

SEQUENTIAL CONFLICTING SELECTION DUE TO MULTISPECIFIC INTERACTIONS TRIGGERS EVOLUTIONARY TRADE-OFFS IN A MONOCARPIC HERB

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Trade-offs are crucial in understanding phenotypic evolution of organisms. A main source of trade-offs is conflicting selection, a phenomenon very likely in complex multispecific scenarios in which many potential selective agents coexist. The main goal of this study is to investigate the selective trade-offs arising due to conflicting selection on female-fitness components in *Erysimum mediohispanicum*. I quantified the selection exerted on 10 plant traits by a mutualistic (pollinators) and antagonistic (gall-makers, predispersal and postdispersal seed predators, mammalian herbivores) multispecific assemblage acting sequentially throughout eight selective episodes of the plant, from floral bud to juvenile production. Variation in lifetime female fitness (quantified as number of juveniles) was related mostly to variation in number of flowers, fruit initiation, and seedling establishment. The direction of selection changed among different selective episode for many traits. Most importantly, conflicting selection was frequent in the study system, with half of the phenotypic traits experiencing opposing selection in different selective episodes. Selection at individual life-cycle stages diverged remarkably from selection based on total fitness. Consequently, the evolution of many traits is determined by the relative importance of each episode of selection, with conflicting selection inevitably yielding evolutionary compromises.

KEY WORDS: Conflicting selection, *Erysimum mediohispanicum*, independent fitness components, multiplicative path analysis, selective episodes, trade-offs.

Trade-offs are crucial in understanding the phenotypic evolution of organisms (Bell 1997; Roff 2002; Mazancourt and Dieckmann 2004). They can occur as a consequence of direct constraints due to resource-allocation compromises expressed as negative correlations between life-history traits/fitness components (Roff 2002). Such trade-offs will affect the evolution of traits that are correlated simultaneously with two negatively correlated life-history traits (Schluter et al. 1991). Trade-offs can also arise due to opposing relationships between several fitness components and a given phenotypic trait, irrespective of the relationship existing between the fitness components themselves. In this case, trade-offs are prompted by conflicting selection pressures (Schluter et al.

1991). Direct trade-offs have been intensely studied both theoretically and empirically as a way to explain optimal life histories, and thus they have been widely documented for many kinds of organisms (Roff 2002; Roff et al. 2006). Trade-offs caused by conflicting selection, in contrast, have been much less reported despite their importance for understanding the evolution of many phenotypic traits (e.g., Roff 2002; Rohr et al. 2003).

Conflicting selection is likely in complex multispecific scenarios, when many potential selective agents impose selection on the same traits (Gómez 2003; Strauss et al. 2005; Irwin 2006). Under these circumstances, the selection resulting from a single selective agent is an inaccurate estimate of the total phenotypic

selection affecting the traits (Preziosi and Fairbairn 2000; Ehrlén 2002, 2003). Instead, because different episodes of selection can interfere with each other and alter net selection, the net outcome of selection is the result of the selection occurring in the different selective episodes over the entire life cycle (Schluter et al. 2001; Ghalambor et al. 2003).

The phenotypes of most plant species have been shaped by the integrated action of many selective agents. Indeed, plants interact throughout their life cycle with many diverse organisms and therefore are under complex selective scenarios caused by the concurrent or sequential actions of different selective agents (Strauss and Irwin 2004; Strauss et al. 2005). If different agents do not share any preference pattern, and their pairwise interactions are mediated by different plant traits, plants will be able to respond independently to most of the selection pressures imposed if no genetic correlation occurs among traits (Stinchcombe and Rausher 2002; Strauss et al. 2005). By contrast, when different agents interact with the same plant traits, the selection imposed is not independent. Furthermore, although such agents can act synergistically, they often cause the selection of the opposite sign on the same phenotypic traits (Karban and Strauss 1993; Niesenbaum 1996; Letihlä and Strauss 1999; Strauss and Irwin 2004; Irwin 2006). A key outcome of these conflicting selection pressures is the emergence of evolutionary trade-offs for some traits (Alcántara and Rey 2003; Gómez 2003, 2004).

In this study, I explore selection exerted on *Erysimum mediohispanicum* (Brassicaceae) phenotypic traits by a mutualistic and antagonistic multispecific assemblage acting sequentially over its life cycle. This plant is an appropriate system to explore these issues because previous information indicates that it interacts with many antagonistic and mutualistic animals that operate as selective agents (Gómez 2003, 2005a,b; Gómez et al. 2006). It has already been shown that some plant traits, such as flower shape, flower size, or flowering-stalk height, are selected by these disparate agents (Gómez 2003, 2005b; Gómez et al. 2006; Gómez and González-Megías 2007). Some interacting organisms disrupt the selection caused by other organisms, whether because they have a similar preference pattern but an opposite effect on fitness, as with ungulates and pollinators (Gómez 2003), or because of opposite preference patterns but similar effect on fitness, as in the case of the gall-maker and predispersal seed predators (Gómez and González-Megías 2007). My main goals in this study are (1) to integrate the effect that these disparate animals acting during different selective episodes have on the total lifetime female fitness (quantified as number of juveniles produced) of *E. mediohispanicum*; (2) to determine the relative importance of variation in different components of female fitness for variation in lifetime female fitness; and (3) to quantify the frequency and intensity of evolutionary trade-offs caused by the occurrence of conflicting selection.

Methods

NATURAL HISTORY OF THE SYSTEM

Erysimum mediohispanicum (Brassicaceae) is a biennial monocarpic herb, found in montane regions of southeastern Spain from 1100 to 2000 m a.s.l., inhabiting forests and subalpine scrublands. Plants usually grow for 2–3 years as vegetative rosettes, and then die after producing one to eight reproductive stalks that can display between a few and several hundred hermaphroditic, slightly protandrous bright-yellow flowers (Gómez 2003). Flowers are visited by many species of beetles, solitary bees, bee flies, and syrphids, with the pollen-beetle *Meligethes maurus* (Nitidulidae) being outstanding in the population studied (Gómez 2005a). Although this crucifer is self-compatible, it requires pollen vectors to produce a full seed set. Selective-exclusion experiments have demonstrated that *M. maurus* are important pollinators of *E. mediohispanicum* at the study site (Gómez 2005a), acting as strong selective agents (Gómez et al. 2006).

