which plants evolve traits to attract certain (efficient) pollinators and pollinators evolve traits to better exploit floral resources of particular plants (Stebbins 1970; Faegri and van der Pijl 1979; Proctor et al. 1996). According to this view, plant–pollinator interactions would evolve towards an increasing degree of specialization. However, multiple empirical studies are showing a different scenario, in which most plants are visited and pollinated by a wide and diverse range of animal species (Waser et al. 1996, and references therein; Herrera 1996; Gómez and Zamora 2006). As a consequence, the ecology and evolution of generalization in pollination interactions has attracted a great deal of attention from pollination ecologists over the last decade (Waser et al. 1996; Herrera 1996; Johnson and Steiner 2000; Aigner

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2001; Fenster et al. 2004; Waser and Ollerton 2006). The recent application of network analysis to the study of mutualistic interactions further supports this scenario of generalization in plant–pollinator interactions. Pollination network studies are showing that most pollination systems are nested, with an asymmetrical structure characterized by a lack of tight interactions between specialist plants and specialist pollinators (Bascompte et al. 2003; Vázquez and Aizen 2003; Jordano et al. 2006). Two important conclusions arise from recent studies on pollination generalization. First, generalization is a pervasive characteristic of pollination systems. Second, plant–pollinator interactions are best understood along a gradient of generalized–specialized relationships (Herrera 1996; Waser et al. 1996).

Spatial variation in pollinators is one of the most important factors contributing to the maintenance of an overall generalized pollination system, especially when between-population gene flow is not restricted (Herrera 1996; Fenster et al. 2004). Accordingly, many studies have reported spatial variation in pollinator abundance and composition (Ollerton and Cranmer 2002; Price et al. 2005; Gómez and Zamora 2006; Ollerton et al. 2006, and references therein). However, very few studies have explicitly tested for spatial variation in pollinator richness or diversity (Herrera 2005; Moeller 2005). These few studies have found between-population variation in the degree of pollination generalization, with conspecific populations ranging from moderately to extremely generalized. A potential consequence of this spatial variation is the occurrence of a geographic structure of specializations/generalization, with plants being pollinated by different numbers of species in different populations.

Despite the effort devoted to characterizing generalization in pollination systems, there is virtually no information about the consequences of pollination generalization on plant populations. From an evolutionary point of view, generalization is considered to decrease the ability of plants to respond to selection imposed by abundant and/or efficient pollinators, consequently decreasing the potential for both microevolution and speciation (Armbruster et al. 2000; Johnson and Steiner 2000; Gómez and Zamora 2006; Sargent and Otto 2006). From an ecological point of view, generalization is considered a positive trait that may favour competitive ability, colonization capacity and invasion ability in plants (Richardson et al. 2000). Nevertheless, empirical ecological studies relating generalization and plant performance or fitness are scarce (Kremen et al. 2002; Klein et al. 2003).

The main goal of this study is to investigate the reproductive and demographic consequences of the spatial variation in the degree of pollination generalization of *Erysimum mediohispanicum* (Brassicaceae), a monocarpic plant with a generalized pollination system (Gómez 2005). To achieve this goal, we quantify between-population variation in pollinator richness, diversity and dominance. Then, we explore the factors contributing to this spatial variation. Finally, we determine the effect of the degree of pollination generalization on seed production and seedling recruitment.

Materials and methods

Study system and populations

Erysimum mediohispanicum Polatschek (Brassicaceae) is a biennial to perennial monocarpic herb that occurs in two separate areas of the Iberian Peninsula, one in the north-east and the other in the south-east. In the latter area, *E. mediohispanicum* is found in montane regions from 1,100 to 2,000 m a.s.l.. Plants usually grow for 2–3 years as vegetative rosettes, and then die after producing one to eight reproductive stalks which may display from a few to several hundred hermaphroditic, slightly protandrous; bright, yellow flowers (Gómez 2003). Although self-compatible, *E. mediohispanicum* requires pollen vectors to produce full seed set (Gómez 2005). Selective exclusion experiments have demonstrated that even minute, unspecialized flower visitors are important pollinators of *E. mediohispanicum* (Gómez 2005), and may exert strong selection on flower traits (Gómez et al. 2006).

