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LETTER

Local adaptation and maladaptation to pollinators in a generalist geographic mosaic

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Abstract

The Geographic Mosaic Theory of Coevolution predicts the occurrence of mosaics of interaction-mediated local adaptations and maladaptations. Empirical support to this prediction has come mostly from specialist interactions. In contrast, local adaptation is considered highly unlikely in generalist interactions. In this study, we experimentally test local adaptation in a generalist plant-pollinator geographic mosaic, by means of a transplant experiment in which plants coming from two evolutionary hotspots and two coldspots were offered to pollinators at the same four localities. Plants produced in the hotspots attracted more pollinators in all populations, whereas coldspot plants attracted fewer pollinators in all populations. Differences in adaptation were not related to genetic similarity between populations, suggesting that it was mainly due to spatial variation in previous selective regimes. Our experiment provides the first strong support for a spatially structured pattern of adaptation and maladaptation generated by a generalist free-living mutualism.

Keywords

Generalist systems, geographic mosaics of coevolution, local adaptation, maladaptation, pollination.

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INTRODUCTION

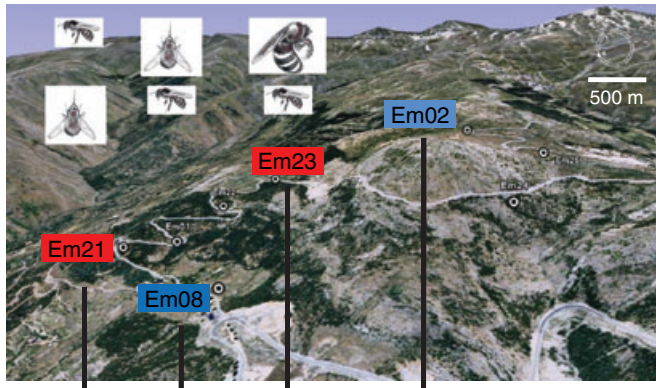
Most species are formed by a collection of genetic and ecologically differentiated populations inserted in a complex landscape. The Geographic Mosaic Theory of Coevolution (GMTC) subsumes this idea and considers that populations differ in evolutionary dynamics due to spatial variation in selective regimes (Thompson 2005). The GMTC argues that the overall coevolutionary dynamics of such interactions are driven by three components of geographic structure: selection mosaics, coevolutionary hotspots, and trait remixing (Thompson 1994; Gomulkiewicz *et al.* 2000). This theory visualizes the landscape as a mosaic of coevolutionary hotspots, populations where reciprocal selection is strong and coevolution is ongoing, embedded in a broader matrix of coevolutionary coldspots, where local selection is weak, non-reciprocal or where only one of the participants occurs (Thompson 2005). The GMTC predicts that these three processes lead to three observable patterns: spatial variation in the traits mediating an interspecific interaction, trait mismatching among interacting species and few species level coevolved traits (Thompson 2005). Consequently, the GMTC suggests the occurrence of a spatial pattern of interaction-mediated local adaptation and maladaptation (defined as deviation from adaptive peaks) (Gomulkiewicz *et al.* 2000, 2007; Nuismer *et al.* 2000; Thompson *et al.* 2002; Thompson 2005).

Theoretical models suggest that the spatial pattern of local adaptations and maladaptations is a complex consequence of the interplay of several non-exclusive factors, including spatially varying selection, patterns of gene flow, but also selection mosaic and relative proportion of hotspots and coldspots (Holt & Gomulkiewicz 1997; Thompson *et al.* 2002; Kawecki & Ebert 2004; Nuismer 2006). Empirical support to these theoretical predictions has come mostly from specialist antagonistic interactions, like parasitism and endophytic herbivory, where the two interacting organisms are tightly engaged in arm races (Van Zandt & Mopper 1998; Hoeksema & Forde 2008). In this kind of system, the outcome of the interaction dramatically changes among localities and causes the occurrence of spatially divergent selection (Kaltz & Shykoff 1998; Mopper & Strauss 1998; Van Zandt & Mopper 1998; Kaltz *et al.* 1999; Kawecki & Ebert 2004). These intense

selection regimes can maintain the levels of local adaptation even under strong gene flow (Kawecki & Ebert 2004).

Contrasting with specialist symbiotic interactions, free-living generalist interactions are formed by multispecies assemblages of interacting organisms that vary spatially in composition but that generate selection with similar strength in all localities. Extreme reciprocal specialization between pairs of species is rare in these interactions (Thompson 1994). In contrast, free-living interactions form interspecific networks (Thompson 2005). Consequently, multispecific selection and diffuse coevolution are prevalent in generalist interactions (Strauss & Irwin 2004; Strauss *et al.* 2005). Under these circumstances, many evolutionary biologists think that local adaptation is less likely in generalist interactions (Lajeunesse & Forbes 2002; Kawecki & Ebert 2004).

In this study, we explore the possibility of local adaptation in a generalist mutualistic system. In 2005, we detected a geographic mosaic of selection occurring for a pollination-generalist plant (*Erysimum mediohispanicum*, Brassicaceae), which inhabits a patchy environment in Sierra Nevada (southeast Spain) (Gómez *et al.* 2009a). This plant is visited by pollinator assemblages differing in composition across nearby populations. Due to between-pollinator differences in preference patterns, this spatial difference in pollinator fauna causes spatially divergent selection on some plant traits (Gómez *et al.* 2008a). As a consequence, the landscape is composed of populations having strong pollinator-mediated selective regimes (evolutionary hotspots, hereafter) mingled with populations having weak pollinator-mediated selective regimes (evolutionary coldspots, hereafter; Gómez *et al.* 2009a). Under these circumstances, we hypothesize that, if gene flow is not high, plants from populations where pollinator-mediated selection is strong (hostpots) will have phenotypes more adapted to attract pollinators than plants from populations were pollinator-mediated selection is weak (coldspots). Note that these hotspots and coldposts are not proper coevolutionary spots but evolutionary spots, as there is not yet information on the reciprocal effects of the plant on the pollinators (Thompson 2005). To investigate local adaptation, we reciprocally translocated plants coming from hotspots and coldspots to quantify their ability in attracting pollinators in each environment. We chose two selective hotspots (Em21 and Em23 populations), and two selective coldspots (Em02 and Em08 populations), where overall pollinator-mediated selection was strong or weak, respectively, during the 2005 selective episode (Fig. 1). Local adaptation was determined by comparing attractiveness of local plants vs. that of foreign plants ('local vs. foreign' criterion) (Lajeunesse & Forbes 2002; Morgan *et al.* 2005). This means that we are considering in this study local adaptation on a population-by-population basis, rather than at the species level.



