

A geographic selection mosaic in a generalized plant–pollinator–herbivore system

J. M. GÓMEZ,^{1,4} F. PERFECTTI,² J. BOSCH,³ AND J. P. M. CAMACHO²

¹Departamento de Ecología, Universidad de Granada, 18071 Granada, Spain

²Departamento de Genética, Universidad de Granada, 18071 Granada, Spain

³Ecologia—CREAF, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain

Abstract. The concept of Selection Mosaic is central to the Geographic Mosaic Theory of Coevolution. Most information on coevolving interactions, however, comes from specialized organisms. In contrast, an accurate understanding of the effect of geographically varying evolutionary dynamics on the evolution of generalist organisms is lacking, although these kinds of organisms are the most frequent in nature. In flowering plants, pollinators and herbivores are important selective agents. In this study we investigate whether a geographic selection mosaic for floral traits in a generalist plant, *Erysimum mediohispanicum* (Brassicaceae), can be mediated by the interplay of mutualistic and antagonistic interacting organisms. In eight populations we quantified the selection exerted by these organisms on several plant traits. We found significant spatial variation in pollinator assemblage. In different populations, the main pollinators belonged to different functional groups (beeflies, large bees, small bees, and beetles). Damage by ungulates also varied among populations. Consequently, we found that different populations were under different selective regimes, and the traits affected by selection depended on the local interaction intensity with pollinators and mammal herbivores. Some traits, such as flower number and stalk height, were selected similarly in most populations. Other traits, such as corolla diameter and tube length, were selected only in some populations. Finally, we found divergent selection for some traits, such as corolla tube width and corolla shape, which were selected in contrasting directions in different localities. This spatial variation in selective scenarios results in populations with strong selective regimes (hot spots) intermingled with populations with weak selective regimes (cold spots). Four important outcomes emerge from the *E. mediohispanicum* selection mosaic. (1) Interactions with generalist organisms may produce strong selection. (2) Spatial changes in main pollinators result in divergent selection across populations. (3) Geographic mosaics depend on a balance between mutualistic and antagonistic selection. (4) Selection mosaics operate at fairly small spatial scales. These findings will surely contribute to expanding the conceptual framework of the Geographic Mosaic Theory of Coevolution.

Key words: corolla shape evolution; diffuse selection; divergent selection; *Erysimum mediohispanicum*; geographic mosaic theory of coevolution; herbivores; multispecific systems; pollinators; structural equation modeling.

INTRODUCTION

According to the Geographic Mosaic Theory of Coevolution, three processes lead coevolutionary dynamics: geographic selection mosaics, intermingled coevolutionary hot and cold spots, and trait remixing (Thompson 2005). The influence of these processes has been supported by theoretical models (Gomulkiewicz et al. 2000, 2007), and empirically validated for some mutualistic and antagonistic systems (Thompson 2005). Most information on coevolving interactions, however, has been collected from specialized systems in which interspecific interactions are tightly fitted (Thompson 2005, Hoeksema and Thompson 2007). In contrast, an

accurate understanding of how geographically varying evolutionary dynamics shape the phenotypic evolution of generalist organisms is still lacking (Lapchin 2002, Thompson 2005), although these kinds of organisms are the most frequent in nature.

Spatial variation in the strength and outcome of the interactions with organisms that act as selective pressures is a primary factor determining the existence of geographic mosaics of selection (Gómez and Zamora 2000, Thompson and Cunningham 2002, Rudgers and Strauss 2004, Thompson 2005, Rey et al. 2006, Thompson and Fernández 2006, Siepielski and Benkman 2007, Toju 2007). In flowering plants, pollinators are a main agent of selection. Plants actually interact with pollinator assemblages that often vary geographically (Aigner 2005, Moeller 2005, 2006, Price et al. 2005). Since different pollinators have different morphologies, foraging behavior, and flower trait prefer-

Manuscript received 12 March 2008; revised 28 April 2008; accepted 13 May 2008. Corresponding Editor (ad hoc): J. N. Thompson.

⁴ E-mail: jmgreyes@ugr.es

ences, a probable consequence of this pollinator variation is the occurrence of a concomitant geographic selection mosaic (Gómez and Zamora 2000, Herrera et al. 2006). Geographic mosaics of selection have effectively been shown for some pollinator-specialist plants. In these systems, spatial variation in interaction is caused either by an overall turnover of the pollinator fauna or by phenotypic variation of the few species pollinating the plant (Thompson and Cunningham 2002, Thompson and Fernández 2006, Anderson and Johnson 2008). The scenario is far more complex for pollinator-generalist plants. In these systems, geographic variation in pollination results from changes in composition, abundance, and diversity of various pollinator groups (Price et al. 2005). That is, different populations share the same pollinator fauna but receive different proportions of the visits from each species or functional group (Fenster et al. 2004). Pollinator-dependent foraging preferences may then cause shifts in the optimal floral phenotypes (Wilson and Thompson 1996, Aigner 2005, 2006). Under these conditions, local adjustment is a consequence of the quantitative spatial structure of the pollinator assemblage, rather than the qualitative spatial turnover of pollinators (Wilson and Thompson 1996, Dilley et al. 2000, Thompson 2005). Although among-population variation in pollinators is common in pollinator-generalist species, very few studies have addressed whether this variation does promote the occurrence of a geographic selection mosaic (Gómez and Zamora 2000, Aigner 2005).

Plants also interact with antagonistic organisms that often cause conflicting selection on pollinator-selected traits (Strauss and Irwin 2004, Gómez and Zamora 2006, Strauss and Whittall 2006). Adaptations to avoid herbivory may then constrain the evolution of plant–pollinator interactions, since the advantages associated with pollination may be countered by the costs associated with herbivore deterrence (Strauss 1997, Strauss and Irwin 2004, Strauss and Whittall 2006). When both kinds of interactors select for similar plant traits, a trade-off between the fitness functions generated by pollinators and herbivores may appear (Strauss et al. 1999, Gómez 2008). Pollination-mediated selection may thus be cancelled by an overwhelming effect of herbivory. Empirical evidence of herbivore–pollinator selective conflicts is increasing, and several plant traits are currently thought of as the evolutionary result of conflicting selective pressures exerted by these two kinds of organisms (Brody 1997, Strauss 1997, Strauss and Whittall 2006). Under these circumstances, the structure of a pollinator-mediated geographic selection mosaic is likely to be also affected by spatial variation of herbivores (Thompson 2005, Thompson and Fernández 2006).

The aim of this study is to investigate whether a geographic selection mosaic for floral traits in a generalist plant can be mediated by the interplay of mutualistic and antagonistic interacting organisms. We

used as a model system the herb *Erysimum mediohispanicum* (Brassicaceae). This species is pollinated by a diverse array of pollinators comprising more than 130 species, including solitary bees, long-tongued bee flies, short-tongued flies, beetles, and butterflies (Gómez 2005a, Gómez et al. 2007). Given the open morphology of *E. mediohispanicum* flowers, insect visitor's contact with the flower's reproductive organs is virtually unavoidable, and therefore most flower visitors act as effective pollinators to some degree (Gómez 2003, 2005b). In addition, pollinators exert significant selection on several floral traits such as stalk height, flower number, corolla size, and corolla shape (Gómez 2003, 2008, Gómez et al. 2006). Even minute flower visitors, such as Nitidulid beetles, may act as strong selective agents in this plant species (Gómez 2003, Gómez et al. 2006). At the same time, *E. mediohispanicum* is attacked by wild and domestic ungulates that have a strong negative effect on fitness (Gómez 2005b). Most important, previous studies have shown that ungulates can exert conflicting selection on some plant traits, thus conditioning pollinator-mediated selection (Gómez 2003, 2008). In this study, we explore the spatial structure of the selective scenario affecting *E. mediohispanicum* as a consequence of the variation in the intensity of its interaction with pollinators and ungulates. First, we establish the identity of the main functional groups in each population and we quantify the among-population variation in pollinator assemblage composition. Second, we determine whether the selective pressures affecting the plant vary among populations as a consequence of spatial variation in the strength and outcome of the interaction with pollinators. Third, we explore the effect of ungulates on the selection caused by pollinators, and the ability of ungulates to modify selection on plant traits across the landscape. Fourth, we determine the spatial structure of the net selection affecting *E. mediohispanicum* phenotype, by exploring the occurrence of sites of intensive selection mediated by pollinators and herbivores. Collectively, these analyses provide evidence of how the structure of selection on a multispecific interaction can vary among environments, thereby providing the crucial first step in assessing how a geographic selection mosaic may develop within networks of interacting species.

MATERIALS AND METHODS

Study system

Erysimum mediohispanicum is a biannual, monocarpic herb endemic to the Iberian Peninsula (see Plate 1). In southeastern Spain, *E. mediohispanicum* is found in montane forests and subalpine scrublands (Table 1). 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. *E. mediohispanicum* is self-compatible, but requires pollen vectors for full seed set

TABLE 1. Location, characteristics, and sampling effort for the eight populations of *Erysimum mediohispanicum* (Brassicaceae) used in this study in southeastern Spain.

Population	Latitude	Longitude	Habitat	Altitude	Ungulate damage (%)†	Sampling effort‡	
						Minutes	Pollinators
Em01	37°8.00'	3°25.69'	forest	1750	11.11	2085	169
Em02	37°7.33'	3°25.86'	shrubland	2099	72.22	2195	118
Em08	37°8.00'	3°25.91'	shrubland	1690	01.11	2395	162
Em21	37°8.07'	3°25.71'	forest	1723	30.00	1720	270
Em22	37°7.86'	3°25.70'	forest	1802	21.11	1955	243
Em23	37°7.74'	3°25.58'	shrubland	1874	36.67	1925	125
Em24	37°7.51'	3°26.14'	forest	1943	43.33	1650	184
Em25	37°7.27'	3°26.05'	shrubland	2064	58.89	1485	117

† Percentage of plants consumed by ungulates.

‡ Minutes invested in pollinator surveys and number of pollinators recorded.

(Gómez 2005a). Mean seed dispersal distance is <20 cm (Gómez 2007).

The study was conducted in Sierra Nevada (Granada, southeastern Spain), spanning the altitudinal range of *E. mediohispanicum* (1600–2300 m). Plants bloom from late May to late June, depending on the altitude. Within a 5 × 2 km area, we selected eight populations with contrasting environments (Table 1; see also Gómez et al. 2007). Populations were at least 200 m apart from each other, with an interpopulation distance of 818 ± 82 m (mean ± SE). In spite of their relative proximity, populations were clearly differentiated from each other, and genetic divergence among populations was high ([fixation index] $G_{st} = 0.22 \pm 0.007$ based on 164 RAPD markers, $F_{st} = 0.32$ based on trnL-trnF cpDNA; authors' unpublished data).

Plant phenotypic traits

Ninety plants per population were marked at the onset of the 2005 flowering period (720 plants in total), using aluminum tags attached to the base of the flowering stalks. For each labeled plant, we analyzed the following phenotypic traits. (1) Stalk height: We measured, to the nearest 0.5 cm, the height of the tallest stalk, from the ground to the top of the highest open flower. (2) Flower number: We counted the entire production of flowers in each plant. (3) Corolla diameter: We measured, with a digital caliper (±0.1 mm error), the distance between the edge of two opposite petals. (4) Corolla tube length: We measured the distance between the corolla tube aperture and the base of the sepals. (5) Corolla tube width: We measured the diameter of the corolla tube aperture as the distance between the bases of two opposite petals. (6) Corolla shape. This variable was determined in each plant by means of geometric morphometric tools, using a landmark-based methodology that eliminates the effect of variation in the location, orientation, and scale of the specimens (Bookstein 1991, Rohlf 2003, Zelditch et al. 2004). We took a digital photograph of one flower per plant using a standardized procedure (front view and planar position). Flowers were photographed at anthesis to avoid ontogenetic effects. We defined 32 coplanar

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 (Roth 1993, 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 (see Gómez et al. [2006] for a detailed description of the landmark locations). We captured the landmarks using the software tpsDig version 1.4 (Stony Brook Morphometrics, available online).⁵ 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 (Rohlf and Slice 1990, Slice 2001). We used this method because of its low bias (Rohlf 2003). This procedure was performed using the software tpsRelw version 1.11 (Stony Brook Morphometrics; see footnote 5). 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 (RWs, principal components of the covariance matrix of the partial warp scores) were computed (Walker 2000, Adams et al. 2004). Unit centroid size was used as the alignment-scaling method and orthogonal 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 RWs (with p equal to the number of landmarks). Each RW is characterized by its singular value, and explains a given variation in shape among specimens. Thus, RWs summarize shape differences among specimens (Adams et al. 2004), and their scores can be used as a data matrix to perform standard statistical analyses (Zelditch et al. 2004).

