The geographic mosaic in predispersal interactions and selection on *Helleborus foetidus* (Ranunculaceae)

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Abstract

We examine the hierarchical geographic structure of the interaction between a plant, *Helleborus foetidus*, and its floral herbivores and pollinators (interactors). Six populations from three distant regions of the Iberian Peninsula were used to examine intra- and inter-regional variation in plant traits, interactors and plant fecundity, and to compare, through selection gradient and path analyses, which traits were under selection, and which interactors were responsible for differential selection. Geographic and temporal congruency in interactor-mediated selection was further tested using a recent analytical approach based on multi-group comparison in Structural Equation Models. Most plant traits, interactors and fecundity differed among regions but not between populations. Similarly, the identity of the traits under selection, the selection gradients (strength and/or the direction of the selection) and the path coefficients (identifying the ecological basis for selection) varied inter- but not intra-regionally. Results show a selection mosaic at the broad scale and, for some traits, a link of differential selection to trait differentiation.

Introduction

Understanding how the evolution of plant traits is influenced by interactions with animals is a major goal in the study of the evolutionary ecology of plant–animal interactions. Plants and animals interact in many different ways (see Herrera & Pellmyr, 2002, for a recent review) involving antagonisms (most frequently herbivory, granivory and parasitism) and mutualisms (pollination, seed dispersal and active defence). Most plant populations and individuals participate in several concurrent interactions, but research programmes on plant–animal interactions have been largely devoted to just one kind (pollination, herbivory, granivory or seed dispersal). During the last decade, however, it has been explicitly recognized that the multiple interactions experienced by plants can lead them through ecological and

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evolutionary pathways that are not easily understood if the effects of each interaction are considered in isolation (Strauss & Armbruster, 1997; Gómez & Zamora, 2000; Herrera, 2000; Herrera *et al.*, 2002a; Gómez, 2003; Strauss & Irwin, 2004). For example, some plant reproductive traits may have evolved as a compromise between conflicting selective pressures exerted by pollinators and herbivores (Brody & Mitchell, 1997; Strauss, 1997; Galen & Cuba, 2001; Irwin *et al.*, 2004). Several mechanisms, including the cancellation of pollinator selection by overwhelming herbivore pressure (Quesada *et al.*, 1995; Gómez & Zamora, 2000; Herrera, 2000; Mothershead & Marquis, 2000) and correlated evolution of traits enhancing pollination and herbivore avoidance (through pleiotropy, developmental associations or correlational selection, see Herrera *et al.*, 2002a and references therein), may occur in plant-pollinator-herbivore systems, both at micro- and macroevolutionary levels.

However, the evolution of species interactions can only be fully understood by considering their variation in space and time (Thompson & Pellmyr, 1992; Thompson, 1994; Travis, 1996; Brody, 1997; Gómez & Zamora, 2000)

because this variation can limit species' responses to selection. In fact, recent advances in coevolutionary theory have arisen from the recognition that the form and incidence of an interaction often vary across geographic landscapes (Thompson & Pellmyr, 1992; Thompson, 1999: Benkman *et al.*, 2001), forming a selection mosaic. This geographic variation forms the basis of the geographic mosaic theory of coevolution (Thompson, 1994, 1997, 1999), and examples now exist that document selection mosaics in plant–animal interactions (Thompson, 1999: Benkman, 1999; Gómez & Zamora, 2000; Benkman *et al.*, 2001; Parchman & Benkman, 2002; Stinchcombe & Rausher, 2002; Zangerl & Berenbaum, 2003). More specifically, the selection mosaic in a specific plant–animal interaction may in part be generated by a concurrent interaction of one of the partners with a third species, which also varies geographically (see Thompson & Pellmyr, 1992; Gómez & Zamora, 2000; Benkman *et al.*, 2001; Stinchcombe & Rausher, 2002). For example, Benkman and co-workers (Benkman, 1999; Benkman *et al.*, 2001; Parchman & Benkman, 2002) have shown that coevolution between crossbills and both lodgepole pine and black spruce (a seed predator–plant interaction) is altered in some regions by red squirrels (*Tamiasciurus hudsonicus*), another seed consumer that outcompetes crossbills. Similarly, in populations of the crucifer *Hormathophylla spinosa* subject to intense herbivory by ungulates, selection by pollinators is annulled or reduced, which leads to a selection mosaic in the interaction between the plant and its pollinators as a consequence of variation in the abundance of ungulates (Gómez & Zamora, 2000). Other aspects of the geographic structure of the plant–animal interactions are, however, poorly known. Thompson (2002) remarked that the hierarchical geographic structure of the species interactions (for example, the shape and scale of the geographic mosaic) have important consequences for the evolutionary dynamics of the interactions. However, we do not know of any attempt to explore such a structure in plant-pollinator-herbivore systems.

Here, we incorporate these new perspectives to the study of the effect of predispersal interactions with animals (pollination, herbivory by insects, and seed predation by rodents) on the maternal fecundity of the perennial herb *Helleborus foetidus* (Ranunculaceae), using the approach suggested by Brody (1997), i.e. using multiple study sites and -years to examine the magnitude, direction and variability of the relationships between a plant and the different sets of animals with which it interacts (termed 'interactors' hereafter). To this end, we adopt a phenotypic selection approach at three widely separated regions spread over most of the geographic range of the species in the Iberian Peninsula. We investigated two populations in each region to examine intra- and inter-regional patterns and to assess the hierarchical geographic structure of these plant–animal interactions.

Phenotypic selection is commonly examined by standard selection gradient analysis (Lande & Arnold, 1983). Standard tests of differences in direct, adaptive, selection involve interaction terms of trait × population (or region) for geographic comparisons, or trait × year for temporal comparisons (Dudley, 1996; Caruso, 2000). When differences in selection are found, path analysis is an excellent way of determining the ecological reasons for the differences (Conner *et al.*, 1996; Conner, 1996). Here, we follow this scheme. First, we look for intra-regional (between populations), geographic (between regions) and temporal differences in phenotypic selection using selection gradient analysis, which will allow us to identify which plant traits are actually under selection and to asses whether differential selection (i.e. variation in the magnitude and direction of the selection) is happening at any of these levels. Then, we use path analysis to identify which interactors are responsible for such selection gradients. The study of selection mosaics caused by herbivores and pollinators in plant fecundity has recently been approached through Structural Equation Models (SEM hereafter) and path analysis using nested models (Gómez & Zamora, 2000). Here, we propose a relatively novel analytical approach using multigroup comparisons of SEMs (Bentler, 1989; see Grace, 2003 for its use in evolutionary biology) that properly allows comparisons, among regions, of each causal link (i.e. paths) between plant traits, interactors and plant fecundity, while keeping the same SEM structure.

Three major questions will be addressed: (i) Does the geographic scale of differentiation in plant traits match the geographic scale of variation in the interaction between the plant and its herbivores and pollinators? (ii) Is there differential selection at regional or geographic level and, if this is the case, are differential selection and trait differentiation matched up? (iii) Does the interaction of *H. foetidus* with its animal interactors exhibit a selection mosaic across a broad geographic scale? We consider a selection mosaic to be caused by interactors when, first, differential selection occurs at some spatial or temporal level and, secondly, differential selection is a consequence of differences, at the same level, in the animal/s responsible for trait selection.