In SE Spain, reproductive individuals of this species are attacked by many different species of herbivores. Some floral buds do not open because they are galled by flies (*Dasineura* sp., Cecidomyiidae). Several species of sap-suckers (primarily the bugs *Eurydema oleraceae*, *E. fieberi*, *E. ornata*, and *Corimera denticulatus*) feed on the reproductive stalks during flowering and fruiting (Gómez and González-Megías 2007). In addition, stalks are bored into by a weevil species (presumably *Lixus ochraceus*, Curculionidae), which consumes the inner tissues, whereas another weevil species (presumably *Ceutorhynchus chlorophanus*, Curculionidae) develops inside the fruits, living on developing seeds and acting as predispersal seed predators (Gómez 2005b, Gómez and González-Megías 2007). The stalks are browsed by Spanish ibex (*Capra pyrenaica*, Bovidae), which consume flowers and mostly green fruits (Gómez 2003, 2005b). Dispersed seeds are consumed by woodmice (*Apodemus sylvaticus*, Muridae), several species of birds (*Fringilla coelebs*, *Serinus serinus*, and *Carduelis cannabina* [Fringillidae], among others), several species of medium-sized granivorous beetles (*Iberozabrus* sp. [Carabidae], among others), and ants (*Lasius niger*, *Tetramorium caespitum*, and probably *Cataglyphis velox* and *Leptothorax tristis*) (Gómez 2005b). These animals feed on the seeds from late August to early April. Seedlings and juveniles are sometime injured by ibex, sheep, wild boars (*Sus scrofa*, Suidae), hares (*Lepus granatense*, Leporidae), and voles (*Pitimus* spp., Arvicolidae), although most seedlings die due to summer drought (Gómez 2005b).

DETERMINATION OF THE SELECTIVE EPISODES

In each of the two years, 2002 and 2003, I tagged 200 plants in a population located in the Sierra Nevada (southeastern Spain, 37°4.8'N 3°27.9'W, 1830 m a.s.l.) at the onset of flowering, when they had produced floral buds but the flowers had not yet opened. During the entire reproductive period, from flowering to seed dis-

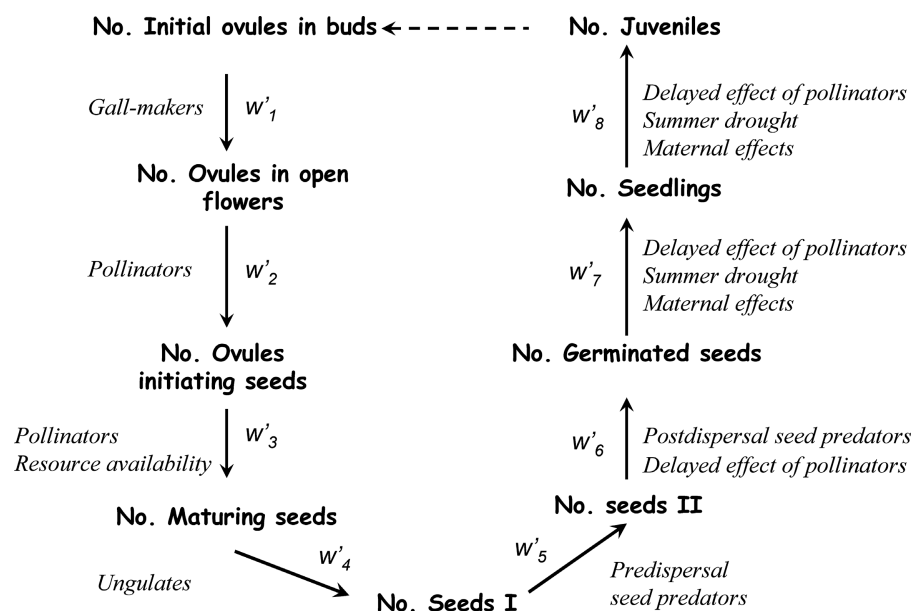


Figure 1. Schematic representation of the *Erysimum mediohispanicum* life cycle, showing each stage and each selective episode (w'_k). Stages are plant based. Putative selective agents acting during the different episodes of selection are indicated in italics.

persal, I tracked each plant individually, recording all the potential agents affecting the reproductive success of the tagged plant. For this, I counted the number of flowers, fruits, and seeds produced per plant. Afterwards, I sowed, in a completely randomized design, 10 seeds per individual plant under natural conditions (the same parent population). In both study years, I checked germination and survival every 15 days the first two months and every three months for one year (see Gómez 2003, 2005a,b; Gómez et al. 2006 for a detailed description of the study site and the labeling methods).