Narrow-sense heritability is significant for all studied traits except flower number ($0.09 < h^2 < 0.50$, $P < 0.02$ in all cases, $n = 332$; authors' unpublished data).

⁵ (<http://life.bio.sunysb.edu/morph/morphmet.html>)

Interaction with pollinators

In 2005 and 2006, we conducted pollinator counts in the eight populations. During peak bloom (10–15 days per population) we conducted 5–7 pollinator surveys per population. In these surveys we noted the number of open flowers in each labeled plant, and the number and identity of pollinators that landed on the flowers during five-minute intervals. Thus, each survey lasted 450 minutes, and we conducted more than 1500 minutes of observation per population and year (Table 1). Pollinators were identified in the field, and some specimens were captured for further identification in the laboratory. Some rare pollinators could not be captured and thus we only identified them to genus or family (see Gómez et al. 2007 for details). The number of samples per population was fitted to the local abundance of pollinators by means of accumulation curves generated with EstimateS software (Colwell 2005). The results indicated that 130–150 pollinators per population provided an accurate estimate of the pollinator assemblage. With this information, in 2006 we resampled each plant population to assess whether pollinator composition was consistent from year to year.

We grouped the insects visiting *E. mediohispanicum* flowers into functional groups (Fenster et al. 2004, Wilson et al. 2004). We define “functional group” as those flower visitors that interact with the flowers in a similar manner. Basically, we used criteria of similarity in size, proboscis length, foraging behavior, and feeding habits. Thus, taxonomically related species were sometimes placed in different functional groups. We established eight functional groups: (1) large bees: mostly pollen- and nectar-collecting females ≥ 10 mm in body length; (2) small bees: mostly pollen- and nectar-collecting females < 10 mm (see Plate 1); (3) wasps: aculeate wasps, large parasitic wasps, and cleptoparasitic bees collecting only nectar; (4) beeﬂies: long-tongued nectar-collecting Bombyliidae (see Plate 1); (5) hoverﬂies: nectar- and pollen-collecting Syrphidae and short-tongued Bombyliidae; (6) beetles: including species collecting nectar and/or pollen; (7) butterﬂies: mostly Rhopalocera, all nectar collectors; (8) others: nectar-collecting ants, small ﬂies, small parasitic wasps, bugs, and grasshoppers.

We conducted several complementary analyses to test for differences among populations in pollinator assemblage. First, we calculated the Morisita-Horn similarity index between pairs of populations (Magurran 2004). Morisita-Horn index ranges from zero (indicating no similarity in community composition between sites) to one (indicating complete overlap), and is considered one of the most robust measures of community similarity (Magurran 2004). Among-population differences in species composition were analyzed with chi-square tests (2×2 contingency tables) with permutation, and calculation of *P* values with Monte Carlo methods (RXC program, provided by G. Carmody, Ottawa, Ontario, Canada). We used 100 000 simulations in each

test. This software implements an efficient algorithm that tolerates cells with zero observations. Spatial autocorrelation and geographical structure of the pollinator assemblage was investigated by means of Moran's *I* coefficient using the R package, version 4.0 (Legendre and Vaudor 2001; *available online*).⁶ Moran's *I* behaves like a Pearson correlation coefficient and calculates the similarity between observations from pairs of locations for each distance class for each independent variable. Pairwise geographic distance was calculated using latitude and longitude coordinates. The number of equidistant distance classes was calculated following Sturges' rule ($\text{classes} = 1 + 3.3 \log_2 n$). We used the Mantel test to determine the occurrence of correlation between spatial distance and differences in pollinator composition among populations (Fortin and Gurevitch 2001).

We used analysis of similarity (ANOSIM) to compare the similarity in pollinator composition between populations occurring in different habitats. ANOSIM is a nonparametric test applied to rank similarity matrices that uses a permutation procedure and tests the null hypothesis that there is no difference in community composition among groups (Magurran 2004). ANOSIM was conducted with R (*available online*).⁷ Significance levels were generated using a randomization process with 1000 permutations.

Interaction with herbivores

In the study area, *E. mediohispanicum* is damaged by Spanish ibex (*Capra pyrenaica*) and domestic sheep. These two species have been interacting with the plant probably for the last two millennia, and previous studies have shown that they can cancel the selection exerted by pollinators on *E. mediohispanicum* (Gómez 2003, 2008). To demonstrate this, we recorded, at the end of the reproductive season, the proportion of plants consumed by ungulates. Ungulates usually consume more than two thirds of the reproductive stalks, sometimes severing all the stalks from their bases (Gómez 2003).

Among-population differences in damage intensity were explored with a generalized linear random model, with a binomial error distribution. In this model, the proportion of plants damaged per population was included as a dependent variable with population as a random factor. Analysis was performed using lme4 library in R (R Development Core Team 2007).

Estimates of plant fitness

For each labeled plant we estimated lifetime female fitness as the number of seeds produced per plant during its entire life (*E. mediohispanicum* is monocarpic). For this, we counted the number of fruits per plant at the end of the reproductive season, and collected five fruits per plant to determine the number of seeds per fruit. Number of seeds per plant was found by multiplying

⁶ (<http://www.bio.umontreal.ca/casgrain/en/labo/R/v4/index.html>)

⁷ (<http://cran.r-project.org/>)

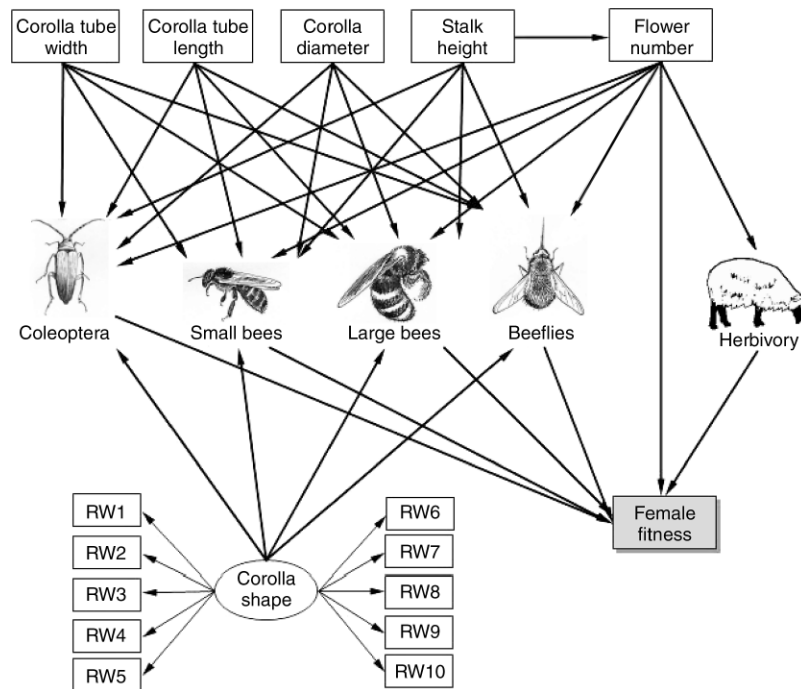


FIG. 1. Saturated model hypothesizing the functional relationships among plant phenotypic traits, pollinators, ungulate damage, and plant female fitness in the study area in southeastern Spain. RWs refer to relative warps, principal components of the covariance matrix of the partial warp scores. (See also Fig. 3.)

number of fruits per plant by number of seeds per fruit (Gómez et al. 2006).

Estimates of selection

We used structural equation modeling (SEM) with latent constructs (Grace 2006) to estimate selection on the phenotypic traits studied through female fitness. We decided to use SEM instead of other, more standard methods, such as selection differential and selection gradients, because SEM allows for the analysis of complex relationships between various variables (pollinators, herbivores, plant phenotype) and fitness. In addition, this method allows consideration of flower shape as a single, inclusive, multidimensional character (Adams and Rosenberg 1998). The total path coefficients generated by the SEMs can be interpreted as the total direct selection acting on each phenotypic trait. Corolla shape was transformed into a latent variable defined by the first 10 RWs obtained in the geometric morphometric analysis and explaining more than 85% of the variance in shape in each population. We built an a priori over-identified saturated model (Fig. 1) using information from previous *E. mediohispanicum* studies (Gómez 2003, 2005a, b, 2008, Gómez et al. 2006). In the saturated model (Fig. 1), plant fitness was directly connected to four floral visitor functional groups (large bees, small bees, bee flies, and beetles), ungulate damage, and number of flowers per plant. We used only these four floral visitor functional groups, because they were, by

far, the most abundant, and because they contacted the flower sexual organs in most visits and thus behaved as legitimate pollinators (Gómez et al. 2007). The rest of the phenotypic traits, including corolla shape, were connected to the pollinator functional groups (Fig. 1). In addition, stalk height was connected to number of flowers, which was connected to herbivore damage (Fig. 1).

To test for spatial variation in selective scenarios, we compared the SEMs among populations by means of multigroup analysis of SEMs (Grace 2006). Multigroup analysis imposes cross-group constraints on the path coefficients, and simultaneously fits the model to the data from each group. The procedure is similar to fitting the model to a single group, except that the constrained paths must have the same coefficient in all groups (i.e., path coefficients of interest are constrained to be equal in the compared groups). We first evaluated the most restrictive hypothesis of equality of all path coefficients, implying homogeneity between groups in the causal relationships among plant traits, interactors, and plant female fitness. If the chi-square value of the goodness-of-fit test showed a significant departure from fit (i.e., $P < 0.05$), then the hypothesis of total equality was rejected, and we proceeded to compare the path coefficients of different groups to identify the origin of overall between-group heterogeneity.

After performing the multigroup comparison, we solved the SEMs in each population separately because

they differed statistically. To do this, we built a set of alternative nested models by constraining some of the causal paths to zero. All models were solved minimizing yield-parameter estimates through an iterative process that uses generalized least squares shifting to maximum likelihood as discrepancy functions. We used maximum-likelihood estimation (MLE) on the variance-covariance matrix to test the goodness of fit of the models. Structural equation modeling was performed with SEPATH procedure in Statistica 7.0 (StatSoft 2004).

To select the best-fitting model(s), we used an information-theoretic approach (Burnham and Anderson 2002). We first selected those models obtaining an appropriate goodness of fit ($P > 0.05$; Grace 2006). From this set of candidate models, we calculated: (1) the Akaike Information Criterion (AIC); (2) the second-order AIC (AIC_c) as

$$\text{AIC}_c = \text{AIC} + \frac{2k(k+1)}{n-k-1}$$

where k is the number of parameters and n the sample size; (3) the AIC_c differences between models (ΔAIC_c); (4) the likelihood of each model, given data ($\zeta(\text{gi}|x) = \exp(-0.5\Delta\text{AIC}_c)$); and (5) the Akaike weights of each model i of the R candidate models as

$$w_i = \frac{e^{-0.5\Delta\text{AIC}_{c,i}}}{\sum_{r=1}^R e^{-0.5\Delta\text{AIC}_{c,r}}}$$

where w_i is taken as the weight of the evidence in favor of a given model i from a set of R candidate models, taking into account that $\sum_{i=1}^R w_i = 1$. All models having $w_i > 0.7$ were considered an appropriate representation of the raw data (Burnham and Anderson 2002).

Landscape variation in selection on plant traits

We tested whether selection on phenotypic traits varied across populations in relation to intensity of interaction with major pollinator groups and herbivores. First, we explored spatial autocorrelation in both phenotypic and interaction traits using Moran's I coefficient (Fortin and Dale 2006). Due to restricted sample size (eight populations) the significance of Moran's I coefficient was tested with a Monte Carlo approach. Since most traits were spatially autocorrelated, we explored the relationship between intensity of interaction and selection using spatially explicit GLM analyses. In these analyses, the spatial component was modeled in the residual terms defined as a fitted semi-variogram. These residuals can be decomposed into spatially structured residuals and pure error terms using Cholesky's decomposition. The spatial structures used were Exponential, Gaussian, and Linear models (Haining 2002, Fortin and Dale 2006). These models were performed using package `gls` of the `nlme` library in R (R Development Core Team 2007).

