

HERITABILITY AND GENETIC CORRELATION OF COROLLA SHAPE AND SIZE IN *ERYSIMUM MEDIOHISPANICUM*

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Flower shape has evolved in most plants as a consequence of pollinator-mediated selection. Unfortunately, no study has explored the genetic variation of flower shape, despite that this information is crucial to understand its adaptive evolution. Our main goal here is to determine heritability of corolla shape in *Erysimum mediohispanicum* (Brassicaceae). Also, we explore heritability of other pollinator-selected traits in this plant species, such as plant size, flower display, and corolla size. In addition, we investigate genetic correlations between all these traits. We found significant heritability for one plant-size trait (stalk height), for number of flowers, for all corolla-size traits (corolla diameter, corolla tube length and corolla tube width), and for corolla shape. Consequently, this species retains a high ability to respond to the selection exerted by its pollinators. Genetic correlation was strong between all functionally related traits and between flower number and plant size, weak between corolla size and plant size and no correlation between corolla shape and any other trait. Thus, selection affecting some *E. mediohispanicum* traits would also indirectly affect other functionally related and unrelated traits. More importantly, the observed genetic correlation seems to be at least partially adaptive because positive correlational selection currently acts on the covariance between some of these traits (Gómez 2003; Gómez et al. 2006).

KEY WORDS: Brassicaceae, corolla shape heritability, *Erysimum mediohispanicum*, genetic correlation, geometric morphometrics, phenotypic integration.

Adaptive floral evolution requires the occurrence of two important factors, phenotypic selection on floral traits caused by pollinator activity and genetic variation for those selected traits (Lynch and Walsh 1998). Pollinator-mediated phenotypic selection has been widely documented for floral traits in many plant species (for recent reviews, see Ashman and Morgan 2004; Harder and Barrett 2006). In contrast, genetic variation and heritability has been much less frequently studied for these types of plant traits (Geber and Griffen 2003; Ashman and Majetic 2006). Furthermore, most quantitative genetic studies on floral traits have focused on variables related to size, such as corolla size, corolla tube length, number of stamens, number of flowers, etc. (Conner and Via 1993; Mitchell and Shaw 1993; Ashman 1999; Kaczorowski

et al. 2008). By contrast, studies on genetic architecture of shape-related floral traits are scarce, and the few examples studying the heritability of floral shape divide shape into several simple linear variables (Venable and Búrquez 1989; Galen and Cuba 2001). However, floral shape is a complex multidimensional trait that can only partially be described by its linear components. Exploring the quantitative genetics of floral shape, thereby, requires a multivariate approach (Monteiro 1999; Klingenberg and Leamy 2001; Monteiro et al. 2002; Klingenberg 2003; Monteiro et al. 2003; Klingenberg and Monteiro 2005). This is probably the main reason explaining the paucity of studies affronting the investigation of the genetic basis of corolla shape. This happens despite that most theoretical and empirical studies on floral evolution postulate

that corolla shape has evolved as a response to strong selection exerted by pollinators (Coen et al. 1995; Schemske and Bradshaw 1999; Endress 2001; Sargent 2004).

Selection on floral traits has both a direct and an indirect component. The relative importance of these two components depends on the strength of the genetic correlation between the target traits and other phenotypic traits. Under these circumstances, response to selection is mediated not only by trait heritability but also by the genetic correlations among traits. We are still far from knowing how strongly the genetic correlations constrain the responses of plant traits to selection (Conner 2002; Ashman and Majetic 2006; Kaczorowski et al. 2008). In addition, genetic correlations also contribute to the phenotypic integration of complex structures. In scenarios in which integration is beneficial, selection should increase the genetic correlation among functionally related traits, leading to the evolution of complex integrated structures (Venable and Búrquez 1990). Genetic correlation is expected to be stronger between functionally and developmentally related traits than between unrelated ones (Berg 1959, 1960). For example, genetic correlation between vegetative and floral traits is expected to be lower than among floral traits or vegetative traits themselves (Armbruster et al. 1999). Correlational selection would cause higher genetic correlation between traits belonging to the same complex structure than between traits from different structures (Sinervo and Svensson 2002; McGlothlin et al. 2005). This enhanced genetic correlation can result through the build up of linkage disequilibrium, by favoring pleiotropic mutations, or through linkage between genes affecting traits under correlational selection (Lynch and Walsh 1998).

Erysimum mediohispanicum (Brassicaceae) is a pollination-generalist plant that shows high phenotypic variation for fitness-related traits (Gómez et al. 2006). Despite its being a generalist, we have found that pollinators exert strong phenotypic selection on many traits, associated mostly with plant size and corolla size and shape (Gómez et al. 2006, 2008a, 2009). Our main objective in this article is to estimate the heritability and genetic correlation for the major pollinator-selected phenotypic traits in this plant species, as a way to predict their response to selection. Estimating the quantitative genetics of size-related traits would require the use of standard methodology. However, it is not adequate to use such methodology to estimate the quantitative genetics of complex multidimensional traits such as corolla shape. For this reason, following the approach proposed by some evolutionary biologists (Monteiro 1999; Monteiro et al. 2002, 2003; Klingenberg 2003; Klingenberg and Monteiro 2005), we have estimated corolla shape heritability and genetic correlation using a multivariate approach that does not break it down into linear components. Very few attempts have been made so far to use this approach to explore the heritability of complex shapes (see Santos et al. 2005; Myers et al. 2006).