Eight sequential selective episodes were identified between the nine life-history stages of the *E. mediohispanicum* life cycle, from the initial production of ovules to the production of 1-year juveniles (Fig. 1). The fitness components associated with these episodes were: w'_1 = proportion of ovules in floral buds surviving gall-maker attacks; w'_2 = proportion of ovules initiating seed development; w'_3 = proportion of initiated seeds not aborting; w'_4 = proportion of ripe seeds surviving ungulate damage; w'_5 = proportion of ripe seeds surviving predispersal seed-predator attack and dispersing; w'_6 = proportion of ripe seeds escaping postdispersal seed predators and germinating; w'_7 = proportion of seedlings surviving first summer; w'_8 = proportion of 4-month-old seedlings surviving into 1-year juveniles. In 2003, I pooled the last three episodes (w'_6 to w'_8) because I was unable to sample all of them properly. However, I could state which agents acted during many episodes of selection, although, during the last three postdispersal episodes, it was possible for more than one agent to affect fitness. Nevertheless, based on both my previous observations as well as on the experimental design to study selection through the last

three life-cycle stages, I could infer which selective agents were important during these episodes. Thus, I observed that no seedling or juvenile had been injured by herbivores. In fact, herbivory is not important at these life-cycle stages for *E. mediohispanicum* (Gómez 2005b). Furthermore, because seeds were sown in a completely randomized design, environmental covariance was minimized. In addition, I detected no strong maternal effect (i.e., larger plants producing larger seeds coping better with abiotic factors), because there was no relationship between seed size and plant size (product-moment correlation, $r = 0.01$, $P = 0.60$, $n = 193$), seedling survival ($r = -0.11$, $P = 0.25$) or juvenile survival ($r = 0.06$, $P = 0.36$). Thus, variation in survival during these episodes may have been due to differences in seed quality caused by variation in the quality of the pollen received (e.g., proportion of self pollen), or in ability to grow and cope with abiotic stress.

FITNESS ESTIMATION

The lifetime fitness through seed production w_t (lifetime female fitness, hereafter) was calculated as the number of juveniles produced per plant at the end of the life cycle, a very inclusive female-fitness estimate (Bell 1997; Roff 2002). In addition, the fitness component during each sequential k th episode of selection was calculated as the proportion of propagules surviving that episode (Wade and Kalisz 1989). The relative fitness w'_k was calculated as the individual absolute fitness per stage k divided by the average fitness in that stage. Only individuals reaching a given transition were included for the calculation of these fitness estimates, and thus w'_k s are independent fitness components (Arnold and Wade 1984a,b; Koenig and Albano 1987; Wade and Kalisz 1989;

Campbell 1991; Koenig et al. 1991). The independent method was used rather than the additive one, because the immediate aim was to determine the selection occurring at each stage independently of selection at other stages, as well as to explore potential evolutionary trade-offs (Koenig et al. 1991). I also calculated the initial number of ovules per plant as one fitness estimate related to fecundity, w_f .

PLANT PHENOTYPIC TRAITS

The following groups of phenotypic traits were determined for each tagged plant (see online Supplementary Appendix S1):

Plant size

Plant size was estimated by means of three raw variables: (1) number of stalks, the number of reproductive stalks growing from the rosette; (2) stalk height, the height of the tallest stalk, measured to the nearest 0.5 cm as the distance from the ground to the top of the highest opened flower; and (3) stalk diameter, determined as the basal diameter of the tallest stalk (measured in millimeters by digital callipers with ± 0.1 mm error).

Number of flowers

This trait was estimated as the total production of flowers of each tagged plant over the entire reproductive season. This phenotypic trait is also an estimate of the potential fecundity of plants, and in this way it can also be considered as an additional fitness component. However, in the current study it will be considered exclusively as a phenotypic trait (preliminary analyses showed that using the number of flower as a phenotypic trait rather than fitness component does not affect the main conclusions).

Flower size

Flower size was estimated by means of three variables: (1) petal length, as the length in millimeters of the visible part of one petal (from petal tip to corolla-tube aperture); (2) flower diameter, estimated as the distance in millimeters between the outer border of two opposite petals; and (3) corolla-tube length, the distance in millimeters between the lower part of the petals and the bottom end of the sepals. These variables were measured by using a digital caliper with ± 0.1 mm of error.

Flower shape

The flower of *E. mediohispanicum* has four petals on two crossed axes, as in all crucifers. Nevertheless, the shape of the corolla is highly variable and affects pollination and reproductive success (Gómez et al. 2006). Flower shape was determined only in 2003 by means of geometric morphometric tools, using a landmark-based methodology that eliminates the effect of variation in location, orientation, and scale of the specimens (Zelditch et al. 2004). I took a digital photograph of one flower per

plant (within-plant variation in flower shape is very low, J. M. Gómez, unpubl. data) using a standardized procedure (front view and planar position). Flowers were photographed at anthesis to avoid ontogenetic effects. I defined 32 coplanar landmarks located along the outline of the flowers and the aperture of corolla tube (see fig. 1 in Gómez et al. 2006). Landmarks were defined by the reference to the midrib, primary veins, and secondary veins of each petal as well as the connection between petals (Gómez et al. 2006). I captured the landmarks using the software tpsDig ver. 1.4 (available in the Stony Brook Morphometrics website at <http://life.bio.sunysb.edu/morph/morphmet.html>). Afterwards, the two-dimensional coordinates of these landmarks were determined for each plant, and the generalized orthogonal least-squares Procrustes average configuration of landmarks was computed using the Generalized Procrustes Analysis (GPA) superimposition method (Rohlf and Slice 1990; Slice 2001). GPA was performed using the software tpsRelw ver. 1.11 (available in the Stony Brook Morphometrics website at <http://life.bio.sunysb.edu/morph/morphmet.html>). In these analyses, I have considered the flower as a nonarticulated structure because the relative position of the petals does not change during their functional life (see Adams 1999 for a discussion on articulated structures). After GPA, the relative warps (RWs, which are principal components of the covariance matrix of the partial warp scores) were computed (Walker 2000; Adams et al. 2004). Each RW is characterized by its singular value, and explains a given variation in shape among specimens. Thus, RWs summarize shape differences among specimens (Adams et al. 2004), and their scores can be saved to be used as a data matrix to perform standard statistical analyses (Zelditch et al. 2004). I selected the first four RWs, because together they explained more than 70% of the variance in flower shape (Gómez et al. 2006). RW1 is correlated with petal parallelism, RW2 with flower zygomorphy, RW3 with enantiomorphy, and RW4 with flower roundness (see Gómez et al. 2006 for details).

