

Floral integration, phenotypic covariance structure and pollinator variation in bumblebee-pollinated *Helleborus foetidus*

C. M. HERRERA,* X. CERDÁ,* M. B. GARCÍA,* J. GUITIÁN,† M. MEDRANO,†
P. J. REY,‡ & A. M. SÁNCHEZ-LAFUENTE*

*Estación Biológica de Doñana, CSIC, E-41013 Sevilla, Spain

†Departamento de Biología Vegetal, Facultad de Farmacia, Universidad de Santiago de Compostela, E-15706 Santiago de Compostela, Spain

‡Departamento de Biología Animal, Vegetal y Ecología, Universidad de Jaén, E-23071 Jaén, Spain

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Abstract

By analysing patterns of phenotypic integration and multivariate covariance structure of five metric floral traits in nine Iberian populations of bumblebee-pollinated *Helleborus foetidus* (Ranunculaceae), this paper attempts to test the general hypothesis that pollinators enhance floral integration and selectively modify phenotypic correlations between functionally linked floral traits. The five floral traits examined exhibited significant phenotypic integration at all populations, and both the magnitude and the pattern of integration differed widely among populations. Variation in extent and pattern of integration was neither distance-dependent nor significantly related to between-population variation in taxonomical composition and morphological diversity of the pollinator assemblage. Patterns of floral integration were closer to expectations derived from consideration of developmental affinities between floral whorls than to expectations based on a pollinator-mediated adaptive hypothesis. Taken together, results of this study suggest that between-population differences in magnitude and pattern of floral integration in *H. foetidus* are probably best explained as a consequence of random genetic sampling in the characteristically small and ephemeral populations of this species, rather than reflecting the selective action of current pollinators.

Introduction

From Darwin's (1862) pioneering work on orchid flowers until relatively recently, studies on plant adaptation to pollinators have mostly focused on adaptive interpretations of the morphological and functional matching frequently existing between flowers and their pollinators. This approach was progressively replaced in the last few decades by more rigorous research programs [see Wilson & Thomson (1996) for historical background]. One approach figuring prominently among modern lines of enquiry on floral adaptation has applied the theory and methods developed to analyse selection on continuous, correlated characters ('phenotypic selection approach';

Lande & Arnold, 1983). These investigations have assessed the fitness consequences of individual variation in floral traits and have evaluated the selection gradients imposed by pollinators on floral traits (e.g. Campbell *et al.*, 1991, 1996; Andersson & Widén, 1993; Herrera, 1993; Andersson, 1994; Maad, 2000).

Another recent, although much less developed line of enquiry in the analysis of floral adaptation focuses on patterns of phenotypic variability in floral characters (Armbruster, 1991; Fenster, 1991; Armbruster & Schwaegerle, 1996; Herrera, 1996; Cresswell, 1998; Armbruster *et al.*, 1999). The significance of studying variability in floral traits as a way of addressing floral adaptation was long ago stressed by Berg (1959, 1960), yet relatively few investigations have approached the study of floral adaptation from this perspective. Adopting an univariate approach, Fenster (1991) and Herrera (1996) examined predictions relating the mean and the variance of corolla depth in species with restrictive

Correspondence: Carlos M. Herrera, Estación Biológica de Doñana, Consejo Superior de Investigaciones Científicas, Avenida de María Luisa s/n, E-41013 Sevilla, Spain.
Tel.: +34 954 232340; fax: +34 954 621125;
e-mail: herrera@cica.es

tubular corollas, and Wolfe & Krstolic (1999) tested a predicted relationship between the type of floral symmetry and variability in flower size. These studies tested predictions derived from the hypothesis that patterns of univariate phenotypic variability have been decisively moulded by selection from pollinators (Cresswell, 1998). Variability in floral traits has been also examined from a multivariate perspective, testing predictions related to patterns of covariation among multiple floral traits. Berg's (1959, 1960) 'correlation pleiades' hypothesis that, as a result of selection from pollinators, floral characters should exhibit greater integration among themselves than vegetative ones has received considerable attention (Armbruster *et al.*, 1999; and references therein). Nevertheless, the selective mechanisms underlying the hypotheses tested in studies of multivariate floral integration have been not always made explicit, perhaps because few of these have examined patterns of covariation of floral traits alone, independently of vegetative traits (but see: Armbruster, 1991; Armbruster & Schwaegerle, 1996; Andersson, 1997; Herrera, 2001).

Discussions on the correlated evolution of traits have traditionally focused on the genetic (e.g. pleiotropy, linkage) and developmental causes of correlation among traits (e.g. Grant, 1975; Lande, 1980; Falconer, 1989). Nevertheless, covariance between traits can also evolve as a consequence of correlational selection (Endler, 1986, 1995). The assumption that pollinators can exert correlational selection on floral traits lies at the core of Berg's (1959, 1960) classical hypothesis on correlation pleiades, and it is also implicit in Stebbins's (1974, p. 50) assertion that 'for adaptation to cross-pollination by animals with specialized habits ... the flower must become a highly integrated structure, with all of its parts precisely adjusted to one another'. Floral parts are functionally linked, that is, their functional effectiveness is determined by their ability to work together, and fitness is determined by the interaction of two or more of them. Pollinators may thus selectively modify the correlation between functionally linked floral traits via correlational selection (Armbruster, 1991; Armbruster & Schwaegerle, 1996; Armbruster *et al.*, 1999). The general adaptive hypothesis tested in this study, namely that geographical variation in the magnitude and pattern of floral integration of *Helleborus foetidus* may reflect variation in the species' pollinators, is based on these premises. As a number of studies have validated, at least in part, Berg's correlation pleiades hypothesis (e.g. Conner & Via, 1993; Waitt & Levin, 1993; Andersson, 1994; Conner & Sterling, 1995, 1996; Armbruster *et al.*, 1999), it seems reasonable to grant some validity to its underlying assumptions. Furthermore, strong indirect support for the assumption that selection by pollinators can effectively change the sign and magnitude of correlations between floral characters has been provided by Stanton & Young (1994) for the bee-pollinated *Raphanus sativus*. In this species, floral traits have not evolved independently although they are

genetically independent. Artificial selection on the petal size/pollen number ratio over just two generations was able to eliminate the positive correlation between petal size and pollen production occurring in nature.

Although the theoretical basis and analytical tools necessary to incorporate the study of correlational selection into studies of phenotypic selection on floral traits have been around for some time (e.g. Lande & Arnold, 1983; Phillips & Arnold, 1989), only a few recent investigations have addressed it directly so far (O'Connell & Johnston, 1998; Gómez, 2000; Maad, 2000). The proximate mechanisms whereby pollinators may come to exert correlational selection on two or more floral traits are essentially identical to those involved in selection on single characters, that is, generating fitness differences among individual plants related to differential flower attractiveness to pollinators, and/or differential success in pollen export and receipt per realized pollinator visit. The first mechanism is illustrated by pollinators discriminating against departures from floral symmetry (Møller & Eriksson, 1995). The second mechanism is illustrated, for example, by the impaired pollen deposition or removal resulting from the mismatch between floral structure and pollinator body size (e.g. Armbruster, 1988, 1991; Armbruster *et al.*, 1999).