Materials and Methods

STUDY SYSTEM

E. mediohispanicum Polatschek (Brassicaceae) is a biennial to perennial monocarpic herb abundant in the N and SE of the Iberian Peninsula. Plants usually grow for 2–3 years as vegetative rosettes, and then die after producing one to eight reproductive stalks which can display between a few and several hundred hermaphroditic, slightly protandrous bright yellow flowers (Gómez 2003). Although this crucifer is self-compatible, it requires pollen vectors to produce a full seed set (Gómez 2005a). Flowers are visited by many different species of insects, from large bees and butterflies to tiny beetles and ants (Gómez et al. 2007, 2008a). Selective exclusion experiments have demonstrated that even minute, unspecialized flower visitors are important pollinators of *E. mediohispanicum* (Gómez 2005a) and can exert strong selective pressure (Gómez et al. 2006, 2008a). Mean seed dispersal distance is extremely short in this species, less than 20 cm (Gómez 2007).

The field study was conducted between 2005 and 2007 in eight *E. mediohispanicum* populations of the Sierra Nevada high mountains (Granada province, SE Spain; Table 1). Genetic differentiation among populations is high, based on both nuclear markers (Bayesian $G_{st} = 0.27 \pm 0.02$ based on 160 RAPDs) and plastidial haplotypes ($F_{st} = 0.35$ based on trnL-trnF cpDNA; Gómez et al. 2009).

Pollination ecology and phenotypic selection of floral traits were previously measured at these same sites (Gómez et al. 2008a,b, 2009). In these populations, flowers are visited by more than 150 insect species, ranging from beetles to bee flies and bees (Gómez et al. 2007).

EXPERIMENTAL DESIGN

Ninety plants were marked in each of the eight populations (720 plants in total), at the onset of the 2005 flowering period (April) using aluminum tags attached to the base of the flowering stalks. Plants were monitored throughout the entire reproductive season. At the end of the season, when seeds are mature but prior to dispersal (September), we collected 30–40 seeds per plant from each of the surviving individuals ($N = 335$ plants; Table 1). Losses are very frequent under natural conditions due to summer drought and ungulate damage (Gómez 2005b).

We planted 10 seeds per surviving maternal plant on October 2005 in a University of Granada (UGR) glasshouse. Seeds were located in individual pots 15 cm apart to avoid competition. To avoid environmental covariance, pots were distributed according to a completely randomized design. Seedlings were transferred to an UGR outdoors common garden when they had produced the cotyledons but before true leaf development. Plants were watered once weekly during winter (October–January), twice weekly during spring (February–May) and daily during summer

Table 1. Location and characteristics of the eight plant populations studied, and sample size of the individuals used in this study.

Code	Latitude	Longitude	Habitat	Altitude	Number of maternal plants	Number of planted seeds	Number of flowering offspring
Em01	37°8.00'N	3°25.69'W	Forest	1750	65	650	334
Em02	37°7.33'N	3°25.86'W	Shrubland	2099	14	140	33
Em08	37°8.00'N	3°25.91'W	Shrubland	1690	65	650	370
Em21	37°8.07'N	3°25.71'W	Forest	1723	41	410	237
Em22	37°7.86'N	3°25.70'W	Forest	1802	57	570	370
Em23	37°7.74'N	3°25.58'W	Shrubland	1874	32	320	97
Em24	37°7.51'N	3°26.14'W	Forest	1943	34	340	147
Em25	37°7.27'N	3°26.05'W	Shrubland	2064	26	260	64

(June–September). The watering regime was identical for all plants. Plants flowered when they were 2 years old, on April–May 2007. In total, 1675 plants belonging to 332 families reached adulthood (Table 1).

QUANTIFICATION OF FLORAL TRAITS

The following phenotypic traits were determined for both the maternal (2005) and the offspring (2007) plants:

- (1) Plant size, estimated by (a) stalk height, quantified as the height of the tallest stalk, measured to the nearest 0.5 cm as the distance from the ground to the top of the highest open flower; (b) Number of stalks; and (c) stalk diameter, quantified as the basal diameter in mm of the tallest stalk. These traits were measured with a digital caliper with ± 0.1 mm resolution. All plant-size-related traits were measured when plants were in full bloom.
- (2) Flower number, counting the entire production of flowers of each plant.
- (3) Corolla size, estimated in one flower per plant by (a) corolla diameter, estimated as the distance in mm between the edges of two opposite petals; (b) corolla tube length, the distance in mm between the corolla tube aperture and the base of the sepals; (c) corolla tube width, the diameter of the corolla tube aperture, estimated as the distance between the bases of two opposite petals. These traits were also measured with a digital caliper.
- (4) Corolla shape, determined in each of the plants by means of geometric morphometric tools, using a landmark-based methodology (Bookstein 1991; Rohlf 2003; Zelditch et al. 2004). We took a digital photograph of the same flower as above using a standardized procedure (front view and planar position). Flowers were photographed at anthesis to avoid ontogenetic effects. We defined 32 coplanar landmarks located along the outline of the flowers and the aperture of the corolla tube, the number of landmarks being chosen to provide comprehensive coverage of the flower shape (Roth 1993; Zelditch et al. 2004). Landmarks were defined by reference to the midrib (landmarks 1, 9, 17, and 25), primary veins (landmarks 2, 8, 10, 16, 18, 24,

