Herbivory Reduces the Strength of Pollinator-Mediated Selection in the Mediterranean Herb *Erysimum mediohispanicum*: Consequences for Plant Specialization

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ABSTRACT: In this study, I tested whether selection occurring on several morphological and floral traits in *Erysimum mediohispanicum* (Cruciferae) is modified by the effects of herbivores. Six plots were established in 1997 in the Sierra Nevada, Spain; three were fenced to exclude native ungulates, and the remaining were open to ungulates. I determined pollinator and ungulate preferences for plant traits and their effect on plant fecundity. Then I compared the selection regimes between plants excluded from herbivores and plants open to them. When ungulates were absent, I found significant selection on flower number, reproductive stalk height, basal diameter of the stalks, petal length, and inner diameter of the flowers. When ungulates were present, selection on floral traits completely disappeared, and selection strength on flower number and morphological traits decreased. This effect was due to the ungulate preference for larger plants and the phenotypic correlations between plant size and floral traits. Results suggest that pollinator-mediated selection can be disrupted by conflicting effects of plant enemies acting during or subsequent to pollination. An accurate picture of the pollinator role as selective pressure requires the consideration of the entire life cycle of the plant as well as the ecological scenario in which the interactions occur.

Keywords: ecological conflicts, environmental manipulation, *Erysimum mediohispanicum*, evolutionary trade-offs, ibex, natural selection.

Because of their impact on plant relative fitness, pollinators can exert selective pressures on many plant traits such as

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flower number; flowering time; floral color, shape and size; and pollen number and size, all of which are traditionally considered the result of pollinator-mediated selection (Faegri and van der Pijl 1979; Wilson and Thomson 1996; Morgan and Conner 2001; Totland 2001). However, the fitness of most plant species is affected not only by pollinators but also, in a complex way, by the simultaneous or sequential actions of several antagonistic organisms. Indeed, the reproductive success of many plants is dramatically reduced by herbivores feeding directly on reproductive tissue or by indirect feeding that damages vegetative tissue and therefore decreases the amount of resources available for flowers, pollen, and seeds (Zamora et al. 1999). As a consequence, herbivores can also represent significant selective pressure on plants, and many other plant traits are thought to be a consequence of the selection imposed by these antagonistic organisms (Marquis 1992).

Strauss and Armbruster (1997) have pointed out that pollination and herbivory rarely operate independently of each other. The ecological effects of pollinators and herbivores may be linked when they affect the same plant traits (Karban and Strauss 1993; Quesada et al. 1995; Niesembaum 1996; Strauss et al. 1996; Lehtilä and Strauss 1999; Gómez and Zamora 2000; Herrera 2000). Growing evidence demonstrates that herbivores can affect phenotypic traits related to pollination such as flowering phenology (Gómez 1993; Strauss et al. 1996), flower display (Karban and Strauss 1993; Gómez and Zamora 2000), floral morphology (Strauss et al. 1996; Lehtilä and Strauss 1999; Leege and Wolfe 2002), quantity and quality of floral nectar (Krupnick et al. 1999), pollen production per flower (Frazee and Marquis 1994; Strauss et al. 1996), and pollen performance (Delph et al. 1997). By affecting these traits, herbivores can shape the interaction occurring between the plants and their pollination mutualists. Some recent studies have shown that damage to plants can alter the

abundance and visitation rate of pollinators at flowers and the subsequent pollination rates (Strauss 1997; Strauss et al. 1999; Hambäck 2001).

When herbivores affect traits relevant to the pollination system, the potential selection imposed by pollinators might be altered by the influence of herbivory (Gómez 1993; Herrera 1993; Armbruster et al. 1997; Fineblum and Rausher 1997; Shykoff et al. 1997). Adaptations to avoid herbivory can constrain the evolution of plant-pollinator interactions, since the advantage associated with adaptation to the latter function are countered by costs associated to the former (Quesada et al. 1995; Armbruster et al. 1997; Brody 1997; Brody and Mitchell 1997; Ehrlén 1997; Strauss 1997; Gómez and Zamora 2000; Galen and Cuba 2001). Indeed, recent findings suggest that several plant traits can be the evolutionary result of conflicting selective pressures exerted by pollinators and herbivores (Euler and Baldwin 1996; Strauss 1997; Galen and Cuba 2001; Lara and Ornelas 2001). Nevertheless, the herbivores' ability to disrupt the selective impact imposed by pollinators depends mainly on their capacity to modify the traits-fitness relationship established by pollinators, not just on their slight effect on plant traits.

The environmental context frequently affects the covariance occurring between fitness and phenotype, modifying the strength and sign of the resulting phenotypic selection (Rausher 1992; Mauricio and Mojonnier 1997; Totland 1998; Stanton et al. 2000). Consequently, there is a prevalence of environment-specific selection regimes in nature (Kalisz 1986; Arnqvist 1992; Caruso 2000; Stanton et al. 2000; Totland 2001). For example, O'Connell and Johnston (1998) have documented that selection on plant height varies between microhabitats in the orchid *Cypripedium acaule*, whereas Roy et al. (1999) found that the ability of enemies to cause directional selection on leaf-hair density in *Sinapis arvensis* greatly depends on some stressful environmental conditions. Similarly, Caruso (2000) has demonstrated that competition with *Castilleja linariaefolia* alters the selection on the *Ipomopsis aggregata* flower shape. According to these ideas, when herbivores interact with the selection imposed by pollinators, it is expected that the pollinator-mediated selective regimes differ between herbivore-free and herbivore-occupied environments (Gómez and Zamora 2000). Despite its potential importance to clarify the role of herbivory as a constraint on plant-pollinator evolutionary relationships, this environmental-focused approach has been largely unexplored up to now, with most studies providing only observational comparisons of the selective regimes occurring in populations suffering different damage intensity (Gómez and Zamora 2000; Galen and Cuba 2001; Sánchez-Lafuente 2002). No experimental manipulation of herbivore presence or abundance has yet been performed to investigate the effect on the pollination-mediated selection regimes.