Plant natural history

Helleborus foetidus L. (Ranunculaceae) is a rhizomatous perennial herb distributed in western and south-western Europe (Werner & Ebel, 1994). In the Iberian Peninsula it typically occurs in clearings, forest edges, and the understory of montane forests. Each plant is formed by one or several ramets that develop a terminal inflorescence after several seasons of vegetative growth. Each inflorescence produces 25–100 flowers over its 1.5–2.0 months flowering period, but these open gradually and are extremely long-lived (up to 20 days). Details

of the floral biology and seed dispersal of the species in the Iberian Peninsula can be found in Herrera *et al.* (2001, 2002a,b) and Garrido *et al.* (2002). Flowering takes place in winter (January to March) and the species is mainly bumblebee-pollinated. Flowers are hermaphroditic, protogynous and self-compatible, although substantial fruit set requires the participation of pollinators. Flowers are apocarpous, with one to five carpels (most commonly two to three) and each carpel contains 8–15 elaiosome-bearing seeds. Fruit maturation and seed shedding take place in June to early July. Flowers and, especially, developing fruits are frequently consumed by rodents and lepidopteran larvae (*Noctua* spp. and *Trigonophora flammea*, Noctuidae). Aphids (most frequently the monophagous *Macrosiphum helebori*, but also sometimes the polyphagous *Brachycaudus cardui*) also feed on flowers and fruits and, although their occurrence is rather unpredictable in time and space, they may become serious damaging agents in some plants, populations or years. Cryptic fruit or seed damage have never been detected.

Methods

This study was conducted during 1998 and 1999 in three widely separated regions, Sierra del Caurel (42°36′N, 7°19′W), Sierra de Cazorla (37°54′N, 2°55′ W) and Sierra de Mágina (37°44′N, 3°15′W), covering a considerable part of the geographic range of *Helleborus* in the Iberian Peninsula. Two of these regions, Sierra de Cazorla and Sierra de Mágina, are south-eastern mountain systems, 100 km apart from each other. The third region was Sierra de Caurel, more than 600 km north-west of the other two localities. Two *Helleborus* populations, located a few kilometres apart from each other, were considered within each region. Data on vegetation type, elevation and climate at each region can be found in Herrera *et al.* (2002a,b) and Garrido *et al.* (2002).

Plant traits, interactors' incidence and maternal fecundity

In each study year, 30–34 flowering plants were haphazardly chosen in each population. Total number of flowers was estimated as the total number of flower buds present at the beginning of the flowering season. Simultaneously, we counted the number of ramets, and measured ramet (inflorescence + supporting stem) length. Plant size was estimated as the product of number of ramets by mean ramet length. The number of carpels per flower and flower size (estimated as the geometric mean of flower length and width) were also measured from five flowers per plant after anthesis. Data on flower traits for Cazorla in 1999 are not available.

We checked each plant on three to five occasions throughout the flowering-to-fruiting period in order to determine the effect of pollinator visitation and herbivore damage on the final number of fruits releasing seeds from each plant. Pollinator visitation rate (pollinator service, hereafter) was evaluated as the probability of a plant being visited by pollinators, based on visitation data obtained during 20–30 3-min censuses per plant (see Herrera *et al.*, 2001 for details). Three major types of herbivory were recorded: damage by rodents, which cut off flowers or fruits by the pedicels; damage by lepidopteran larvae, which externally fed on flowers or, more frequently, carpels; and presence of aphids, which occur on both flowers and maturing carpels. Total fruit losses caused by rodents and lepidoptera were assessed as the cumulative number of flowers or fruits damaged by these herbivores recorded on different occasions. As direct effects of aphids on plants were not so evident, their incidence was evaluated as the maximum number of flowers infested in successive inspections. Fruit losses caused by abortion or abiotic damage were also recorded. At the end of the fruiting period, in late June and early July, and just before follicle dehiscence, we estimated for each plant the total number of fruits releasing seeds by counting the number of mature and intact fruits. These figures will be used here as estimates of maternal fecundity. Number of intact fruits per plant is strongly correlated with total final number of seeds (r-values ranging from 0.97 in Cazorla to 0.99 in Mágina, $P < 0.001$).

To estimate variance components (between regions, between populations within regions and between plants within populations) in plant traits, interactors' incidence and fecundity, we considered both region and population nested within region as random effects, as required for variance partitioning, and analyses were conducted with the MIXED procedure of SAS (SAS Institute, 1999). However, provided that only three regions were chosen for study, it is difficult to assume that they represent an adequate random sample of all regions in which the species appears. Therefore, to test for differences between regions and populations in the variables considered, we treated region as a fixed effect and conducted generalized linear mixed models. Error distributions and link functions were chosen according to the nature of data (Poisson error and log link function with variables involving counts, binomial error and logit link function with proportions or dichotomous variables, and normal error and identity with continuous variables). Analyses were conducted with SAS macro GLIMMIX (Littell *et al.*, 1996).

Study of phenotypic selection

Phenotypic selection at each region and year was examined by Lande-Arnold selection gradient models (Lande & Arnold, 1983). Multiple regressions for these models were conducted with relative fitness (estimated by dividing the maternal fecundity of each individual by the population mean) and standardized variables

(standardization of plant traits to mean 0 and variance 1). We obtained 95% confidence limits for selection coefficients by bootstrapping (bias-corrected percentile limit estimates; Manly, 1997). The coefficients were considered significant if their 95% confidence interval did not include zero. Regressions were conducted with SAS REG procedure and the bootstrap estimates with the SAS macro JACKBOOT (SAS Institute, 1999). Previous analyses including nonlinear terms, show that quadratic partial regression coefficients were not significant at any region or year (data not shown), suggesting that, when present, selection on each particular trait was predominantly directional and linear. Similarly, bivariate terms for correlational selection were not significant in most cases, the only exception being Caurel in 1999, where a negative correlational gradient ($b = -0.15$, $P < 0.01$) appeared for the interaction between plant size and number of flowers. Consequently, we consider here only direct linear selection components.

We looked at differential selection between populations within region, between regions, and between years within region, in the traits being selected. In each case, we conducted an ANCOVA analysis including, as adequate, population × trait, region × trait or year × trait interactions. Traits were transformed as appropriate for normality in the ANCOVA analyses.

A causal model of the phenotypic selection by interactors
To explain the ecological basis of the phenotypic selection found at each region and year, an overall SEM was built hypothesizing the causal relationships between three groups of variables: maternal fecundity (target variable), interactors' incidence (intermediate variables directly influencing plant fecundity), and plant traits (independent variables) (see Mitchell, 1992, 1993; Shipley, 1997; Gómez & Zamora, 2000 for similar approaches; see also Scheiner *et al.*, 2000, for a recent review of the use of path analysis to measure natural selection). As all plant traits considered here might influence the incidence of each interactor on plant fitness, paths from each trait to each interactor were allowed in the model. We found each interactor to significantly affect relative fitness in some region or year (data not shown), thus paths from each interactor to maternal fecundity were included. Finally, as *Helleborus* is self-compatible and autonomous self-pollination occurs at all study regions (Herrera *et al.*, 2001), we also included the number of flowers as a predictor of the maternal fecundity. We could not build the SEM for Cazorla in 1999 because of the absence of information on floral traits. A previous inspection of the correlation matrices showed poor correlations among interactors. Only three of 30 correlations (six from each path diagram) were significant: rodent damage was positively correlated with pollinator service in Cazorla in 1998 and Mágina in 1999 ($r = 0.26$, $P < 0.05$, $n = 62$; $r = 0.29$, $P < 0.05$, $n = 64$ respectively), and aphid damage was positively correlated with

Lepidoptera damage in Cazorla in 1998 ($r = 0.43$, $P < 0.001$). Entering paths for correlations between interactors only improved the model fit in Cazorla in 1998, while in the other regions the model became overspecified. By these reasons, we included such paths only in Cazorla.