The study was conducted in the Sierra Nevada mountains (Granada province, south-east Spain), spanning the complete altitudinal range of *E. mediohispanicum* (1,600–2,300 m). In this area, *E. mediohispanicum* is found in two main habitats, the understory of pine (*Pinus nigra* and *Pinus sylvestris*) forests, and montane species-rich shrublands, formed mainly by *Berberis vulgaris*, *Juniperus communis*, *Astragalus granatense*, *Vella spinosa*, and *Ononis aragonensis*.

We selected eight populations within a 5 × 2-km area (Table 1). Populations were at least 150 m from each other, with a mean inter-population distance of 818 ± 82 m (±1 SE). For each population we determined: (1) overall habitat (shrubland or forest); (2) geographical location (GPS georeference); (3) altitude (GPS); and (4) size (we ranked populations according to both *E. mediohispanicum* density and cover area).

Determination of pollinator assemblage

E. mediohispanicum started to flower in mid May in the lower populations and reached full bloom in early July in the higher populations. The between-population variation in flowering time afforded us with the opportunity to sample thoroughly the flower visitor assemblage in each

Table 1 Location, characteristics and sampling effort of the eight *Erysimum mediohispanicum* populations studied during 2005

Population	Population characteristics					Sampling effort			
	Latitude	Longitude	Altitude	Habitat	Size[a]	Plants	Minutes	Flowers	Pollinators
01	37°8.00′	3°25.69′	1,750	Forest	2	90	2,395	3,024	162
02	37°7.33′	3°25.86′	2,099	Shrubland	7	90	1,720	2,486	270
08	37 8.00′	3°25.91′	1,690	Shrubland	6	90	2,085	2,642	169
21	37°8.07′	3°25.71′	1,723	Forest	5	90	1,955	1,826	243
22	37°7.86′	3°25.70′	1,802	Forest	3	90	1,925	1,939	125
23	37°7.74′	3°25.58′	1,874	Shrubland	8	90	1,650	2,266	184
24	37°7.51′	3°26.14′	1,943	Forest	1	90	1,485	1,927	117
25	37°7.27′	3°26.05′	2,064	Shrubland	4	90	2,195	1,710	118

[a] Population size is ranked based on *E. mediohispanicum* density and cover area

population. In 2005 we labelled 90 *E. mediohispanicum* plants per population at the onset of the flowering period. Then, throughout the peak bloom (10–15 days per population), we conducted five to seven pollinator censuses per population. Censuses were performed under sunny conditions with no wind. In each census we noted the number of open flowers in each labeled plant, and the number of pollinators that landed on their flowers during 5 min. Thus, each census lasted 450 min, and we conducted more than 1,500 min of observation per population (Table 1). The number of censuses per population was fitted to the local abundance of insects by means of accumulation curves (Magurran 2004). Using these data, we used rarefaction curves (see below) to determine the number of pollinators necessary to obtain an adequate estimate of the pollinator assemblage for each population. Our results indicated that 130–150 pollinators per population provided an accurate estimate of the pollinator assemblage.

Most individual pollinators were identified in the field, but some specimens were captured and sent to specialists. Some rare pollinators could not be captured and thus we only identified them to genus or family.

Two traits of the pollinator assemblage visiting *E. mediohispanicum* flowers are considered in this study: abundance and diversity (Magurran 2004). Abundance of pollinators was estimated by standardizing the number of visits per open flower and time unit (expressed as visits flowers^{-1} h^{-1}). We assessed pollinator diversity by calculating richness (S_{obs}), diversity, evenness, and dominance. S_{obs} was calculated as the number of pollinator species found visiting flowers in each population. In addition, we used EstimateS software (http://www.purl.oclc.org/estimates) (Colwell 2005) to calculate two asymptotic richness estimates, the incidence coverage estimator (S_{ICE}) and the Michaelis–Menten index (S_{MM}). These are two robust estimates used to evaluate sample-size adequacy in calculation of diversity indices (Hortal et al. 2006). Sampling is considered adequate when the sample-based rarefaction curves and the two estimators converge closely at the highest observed values (Longino et al. 2002; Colwell et al. 2004). Dominance was calculated as the relative abundance of the most abundant pollinator species. We used pollinator rank-abundance plots as a way to visualize the structure of the pollinator communities (Magurran 2004). Diversity was calculated as the Shannon–Wiener index and Hulbert's PIE (Colwell 2005). Hulbert's PIE is the probability that two randomly sampled individuals from the community pertain to two different species. It is an evenness index that combines the two mechanistic factors affecting diversity: dominance and species abundance. All these indexes were generated by a randomization process using EcoSim (http://www.homepages.together.net/~gentsmin/ecosim.htm)(Gotelli and Entsminger 2005).