RESULTS

Overview of pollinator assemblage

In total, 1920 individuals belonging to 138 species and six orders were observed visiting the flowers of *Erysimum mediohispanicum* in the eight populations during the two years of the study. Most species belonged to the Hymenoptera (55 species) and Coleoptera (36; see Appendix A). Of the 138 species, 14 could not be told apart from closely related species in the field. Therefore some of the 124 taxa identified and used in the statistical analysis throughout the study actually include more than one species (see Appendix A).

Overall, the pollinator assemblage of *E. mediohispanicum* was highly generalized. It included species in a wide range of body size, mouthparts length, and foraging behavior. Body size ranged from 0.3 mg in *Melighetes minutus* (Nitidulid beetle) to 130 mg in *Anthophora aestivalis* (solitary bee). Mouthparts length ranged from <1 mm in several beetles to several cm in some butterflies. Most pollinators had mouthparts shorter than the corolla tube depth (10 mm) of *E. mediohispanicum*. Only a few species (11 species of large bees, eight species of bee flies, and 12 species of butterflies) had mouthparts of length comparable to or longer than the corolla tube depth of *E. mediohispanicum*. Some pollinators visited *E. mediohispanicum* flowers mostly for nectar (bee flies, butterflies, wasps, male bees), while others collected large amounts of pollen (beetles, female bees). Only two species, the solitary bees *Osmia brevicornis* and *Andrena agillissima* were crucifer specialists.

Most pollinator species were scarce. In 2005, >23% of the species identified were recorded just once. Only six species accounted for >5% of the total visits (Appendix A): two large bees (*Anthophora aestivalis*, *O. brevicornis*), two small bees (*Lasioglossum aeratum*, *L. interruptum*), and two bee flies (*Bombylius major*, *Bombylius* sp.1). Together, these species comprised >39% (512 individuals) of the total pollinator abundance (Appendix A). In 2006, five species accounted for >5% of the total visits, *A. aestivalis*, *Bombylius* sp. 1, *B. major*, and two small beetles (*Malachius laticollis* and *Dasytes subaeneus*). Thus, even when considering only the dominant species, the pollinator assemblage of *E. mediohispanicum* included large bees, small bees, bee flies, and small beetles, and therefore should be considered generalized.

Spatial variation in the intensity of interaction with pollinators

Populations differed in pollinator assemblage composition, both at the functional group level ($\chi^2 = 2458.09$, $P < 0.0001$, Monte Carlo contingency test) and at the species level ($\chi^2 = 418.05$, $P < 0.0001$). Consequently, the pairwise Morisita-Horn coefficients were low, ranging between 0.21 and 0.73, and averaging 0.43 ± 0.15 (mean \pm SE). The most abundant pollinator functional groups were bee flies and bees in populations Em01, Em08,

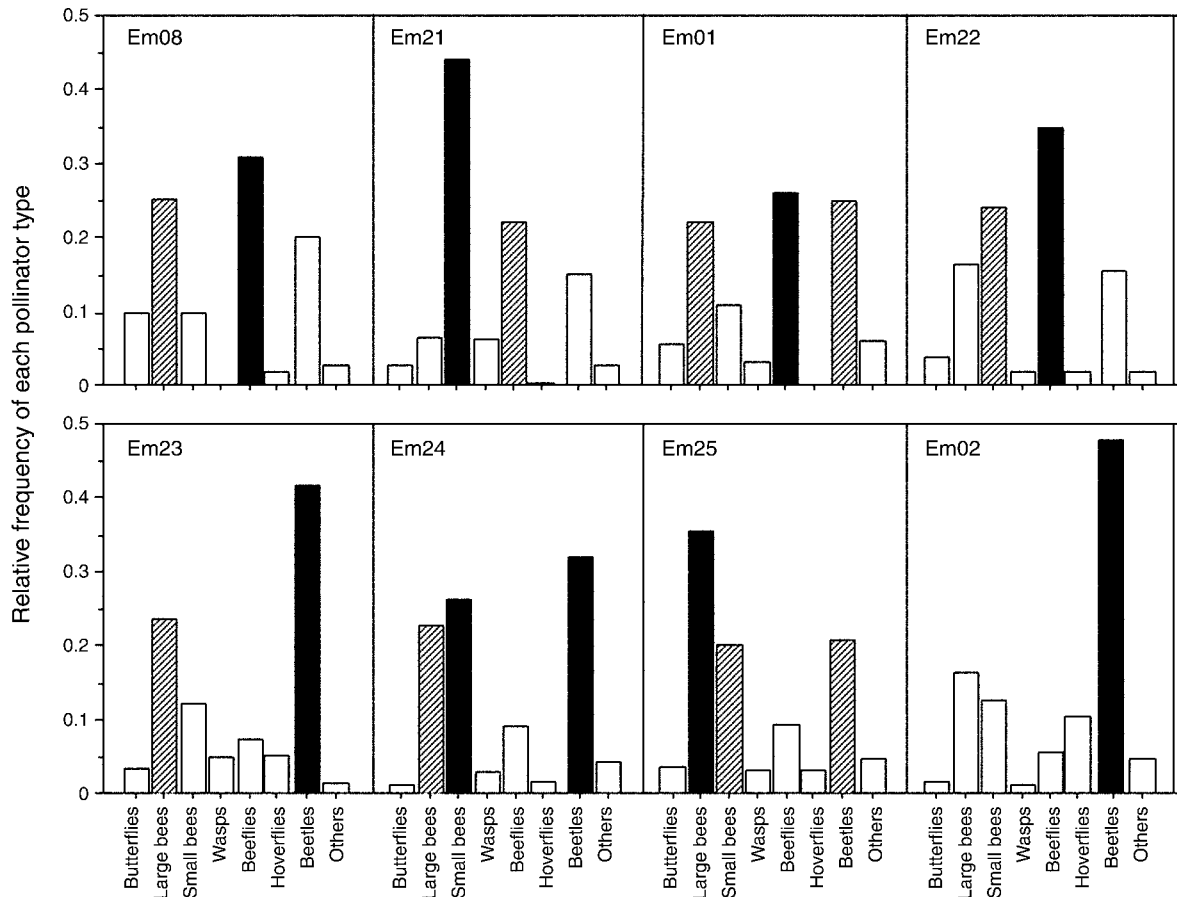


FIG. 2. The relative abundance of pollinator functional groups. Hatched bars indicate functional groups accounting for >20% of the pollinator visits, and solid bars indicate the most abundant functional group in each population (see Table 1 for details about population location).

Em21, and Em22, large bees and beetles in populations Em23 and Em25, beetles and small bees in population Em24, and beetles in population Em02 (Fig. 2).

The pollinator assemblage composition remained similar within populations between the two study years (Appendix A). Thus, pollinator assemblage similarity was significantly higher in within-population between-year (2005 vs. 2006) comparisons (Morisita-Horn similarity index; median = 0.742, rank = 0.449–0.879), than in between-population paired comparisons (median = 0.561, rank = 0.127–0.931; $Z = 2.002$, $P = 0.04$; nonparametric median test).

Neither pollinator abundance nor the relative frequency of the major functional groups were spatially autocorrelated ($P > 0.1$ for all distance classes and all descriptors, Moran's I test). Altitude was correlated only with the abundance of butterflies ($r^2 = -0.63$, $P < 0.01$, $n = 8$ populations, product-moment correlation), beflies ($r^2 = 0.76$, $P < 0.005$), and hoverflies ($r^2 = 0.45$, $P < 0.05$). Habitat type (forest vs. shrubland) did not affect the degree of similarity between populations in pollina-

tor assemblage composition ($R = 0.26$, $P = 0.076$, ANOSIM).

Spatial variation in the intensity of interaction with herbivores

We found significant between-population differences in ungulate damage intensity (likelihood ratio test = 165.28, $P < 0.0001$, GLM with binomial response distribution), ranging between 1% and 72% plants attacked per population (Table 1). There was a significant increase in damage with altitude ($\beta = 0.14 \pm 0.02$, $t = 6.82$, $P < 0.00001$, $R^2 = 0.85$, linear regression).

Damage correlated negatively with abundance of beflies ($\beta = -0.54 \pm 0.17$, $t = 3.20$, $n = 8$, $P < 0.01$, linear regression) and butterflies ($\beta = -0.13 \pm 0.04$, $t = 3.15$, $n = 8$, $P < 0.01$). This correlation can be explained by the low abundance of these two pollinator groups at high elevations, where herbivore damage was highest. There was no correlation with the remaining pollinator groups. Finally, herbivore damage in 2005 correlated negatively with pollinator richness in 2006 ($\beta = -0.01 \pm 0.006$, $t = 2.55$, $n = 8$, $P = 0.05$).

TABLE 2. Among-population differences in phenotypic traits (mean \pm SE) of *E. mediohispanicum*.

Population	Stalk height (cm)	Corolla diameter (mm)	Corolla tube width (mm)	Corolla tube length (mm)	Flower number	RW1
Em01	35.41 \pm 1.36	10.77 \pm 0.16	0.48 \pm 0.10	10.46 \pm 0.14	43.63 \pm 3.87	-0.02 \pm 0.01
Em02	25.87 \pm 1.51	10.52 \pm 0.17	0.21 \pm 0.08	9.33 \pm 0.15	51.20 \pm 6.62	-0.01 \pm 0.01
Em08	44.47 \pm 1.36	11.23 \pm 0.17	0.43 \pm 0.10	10.84 \pm 0.14	40.98 \pm 2.69	-0.01 \pm 0.01
Em21	39.88 \pm 1.41	10.16 \pm 0.18	0.66 \pm 0.09	9.73 \pm 0.16	36.22 \pm 2.27	0.02 \pm 0.01
Em22	29.05 \pm 1.35	10.55 \pm 0.15	1.09 \pm 0.07	9.88 \pm 0.15	35.09 \pm 2.72	-0.01 \pm 0.01
Em23	28.00 \pm 1.39	10.46 \pm 0.17	0.51 \pm 0.08	10.16 \pm 0.16	42.56 \pm 4.30	0.02 \pm 0.01
Em24	31.33 \pm 1.40	10.59 \pm 0.17	0.15 \pm 0.10	10.66 \pm 0.16	35.46 \pm 2.69	0.05 \pm 0.01
Em25	30.34 \pm 1.54	10.97 \pm 0.19	0.50 \pm 0.10	10.57 \pm 0.17	32.32 \pm 1.86	-0.04 \pm 0.01
<i>F</i> ratio	20.78****	3.73***	8.07****	11.43****	2.79**	5.66*****
<i>R</i> ²	0.18	0.03	0.07	0.09	0.01	0.06

Notes: RW1–RW4 are the first four relative warps obtained in the geometric morphometric analysis of flower shape (see *Materials and Methods: Plant phenotypic traits*). *F* ratios refer to one-way ANOVA. Total sample size is 720 (90 plants per population).

** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, ***** $P < 0.00001$; NS, not significant.

Among-population differences in phenotypic traits

We found significant among-population differences in all plant phenotypic traits studied (Table 2). Thus, plants were tallest in populations Em08 and Em21, and shortest in Em22 and Em02 (Table 2). Flowers were largest in populations Em08 and Em25, and smallest in Em22 and Em23. On the other hand, the length of the corolla tube was longest in Em08 and shortest in Em02, Em21, and Em22. Plants in populations Em02, Em01, and Em23 produced many flowers, whereas those in Em25 produced few flowers.

The geometric morphometric analysis produced 60 shape variables (RWs), but only the first four explained $>5\%$ of the variance in flower shape (RW1 = 35.2%, RW2 = 19.2%, RW3 = 9.7%, RW4 = 6.2%). RW1 was associated with the relative position of adjacent petals (from perpendicular to almost parallel), RW2 with dorsoventral asymmetry (and therefore true zygomorphy), RW3 with lateral asymmetry, and RW4 with petal shape (from elongated to rounded; Fig. 3). As shown in Table 3, there were among-population differences in RW1, RW2, and RW4. Differences among populations in flower shape were also significant when all 60 RWs were considered (Wilk's $\lambda = 0.14$, $F_{413,4528,12} = 3.61$, $P < 0.00001$; MANOVA).