In this study, I tested whether the selection occurring on some floral and morphological traits in the Mediterranean herb *Erysimum mediohispanicum* (Cruciferae) is modified by the effects of herbivores. I experimentally excluded ungulates, a main herbivore of *E. mediohispanicum* in the study site, from part of a plant population and then compared the selection regimes between plants excluded from herbivores and plants open to them. This approach circumvents some potential problems when comparing the selection in different populations, specifically, the decrease of the likelihood that any between-population difference might be due to any other unmeasured factor than herbivore presence or absence.

Methods

Plant Natural History

Erysimum mediohispanicum is a perennial monocarpic herb found in many montane regions of southeast Spain (Nieto Feliner 1993), where it is distributed 1,100–2,000 m above sea level (Blanca et al. 1991) and inhabits forests, scrublands, and shrublands. In the Sierra Nevada, plants usually grow for 2–4 yr as vegetative rosettes and then die after producing one to eight reproductive stalks that can display between a few and several hundred bright yellow, hermaphroditic, slightly protandrous flowers (Nieto Feliner 1993). Flowers are visited in the study site by several species of insects, particularly the pollen beetles *Meligethes maurus* (Nitidulidae) and several species of beetles, bumblebees, solitary bees, and sirphids (J. M. Gómez, unpublished data). Although this crucifer is self-compatible, it needs pollen vectors to produce full seed set. In fact, plants experimentally excluded from pollinators set only 16% of the fruits set by control plants (J. M. Gómez, unpublished data).

At the study site, reproductive individuals are fed on by many different species of herbivores. Several species of sapsuckers (particularly the bugs *Corimeris denticulatus*, *Eurydema fieberi*, and *E. ornata*) feed on the reproductive stalks during both flowering and fruiting. In addition, stalks are bored into by a weevil species that consumes the inner tissues, whereas another weevil species develops inside the fruits and lives on developing seeds. However, the main herbivore of *E. mediohispanicum* in the study zone is the Spanish ibex (*Capra pyrenaica*), an ungulate that consumes flowers and green fruits while browsing on the reproductive stalks.

Experimental Design

In 1997, I established six plots (∼1,000 m² each) in a large plant population located at 1,900 m above sea level in the Sierra Nevada National Park (Granada, Spain). Three plots were fenced to exclude ungulates from the plants, leaving the other three plots open to ungulates. Since establishing the plots, I have monitored several life-history, interactive, and populational traits of *E. mediohispanicum* inside and outside the enclosures. Each year, I studied between 300 and 500 reproductive individuals (50–100 individuals per plot), depending on the annual abundance of plants.

Traits Measured

The following phenotypic traits were quantified for each plant: (1) length of the showy part of one petal (measured in millimeters by digital calipers with ±0.1 mm error; three flowers per plant) as an estimate of corolla size (Conner and Rush 1996); (2) inner diameter of the corolla tube (in millimeters), which refers to the space existing between the petal basis and where reproductive organs are located (quantified as the distance between the outer border of two opposite petals less the length of each petal); (3) corolla tube length or the claw length (*E. mediohispanicum*, like most other crucifers, does not have a true fused corolla tube but instead has a functional tube; Conner et al. 1995; Gómez 1996); (4) reproductive stalk height (measured to the nearest 0.5 cm), the distance from the ground to the top of the highest opened flower; (5) number of stalks growing from each rosette; (6) basal diameter of the stalk (measured in millimeters by digital calipers with ±0.1 mm error; if more than one stalk, I measured the diameter of the tallest one); (7) number of flowers, quantified as the total number of flowers produced by the plant; (8) number of ovules per flower, quantified in the lab under a magnifying glass on a sample of ripe fruits; and (9) microhabitat, classified as "under shrub" when the plant was growing in the middle of any species of shrub inhabiting the study zone, "medium shrub" when the plant was growing in the border of the shrub (thus available to the ungulates), and "open" when the plant was growing in open areas located between shrubs. Traits 4–9 were quantified every year from 1997 to 2002 for all labeled plants; traits 1–3 were monitored only in 2001 and 2002 on 100 randomly selected plants inside the enclosures and another 100 outside the enclosures per year.

I correlated all quantitative phenotypic traits by product moment correlations (PROC CORR; SAS Institute 1997), using only the values from the last 2 yr, when I had data from all phenotypic traits. I transformed those variables that departed significantly from normality in all previous analyses. To control for experiment-wise Type I errors produced by multiple comparisons, I adjusted the probability of error to $\alpha = 0.05$ by using the sequential Bonferroni technique (Rice 1989).

Female Fitness Measures

On each plant I quantified the following sequential estimates of the female reproductive success: the number of fruits produced per plant, counting only those surviving ungulate damage; the percentage of flowers ripening to successful fruit (fruit set); the number of seeds produced per ripe fruit, quantified in the lab under a magnifying glass (×10) for three fruits per plant; the percentage of ovules ripening to seeds in each successful fruit (seed-ovule ratio); and female fecundity, or total number of seeds produced per plant. Female fecundity represents an accurate estimate of the lifetime female fitness of the individuals since this species is monocarpic and reproduces only once during its life. Relative fitness was estimated by dividing the absolute fecundity of each individual by the population mean (Falconer 1989); it was calculated separately for the grazed and ungrazed plots.

Ungulates as Selective Agents: Effect on Plant Fitness and Preference Patterns

I determined the damage inflicted by ungulates for all plants labeled since 1997 by quantifying at the end of the reproductive season the number of stalks browsed and the number of flowers and fruits consumed per plant. This damage is easily discovered since the mammals usually removed more than two-thirds of the reproductive stalks when they browsed on the plants, in some cases removing all the stalks from their bases.

As an estimate of ungulate preference pattern, I tested whether the probability of the plants of being attacked by ungulates depended on the morphological traits of the plants by means of logistic regressions (PROC LOGISTIC; SAS Institute 1997), introducing as dependent the presence or absence of damage to the plants and as independent the values of each trait.

I experimentally determined whether ungulates affected plant fecundity by comparing every reproductive parameter inside versus outside the fences by means of one-way ANOVAs (PROC GLM; SAS Institute 1997).