Selection strength	Strong	Weak	Strong	Weak
Stalk height	**0.163**	**0.442**	**0.229**	0.032
N flowers	**0.499**	**0.622**	**0.036**	0.083
Corolla diameter		–0.033	0.022	
Corolla tube length			**0.061**	
Corolla tube width	**0.079**		0.041	
Corolla shape	**0.149**		**0.256**	
RW1 component	0.012		0.040	
RW2 component	**0.038**		0.009	
RW3 component	0.005		0.001	
RW4 component	**0.039**		**0.079**	

Figure 1 Spatial location of the two hot spots (in red) and the two cold spots (in blue) in Sierra Nevada. Pollinators acting as main selective agents were small bees and beeflies in Em21, beeflies and small bees in Em08, and large and small bees in Em23. Table shows selection strength per population, measured as path coefficient of phenotypic traits on fitness (significant selection is shown in bold).

METHODS

Study system

Erysimum mediohispanicum is a biannual, monocarpic herb endemic to the Iberian Peninsula. In southeast Spain, *E. mediohispanicum* is found in montane forests and subalpine scrublands. Individual plants grow for 2–3 years as vegetative rosettes, and then die after producing 1–8 reproductive stalks bearing up to several hundred hermaphroditic, bright yellow flowers. *Erysimum mediohispanicum* is self-compatible, but requires pollen vectors for full seed set (Gómez 2005). Mean seed dispersal distance is lower than 20 cm (Gómez 2007).

Experimental design

We assessed local adaptation to pollinators using reciprocal field translocations based on previous information on the strength of pollinator-mediated selection across several localities of a selection mosaic (Gómez *et al.* 2009a). Local adaptation was tested for by comparing pollinator attractiveness (pollinator visitation rate) with the experimental plants in each population of destination. Pollinator attractiveness is appropriate to test for local adaptation in *E. mediohispanicum* due to several non-exclusive reasons. First, pollinator abundance at flowers is conceptually similar to parasite abundance at hosts or parasite load, two major metrics to test for local adaptation in host–parasite systems (Kawecki & Ebert 2004; Hoeksema & Forde 2008). Furthermore, pollinator visitation rate is a good proxy of *E. mediohispanicum* fitness in the study area, as it significantly correlates with the overall seed production (Gómez *et al.* 2006, 2008a, 2009a). In fact, we found a significant relationship between pollinator visitation rate and overall number of seeds produced per plant in the four experimental populations [log (seed/plants) = 4.705 + 0.169*visits/hour, $F = 3.46$, $n = 179$ plants, $P = 0.0007$; General Linear Model]. This relationship was similar across the four populations, as the interaction between pollinator abundance and population was not significant ($F = 0.19$, $P = 0.903$; General Linear Model). In addition, pollinator visitation rate is a good estimate of female fitness as, in the study area, *E. mediohispanicum* seed production is pollen-limited. Thus, in an experiment conducted in the studied populations, pollen-supplemented flowers had higher fitness (15.46 ± 0.75 seeds per flower, $n = 136$ plants) than both control (12.67 ± 0.64, $n = 139$ plants) and procedural control flowers (10.77 ± 1.18, $n = 55$ plants; $F = 6.66$, df = 2, 327, $P = 0.0001$; one-way ANOVA; author's unpublished data). Finally, several studies have shown that pollinators are important selective agents for many *E. mediohispanicum* floral traits (Gómez *et al.* 2006, 2008a,b, 2009a).

As focal populations for our experiment, we selected four populations: two populations where overall pollinator-mediated selection was strong during the 2005 selective episode (hotspots, Em21 and Em23), and two populations where overall selection was weak during the 2005 selective episode (coldspots, Em02 and Em08) (Gómez *et al.* 2009a). Em08 was considered as a pollinator-mediated selective coldspot because the observed selection acting on the number of flowers was exclusively due to its direct effect on seed production, rather than its pollinator-mediated effects. In addition, in this latter population much of the selection acting on stalk height was due to its correlation with the number of flowers rather than the pollinator effect (Gómez *et al.* 2009a).

At the end of the 2005 reproductive season, we collected seeds from the plants where selection had been determined in these four populations. The seeds were sowed during autumn 2005 in individual pots and randomly located in a common garden at the Facultad de Ciencias, University of Granada (720 m altitude). Seedlings started to emerge quickly that autumn and were growing until spring 2007, when they started to flower. Then, we randomly selected 20 plants per population (80 in total). Flowering plants were carried out together to the four populations (pollination scenarios) from which the seeds had been collected. Flowering period of the experimental plants overlapped with the natural flowering period of the species in the study area. We used the same 80 plants throughout the experiment, to control for individual phenotype effect in pollinator attraction. In each pollination scenario, the 80 experimental plants were arranged at random in a grid of 20×4 plants separated from each other by 0.5 m. The experiment was conducted during the whole flowering period of the experimental plants (15 days), and plants were in the field every two days, until some experimental plants started to fruit and lose many flowers. The experimental trials started at 10 am, and the plants were offered to pollinators for a 2-h period per pollination scenario, for an overall daily period of 8 h. We randomly changed the order in which each pollination scenario was tested to avoid biases due to circadian differences in pollinator identity and activity. All insects landing on the flowers and contacting anthers and stigma, but not those thieving nectar from below the corolla, were recorded. We visually identified almost all floral visitors to the species level, using previous information gathered during the last 11 years (Gómez *et al.* 2008a,b, 2009a).

Quantification of floral traits

Plant phenotype was characterized in the greenhouse, before the beginning of the experiment, to avoid any putative plastic response of the experimental plants to the new environments. The following phenotypic traits were determined for the experimental plants.