The adequacy of the hypothesis expressed in the SEM model is obtained from a goodness-of-fit test comparing the observed covariance matrix with that expected if the model were true (Mitchell, 1994; Shipley, 1997). The SEM was analysed with SEPATH module (StatSoft, 2000). The variables were transformed for linearity and multinormality (log transformed or square root transformed as appropriate, Zar, 1999), although they sometimes remained non-normal after transformation. We used Generalized Least Squares (GLS) as discrepancy function because GLS is preferred to maximum likelihood when multivariate normality is not met (see SAS/STAT User's guide, SAS Institute, 1990). In any case, we stress that the adequacy of the hypothesis of our causal model is not, in fact, a major issue in this paper. Instead, our interest is to build an overall model accounting for all the relevant relationships possibly occurring in any population, so that such relationships can be readily compared among populations, regions and years. The analytical method for such comparison is described below.

Spatial and temporal congruency in the causal model
An objective of this study was to evaluate the extent of spatial and temporal congruency in the constellation of selective pressures potentially exerted by animal interactors on plant traits. This implied comparing SEMs structure and path coefficients between years, regions and populations within region. The few studies that have so far assessed the spatio-temporal congruency of selective pressures by means of SEM have used nested models (Mitchell, 1994; Gómez & Zamora, 2000; Sánchez-Lafuente, 2002). This approach looks for the most parsimonious model adequately explaining the causal relationships among variables. However, it does not explicitly compare the magnitude and direction of a path coefficient between population or years, a goal that can be accomplished by means of multigroup analysis of SEMs (Bentler, 1989; Grace, 2003; see Bishop & Schemske, 1998, for an application to plant-pollinator systems). A multigroup analysis allows to ask whether sets of parameters in the model differ between groups (Bentler, 1989). Multigroup analysis is carried out by imposing cross-group constraints on the path coefficients, and simultaneously fitting 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. paths 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,

meaning homogeneity between groups in the causal relationships among plant traits, interactors and plant fecundity. If the chi-square value of the goodness of fit test shows significant departure of fit (i.e. $P < 0.05$), then the hypothesis of total equality can be rejected and it is pertinent to compare the path coefficients of different groups to identify the origin of overall between-groups heterogeneity. Multiple equality constraints containing $n > 2$ parameters or path coefficients are tested in n successive steps, each assuming the release of one of the equality-constrained parameters. Those constraints for which the relaxation of the equality assumption causes a significant decrease in the chi-square value indicate significantly different path coefficients (Bentler, 1989, see also SAS/STAT User's Guide, SAS Institute, 1990). These multi-group analyses were conducted with the SEPATH module (StatSoft, 2000).

Results

Geographical variation in plant traits, interactors' incidence and fecundity

For most variables considered in this study, a partition of variance shows greater variance among regions than among populations within regions (Fig. 1). Plant traits frequently varied significantly among regions (Fig. 1 and Table 1) and much variance remained among plants within populations. However, plant traits did not vary significantly between populations of the same region. The mean number of flowers produced per plant was considerably higher in Mágina than in the other two regions in both study years (Table 1). Flowers in Cazorla produced fewer carpels than in Caurel or Mágina and, at least in 1998, flowers were larger in Mágina than in the other two regions.

Similarly, the relative incidence of most animal inter-actors on *Helleborus* plants varied significantly among regions (Table 1, Fig. 1) in both study years, but not between the populations of the same region. In any case, the incidence of each interactor on *Helleborus* plants varied widely among individuals within populations (Fig. 1). Pollinator service was particularly low in Mágina and high in Caurel in both years, and this was largely responsible for the observed variation among regions. Rodent damage tended to be high in relative terms, especially at Mágina in 1998 and Cazorla in 1999, and differed between regions only in the second year. Aphids were never detected in Caurel, but their incidence was high in the other two regions during 1998, particularly in Cazorla where on average more than 35% of the flowers were infected that year. Aphid frequency was negligible during 1999 in all the regions. Damage by Lepidoptera was particularly high in Cazorla, where they devastated the inflorescences of many plants.

The spatial pattern of variation in plant fecundity matched that of plant traits and interactors' incidence,
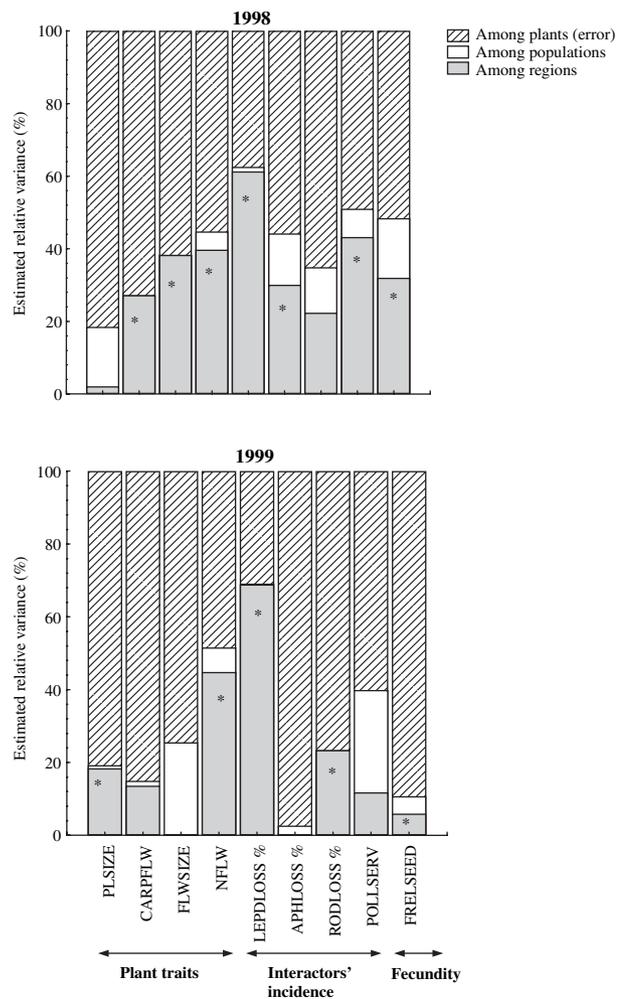

Fig. 1 Variance components (among regions, among populations within region and among plants within populations) for the variables considered in this study (see Methods). Statistically significant variation ($P < 0.05$ or better) among regions for a variable is indicated with an asterisk within the corresponding bar (see also Table 1).

being widely variable among regions in both years, and among individuals within populations, but not differing between populations of the same region (Fig. 1). As shown in Table 1, plants in Caurel had higher fecundity in both years than those from Cazorla or Mágina. Average fecundity was particularly low in Cazorla in 1998, when many plants produced few or no seeds.