Pollinators as Selective Agents: Effect on Plant Fitness and Preference Patterns

The abundance and composition of the *E. mediohispanicum* pollinator assemblage was determined by counting all insects feeding on the flowers of the labeled plants. Only those visitors making contact with both male and female

reproductive structures of the flowers were considered to be pollinators. The censuses, each corresponding to 2 min observation per plant, were performed from sunrise to sunset, evenly distributed over the entire flowering period of each experimental plant (May–June). The censuses were made ~1 m from the flowering plant in order to observe all the visitors without alarming them or disturbing their foraging behavior. In total, I made 1,800 censuses (60 h of observations in total), registering a total of 1,519 insects.

As an estimate of pollinator preference pattern, I tested whether the visitation rate of pollinators per plant depended on the morphological traits of the plants. For this, I regressed the pollinator visitation rate against the phenotypic plant traits by the mean of a multiple linear regression and univariate regressions (PROC GLM; SAS Institute 1997) and showed results from this latter test only when differing from the multivariate one.

To estimate the effect of pollinators on plant fitness, I correlated the pollinator visitation rate received per plant with their relative fecundity and separated the plants located inside and the plants located outside the fences.

Linear, Nonlinear, and Correlational Selection Gradients

I estimated selection gradients, a multivariate technique that reveals the direction and magnitude of selection for each quantitative trait independent of the other traits (Lande and Arnold 1983). The linear selection gradient, β, was computed from the standardized partial-regression coefficients of a linear regression of relative fecundity on all the traits, whereas the nonlinear selection gradient, γ, was calculated from the second-order standardized coefficient in a quadratic regression of relative fecundity (Lande and Arnold 1983). In addition, in this last quadratic model, I partitioned selection into direct selection gradients for the character i (γ_{ii}) and gradients that describe selection acting on the correlation between characters i and j (γ_{ij}) and introduced multiple peaks in the model to improve the method for estimating selection surfaces (Mitchell-Olds and Shaw 1987; Schluter 1988). The multivariate models of selection gradients were built introducing as independent variables the raw data of the original phenotypic traits and as a dependent variable the estimate of the lifetime relative fecundity of the plants. To control for potential biases produced by environmental covariances, I also introduced as an independent variable in the phenotypic-selection models the microhabitat occupied by the experimental plants since it can affect the interaction with pollinators and herbivores, the expression of some phenotypic traits, and the reproductive success of the plants (J. M. Gómez, unpublished data). Microhabitat was introduced as a dummy variable only in the linear models (Rawling et al. 1998). I performed this analysis twice, once using only the phenotypic traits as predictor variables and once more to introduce the pollinator visitation rate as an additional independent variable. This later analysis was done to discover the amount of direct selection acting on the phenotypic traits without the influence of pollinators. The sequential Bonferroni technique was used to control for experiment-wise Type I errors.

To determine the effect of each phenotypic trait on fecundity while removing the confounding effects of the other traits, I used the partial regression leverage plots of each trait on fecundity residuals (Rawling et al. 1998). Leverage plots were made by regressing each variable residual against the residuals from the regression of the dependent variable on all the remaining independent variables. The slope of the linear regression is the partial regression coefficient for that independent variable in the full model (Rawling et al. 1998). This plot also calculates a confidence function with respect to each variable, from which it can give the sign and percentage of the variability in fecundity explained by each variable.

I also fitted a projection pursuit regression to the data by using the multivariate cubic spline method (Schluter and Nychka 1994). However, I have decided not to show the results from this analysis, since they were virtually identical to those obtained with the previous technique.

Results

Table 1 shows the correlations and covariances between phenotypic traits used in this study. There were highly significant correlations between the three floral characters, between the number of flowers and the three morphological characters, and between basal diameter and stalk height. Furthermore, there were significant positive correlations between petal length and stalk height, between basal diameter and the number of flowers, and between corolla length and basal diameter and the number of flowers. These correlations suggest that larger plants also usually produce larger flowers.

The microhabitat in which the plants were growing did not affect any of the floral traits (petal length: $F = 0.90$, df $= 2, 197$, $P = .409$; corolla diameter: $F = 1.21$, df $= 2, 197$, $P = .300$; corolla tube length: $F = 1.47$, df $= 2, 197$, $P = .232$; one-way ANOVAs). In addition, there was a marginal effect of microhabitat on female fecundity ($F = 2.74$, df $= 2, 97$, $P = .07$; only plants excluded from ungulates), with plants located under shrubs producing more seeds (929.6 ± 46.5 seeds/plants) than those in medium shrub (761.1 ± 169.2) and open sites (648.7 ± 94.2).

As observed in table 2, most phenotypic characters were similar inside and outside the enclosures. Only stalk height

Table 1: Phenotypic correlations between plant traits

	Stalk height	Basal diameter	No. stalks	No. flowers	Petal length	Inner diameter	Corolla tube length
Stalk height	309.157	.462[b]	.045	.382[b]	.123[a]	−.033	.168
Basal diameter	7.890	.942	.016	.603[b]	.144[a]	−.010	.215[b]
No. stalks	.920	.018	1.376	.564[b]	−.015	.079	−.043
No. flowers	238.691	20.812	23.506	1,263.953	.100[a]	.022	.158[a]
Petal length	2.010	.130	−.017	3.321	.868	−.530	.554[b]
Inner diameter	−.635	−.011	.103	.866	1.190	1.216	.082
Corolla tube length	3.851	.272	−.066	.674	.674	1.225	1.704

Note: Above diagonal are product-moment correlations, below diagonal are covariances, and in diagonal is the variance. Plants from inside and outside the enclosures have been pooled; $n = 200$.

[a] Correlations values $\alpha < 0.05$.

[b] Correlations values $\alpha < 0.01$, after Bonferroni corrections.

was significantly greater, and petal length was significantly smaller, inside the enclosures compared with outside.