(1) Stalk height, quantified as the height of the tallest stalk, measured to the nearest 0.5 cm as the distance from the ground to the top of the highest open flower.
(2) Flower number, counting the entire production of flowers of each plant.
(3) Corolla diameter, estimated as the distance in millimetre between the edges of two opposite petals.
(4) Corolla tube length, the distance in millimetre between the corolla tube aperture and the base of the sepals. These two latter traits were also measured with a digital caliper with ±0.1 mm resolution.
(5) Corolla tube width, the diameter of the corolla tube aperture, estimated as the distance between the bases of two opposite petals.
(6) Corolla shape, determined in each of the plants by means of geometric morphometric tools, using a landmark-based methodology (Zelditch *et al.* 2004).

We took a digital photograph of the same flower as before using a standardized procedure (front view and planar position). The flowers were photographed at anthesis to avoid ontogenetic effects. We defined 32 co-planar landmarks located along the outline of the flowers and the aperture of the corolla tube, the number of landmarks being chosen to provide comprehensive coverage of the flower shape (Zelditch *et al.* 2004). Landmarks were defined by reference to the midrib, primary veins, and secondary veins of each petal as well as the connection between petals. We captured the landmarks using the software tpsDig vs. 1.4 (available in the Stony Brook Morphometrics website at: http://life.bio.sunysb.edu/morph/morphmet.html). Afterwards, the two-dimensional coordinates of these landmarks were determined for each plant, and the generalized orthogonal least-squares Procrustes average configuration of landmarks was computed using the Generalized Procrustes Analysis (GPA) superimposition method. This procedure was performed using the software tpsRelw vs. 1.11 (available in the Stony Brook Morphometrics website at: http://life.bio.sunysb.edu/morph/morphmet.html). In these analyses, we considered the flower as a non-articulated structure because the relative position of the petals does not change during their functional life. After GPA, the relative warps (RW, which are principal components of the covariance matrix of the partial warp scores) were computed (Adams *et al.* 2004). Unit centroid size was used as the alignment-scaling method and the orthogonal projection as the alignment projection method. This procedure generates a consensus configuration, the central trend of an observed sample of landmarks, which is similar to a multidimensional average. In addition, this procedure generates $2p - 4$ orthogonal RW (p is the number of landmarks). Each RW is characterized by its singular value, and explains a given variation in shape among specimens. Thus, RW summarize shape differences among specimens (Adams *et al.* 2004), and their scores can be saved to be used as a data matrix to perform standard statistical analyses (Zelditch *et al.* 2004).

Analysis of pollinator assemblages

We determined pollinator assemblage in the four studied populations during four years (2005–2008), including the year in which we performed the translocation experiment. For this, we recorded all insects visiting the flowers of *E. mediohispanicum* during 2 h samplings per population. These samplings were distributed throughout the entire flowering period (10–15 days per population). Only insects landing on the flowers and contacting anthers and stigma, but not those thieving nectar from below the corolla, were recorded. In total, we sampled each population at least for 10 h per reproductive season. In total, 3662 insects were recorded in these four populations during the four years.

Pollinators were identified in the field, and samples of each pollinator species were captured for further identification in the laboratory (Gómez *et al.* 2009a). Nevertheless, we did not collect many specimens, to avoid a depletion of the insect populations. Some closely related species (notably among Apiformes) could not be told apart in the field, and thus some of our taxa include more than one species. We grouped pollinator species into seven functional groups according to their similarity in size, foraging behaviour and feeding habits: (i) large bees, mostly pollen- and nectar-collecting females measuring 10 mm in body length or larger; (ii) small bees, mostly pollen- and nectar-collecting females smaller than 10 mm; (iii) beeflies, long-tongued nectar-collecting Bombyliidae; (iv) hoverflies, nectar- and pollen-collecting Syrphidae and short-tongued Bombyliidae; (v) beetles, including species collecting nectar and/or pollen; (vi) butterflies, mostly Ropalocera, all nectar collectors; and (vii) Others, mostly nectar-feeding Muscoid flies, ants and bugs.

Genetic analyses

We analysed molecular markers in order to evaluate the genetic differentiation and infer the historic gene flow among the experimental populations. Sixty-five milligram of silica-gel-dried leaf tissue per individual was disrupted in liquid nitrogen. DNA was extracted using the GenElute™ Plant Genomic DNA Miniprep Kit (Sigma-Aldrich, St Louis, MO, USA).

We determined randomly amplified polymorphic DNA (RAPD) in 10 individuals from each plant population (40 individuals in total). RAPD amplification was performed using Operon primers (Operon Technologies, Alameda, CA, USA): OPA01 (5′-CAGGCCCTTC-3′), OPA02 (5′-TG CCGAGCTG-3′), OPA03 (5′-AGTCAGCCAC-3′), OPA04

(5′- AATCGGGCTG-3′), OPA06 (5′-GGTCCCTGAC-3′), OPA07 (5′-GAAACGGGTG-3′), OPA08 (5′-GTGACG-TAGG-3′), OPA09 (5′-GGGTAACGCC-3′), OPA10(5′-GTGATCGCAG-3′), OPA11 (5′-CAATCGCCGT-3′), OPA12 (5′-TCGGCGATAG-3′), OPA13 (5′-CAGCACC-CAC-3′), OPA14 (5′-TCTGTGCTGG-3′), OPA19 (5′-CAAACGTCGG-3′), OPA20 (5′-GTTGCGATCC-3′).

The following 13 primer combinations were used: OPA01–OPA01, OPA01–OPA03, OPA01–OPA06, OPA01–OPA07, OPA02–OPA08, OPA04–OPA14, OPA07–OPA14, OPA08–OPA13, OPA09–OPA12, OPA09–OPA19, OPA10–OPA10, OPA11–OPA12 and OPA19–OPA20. Polymerase chain reaction (PCR) was performed in 25 μL final volume containing 2.5 μL of 10× buffer (New England Biolabs, Beverly, MA, USA), 2.5 μL of 2 mM dNTP (Sigma-Aldrich), 1.25 μL of each 10 μM primer, 0.4 μL of 5 U μL^{-1} Taq-polymerase (New England BioLabs) and 1 μL of 10 ng μL^{-1} DNA. PCR was performed using a MJ Mini Personal Thermal Cycler (Bio-Rad, Richmond, CA, USA) with the following profile: 2 min at 94 °C, followed by 38 cycles of 15 s at 94 °C, 15 s at 36 °C, 30 s at 72 °C, followed by 2 min at 72 °C. RAPD products were electrophoresed in 1.5% agarose gels containing SYBR-Green in 1× TBE buffer at 4.7 V cm^{-1}, and photographed with a Gel Doc ™ XR (Bio-Rad). We scored 160 different markers, determined by using a standard ladder (50 bp; Sigma) with the Quantity One-4,6 1D Analysis Software. Weaker bands were omitted from the scoring protocol and some of the amplifications were repeated to control for repeatability. The bands were scored for each primer as either present (1) or absent (0). Nei genetic distances were determined with GenAlEx 6.1 (http://www.anu.edu.au/BoZo/GenAlEx/) and Gst with Hickory (Holsinger *et al.* 2002).