Differential selection on plant traits

Selection did not differ between populations at any region in each year, as suggested by nonsignificant trait × population interactions in within-region ANCOVA analyses ($P > 0.1$ for each trait in each region, data not

Table 1 Regional variation in plant traits, interactors' incidence and plant fecundity in the two study years.

Variables	1998			1999		
	Caurel	Cazorla	Mágina	Caurel	Cazorla	Mágina
Nflw	**27.7ᵃ (14.6–52.6)**	**31.2ᵃ (17.3–56.2)**	**83.5ᵇ (54.1–129.0)**	**39.4ᵃ (23.1–67.3)**	**38.0ᵃ (22.5–64.4)**	**105.8ᵇ (74.1–151.1)**
Plsize	60.7 (34.7–106.3)	90.6 (53.7–152.8)	80.3 (47.1–136.8)	89.7 (62.0–129.9)	120.8 (90.8–160.7)	150.4 (118.1–191.6)
Carpflw	**2.3ᵃ (2.1–2.4)**	**1.9ᵇ (1.8–2.1)**	**2.2ᵃ (2.1–2.3)**	2.4 (2.1–2.7)	–	2.5 (2.2–2.8)
Flwsize	**14.3ᵃ (14.1–14.5)**	**14.8ᵇ (14.6–15.0)**	**15.6ᶜ (15.4–15.8)**	15.1 (13.5–16.9)	–	15.2 (13.7–1 7.0)
Leploss (%)	**1.8ᵃ (0.2–15.6)**	**37.4ᵇ (7.2–82.1)**	**4.2ᵃ,ᵇ (0.5–27.1)**	**1.6ᵃ (0.3–9.7)**	**22.4ᵇ (6.8–53.4)**	**3.3ᵃ (0.7–14.3)**
Aphids* (%)	0	35.5 (2.6–92.0)	11.0 (0.5–75.8)	0	0.6 (0.01–16.7)	0.3 (0.01–15.6)
Rodloss (%)	5.6 (0.3–55.1)	11.2 (0.7–69.0)	32.6 (2.7–89.2)	**2.4ᵃ (0.5–9.6)**	**21.8ᵇ (10.0–41.2)**	**11.3 (4.6–25.3)**
Pollserv	**0.26ᵃ (0.06–0.56)**	**0.05ᵃ,ᵇ (0.01–0.22)**	**0.02ᵇ (0.003–0.11)**	0.20 (0.02–0.63)	0.09 (0.01–0.43)	0.04 (0.005–0.30)
Frelseed†	**21.9ᵃ (10.5–36.8)**	**2.9ᵇ (1.1–7.7)**	**15.8ᵃ (7.3–34.0)**	**40.2ᵃ (25.2–64.3)**	**20.4ᵇ (12.3–33.7)**	**20.6ᵇ (12.5–33.9)**

Data shown are back-transformed least squares mean values and 95% confidence intervals predicted by generalized linear models with region and population nested within region as fixed and random effects respectively (see Methods), although only the region effect is shown because the population effect was not significant for any variable. See also Fig. 1 for variance components (among populations and regions) for each variable. Variables showing significant variation are in boldface and the localities differing significantly after post-hoc tests are identified with different superscripts.

Plsize = plant size, Carpflw = number of carpels per flower, Flwsize = flower size, Nflw = number of flowers, Leploss = fruit loss to Lepidopetera, Aphloss = fruit infestation by aphids, Rodloss = fruit loss to rodent, Pollserv = pollinator service, Frelseed = fruits releasing seeds.

*Tests comparing aphid abundance refer only to Mágina and Cazorla, as aphid infestation was not observed in Caurel in any year.

†After controlling for total number of flowers produced by each individual plant (incorporated to the model as a covariate).

Note that no data on flower size and number of carpels per flower were available in Cazorla in 1999.

shown). Consequently, all subsequent analyses were conducted merging data from populations of the same region.

Selection gradient analyses identified the traits subjected to selection, and the sign and magnitude of the selection, at each region and year (see coefficients in Table 2). In Caurel, selection was positive and significant on flower number and carpels per flower and marginally significant on plant size during 1998. In 1999, we detected again positive selection on flower number; however, that time, selection on plant size and carpel number per flower did not differ from zero. Instead, we detected significant positive selection on flower size. Consequently, an ANCOVA analysis detected variation between years in traits subjected to selection in Caurel (Table 3). In Mágina, selection was positive and

significant on flower number and flower size during 1998. The second year, we detected again significant positive selection on flower number (being higher than in the previous year), but not on flower size; instead, we found positive selection on carpel number per flower. Consequently, an ANCOVA analysis also detected between years variation in the traits subjected to selection in this region (Table 3). Finally, in Cazorla, only carpel number per flower resulted significantly selected in the year with data for this region, and its selection was in the direction of decreasing the carpel number (negative selection).

As expected from the selection gradient analyses above, ANCOVA analyses showed that, during both years of study, selection differed significantly between regions (Table 4). In 1998, differential selection occurred on carpel number per flower and flower size (i.e. significant

Table 2 Selection gradient analyses for each region and year.

	1998 (b ± SE)			1999 (b ± SE)	
	Caurel	Cazorla	Mágina	Caurel	Mágina
Plsize	0.10 ± 0.06*	0.13 ± 0.33	−0.07 ± 0.22	−0.04 ± 0.04	−0.07 ± 0.21
Carpflw	**0.09 ± 0.04**	**−0.36 ± 0.17**	−0.12 ± 0.14	−0.01 ± 0.02	**0.36 ± 0.07**
Flwsize	−0.02 ± 0.04	−0.04 ± 0.12	**0.23 ± 0.11**	**0.06 ± 0.02**	0.04 ± 0.08
Nflw	**0.39 ± 0.06**	0.35 ± 0.31	**0.41 ± 0.19**	**0.45 ± 0.03**	**0.60 ± 0.22**
Adjusted-R^2	0.66	0.20	0.24	0.92	0.61

Only linear selection gradients were considered (see Methods). Results show the standardized selection coefficient (b) and its standard error (SE) estimated by bootstrapping. Significant coefficients (those whose 95% confidence interval did not include 0) are in boldface. Marginally significant coefficients (P < 0.1) are marked with an asterisk. Independent variables were standardized to mean 0 and variance 1 and the dependent variable was relative fitness.

Table 3 ANCOVA analyses comparing patterns of selection on plant traits between years for the same region.

Effect	d.f.	Caurel		Mágina	
		$F_{(110)}$	P	$F_{(113)}$	P
Intercept	1	11.49	<0.001	2.43	0.12
Year	1	2.31	0.13	0.03	0.87
Plsize	1	**4.93**	**0.03**	3.20	0.08
Carpflw	1	3.49	0.06	0.92	0.34
Flwsize	1	0.38	0.54	**6.30**	**0.01**
Nflw	1	**196.32**	**<0.001**	**28.68**	**<0.001**
Year × Plsize	1	**9.52**	**0.003**	0.77	0.38
Year × Carpflw	1	**4.52**	**0.04**	**12.95**	**<0.001**
Year × Flwsize	1	2.88	0.09	3.28	0.07
Year × Nflw	2	0.004	0.95	0.01	0.92

Dependent variable was relative fitness. Significant ($P < 0.05$ or better) are in boldface. Particular interest is paid to the interaction effects.