There was no spatial autocorrelation in the phenotypic traits measured (Mantel $r = -0.05$, $P = 0.55$), except for flower shape, whose autocorrelation was marginally significant (Mantel $r = 0.312$, $P = 0.057$).

Phenotypic correlations among traits

There was a significant positive phenotypic correlation between number of flowers and stalk height, corolla diameter, and corolla tube length (Table 3). In addition, stalk height was correlated with corolla diameter and corolla tube length. Corolla diameter was correlated with corolla tube length, corolla tube width, RW1, and RW4. Finally, corolla tube width was correlated with RW4 (Table 3).

Geographical variation in selection on plant traits

The model selection outcomes yielded one single best model for each population except for Em08, with two best models (Table 4; see Appendix B). The structural equation modeling (SEM) multigroup comparison

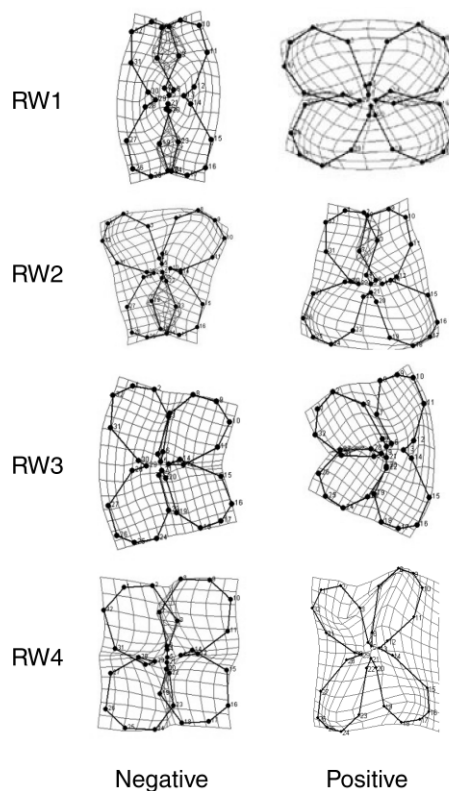


FIG. 3. A summary of the geometric morphometric analysis ($n = 720$ plants) showing the variation in flower morphology produced by relative warps (RWs) explaining $>5\%$ of the overall variation in shape (RW1 = 35.17%, RW2 = 19.22%, RW3 = 9.67%, RW4 = 6.23%). The figures represent the theoretical shape of flowers with extreme positive or negative values of each individual RW, while maintaining constant the other RWs (see Gómez et al. [2006] for details).

TABLE 2. Extended.

RW2	RW3	RW4
0.01 ± 0.01	0.006 ± 0.01	-0.014 ± 0.004
-0.02 ± 0.01	0.009 ± 0.01	0.011 ± 0.005
0.01 ± 0.01	-0.002 ± 0.01	0.023 ± 0.006
0.02 ± 0.01	-0.003 ± 0.01	-0.019 ± 0.005
0.01 ± 0.01	-0.002 ± 0.01	-0.018 ± 0.005
-0.02 ± 0.01	-0.019 ± 0.01	0.012 ± 0.006
0.01 ± 0.01	0.005 ± 0.01	0.017 ± 0.005
-0.02 ± 0.01	0.008 ± 0.01	-0.012 ± 0.005
3.14***	1.84 NS	10.72****
0.02	0.008	0.05

showed heterogeneity among populations in the solved models ($\chi^2 = 1072.6$, $df = 464$, $P < 0.00001$). That is, the most parsimonious models varied across populations, with different traits and pollinators affecting female fitness in different populations (Fig. 4). Thus, bee fly abundance was positively related to fitness in five populations (plants most visited by bee flies had highest fitness), although only in Em08 and Em01 was this relationship significant (Fig. 4). Large bees were related to fitness, positively, in three populations, although only in Em23 was this relationship significant. Small bees were related to fitness in six populations, being significant in populations Em08, Em21, Em01, Em23, and Em24. Finally, beetles were related to fitness in three populations, although this relationship was only significant in Em01 and Em22. However, the effect of small bees and beetles on fitness varied across populations, being positive in some populations and negative in others (Fig. 4). Ungulate damage negatively and significantly affected fitness in all populations but three: Em08, Em22, and Em23 (Fig. 4), which are among the five populations least affected by herbivory (Table 1).

The selected traits also varied across populations (Fig. 4, Table 5). Only two traits, flower number and stalk height, were related to fitness in all populations, although the intensity of selection differed among them. Thus, flower number was significantly and positively related with fitness in six populations (Table 5). In addition, the SEM indicates that number of flowers affected *E. mediohispanicum* female fitness indirectly

through its effect on pollinator visitation in all populations and directly in six populations (Fig. 4). Only in populations Em23 and Em02, was the effect of flower number totally mediated by pollinators (Fig. 4). As for stalk height, taller plants had higher female fitness in most populations (Fig. 4). However, the pattern of selection on this trait was complex. On the one hand, selection on stalk height through its association with flower number was ubiquitous and always positive (Table 5). On the other hand, the sign of pollinator-mediated selection on stalk height varied across populations due to its different effect on different pollinator functional groups. Thus, stalk height was positively selected by bee flies in Em08, but negatively selected by large and small bees in Em23 and Em21. In these two populations, however, total selection was positive due to the association between stalk height and flower number (Table 5).

Geographical variation in the selective scenarios of *E. mediohispanicum* in Sierra Nevada was also evident for traits related to corolla size and shape. For some traits, such as corolla tube length, selection was uniformly positive, but significant in only some populations (Table 5). Thus, corolla tube length was selected in Em01, Em22, Em23, Em24, and Em25, mostly mediated by the action of large and small bees (Fig. 3, Table 5). Instead, corolla tube length was negatively selected through small bees in Em01, but the net selection on this trait was positive due to the negative effect of small bees on plant fitness (Fig. 4).

Corolla diameter, corolla tube width, and corolla shape were selected in different ways in different populations, suggesting the occurrence of divergent selection for these traits (Table 5). Corolla diameter was positively selected through small bees in Em23, and negatively selected through bee flies and large bees in Em08, Em01, and Em25 (Fig. 3). Similarly, selection favored narrow corolla tubes in Em21 and Em25, but wide corolla tubes in Em01 and Em23 (Fig. 4).

Corolla shape was significantly selected in five populations (Table 5), but, interestingly, different shape components were selected in different populations. In addition, for some shape components, selection acted in

TABLE 3. Phenotypic correlations (product-moment correlations) between *E. mediohispanicum* phenotypic traits in the Sierra Nevada (southeastern Spain).

Trait	Flower number	Corolla diameter	Corolla tube length	Corolla tube width	RW1	RW2	RW3	RW4
Stalk height	0.468****	0.350****	0.367****	0.007	-0.075	0.050	0.059	-0.006
Flower number		0.215***	0.211***	-0.009	-0.014	-0.032	0.074	0.041
Corolla diameter			0.517****	0.344****	-0.152***	-0.087	-0.023	-0.213***
Corolla tube length				-0.042	-0.072	0.055	0.030	0.017
Corolla tube width					0.014	0.006	-0.021	-0.210***
RW1						0.023	-0.002	-0.001
RW2							0.018	0.007
RW3								-0.005
RW4								

Notes: All populations pooled ($n = 720$ plants). Significance levels are after Bonferroni corrections. *** $P < 0.001$; **** $P < 0.0001$.

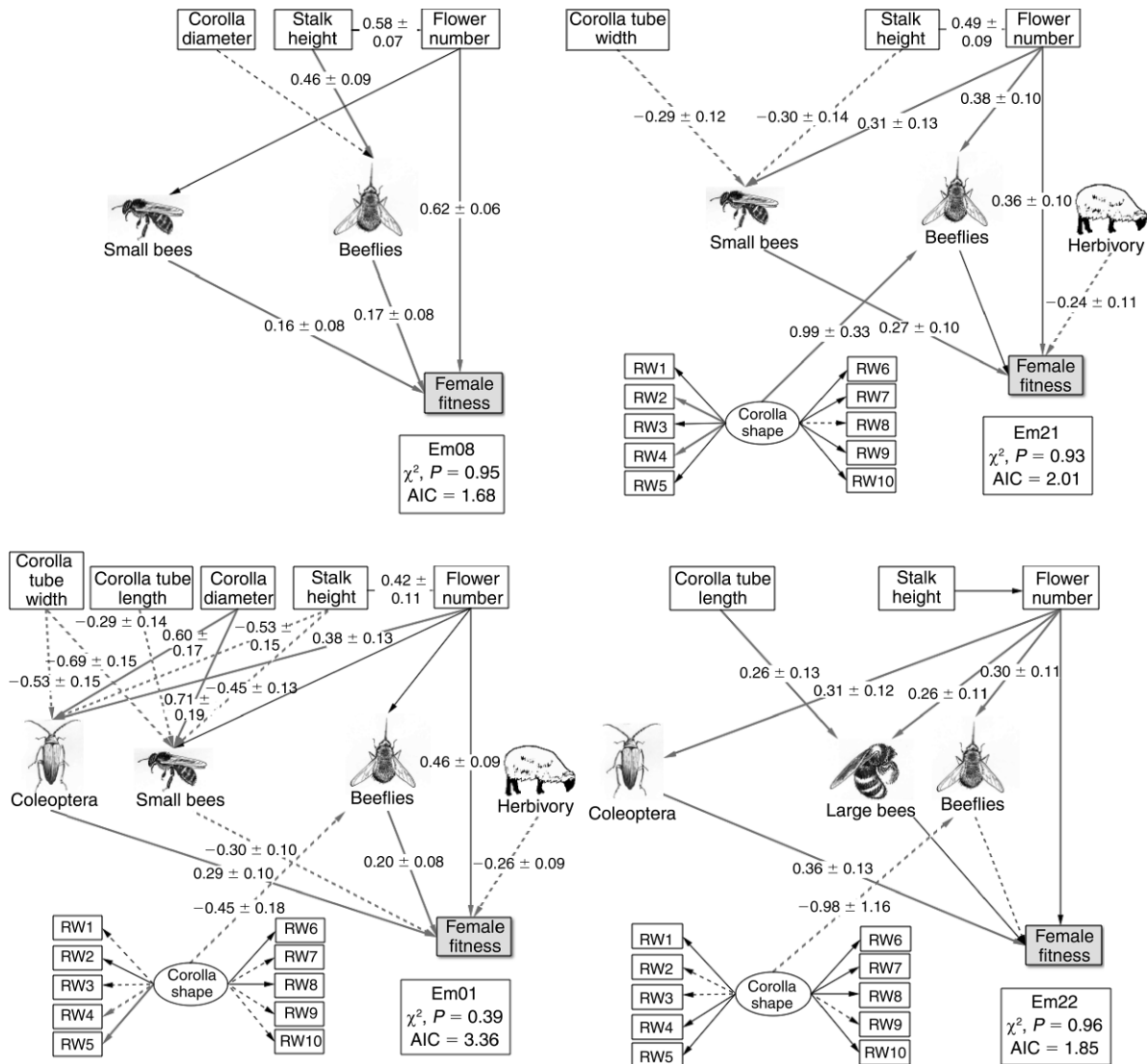


FIG. 4. The best structural equation model (EM) for each population, showing the causal relationships between the set of indicator variables (boxes), the latent variable (sphere), the main pollinators, and the female fitness estimates (see Fig. 1 for the saturated model). Only the values of the path coefficients (mean \pm SE) that were significant in the best models are shown. Positive effects are indicated by solid lines, and negative effects by broken lines. RW1–RW10 refer to the first 10 relative warps in the geometric morphometric analysis of flower shape.

different directions depending on the population (Fig. 4). Thus, zygomorphic flowers (positive RW2) were selected through bees in Em21 (Fig. 4), while flowers with narrow petals (positive RW4) were selected in Em01, Em21, Em23, and Em25. Instead, in Em22, selection through befly visitation favored flowers with rounded corollas (negative RW4; Fig. 4). As a consequence of these interactions, selected corolla shapes varied among populations in agreement with the main pollinators acting as selective agents (Fig. 4).