26, and 32), and secondary veins (landmarks 3, 4, 6, 7, 11, 12, 14, 15, 19, 20, 22, 23, 27, 28, 30, and 31) of each petal as well as the connection between petals (landmarks 5, 13, 21, and 29; see Fig. 1). We captured the landmarks using the software tpsDig version 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). We used this method because of its low bias (Rohlf 2003). This procedure was performed using the software tpsRelw version 1.11 (available in the Stony Brook Morphometrics website at

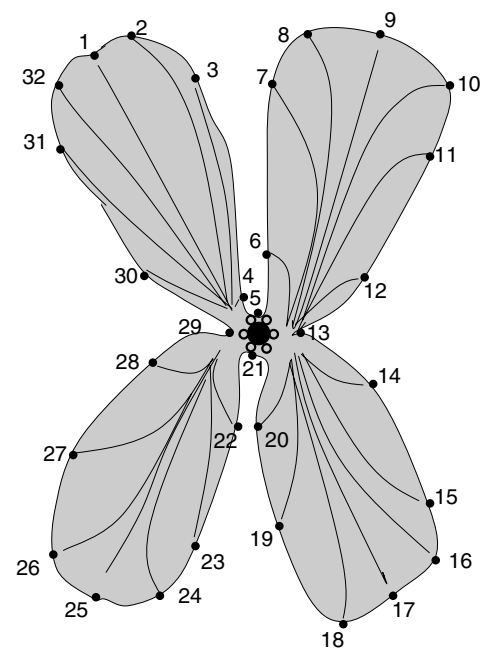


Figure 1. A schematic planar view of the *Erysimum mediohispanicum* corolla, showing the location of the 32 landmarks used in the geometric morphometric analysis.

<http://life.bio.sunysb.edu/morph/morphmet.html>). In these analyses, we considered the flower as a nonarticulated structure because the relative position of the petals does not change during their functional life. 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). Unit centroid size was used as the alignment-scaling method and the orthogonal projection as the alignment-projection method. This procedure generates a consensus configuration, the central trend of an observed sample of landmarks, which is similar to a multidimensional average. In addition, this procedure generates $2p - 4$ orthogonal RWs (p = number of landmarks). Each RW is characterized by its singular value, and explains a given variation in shape among specimens. Thus, RWs summarize shape differences among specimens (Adams et al. 2004), and their scores can be saved to be used as a data matrix to perform standard statistical analyses (Zelditch et al. 2004).

ESTIMATION OF HERITABILITY AND GENETIC CORRELATIONS

Heritability of plant and corolla size

Heritability was quantified using a mother–offspring regression (Falconer and Mackay 1996) as

$$h_{op}^2 = 2B,$$

where B is the slope of the regression of offspring trait values on the mother trait values. However, h_{op}^2 is dependent not only on the additive genetic variation in the parent generation, but also on the additive genetic variation in the offspring generation grown in the greenhouse (see appendix by R. Lande in Coyne and Beecham 1987). Under these circumstances, estimates of h_{op}^2 may not be accurate estimates of the heritabilities in the natural population. Therefore, we also used a method developed by Riska et al. (1989) to calculate the heritability in the natural population from the offspring–parent regression, which corrects for the additive genetic variation of the offspring generation in the greenhouse,

$$h_{RISKA}^2 = 4B^2 \left(\frac{\sigma_{PP}^2}{\sigma_{GO}^2} \right)$$

where σ_{PP}^2 is the phenotypic variance in the natural population, estimated from the parental plants, and σ_{GO}^2 is the additive genetic variance of the offspring in the greenhouse (Kleunen and Ritland 2004). The squared coefficient of parent–offspring regression is multiplied by 4 following the suggestions by Riska et al. (1989) when information exists only for mothers. We estimated σ_{GO}^2 from the analysis of variance on the offspring plants under the conservative assumption that offspring of the same seed family are half-siblings. Consequently, the Riska estimator is a minimum

estimate of the actual heritability and therefore it yields low values of heritability. Standard errors and significance levels of the Riska estimator of heritability were calculated by bootstrapping, producing 1000 bootstrap replicates for each phenotypic trait in the “boot” package of R (R Development Core Team 2008).

Because we had unequal family sizes, we used weighted least-squares regression to find h_{OP}^2 and h_{RISKA}^2 (Lynch and Walsh 1998). Weight was the inverse of the residual sampling variance of family means about the mother–offspring regression (Lynch and Walsh 1998). Because of the hierarchical nature of the design, all the regressions were performed on residuals from an analysis of variance that included the population as the random factor (Campbell 1996). Considering population as random avoids problems associated to heritability overestimation when fixed factors are included in the models (Wilson 2008). Nevertheless, to check whether this hierarchical design could affect our conclusions on heritability, we determined heritability separately for the only three populations for which we obtained information from more than 50 families: Em01, Em08, and Em22.