Ungulates as Selective Agents: Effect on Plant Fitness and Preference Patterns

The percentage of plants damaged by ungulates ranged between 24% in 1997 and 47% in 2001 ($\chi^2 = 13.09$, df $= 5$, $P = .01$). In addition, herbivory intensity also fluctuated spatially ($\chi^2 = 26.77$, df $= 2$, $P < .0001$), with percentage of plants damaged being 38% in one plot, 20% in another plot, and 5% in the remaining one. Ungulate herbivory significantly affected plant reproductive output. In fact, most estimates of plant reproductive success were much higher during 2001 when ungulates were excluded than when they were present (table 2). Consequently, absolute female fecundity, estimated as number of seeds produced per plant, was much higher in plants excluded from ungulates (736.5 ± 72.0 seeds per plant) than in nonexcluded plants (389.3 ± 52.0).

Ungulates preferentially consumed large plants. In fact, the probability of a plant of being attacked by the ungulates significantly increased with stalk diameter, number of flowers, and number of stalks (table 3). Probability of being damaged varied among microhabitats ($\chi^2 = 51.16$, df $= 2$, $P < .0001$), being higher for plants located in open sites (45% of plants damaged) and medium shrubs (32%) than under shrubs (11%).

Pollinators as Selective Agents: Effect on Plant Fitness and Preference Patterns

The most abundant flower visitor during the study years was the beetle *Meligethes maurus*, which made 90% of the total visits to the flowers. This beetle acted as a pollinator because when it entered the corolla tube of the flower for pollen, it contacted both the anthers and the stigma of the flower, filling up on pollen grains. A beetle transported an

average of 273 ± 182 pollen grains on its body (range: 32–1,180; $n = 6$ beetles), most of them in the lower part of the pronotum and in the head. The multiple regression between pollinator visitation rate and plant traits was highly significant ($F = 10.06$, df $= 7, 186$, $P < .0001$, $R^2 = 0.25$). Pollinator visitation rate per plant related negatively with corolla tube length and positively with stalk height and number, three traits that together explained about 25% of the between-plant variability in insect abundance (table 3). This result suggests that pollinators preferred taller plants with shorter corollas. Nevertheless, when tested by univariate regressions, pollinator visitation rate was also positively correlated with number of flowers (table 3).

There was a significant relationship between pollinator visitation rate per plant and relative female fecundity inside the enclosures, with plants more visited by pollinators producing more seeds than plants less visited ($\beta \pm 1$ SE $= 1.39 \pm 0.60$, $F = 5.40$, df $= 1, 88$, $P = .02$). However, this positive effect of pollinators disappeared when ungulates were present, since outside the fences there was no relationship between pollinator visitation rate and relative female fecundity ($\beta \pm 1$ SE $= -0.52 \pm 0.85$, $F = 0.38$, df $= 1, 60$, $P = .54$).

Linear, Nonlinear, and Correlational Selection Gradients

When ungulates were excluded, the phenotypic traits together explained more than 70% of the variance in lifetime female fecundity both in the linear ($R^2 = 0.73$, $F = 35.84$, df $= 7, 92$, $P < .0001$) and the nonlinear model ($R^2 = 0.71$, $F = 9.77$, df $= 28, 71$, $P < .002$). Inside the enclosures, there was a significant linear selection on four phenotypic traits: stalk height, number of flowers, petal length, and inner diameter of the corolla (table 4). This means that selection is favoring those tall plants bearing many large flowers with petals arranged closely together. According to the regression between the residuals, flower

Table 2: Values of the phenotypic and fecundity-related characters for the *Erysimum mediohispanicum* plants located inside and outside the enclosures, respectively

Character	Inside (n = 100 plants)		Outside (n = 100 plants)		
	Mean ± 1 SE	Range	Mean ± 1 SE	Range	F ratio
Stalk height (cm)	52.3 ± 1.9	21–123	44.2 ± 14.2	19–86	11.35[a]
Stalk diameter (mm)	2.4 ± .1	.6–4.5	2.6 ± .1	.9–5.7	.46
No. stalks	1.6 ± .1	1–9	1.3 ± .1	1–7	4.53
No. flowers	51.4 ± 3.7	7–219	44.9 ± 3.3	4–196	1.69
Petal length (mm)	5.4 ± .1	3.0–7.4	5.8 ± .1	3.7–9.9	11.64[a]
Inner diameter (mm)	.8 ± .9	0–3.4	.4 ± 1.2	0–3.0	4.42
Corolla tube length (mm)	10.0 ± .1	5.7–12.7	10.5 ± .1	7.2–15.0	7.08
Ovules/flower	35.4 ± .7	18–56	36.4 ± 1.0	20–62	.61
Fruit set (%)	62.5 ± 1.4	34.2–96.7	38.9 ± 3.1	0–93.3	47.68[a]
Number of fruits	32.2 ± 2.4	4–132	19.3 ± 2.4	0–129	14.23[a]
Seed-ovule ratio (%)	61.6 ± 1.9	4.5–95.2	52.9 ± 2.4	8.1–87.3	7.77[a]
Seeds/fruit	21.7 ± .8	2–43	19.4 ± 9.2	3–42	2.88
Female fecundity	736.5 ± 72.0	28–4,356	389.3 ± 52.3	0–2,752	15.21[a]

Note: Only 2001 data are shown.

[a] Significant at $\alpha < 0.05$ after Bonferroni correction.

number explained 41.0% of the variability in fecundity, whereas stalk height, petal length, and inner diameter explained 11.9%, 4.9%, and 6.0%, respectively (fig. 1). There was also a positive nonlinear selection gradient for number of flowers and stalk height, indicating that the relationship between these traits and female fecundity was nonlinear. Finally, a significant positive correlational selection gradient was found for the interaction between the number of flowers, stalk height, and basal diameter (table 4). The full linear phenotypic selection model built by introducing the pollinator visitation rate as an independent variable was also significant ($R^2 = 0.77$, $F = 28.92$, df = 8, 91, $P < .0001$), although the only phenotypic trait obtaining a significant selection gradient was number of flowers ($\beta = 0.800 \pm 0.101$, $P < .0001$). Pollinator visitation rate also correlated significantly with female fecundity ($\beta = 0.36 \pm 0.06$, $P < .02$). No significance was obtained in the nonlinear full model ($P > .05$ in all cases).