Factors affecting selection intensity across populations

We found spatial autocorrelation in the selection exerted on stalk height, corolla tube length, and flower

number. In all cases this autocorrelation was positive for the smaller distance classes (Moran's $I > 0.437$, $P < 0.001$ for all traits) and negative for the larger distance classes ($I < -0.750$, $P < 0.009$). Similarly, we found spatial autocorrelation for herbivore damage intensity ($I = 0.615$, $P = 0.002$ for small distance classes; $I = -1.038$, $P < 0.0001$ for large distance classes) and befly abundance ($I = 0.515$, $P = 0.002$ for small distance classes; $I = -0.836$, $P < 0.001$ for large distance classes). No spatial autocorrelation was found for any other phenotypic trait or pollinator group.

The intensity of total selection on two traits, stalk height and flower number, significantly depended on the local intensity of herbivore damage (Table 6, Fig. 5).

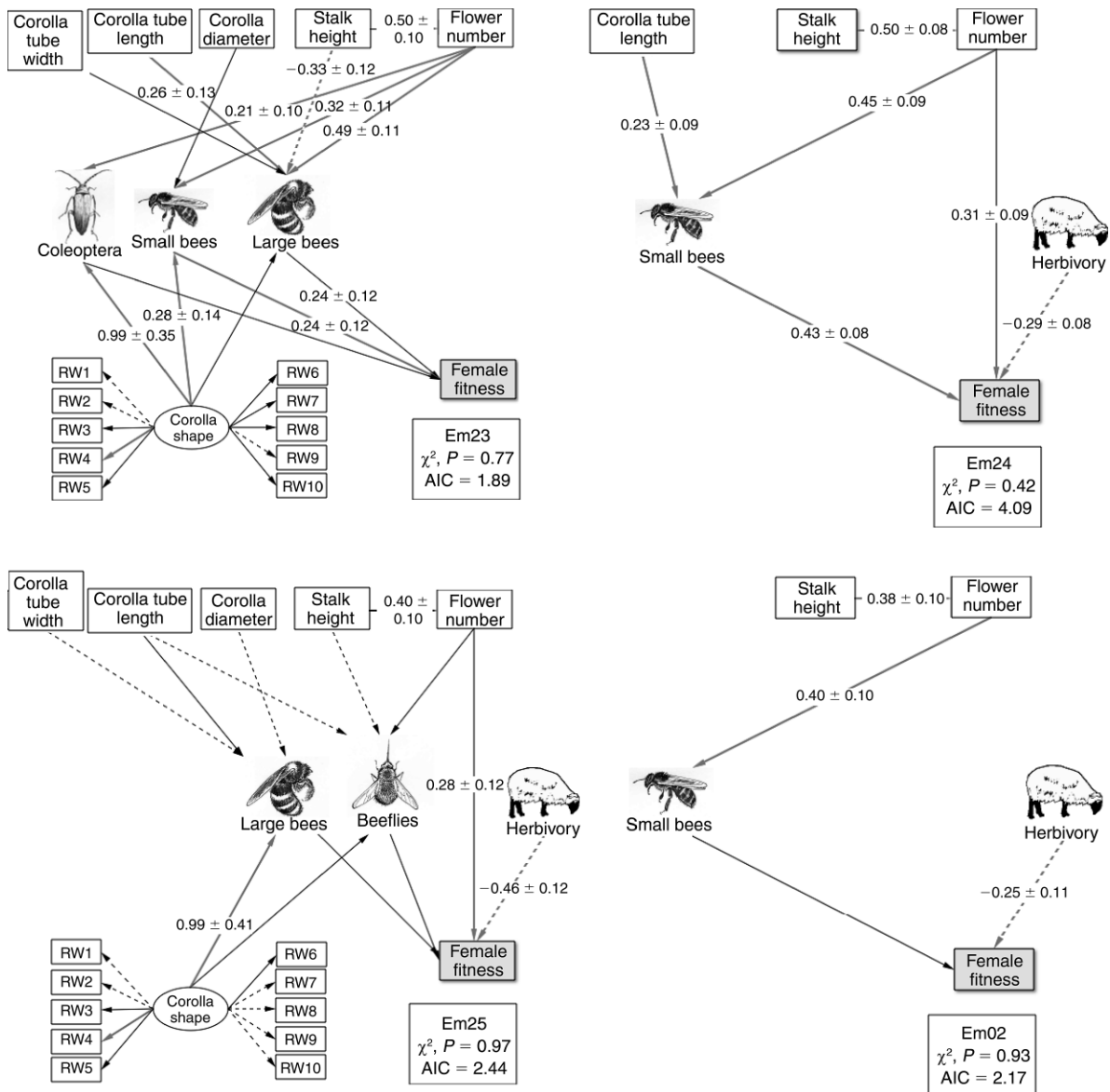


FIG. 4. Continued.

Total selection on these two traits was weaker in populations in which damage was higher. Total selection on corolla tube length was positively related to local abundance of beeflies and large bees, but not to herbivore damage (Table 5). Finally, selection on flower shape was positively related to local abundance of large bees but negatively related to local abundance of beeflies (Table 6).

DISCUSSION

Generalization of the E. mediohispanicum pollination system

The pollinator assemblage visiting the flowers of *Erysimum mediohispanicum* was extremely generalized.

It was composed of more than 130 species belonging to six orders and over 30 families with disparate morphology, body size, mouthparts length, and foraging behavior. In addition, most taxa belonged to highly generalist pollinator groups (Faegri and van der Pijl 1979, Proctor et al. 1996). 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 from the corolla aperture is bound to contact both stigma and anthers. Even one of the smallest insects recorded on *E. mediohispanicum* (*Melighetes maurus*, ~2 mm in length) has been shown to be an

TABLE 4. Selection of the different structural equation models (SEMs) tested for each *E. mediohispanicum* population.

Model	Em01	Em02	Em08	Em21	Em22	Em23	Em24	Em25
0	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000
1	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.000000	0.018868
2	0.000000	0.000000	0.000000	0.000000	0.000000	0.000074	0.000000	0.981132
3	0.000000	0.000000	0.000000	0.000000	0.000000	0.001066	0.000000	
4	0.000000	0.000000	0.000000	0.000005	0.000000	0.010385	0.000000	
5	0.000000	0.000000	0.000028	0.000400	0.000006	0.098746	0.000000	
6	0.000000	0.000000	0.010605	0.003035	0.000062		0.000000	
7	0.000000	0.000000	0.411348	0.996560	0.999931		0.000000	
8	0.000001	0.000910	0.578019	0.000000			0.000000	
9	0.000045	0.137915					0.000008	
10	0.000007	0.291695					0.002153	
11	0.009713	0.569480					0.997839	
12	0.205363							
13	0.784870							

Notes: Values represent the AIC weights, w_i (Burnham and Anderson 2002). Different numbers of models were tested for each population. See Appendix B for further details.

effective pollinator of *E. mediohispanicum* (Gómez 2005b). Thus, it is fair to assume that all of the species recorded acted as effective pollinators to some degree.

Spatial variation in the interaction with mutualistic and antagonistic organisms

There was significant spatial variation in the pollinator assemblage of *E. mediohispanicum*, since the identity and the relative abundance of the flower visitors varied across plant populations. A main consequence of the spatial variation in pollinator abundance is the low similarity in pollinator assemblage composition among populations. Differences between plant populations in pollinator groups have been found in many other systems (e.g., Fishbein and Venable 1996, Gómez and Zamora 1999, Thompson 2001, Boyd 2004, Moeller 2005, 2006, Price et al. 2005). The magnitude of spatial variation in pollinators was high in our system, however, since the pollinators maintaining the most intense interactions with the plants belonged to different functional groups in each population (beeflies, large bees, small bees, and beetles). These differences were consistent across the two study years, since within-population similarity indices were significantly higher than between-population similarity indices. If this

temporal consistency is maintained for long periods, then different *E. mediohispanicum* populations in Sierra Nevada are basically interacting with different pollinator assemblages. It is remarkable that the spatial structure in *E. mediohispanicum* pollination system occurred despite plant populations being close enough to allow pollinator movement between populations (interpopulation distance = 818 ± 82 m [mean \pm SE]). In fact, there was no spatial autocorrelation in the pairwise similarity indices, indicating that close populations were no more similar in pollinator assemblage composition than distant populations. Thus, the overall pollinator assemblage was distributed as a mosaic among the studied populations. These findings suggest that the sign, intensity, and outcome of the interspecific interactions maintained by *E. mediohispanicum* vary across the landscape due to differences in the community context (Siepielski and Benkman 2007).

Spatial variation in the intensity of interactions with organisms that exhibit contrasting preference patterns is a primary factor causing a geographic selection mosaic (Gómez and Zamora 2000, Thompson and Cunningham 2002, Thompson 2005, Rey et al. 2006, Thompson and Fernández 2006, Toju 2007). In agreement with this idea, different *E. mediohispanicum* pollinators exhibit

TABLE 5. Total direct selection on phenotypic traits in each *E. mediohispanicum* population according to the structural equation models.

Phenotypic trait	Em01	Em02	Em08	Em21	Em22	Em23	Em24	Em25
Stalk height	0.217	0.032	0.442	0.163	0.019	0.229	0.252	0.316
Flower number	0.556	0.083	0.622	0.499	0.084	0.036	0.503	0.107
Corolla diameter	-0.037		-0.033			0.022		-0.005
Corolla tube length	0.087				0.035	0.061	0.100	0.011
Corolla tube width	0.058			-0.079		0.041		-0.001
Corolla shape	-0.091			0.149	0.208	0.256		0.066
RW1	0.001			0.012	0.037	0.040		-0.011
RW2	-0.019			0.038	-0.005	0.009		-0.004
RW3	0.012			0.005	-0.005	0.001		0.007
RW4	0.015			0.039	-0.020	0.079		0.009

Notes: Values indicate the total path coefficient relating each phenotypic trait and plant fitness. Traits with significant ($P < 0.05$) links to fitness are shown in boldface type.

TABLE 6. Results of the spatially explicit generalized linear models (GLMs) testing for the effects of herbivore damage (quantified as the percentage of plants consumed) and the abundance of each major pollinator group on the total selection of each phenotypic trait across populations.

Model†	Phenotypic trait					
	Stalk height	Flower number	Corolla diameter	Corolla tube length	Corolla tube width	Corolla shape
Herbivory	-0.005 ± 0.001	-0.011 ± 0.002	0.001 ± 0.002	0.002 ± 0.004	0.001 ± 0.002	-0.003 ± 0.002
Beeflies	0.216 ± 0.310	-1.524 ± 1.129	1.917 ± 1.051	0.800 ± 0.179	0.856 ± 0.652	-0.887 ± 0.234
Large bees	0.425 ± 0.338	-0.742 ± 1.358	1.914 ± 1.282	1.449 ± 0.302	-0.794 ± 0.697	1.013 ± 0.306
Small bees	0.165 ± 0.196	0.006 ± 1.419	1.423 ± 1.338	0.716 ± 0.264	-0.429 ± 0.421	0.239 ± 0.158
Beetles		-0.161 ± 1.692	1.104 ± 1.593			
AIC	2.867	5.050	4.793	9.403	7.234	3.988
BIC	-3.443	-5.405	-5.662	3.093	0.925	-2.321
Log-likelihood	5.566	5.475	5.604	2.298	3.827	5.006

Notes: Values indicate maximum-likelihood estimates of parameters (mean ± SE). Significance at $\alpha < 0.05$, after Bonferroni correction, is indicated by boldface type. Beetles were removed from some models because they generated singular matrices. All models were fitted assuming an exponential covariance matrix of the residuals. AIC is the Akaike Information Criterion; BIC is the Bayesian Information Criterion. Differences were nonsignificant when using other covariance matrices.