Table 4 ANCOVA analyses comparing patterns of selection on plant traits between regions.

Effect	d.f.	1998		1999	
		$F_{(168)}$	P	$F_{(117)}$	P
Intercept	1	1.10	0.30	3.15	0.08
Region	2	1.58	0.21	0.54	0.46
Plsize	1	0.39	0.44	1.98	0.16
Carpflw	1	3.71	0.056	**14.87**	**<0.001**
Flwsize	1	1.01	0.32	1.77	0.19
Nflw	1	**28.90**	**<0.001**	**90.19**	**<0.001**
Region × Plsize	2	2.87	0.059	0.005	0.95
Region × Carpflw	2	**4.36**	**0.014**	**17.72**	**<0.001**
Region × Flwsize	2	**3.15**	**0.045**	0.07	0.79
Region × Nflw	2	1.76	0.175	**6.40**	**0.01**

Dependent variable was relative fitness. Data from the two populations of each region were merged after demonstrating the absence of significant differences on selection on any trait among populations of each region. Significant effects are in boldface ($P < 0.05$ or better). Note that during 1999 only Caurel and Mágina were compared. Particular interest is paid to the interaction effects.

trait × region interactions in Table 4) and it was marginally significant on plant size, while in 1999 selection on flower number and carpel number per flower varied inter-regionally.

Ecological basis for the phenotypic selection at each region (path analyses)

Chi-square tests of goodness of fit indicate that three of five data sets examined (Caurel 1998: $\chi_6^2 = 7.8$, $P = 0.26$; Cazorla 1998: $\chi_3^2 = 2.4$, $P = 0.50$; and Mágina 1999: $\chi_9^2 = 16.8$, $P = 0.06$) were adequately described by the hypothesized model of causal relationships, whereas in the other two cases (Mágina 1998: $\chi_9^2 = 23.5$, $P < 0.01$; and

Caurel 1999: $\chi_8^2 = 13.2$, $P < 0.05$) the fit was significant. Anyway, all the tests showed χ^2/d.f. ratios lower than 5 (2.6 in Mágina 1998, 1.7 in Caurel 1999), the threshold value suggested by Wheaton et al. (1977) (see also Pedhazur, 1982; Mitchell, 1992). We therefore consider that the model depicts satisfactorily the overall relationship between plant traits, interactors' incidence and plant fecundity. The model explained more than 90% of variance in individual fecundity in Caurel, around 60% in Mágina, and 30% in Cazorla (see variance errors of the target variable, fruits releasing seeds, in Fig. 2).

In Caurel 1998, rodents and Lepidoptera were responsible for most of the phenotypic selection detected in previous selection gradients analysis. Rodents were responsible for the positive selection gradient on carpel number per flower, through negatively affecting maternal fecundity and preferring plants with few carpels per flower. Lepidoptera seem responsible for the marginal positive selection gradient on plant size through its negative effect on maternal fecundity and its higher consumption on smaller plants. Finally, the positive selection on flower number was a consequence of the direct and positive effect of this trait on maternal fecundity; this positive effect largely overcomes a negative selection on this trait by Lepidoptera that, feeding preferentially on plants with more flowers, decreased the maternal fecundity. Next year, rodents and Lepidoptera were again partially responsible for the phenotypic selection at this region but this time pollinators contributed too. Positive selection gradient on flower number was again mainly due to a direct positive effect on maternal fecundity which overcomes the negative selection induced, this time, by rodents (that fed preferentially on plants with more flowers, affecting negatively fecundity) and to a lesser extent by pollinators. Pollinators seem responsible for the positive selection gradient on flower size by visiting more frequently plants with smaller flowers and affecting negatively the maternal fecundity. Finally, we found a negative selection on plant size by Lepidoptera (and a negative selection on carpel number per flower by pollinators), not emerging in the selection gradient analysis.

In Cazorla, path analysis confirmed that the negative selection gradient on carpel number per flower was mostly because of rodents, whose activity was positively correlated with fecundity, and preferred plants with fewer carpels per flower. Pollinators contribute also, but to a lesser extent, to this gradient visiting plants with few carpels per flower and affecting positively fecundity. In this region, fruit loss to rodent and Lepidoptera were negatively correlated. It means that plants suffering more fruit loss to rodents were less attacked by Lepidoptera, the overall effect of rodent on maternal fecundity being positive, much probably because it prevented from heavier attack by Lepidoptera larvae.

In Mágina, in 1998 selection by aphids and rodents contributed to explain the significant selection gradients
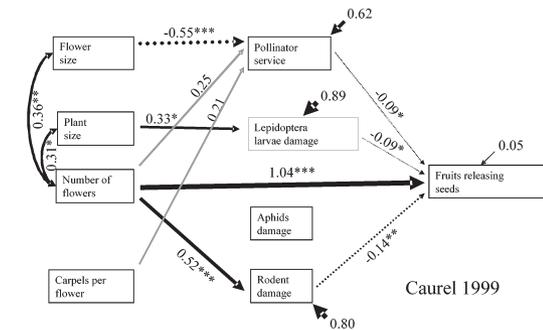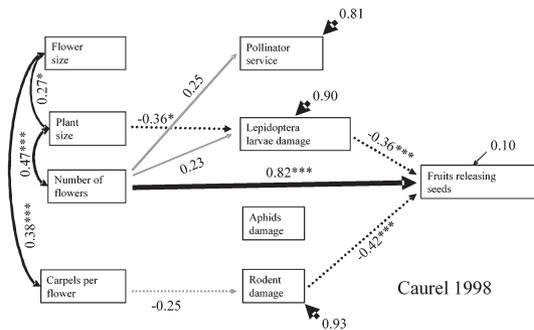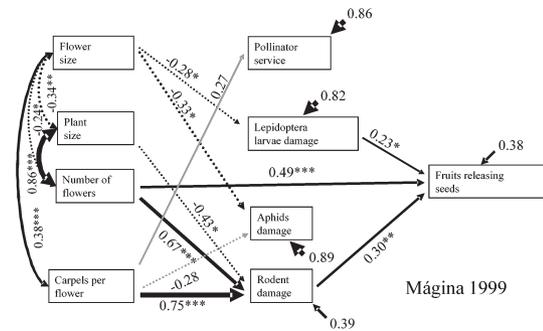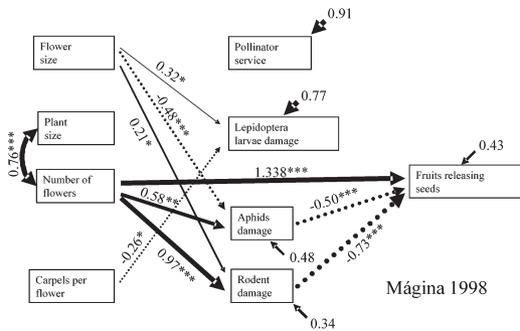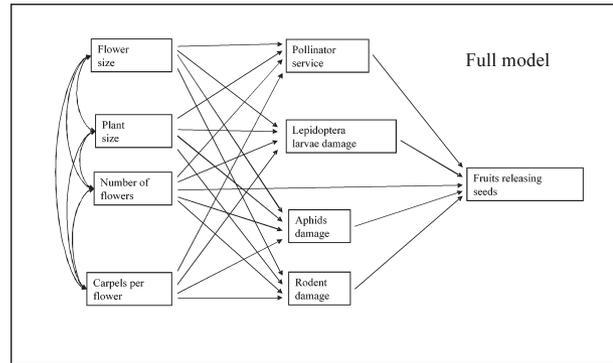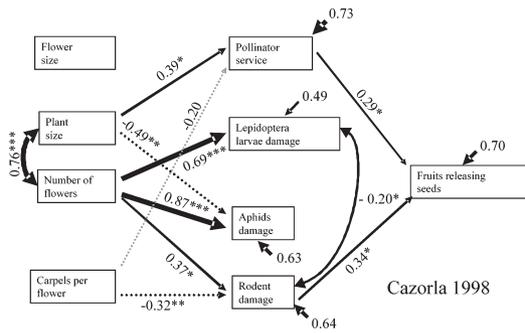
Fig. 2 SEM diagrams for each region and year. Only significant paths (*P* < 0.05, black arrows) or marginally significant (*P* < 0.1, grey arrows) are represented. Arrow widths are proportional to the path coefficients values which are also indicated over the corresponding arrow. Negative effects are indicated with dashed arrows, positive effects with continuous arrows. Error values of endogenous variables (those being affected by other variables) are also indicated. Phenotypic selection mediated by interactors is identified by significant (or marginally significant) pathways from traits to interactors and from interactors to maternal fecundity (number of fruits releasing seeds) in the path diagrams. The direction (positive or negative) of such selection is determined by the sign resulting from multiplying the path coefficients involved in each pathway. Correlations between plant traits are shown by two-headed arrows. Note that for the Caurel region there was no link between aphid damage and any other variable because of absence of infestation in this region in both study years. Note also that the SEM for Cazorla 1999 was not available because of the absence of information on floral traits in this region during this year.