Plant reproduction and recruitment

We quantified female reproductive success of the 720 labelled plants by means of the following sequential estimates:

1. Number of flowers. All flowers produced by each labeled plant were counted.
2. Female fertility. The proportion of ovules setting seeds in each plant was estimated. The number of fruits produced per plant were counted to obtain the fruit set as the proportion of flowers setting fruits. Then, on three fruits per plant, seed set was estimated as the proportion of ovules ripening to seeds in each successful fruit. Female fertility was obtained by multiplying fruit set by seed set.
3. Female fecundity. The total number of seeds produced per plant was estimated by multiplying the number of fruits per plant by the number of seeds per fruit. *E. mediohispanicum* is monocarpic, reproducing only once in its lifetime.

4. Seedling emergence. The proportion of seeds germinating and emerging as seedlings was estimated. In autumn 2005 ten seeds of each of 16 randomly selected plants per population were sown in a common garden (n = 1,280 seeds), and the proportion of these seeds emerging in spring 2006 was recorded.

Data analysis

Individual plants are treated as sampling units in all analyses. Plants receiving no pollinator visits during the censuses were included in the analyses (Colwell 2005). Among-population differences in pollinator abundance and plant reproductive success were analysed with one-way ANOVA, considering population as a random factor. Richness, dominance and diversity were compared among populations with individual-based rarefaction curves generated by permutation with EcoSim using the Coleman method (Gotelli and Colwell 2001). Rarefaction allows for estimation of the number of species (S) expected in a random sample of n individuals taken from a larger collection made up of N individuals and S species (Gotelli and Entsminger 2005). Spatial autocorrelation and geographical structure of the pollinator assemblage was investigated for all pollinator assemblage descriptors with two indices, Moran's I coefficient and Mantel r (R package, version 4.0; http://www.bio.umontreal.ca/casgrain/). Moran's I behaves like a Pearson correlation coefficient and calculates the similarity between observations from pairs of locations for each distance class and each independent variable. The number of equidistant distance classes was calculated following Sturge's rule (N classes=1 + 3.3log$n$). Regular Bonferroni corrections were applied to determine whether there was significant spatial structure in the different explanatory and dependent variables. Then, we used Mantel partial r (Fortin and Gurevitch 2001) to test the correlation between altitude and generalization degree for those estimates that were spatially autocorrelated. We used product–moment correlations for the remaining estimates. The effect of pollinator diversity on plant reproduction and recruitment was explored with simple regressions, both linear and quadratic. We retained the regression providing the best fit (highest R-^{2} and lowest P-values). Since the power of these analyses is low due to the low number of populations, we kept relationships with P-values which were marginally significant (<0.10), provided the R^2 was higher than 50%.

Results

A total of 1,324 insects belonging to 112 species in six orders were observed visiting the flowers of *E. mediohispanicum* in the eight populations. Voucher specimens are deposited in the CREAF insect collection. The majority of species were Hymenoptera (49 species) and Coleoptera (30 species). Most species were scarce. Only six species made up more than 5% of the total visits. These species were two large bees (*Anthophora aestivalis*, *Osmia brevicornis*), two small bees (*Lasioglossum aeratum*, *Lasioglossum interruptum*,) and two bee-flies (*Bombylius major*, *Bombylius* sp.). Together, these dominant species comprised 39% of the total abundance. Only two species (*O. brevicornis* and *Andrena agilissima*) were crucifer oligoleges. Considering only the most abundant pollinator species (Fig. 1), body size ranged from ca. 0.3 mg in *Meligethes minutus* to ca. 130 mg in *A. aestivalis*. Mouthpart length ranged from <1 mm in *M. minutus* and *Anthaxia funerula* to 6–8 mm in *Bombylius* spp., and 10–12 mm in *A. aestivalis*. Overall, the flower visitor assemblage was composed mostly of species with short mouthparts. Only 27 species (nine bee-flies, six large bees and 12 butterflies) had mouthparts with a length comparable to *E. mediohispanicum* corolla depth. Some pollinators visited *E. mediohispanicum* flowers mostly for nectar, while others collected large amounts of pollen.