† The first column includes both factors in the models (herbivory, insect visitors) and model fit (AIC, BIC, log-likelihood).

different preference patterns for plant traits. The only trait consistently preferred by all pollinator functional groups is flower number (Gómez 2003, Gómez et al. 2006, 2008a). The rest of the plant traits showed differing preference by different pollinator functional groups. Thus, only large bees favored plants with deep corollas and large flowers (Gómez et al. 2008a), a preference observed in other systems (Galen et al. 1987, Campbell 1989, 1991, Galen 1989, Gómez and Zamora 2000, Martin 2004, Wilson et al. 2004). In contrast, short-tongued hoverflies forage in plants with short corollas, providing an easier access to reward (Branquart and Hemptinne 2000, Colley and Luna 2000). On the other hand, beesflies, beetles, and small bees did not show discrimination for these plant traits. A recent study in which paper model flowers were used, has shown differences between the main *E. mediohispanicum* pollinator groups in preference for corolla shape traits (Gómez et al. 2008b). Large and small bees chose model flowers with narrow petals, whereas beesflies selected flowers with rounded petals and zygomorphic shape (Gómez et al. 2008b).

Damage by ungulates also varied among populations. Damage to *E. mediohispanicum* was caused mostly by sheep, and to a lesser extent by Spanish ibex. In the study area, medium to large herds of both species usually move from lowland to upland pastures, using similar paths every year (Alados and Escós 1987). Most damage to *E. mediohispanicum* is produced during this altitudinal migration, which coincides with the flowering and fruiting period of the plant. For this reason, damage likelihood, although more related to large-scale foraging decisions than to specific plant population properties, is similar between years across populations (Gómez 2003, 2005a).

Pollinator-mediated geographic selection mosaic

Our study suggests the occurrence of spatial variation in the strength and outcome of the interactions of *E. mediohispanicum* with pollinators, a primary factor

determining the existence of a geographic selection mosaic on this plant's floral traits. We found selection on most of the phenotypic traits studied in at least one population. Structural equation modeling (SEM) suggests that the selection detected on these traits was mostly mediated by pollinators, an outcome that agrees with studies on other *E. mediohispanicum* populations (Gómez 2003, Gómez et al. 2006). Thus, only two traits, flower number and stalk height, were related to fitness without the concurrence of pollinators. Many studies

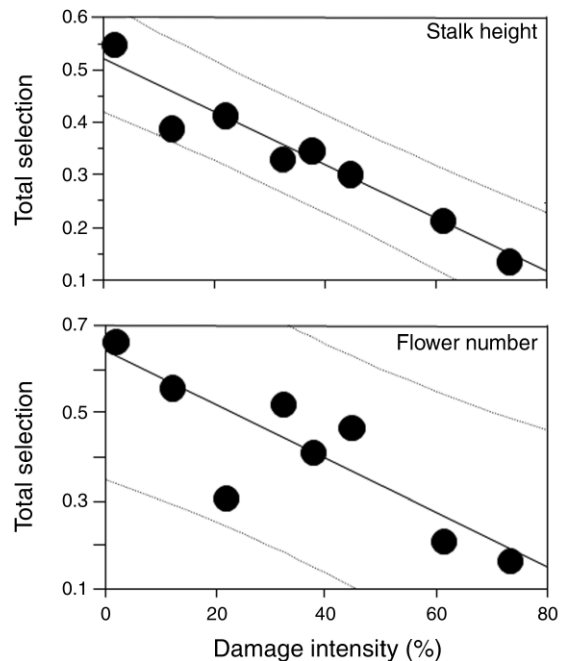


FIG. 5. Response curves showing the effect of ungulate damage intensity in each population (quantified as the percentage of plants consumed) on the total selection on stalk height and flower number. Each solid circle represents a plant population, and the gray dotted lines represent 95% confidence intervals.



PLATE 1. Two pollinators of *E. mediohispanicum*: (left) *Lasioglossum xanthopus* and (right) *Bombylius major*. Photo credits: *L. xanthopus*, J. M. Gómez; *B. major*, F. Perfectti.

suggest that selection on flower number is mediated by two non-exclusive mechanisms: first, through its positive effect on pollinator visitation rate (Conner et al. 1996, Vaughton and Ramsey 1998, Thompson 2001, Benítez-Vieyra et al. 2006, Makino and Sakai 2007); second, through its positive direct association with ovule number and thus potential reproduction (Conner and Rush 1996, Gómez 2000, Gómez and Zamora 2000). We have been able to statistically disentangle these two pathways by using SEM. These analyses suggest that both mechanisms are operating in *E. mediohispanicum* populations. Only in two populations (Em02 and Em23) was there no direct effect of flower number on female fitness. Selection on flowering stalk height is similarly complex. The effect of stalk height on fitness was mediated in all the populations studied, through its positive association with flower number. In addition, this trait affected fitness through its effect on pollinators in five populations. However, this pollinator-mediated selection was positive only in population Em08, mediated by the effect of this trait on bee fly preference (Gómez et al. 2008a). Consequently, the total selection on stalk height was greatly diminished in all populations except Em08, which showed a strong selection for taller plants (Figs. 4 and 5). It is remarkable that plants in Em08 were those with the tallest flowering stalks (Table 2).

Geographical variation in the selective scenarios of *E. mediohispanicum* in Sierra Nevada was also evident for traits related to corolla size and shape. Contrary to flower number or stalk height, selection for traits related to corolla size and shape was not autocorrelated. In agreement with the Geographic Mosaic Theory of Coevolution (Gomulkiewicz et al. 2000, Nuismer et al. 2000, Thompson 2005), these results suggest the existence of selective hotspots intermingled with cold-

spots for each *E. mediohispanicum* trait studied, although those hotspots and coldspots may not necessarily generate a coevolutionary response in this interaction.

Selection was significant only in some populations, thereby producing evidence for a selection mosaic. For example, selection for corolla diameter was only significant in population Em01, in which plants with smaller corollas produced more seeds. This outcome is remarkable, since two main selective agents in that population, beetles and small bees, actually selected for large corollas. This counterintuitive outcome resulted from the intense negative effect of small bees on fitness in that population (Fig. 4). Similarly, corolla tube length was significantly selected only in four populations in which plants with deeper flowers produced more seeds. Interestingly, in three of these populations (Em22, Em23, and Em24) long corolla tubes were favored by a positive selection mediated by bees, whereas in population Em01 this trait was favored because it was negatively associated with small bees, which prefer shallow flowers (Gómez et al. 2008a), but negatively affected plant fitness in this population. In addition, three of these populations (Em01, Em23, and Em24) ranked highest in corolla tube length (Table 2), in agreement with the selective pressures exerted on this trait.

An important result of our study is that some plant traits, such as corolla diameter, and above all corolla tube width and corolla shape, show divergent selection (are selected in opposite directions in different populations). It is important to know what factors are producing this pattern of selection. Our results suggest that it is caused by the conflicting selection exerted by different pollinators. Corolla tube width was negatively

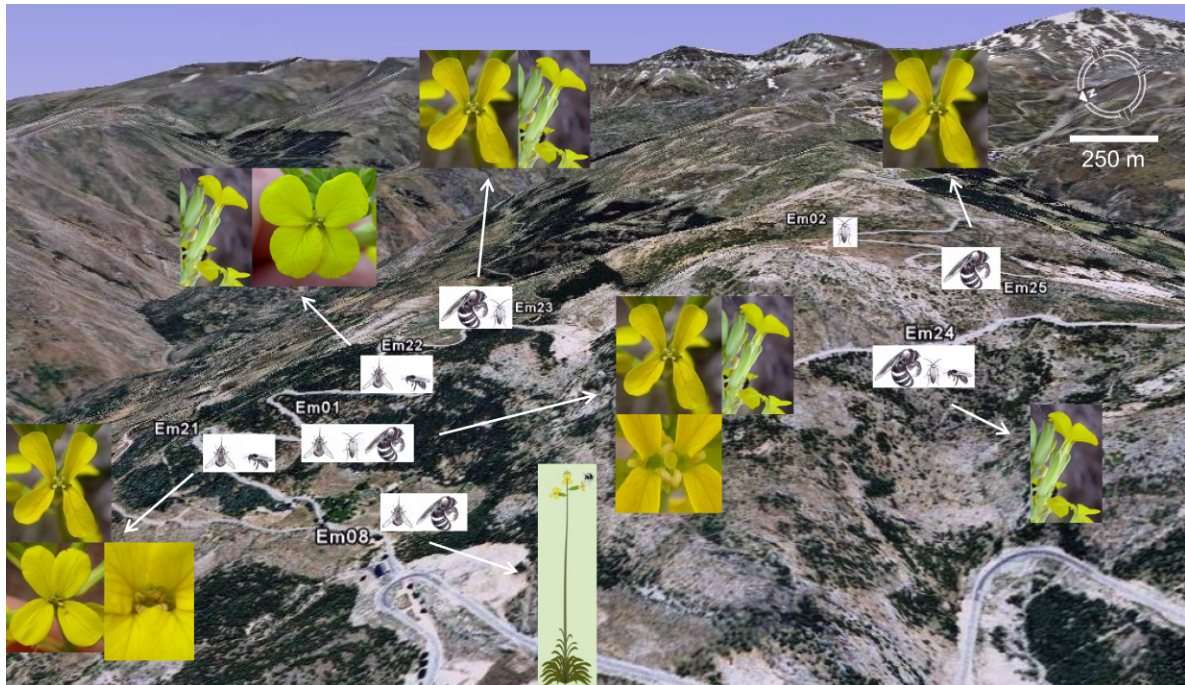


FIG. 6. Geographically varying selective landscape in *E. mediohispanicum*, indicating the spatial distribution of the pollinator-mediated selective hotspots for plant traits across the landscape. The pictures show the fit floral phenotype in each population, according to our geographic selection study. Only pollinators representing >20% of the visits in a given population are shown (see Fig. 1). Photos by J. M. Gómez; drawings by D. Nesbitt.

selected by small bees in all populations. However, since these insects have contrasting effects on fitness in different populations, we found opposing selection on tube width. Plants with wide corolla tubes were selected in population Em01, as opposed to plants with narrow corolla tubes in population Em21 (Fig. 6). Divergent selection was also found for corolla shape. In this case, the role of pollinators was even stronger. Our spatially explicit GLM analysis indicates that divergent selection in corolla shape in *E. mediohispanicum* results mostly from the opposing effects of bee flies and bees. Interestingly, the selected corolla shape was related to the main pollinator group in each location (Fig. 6). That is, the fittest plants had flowers with narrow petals in populations mostly visited by large bees, but rounded or zygomorphic flowers in populations mostly visited by bee flies (Fig. 6). Also interestingly, these flower shapes coincide with preferences shown by large bees and bee flies, respectively, on experimental arrays of paper flowers of various shapes (Gómez et al. 2008b).

The pollinator-mediated divergent selection for floral traits in *E. mediohispanicum* is a prerequisite for the occurrence of pollinator-mediated local adaptation and speciation (Wilson and Thomson 1996, Streisfeld and Kohn 2007). This study has found strong local selection in some populations. In addition, and although further studies are needed, our preliminary results on 164 RAPD markers point to strong genetic structure and low gene flow between populations. These findings

suggest that local adaptation is possible in *E. mediohispanicum* even at the small spatial scale investigated.

The role of herbivores in shaping the pollinator-mediated selection mosaic

The structure of the *E. mediohispanicum* geographic selection mosaic was also affected by the interaction with ungulates. We detected herbivore-mediated negative selection on two plant traits, flower number and stalk height. In addition, the spatially explicit GLM analyses show that the intensity of selection on these two traits clearly decreased with increasing damage intensity. These findings suggest that the conflicting selection exerted by ungulates may weaken or even cancel out the pollinator-mediated selection occurring on these two traits. This outcome agrees with previous studies, which show that ungulates preferentially feed on large, many-flowered *E. mediohispanicum* plants, thus disrupting the positive selection caused by pollinators on flower number and stalk height (Gómez 2003, 2005a, b, 2008). In fact, conflicting selection produced by the sequential action of mutualistic and antagonist interacting organisms is frequent in *E. mediohispanicum* (Gómez 2008).