Where ungulates were present, the percentage of variance explained by the multivariate models was about 47% for the linear model ($R^2 = 0.47$, $F = 3.13$, df = 7, 92, $P < .0001$) and 75% for the nonlinear model ($R^2 = 0.76$, $F = 12.30$, df = 28, 71, $P < .0002$). Outside the enclosures, there was significant linear selection only for number of flowers (table 4), with plants displaying more flowers also having higher relative fecundity. Nevertheless, the value of the selection gradient differed significantly from that found inside the enclosure ($F_{enclosure \times flowers} = 5.21$, df = 1, 196, $P = .02$, ANCOVA; fig. 1), and the number of flowers outside the enclosures explained only 13.4% of the variability in fecundity, according to leverage plots. Stalk height also related to fecundity outside the enclosure,

but the relationship was negative and nonlinear (table 1), indicating a stabilizing selection against tall plants (fig. 1). In fact, the value of the linear and nonlinear selection gradient also differed significantly outside versus inside the enclosure ($F_{enclosure \times stalk\ height} = 4.47$, df = 1, 196, $P = .03$; $F_{enclosure \times (stalk\ height)^2} = 6.38$, df = 1, 196, $P = .01$, ANCOVA; fig. 1), and stalk height outside the enclosures explained about 1.0% of the variability in fecundity. There was no significant relationship, either linear or nonlinear, on any floral trait (table 4); the percentage of variability of fecundity fell below 2% in the best case (fig. 1). Very interestingly, however, when I introduced pollinator visitation rate as an independent variable, the full linear model was also significant outside the enclosure ($R^2 = 0.33$, $F = 2.65$, df = 8, 91, $P < .014$), and no phenotypic trait, even the number of flowers, obtained a significant selection gradient. In addition, the nonlinear model was not significant ($P > .05$).

Discussion

A Preliminary Cautionary Tale

Selection estimates based exclusively on phenotypic covariances between traits and fitness may be biased by unmeasured environmental factors (Rausher 1992; van Tienderen and de Jong 1994; Mauricio and Mojonnier 1997). To eliminate such bias is extremely difficult, if not impossible, when working in natural populations. However, I have tried to reduce the effect of environmental covariances by two complementary methods. First, I have performed experimental manipulations to decrease environ-

Table 3: Summaries of the analyses testing the preference pattern of pollinators and ungulates

Phenotypic traits	Pollinator preference				Herbivore preference		
	$\beta \pm 1$ SE	t	P	Partial R^2	Coefficient ± 1 SE	χ^2	P
Stalk height	$.07 \pm .01$	4.73	.0001	.10	$.01 \pm .01$	.14	.603
Stalk diameter	$.14 \pm .35$	.40	.690	.001	$.67 \pm .11$	35.99	.0001
No. stalks	$.55 \pm .21$	2.60	.009	.03	$.21 \pm .07$	7.6	.006
No. flowers[a]	$.01 \pm .01$	.50	.878	.001	$.01 \pm .00$	11.45	.0001
Petal length	$-.04 \pm .41$	$-.11$	.909	.01	$.03 \pm .00$	.27	.818
Inner diameter	$.00 \pm .18$	.02	.981	.02	$.07 \pm .09$	.59	.444
Corolla tube length	$-.88 \pm .17$	-5.04	.0001	.12	$.04 \pm .10$	.14	.706

Note: The pollinator preference was analyzed by a multiple regression of pollinator visitation rate per plant (expressed as number of insect plant^{-1} 2 min^{-1}) against all morphological traits. The herbivore preference was analyzed by logistic regression of herbivore damage per plant (expressed binomially as damaged or undamaged) against all morphological traits. Partial R^2 for each plant trait was obtained by partial regression leverage plots of each trait on fecundity residual (Rawling et al. 1998). Maximum-likelihood estimation of χ^2 was used to test the effect of each plant trait on ungulate probability of attack.

[a] When analyzed with univariate regression, there was a positive effect of number of flowers on pollinator visitation rate ($\beta \pm 1$ SE $= 0.02 \pm 0.01$, $t = 2.08$, $P = .039$, $R^2 = 0.03$)

mental covariance between some trait values and fitness. Thus, herbivores were experimentally excluded from part of a plant population, which ensures that the environmental conditions were very similar between the two groups of experimental plants. By doing this, local to regional covariation in other important factors such as climate or pollinator composition and abundance can be controlled. In addition, it is not probable that the selection found in fenced plots is due to ungulates eliminating environmental bias due to spatial distribution pattern of the plants, since while plant fecundity varied between microhabitats, ungulates did not prefer the plants located in the microhabitats where fecundity was highest (under shrubs).

As a second method to decrease environmental bias, I included in the multiple regression model some variables like microhabitat that, without being phenotypic traits of the plants, can affect both their fecundity and the fecundity-trait relationships. By doing so, the selection gradients were obtained by statistically controlling for these environmental variables. I believe that although it is impossible to control fully the environmental covariance affecting our estimates of phenotypic selections, by using these methods, it is possible to obtain more accurate estimates of selection with the phenotypic Lande-Arnold method (Mauricio and Mojonnier 1997).

Phenotypic Selection without Herbivores

Two factors indicate that pollinators have the potential to act as selective agents on *Erysimum mediohispanicum* when ungulates were excluded. First, pollinator visitation rate was positively correlated to the lifetime female reproductive success of plants, which suggested that pollinators

significantly affect plant fecundity and that plants most visited produced more seeds than plants less visited. Second, pollinators selected plants depending on several phenotypic traits, which can cause the occurrence of a significant relationship between fecundity and trait values.