E. mediohispanicum pollination system is also generalized at the local scale. The observed species richness per population (S_{obs}) ranged from 30 to 41 (Table 2), the expected pollinator richness according to asymptotic estimates was well above 40 in all populations, and the Hulbert's PIE indices were always higher than 0.9 (more than 90% probability of two randomly selected insects belong to different species). The pollinator assemblage structure was similar in all populations, with few abundant species and a high number of scarce species (Fig. 1). Dominance was consistently low, ranging from 11.9% in population 25 to 21.2% in population 23 (Table 2). In addition, the number of species scoring a relative abundance higher than 10% was low (1–4), in all populations (Fig. 1).

Populations varied significantly in the composition of pollinator assemblage. The most abundant species in different populations often belonged to different insect orders, and had different feeding habits (Fig. 1). Dominant species included bee-flies (*Bombylius*), large bees (*Anthophora, Osmia*), small bees (*Ceratina, Lasioglossum*), and small beetles (*Meligethes, Mordellistena, Aplocnemus, Phalacrus*). There was also significant between-population variation in pollinator abundance. Visitation rates were more than twice as high in population 21 as in population 1 (Table 2). Populations also differed in their degree of generalization. Randomization analyses showed that populations differed in S_{obs} (Table 2). Asymptotic richness estimates (S_{MM} and S_{ICE}) also reflect among-population differences in estimated species richness (Table 2). There

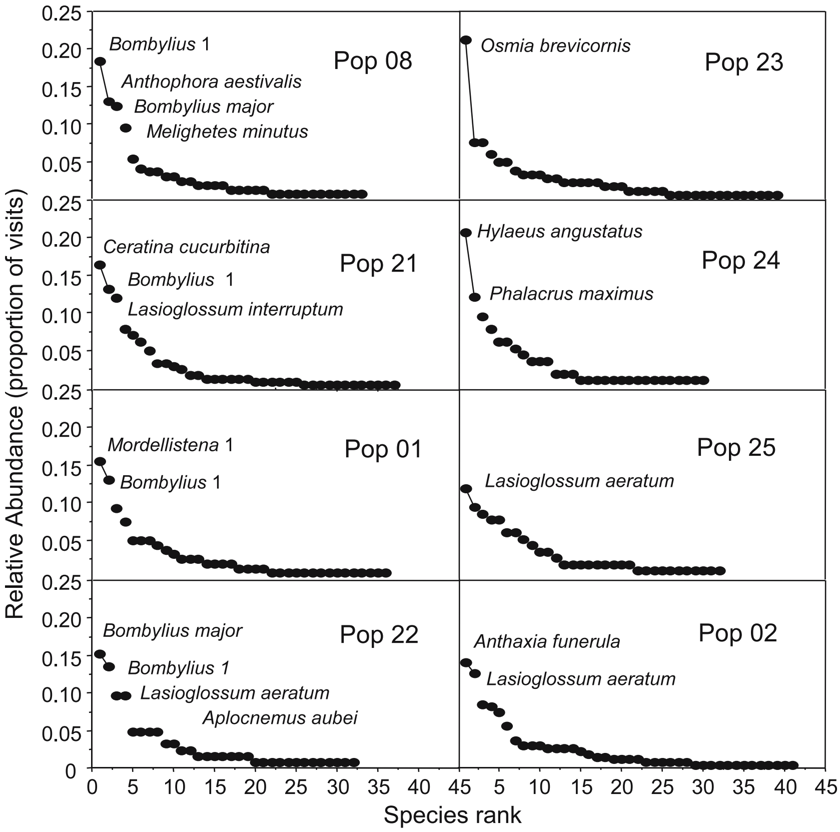

Fig. 1 Rank–abundance curves of pollinator species visiting the eight *Erysimum mediohispanicum* populations in the Sierra Nevada. Names of species accounting for at least 10% of the visits at a given population are provided