Heritability of corolla shape

Because shape is an inherently multivariate concept, estimating heritability of corolla shape is not possible by using the above-described standard univariate methods (Monteiro et al. 2002, 2003; Klingenberg 2003). Monteiro et al. (2002) recommended the calculation of shape heritability as the ratio of the total variances of the **G** and **P** matrices from the relative warps. We first used this method to calculate the overall heritability for corolla shape. However, this approach does not consider the directionality of variation in **G** and **P** (Klingenberg and Monteiro 2005). Some authors have proposed the use of a multivariate approach, such as generalized multivariate regression (GMR), to overcome this problem and to accurately estimate heritability of shape (Monteiro 1999; Klingenberg and Leamy 2001; Klingenberg 2003; Klingenberg and Monteiro 2005). Here, we followed this suggestion and estimated heritability on flower shape by means of a GMR, including as dependent variables the 60 RWs of the offspring, averaged per family, and as independent variables the 60 RWs of the mother plants. The GMR measures only the magnitude of shape differences, but ignores their direction, and consequently no coefficient is associated to this analysis (Klingenberg 2003). The significance of the whole shape heritability was performed by a Wilks’ Lambda (Zelditch et al. 2004). In addition, we calculated a multivariate regression coefficient for each of the first four RWs because each of these explained more than 5% of the variance in corolla shape (over 70% of variability all together, see below). These multivariate coefficients are multivariate analogues to the standard univariate regression coefficients; they represent the association between each parent and offspring pair of RWs while controlling for the remaining RWs in the matrix. After this,

following the procedure explained above, we found the h^2_{RISKA} for the shape components.

Genetic correlation

Genetic correlations r_A between traits x_i and x_j were calculated as

$$r_A = \frac{\text{Cov}(x_i, x_j)}{\sqrt{\text{Var}(x_i) \times \text{Var}(x_j)}}.$$

Where $\text{Cov}(x_i, x_j)$ are additive covariances and $\text{Var}(x_i)$ are additive variances (Falconer and Mackay 1996; Lynch and Walsh 1998). However, because we studied the parent population in the wild and the offspring population in the greenhouse, we also estimated genetic correlations between traits x_i and x_j as

$$r_A^* = \frac{0.5 [\text{Cov}(x_{i,O}x_{j,P}) + \text{Cov}(x_{j,O}x_{i,P})]}{\sqrt{\text{Cov}(x_{i,O}x_{i,P}) \times \text{Cov}(x_{j,O}x_{j,P})}},$$

where O and P refer to offspring and parent values, respectively. This method allows the estimation of genetic correlation without any previous knowledge concerning the relatedness between measured individuals in the natural population (Lynch 1999; Kleunen and Ritland 2004). Because r_A^* is not a product-moment correlation, it can sometimes be estimated out of the ± 1 boundary (Lynch and Walsh 1998).

The standard error for the mean of the genetic correlation was calculated as

$$\text{SE} = \left[\frac{1 - r_A^*}{\sqrt{2}} \right] \sqrt{\frac{\sigma_{h_x^2} \sigma_{h_y^2}}{h_x^2 h_y^2}},$$

Falconer and Mackay (1996).

Results

HERITABILITY OF TRAITS

Phenotypic values for floral traits were very similar in parental and offspring plants, except for flower number that was almost twice as high in the offspring plants (Table 2). The number of flowers, stalk diameter, and number of stalks had nonsignificant heritability values (Table 3). The heritability of these traits was also low in each of the three populations analyzed separately (Appendix S1). By contrast, stalk height presented a significant heritability, which remained high even when estimated using the Riska method (Table 3).

Heritability was significant for the traits related to flower size, such as corolla diameter, corolla-tube length and corolla-tube width (Table 3). Corolla diameter and tube length displayed higher R^2 value than did corolla tube width (Table 3). Corolla diameter heritability was significant in two of the populations whereas corolla tube length and width heritability was significant in one of the populations analyzed separately (Appendix S1).

The main components describing the variation in corolla shape were similar for parental as well as offspring generations (Fig. 2). Thus, the first four RWs explained more than 70% of the variance in corolla shape in these two groups of plants (Appendix S2), and were associated with the same patterns of shape variation (Fig. 2). Thus, RW1 was associated with changes in petal parallelism, RW2 was associated with changes in corolla zygomorphy, RW3 was associated with lateral symmetry, and RW4 was associated with corolla roundness.

Table 2. Descriptive statistics of floral traits in *Erysimum mediohispanicum* ($N=332$ families).