Seed weight

I collected 20 seeds from each tagged plant, and weighed them in the laboratory with a precision digital microbalance (0–5 g \times 2 μ g).

PHENOTYPIC SELECTION ESTIMATES

Selection during each episode was estimated by selection differentials and gradients. Selection differentials indicate the magnitude and direction of the total selection occurring on each phenotypic trait during an episode (Lande and Arnold 1983; Arnold and Wade 1984a,b), and were estimated by the covariance between that trait and the values of fitness ($s = \text{cov}(w, z)$; Rice 2004). Selection gradients reveal the direction and magnitude of direct selection occurring on each quantitative trait independently of the

other traits included in the model (Lande and Arnold 1983). Linear selection gradients, β , were estimated from the standardized partial-regression coefficients of a linear regression of each fitness component on all the traits.

Finally, the approach proposed by Conner (1996) of combining structural equation modeling (SEM) with the independent fitness components was used to detect the relative importance of each episode of selection and to understand the causes of selection occurring over the life cycle (Conner 1996). I built an a priori saturated model connecting all plant phenotypic traits to each independent fitness component, and these components to lifetime fitness (Conner 1996). The number of flower was also connected in this inclusive model directly to lifetime fitness, because it has been demonstrated several times that this direct relationship is significant in this species (Gómez 2003; Gómez et al. 2006). Correlation among exogenous variables (=plant traits) was automatically specified during the solving process, and thus SEM was based on selection gradients. I built several alternative nested models in which some of the causal paths were constrained to zero. The models were solved by minimizing yield-parameter estimates through an iterative process that used generalized least squares shifting to maximum likelihood as discrepancy functions. I used maximum-likelihood estimation (MLE) on the variance-covariance matrix to test the goodness of fit of the models and to calculate the Akaike Information Criterion (AIC). To select the best-fitting model, I chose the one with the highest *P*-value and the lowest chi-squared and AIC values (Shiple 2000; Grace 2006). SEM was performed with the SEPATH procedure in Statistica 7.0 (StatSoft 2004).

Results

CORRELATION BETWEEN PHENOTYPIC TRAITS

The structures of the phenotypic correlation matrices were similar in both study years (Table 1). In general, plant-size variables were positively correlated with flower-size variables, meaning that bigger plants produced larger flowers. The number of flowers was also positively correlated with plant size. The plant-size variables just barely and negatively correlated in 2002 but did not correlate at all in 2003. By contrast, the flower-size variables were highly and positively correlated. Seed weight was positively correlated in 2002 with one flower-size variable (petal length) and negatively correlated with the number of flower in 2003 (Table 1). In 2003, one flower-shape variable, RW1, was negatively correlated with flower size, larger flowers having more parallel petals. RW2 was positively correlated with seed weight, signifying that flowers with over development of lower petals produced bigger seeds. RW4 was positively correlated with flower diameter, so that the petals of larger flowers did not overlap (Table 1).

Table 1. Phenotypic correlations (product-moment correlations) between the phenotypic traits used in this study.

	Stalk diameter	Stalk height	Number of stalks	Number of flowers	Petal length	Flower diameter	Corolla length	RW1	RW2	RW3	RW4	Seed weight
Stalk diameter	0.188	-0.186		0.566	0.262	0.202	0.262					-0.012
Stalk height	0.151	-0.061		0.121	0.270	0.278	0.132					0.060
Number of stalks	-0.097	-0.039		0.319	-0.052	-0.049	-0.010					0.097
Number of flowers	0.511	0.233	0.163		0.071	0.077	0.057					-0.075
Petal length	0.160	0.295	0.029	0.189		0.784	0.650					0.178
Flower diameter	0.189	0.290	0.063	0.178	0.794		0.455					0.111
Corolla length	0.119	0.298	0.154	0.064	0.539	0.500						0.141
RW1	-0.025	-0.074	0.067	-0.068	-0.150	-0.179	0.004					
RW2	-0.064	0.104	0.032	-0.030	-0.030	-0.024	0.015	0.009				
RW3	-0.102	0.038	0.061	0.077	0.007	0.001	-0.042	0.007	-0.019			
RW4	0.050	-0.102	-0.155	-0.034	0.072	0.171	0.067	-0.005	-0.013	0.004		
Seed weight	-0.06	-0.037	-0.094	-0.184	-0.042	-0.045	-0.104	0.034	0.149	-0.083	-0.024	

Above diagonal, 2002. Below diagonal, 2003. *N*=200 each year. Flower shape was studied only in 2003. Bold figures refer to *P*<0.05, and bold and underlined figures refer to *P*<0.0001 after sequential Bonferroni corrections.

Table 2. Phenotypic correlations (product-moment correlations) between the different fitness components.

	w'_1	w'_2	w'_3	w'_4	w'_5	w'_6	w'_7	w'_8
w'_1		0.000	0.000	0.000	0.000	0.000	0.000	0.000
w'_2	0.142		-0.004	0.270	0.003	-0.006	0.109	0.033
w'_3	0.057	0.038		0.071	0.061	-0.060	0.013	-0.046
w'_4	0.023	0.118	0.009		-0.145	-0.015	0.026	-0.238
w'_5	0.134	0.027	0.053	-0.098		0.070	0.178	-0.131
w'_6							-0.205	0.159
w'_7								-0.111
w'_8	0.099	0.156	-0.060	-0.146	0.108			

In 2003 the w'_6 , w'_7 and w'_8 were pooled (see Methods).

Above diagonal, 2002. Below diagonal, 2003. $N=113$ for 2002 and 188 for 2003.

Bold figures refer to $P < 0.05$ after sequential Bonferroni corrections.

CORRELATION BETWEEN FITNESS COMPONENTS

There were no strong negative correlations between fitness components (Table 2). Three significant correlations were found in 2002, although two of these vanished after Bonferroni correction. Thus, the only remaining correlation was between the proportion of flowers pollinated (w'_2) and ungulate damage (w'_4), and was positive ($r = 0.27$, $P = 0.002$). In 2003, no single significant correlation was found between any pair of fitness components (Table 2).