Different pollinators may select on different floral characters, or in different ways on the same character, and this applies even to pollinators that differ only slightly in body size, behaviour, and size of mouthparts (e.g. Herrera, 1987; Wilson & Thomson, 1991, 1996; Conner *et al.*, 1995). Differences between pollinator species in the nature and magnitude of correlational selection on floral characters are also to be expected, although this aspect has not been directly addressed by any investigation. In the same way as geographical variation in pollinator composition sometimes gives rise to contrasting directional selection on individual characters which may eventually lead to population divergence in mean floral traits (e.g. Armbruster, 1985, 1988; Robertson & Wyatt, 1990; Galen, 1996), it is also theoretically possible that between-population differences in pollinator composition may select for population differentiation in patterns of floral trait covariance structure via divergent correlational selection on floral traits. Furthermore, between-population variation in the morphological and behavioural diversity of pollinators may translate into differences in the net correlational selection exerted by the whole pollinator assemblage. It seems intuitive to expect that increasing morphological and behavioural homogeneity of pollinators would lead to more consistent correlational selection on relevant floral characters and, consequently, to tighter floral integration.

There are at least four additional reasons why the study of multivariate patterns of phenotypic integration and covariation of floral traits of individual species at the within- and between-population levels may complement

investigations based on analyses of phenotypic selection. First, testable predictions can be more easily generated than in phenotypic selection studies. Secondly, whereas phenotypic selection approaches may provide insight into the mechanisms and *potential* consequences of pollinator selection, patterns of phenotypic integration provide a short cut to studying some *realized* phenotypic consequences of such selection. Thirdly, studies on interspecific patterns of floral variability and character integration have provided equivocal or contradictory support to adaptive hypotheses (e.g. Fenster, 1991; Herrera, 1996; Armbruster *et al.*, 1999; Wolfe & Krstolic, 1999), and we remain largely ignorant on whether interspecific patterns represent a scaled-up version of those occurring across populations and individuals within species. This aspect is essential to distinguishing between the 'constraint' and 'adaptive' models of morphological diversification, and to link micro and macroevolutionary processes in floral evolution (Armbruster, 1991; Andersson, 1993, 1997; Björklund & Merilä, 1993; Armbruster & Schwaegerle, 1996). Fourthly, recent investigations have corroborated for plants Cheverud's (1988) conjecture, and found that phenotypic correlation matrices closely reflect genetic correlation matrices (Waitt & Levin, 1998; see also Roff, 1996). With some caution, therefore, phenotypic covariance matrices of floral traits may be interpreted in relation to genetic covariance matrices.

This paper has two main objectives. The first is to analyse between-population variation in magnitude and pattern of multivariate phenotypic integration and character covariance structure in floral morphometric traits of the bumblebee-pollinated herb *Helleborus foetidus* L. (Ranunculaceae). Our second general objective is to assess the relative importance of variation in pollinator composition, geographical distance between populations, and possible developmental constraints affecting floral parts, as explanatory factors for between-population variation in multivariate covariance structure of floral traits. A number of studies have examined the relationship between population differences in floral characteristics and associated variation in the composition and/or morphology of pollinators (e.g. Armbruster, 1985; Robertson & Wyatt, 1990; Johnson, 1997; Johnson & Steiner, 1997; Hansen *et al.*, 2000). The present study differs from most of them (but see Armbruster, 1985, 1991) in that we emphasize multivariate patterns of within-population covariation among floral traits rather than population means. The following specific questions will be addressed: (1) Do populations of *H. foetidus* differ in the magnitude and pattern of integration of morphometric floral traits? (2) Are population differences in magnitude and pattern of floral integration related to geographical distance or to variation in the taxonomical composition and/or morphological characteristics of the pollinator assemblage? and (3) Do observed patterns of floral integration depart significantly from null-model

expectations derived from consideration of developmental affinities between distinct floral whorls?

Methods

Study species

Helleborus foetidus is a perennial herb widely distributed in western and south-western Europe (Werner & Ebel, 1994). In the Iberian Peninsula, it typically occurs in clearings, forest edges, and the understory of montane forests. Flowering mainly takes place during January–March. Flowers are protogynous and self-compatible, and although some spontaneous autogamy occurs when pollinators are excluded experimentally, successful reproduction requires insect pollination (Herrera *et al.*, 2001). In comparison with other species of the genus *Helleborus*, *H. foetidus* has a comparatively 'specialized' pollination system, characterized by a restrictive floral morphology and dependence on a taxonomically restricted array of pollinators for seed production. Our observations at six Iberian populations show that a few species of similarly sized bumblebees are the main pollinators at all sites (Herrera *et al.*, 2001; and results below). This low diversity of pollinators may be attributed to the morphological and structural differentiation of its flowers relative to the species congeners. As in other species of the genus *Helleborus*, the petals of *H. foetidus* have become modified into nectaries (Tamura, 1993), which are shaped more or less like flattened horns (Fig. 1) and produce abundant nectar (Herrera & Soriguer, 1983; Vesprini *et al.*, 1999). The five sepals have taken over the function of the petals. They are enlarged, pale green, and their length, disposition, and curvature determine the overall size and shape of the flowers. In contrast to the fully exposed nectaries of other *Helleborus* species with open, bowl-shaped corollas, the nectaries of *H. foetidus* are deeply hidden inside the globose or subcylindrical pendent corolla (Fig. 1). The relatively narrow entrance of the corolla makes the nectaries accessible only to relatively large-bodied, long-tongued insects. The nectaries themselves are long and narrow structures of reduced accessibility. Further, because of the radial arrangement of the concealed nectaries and the pendent disposition of flowers, efficient exploitation of the nectar presumably requires a certain degree of learning ability on the part of visitors, thus adding behavioural restrictions to morphological ones.

Study sites and methods

This study was conducted from February to June (1998–99) at nine *H. foetidus* populations widely distributed over the Iberian Peninsula (Fig. 2). Five flowers were collected from each of 30–62 plants in each population ($n = 368$ plants, the nine populations combined), and preserved in 2.5–2.5–95% formaldehyde–acetic

acid-ethyl alcohol solution until their dissection and measurement. Individual flowers of *H. foetidus* last for up to 3 weeks (Vesprini *et al.*, 1999), and shape and dimensions vary slightly with flower age. To avoid biases derived from possible differences between populations in age structure of flowers, these were collected in the early male stage (20–40% of anthers dehisced), which roughly represents the midpoint in a flower's life.