	Parental generation ($N=332$)		Offspring generation ($N=1665$)		
	Mean \pm 1 SE	V_p	Mean \pm 1 SE	V_p	V_g
Stalk diameter (mm)	1.85 \pm 0.03	0.32	2.40 \pm 0.09	13.70	1.15
Number of stalks	1.35 \pm 0.98	0.95	1.86 \pm 0.84	2.29	1.23
Stalk height (cm)	36.97 \pm 0.85	239.73	38.06 \pm 0.35	207.84	148.05
Number of flowers	40.90 \pm 0.85	926.17	78.84 \pm 1.96	6435.13	6428.12
Corolla diameter (mm)	10.89 \pm 0.09	2.64	12.41 \pm 0.04	2.77	1.05
Corolla tube length (mm)	10.52 \pm 0.08	1.94	10.65 \pm 0.03	1.98	1.06
Corolla tube width (mm)	0.52 \pm 0.05	0.95	1.47 \pm 0.02	0.64	0.18
Corolla shape ($\times 10^2$)		2.82		2.64	1.11
RW1 ¹	0.90 \pm 0.68	1.54	0.17 \pm 0.29	1.39	0.64
RW2 ¹	0.45 \pm 0.47	0.74	0.02 \pm 0.19	0.63	0.25
RW3 ¹	0.58 \pm 0.34	0.38	0.31 \pm 0.15	0.39	0.13
RW4 ¹	0.10 \pm 0.31	0.31	0.15 \pm 0.11	0.21	0.09

¹RWs refer to the first four relative warps obtained from the geometric morphometric analysis of corolla shape.

V_p , phenotypic variance (calculated as the among-individual variance, $N=332$ parental plants and 1665 offspring plants), V_g , genetic variance (calculated as the among-family variance, $N=332$ families)

Table 3. Heritability of floral traits in *Erysimum mediohispanicum* ($N=332$ families). Significant heritabilities are shown in bold.

Plant traits	h_{OP}^2 ⁵					h_{Riska}^2 ⁶
	Values \pm 1 SE	R^{24}	Wilk's λ	F	P	Value \pm 1 SE _b ⁷
Stalk diameter ¹	0.180 \pm 0.110	0.020		0.86	0.389	0.009 \pm 0.028
Number of stalks ¹	0.002 \pm 0.063	0.001		0.16	0.876	0.004 \pm 0.014
Stalk height ¹	0.190\pm0.043	0.015		2.21	0.027	0.361\pm0.162
Number of flowers ¹	0.001 \pm 0.149	0.001		0.14	0.887	0.050 \pm 0.199
Corolla diameter ¹	0.270\pm0.033	0.046		3.97	0.0001	0.239\pm0.199
Corolla tube length ¹	0.392\pm0.039	0.071		5.00	0.0001	0.267\pm0.152
Corolla tube width ¹	0.094\pm0.024	0.011		1.94	0.050	0.019\pm0.094
Corolla shape ²	0.423		0.642		0.0001	
RW1 ³	0.184\pm0.056	0.277	0.917		0.002	0.045 \pm 0.059
RW2 ³	0.038\pm0.055	0.363	0.933		0.018	0.001 \pm 0.047
RW3 ³	0.004 \pm 0.055	0.312	0.967		0.407	0.012 \pm 0.034
RW4 ³	0.500\pm0.061	0.415	0.917		0.003	0.001 \pm 0.024

¹Models including population and population \times phenotypic trait as random factors were solved by REML and were weighted by the inverse of the variance of the residuals.

²Overall heritability for corolla shape was calculated as the ratio of the total variances of the G and P matrices (Monteiro et al. 2002), and its significance was estimated by a generalized multivariate regression (GMR) between the 60 parental RWs and the 60 offspring RWs, including population as the random factor.

³Heritability values of each shape component were the multivariate regression coefficients resulting from the GMR.

⁴ R^2 was multivariate for corolla shape components and univariate for the remaining traits.

⁵ $h_{OP}^2 = 2B$, where B is the slope of the regression of offspring-trait values on the mother trait values.

⁶ $h_{Riska}^2 = \gamma^2 h^2 = 4B^2 (\frac{\sigma_{PP}^2}{\sigma_{GO}^2})$.

⁷The standard error and the significance level of the Riska estimator of heritability were found by bootstrapping.

The multivariate regression indicates that heritability was also significant for corolla shape (Table 3). Specifically, three shape components had significant heritability, namely RW1, RW4, and to a lesser extent RW2 (Table 3). Thus, nonparallel petals and narrow petals were highly heritable. In these cases, the multivariate R^2 were very high, consistently above 25% of the variance explained (Table 3). When the three populations were studied separately, we found significant heritability for corolla shape in two populations, due mostly to RW4 and RW1 components (Appendix S1).

GENOTYPIC CORRELATIONS AMONG TRAITS

There was a significant and high positive genetic correlation among all plant-size-related phenotypic traits (number of stalks, stalk diameter, and stalk height), irrespective of the method used to quantify it (Table 4). Many of the genetic correlations, when quantified using the standard method, remained significant when the three populations were analyzed separately (Appendix S1). Furthermore, number of flowers was positively correlated with all these plant-size traits, both when all populations were analyzed together (Table 4) as well as when the three populations were studied separately (Appendix S1).

Flower-size-related traits (corolla diameter, corolla tube length, and corolla tube width) were also positively correlated

among them according to the standard r_A . However, according to r_A^* the correlations between corolla tube width and the other two flower-size-related traits vanished (Table 4). When studied separately, there was again significant genetic correlation, when estimated as r_A , between corolla diameter and the other two floral-size-related traits (Appendix S1). Flower-size traits were significantly correlated with plant-size traits when estimated as r_A^* (Table 4). However, most of this correlation disappeared when populations were studied separately (Appendix S1).