Analysis of cpDNA trnL-F region was performed in five individuals per population (20 individuals in total), using tabC and tabF primers (Taberlet *et al.* 1991). PCR was carried out in a final volume of 50 μL containing 5 μL of 10× buffer (New England BioLabs), 0.1 mM of each dNTP (Sigma-Aldrich), and 0.02 U μM^{-1} of *Taq* (New England BioLabs). PCR was carried out on a Gradient Master Cycler Pro S (Eppendorf, Westbury, NY, USA) as follows: one cycle at 94 °C for 3 min, 35 cycles of 94 °C for 15 s, 58 °C for 30 s, and 72 °C for 1 min 30 s, and a final cycle at 72 °C for 3 min; then, 4 °C until reactions were taken off the thermocycler. PCR products were electrophoresed on 1.5% agarose gel to check the results and then the amplification products were purified by centrifugation at 4 °C with 0.15 volume sodium acetate 3 M (pH 4.6) and three volumes of 95% ethanol. Sequencing was done with BigDye-Terminator-v3.1 (Applied Biosystems, Foster City, CA, USA), using tabF primer and the fragments were visualized on an ABI PRISM 3100-avant automated sequencer. Chromatograms were visualized with Finch TV version 1.4.0 (Geospiza Inc., Seattle, WA, USA). Sequences were adjusted and aligned by hand, using bioedit version 7.0.5.3 (Hall 1999). All positions, including indel regions, were used as input to estimate distances among populations with Arlequin version 3.1 (CMPG, University of Berna). We used this region because of its high variability among individuals, even at the intrapopulation level.

Data analysis

To test for local adaptation, we used a Generalized Mixed Model where we included the type of population of origin (hotspot vs. coldspot), destination and their interaction as fixed factors, population identity as random factor nested in the two previous fixed factors, and visitation rate of pollinators as the dependent variable. The models were performed for all pollinators pooled together and for each pollinator functional type separately. All analyses were performed using the package lme4 in R (R Development Core Team 2008).

We performed a canonical discriminant function analysis to explore between-population differences in plant phenotype. In this analysis, the different phenotypic traits quantified for each experimental plant was included as the dependent variable. A discriminant function analysis was also used to classify the four studied populations according to the composition, in terms of functional groups, of their pollinator fauna. In this analysis, we included the abundance of each pollinator functional group as dependent variables, population as independent variable and the annual data as observation units. After that, using the Mahalanobis distances, we predicted the probability of each experimental population of destination belonging to the actual population where experimental plants were located according to the pollinator assemblage composition.

The effect of plant phenotype on pollinator attraction was also analysed with a Generalized Linear Model, including all phenotypic traits and the type of population of origin as explanatory variables and visitation rate of pollinators as the dependent variable. As the dependent variable was, in all cases, the number of insects per experimental plant, we fitted its residuals to a Poisson distribution with log as the link function. The models were performed for all pollinators pooled together and for each pollinator functional type separately. All analyses were performed using the package lme4 in R (R Development Core Team 2008).

The relationship between genetic distances and pollinator attractiveness differences across populations was tested by Mantel test using the package vegan in R (R Development Core Team 2008). Between-population differences in attractiveness were calculated as the Euclidean pairwise differences in the number of pollinators visiting the plants at each experimental population.

RESULTS

There were between-population differences in the phenotype of the experimental plants (Wilk's $\lambda = 0.26$, df = 24, 186.22, $P < 0.0001$, discriminant analysis). Hotspot plants were clearly different from the coldspot ones (Fig. 2). Specifically, Em21 plants had short flowering stalks, with small flowers having long and narrow corolla tubes and positive corolla-shape RW1 component, whereas Em23 plants had short flowering stalks and large flowers, Em02 plants had flowers with wide corolla tubes and Em08 plants had long flowering stalks and flowers with positive corolla-shape RW3 component (Fig. 2; see Table S1).

The four populations used in this study differed in the composition of the pollinator assemblage during the period 2005–2008 (Wilk's $\lambda = 4.81 \times 10^{-9}$, df = 27, 3.56, $P < 0.0001$; discriminant analysis). In general, although some interannual differences were found, pollinator assemblage was dominated by beetles and others (mostly Muscoid flies) in Em02, beeflies, beetles, and large bees in Em08, small bees, large bees, and others in Em21 and large bees and beetles in Em23 (see Fig. S1).

Genetic differentiation among populations was also high, based on both nuclear markers (Bayesian $G_{st} = 0.27 \pm 0.02$ based on 160 RAPD) and plastidial haplotypes ($F_{st} = 0.35$ based on trnL-trnF cpDNA).

We recorded a total of 1158 visits from 52 insect species to the experimental plants (Table S2). The pollinator fauna visiting the experimental plants differed between populations of destination ($\chi^2 = 4100.5$, $P < 0.0001 \pm 0.00001$; Monte Carlo contingency test based on 1 00 000 permutations). The most abundant floral visitors were Muscoid flies and beetles in Em02 (78.4% and 19.8% of visits, respectively), beeflies and large bees in Em08 (32.1% and 28.4%, respectively), large and small bees in Em21 (40.0% and 33.3%), and large bees in Em23 (82.9%). The identity of the main pollinators was similar to that found in the same localities in the previous years ($P > 0.95$; discriminant analysis, see Fig. S2).