Ungulates may also weaken selection on plant phenotype indirectly, by causing a decline in pollinator visitation (Gómez 2005b). In this study, we found a negative correlation between ungulate damage and butterfly and, more importantly, bee fly abundance. We have demonstrated that bee flies are important selective

agents of *E. mediohispanicum* in some populations, in which they select, among other traits, for taller plants (Gómez et al. 2008a; and this study). Thus, by causing a decrease in bee fly abundance, ungulates are probably also diminishing the strength of selection on stalk height mediated by bee flies. Moreover, we observed a negative relationship between the number of pollinator species and the ungulate damage experienced by *E. mediohispanicum* populations the previous year. This delayed effect on pollinator richness could be produced by concomitant decrease in plant abundance and density in those populations undergoing severe ungulate damage. It is important to note that ungulates not only consume full-grown *E. mediohispanicum* individuals, but may also trample seedlings and juveniles, a phenomenon that directly and negatively affects the abundance and density of this herb (Gómez 2005a). Other studies have shown plant abundance and density to be correlated with pollinator abundance and diversity (Conner and Neumeier 1995, Kunin 1997).

Geographic selection in generalized, multispecific systems operating at small scales

As a consequence of being a multispecific system, the net selection on some *E. mediohispanicum* traits across the landscape results from a balance between the selection mediated by mutualistic organisms and the selection exerted by antagonistic organisms. Other studies have shown the disrupting effect of a wide variety of antagonistic organisms, such as seed predators, nectar robbers, folivores, and florivores, on plant–pollinator interactions (Krupnick and Weiss 1999, Gómez and Zamora 2000, Herrera 2000, Irwin and Brody 2000, Cariveau et al. 2004, Althoff et al. 2005). As a result, it is widely assumed that the phenotypic evolution of plants is a consequence of the combined effect of herbivores and pollinators (Irwin 2006, Strauss and Whittall 2006). However, very few studies have reported a geographical structure in selective scenarios caused by the simultaneous action of these two kinds of interacting organisms (e.g., Gómez and Zamora 2000, Rey et al. 2006). We believe that this outcome may prove more frequent than previously suspected. Further studies considering the simultaneous geographic variation in the outcome of the interactions with both antagonistic and mutualistic generalist organisms will be necessary to know how frequent this phenomenon is in natural systems (Thompson 2005).

It is widely assumed that, in generalist systems, the selection imposed by multiple organisms may be mostly diffuse rather than pairwise, since the response to selection by one interacting organism on a trait is probably altered by the presence of another community member (Strauss et al. 2005, Leimu and Koricheva 2006). This study effectively shows that diffuse selection is occurring in *E. mediohispanicum*, since two of the three criteria for diffuse evolution are met in our system (Iwao and Rausher 1997, Strauss et al. 2005). First, we

found that the interaction with one species or functional group was affected by the presence of other interacting organisms. Second, the effect on fitness and the selection imposed by some pollinators was altered in some populations by the presence of other pollinators or herbivores. However, our study also suggests that we should go beyond simplistic statements of “diffuse selection,” since even in generalist systems composed of multiple interacting organisms it is possible to find selection caused by some specific components of the community. Furthermore, since there was spatial variation in the interacting organisms, the identity of the selective agents also varied across populations. In generalized systems there are no co-pollinators diluting the selection exerted by one single adapted pollinator (e.g., Thompson and Cunningham 2002), but assemblies of species changing spatially. It is easy to envision in such a system how the interaction with a subgroup of organisms exerting strong selection that varies among populations may cause the appearance of a selective hotspot and even lead to divergent phenotypic selection regimes.

Our study shows that the overall selection regime affecting *E. mediohispanicum* and the potential for selective hotspots and coldspots may change even over very local scales, since our populations are located very close to each other. This outcome contrasts with many other systems that have detected mosaics at a larger spatial scale (e.g., Benkman 1999, Mezquida and Benkman 2005, Rey et al. 2006, Hoeksema and Thompson 2007). For example, Benkman et al. (2003) report on a selection mosaic in *Pinus contorta* produced by the presence and activity of crossbills (*Loxia curvirostra*) and squirrels (*Tamasciurus hudsonicus*) across populations located more than 1000 km apart. Similarly, Rey et al. (2006) document a selection mosaic on *Helleborus foetidus* produced by pollinators and floral herbivores across regions located 100–600 km apart. As pointed out by Thompson (2005), studies designed at geographical scales greater than the scale of local adaptation may mask the pattern of evolutionary process. Gómez and Zamora (2000) found that selection on several *Hormathophylla spinosa* traits produced by pollinators and herbivores varied geographically between close populations being 1–5 km apart. Laine (2005, 2006) has recently shown a selection mosaic between *Plantago lanceolata* and the mildew fungus *Podosphaera plantagini* propitiated by small-scale changes in temperature and humidity. Similarly, Capelle and Neema (2005) have reported a microgeographic mosaic between *Phaseolus vulgaris* and the fungus *Colletotrichum lindemuthianum* as a consequence of a very limited gene flow. Our study is the first report of a microscale geographic selection mosaic for a generalist system. We believe that small-scale geographic mosaics are more prevalent on generalized systems than on specialized systems, since a slight modification in the community of organisms interacting with generalist species can have intense effects on the

overall interaction outcome. We also believe that fine-scale variations are more common for such generalist assemblages, since in specialized assemblages the tight association between the interacting organisms decreases or precludes the probability of small-scale variations. Further studies, however, are necessary to confirm this statement.

In conclusion, four important outcomes emerge from our study of the *E. mediohispanicum* geographic selection mosaic. (1) The interactions with generalist organisms may produce significant selection. (2) The structure and function of geographic mosaics depend on a balance between mutualistic and antagonistic selection. (3) Spatially varying multispecific, generalist systems may trigger divergent selection across populations. (4) Selection mosaics may operate at fairly small spatial scales. We presume that these four properties are common to other generalist systems. Considering them in future empirical and theoretical studies will surely contribute to broadening the conceptual framework of the Geographic Mosaic Theory of Coevolution.

ACKNOWLEDGMENTS

We thank several specialists who kindly identified pollinator specimens: M. A. Alonso Zarazaga, R. Constantin, S. Fernández Gayubo, M. Goula, F. Gusenleitner, J. Háva, P. Leblanc, M. A. Marcos, J. C. Otero, A. Sánchez Ruiz, A. Sánchez Terrón, M. Schwarz, A. Tinaut, F. Vallhonrat, and D. Ventura. The Ministerio de Medio Ambiente and Consejería de Medio Ambiente of the Junta de Andalucía granted permission to work in the Sierra Nevada National Park. This study was partially supported by the Spanish MCyT (GLB2006-04883/BOS) and Junta de Andalucía PAI (RNM 220 and CVI 165).

LITERATURE CITED

- Adams, D. C., F. J. Rohlf, and D. E. Slice. 2004. Geometric morphometrics: ten years of progress following the 'revolution.' *Italian Journal of Zoology* 71:5–16.
- Adams, D. C., and M. S. Rosenberg. 1998. Partial warps, phylogeny, and ontogeny: a comment on Fink and Zelditch (1995). *Systematic Biology* 47:168–173.
- Aigner, P. A. 2005. Variation in pollination performance gradients in a *Dudleya* species complex. Can generalization promote floral divergence? *Functional Ecology* 19:681–689.
- Aigner, P. A. 2006. The evolution of specialized floral phenotype in a fine-grained pollinator environment. Pages 23–46 in N. M. Waser and J. Ollerton, editors. *Plant–pollinator interactions, from specialization to generalization*. University of Chicago Press, Chicago, Illinois, USA.
- Alados, C. L., and L. Escós. 1987. Relationships between movement rate, agonistic displacements and forage availability in Spanish ibexes (*Capra pyrenaica*). *Biological Behavior* 12:245–255.
- Althoff, D. M., K. A. Segraves, and O. Pellmyr. 2005. Community context of an obligate mutualism: pollinator and florivore effects on *Yucca filamentosa*. *Ecology* 86:905–913.
- Anderson, B., and J. D. Johnson. 2008. The geographical mosaic of coevolution in a plant–pollinator mutualism. *Evolution* 62:220–225.
- Benítez-Vieyra, S., A. M. Medina, E. Glinos, and A. A. Cocucci. 2006. Pollinator-mediated selection on floral traits and size of floral display in *Cyclopogon elatus*, a sweat bee-pollinated orchid. *Functional Ecology* 20:948–957.
- Benkman, C. W. 1999. The selection mosaic and diversifying coevolution between crossbills and lodgepole pine. *American Naturalist* 153:S75–S91.
- Benkman, C. W., T. L. Parchman, A. Favis, and A. M. Siepielski. 2003. Reciprocal selection causes a coevolutionary arms race between crossbills and lodgepole pine. *American Naturalist* 162:182–194.
- Bookstein, F. L. 1991. *Morphometric tools for landmark data: geometry and biology*. Cambridge University Press, Cambridge, UK.
- Boyd, A. E. 2004. Breeding system of *Macromeria viridiflora* (Boraginaceae) and geographic variation in pollinator assemblages. *American Journal of Botany* 91:1809–1813.
- Branquart, E., and J. L. Hemptinne. 2000. Selectivity in the exploitation of floral resources by hoverflies (Diptera: Syrphinae). *Ecography* 23:732–742.
- Brody, A. K. 1997. Effects of pollinators, herbivores, and seed predators on flowering phenology. *Ecology* 78:1624–1631.
- Burnham, K. P., and D. R. Anderson. 2002. *Model selection and multimodel inference: a practical-theoretic approach*. Springer, Heidelberg, Germany.
- Campbell, D. R. 1989. Measurements of selection in a hermaphroditic plant: variation in male and female reproductive success. *Evolution* 43:318–334.
- Campbell, D. R. 1991. Effects of floral traits on sequential components of fitness in *Ipomopsis aggregata*. *American Naturalist* 137:713–737.
- Capelle, J., and C. Neema. 2005. Local adaptation and population structure at a micro-geographical scale of a fungal parasite on its host plant. *Journal of Evolutionary Biology* 18:1445–1454.
- Cariveau, D., R. E. Irwin, A. K. Brody, L. S. Garcia-Mayeya, and A. von der Ohe. 2004. Direct and indirect effects of pollinators and seed predators to selection on plant and floral traits. *Oikos* 104:15–26.
- Colley, M. R., and J. M. Luna. 2000. Relative attractiveness of potential insectary plants to aphidophagous hoverflies (Diptera: Syrphidae). *Environmental Entomology* 29:1054–1059.
- Colwell, R. K. 2005. EstimateS—statistical estimation of species richness and shared species from samples. Version 7.5. User's guide and application. (<http://viceroy.eeb.uconn.edu/estimates>)
- Conner, J. K., and R. Neumeier. 1995. Effects of black mustard population size on the taxonomic composition of pollinators. *Oecologia* 104:218–224.
- Conner, J. K., and S. Rush. 1996. Effects of flower size and number on pollinator visitation to wild radish, *Raphanus raphanistrum*. *Oecologia* 105:509–516.
- Conner, J. K., S. Rush, and P. Jennetten. 1996. Measurements of natural selection on floral traits in wild radish (*Raphanus raphanistrum*). 1. Selection through lifetime female fitness. *Evolution* 50:1127–1136.
- Dilley, J. D., P. Wilson, and M. R. Mesler. 2000. The radiation of *Calochortus*: generalist flowers moving through a mosaic of potential pollinators. *Oikos* 89:209–222.
- Faegri, K., and L. van der Pijl. 1979. *The principles of pollination ecology*. Pergamon, London, UK.
- Fenster, C. B., W. S. Armbruster, P. M. Wilson, R. Dudash, and J. D. Thompson. 2004. Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution, and Systematics* 35:375–404.
- Fishbein, M., and D. L. Venable. 1996. Diversity and temporal change in the effective pollinators of *Asclepias tuberosa*. *Ecology* 77:1061–1073.
- Fortin, M. J., and M. Dale. 2006. *Spatial analysis: a guide for ecologists*. Cambridge University Press, Cambridge, UK.
- Fortin, M. J., and J. Gurevitch. 2001. Mantel test: spatial structure in field experiments. Pages 308–326 in S. M. Scheiner and J. Gurevitch, editors. *Design and analysis of*