There was no significant genetic correlation, either r_A or r_A^* , among flower-shape components. Also remarkable was the low correlation observed between corolla shape and other plant traits (Table 4). Only number of flowers significantly correlated with flower shape (Table 4). In addition, number of stalks was negatively correlated with floral roundness (RW1), stalk height was positively correlated with floral zygomorphy (RW2), and number of stalks and flower size were correlated with floral asymmetry (RW3; Table 4). However, when we calculated the genetic correlations separately for the populations Em01, Em08, and Em22, we found some striking outcomes. Thus, different shape components correlated significantly with different traits in each population (Appendix S1). RW1 correlated positively with some plant-size traits in Em01, but negatively in the other two populations, whereas RW3 correlated with plant-size traits only in Em08, and

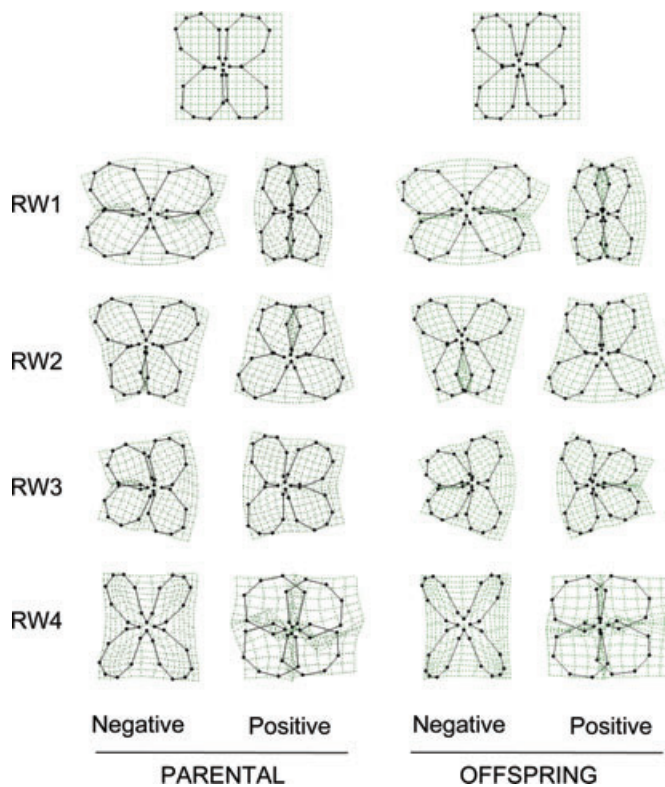


Figure 2. Summary of the geometric morphometric analysis ($N = 720$ plants in the parental group and 1635 in the offspring group) showing the consensus morphology (uppermost panels) and the variation in flower morphology produced by the Relative Warps explaining more than 5% of the overall variation in shape (see Appendix S1). The distribution of each RW statistically fitted a normal distribution with mean = 0 ($\chi^2 < 0.993$, $P > 0.34$ in all cases, Shapiro–Wilks' W test).

the other two shape components did not correlate with any trait (Appendix S1).

Discussion

HERITABILITY OF SIZE AND SHAPE

We found that traits associated with pollinator attraction and plant fitness (Gómez et al. 2006, 2008a, 2009), such as stalk height, corolla diameter, corolla tube length, and corolla tube width, showed high levels of heritability. By contrast, traits not associated with fitness, like number of flowering stalks and diameter of the flowering stalks, showed low heritability. This outcome partly agrees with a recent review on heritability comprising more than 60 systems (Ashman and Majestic 2006). However, contrasting with the above-mentioned review, we found no heritability for number of flowers per plant. As stated above, *E. mediohispanicum* is self-compatible (Gómez 2005a), and according to Ashman and Majestic's review, heritability tends to be lower in self-compatible

than in self-incompatible species. In any case, our finding suggests that variation in *E. mediohispanicum* flower number is partially caused by environmental factors. Many studies have indeed found that additive genetic variation is more common for floral traits than for flower number (Campbell 1997; Elle 1998; Worley and Barrett 2000). Remarkably, genetic variance for flower number was much higher than the phenotypic variance of the parental generation, suggesting that flower number is very similar among different genotypes in field conditions as a consequence of some environmental factors constraining its variation.

We also found significant heritability for corolla shape, both for the overall trait and for three of the four main shape components. To our knowledge, this is the first study that has demonstrated heritability corolla shape considering it as a complex and single multidimensional trait rather than decomposing it in its linear components. Indeed, as far as we know, Galen and Cuba (2001)'s study on *Polemonium viscosum* is the only study exploring corolla shape heritability to date. However, these authors estimated corolla shape by two linear surrogates, corolla flare and length. Our findings suggest that not only size-related floral traits but also shape-related traits have enough genetic variation to respond to selection exerted by pollinators.