found for flower size and number. Aphids were responsible for the positive selection on flower size because they prefer plants with smaller flowers and affected negatively maternal fecundity. This positive selection was somewhat lessened by negative selection by rodents, which tended to select larger flowers and also affected negatively fitness. Flower number affected directly fitness but it was also negatively selected by the activity of herbivores, in particular aphids and rodents, which preferred plants with more flowers. The positive selection gradient on this

trait occurred because of the increased potential for reproduction with higher flower number that overcomes the negative selection by herbivores. Herbivores, specifically rodents, seem also involved in the significant selection gradients detected in 1999. Although rodents consumed more in plants with more carpels per flower, still their activity was positively correlated with fitness. Thus, in spite of the activity of rodents there was positive selection on this trait. Rodents were also involved in the positive selection gradient on number of flower this year,

reinforcing the direct positive effect of flower number on fecundity. Other interactor-mediated selections emerging from path analysis (e.g. the negative selection on plant size caused by rodents) were, however, not apparent from the selection gradients. Finally, the unexpected positive effects of rodents and Lepidoptera on maternal fecundity were negatively correlated with fruit abortion ($r = -0.40$, $P < 0.001$; $r = -0.26$, $P < 0.05$, respectively; correlations after controlling for number of flowers), which in turn affected maternal fecundity ($r = -0.40$; $P < 0.001$, $n = 64$) and accounted for much fruit loss in this region ($46.2 \pm 2.4\%$ of total flower production; mean and 1 SE). It means that fruit consumption by these herbivores might compensate for late fruit abortion, turning their effects on maternal fecundity to positive.

Geographic and temporal congruency in interactor-mediated selection

Multigroup tests revealed significant regional variation of the SEM structure, as the hypothesis of total equality of path coefficients was rejected in both study years ($\chi^2_{53} = 106.7$, $P < 0.001$ in 1998, for the three regions considered; $\chi^2_{31} = 68.96$, $P < 0.001$ in 1999, for only two regions). There was also a marked temporal inconsistency in the structure of the SEMs for the two regions with data for 1998 and 1999 (Caurel: $\chi^2_{31} = 60.6$, $P < 0.001$; Mágina: $\chi^2_{44} = 90.4$, $P < 0.001$). Comparisons of path coefficients confirmed the existence of significant differences among regions each year and between years in each region (Table 5)

Consideration of the variation of some particular paths illustrates by way of example the sort of variation underlying the spatio-temporal inconsistencies in SEMs. Rodent damage was, almost invariably, positively related to number of flowers, but the intensity of these relationships frequently differed (see Table 5). The signs of the relationships between other plant traits and the different interactors were, however, largely inconsistent across time and space. For example, carpels per flower influenced positively rodent damage in Mágina in 1999 but negatively in Cazorla in 1998 and Caurel 1998; Lepidopteran damage was negatively related to plant size in Caurel in 1998 but positively in 1999. Likewise, the sign of the effect of most interactors on plant fecundity was frequently inconsistent in time and space. For example, although the effect of rodent damage on plant fecundity was negative in Caurel both years, in Mágina it shifted from negative in 1998 to positive in 1999, and in Cazorla was positive. Aphid damage affected plant fecundity negatively in

Table 5 Multigroup comparison of SEM showing decrease in the χ^2 value (i.e. improvement of the model fit) when releasing each path coefficient from the cross-groups equality constraint (see Methods for details).

H		χ^2 comparison (between regions)				χ^2 comparison (between years)	
Independent variable	Dependent variable	Caurel vs. Cazorla (1998)	Caurel vs. Mágina (1998)	Cazorla vs. Mágina (1998)	Caurel vs. Mágina (1999)	1998 vs. 1999 (Caurel)	1998 vs. 1999 (Magina)
Plsize	Leploss	2.61	3.29(*)	0.06	1.06	**4.39**	0.00
Carpflw	Leploss	0.13	0.97	2.51	0.02	0.38	**4.35**
Flwsize	Leploss	0.47	**4.37**	2.78	1.42	1.38	1.84
Nflw	Leploss	**11.24****	1.00	**4.07**	0.13	0.69	0.54
Plsize	Aphloss	***	–	0.45	–		1.87
Carpflw	Aphloss	–	–	0.12	***		0.00
Flwsize	Aphloss	–	***	0.25	***		0.30
Nflw	Aphloss	***	***	0.45	–		**5.13**
Plsize	Rodloss	1.51	0.41	0.01	1.55	0.22	0.35
Carpflw	Rodloss	0.54	2.20	**4.05**	**6.65**	1.35	**7.70**
Flwsize	Rodloss	0.05	0.93	0.02	0.17	1.10	**5.59**
Nflw	Rodloss	**7.60**	**8.94**	0.001	0.02	2.57	3.02(*)
Plsize	Pollserv	1.08	0.18	0.22	1.29	1.19	0.68
Carpflw	Pollserv	2.11	0.19	0.87	0.45	2.22	0.12
Flwsize	Pollserv	2.25	0.00	0.3	**4.81**	8.33	0.2
Nflw	Pollserv	0.27	0.06	0.43	3.58(*)	0.00	0.35
Nflw	Frelseed	**5.21**	0.05	15.42	**4.09**	1.63	0.35
Leploss	Frelseed	0.67	**9.71**	1.00	**6.19**	9.16	0.50
Aphloss	Frelseed	–	***	3.73(*)	–	–	3.49(*)
Rodloss	Frelseed	13.10***	0.11	1.48	0.51	1.76	**7.50**
Pollserv	Frelseed	0.21	0.86	2.14	0.51	0.08	0.80

Significant figures are in boldface: ***$P < 0.001$,**$P < 0.01$, *$P < 0.05$, (*)$P < 0.1$. Variable names coded as in Table 1. Comparisons of paths involving aphids are not possible with Caurel because there was no aphid infestation there, but significant differences in such paths are presumed when they significantly differ from cero in the other regions (***). Models for multi-group comparisons did not consider the correlations between interactors (only found to be significant in Cazorla) because the same model is required for such comparisons.