SELECTION THROUGH DIFFERENT EPISODES

All selection analyses showed that different plant traits were selected during different selective episodes of the plant life cycle (Tables 3 and 4). No trait was selected during the first, third, or seventh selective episode in 2002, whereas no selection occurred during the fourth episode in 2003 (Table 3). Six traits (stalk height, number of stalks, petal length, flower diameter, corolla-tube length, and seed weight) showed significant selection differential during at least one episode in 2002 (Table 3). In 2003, stalk diameter, stalk height, number of flowers, flower diameter, corolla-tube length, corolla zygomorphy (RW2), and corolla roundness (RW4) were selected during at least one episode (Table 3).

By comparing selection differentials versus selection gradients, I found that the selection occurring in some traits was indirect rather than direct. For example, petal length and flower diameter were indirectly selected in 2002 (Table 3), probably due to their significant correlation with corolla-tube length. By contrast, in 2003, I noted that direct selection occurring on some traits, such as petal length and corolla-tube length through w'_2 and w'_3 , did not lead to total selection as suggested by the absence of significant selection differentials (Table 3).

The most parsimonious SEM models are offered in Figure 2 and Table 4 (see online Supplementary Appendix S2 to see all the alternative models tested). The results suggest that variation in all fitness components except the proportion of ovules surviving gall-maker attack (w'_1) and ungulate damage (w'_4) substantially affect

lifetime female fitness (Fig. 2). The latter two fitness components varied very little among plants (see online Supplementary Appendix S1). The SEM models showed that the number of flowers also directly and significantly affected lifetime fitness in the both years of the study (Fig. 2).

TRADE-OFFS AND CONFLICTING SELECTION OVER THE PLANT LIFE CYCLE

Most phenotypic traits were related to fitness in opposite ways during some selection episodes, although only stalk height and flower diameter had significant opposing selection differentials and gradients. Stalk height was positively related to fitness through w'_2 , w'_3 , and w'_4 but negatively through w'_5 (Table 3). Taller plants had more pollinated ovules, underwent lower abortion, and were less attacked by ungulates, but were more attacked by predispersal seed predators. This result was consistent, because it appeared in both the years and for both kinds of selection estimates (Table 3). There were conflicting selection differentials on flower diameter only during 2002 (Table 3). This trait was negatively selected through w'_5 but positively selected through w'_6 , indicating that plants with larger flowers produced seeds that were more heavily attacked by granivorous weevils but had higher germinability.

The SEM demonstrated that, when considering only the significant paths, five traits were under conflicting selection during some selective episodes in at least one study year: stalk height, stalk diameter, number of flowers, flower diameter, and seed weight (Table 4 and Fig. 2). Conflicting selection on stalk height and flower diameter was similar to those found with selection differentials and gradients. However, SEM detected conflicting selection also on number of stalks, stalk diameter, number of flowers, and seed weight in at least one year of the study. The number of flowers was negatively selected through proportion of ovules pollinated although it was related positively and directly with lifetime female fitness in 2003 (Fig. 2). Stalk diameter and number were positively related to number of flower but negatively related to the proportion of nonaborted seeds and proportion of

Table 3. Selection differentials and gradients (± 1 standard error) on each phenotypic trait through each independent fitness component (w_k), the fecundity estimate of fitness (w_f), and the lifetime female fitness (w_t).