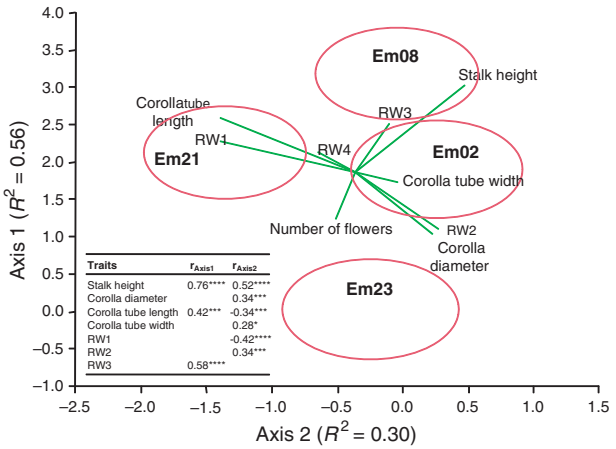


Figure 2 Outcome of the canonical discriminant analysis showing between-population differences in plant phenotype. The circles indicate the 95% confidence interval for each population. Significant Pearson correlations between traits and eigenvalues of the two main axes are shown. For a given variable, the length and direction of each ray indicate the correlation magnitude and sign of that variable with both the axes.

Our experiment showed that the attractiveness of the plants (estimated as insect visits per flower per hour) to all pollinators, pooled together, depended on the pollination scenario (Table 1). Plants were visited more when placed in hotspots than when placed in coldspots (Fig. 3). We found that pollination scenario significantly affected the visitation rate of all pollinator functional groups when analysed separately, except beeflies (Table 1). But, in this case, different pollinator groups were differentially attracted in different scenarios. Large and small bees visited the experimental plants mostly in hotspots, whereas hoverflies, beetles and Muscoid flies visited plants mostly in coldspots (Fig. 3; see Fig. S3 for details).

Most importantly, our experiment showed that plant attractiveness to all pollinators, pooled together, depended on origin (Table 1). Hotspot plants were more visited in both hotspot and coldspot scenarios (Fig. 3; see details on individual populations in Fig. S2). In all cases except Muscoid flies, when hotspot plants were local, they were more attractive than foreign plants. When coldspot plants were local, however, they were generally less attractive than foreign plants for all pollinators. Differences in attractiveness between populations were not associated with genetic distances ($r_m = -0.54$, $P = 0.83$ for RAPD and $r_m = -0.45$, $P = 0.89$ for cpDNA trnF-L, Mantel tests).

We found that some phenotypic traits of the experimental plants were positively associated with the visitation rate of large bees, small bees, and beeflies (Table 2). Large bees were significantly attracted to plants with large number of flowers, deep corolla tubes and negative values of the corolla-shape RW2 component (Table 2). Small bees chose plants with short flowering stalks, many flowers, deep and narrow corolla tubes, positive values of corolla-shape RW1 component, and negative values of corolla-shape RW2 component (Table 2). Finally, beeflies chose plants with many flowers, short flowering stalks, and large flowers (Table 2).

DISCUSSION

There were significant differences in the phenotype of the plants from different experimental populations. In addition,

Table 1 Outcome of the Generalized Linear Mixed Models testing the effect of origin and destination in plant attractiveness to main pollinator functional groups, quantified as number of visits per flower and 4 h. Values are likelihood ratio tests. Dependent variable was fitted to a Poisson distribution (log as link function), and all variables were standardized variables prior analyses

Source	df	Total	Large bees	Small bees	Beeflies	Hoverflies	Muscoids	Beetles
Origin	1	14.69****	10.84****	5.36**	5.71**	4.7*	0.97	5.19**
Destination	1	10.85****	36.46****	6.43**	0.57	5.86**	23.71****	10.18***
Origin*destination	1	1.83	3.88*	3.22	0.88	3.37	1.02	3.73*
Population [origin]	2	2.88	1.85	0.93	0.27	1.20	2.64	0.35
Population [destination]	2	11.46**	10.61**	22.43***	27.21***	21.26****	0.78	8.66*
Deviance	300	2.28***	0.57****	0.28****	0.16****	0.01	0.0099	0.04***

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.