Our results suggest that *E. mediohispanicum* traits under strong pollinator-mediated selection had high heritability (Gómez et al. 2008a, 2009). In fact, the only highly selected trait showing low heritability was number of flowers. This is counterintuitive because a wide amount of information suggests that those traits more tightly related with fitness have low heritability (Merilä and Sheldon 2000). In our case, several nonexclusive factors can promote enough genetic variation even in pollinator-selected traits. First, herbivore–pollinator conflicting selection occurs on many *E. mediohispanicum* traits, weakening the strength of the net selection exerted by the pollinators (Gómez 2003, 2005a, 2008). Another possible explanation for this phenomenon is that the selection acting on these traits is relatively recent and it has not yet exhausted all deleterious variation. Finally, the high values of heritability could be related to our experimental design. We estimated the phenotypic traits of the progeny in a common garden rather than in its natural environment in which the traits of the parents were studied. This approach may overestimate heritability because the environmental-variance component is much higher in the field than in the greenhouse (Schoen et al. 1994; Conner et al. 2003). We tried to circumvent this pitfall by estimating heritability following the Riska method (Riska et al. 1989; Kleunen and Ritland 2004). In fact, heritability estimates were smaller when using this method than when using the standard method (Table 2), suggesting that heritability can actually be overestimated when quantified in the greenhouse. Nevertheless, Young et al. (1994) found for *Raphanus sativus* (Brassicaceae) that heritability on several floral traits is similar both in the greenhouse and the field.

Table 4. (A) Genetic correlations among *E. mediohispanicum* phenotypic traits ($N=332$ families) calculated by the standard r_A (below diagonal) and alternative r_A^* method (above diagonal). Functionally related traits appear in gray. * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$. (B) Standard errors of the genetic correlations.

	Number of stalk	Diameter of stalk	Stalk height	Number of flower	Corolla diameter	Corolla tube length	Corolla tube width	RW1	RW2	RW3	RW4
A											
Number of stalk		0.990****	0.990****	0.980****	-0.049	-0.399***	0.980****	-0.010	0.004	-0.005	0.002
Diameter of stalk	0.109*		0.999****	0.980****	0.698****	-0.138	0.599****	0.001	-0.005	-0.002	0.000
Stalk height	0.145**	0.255****		0.551****	0.323***	0.351***	-0.034	0.067	-0.067	-0.003	-0.033
Number of flower	0.703****	0.311****	0.458****		0.143	0.144	-0.109	-0.528****	0.194*	-0.515****	-0.034
Corolla diameter	-0.004	0.078	0.122*	0.115*		0.579****	-0.092	-0.001	0.000	-0.005	0.000
Corolla tube length	-0.094	0.037	-0.021	0.023	0.542****		0.023	0.000	0.003	-0.002	0.002
Corolla tube width	-0.095	-0.029	-0.060	-0.016	0.237****	0.486****		0.002	-0.000	-0.001	0.001
RW1	-0.110*	0.017	0.068	-0.082	-0.008	0.005	0.052		-0.000	-0.000	0.000
RW2	0.078	-0.094	-0.109*	0.048	0.010	0.067	-0.018	-0.012		-0.000	-0.000
RW3	-0.126*	-0.047	-0.007	-0.180***	-0.123*	-0.048	-0.053	-0.099	-0.002		0.001
RW4	0.045	0.003	-0.089	-0.014	0.015	0.057	0.072	0.092	-0.047	-0.014	
B											
Number of stalk		0.061	0.005	0.264	0.396	0.477	0.011	0.602	1.294	4.025	0.376
Diameter of stalk	5.401		0.004	1.938	0.838	2.849	1.608	4.374	9.597	29.491	2.772
Stalk height	0.439	2.813		3.687	0.159	0.138	0.351	0.346	0.864	2.502	0.243
Number of flower	3.916	66.755	4.451		5.172	4.661	9.673	14.552	16.739	96.978	6.235
Corolla diameter	0.379	2.558	0.206	5.341		0.066	0.273	0.273	0.595	1.842	0.173
Corolla tube length	0.373	2.411	0.217	5.320	0.071		0.220	0.246	0.535	1.657	0.155
Corolla tube width	0.598	4.127	0.360	8.862	0.191	0.116		0.393	0.860	2.652	0.249
RW1	0.661	4.304	0.346	10.304	0.275	0.245	0.374		0.939	2.893	0.272
RW2	1.198	10.447	0.898	19.771	0.589	0.501	0.875	0.950		6.309	0.594
RW3	4.510	30.815	2.512	75.534	2.059	1.733	2.790	3.179	6.322		1.830
RW4	0.360	2.764	0.256	6.114	0.170	0.147	0.232	0.247	0.622	1.857	

Another important caveat of our experimental design is related to the fact that it does not consider maternal effect explicitly. Maternal effects may overestimate heritability estimates because it inflates the parent–offspring regression coefficients. Because we did not control the sire in our experiment, it is not possible to compare maternal covariance to paternal covariance to estimate the maternal effects (Roff 1998). Nevertheless, we tried to minimize the maternal effects by randomly distributing the seeds in the greenhouse to lower the within-family environmental correlation. In addition, most studies have shown that maternal effects have a stronger influence on juvenile traits than on adult traits (Shaw and Byers 1998). Because all phenotypic traits considered in this study are displayed during flowering, we presume that maternal effects are not very important. Finally, including in the same analysis families from eight populations could also affect our heritability estimates. Nevertheless, we believe that our estimates are good proxy of real heritability estimates because we include in the analysis population as a random effect, and furthermore we repeated the analysis in three populations independently and the outcomes were similar.