Traits	Selection	w_f	Fitness components										w_t
			w_1	w_2	w_3	w_4	w_5	w_6	w_7	w_8	w_t		
2002													
Stalk	Differential	0.60	0.000	-0.024	0.012	0.012	-0.018	0.003	-0.057	0.019	-0.240	0.01	
diameter	Gradient	0.12±0.03	0±0	-0.028±0.037	0.031±0.037	-0.042±0.042	0.009±0.026	-0.025±0.059	0.197±0.123	-0.343±0.229	-0.36±0.30		
Stalk	Differential	0.07	0.000	0.059	0.051	0.002	-0.066	0.005	0.038	0.187	0.30		
height	Gradient	0.03±0.03	0±0	0.067±0.026	0.045±0.025	0.106±0.028	-0.054±0.017	-0.017±0.039	0.022±0.08	0.224±0.161	0.31±0.21		
Number of stalks	Differential	0.23	0.000	0.007	0.000	-0.005	0.006	0.072	-0.075	0.502	0.99		
Number of flowers	Gradient	0.01±0.03	0±0	0.001±0.029	0.011±0.029	0.001±0.034	-0.015±0.021	0.023±0.045	-0.055±0.094	0.400±0.181	0.71±0.24		
Petal length	Differential	0.95	0.000	-0.049	-0.006	0.008	0.003	-0.033	-0.003	0.115	0.77		
Flower diameter	Gradient	0.43±0.03	0±0	-0.056±0.036	-0.015±0.036	0.035±0.041	-0.002±0.026	-0.027±0.056	-0.068±0.117	0.104±0.219	0.72±0.30		
Corolla tube length	Differential	0.13	0.000	0.028	0.004	-0.021	-0.029	0.121	-0.245	-0.008	-0.06		
Seed weight	Gradient	0.01±0.05	0±0	0.073±0.048	0.002±0.048	-0.001±0.053	-0.001±0.033	-0.057±0.075	-0.170±0.156	-0.414±0.293	-0.50±0.39		
Flower	Differential	0.12	0.000	-0.014	0.022	-0.017	-0.044	0.136	-0.225	0.156	0.08		
diameter	Gradient	0.01±0.04	0±0	-0.070±0.040	-0.015±0.040	0.003±0.045	-0.026±0.028	0.113±0.062	-0.089±0.130	0.383±0.254	0.27±0.33		
Corolla tube length	Differential	0.14	0.000	0.034	0.022	-0.004	-0.014	0.130	-0.155	0.127	0.12		
Seed weight	Gradient	0.08±0.03	0±0	0.014±0.034	0.057±0.034	-0.027±0.038	-0.001±0.024	0.114±0.053	0.090±0.111	0.235±0.206	0.34±0.28		
2003	Differential	-0.07					0.025	0.105	-0.170	0.078	0.04		
Stalk diameter	Gradient	-0.04±0.03				0.024±0.021	0.134±0.042	-0.063±0.088	0.087±0.176	0.03±0.22			
Stalk	Differential	0.49	-0.007	-0.002	-0.030	-0.033	-0.047	0.042	0.042	0.32			
height	Gradient	0.08±0.03	-0.011±0.004	-0.044±0.033	-0.035±0.053	-0.097±0.039	-0.050±0.029	0.098±0.147	0.12±0.20				
Number of stalks	Differential	0.23	0.005	0.021	0.084	0.042	-0.075	-0.066	0.39				
Number of flowers	Gradient	0.05±0.02	0.004±0.003	0.063±0.031	0.104±0.048	0.015±0.035	-0.086±0.025	-0.067±0.129	0.13±0.18				
Petal length	Differential	0.19	0.002	0.005	-0.080	0.035	0.014	-0.097	-0.14				
Number of flowers	Gradient	0.05±0.02	-0.001±0.003	-0.028±0.029	-0.101±0.047	-0.004±0.034	-0.001±0.025	-0.092±0.125	-0.25±0.17				
Number of flowers	Differential	0.95	-0.001	-0.000	-0.155	0.038	-0.023	-0.155	0.32				
Petal length	Gradient	0.54±0.03	0.006±0.004	0.057±0.034	-0.081±0.054	0.075±0.038	0.029±0.028	-0.091±0.143	0.25±0.20				
Flower diameter	Differential	0.19	0.001	-0.005	-0.009	0.019	-0.037	-0.142	0.18				
Number of flowers	Gradient	0.04±0.04	-0.002±0.005	-0.128±0.049	-0.170±0.078	-0.080±0.055	-0.004±0.040	-0.462±0.202	-0.52±0.29				
Flower diameter	Differential	0.20	0.004	0.012	0.073	0.047	-0.013	0.055	0.36				
Corolla tube length	Gradient	-0.02±0.04	-0.002±0.005	0.093±0.049	0.144±0.077	0.064±0.056	-0.029±0.041	0.363±0.204	0.52±0.29				
Corolla tube length	Differential	0.06	-0.001	0.003	0.005	0.034	-0.042	0.029	0.38				
Number of flowers	Gradient	-0.01±0.03	0.004±0.004	0.072±0.035	0.151±0.055	0.074±0.040	0.038±0.029	0.160±0.149	0.36±0.21				

Continued

Table 3. Continued.

Traits	Selection	Fitness components									
		w_f	w_1	w_2	w_3	w_4	w_5	w_6	w_7	w_8	w_t
Parallelism (RW1)	Differential	-0.06	0.001	-0.001	0.055	-0.020	0.001	0.084	-0.05		
	Gradient	0.00±0.02	-0.001±0.003	-0.001±0.028	0.044±0.045	-0.031±0.032	-0.018±0.023	0.079±0.119	0.01±0.17		
Zygomorphy (RW2)	Differential	-0.04	0.007	-0.007	0.026	-0.028	0.007	0.271	0.45		
	Gradient	0.02±0.03	0.005±0.003	0.032±0.028	0.042±0.044	-0.035±0.034	0.008±0.024	0.284±0.124	0.44±0.17		
Enantiomorphy (RW3)	Differential	0.06	-0.002	0.010	0.009	0.056	0.004	-0.053	-0.06		
	Gradient	0.03±0.02	-0.003±0.003	-0.018±0.029	-0.006±0.045	0.042±0.032	-0.008±0.024	0.029±0.122	0.09±0.17		
Roundness (RW4)	Differential	-0.04	0.003	-0.017	-0.084	-0.012	0.024	0.193	0.08		
	Gradient	0.07±0.02	0.004±0.003	-0.011±0.029	-0.104±0.046	-0.006±0.034	0.004±0.025	0.168±0.128	-0.02±0.17		
Seed weight	Differential	-0.19					-0.011	0.156	-0.01		
	Gradient	-0.02±0.03					-0.013±0.024	0.086±0.118	0.01±0.17		

The phenotypic traits were standardized before analysis. No standard error exists for differentials because they are found by means of the covariances between fitness and traits. Significant trait-fitness covariance indicated in bold, marginal significance in italics (0.05 < P < 0.1). All P-values after sequential Bonferroni correction. Traits in boldface indicate potential conflicting selection (significant opposite-selection regimes during different episodes of selection).

seeds escaping predispersal seed predation (Fig. 2). Finally, seed weight was negatively related to proportion of seeds ripening but positively related to seed germination (Fig. 2).

TOTAL SELECTION ON PHENOTYPIC TRAITS

Selection differential on lifetime female fitness indicated a significant positive selection for number of stalks and number of flowers in 2002, and stalk height, number of flowers, flower diameter, corolla-tube length, and corolla zygomorphy in 2003 (Table 3). That is, plants with more stalks and flowers produced more juveniles in 2002, whereas taller plants with more and larger, zygomorphic flowers with longer corollas produced more juveniles in 2003.

Selection gradients suggest that both the number of stalks and number of flowers were under direct selection in 2002, whereas only corolla zygomorphy was under direct selection in 2003 (Table 3).

The SEMs showed similar results as differentials and gradients, because number of flowers and number of stalks were the traits registering the highest total effects on fitness in 2002, whereas flower diameter, petal length, number of flowers and zygomorphy had the highest total effects in 2003 (Table 4). It is noteworthy that the total effects were markedly lower for those plant traits undergoing conflicting selections: 0.007 and 0.04 for stalk height in 2002 and 2003, respectively, -0.003 for flower diameter in 2002, 0.19 for number of flowers in 2003, 0.01 for stalk diameter in 2003, and -0.01 for seed weight in 2002 (Table 4).