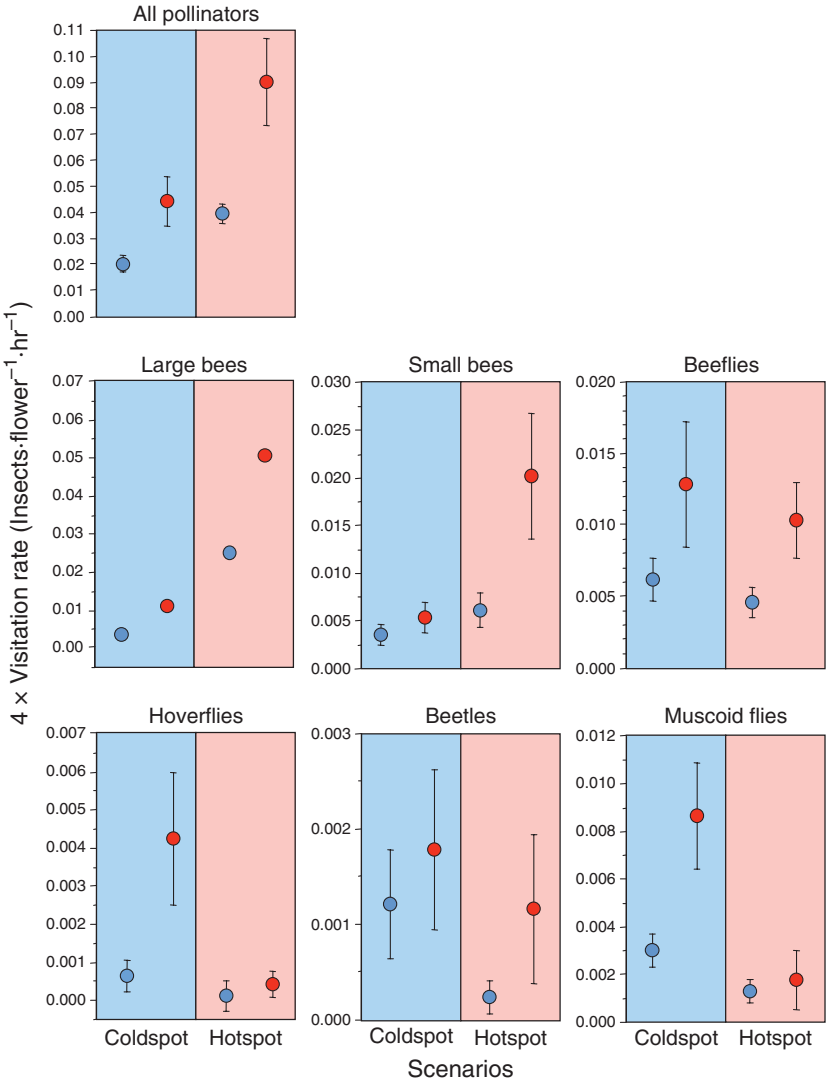


Figure 3 Outcome of the translocation experiment. Charts show the attractiveness (estimated as mean ± 1SD pollinator visitation rate) of plants from different origin (blue dots, coldspots; red dots, hotspots) in each of the two scenarios (blue background, coldspots; red background, hotspots). Note the difference in *Y*-scale between the panels. The log-likelihood ratio tests from the Generalized Linear Mixed Models are also shown (*O*, population of origin; *D*, population of destination; $O \times D$: interaction term; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$).

Table 2 Outcome of the Generalized Linear Mixed Models relating the phenotype of the experimental plants with the visitation rate by the main pollinators. The dependent variable was fitted to a Poisson distribution, because it was referred as number of insects. All the independent variables were standardized to mean = zero and SD = 1. The factor origin was tested by deviance differences between the full and partial models

	Large bees		Small bees		Beeflies		Beetles		Hoverflies		Muscoid flies	
	β ± SE	χ^2	β ± SE	χ^2	β ± SE	χ^2	β ± SE	χ^2	β ± SE	χ^2	β ± SE	χ^2
Origin		29.81*****		15.02***		15.45***		9.12*		4.95		2.32
Stalk height	−0.09 ± 0.06	2.90^{ns}	−0.38 ± 0.10	14.20****	−0.45 ± 0.10	21.78****	−0.17 ± 0.24	0.56	−0.44 ± 0.24	3.68ms	−0.19 ± 0.15	1.66
Number of flowers	0.15 ± 0.05	8.00***	0.35 ± 0.08	15.98****	0.38 ± 0.08	18.77****	0.36 ± 0.20	2.91^{ns}	0.16 ± 0.22	0.47	0.24 ± 0.13	3.51^{ns}
Corolla diameter	−0.02 ± 0.03	0.23	−0.07 ± 0.09	0.62	0.19 ± 0.09	4.15*	0.29 ± 0.23	1.54	0.27 ± 0.20	1.74	0.16 ± 0.13	1.36
Corolla tube length	0.12 ± 0.06	4.96*	0.31 ± 0.10	10.21***	0.17 ± 0.10	2.87^{ns}	0.07 ± 0.25	0.07	0.03 ± 0.22	0.01	−0.03 ± 0.14	0.05
Corolla tube width	−0.06 ± 0.05	1.51	−0.31 ± 0.08	17.29****	−0.11 ± 0.08	1.55	0.23 ± 0.19	1.52	−0.15 ± 0.19	0.67	−0.35 ± 0.12	9.12**
RW1	0.42 ± 0.68	0.38	0.27 ± 0.11	6.81***	0.14 ± 0.11	1.47	−0.29 ± 0.28	1.06	0.15 ± 0.23	0.44	−0.15 ± 0.15	1.00
RW2	−0.25 ± 0.08	8.81***	−0.28 ± 0.13	4.47*	−0.12 ± 0.14	0.73	0.31 ± 0.35	0.77	0.20 ± 0.31	0.01	0.24 ± 0.20	1.41
RW3	−0.32 ± 0.11	0.08	−0.27 ± 0.16	3.17ms	0.80 ± 0.18	0.19	0.27 ± 0.54	0.25	−0.21 ± 0.43	0.24	−0.63 ± 0.27	0.01
RW4	−0.17 ± 0.16	1.08	−0.33 ± 0.25	1.69	0.31 ± 0.25	1.52	−0.12 ± 0.06	3.96	−0.32 ± 0.55	0.33	−0.25 ± 0.37	0.88

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$, ms = marginally significant.

there were also between-population differences in the pollinator assemblages visiting the experimental plants, an outcome suggesting that the populations of destination used in our experiment can be considered as different selective scenarios even during the year we performed the experiment.

Our translocation experiment has shown that hotspot plants were more visited than coldspot plants both in local and foreign scenarios. Because pollinator visitation rate is significantly related with plant fitness, *E. mediohispanicum* seems to be pollen-limited in the study area, and pollinators are important selective agents in our plant species (Gómez *et al.* 2008b, 2009a). This outcome strongly suggests local adaptation for hotspot plants and maladaptation for coldspot plants. In addition, our study has shown that differences in attractiveness between populations were not associated with genetic distances, indicating that genetic similarity is not a main factor explaining the observed local pattern of adaptation, as gene flow is probably not high enough to swamp local selection. Consequently, the attractiveness of a floral phenotype seems to be linked almost exclusively to the occurrence of a selective hotspot. Our experiment was able to detect the specific phenotypic traits mediating *E. mediohispanicum* local adaptation to its pollinators. In fact, some phenotypic traits of the experimental plants were positively associated with the visitation rate of main *E. mediohispanicum* pollinators, such as large bees, small bees, and beeflies. In general, the preference pattern of these pollinators coincides with the previously reported pattern for these floral visitors (Gómez *et al.* 2008a,b). Remarkably, most traits preferred by bees and beeflies, two very effective pollinators for many plant species (Proctor *et al.* 1996), were also those discriminating between hotspots and coldspots in *E. mediohispanicum*. In addition, many traits preferred by these pollinators were also under significant pollinator-mediated selection during the 2005 selective episode (Gómez *et al.* 2009a). These findings suggest that the plant traits conferring attractiveness to the experimental plants were those selected by pollinators during the parental selective episode. All of this suggests a correspondence between the adaptation degree displayed by the experimental plants to pollinators and the overall selection strength experienced by their mother plants. Plants coming from more selective environments presumably produced better adapted offspring. The fast response of *E. mediohispanicum* to the selection exerted by pollinators is favoured by the significant heritability found for the phenotypic traits in the study populations (Gómez *et al.* 2009b).