Five morphometric floral characters will be considered in this study (abbreviations used to denote them hereafter are shown in parentheses): corolla length (CLen), corolla width (Cwid), corolla aperture (CApe), nectary length (NLen), and nectary aperture (NApe) (Fig. 1). The first three measurements were taken with a digital caliper, and the other two under a dissecting microscope using an ocular micrometer. A single nectary, chosen at random, was measured per flower. All the flowers considered in this study ($n = 1686$) were measured by the same person. The five floral traits considered are potentially relevant to the interaction of *H. foetidus* with its pollinators, and their variation among individuals or populations may result in differential flower visitation rate, per-flower pollination success, or both. Variations in

corolla length, width and aperture may influence flower attractiveness to pollinators, the accessibility of nectaries, and the disposition and orientation of pollinators' bodies relative to the anthers and stigmas. The latter, in turn, may affect the placement of pollen grains on insect bodies and the probability of deposition on stigmas in subsequent visits to receptive flowers. Variation in length and aperture of nectaries will presumably influence nectar accessibility, extraction efficiency and handling time by pollinators.

Detailed observations on pollinator composition and behaviour were conducted during the reproductive seasons of 1998 and 1999 simultaneously at six of the nine populations studied (populations 1, 2, 5, 6, 7 and 8 in Fig. 2). At each site, 20–25 three-minute censuses were performed every year on each of the same individually marked plants from which flowers had been collected for measurement. The information recorded in each census included the identity of flower visitors and the number of flowers visited. Analyses of pollinator censuses have been presented elsewhere (Herrera *et al.*, 2001), and only a summary of results is presented here to document pollinator composition at the study populations. At each site, only minor differences existed between years in pollinator composition and abundance, and data from the two study seasons are combined into a single sample. Measurements of

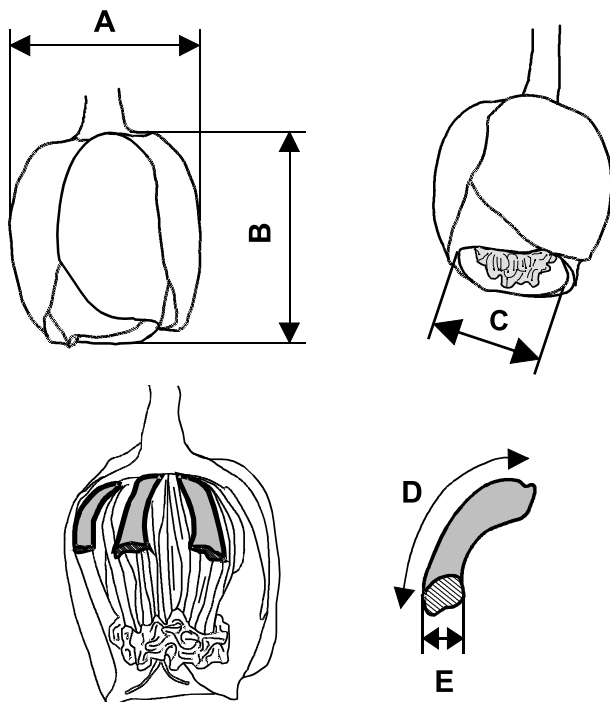


Fig. 1 Schematic representation of the pendent flowers of *Helleborus foetidus*. Two sepals have been omitted from the bottom left flower to show the position of nectaries (shaded, funnel-like structures). Arrows denote the five measures used in this study (abbreviations used are shown in parentheses): (A) corolla width (Cwid); (B) corolla length (CLen); (C) corolla aperture (CApe); (D) nectary length (NLen); (E) nectary aperture (NApe).

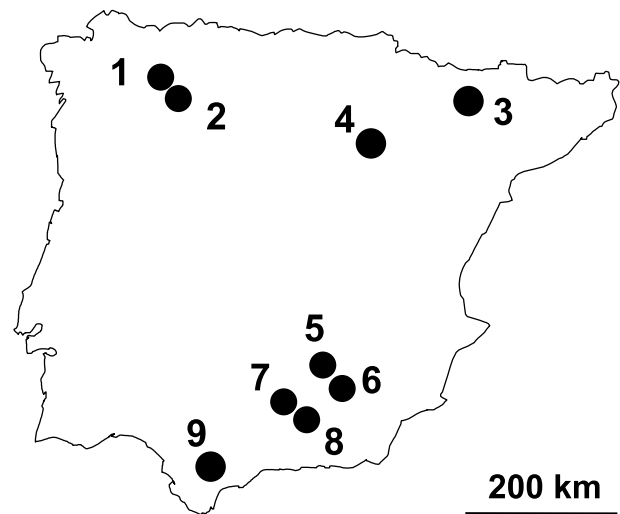


Fig. 2 Location in the Iberian Peninsula of the nine populations of *Helleborus foetidus* studied. Number of plants (N_p) and flowers (N_f) sampled at each site in parentheses. 1, Entretaros, Sierra del Caurel ($N_p = 62$, $N_f = 285$); 2, Mostad, Sierra del Caurel ($N_p = 56$, $N_f = 279$); 3, Jaca, Pirineos ($N_p = 30$, $N_f = 150$); 4, Alcalá del Moncayo, Sierra del Moncayo ($N_p = 32$, $N_f = 159$); 5, Arroyo de la Hiedra, Sierra de Cazorla ($N_p = 33$, $N_f = 119$); 6, Roblehondo, Sierra de Cazorla ($N_p = 34$, $N_f = 110$); 7, Puerto de la Mata, Sierra Mágina ($N_p = 41$, $N_f = 205$); 8, Pasailla, Sierra Mágina ($N_p = 45$, $N_f = 221$); 9, Pinsapar, Sierra de Grazalema ($N_p = 35$, $N_f = 158$).

body length and proboscis length of all major pollinator taxa were taken on specimens collected at the study regions. We found no evidence of geographical variation in body size or proboscis length of the main pollinators, thus each species was characterized by a single average value.

Statistical analyses

Measurements from flowers of the same plant were averaged to obtain a single set of values, and these plant means formed the raw database for the analyses. Unless otherwise stated, all statistical analyses were performed using the SAS statistical package (SAS Institute, 1990). Bootstrap estimates of standard errors and confidence limits were computed using functions written by CMH for the S-Plus 2000 statistical environment (Mathsoft, 1999). Bootstrap estimates were obtained using the bias-corrected and accelerated percentile method (BCa; Efron & Tibshirani, 1993) with 1000 repetitions.

Magnitude and pattern of integration

The magnitude and statistical significance of floral integration was assessed for each population using the variance of the eigenvalues (λ_i , $i = 1, 5$) of its character correlation matrix (Integration index = $\text{Var} [\lambda_i]$, INT hereafter) (Wagner, 1984; Cheverud *et al.*, 1989). Standard errors and confidence intervals of INT were obtained by bootstrapping. Character correlation matrices for different populations were based on a different number of plants, thus the expected eigenvalue variance on the hypothesis of random covariation among characters [$\text{Exp(INT)} = (\text{number of characters} - 1)/\text{number of plants}$; Wagner, 1984] varied among populations. For the purpose of comparisons among populations in the magnitude of integration, empirical integration indices were thus corrected by subtracting the population-specific expected value. The (corrected) INT for a population was considered to reflect significant floral integration if its 95% confidence interval did not include zero.