Mágina in 1998, where some plants were heavily damaged, but not in other years or regions with more homogeneous levels of aphid infestation. Lepidoptera negatively affected plant fecundity in Caurel both years, but positively in Mágina 1999.

As a consequence of the differences in path coefficients (see Table 5), there was no interactor-mediated phenotypic selection on plant traits geographically or temporally consistent (compare pathways from traits to fecundity in Fig. 2). For example, rodent-mediated phenotypic selection on number of flowers occurred frequently, but in 1998 it shifted from positive in Cazorla to negative in Mágina, and in 1999 from negative in Caurel to positive in Mágina. Similarly, it shifted between years in a same region (Mágina) from negative in 1998 to positive in 1999. The only consistent, predictable effect on plant fecundity was a direct effect (not mediated by interactors) of number of flowers, which always had a positive effect and was statistically significant in four of five possible scenarios.

Discussion

By hierarchically exploring (intra- and inter-regionally) the interaction between *H. foetidus* and its animal interactors, we intended to depict part of the hierarchical geographic structure of such interactions (Thompson, 2002). Though the generality of our conclusions is limited by the small number of populations considered in each region, results clearly support the existence of a large-scale geographic and temporal mosaic involving the different interactions between *H. foetidus* and animals. *Helleborus foetidus* interacts with several herbivores (rodents, lepidopteran larvae and aphids) and pollinators (*Bombus* spp., Herrera *et al.*, 2001) during flowering and fruiting, and although there was remarkably little variation in the taxonomic identity of interacting species in the three widely separated regions considered, the main agents of selection did differ between regions. Differential selection on plant traits occurred at regional and temporal scales and it was linked to distinct subsets of interactors exerting phenotypic selection in different regions or years (see also Gómez & Zamora, 2000). The selection mosaic (Thompson, 1994, 1997, 1999) emerging in the interaction between *H. foetidus* and its animal counterparts, and its causes and likely evolutionary consequences, are discussed below.

Geographic variation in plant traits and interaction outcomes

In plant–animal interactions, variation among populations and across geographic ranges in traits and interaction outcomes seems to be the norm, and such variation is being emphasized by recent studies because it contributes to the evolution of interspecific interactions (Thompson & Pellmyr, 1992; Thompson, 1997; Holland & Fleming, 1999; Caruso, 2000; Garrido *et al.*, 2002; Sánchez-Lafuente, 2002). Our results for *H. foetidus* show that significant variation existed only at the larger regional geographic scale, but not intra-regionally. The absence of intra-regional variation in plant traits, interactors' incidence and average maternal fecundity suggests that there was no population divergence or local differentiation within regions in the plant traits considered, and that this may be a consequence of similarity in the selective pressures exerted by interactors on the populations of the same region leading to similar patterns of phenotypic selection. The lack of differential selection on plant traits at this level and the fact that the multigroup comparisons of SEM from populations of the same region consistently failed to refute the hypothesis of total equality in path coefficients (data not shown) support this explanation and reinforce the conclusion that interactors exerted comparable selective pressures on populations located in the same region.

Substantially different to the intra-regional pattern was the inter-regional pattern. The variation in abundance and/or incidence of herbivores and pollinators could be expected from the very different climatic conditions and abundance of alternative resources (the latter is valid also for annual variation). For example, rodent damage was infrequent in Caurel in both years, where rodents have an abundant supply of acorns and fruits of other species, but considerably higher at Cazorla and Mágina where availability of these resources was extremely low during the study. Overall, the variable outcome of the interaction at broad but not small geographic scales paralleled the differences in abundance of interactors, and/or in their dependence on the flowers or fruits of *H. foetidus*, at these different scales. The distinct phenotypic selection patterns exerted by interactors in different regions may set the stage for subsequent divergence of plant traits among regions (Galen, 1996; Wilson & Thomson, 1996; Gómez & Zamora, 2000). This possibility is examined below.

The roles of pollinators and herbivores on selection

Pollinators had a negligible effect on maternal fecundity in most regions and years. Significant contribution of pollinators to maternal fecundity was only detected in Cazorla 1998 and in Caurel 1999, but in the latter the effect was negative. Furthermore, these effects barely translated into significant selection gradients. This scarce contribution of pollinators is probably related to the fact that, except in Cazorla, maternal fecundity in *Helleborus* is only weakly or not pollen limited (Herrera *et al.*, 2001) and substantial autonomous self-pollination occurs in all regions. In the absence of pollen limitation, changes in pollinator visitation have no effect on relative fitness (Cariveau *et al.*, 2004). Some caution is, however, needed in the interpretation of pollinator role in the phenotypic selection. First, because we measured plant

reproduction via maternal fecundity only, we remain ignorant of the effects each trait had on male-fitness components. Pollinators could still select traits that influence pollen export or donation, estimates which are more sensitive to changes in pollinator visitation than female function (Young & Stanton, 1990). Secondly, pollinator service was probably measured less accurately than herbivore damage, as observations conducted over only 3–5 days might not sufficiently characterize individual differences in pollination regime.

In contrast, herbivores seemed responsible for most phenotypic selection in this study, as the phenotypic selection mediated by their activity was linked to the selection gradients and to the differential selection between regions. Other studies have found similar results (Gómez & Zamora, 2000; Gómez, 2003; Cariveau et al., 2004). Trade-offs in the evolution of plant characters may arise when trait preferences by herbivores and pollinators are the same and their activity limits fecundity (Gómez, 2003; Cariveau et al., 2004). The extensive selection exerted by herbivores in this study and their preference for some traits (flower number and, to a lesser extent, plant size or carpel number), similar to that of pollinators, suggest opportunity for trade-offs, though they only will arise when pollen limitation occur.

Differential selection, agents of selection and trait differentiation at the broad scale

Strong linking of the detected phenotypic selection to the activity of interactors and from the phenotypic selection to trait differentiation are impeded by several reasons: (i) selection estimates based exclusively on phenotypic covariances between traits and fitness may be biased by unmeasured environmental factors (Rausher, 1992; Scheiner et al., 2002; Kruuk et al., 2003; Winn, 2004); (ii) absence of information on trait heritability and genetic differentiation prevents quantification of the response to selection; and (iii) inconsistency at each region in the selection gradients that, except for flower number, were not significantly different from zero in both study years (although opposite selection on a trait was not found between years in any region either). Despite this, however, findings of this study deserve an attempt at linking selection and regional differentiation for some traits.