Different *E. mediohispanicum* populations are, in the study area, undergoing different selective regimes. This spatial variation generates the occurrence of a geographic mosaic of selection, where some populations are selective hotspots

whereas others are selective coldspots (Gómez *et al.* 2009a). Under these circumstances, as the current experiment suggests an association between selection strength and adaptation, the landscape can be visualized for *E. mediohispanicum* as a mosaic of populations, some of them constituted by plants displaying attractive phenotypes and others composed of plants with non-attractive phenotype. Long-term persistence of this spatial pattern of adaptation requires, nevertheless, temporal stability in the pollinating selective agents across localities (Nuismer *et al.* 2000). This situation has been demonstrated for some highly specialist mutualisms like the interaction between *Greya politella* and *Lithophragma parviflorum* (Thompson & Fernandez 2006) and between mycorrhizal fungi and nitrogen-fixing bacteria (Parker 1999). However, reaching this kind of stability in the spatial pattern of adaptation and maladaptation is unlikely in generalist systems, as in these systems there is frequent temporal variation in the identity and abundance of the most important selective agents that causes strong fluctuations in the selective regimes (Waser *et al.* 1996; Gómez & Zamora 2006; but see Alcántara *et al.* 2007). In contrast, we postulate that in generalist systems, the occurrence of spatially and temporally shifting patterns of local adaptations and maladaptations is more probable. However, further studies are necessary to test this possibility.

Spatially structured adaptation has been detected mostly at large spatial scales (Lajeunesse & Forbes 2002; Alcántara *et al.* 2007). However, in this study we have found local adaptation at a very small spatial scale, as experimental populations were separated by only hundreds of metres. Small spatial scale local adaptation is favoured when gene flow, a main factor preventing local adaptation (Lajeunesse & Forbes 2002; Savolainen *et al.* 2007), is restricted. In fact, small-scale local adaptation has been found only for pathosystems where gene flow is very limited (Capelle & Neema 2005). In *E. mediohispanicum,* gene flow via seed is probably low, as seed dispersal is very short (usually less than 0.5 m, Gómez 2007). We do not have information about the extent of pollen flow in this plant species, as it is pollinated by many different insects, some of them showing high movement ability (i.e. *Anthophora* spp., *Osmia* spp., *Macroglossum stellatarum*, etc.), but many others displaying narrow home ranges (i.e. *Dasytes* spp., *Bombylius* spp., *Malachius* spp., etc.). Nevertheless, the high genetic differentiation detected among populations suggests that, in spite of their relative proximity, gene flow among experimental populations is low.

Two important consequences emerge from our study. First, local adaptation is not exclusive of specialist interactions. A geographically structured pattern of local adaptation and maladaptation can also be associated to generalist facultative mutualisms where multispecific selection is prevalent (Gómez *et al.* 2009a). In these systems, despite a species interacting simultaneously with multiple species, the interaction with a spatially variable subset of organisms exerting strong selection may eventually lead to divergent selection (Thompson 2005; Gómez *et al.* 2009a). Second, the spatial pattern of adaptation can operate at fairly small spatial scales, highlighting the importance of considering the microscale as a relevant template to study evolutionary processes in generalist systems. We presume that this outcome is indeed more likely in generalist than in specialist systems, as a slight modification in the community of organisms interacting with generalist species can have intense effects in the overall interaction outcome. Considering these two consequences in future, empirical and theoretical studies will surely contribute to broadening the conceptual framework of the geographical mosaic of coevolution.

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REFERENCES

Adams, D.C., Rohlf, F.J. & Slice, D.E. (2004). Geometric morphometrics: ten years of progress following the 'revolution'. *Ital. J. Zool.*, 71, 5–16.

Alcántara, J., Rey, P., Manzaneda, A., Boulay, R., Ramírez, J. & Fedriani, J. (2007). Geographic variation in the adaptive landscape for seed size at dispersal in the myrmecochorus *Helleborus foetidus*. *Evol. Ecol.*, 21, 411–430.

Capelle, J. & Neema, C. (2005). Local adaptation and population structure at a micro-geographical scale of a fungal parasite on its host plant. *J. Evol. Biol.*, 18, 1445–1454.

Gómez, J.M. (2005). Non-additive effects of pollinators and herbivores on *Erysimum mediohispanicum* (Cruciferae) fitness. *Oecologia*, 143, 412–418.

Gómez, J.M. (2007). Dispersal-mediated selection on plant height in an autochorously-dispersed herb. *Plant. Syst. Evol.*, 268, 119–130.

Gómez, J.M. & Zamora, R. (2006). Ecological factors that promote the evolution of generalization in pollination systems. In: *Plant-pollinator interactions, from specialization to generalization* (eds Waser, N.M. & Ollerton, J.). Univ of Chicago Press, Chicago, pp. 145–165.

Gómez, J.M., Perfectti, F. & Camacho, J.P.M. (2006). Natural selection on *Erysimum mediohispanicum* flower shape: insights into the evolution of zygomorphy. *Am. Nat.*, 168, 531–545.

Gómez, J.M., Bosch, J., Perfectti, F., Fernández, J.D., Abdelaziz, M. & Camacho, J.P.M. (2008a). Spatial variation in selection on corolla shape in a generalist plant is promoted by the preference of its local pollinators. *Proc. R. Soc. Lond. B*, 275, 2241–2249.

Gómez, J.M., Bosch, J., Perfectti, F., Fernández, J.D., Abdelaziz, M. & Camacho, J.P.M. (2008b). Association between floral traits and reward in *Erysimum mediohispanicum* (Brassicaceae). *Ann. Bot.*, 101, 1413–1420.

Gómez, J.M., Perfectti, F., Bosch, J. & Camacho, J.P.M. (2009a). A geographic selection mosaic in a generalized plant–pollinator–herbivore system. *Ecol. Monogr.*, 79, 245–263.

Gómez, J.M., Abdelaziz, M., Muñoz-Pajares, A.J. & Perfectti, F. (2009b). Heritability and genetic correlation of corolla shape and size in *Erysimum mediohispanicum*. *Evolution*, in press.

Gomulkiewicz, R., Thompson, J.N., Holt, R.D., Nuismer, S.L. & Hochberd, M.E. (2000). Hotspots, coldspots, and the geographic mosaic theory of coevolution. *Am. Nat.*, 156, 156–174.