Character covariance matrices can share more complex relationships between one another than just being equal or unequal (e.g. Flury, 1988; Stepan, 1997a), hence we tested the similarity of different within-population floral covariance structures by applying Flury's (1988) hierarchical method. This approach can reveal shared similarities across covariance matrices that go beyond the simple question of matrix equality (Phillips & Arnold, 1999). The 'jump-up' approach of Phillips & Arnold (1999) was adopted. The model assuming heterogeneity of covariance matrices [or 'heteroscedastic' model in Mathsoft, (1999) terminology] was sequentially compared using log-likelihood ratio tests with models that specify different relationships among the populations covariance matrices (i.e. common principal components, partial principal components, proportionality, and equality), until a statistically significant deviation was encountered.

We also compared the heteroscedastic model with one assuming that populations have a common correlation structure, but different variances. This model corresponds to the 'equal correlation' structure in the 'Classical' family of discriminant models in S-Plus (Mathsoft, 1999). Although this model does not properly belong in Flury's hierarchy (Phillips & Arnold, 1999), it was worth considering here too to test explicitly for a possible homogeneity of character correlation matrices in spite of inequality of variances. Tests related to Flury's hierarchy were carried out using the CPC program written by P. Phillips, University of Oregon (available over the World Wide Web at <http://www.uoregon.edu/~pphil/software.html>). Comparisons involving models in the classical family of discriminant models were carried out with S-Plus functions.

Developmental vs. functional integration hypotheses

As noted above, the petals of *H. foetidus* have become modified into nectaries and the five sepals have taken over the function of the petals. In the absence of correlational selection from pollinators favouring coordinated variation of floral parts and enhancing intrafloral integration, nectary-related (i.e. petal-related) and sepal-related traits should tend to vary as relatively independent suites of characters because of their origin from different floral whorls and distinct developmental origins. Alternatively, if correlational selection from pollinators has enhanced the functional integration of different floral whorls, it may be hypothesized that petal-related and sepal-related traits should vary more coordinately. The first alternative (developmental hypothesis) may be seen as a null-type hypothesis, while the second one (functional hypothesis) represents a pollinator-mediated adaptive hypothesis.

Dow & Cheverud's (1985) approach for comparing the observed correlation structure (\mathbf{R} matrix) with alternative hypothetical integration patterns was used to discriminate between the two possibilities (see also Cheverud *et al.*, 1989; Waitt & Levin, 1993; for details and applications). An hypothetical integration matrix corresponding to the expectation under the developmental hypothesis (\mathbf{T}_a matrix) was constructed by entering 1's at matrix cells corresponding to correlations among variables related to the same whorl-based subset (CLen, CWid and CApe, on one side; NLen and NApe on the other), and entering 0's at cells corresponding to correlations across the two sets. The hypothetical matrix under the functional hypothesis (\mathbf{T}_b matrix) was constructed by entering 1's at matrix positions corresponding to correlations within subsets, and 0.5's at positions corresponding to correlations between subsets. To determine which of the two hypotheses best fitted the observed data and whether their difference in fit was statistically significant, the difference matrix $\mathbf{Z} = \mathbf{T}_a - \mathbf{T}_b$ was obtained, and a matrix permutation test was

then performed to compare similarity in structure between **Z** and **R**. A significant positive matrix correlation would indicate that observations fit **T_a** better than **T_b**, while a significant negative matrix correlation would indicate that **T_b** fits significantly better than **T_a** (Cheverud *et al.*, 1989).

Pollinator similarity and geographical distance

The morphological diversity of local pollinator assemblages was assessed for the six populations with pollinator census data available. Coefficients of variation of pollinator body length and proboscis length were obtained for each site using the relative abundance of taxa as weighing factors. A similarity matrix between populations in pollinator composition was computed using Renkonen's percentage similarity index (Krebs, 1989) on percent flower visitation data. A similarity matrix in the pattern of floral integration was obtained using pairwise matrix correlations between populations (correlation coefficients between the corresponding elements of the two matrices; e.g. Merilä & Björklund, 1999). Statistical significance of the relationship between the two matrices was then evaluated using a permutation test (e.g. Smouse *et al.*, 1986; in this and all other instances of matrix permutation tests, 1000 repetitions were used).

Distance-dependent geographical patterns at the scale of the Iberian Peninsula were tested by correlating the matrix of geographical distances among populations with either similarity or distance matrices among populations for pollinator composition, average floral morphology, and pattern of floral integration. Statistical significance was tested using matrix permutation tests.

Results

Pollinators

The proportion of flower visits by different insect taxa at the six intensively studied populations are summarized in Table 1. Bumblebees contributed most visits at all populations. Anecdotal observations at the other three populations revealed that bumblebees were also the most frequent floral visitors. *Bombus pratorum* predominated at the two northernmost sites, whereas *B. terrestris* was dominant at the four southern ones. The pollinator assemblage was taxonomically most diverse at the northern localities where, in addition to bumblebees, *Anthophora acervorum* and honeybees contributed a significant proportion of visits. *Andrena* sp. and halictid bees surely played a minor role, if any at all, in the pollination of *H. foetidus* flowers, as these small-sized bees very rarely contacted the stigmas. Bumblebees, honeybees and *A. acervorum*, in contrast, almost invariably contacted anthers and stigmas of visited flowers.

There was a significant distance-dependent geographical pattern in the species composition of *H. foetidus*

pollinators at the scale of the Iberian Peninsula. The matrix of geographical distances between the six populations with pollinator census data was negatively correlated with the similarity matrix between these populations in pollinator composition ($r = -0.94$, $P = 0.005$, matrix permutation test).

Population means

The *H. foetidus* populations studied differed significantly in mean floral traits, both for traits considered individually (Fig. 3) and when all characters were treated simultaneously in a multivariate analysis of variance (Wilk's $\lambda = 0.128$, $F_{40,1550} = 23.4$, $P < 0.0001$). Variation among populations in mean floral morphology was not related significantly to either geographical location or pollinator composition. The matrix of Euclidean distances between multivariate population means (after standardization of original measurements to mean zero and variance unity to weigh all traits equally) was not significantly related to the matrices of geographical distances between populations or similarity in pollinator composition ($P = 0.72$ and 0.34 , respectively; matrix permutation tests).

Variation among population means was relatively minor in comparison with differences among individual means within populations. Ranges of population means for all characters were considerably narrower than the corresponding within-population ranges (Fig. 3). Furthermore, most variance in floral traits occurred within local populations. For all characters examined, the between-population variance component (range = 23–34% of total variance) was relatively unimportant in comparison with the within-population component (range = 66–77% of total variance).

There was almost no consistency among floral traits in the pattern of variation of their respective means across populations, as reflected by the extremely low Kendall coefficient of concordance of trait mean ranks across populations ($W = 0.017$, d.f. = 6, $P > 0.95$). Different characters varied in different ways among populations, as illustrated in Fig. 4 by the numerous crossing-overs of lines for different traits. Had all traits varied in unison across populations, all lines would have looked roughly parallel to each other.