Table 2 Among-population differences in pollinator abundance and diversity. Means followed by *different letters* are statistically different at α 0.05. S_{obs} Observed number of pollinator species censused per population, S_{ICE} estimate of the expected number of pollinator species per population according to the incidence coverage estimate, S_{MM} estimate of the expected number of pollinator species per population according to the Michaelis–Menten estimate, *Dominance* percentage of the most abundant species within a population

Population	Abundance[a] ± 1 SE	S_{obs}[b] (±95% CI)	S_{ICE}	S_{MM}	Dominance	Shannon–Wiener H'[b]	Hulbert PIE
1	0.64 ± 0.07 c	36 c,d (25.4–46.7)	67.54	46.32	15.4 d	3.03 d,e	0.93 a,d
2	1.30 ± 0.12 a,b	41 a,d (31.1–50.9)	55.24	48.33	14.1 a,d	3.09 a,c,d	0.94 a
8	0.77 ± 0.07 c	33 a,b (22.6–41.4)	43.48	39.74	18.3 b	2.85 a,b,d	0.92 b,c
21	1.60 ± 0.14 a	37 b (27.3–46.7)	49.80	43.15	16.5 b,d	2.91 b	0.92 b,d
22	0.77 ± 0.07 c	32 c,d (22.7–41.3)	47.69	43.09	15.2 d	2.95 c,d	0.93 a,c
23	0.97 ± 0.12 b,c	39 c,e (29.1–48.9)	52.08	50.40	21.2 c	3.10 c,e	0.93 a,b,d
24	0.73 ± 0.10 c	30 a,b,c (20.1–39.5)	52.64	44.83	20.5b,c	2.83 b	0.92 b,d
25	0.82 ± 0.10 c	32 d,e (22.8–41.2)	43.14	46.09	11.9a	3.08 c	0.95 e

[a] Abundance is expressed as visits flower^{-1} h^{-1}. Abundance was compared by a Tukey HSD post-hoc test following one-way ANOVA ($F = 10.59$, $df = 7{,}714$, $P < 0.0001$).

[b] Richness (S_{obs}) and diversity (H) indices were compared by means of a randomized rarefaction procedure using EcoSim software

were also among-population differences in the two diversity indices considered, the Shannon-Wiener H' and the Hulbert's PIE (Table 2). Overall, the populations having the most diverse pollinator assemblage were populations 1, 2, 23 and 25 (Table 2).

Between-population differences in pollinator abundance or diversity were not autocorrelated ($P > 0.1$ for all distance classes, Moran's *i*), suggesting that closer populations were not more similar in the diversity of pollinators visiting their flowers. Pollinator abundance did not correlate with any of the plant population factors considered (habitat, altitude, population size, all $P > 0.1$, one-way ANOVA, linear regression or partial Mantel test). Two factors affected species richness at the population level: pollinator abundance and plant population size. Populations with higher pollinator abundance were those with higher pollinator richness ($F = 19.12$, $df = 1,6$, $P = 0.005$, $R^2 = 0.72$). Pollinator richness also increased with plant population size ($F = 6.70$, $df = 1,6$, $P = 0.04$, $R^2 = 0.52$). However, pollinator abundance and plant population size did not correlate with any of the diversity indices ($P > 0.1$, linear or quadratic regressions).

The eight populations studied differed significantly in flower number per plant ($F = 2.79$, $df = 7,712$, $P = 0.007$), female fertility ($F = 12.70$, $df = 7,529$, $P = 0.0001$), female fecundity ($F = 10.51$, $df = 7,529$, $P = 0.0001$), and percent seedling emergence ($F = 3.66$, $df = 7,114$, $P = 0.001$, one-way ANOVAs). Flower number was not related to plant reproductive success or to pollinator diversity ($P > 0.1$, linear and nonlinear regression analyses). Similarly, none of the reproductive success estimates considered (fertility, fecundity and seedling emergence) was related to pollinator abundance ($P > 0.2$, linear and nonlinear regression analyses). Instead, pollination generalization was significantly related to population reproductive success. This relationship was not linear but quadratic (Fig. 2). We found that populations with an intermediate level of generalization were those with highest seed production and seedling emergence. This relationship was consistent irrespective of the generalization estimate (richness or diversity) and the reproductive success estimate considered (Fig. 2).