Discussion

This study demonstrates that the selective regimes varied considerably between different *E. mediohispanicum* life-cycle stages, with different traits being selected in different selective episodes. This separation in the time in which each trait was selected may at least partly be a consequence of different agents with different preference patterns acting during different episodes. Thus, gall-makers, acting early in the life cycle of the plants, seem to preferentially attack plants based on some vegetative traits such as stalk diameter (Gómez 2005b; Gómez and González-Megías 2007), whereas pollinators acting later apparently use several floral traits (Gómez 2003; Gómez et al. 2006), and predispersal seed predators acting even later appear to use stalk height as cues to select plants (Gómez 2005b; Gómez and González-Megías 2007).

Despite the fact that most phenotypic traits considered in this study were selected at least during one episode of selection, only a small group of these traits were still under selection when using inclusive estimates of female fitness, such as juvenile production. Thus, lifetime female fitness was significantly, and positively, correlated to only two traits (number of stalks and number of flowers) in 2002 and five traits (stalk height, number of flowers, flower

Table 4. Summary of the structural-equation modeling of phenotypic traits on fitness components and total fitness both years of study for the most parsimonious models. Figures refer to path coefficients ± 1 SE.

	Indirect effect								N flowers	Direct effect w_t	Total effect
	w'_1	w'_2	w'_3	w'_4	w'_5	w'_6	w'_7	w'_8			
2002											
w'_1										0	0
w'_2										0.17 \pm 0.07*	0.17
w'_3										0.17 \pm 0.06**	0.17
w'_4										0	0
w'_5										0.23 \pm 0.07**	0.23
w'_6										0.20 \pm 0.07***	0.20
w'_7										0.25 \pm 0.06****	0.25
w'_8										0.51 \pm 0.06****	0.51
No flowers										0.39 \pm 0.07****	0.39
Stalk height		0.24 \pm 0.10**	0.19 \pm 0.10*		-0.35 \pm 0.10****						0.007
Stalk diameter						-0.24 \pm 0.10*					-0.08
Number of stalks							0.36 \pm 0.09****				0.14
Petal length		0.37 \pm 0.16*									0.06
Flower diameter		-0.42 \pm 0.17*				0.34 \pm 0.10****					-0.003
Corolla tube length											
Seed weight			-0.38 \pm 0.09***			0.26 \pm 0.09***					-0.01
2003											
w'_1										0	0
w'_2										0.16 \pm 0.05****	0.16
w'_3										0.15 \pm 0.05****	0.15
w'_4										0	0
w'_5										0.08 \pm 0.05	0.08
w'_{6-8}										0.75 \pm 0.03****	0.75
No flowers		-0.28 \pm 0.07****								0.24 \pm 0.05****	0.19
Stalk height		0.12 \pm 0.08			-0.23 \pm 0.08				0.16 \pm 0.06**		0.04
Stalk diameter		-0.14 \pm 0.08*	-0.11 \pm 0.07		-0.14 \pm 0.08				0.52 \pm 0.06****		0.01
Number of stalks									0.25 \pm 0.06****		0.04
Petal length										-0.31 \pm 0.12*	-0.23
Flower diameter									0.30 \pm 0.12**		0.22
Corolla tube length		0.14 \pm 0.07*									0.02
Parallelism (RW1)		0.12 \pm 0.07									0.02
Zygomorphy (RW2)											0.13
Enantiomorphy (RW3)									0.18 \pm 0.08*		
Roundness (RW4)		-0.14 \pm 0.07									-0.04
Seed weight			-0.37 \pm 0.07****								-0.05

Path coefficients showed only for paths maintained in the most parsimonious models (see Fig. 2). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$. Traits in boldface indicate potential conflicting selection (significant opposite selection regimes in different components).

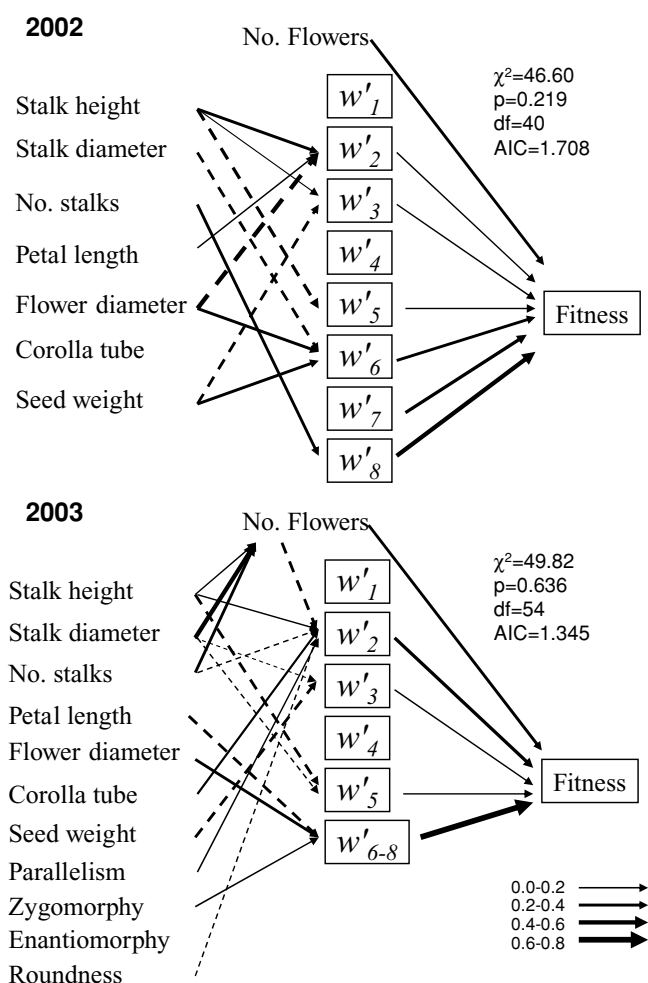


Figure 2. Results of the structural equation modeling relating the phenotypic traits, the independent fitness components, and the lifetime fitness of *E. mediohispanicum*; w'_i 's refer to the independent fitness components (see Fig. 1). Only the most parsimonious path models are shown for each year. Black lines refer to positive relationships, whereas broken lines refer to negative relationships. See online Supplementary Appendix S1 for a list of all the alternative models.