Floral integration: magnitude

Phenotypic correlation matrices between floral characters for the nine populations are summarized graphically in Fig. 5. There was no indication of morphological tradeoffs between characters, as the vast majority of correlations were positive, and the few negative ones were nonsignificant and small in absolute value (Fig. 5). The strength of correlations varied widely for different character pairs. CLen and CWid, CLen and NLen, and CWid and CApe, tended

Table 1 Importance of different bee taxa as visitors of *Helleborus foetidus* flowers at six of the populations studied (see Fig. 2 for locations). Taxa marked with asterisks are unlikely to perform pollination, for reasons given in text. Data for years 1998 and 1999 combined, observation effort 59–66 h population⁻¹. *n* = Total number of flower visits recorded in each population.

Bee taxa	Mean body length (mm)	Mean proboscis length (mm)	Proportion of flower visits (%)					
			Population					
			1 (<i>n</i> = 494)	2 (<i>n</i> = 784)	5 (<i>n</i> = 225)	6 (<i>n</i> = 343)	7 (<i>n</i> = 17)	8 (<i>n</i> = 151)
<i>Andrena</i> sp.*	10.2	2.4	6.1	0.3	0.9	4.1	17.6	26.5
<i>Anthophora acervorum</i>	15.8	9.2	0.4	24.5	4.0	1.2		5.3
<i>Apis mellifera</i>	13.0	3.6	13.8	21.7				
<i>Bombus pascuorum</i>	16.9	8.6	3.6	3.1				
<i>Bombus pratorum</i>	17.3	8.6	54.9	45.9	13.8	12.0		
<i>Bombus terrestris</i>	17.8	7.1	21.3	4.5	74.2	80.7	70.6	66.2
<i>Halictidae</i> gen. sp.*	8.3	1.6					11.8	2.0
Other*					4.4	2.0		

*Unlikely to perform pollination.

consistently to be the three trait pairs most closely correlated at all populations. In general, there was considerable variation between populations in the magnitude of the correlation exhibited by any given character pair, as shown by the vertical spread of points in Fig. 5. For example, the ranges for the correlation coefficients CLen–CWide, CLen–NLen, and CWide–CApe were 0.23–0.91, 0.37–0.75 and 0.29–0.73, respectively. Similarly broad ranges are exhibited by other character pairs.

The five floral traits examined were significantly integrated at all populations (Table 2). The magnitude

of the integration, however, differed widely among populations, with INT values ranging between 0.28 and 1.53, or 5.5–30.7% of the maximum possible integration attainable for a five-character matrix ($INT_{max} = 5$). Differences between populations in the magnitude of integration were statistically significant, as revealed by the nonoverlapping confidence intervals of INT for populations in or near the lower and upper extremes of the range of integration values (Table 2).

In the subset of populations with pollinator census data, the magnitude of floral integration (as measured with INT; Table 2) was not significantly correlated across

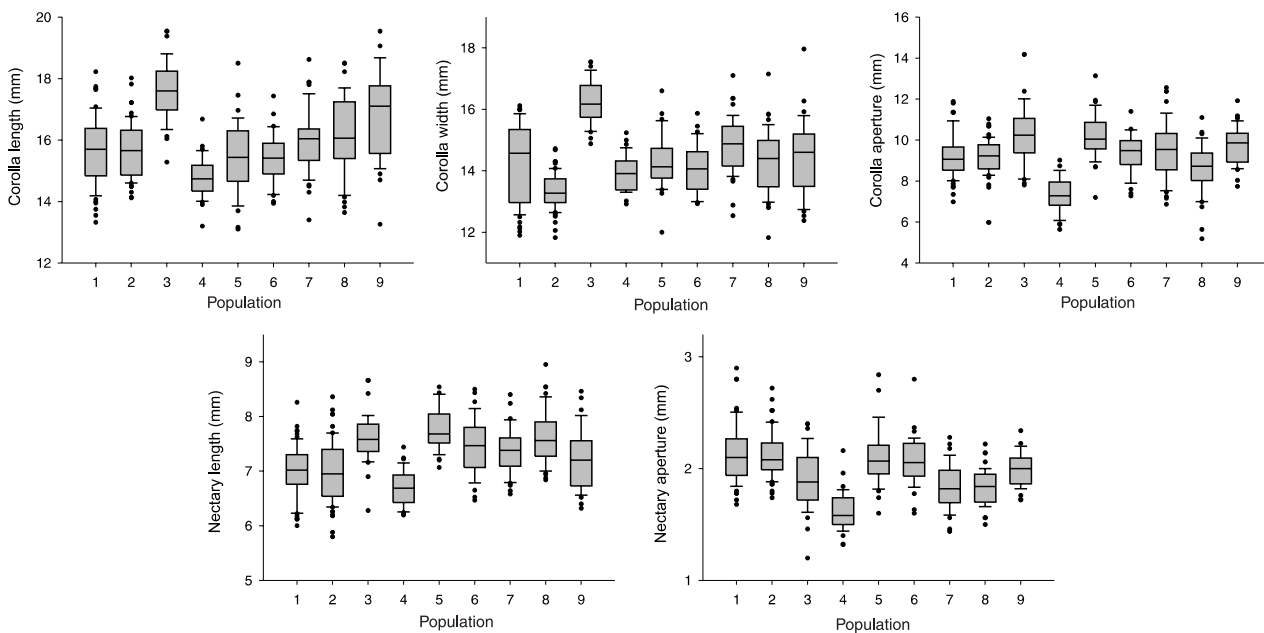


Fig. 3 Variation among *Helleborus foetidus* populations in individual plant means of the five floral traits considered. Box plots show the 10, 25, 75 and 90% percentiles of the distributions of plant means. Plant means beyond the 10–90% range are shown as dots. Univariate analyses of variance testing for heterogeneity among populations in trait means are all statistically significant ($F_{8,359} \geq 18.5$, $P < 0.0001$ in all cases). Refer to Fig. 2 for population locations and sample sizes.

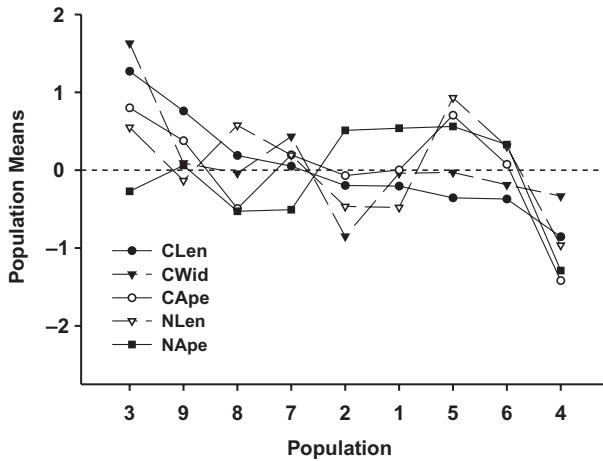


Fig. 4 Population means of standardized values of the five floral traits considered. Original values for each character were standardized to mean zero and variance unity to facilitate between-trait comparisons. Populations have been ranked on the horizontal axis in decreasing order of corolla length. Abbreviations used to denote floral traits as defined in Fig. 1.