- ecological experiments. Chapman and Hall, New York, New York, USA.
- Galen, C. 1989. Measuring pollinator-mediated selection on morphometric floral traits: bumblebees and the alpine sky pilot, *Polemonium viscosum*. *Evolution* 43:882–890.
- Galen, C., K. A. Zimmer, and M. E. Newport. 1987. Pollination in floral scent morphs of *Polemonium viscosum*: a mechanism for disruptive selection on flower size. *Evolution* 41:599–606.
- Gómez, J. M. 2000. Phenotypic selection and response to selection in *Lobularia maritima*: importance of direct and correlational components of natural selection. *Journal of Evolutionary Biology* 13:689–699.
- Gómez, J. M. 2003. Herbivory reduces the strength of pollinator-mediated selection in the Mediterranean herb *Erysimum mediohispanicum*: consequences for plant specialization. *American Naturalist* 162:242–256.
- Gómez, J. M. 2005a. Ungulate effect on the performance, abundance, and spatial structure of two montane herbs: a seven-year experimental study. *Ecological Monographs* 75: 231–258.
- Gómez, J. M. 2005b. 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 Systematics and Evolution* 268:119–130.
- Gómez, J. M. 2008. Sequential conflicting selection due to multispecific interactions triggers evolutionary trade-offs in a monocarpic herb. *Evolution* 62:668–679.
- Gómez, J. M., J. Bosch, F. Perfectti, J. Fernández, and M. Abdelaziz. 2007. Pollinator diversity affects plant reproduction and recruitment: the tradeoffs of generalization. *Oecologia* 153:597–605.
- Gómez, J. M., J. Bosch, F. Perfectti, J. D. Fernández, M. Abdelaziz, and J. P. M. Camacho. 2008a. Association between floral traits and reward in *Erysimum mediohispanicum* (Brassicaceae). *Annals of Botany* 101:1413–1420.
- Gómez, J. M., J. Bosch, F. Perfectti, J. D. Fernández, M. Abdelaziz, and J. P. M. Camacho. 2008b. 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 B* 275:2241–2249.
- Gómez, J. M., F. Perfectti, and J. P. M. Camacho. 2006. Natural selection on *Erysimum mediohispanicum* flower shape: insights into the evolution of zygomorphy. *American Naturalist* 168:531–545.
- Gómez, J. M., and R. Zamora. 1999. Generalization vs. specialization in the pollination system of *Hormathophylla spinosa* (Cruciferae). *Ecology* 80:796–805.
- Gómez, J. M., and R. Zamora. 2000. Spatial variation in the selective scenarios of *Hormathophylla spinosa* (Cruciferae). *American Naturalist* 155:657–668.
- Gómez, J. M., and R. Zamora. 2006. Ecological factors that promote the evolution of generalization in pollination systems. Pages 145–165 in N. M. Waser and J. Ollerton, editors. *Plant–pollinator interactions, from specialization to generalization*. University of Chicago Press, Chicago, Illinois, USA.
- Gomulkiewicz, R., D. M. Drown, M. F. Dybdahl, W. Godsoe, S. L. Nuismer, K. M. Pepin, B. J. Ridenhour, C. I. Smith, and J. B. Yoder. 2007. Dos and don'ts of testing the geographic mosaic theory of coevolution. *Heredity* 98:249–258.
- Gomulkiewicz, R., J. N. Thompson, R. D. Holt, S. L. Nuismer, and M. E. Hochberg. 2000. Hot spots, cold spots, and the geographic mosaic theory of coevolution. *American Naturalist* 156:156–174.
- Grace, J. B. 2006. Structural equation modeling and natural systems. Cambridge University Press, Cambridge, UK.
- Haining, R. 2002. *Spatial data analysis*. Cambridge University Press, Cambridge, UK.
- Herrera, C. M. 2000. Measuring the effects of pollinators and herbivores: evidence for non-additivity in a perennial herb. *Ecology* 81:2170–2176.
- Herrera, C. M., M. C. Castellanos, and M. Medrano. 2006. Geographical context of floral evolution: towards an improved research programme in floral diversification. Pages 278–294 in L. D. Harder and S. C. H. Barrett, editors. *Ecology and evolution of flowers*. Oxford University Press, Oxford, UK.
- Hoeksema, J. D., and J. N. Thompson. 2007. Geographic structure in a widespread plant–mycorrhizal interaction: pines and false truffles. *Journal of Evolutionary Biology* 20: 1148–1163.
- Irwin, R. E. 2006. The consequences of direct versus indirect species interactions to selection on traits: pollination and nectar robbing in *Ipomopsis aggregata*. *American Naturalist* 167:315–328.
- Irwin, R. E., and A. K. Brody. 2000. Consequences of nectar robbing for realized male function in a hummingbird-pollinated plant. *Ecology* 81:2637–2643.
- Iwao, K., and M. D. Rausher. 1997. Evolution of plant resistance to multiple herbivores: quantifying diffuse coevolution. *American Naturalist* 149:316–335.
- Krupnick, G. A., and A. E. Weiss. 1999. The consequences of floral herbivory for pollinator service to *Isomeris arborea*. *Ecology* 80:125–134.
- Kunin, W. E. 1997. Population size and density effects in pollination, pollinator foraging and plant reproductive success in experimental arrays of *Brassica kaber*. *Journal of Ecology* 85:225–234.
- Laine, A. L. 2005. Spatial scale of local adaptation in a plant–pathogen metapopulation. *Journal of Evolutionary Biology* 18:930–938.
- Laine, A. L. 2006. Evolution of host resistance: looking for coevolutionary hotspots at small spatial scale. *Proceedings of the Royal Society of London B* 273:267–273.
- Lapchin, L. 2002. Host–parasitoid association and diffuse coevolution: When to be a generalist? *American Naturalist* 160:245–254.
- Legendre, P., and A. Vaudor. 2001. *The R package: multidimensional analysis, spatial analysis*. University of Montreal, Montreal, Quebec, Canada.
- Leimu, R., and J. Koricheva. 2006. A meta-analysis of genetic correlations between plant resistances to multiple enemies. *American Naturalist* 168:E15–E37.
- Magurran, A. E. 2004. *Ecological diversity and its measurements*. Second edition. Princeton University Press, Princeton, New Jersey, USA.
- Makino, T. T., and S. Sakai. 2007. Experience changes pollinator responses to floral display size: from size-based to reward-based foraging. *Functional Ecology* 21:854–863.
- Martin, N. H. 2004. Flower size preferences of the honeybee (*Apis mellifera*) foraging on *Mimulus guttatus* (Scrophulariaceae). *Evolutionary Ecology Research* 6:777–782.
- Mezquida, E. T., and C. W. Benkman. 2005. The geographic selection mosaic for squirrels, crossbills and Aleppo pine. *Journal of Evolutionary Biology* 18:348–357.
- Moeller, D. A. 2005. Pollinator community structure and sources of spatial variation in plant–pollinator interaction in *Clarkia xantiana* spp. *xantiana*. *Oecologia* 142:28–37.
- Moeller, D. A. 2006. Geographic structure of pollinator communities, reproductive assurance, and the evolution of self pollination. *Ecology* 87:1510–1522.
- Nuismer, S. L., J. N. Thompson, and R. Gomulkiewicz. 2000. Coevolutionary clines across selection mosaics. *Evolution* 54: 1102–1115.
- Price, M. V., N. M. Waser, R. E. Irwin, D. R. Campbell, and A. K. Brody. 2005. Temporal and spatial variation in

- pollination of a montane herb: a seven-year study. *Ecology* 86:2106–2116.
- Proctor, M., P. Yeo, and A. Lack. 1996. *The natural history of pollination*. Timber Press, Portland, Oregon, USA.
- R Development Core Team. 2007. R: a language and environment for statistical computing. R Foundation for Statistical Computing. (<http://www.R-project.org>)
- Rey, P. J., C. M. Herrera, J. Guitián, X. Cerdá, A. M. Sánchez-Lafuente, M. Medrano, and J. L. Garrido. 2006. The geographic mosaic in predispersal interactions and selection on *Helleborus foetidus* (Ranunculaceae). *Journal of Evolutionary Biology* 19:21–34.
- Rohlf, F. J. 2003. Bias and error in estimates of mean shape in geometric morphometrics. *Journal of Human Evolution* 44: 665–683.
- Rohlf, F. J., and D. E. Slice. 1990. Extensions of the Procrustes method for the optimal superimposition of landmarks. *Systematic Zoology* 39:40–59.
- Roth, V. L. 1993. On three-dimensional morphometrics, and on the identification of landmark points. Pages 41–62 in L. F. Marcus, E. Bello, and A. Garcia-Valdecasas, editors. *Contributions to morphometrics*. Museo de Ciencias Naturales, Madrid, Spain.
- Rudgers, J. A., and S. Y. Strauss. 2004. A selection mosaic in the facultative mutualism between ants and wild cotton. *Proceedings of the Royal Society of London B* 271:2481–2488.
- Siepielski, A. M., and C. W. Benkman. 2007. Convergent patterns in the selection mosaic for two North American bird-dispersed pines. *Ecological Monographs* 77:203–220.
- Slice, D. 2001. Landmarks aligned by Procrustes analysis do not lie in Kendall's shape space. *Systematic Biology* 50:141–149.
- StatSoft. 2004. STATISTICA for Windows (program manual). StatSoft, Tulsa, Oklahoma, USA.
- Strauss, S. Y. 1997. Floral characters link herbivores, pollinators, and plant fitness. *Ecology* 78:1640–1645.
- Strauss, S. Y., and R. E. Irwin. 2004. Ecological and evolutionary consequences of multispecies plant–animal interactions. *Annual Review of Ecology, Evolution, and Systematics* 35:435–466.
- Strauss, S. Y., H. Sahli, and J. K. Conner. 2005. Toward a more trait-centered approach to diffuse (co)evolution. *New Phytologist* 165:81–90.
- Strauss, S. Y., D. H. Siemens, M. B. Decher, and T. Mitchell-Olds. 1999. Ecological costs of plant resistance to herbivores in the currency of pollination. *Evolution* 53:1105–1113.
- Strauss, S. Y., and J. B. Whittall. 2006. Non-pollinator agents of selection on floral traits. Pages 120–138 in L. D. Harder and S. C. H. Barrett, editors. *The ecology and evolution of flowers*. Oxford University Press, Oxford, UK.
- Streisfeld, M. A., and J. R. Kohn. 2007. Environment and pollinator-mediated selection on parapatric floral races of *Mimulus aurantiacus*. *Journal of Evolutionary Biology* 20: 122–132.
- Thompson, J. D. 2001. How do visitation patterns vary among pollinators in relation to floral display and floral design in a generalist pollination system? *Oecologia* 126:386–394.
- Thompson, J. N. 2005. *The geographic mosaic of coevolution*. University of Chicago Press, Chicago, Illinois, USA.
- Thompson, J. N., and B. M. Cunningham. 2002. Geographic structure and dynamics of coevolutionary selection. *Nature* 417:735–738.
- Thompson, J. N., and C. C. Fernández. 2006. Temporal dynamics of antagonism and mutualism in a geographically variable plant–insect interaction. *Ecology* 87:103–112.
- Toju, H. 2007. Interpopulation variation in predator foraging behavior promotes the evolutionary divergence of prey. *Journal of Evolutionary Biology* 20:1544–1533.
- Vaughton, G., and M. Ramsey. 1998. Floral display, pollinator visitation and reproductive success in the dioecious perennial herb *Wurmbea dioica* (Liliaceae). *Oecologia* 115:93–101.
- Walker, J. A. 2000. The ability of geometric morphometric methods to estimate a known covariance matrix. *Systematic Biology* 49:686–696.
- Wilson, P., M. C. Castellanos, J. N. Hogues, J. D. Thomson, and W. S. Armbruster. 2004. A multivariate search for pollination syndrome among penstemons. *Oikos* 104:345–361.
- Wilson, P., and J. D. Thomson. 1996. How do flowers diverge? Pages 88–111 in D. Lloyd and S. C. H. Barrett, editors. *Floral biology*. Chapman and Hall, New York, New York, USA.
- Zelditch, M. L., D. L. Swiderski, H. D. Sheets, and W. L. Fink. 2004. *Geometric morphometrics for biologists: a primer*. Elsevier Academic Press, San Diego, California, USA.

APPENDIX A

Specific and functional composition of the pollinator assemblage of *Erysimum mediohispanicum* (*Ecological Archives* M079-009-A1).

APPENDIX B

Summary of the selection model of each population (*Ecological Archives* M079-009-A2).