Discussion

The pollination system of *E. mediohispanicum* was extremely generalized. Its flowers were visited during 2005 by more than 100 species of insects with very different morphology, size and behaviour. Given the morphology of *E. mediohispanicum* flowers, contact of the insect visitor with the flower's reproductive organs is virtually unavoidable. Stigma and anthers are located at the opening of the corolla tube, which is only 1–3 mm in diameter. Therefore, any insect attempting to reach the nectaries is bound to contact both stigma an anthers. Even one of the smallest insects recorded (*Meligethes maurus*, ~2 mm in length) has been shown to be an effective pollinator of *E. mediohispanicum* (Gómez 2005; Gómez et al. 2006). A few species (small bees in the genera *Nomada* and *Lasioglossum*, and small ants) sometimes behaved as nectar thieves, sipping nectar from lateral gaps between the basal part of the sepals, but behaved as legitimate pollinators other times. Thus, it is fair to assume that all species recorded acted as effective pollinators to some degree. This taxonomical, morphological and behavioural diversity agrees with results from a close (10 km) population, in which more than 30 pollinator species belonging to five orders were recorded with a different sampling methodology (Gómez 2005).

Beyond this high degree of generalization, we found a significant spatial variation among populations in pollinator assemblage richness, diversity and dominance. Observed richness varied between 30 and 41 species, and estimated richness between 43 and 67 species. It is important to note that these differences occurred at a small spatial scale, since populations were less than 1 km apart. In addition, there was no spatial autocorrelation in pollinator richness or diversity, indicating that close populations were no more similar in pollination generalization than distant populations. This outcome depicts a mosaic of generalized populations in our study area.

Two factors were correlated positively with pollinator richness: pollinator abundance and plant population size. The abundance–richness relationship is frequent in pollinator assemblages (Steffan-Dewenter et al. 2002; Steffan-Dewenter 2003). The positive relationship between pollinator richness and plant population size has been found in other studies (Conner and Neumeier 1995; Kunin 1997), and it is expected as a consequence of the species richness–area relationship. Interestingly, and in contrast to many studies that have shown a decrease in pollinator richness with altitude (Arroyo et al. 1982; Medan et al. 2002; Devoto et al. 2005; Hodkinson 2005), we found no effect of altitude on pollinator richness or diversity.

We found no effect of overall pollinator abundance on plant reproduction. Instead, we found a significant relationship between pollination generalization and plant reproductive success (fertility, fecundity and seedling emergence). In *Coffea arabica*, reproductive success increased with pollinator diversity, but not with pollinator abundance (Klein et al. 2003). Different pollinators have different pollinating effectiveness (Motten et al. 1981; Herrera 1987a; Gómez and Zamora 1999). Different pollinators also differ in flight distances between consecutively visited plants and/or in numbers of flowers visited per individual plant (Schmitt 1980; Herrera 1987b; Bosch and Blas 1994). Therefore, high pollinator diversity is expected to result in increased diversity of stigma pollen loads, with pollen grains from a range of donors. Increased pollinator diversity is associated with decreased pollination limitation in many plants (Ashman et al. 2004; Knight et al. 2005). However, the relationship between pollinator diversity in *E. mediohispanicum* is quadratic. Maximum reproductive success was found at intermediate levels of pollinator diversity. The fact that seedling emergence is highest at intermediate diversity levels suggests that the "quality" of pollen grains deposited on the stigmas decreases with increased diversity. This situation would occur if increased pollinator diversity resulted mostly from the addition of pollinators depositing low-quality pollen grains. Increased pollination diversity could be achieved through visitation of pollinators depositing mostly geitonogamous pollen, or