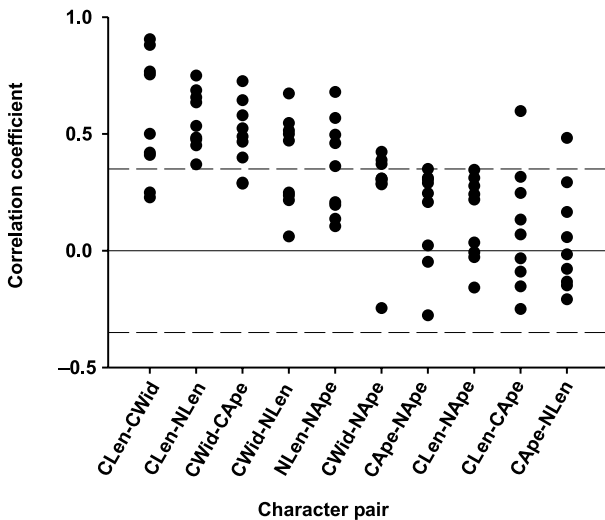


Fig. 5 The sign and magnitude of the correlations between floral traits (Pearson r) vary among populations and among character pairs. For each character pair, the different points shown correspond to the nine populations studied. Character pairs have been ranked in decreasing order of mean correlation coefficients. Dashed horizontal lines denote the critical values for correlation coefficients for $P < 0.05$ and $n = 30$. Abbreviations used to denote floral traits as defined in Fig. 1.

populations with the morphological diversity of pollinators in either body length ($r = 0.303$, $n = 6$, $P = 0.57$) or proboscis length ($r = 0.371$, $n = 6$, $P = 0.43$; P -values determined by randomization).

Table 2 Between-population variation in the extent of phenotypic integration among morphometric traits, as measured with the variance of eigenvalues of phenotypic correlation matrices. Populations are listed in increasing order of integration to facilitate comparisons.

Population	Integration index*	Standard error	95% Confidence interval
3	0.275	0.134	0.087–0.373
4	0.380	0.178	0.097–0.656
7	0.429	0.148	0.180–0.607
5	0.453	0.165	0.168–0.682
2	0.631	0.180	0.380–1.008
1	0.882	0.155	0.589–1.156
6	0.971	0.326	0.497–1.742
9	0.991	0.246	0.542–1.423
8	1.534	0.287	0.993–2.102

*Values shown are those obtained after adjusting for differences among populations in the number of plants entering in the computations of correlation matrices. Adjustment was made by subtracting from the empirically determined index the expected value under the assumption of complete independence among characters. Standard errors and confidence intervals were obtained by bootstrapping.

Floral integration: pattern

Developmental vs. functional hypotheses

For each population, we tested pattern similarity between the observed character correlation matrix and each of the two hypothetical matrices describing the developmental and functional hypotheses of floral integration (Table 3). Evidence favouring the developmental hypothesis was found for populations 1, 4 and 9, where the pattern of integration among floral traits was significantly (populations 4 and 9) or nearly significantly (population 1) closer to the expectation under the developmental than under the functional hypothesis ($r_{diff} > 0$). Neither the developmental nor the functional hypotheses were significantly supported in the remaining six populations, yet in all instances the comparison between predictions pointed consistently in the direction ‘developmental better than functional’ (i.e. $r_A > r_B$ and $r_{diff} > 0$).

Geographical variation

There was significant heterogeneity among populations in the structure of phenotypic covariance matrices of floral traits. The heteroscedastic model could not be rejected against the next model in the Flury’s hierarchy, namely one having at least one common principal component ($\chi^2_{32} = 85.2$, $P < 0.0001$). Furthermore, the likelihood ratio test for the comparison between the heteroscedastic and the equal correlation models provided strong evidence that the hypothesis of unrelated covariance matrices could not be rejected in favour of one assuming a common correlation structure ($\chi^2_{80} = 201.4$, $P < 0.0001$).

Table 3 Comparison of observed character correlation matrices with two different hypotheses of floral integration. The 'developmental hypothesis' (Hypothesis A) considers two groups of independently covarying traits defined on the basis of membership in floral whorls. The 'functional hypothesis' (Hypothesis B) postulates extensive integration among all traits. r_A , r_B and r_{diff} = element-wise Pearson correlation coefficients between the observed character correlation matrix (**R** matrix) and hypothetical matrices *A* and *B*, and their difference matrix (**Z** matrix), respectively. Statistical significance of the difference between hypotheses was tested by comparing **R** and **Z** matrices using permutation tests. See 'Methods: Statistical Analyses' for further details.

Population	Hypothesis A r_A	Hypothesis B r_B	Comparison between hypotheses:	
			r_{diff}	<i>P</i>
1	0.463	0.199	0.475	0.07
2	-0.119	-0.355	0.077	0.92
3	0.229	0.005	0.297	0.56
4	0.464	-0.205	0.743	0.03
5	-0.162	-0.421	0.063	0.74
6	0.368	0.072	0.434	0.15
7	0.167	-0.092	0.279	0.38
8	0.458	0.242	0.442	0.14
9	0.319	-0.334	0.636	0.02

Values in bold correspond to statistically significant or nearly significant tests.

We tested whether variation among populations in covariance structure was distance-dependent. As an index of similarity among populations in covariance structure we used the matrix correlation coefficient. All possible pairwise matrix correlation coefficients between populations were obtained, and then transformed to a Euclidean metric to reflect distances rather than similarity using Orloci's method (Sneath & Sokal, 1973, pp. 139–140). The resulting matrix was then related to the matrix of geographical distances using a matrix permutation test. The two matrices were not significantly related ($P = 0.55$).

In the subset of populations with pollinator census data, similarity between populations in pattern of floral integration (measured using the matrix correlation coefficient) and in pollinator composition (measured using Renkonen's percent similarity index on percent flower visitation data) were not significantly correlated ($P = 0.74$, matrix permutation test).

Discussion

Underlying assumptions

Expectations tested in this study were predicated on the assumption that phenotypic correlations between floral characters are an adequate representation of

underlying genetic correlations. This assumption is shared with previous studies that have examined multivariate patterns of phenotypic variation in plants from an evolutionary perspective (e.g. Schlichting, 1989; Waitt & Levin, 1993; Armbruster *et al.*, 1999). Phenotypic covariances may not be equivalent to genetic covariances (e.g. Kohn & Atchley, 1988; Willis *et al.*, 1991), but considerable evidence shows that they are typically similar, and that the phenotypic correlation may be a suitable substitute for the genetic correlation (reviews in Cheverud, 1988; Roff, 1995, 1996; Waitt & Levin, 1998). This seems to apply particularly to morphological data of the kind analysed here (Roff, 1996).