The selection gradient and ANCOVA analyses showed that all the plant traits examined were under directional selection in some region or year, but also that differential selection, either between regions or between years, occurred for every trait. The most consistently selected trait was flower number which, except for Cazorla in 1998, was always positively selected. Flower number commonly affects plant fitness, both indirectly by increasing the visitation rate of pollinators (e.g. Conner & Rush, 1996; Conner et al., 1996; Vaughton & Ramsey, 1998; Thompson, 2001) and directly by increasing

potential reproduction, irrespective of the effect on pollinator behaviour (Herrera, 1993; Conner & Rush, 1996; Gross et al., 1998; Gómez, 2003; Gómez & Zamora, 2000; Cariveau et al., 2004). We found that, except for Cazorla, flower number consistently affected fitness directly, this path making a major contribution to the total effect of flower number on maternal fecundity. Flower number did not affect fitness through pollinator preference at any ecological scenario, but herbivores (aphids, Lepidoptera and rodents) exerted selection on this trait, sometimes reinforcing it, sometimes reducing it. Irrespective of the type of herbivore, they were frequently attracted to plants with more flowers. The positive effects of flower production on the intensity of predispersal seed predation are well documented (Brody & Mitchell, 1997; Ohashi & Yahara, 2000; Cariveau et al., 2004).

In spite of the consistent positive selection on flower number, differences in the strength of selection appeared in the second year of study, with a stronger selection gradient in Mágina than in Caurel (see Table 2). Furthermore, although in 1998 there was no differential selection on this trait, the coefficient did not differ significantly from zero in Cazorla. Plants in Mágina had significantly more flowers than those in the other two regions, which is congruent with the strongest selection for increased flower number in Mágina. Thus, a link of differential selection to flower number differentiation was apparent.

Of major interest for regional trait divergence was the different direction on selection on carpel number per flower. This trait was negatively selected in Cazorla, whereas positive albeit inconstant episodes of selection were detected in Caurel and Mágina. This pattern of differential selection is congruent with the regional differentiation in this trait, since the carpel number per flower was significantly lower in Cazorla. Current negative selection and the regional differentiation in carpel number thus seem to match. Selection on this trait was always because of rodents, but their preferences on this trait varied regionally, and the consequences of their activity on fitness also varied. Follicle walls of fruits of this species are highly toxic to vertebrates and invertebrates (Holliman & Milton, 1990, J.M. Fedriani, pers. comm.) and rodents forage on fruits looking for seeds (Fedriani, 2005). Rodents can reduce their exposition to the poisonous walls of the fruits by increasing the reward obtained per seed rather than by foraging on flowers with more carpels. At least in Cazorla, seed number per carpel is independent of carpel number per flower but mean seed size is inversely related to carpel number (C.M. Herrera, unpubl. data). Thus, we would expect rodents to prefer fruits with individually heavier seeds and to forage on plants with flowers with low carpel number. This was, in fact, the rodent preference in Caurel and Cazorla but the opposite happened in Mágina, perhaps because of aphids preferentially infecting flowers with few carpels in this region (Fig. 2).

When significantly selected, the direction of selection on flower size was always positive (Mágina 1998 and Caurel 1999). To some extent, the larger selection gradient in Mágina was congruent with the significantly larger flowers in this region in 1998. Selection on this trait in Mágina was the balance between a positive effect by aphids and negative by rodents. Rodents preferred larger flowers, but they affected positively fecundity. In this region, rodents frequently consumed flower nectar after cutting the flowers, which may explain their preference as the mean nectar volume per flower is correlated across plants with the mean flower size ($r = 0.28$, $P < 0.05$, $n = 192$ plants, all the regions pooled). In Caurel, however, pollinators seemed responsible for the selection on flower size. Keeping other traits constant they more frequently visited plants with smaller flowers, but negatively affected fitness, perhaps because more visited plants also suffered higher herbivore damage ($r = 0.50$, $n = 58$, $P = 0.09$, rodent and lepidopteran damage pooled).

Plant size was the trait showing less differentiation among regions and its selection was only partially confirmed in Caurel 1998 (only marginally significant). Differential selection among regions was also only partially confirmed. Preferences of herbivores and pollinators for larger plants are common (Gómez, 2003; Cariveau *et al.*, 2004). In our study, preferences for plant size were inconsistent among regions for each interactor and not coincident at the same region between pollinators and herbivores. The effect of plant size on maternal fecundity is commonly via its relationship with flower number (Herrera, 1993; Gómez, 2003), thus it is not surprising that, when controlling for flower number in selection gradient and path analyses, its effect disappeared.

The selection mosaic

The SEMs in Fig. 2 illustrate two components that potentially influence the evolutionary outcome of the interactions between *H. foetidus* and its animal interactors, namely effective selective pressures (significant paths linking an animal interactor to plant fecundity, implying a relationship between variation in incidence of an interactor and variation in plant fecundity) and interactor-mediated phenotypic selection (corroborated by a significant pathway, i.e. significant paths from plant traits to an interactor and from that interactor to fecundity). Our study system proved to be geographically and temporarily heterogeneous regarding these two components because no pathway remained significant and/or had the same sign at all region × year combinations, and because the most important selective pressures were often exerted by different sets of animal interactors (see also Gómez & Zamora, 2000).

Inconsistency in the identity of the particular interactor(s) that act as selective agent(s) and cause phenotypic selection in different regions or years, along with concomitant regional or annual variation in the phenotypic trait(s) being subjected to selection, strongly support the idea of a selection mosaic on *H. foetidus*'s reproductive traits which is generated by its interactors. An increasing number of studies have recently shown that plant–animal interactions are geographically structured into selection mosaics (see references in Introduction)

The results of this study are also relevant to the shape and scale of the geographic mosaic (Thompson, 1999) of the interaction between *H. foetidus* and its animal interactors. Both shape and scale of the geographic mosaic probably have important consequences for the evolutionary dynamics of the interactions involved (Thompson, 1999). The mosaic described here is of a broad-scale type, and its shape better fits a gradual variation in traits and outcomes over geographic ranges. Whether the pattern described here would hold if more populations (and environments) were sampled in each region, this study cannot ascertain. Should it hold, the evolutionary dynamics of the interaction of *H. foetidus* with pollinators and herbivores would be built on a large-scale geographic gradient rather than on a sharp landscape of highly differentiated contiguous populations.

The geographic mosaic in the plant-pollinator-herbivore system described here differs from others already described. For example, the interaction between the crucifer *Hormathophylla spinosa* and its pollinators in Sierra Nevada (southern Spain) conforms to a selection mosaic where nearby populations are subject to very different intensities of ungulate herbivory and are apparently differentiated in number and density of flowers and in their physical defences according to the abundance of ungulates (Gómez & Zamora, 2000, but see Gómez & Zamora, 2002, for the possibility of inducibility of such defences). Comparison of such results with the pattern found here reflects the absence of generality in plant-pollinator-herbivore systems and emphasizes that we need more studies addressing the geographic structure of the plant–animal interactions before selection on plant traits is fully understood.

Acknowledgments

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