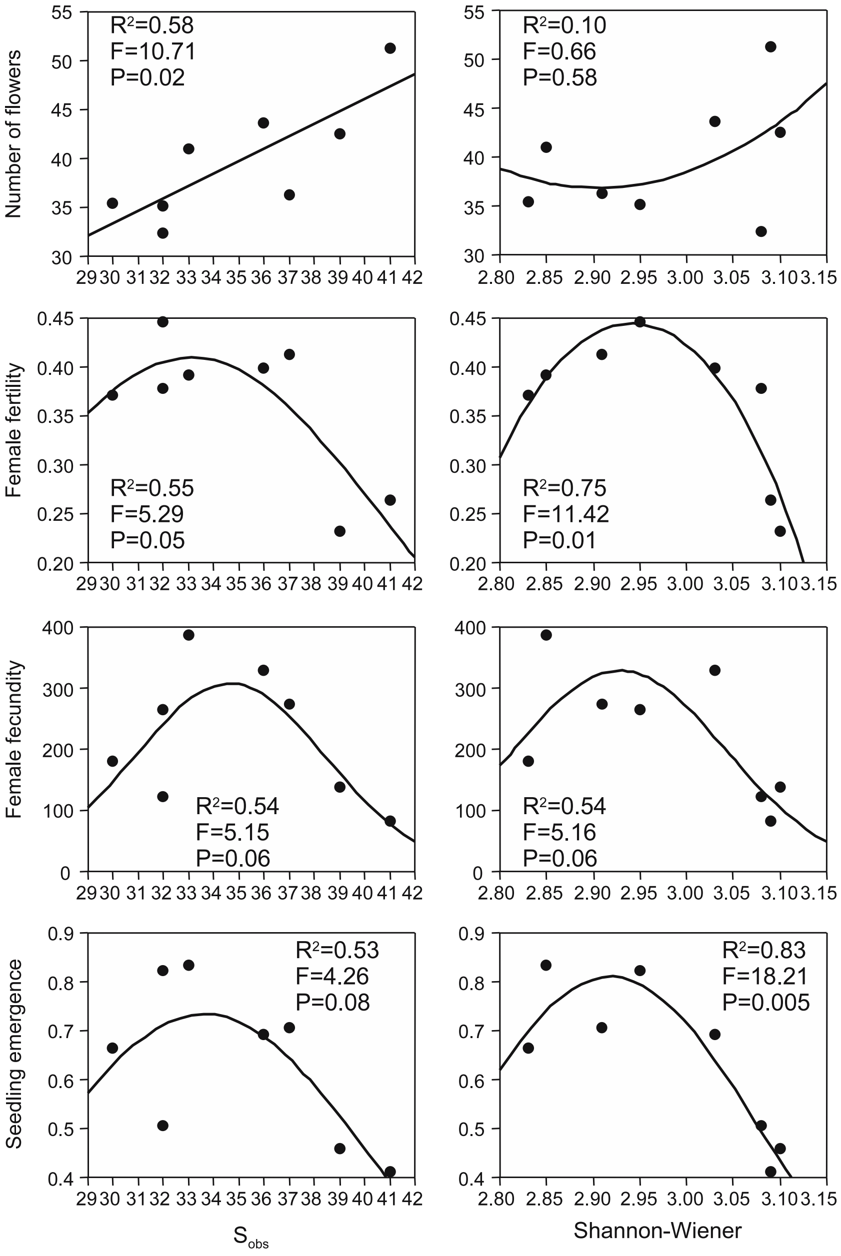

Fig. 2 Relationship between pollinator generalization [estimated as richness (S_{obs}) and diversity (Shannon–Wiener H')] and plant performance in *E. mediohispanicum* populations. The equation fitting pollinator diversity versus plant reproductive success is: $y = \mathrm{e}^{a+bx+c(x-\bar{x})^2}$

through visitation of pollinators with low flower constancy, which could result in heterospecific pollen deposition and, eventually, stigma clogging. Sahli and Conner (2006) describe a similar scenario in which pollinator richness increases by the addition of species with low visitation rates or low pollinating effectiveness, with little effect on plant reproduction.

Our results have to be taken cautiously, since they are from observations made in only eight populations. Nevertheless, the absence of a significant relationship between flower number per plant and pollinator diversity on the one hand and plant reproductive success on the other indicates that the pollinator generalization–plant performance relationship does not result from differences in flower production among populations. Moreover, since no relationship was found between pollinator diversity and altitude or habitat, we do not think that the pollination diversity–plant performance relationship is a side effect of a hidden environmental gradient.

In summary, this study has shown the existence of an optimal level of pollinator diversity even for generalized plants. The exact position of the optimum along the specialization–generalization gradient will surely depend on many extrinsic and intrinsic factors, such as plant phenotype, plant absolute and relative abundance, presence of co-flowering plant species, distribution of pollinator effectiveness, and presence of low-efficiency pollinators and nectar thieves. Further experimental studies are mandatory to confirm whether the pattern found in this study is widespread and to explore its underlying mechanisms.

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