diameter, corolla tube length, and zygomorphy) in 2003. Furthermore, according to the selection gradients, selection was direct only for some traits, such as number of stalks, number of flowers, and corolla zygomorphy. Thus, caution is needed when inferring selection on a trait from some intermediate fitness components, because it is not always appropriate to use fitness components as surrogates for total fitness (Campbell 1991; Louda and Potvin 1995; Ehrlén 2003).

It is important to ascertain which factors decrease or even cancel out the total net selection occurring on some *E. mediohispanicum* phenotypic traits. Two factors appear to be important. First, total selection would be stronger on those traits selected during the selective episodes most correlated to lifetime female fitness,

whereas traits selected during low important selective episodes will suffer weak total selection (Conner et al. 1996a, b). This study has shown that the selective episodes related to pollination, pre-dispersal seed predation, germination, and seedling establishment determined more strongly the lifetime female fitness of the plants, whereas those related to damage by gall makers or by ungulates determined only weakly the lifetime female fitness of the plants. The more intensely selected traits, such as number of stalks, corolla zygomorphy, stalk height, flower diameter, corolla tube length, were directly related to some of those important episodes.

A second factor accounting for the observed cancellation of total net selection in some traits is the occurrence of between-episode trade-offs provoked by conflicting selection. Thus, total selection was very low for those traits exhibiting conflicting selection, as demonstrated both by the selection differential and gradient analyses as well as by the SEMs. Irwin (2006) has recently shown that some traits such as number of flowers or plant height have low total effect on *Ipomopsis aggregata* fitness as a consequence of conflicting selection.

Conflicting selection is frequently caused by the similarity in preference exhibited by different agents having opposite effects on fitness. This is evident for two specific traits undergoing conflicting selection in *E. mediohispanicum*, stalk height and flower diameter (flower size). These two traits were positively selected through the effect on pollinators, but negatively selected through the effect on seed predators, according to both selection differentials and gradients and SEM. Pollinator preference for large flowers is common to many other plant species (Conner et al. 1996b; Lloyd and Barrett 1996; Shykoff et al. 1997; and references therein), and has been previously demonstrated in *E. mediohispanicum* (Gómez et al. 2006). Similarly, *E. mediohispanicum* pollinators visit taller plants more frequently than shorter plants, because the former are probably easier to find (Gómez 2003; Gómez et al. 2006). In this study, the proportion of flowers initiating fruit development (w'_2) increased with stalk height, suggesting that short plants are more pollen-limited than tall plants. This pollination advantage of being tall has been shown for other plant species such as *Verbascum thapsus* (Lortie and Aarssen 1999; Carronero and Hamrick 2005), *Ranunculus acris* (Totland 2001), *Antirrhinum majus* (Jones et al. 1998), *Chiloglottis trilaba* (Peakall and Handel 1993), *Erysimum strictum* (Rautio et al. 2005), and *I. aggregata* (Irwin 2006). However, it seems that *E. mediohispanicum* seed predators used the same cues as pollinators to select individual plants. Thus, they more frequently attacked tall plants with large flowers (Gómez and González-Megías 2007). Similarity in pollinator and seed-predator preferences for plant traits has been previously shown in some plant species (e.g., Brody 1997; Cariveau et al. 2004).

It is probable that other agents in addition to pollinators and herbivores caused variation in fitness at life-cycle stages, such as seed germination or juvenile recruitment. In some cases, these

hidden agents could be abiotic, as for example summer drought. Nevertheless, it is important to note that to be a selective agent, it is necessary not only to affect fitness but also to cause trait-fitness covariance (Strauss et al. 2005). One way that abiotic factors can act as selective agents is through between-family differences in seed size. Many studies have indeed reported that heavy seeds can germinate and survive summer drought better than light seeds (Castro et al. 2006; Moles and Westoby 2006). Seed size could be a consequence of several factors, including pollination quality and maternal effects via environmental covariance (Castro et al. 2006). Unfortunately, in the present study it is difficult to disentangle the two factors.

In brief, this study suggests that conflicting selection is frequent along the life cycle, and thereby that selection at different stages of the life cycle can diverge remarkably from selection based on total fitness. I presume that the result found for *E. mediohispanicum* is common to many other plants, where traits are under selection imposed by several co-occurring or sequential agents ("ecological pleiotropy," Strauss and Irwin 2004). This idea is supported by recent studies showing intense interaction between the selective pressures exerted by different organisms on the same plant traits (Ehrlén et al. 2002; Strauss and Irwin 2004; Strauss et al. 2005, and reference therein). For a given agent to exert selection on a given trait, its impact on the relationship between the trait and a fitness component needs to persist throughout the life cycle of the plant. However, in these multispecific selective scenarios, intense selection caused by a given agent during one episode can be cancelled by conflicting selection acting during other episodes. Consequently, the evolution of many traits would be determined by the relative importance of each episode of selection, conflicting selection surely yielding evolutionary compromises.

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Supplementary Material

The following supplementary material is available for this article:

Appendix S1. Mean \pm 1 standard error [range] of the phenotypic traits and fitness components considered in this study.

Appendix S2. Summary of the goodness-of-fit tests (χ^2) and the Akaike Information Criterion (AIC) of the saturated and nested models.

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