Waitt & Levin's (1998) recent review concluded that phenotypic correlations in plants are a good reflection of their genetic counterparts. As their analyses largely relied on pairs of genotypic and phenotypic correlation matrices from different species, their conclusions might not apply in intraspecific contexts. Nevertheless, the few studies in their review providing intraspecific replicates suggest that the relationship between genetic and phenotypic correlations also holds across populations and/or environments of a given species (see also Roff, 1995; for animals). Waitt and Levin's database was dominated by studies focusing on characters of agronomic importance, and included relatively few investigations examining floral characters. Separate consideration of these latter studies (Soltis, 1986; Harding *et al.*, 1990; Conner & Via, 1993; Mitchell & Shaw, 1993), along with others not included in their review (Campbell, 1996; Klinkhamer & van der Veen-van Wijk, 1999) indicates, however, that floral traits are not different from other plant characters with regard to the similarity of their genetic and phenotypic correlations, which provides justification for interpreting phenotypic correlations among floral characters in an evolutionary context.

Tests of expectations

Given the restrictive floral morphology of *H. foetidus*, its close dependence on a reduced set of pollinators, and the remarkable geographical constancy in pollinator composition, we expected to find that the floral phenotype of this species would exhibit little between-population variation in both mean values and patterns of multivariate covariation among floral characters, and that such variation could be explained by the relatively minor geographical differences in pollinator composition. Our results only partly support the expectation of relative geographical constancy in mean trait values, and clearly negate both the expected constancy in pattern and magnitude of character covariation, and the presumed relationship between pollinator composition and univariate and multivariate aspects of the floral phenotype.

Between-population variation in mean floral traits was statistically significant, but it was quantitatively minor and may be biologically irrelevant from the viewpoint of

the interaction between *H. foetidus* and its pollinators. First, most variance in floral traits occurred within local populations and was accounted for by individual differences and secondly, the range of variation of population means for most characters was narrower than the local ranges of variation in body size or proboscis length exhibited by *B. terrestris* and *B. pratorum*, the two main pollinators (C.M. Herrera, unpublished observations). From a biological viewpoint, the mean floral phenotype of *H. foetidus* may perhaps be considered as roughly constant over the Iberian Peninsula, with small local variations representing just random deviations around the mean with no particular adaptive significance in relation to pollinators. Consistent with this interpretation is the finding that the matrix of Euclidean distances between multivariate population means was not significantly related to either the geographical or pollinator composition distance matrices.

In contrast to the small between-population variation in mean floral phenotype, populations of *H. foetidus* did differ widely in both the magnitude and pattern of multivariate phenotypic integration of flowers, and these differences were unrelated to geographical location and to variation in composition or morphological diversity of pollinators. These results are discussed in the following sections.

Magnitude of integration

The magnitude of floral integration varied widely between populations, between 5 and 31% of the maximum possible. In those populations exhibiting the lowest floral integration, therefore, the floral characters considered varied nearly independently of each other across individual plants. Both the range of variation and the shape of the frequency distribution of floral integration values found in this study for our set of *H. foetidus* populations are remarkably similar to those obtained from the literature for 13 different species (Fig. 6). To our knowledge, no study has so far examined variation in the magnitude of phenotypic floral integration across natural populations of any plant species, thus it is not possible to decide whether our results for *H. foetidus* are representative of insect-pollinated plants in general. The degree of between-population variation in the magnitude of morphometric integration does seem to be broader than in some animal species. Merilä & Björklund (1999), for example, found that integration of metric skeletal traits ranged between 11 and 21% of maximum possible in European populations of the finch *Carduelis chloris* encompassing a much broader geographical range than that considered here for *H. foetidus*. Pending corroboration by future studies on other insect-pollinated plants, our findings serve at least to suggest that the assumption of intraspecific constancy in patterns of floral integration that is ordinarily implicit in interspecific comparisons may be unwarranted. Interspecific comparisons of floral

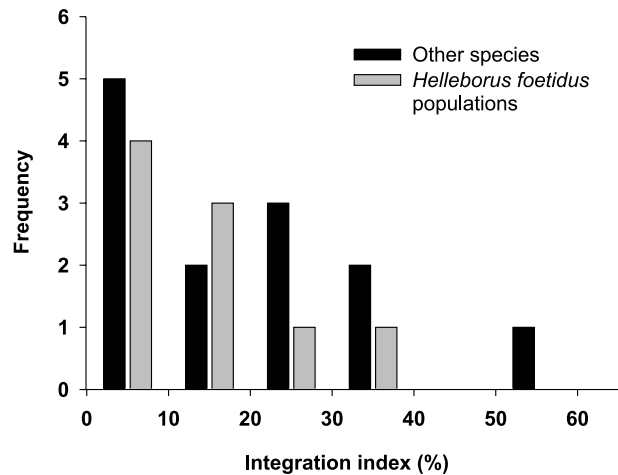


Fig. 6 A comparison of the frequency distributions of the phenotypic integration index for morphometric floral traits in the nine Iberian populations of *Helleborus foetidus* analysed in this study (shaded bars; values from Table 2) and in 13 other plant species (filled bars). The integration index was computed as the variance of the eigenvalues of the phenotypic correlation matrix, then corrected by subtracting the expected variance (a function of the number of traits and plants entering into the computation of the correlation matrix), and finally expressed as percent of the maximum possible value (number of traits considered). Figures for species other than *H. foetidus* were computed from phenotypic correlation matrices for morphometric floral traits in Harding *et al.* (1990), Conner & Via (1993), Mitchell & Shaw (1993), Campbell (1996), Armbruster *et al.* (1999), and Klinkhamer & van der Veen-van Wijk (1999).

integration based on single populations of each species may be subject to a significant chance component because of insufficient sampling of intraspecific variability. This may lead to distorted representations of interspecific differences, and contribute to explain the equivocal nature of some results obtained (e.g. Armbruster *et al.*, 1999).

Phenotypic covariance structure

Studies examining patterns of floral integration as a way of testing evolutionary hypotheses related to plant-pollinator interactions have been generally motivated by Berg's (1959, 1960) notion of correlation pleiades (see Armbruster *et al.*, 1999; for review). These investigations rely on the assumption that pollinators may select for particular character associations, and that variation in the magnitude and pattern of phenotypic integration of floral traits may reflect variation in the pollinator-mediated selective regime on floral traits. Although some studies have found support for the original Berg's prediction, other investigations have failed to verify it or some of its derived expectations. Armbruster *et al.* (1999) studied patterns of phenotypic variation and covariation of floral and vegetative traits in nine species of Neotropical plants,

and tested seven specific predictions of Berg's original hypothesis. Their results provided only equivocal support for the correlation pleiades concept. They concluded that the evolution of floral integration may be too complicated to allow broad generalizations, and that it may prove more profitable to make explicit predictions on a species-by-species basis that take into consideration relevant aspects of pollination ecology. By trying to relate intraspecific variation in floral integration to variation in pollinator composition, the present study has adopted such approach, yet our results provide no evidence of the presumed connection between floral integration and pollinators in *H. foetidus*. We found significant heterogeneity in the structure of phenotypic correlation matrices among populations in face of a relative constancy in pollinator composition, and such between-population variation was unrelated to either geographical location or variation in pollinator composition and morphology.