GENETIC CORRELATIONS AMONG TRAITS

There was a strong genetic correlation between functionally related traits (except between corolla-shape components because a nonzero correlation between them is possible only when phenotypic covariance matrices do not conform to the model of common principal components), both for all plant populations analyzed together and for the three populations studied separately. This outcome suggests the occurrence of phenotypic integration for plant size and for flower size in *E. mediohispanicum*.

Number of flowers was significantly and positively correlated with plant-size traits, also whether analyzing all populations together or separately. This relationship, reported for a wide number of species, is shown as a typical example of environmental covariation among traits. First, being modular organisms, bigger plants produce more modules, which means more flowers. Second, plants located in high-quality microsites have more resources to produce both more vegetative and reproductive tissue, resulting in a spurious correlation between them. Our outcomes suggest, nonetheless, that the relationship between plant size and flower number may also be genetic, at least in *E. mediohispanicum*.

There was also positive genetic correlation between flower-size traits and plant-size traits. In fact, five out of the nine potential correlations between these groups of traits were positive and significant. Nevertheless, most of these correlations vanished for the populations studied separately, suggesting that floral-size traits are not actually coupled with vegetative traits in *E. mediohispanicum*. This finding agrees with most studies, which have shown that genetic and phenotypic correlations between floral

and vegetative character suites are low and mostly statistically nonsignificant (Conner and Via 1993; Conner and Sterling 1996; Waitt and Levin 1998; Armbruster et al. 1999; Worley and Barrett 2000; Juenger et al. 2005; Ashman and Majetic 2006).

Theoretical models predict a trade-off between flower number and size (Sakai 1995; Schoen and Ashman 1995; Harder and Barrett 1996; de Jong and Klinkhamer 2005; Sargent et al. 2007). However, many empirical studies have failed to find such a negative genetic correlation between these plant traits (Mazer 1989; Meagher 1992; Andersson 1996; Elle 1998; Worley and Barrett 2000, 2001; Ashman and Majetic 2006; Caruso 2006; Lehtilä and Holmén Bränn 2007; but see Caruso 2004). Worley and Barrett (2000, 2001) even showed for *Eichhornia paniculata* that genetic correlations for flower-size number can range from negative to positive in different localities. In our case, we have found no correlation, whether positive or negative, between flower number and flower size, both for all populations analyzed together and for the three populations studied separately (except between flower number and corolla tube length in Em08, where correlation was significantly negative). Worley and Barrett (2000) suggest that a potential cause of the absence of a genetic correlation between plant size, flower number, and flower size may be the genetic variation in module size and resource status. That is, the genetic correlation between module size and flower size and between module size and flower number disrupted any potential for flower size and number genetic trade-off. We do not have enough information to test this hypothesis, although we believe that it could also apply to *E. mediohispanicum* because we found a high positive correlation between plant size and flower number, and between plant size and flower size. Nevertheless, we also think that our analyses had low power to detect negative correlations between flower number and flower size (power < 0.5 in all analyses). In fact, although not significant, all estimates of correlations between these traits were negative in Em01, Em08, and Em22.

Remarkably, corolla shape was only slightly correlated with flower number, flower size, and plant-size traits. This absence of strong correlation suggests that corolla shape is genetically decoupled from other floral traits and from vegetative traits in *E. mediohispanicum*. The decoupling found in this study suggests that this complex trait can respond to pollinator-mediated selection without any constraint by indirect selection through other plant traits. Specialized, zygomorphic flowers tend to have higher phenotypic integration and more decoupling between different trait suites than generalist and actinomorphic flowers (Ashman and Majetic 2006). Our findings show that *E. mediohispanicum* corolla shape but not size is decoupled from other traits, behaving more as zygomorphic than as actinomorphic species. Actually, zygomorphy and specialized flower shapes are currently selected by pollinators in several *E. mediohispanicum* populations (Gómez et al. 2006; Gómez 2008).

CONCLUDING REMARKS

This study has shown that most *E. mediohispanicum* floral traits, even complex traits such as corolla shape, can have significant heritability. Consequently, this species retains a high ability to respond to the selection exerted by its pollinators. Furthermore, this study has also shown genetic correlation between flower number, flower size, and plant size. Under these circumstances, selection affecting a given *E. mediohispanicum* trait would also indirectly affect other traits, functionally related and unrelated traits. This finding agrees with the frequent indirect selection detected in this plant (Gómez 2003; Gómez et al. 2006; Gómez 2008; Gómez et al. 2008a, 2009). More importantly, genetic correlation, if beneficial, can be maintained and even promoted by correlational selection (Sinervo and Svensson 2002; McGlothlin et al. 2005). In fact, we have previously reported positive correlational selection acting on the covariance between flower number and plant size (Gómez 2003) and between flower number and flower size (Gómez et al. 2006) of *E. mediohispanicum*. This suggests that the genetic correlation detected between *E. mediohispanicum* traits could be at least partially adaptive.

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Supporting Information

The following supporting information is available for this article:

Appendix S1. Heritability and genetic correlations (calculated by the standard r_A below diagonal and alternative r_A^* method above diagonal) for the three populations with enough number of families studied (>50 families).

Appendix S2. Outcome of the geometric morphometric analysis describing corolla shape in the parental (720 plants) and offspring generations (1635 plants).

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

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