The results revealed the existence of a strong positive directional selection on several plant traits. Plants with more flowers produced more seeds and had higher relative fecundity than did conspecifics with fewer flowers. Since I have also found a significant nonlinear selection gradient for flower number, the relationship between number of flowers and fecundity in *E. mediohispanicum*, although directional, is probably not linear. Flower number is a trait that affects the fitness of many plant species, both indirectly by increasing the visitation rate of many pollinators (e.g., Klinkhamer et al. 1989; Eckhart 1991; Conner and Rush 1996; Conner et al. 1996; Ohashi and Yahara 1998; Rademaker and de Jong 1998; Vaughton and Ramsey 1998; Thompson 2001) and directly by increasing potential reproduction (total number of ovules per plant), irrespective of the effect on pollinator behavior (e.g., Herrera 1993; Conner and Rush 1996; Gross et al. 1998; Gómez 2000; Gómez and Zamora 2000). In this system, I presume that the positive effect of flower number on fecundity was produced by the two causes acting together, as suggested by several pieces of my data. Thus, when using univariate regressions, I found a significant preference of pollinators for many-flowered plants (table 3). In addition, in the phenotypic selection analysis performed by considering pollinator visitation rate as another independent variable the number of flowers still obtained a significant selection gradient. This selection gradient was the consequence of the direct effect of number of flowers on fecundity without

Table 4: Analysis of linear, curvature, and correlational phenotypic selection through lifetime female fecundity on seven characters in *Erysimum mediohispanicum* for the populations located inside and outside the enclosure

Character i	$\beta \pm$ SE	$\gamma_{ii} \pm$ SE	Character j					
			Basal diameter $\gamma_{ij} \pm$ SE	No. stalks $\gamma_{ij} \pm$ SE	No. flowers $\gamma_{ij} \pm$ SE	Petal length $\gamma_{ij} \pm$ SE	Inner diameter $\gamma_{ij} \pm$ SE	Corolla tube length $\gamma_{ij} \pm$ SE
Inside:								
Stalk height	.150 ± .001[a],**	.001 ± .001*	.000 ± .000	−.002 ± .000	.001 ± .000[a],**	.001 ± .001	−.002 ± .001*	−.001 ± .000
Basal diameter	.151 ± .012	−.004 ± .019		−.019 ± .019	−.001 ± .010[a],**	−.024 ± .070	−.026 ± .028	.014 ± .033
No. stalks	.042 ± .009	.016 ± .010			−.001 ± .001	.034 ± .047	−.029 ± .026	.004 ± .032
No. flowers	.721 ± .0001[a],****	.0001 ± .000[a],***				.003 ± .002	−.001 ± .001	−.001 ± .001
Petal length	.208 ± .020[a],**	−.041 ± .063					.035 ± .045	−.025 ± .040
Inner diameter	−.222 ± .010[a],**	−.015 ± .012						.019 ± .024
Corolla tube length	.024 ± .010	−.001 ± .012						
Microhabitat	.217 ± .011[a],***							
Outside:								
Stalk height	.013 ± .001	−.0001 ± .000[a],**	.002 ± .003	−.000 ± .001	.001 ± .000[a],**	.001 ± .001	−.001 ± .001	.003 ± .002
Basal diameter	.113 ± .030	.059 ± .043		.005 ± .064	−.005 ± .002*	−.045 ± .109	.058 ± .066	−.004 ± .039
No. stalks	−.151 ± .028	.005 ± .041			−.001 ± .002	−.153 ± .101	.076 ± .065	−.053 ± .062
No. flowers	.619 ± .001[a],****	.0001 ± .000[a],**				.002 ± .004	−.002 ± .002	−.001 ± .002
Petal length	.115 ± .029	.057 ± .031					−.078 ± .058	−.088 ± .050
Inner diameter	−.070 ± .020	.023 ± .025						.042 ± .031
Corolla tube length	.153 ± .017	.015 ± .013						
Microhabitat	−.040 ± .027							

[a] Significant after Bonferroni correction at $\alpha < 0.05$.

* $P < .05$.
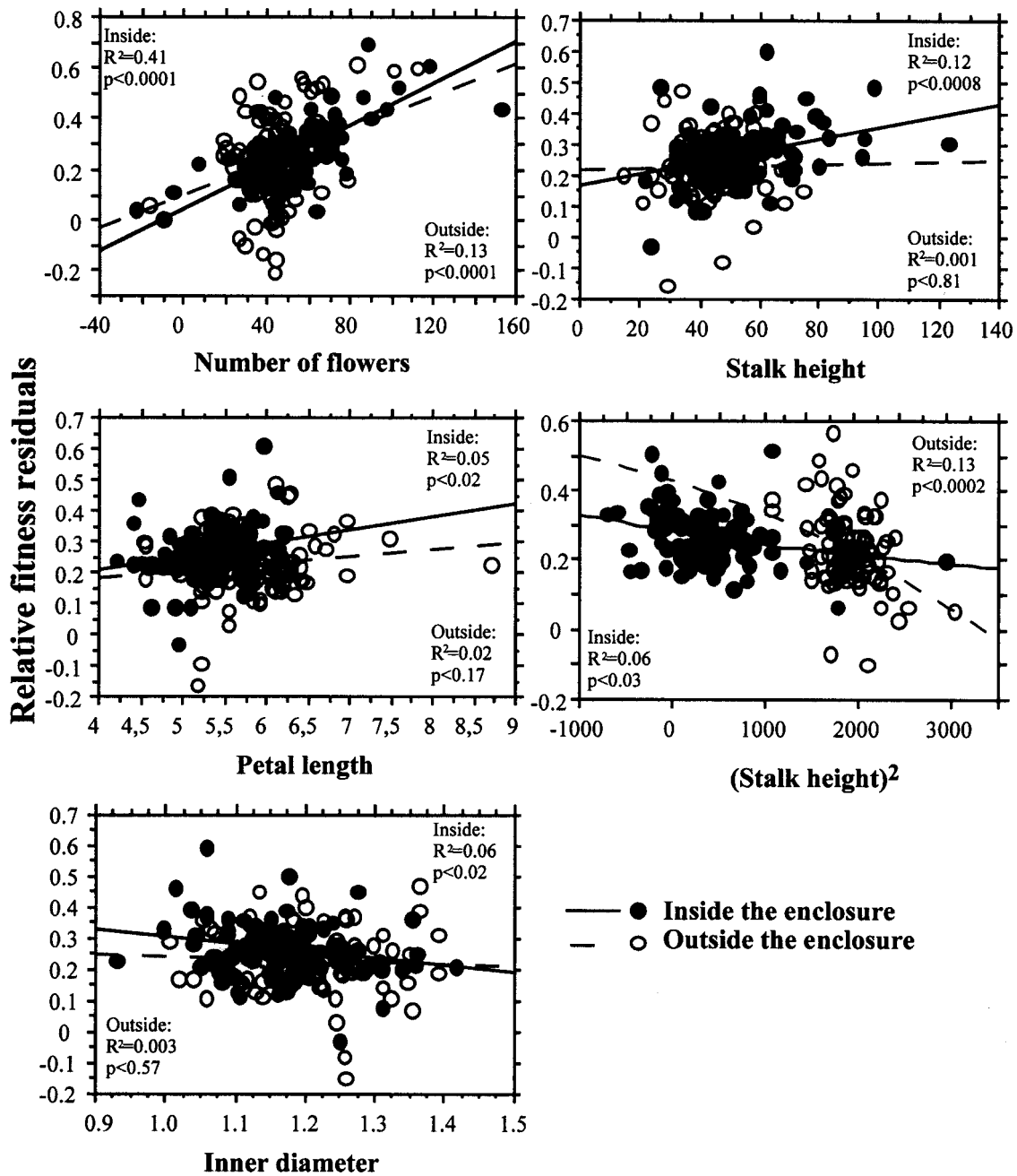
** $P < .01$.