Gomulkiewicz, R., Drown, D.M., Dybdahl, M.F., Godsoe, W., Nuismer, S.L., Pepin, K.M. *et al.* (2007). Dos and don'ts of testing the geographic mosaic theory of coevolution. *Heredity*, 98, 249–258.

Hall, T.A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acid. Symp. Ser.*, 41, 95–98.

Hoeksema, J.D. & Forde, S.E. (2008). A meta-analysis of factors affecting local adaptation. *Am. Nat.*, 171, 275–290.

Holsinger, K.E., Lewis, P.O. & Dey, D.K. (2002). A Bayesian method for analysis of genetic population structure with dominant marker data. *Mol. Ecol.*, 11, 1157–1164.

Holt, R.D. & Gomulkiewicz, R. (1997). How does immigration influence local adaptation? A reexamination of a familiar paradigm. *Am. Nat.*, 149, 563–572.

Kaltz, O. & Shykoff, J.A. (1998). Local adaptation in host–parasite systems. *Heredity*, 81, 361–370.

Kaltz, O., Gandon, S., Michalakis, Y. & Shykoff, J.A. (1999). Local maladaptation in the anther-smut fungus *Microbotryum violaceum* to its host plant *Silene latifolia*: evidence from a cross-inoculation experiment. *Evolution*, 53, 395–407.

Kawecki, T.J. & Ebert, D. (2004). Conceptual issues in local adaptation. *Ecol. Lett.*, 7, 1225–1241.

Lajeunesse, M.J. & Forbes, M.R. (2002). Host range and local parasite adaptation. *Proc. R. Soc. Lond. B*, 269, 703–710.

Mopper, S. & Strauss, S.Y. (1998). *Genetic Structure and Local Adaptation in Natural Insect Populations: Effects of Ecology, Life History, and Behavior*. Chapman and Hall, New York.

Morgan, A., Gandon, S. & Buckling, A. (2005). The effect of migration on local adaptation in a coevolving host–parasite system. *Nature*, 437, 253–256.

Nuismer, S.L. (2006). Parasite local adaptation in a geographic mosaic. *Evolution*, 60, 24–30.

Nuismer, S.L., Thompson, J.N. & Gomulkiewicz, R. (2000). Coevolutionary clines across selection mosaic. *Evolution*, 54, 1102–1115.

Parker, M.A. (1999). Mutualism in metapopulations of legumes and rhizobia. *Am. Nat.*, 153, S48–S60.

Proctor, M., Lack, A. & Yeo, P. (1996). *The Natural History of Pollination*. Harper Collins, New York.

R Development Core Team (2008). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna; Url: http://www.R-project.org.

Savolainen, O.T., Häjärvi, P. & Knürr, T. (2007). Gene flow and local adaptation in trees. *Ann. Rev. Ecol. Evol. Syst.*, 38, 595–619.

Strauss, S.Y. & Irwin, R.E. (2004). Ecological and evolutionary consequences of multispecies plant–animal interactions. *Ann. Rev. Ecol. Evol. Syst.*, 35, 435–466.

Strauss, S.Y., Sahli, H. & Conner, J.K. (2005). Toward a more trait-centered approach to diffuse (co)evolution. *New Phytol.*, 165, 81–90.

Taberlet, P., Gielly, L., Pautou, G. & Bouvet, J. (1991). Universal primers for amplification of three non-coding regions of chloroplast DNA. *Mol. Plant Biol.*, 17, 1105–1109.

Thompson, J.N. (1994). *The Coevolutionary Process*. The University of Chicago Press, Chicago, USA.

Thompson, J.N. (2005). *The Geographic Mosaic of Coevolution*. The University of Chicago Press, Chicago, USA.

Thompson, J.N. & Fernandez, C.C. (2006). Temporal dynamics of antagonism and mutualism in a geographically variable plant–insect interaction. *Ecology*, 87, 103–112.

Thompson, J.N., Nuismer, S.L. & Gomulkiewicz, R. (2002). Coevolution and maladaptation. *Integr. Comp. Biol.*, 42, 381–387.

Van Zandt, P.A. & Mopper, S. (1998). A meta-analysis of adaptive deme formation in phytophagous insect populations. *Am. Nat.*, 152, 595–604.

Waser, N.M., Chitkka, L., Price, M.V., Williams, N.M. & Ollerton, J. (1996). Generalization in pollination systems, and why it matters. *Ecology*, 77, 1043–1060.

Zelditch, M.L., Swiderski, D.L., Sheets, H.D. & Fink, W.L. (2004). *Geometric Morphometrics for Biologists: A Primer*. Elsevier Academic Press, San Diego.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1 Proportion of visits made by insects, belonging to each functional group, to natural plants of *Erysimum mediohispancum* during 4 years (2005–2008) and to experimental plants used in the transplant experiment. Others were Muscoid flies in the experimental plants, but comprise muscoid flies, ants, bugs, and some grasshoppers in the natural populations.

Figure S2 Outcome of the canonical discriminant analysis showing between-population differences in pollinator assemblages. The circles indicate the 95% confidence interval for each population. For a given variable, the length and direction of each ray indicate the correlation magnitude and sign of that variable with both the axes. The red circles refer to the position in the space of the four experimental populations. The squared Mahalanobis distances from the position of each experimental population to the centroid of its corresponding population is shown in the graph.

Figure S3 Outcome of the translocation experiment considering the four studied population. Figures show the mean (±1SD) visitation rate by pollinators to plants from different

origin in each of the four populations of destination. The uppermost rightmost panel shows the average visitation rate to plants from different origin, pooling together all populations of destination. Different superscript letters indicate statistical significance at $\alpha < 0.05$. Red dots indicate hot spots, whereas blue dots indicate cold spots. Note the difference in *Y*-scale between panels. The log-likelihood ratio tests from the Generalized Mixed Models are also shown (*O*, population of origin; *D*, population of destination; $O \times D$: interaction term; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$).

Table S1 Among-population differences in plant phenotypic traits ($n = 20$ plants per population). Figures are mean ± 1SE.

Table S2 Composition of the pollinator assemblage visiting the flowers of *Erysimum mediohispanicum* during the experiment. Figures represent the number of visits of each pollinator species to plants from different origin (pooling together all populations of destination).

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