*** $P < .001$.

**** $P < .0001$.

Figure 1: Partial regression leverage plots of the traits that significantly affect *Erysimum mediohispanicum* lifetime female fecundity according to the multiple regression models (table 4). The percentage of the variability in fecundity explained by each trait (R^2) and the significance value for the slope of the function are also shown (see table 4 for details).

the action of pollinators, since I controlled for their abundance in this specific phenotypic selection model.

There was also significant directional selection on flowering stalk height inside the enclosure, which suggested that taller plants were selected for. Again, two nonexclusive

causes can produce this linear selection on stalk height. First, this morphological trait can be under direct selection by pollinators, since they were significantly attracted to taller plants (table 3) and the selection gradient on stalk height lost its significance when the pollinator visitation

rate was introduced in the model. This preference for tall plants has been repeatedly reported for many different kinds of pollinators, from flies to wasps and bees (e.g., Peakall and Handel 1993; O'Connell and Johnston 1998; Lortie and Aarssen 1999; Totland 2001). In addition, the correlation between stalk height and flower number can also indirectly contribute to the observed selection pattern on the former trait.

Several floral traits of *E. mediohispanicum* were also under natural selection when ungulates were not present. Plant individuals with small spaces in the petal basis, the inner diameter, had higher relative fecundity. The mechanism promoting this selection is presumably related to the mechanical fit of pollinators with the plant rather than an increase in pollinator visitation rate, since I did not find a significantly higher visitation rate to plants with smaller inner diameter. Large inner diameter allows small flower visitors, such as the pollen beetles visiting *E. mediohispanicum* flowers, to reach the pollen and nectar without touching the flower's reproductive organs. By contrast, a smaller diameter results in a higher probability of the pollinators making contact with the stigma and delivering some pollen while visiting the flowers. I also found that plants with larger petals had higher fecundity than did plants with small petals, which suggested that large flowers are selected in this plant species. Although I did not find any pollinator preference for larger flowers, this selection may be a consequence of the widespread preference that many pollinator species show for large flowers (Galen 1989, 2000; Johnson et al. 1995; Conner and Rush 1996). Indeed, fecundity significantly increases with flower size in many species of plants (e.g., Galen 1989, 2000; Andersson and Widén 1993; Eckhart 1993; Meagher 1994; Johnson et al. 1995; Conner et al. 1996; Gómez 2000; Gómez and Zamora 2000; Maad 2000; Totland 2001). In addition, the fact that the significance of the selection gradients on these floral traits disappears when introducing the model pollinator attraction suggests that the selection acting on these traits is mainly pollinator mediated.

Ungulate Disruption of Pollinator-Mediated Selection

Ungulates affected the ability of pollinators to act as selective agents of *E. mediohispanicum*, since the positive effect of pollinator visitation rate on plant reproductive output found inside the enclosures disappeared outside the enclosures. Furthermore, a major result of my study is that the exclusion of ungulates also affected the phenotypic selection regime occurring on *E. mediohispanicum*. Thus, although selection for flower number proved significant in both the presence and absence of ungulates, it was much stronger when ungulates were experimentally excluded. The selection gradient that was found for the

flower number on fecundity was much higher inside (0.721) than outside the enclosures (0.619). Similarly, the proportion of the variance in fecundity explained by flower number was much higher inside (41%) than outside (13%) the enclosures. In addition, the selection gradient acting on flower number lost its significance when adding into the phenotypic selection analysis the pollinator visitation rate, which suggests that after controlling for the positive effect of pollinators, the detrimental effect of ungulates was so strong that it canceled the selection on flower number. Something similar occurred with stalk height; the directional selection on stalk height found inside the enclosure disappeared when ungulates were present, with the proportion of the variation explained in fecundity by this trait decreasing from 12% inside the enclosures to barely 1% outside the enclosures. In this case, there was even a significant stabilizing selection with opposite sign outside the enclosure, which suggested that selection was acting against very tall plants. The weaker selection for flower number and the cancellation of the selection on stalk height when ungulates were present was probably a direct consequence of the significant preference of these mammals for browsing on larger plants bearing many flowers (table 3). Similar preference for high flower number has been reported in many other plants, such as *Ipomopsis aggregata* (Hainsworth et al. 1984), *Bartsia alpina* (Molau et al. 1989), *Lathyrus vernus* (Ehrlén 1997), and *Hormathophylla spinosa* (Gómez and Zamora 2000). My findings imply that when ungulates are present, large size and many flowers in *E. mediohispanicum* plants provide not only a benefit but also a cost in the form of heightened risk of being damaged by these herbivores. These results depict a conflict in *E. mediohispanicum* with respect to the optimal height and number of flowers to produce, since large size and abundant flower production strengthens fecundity via pollination but weakens it by attracting ungulates.

Not only the selection for morphological traits but also the selection for floral traits was affected by the ungulate presence. Thus, the selection occurring for petal length and inner diameter of the flowers disappeared outside the enclosures. This finding suggests that ungulates, by browsing on plants, can cancel the selective impact that the pollinators could impose on these floral traits. This disruption of the selective pressures for floral traits is a consequence both of the relationship between the number of flowers and the floral traits in *E. mediohispanicum* and of the foraging pattern of the ungulates. First, larger plants bear the larger flowers with smaller inner diameter. In addition, ungulates preferentially browsed on larger plants with many flowers. For these reasons, ungulates are indirectly damaging plants with large petals and small inner diameter more frequently than those with small petals and

large inner diameter, indirectly selecting against these traits. Consequently, the evolution of floral traits in this crucifer presumably depends on the severity of ungulate attacks. Other studies have also described herbivore-pollinator conflicts that affect the evolution of plant traits. Brody (1997) suggested that the flowering phenology of many plant species reflects an evolutionary compromise against pollinators and enemies. Galen and Cuba (2001) recently reported that the nectar-robbing ants visiting the *Polemonium viscosum* flowers show a preference pattern very similar to that shown by the plant's main pollinators; they conclude that the flower shape in this species is the result of an ecological conflict between flower predation by ants and pollination by bumblebees. In a similar way, Lara and Ornelas (2001) have shown that both nectar-robbing and pollinating hummingbirds select for long corollas in *Salvia mexicana*, *Salvia iodantha*, and *Ipomoea hederifolia*, which suggested that the evolution of corolla length in these taxa may be the result of a trade-off to favor pollination while discouraging nectar robbers.

All the results shown here indicate that selection on several morphological and floral traits of *E. mediohispanicum* is less strong outside the enclosure than inside and is acting in opposite directions outside for some traits like stalk height. This by itself constitutes a very interesting result, since experimental evidence of conflicting selection is very scarce. However, to ascertain whether conflicting selection will have any biological meaning is a little harder, and a long-term study of the response to selection in the two environments and an analysis of the heritability of traits will help to clarify this issue.

Consequences for Floral Evolution and Plant-Pollinator Specialization

Evolutionary theory predicts that the pollinator-mediated selection should modify the phenotypes of the plants in order to increase the frequency of their interaction with pollinators that confer the best services and thereby encourage plant specialization toward the most effective pollinators (Johnson and Steiner 2000). This idea is supported by the widespread occurrence of pollinators as significant selective agents of plant traits (Galen 1989; Herrera 1993; Wilson and Thomson 1996; Morgan and Conner 2001; Totland 2001). Paradoxically, contrary to the theoretical expectations, generalization is frequent in natural pollination systems (Herrera 1996; Ollerton 1996; Waser et al. 1996; Gómez and Zamora 1999; Dilley et al. 2000; Thompson 2001). Several ecological factors have been proposed to reconcile the theoretical predictions with the empirical evidence, such as the spatiotemporal variability in the identity and abundance of the most effective pollinators or the similarity in effectiveness between pollinators (Her-

rera 1996; Waser et al. 1996; Gómez and Zamora 1999; Gómez 2002). The results in this study suggest that the herbivore damage during or subsequent to pollination can also counteract specialization by hindering the plants from responding to the phenotypic selection by pollinators. Thus, as shown for *E. mediohispanicum*, herbivores can, by imposing conflicting selection for some traits, cancel the pollinator-mediated selection and thereby the ability of plant adaptive responses. The conflict between herbivore- and pollinator-mediated selection requires a non-random herbivore-provoked variation in recruitment that should trigger a trade-off between pollinator and herbivore effects (Queller 1997). Trade-offs between mutualistic and antagonistic organisms can occur when herbivores have a preference pattern similar to that of pollinators (Ehrlén 1997; Galen and Cuba 2001; Lara and Ornelas 2001). In the system studied here, it seems that both pollinators and herbivores prefer larger plants. In addition, herbivory can also indirectly disrupt the pollination-mediated selection occurring in plant traits correlated with the ones directly selected by the herbivores, as in *E. mediohispanicum* flower size or inner diameter. This indirect effect may be common in nature (e.g., Meagher 1994; Gómez 2000) since most plant phenotypes are highly integrated and genetic and phenotypic correlations between traits are thereby extremely frequent (Schliting and Pigliucci 1998).

The disruption by herbivores increases in importance when one considers that a common trait of many plant-herbivore interactions is the significant spatiotemporal variability of the herbivory intensity, as shown for *E. mediohispanicum*. When the herbivory intensity within a single plant population fluctuates between years, even with an interannually constant pollinator assemblage, temporal variability can occur in selection regimes. Any pollinator-mediated selection will presumably weaken more during years of intense herbivory compared with years of low herbivory. Similarly, when herbivory varies spatially within a population, different plants can undergo different selection regimes even with a spatially homogeneous pollinator assemblage. This signifies that the strength of selection for floral traits may strongly depend not only on variations in pollinator abundance but also on variation in the abundance of some enemies.

Concluding Remarks

Pollinator effectiveness and plant fitness are usually estimated by isolating the plant from the community in which it develops and measuring only some predispersal components of its reproductive success, such as seed production, pollen removal, or pollen receipt (e.g., Thompson and Pellmyr 1992; Vaughton 1992; Olsen 1997; Gómez and Zamora 1999). However, like other organisms, plants

have complex life cycles, and therefore a fitness measure should take into account the entire life cycle (Kozlowski 1999) as well as the recruitment process (the populational projection of the life cycle) of its propagules (Bell 1997). Only when the impact of pollinators on the relationship between a trait and a fitness component persists throughout the life cycle of the plant can we be sure that pollinators act as a true selective pressure (Campbell 1991). However, the empirical evidence in this study suggests that intense pollinator-mediated selection can be disrupted or canceled by conflicting factors acting at any other stage. These findings highlight the necessity of considering the entire life cycle of the plant as well as the complete ecological scenario in which the interactions take place to obtain an accurate view of the pollinator role as selective pressure. I believe that the approach followed in this study, the experimental manipulation of certain environmental factors in order to produce alternative selective scenarios where the selection exerted by pollinators can be quantified, will help to unravel the intricacies in the evolutionary relationships between plants and pollinators.

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