Inconsistency in phenotypic covariance structure comparable with that found here for *H. foetidus* flowers is not exceptional among animals. For example, Riska (1985) examined 34 local populations of the aphid *Pemphigus populicaulis* and found both a significant heterogeneity and an absence of geographical pattern in phenotypic correlation matrices of morphological variables. Stepan (1997a), studying a set of species, subspecies and populations of *Phyllotis* mice, likewise found statistically significant divergence in phenotypic covariance structure at all levels of the phylogeny. There is, however, little information on patterns of intraspecific variation in phenotypic integration and covariance structure in natural plant populations, as most studies have been conducted on single populations and/or have adopted univariate approaches. Pigliucci *et al.* (1991) found significant variation among populations of *Castanea sativa* in both the pattern and magnitude of integration of leaf and fruit traits.

Population-level differentiation in phenotypic covariance structure of floral traits in *H. foetidus* may be the result of different causes. One of these, sampling related error in the estimation of covariances, cannot be responsible for observed differentiation, as this is adequately accounted for in common principal components analyses (Stepan, 1997a). Among biological causes, heterogeneity of covariance structures may (1) mainly reflect geographical variation in environmental covariances, with the genetic covariance structure remaining essentially constant among populations; (2) actually reflect differentiation in genetic covariance structure, either because of changing selection by pollinators (2.1) or because of random genetic sampling in small populations (genetic drift; 2.2). Under (1) above, one would expect variation in phenotypic covariance structure to be distance-dependent, as major environmental sources of variation most likely are patterned geographically. Results are not consistent with this interpretation, as

phenotypic covariance structures are not predictably related to geographical location. It must be noted, however, that our distance-based test for the effect of environmental heterogeneity is admittedly crude, and there could still be environmental effects on phenotypic variances and covariances if the factors involved vary at small spatial scales. Interpretation (2.1) would be supported if either: (a) some relationship existed between variation in pollinator composition and covariance structure, which is not the case in our results; or (b) at a significant number of populations, patterns of integration were closer to those expected under the functional (pollinator-driven) than under the developmental hypothesis. This is clearly contradicted by the results, as the developmental hypothesis tended to explain integration patterns consistently better than the functional hypothesis. By exclusion, therefore, the genetic drift scenario (2.2) would thus seem the most likely one.

A genetic drift scenario was also suggested by Riska (1985) to explain the significant heterogeneity in phenotypic covariance structures in the aphid *Pemphigus populicaulis*, a species with which *H. foetidus* shares a significant number of life-history traits. Regional populations of *H. foetidus* are typically made up of disjunct local nuclei each with fewer than 100 reproductive individuals. These populations are subject to a marked dynamic of colonization, building up and extinction operating at a temporal scale of 10–20 years (C.M. Herrera & X. Cerdá, unpublished data). This local dynamics is probably nested within a similar one taking place at the regional level and a longer time scale. In the Sierra de Cazorla region, where our populations 5 and 6 are located, *H. foetidus* is currently a widespread species found in a broad range of elevations and habitat types, yet it was deemed a rare species 50 year ago (Heywood, 1962). Under these conditions of population instability, genetic sampling and associated genetic drift must probably be the rule, thus explaining unpatterned geographical variation in both character means and covariance structure (Riska, 1985; Stepan, 1997b). The virtually uncoupled variation of floral trait means across *H. foetidus* populations adds further support to the hypothesis conferring to genetic drift a prevailing role in population differentiation in phenotypic covariance structure. It must also be noted that attributing to genetic drift the differences in phenotypic correlations among *H. foetidus* populations is not at odds with what is known about patterns of intra- and interspecific variations of genetic correlations. For these, the results of Roff's (2000) survey suggest that most of the differentiation observed between populations may be the result of drift rather than selection.

Specialization and adaptedness

Recent studies indicate that generalization seems to be the rule in plant–animal pollinator systems, with most

plant species being serviced by taxonomically and morphologically diverse arrays of pollinators whose composition fluctuates in time and space (Herrera, 1988, 1996; Waser *et al.*, 1996). These plants can be at most only weakly adapted to each of their pollinators, which in turn suggests an association between generalization and weak adaptedness to particular pollinators in such pollination systems. Standing as conspicuous counterpoints to the prevailing trend of generalization among entomophilous plants, however, are notorious examples of 'specialized' species with morphologically very restrictive corollas that have a close dependence on just one or a few species of pollinators (e.g. Nilsson *et al.*, 1985; Herrera, 1993; Johnson, 1994; Johnson & Steiner, 1997). *Helleborus foetidus* clearly belongs to this minority category, as it is pollinated by a few species of insects whose composition varies remarkably little among populations and years. Spatio-temporal consistency in pollinator composition extends well beyond the Iberian Peninsula. In the British Isles, the main pollinators are also bumblebees, honeybees and *Anthophora* bees (Proctor & Yeo, 1973), and bumblebees (including *B. terrestris*) are also the main pollinators in central Italy (Vesprini *et al.*, 1999). This low diversity of pollinators over most of the species range confirms our interpretation that it is a species-specific feature related to the restrictive morphology of flowers. The latter is also a species-specific distinguishing feature which contrasts sharply with the open corollas and exposed nectaries of the majority of *Helleborus* species (Mathew, 1989; Rice & Strangman, 1993), accessible to a broad variety of insect pollinators (Vesprini *et al.*, 1999). The closed flowers and hidden nectaries of *H. foetidus* most likely are a derived feature in the genus *Helleborus*, as phylogenetically closely related genera (*Nigella*, *Eranthis*; Ro *et al.*, 1997) have, like the majority of *Helleborus* species, open flowers with exposed nectaries.

All else being equal, it is among specialized (as used here) plant-pollinator systems where consistent selective pressures from only one or a few pollinators on plants, and thus pollinator-driven character evolution (including character variances and covariances), should most likely occur (Stebbins, 1974; Fenster, 1991; Herrera, 1996; Armbruster *et al.*, 1999). Results of this study, however, tend to suggest that specialization does not necessarily imply that patterns of within- and between-population variation in floral phenotypes can be explained by the selective action of current pollinators. This result is similar to those of Herrera (1993) for *Viola cazorlensis*, an endemic violet with a very long spur and pollinated by a single hawkmoth species. Although the nearly perfect matching between mean hawkmoth tongue length and mean spur length were suggestive of close floral adaptation to the species single pollinator, spur length was the only floral trait for which no evidence of phenotypic selection was found. Taken together, these findings suggest that a combination of specialized floral morphology with close dependence on a reduced set of

pollinators is not a safe indication that selection by current pollinators has been decisive in shaping floral phenotypes.

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References

- Andersson, S. 1993. Population differentiation in *Crepis tectorum* (Asteraceae): patterns of correlation among characters. *Biol. J. Linn. Soc.* **49**: 185–194.
- Andersson, S. 1994. Floral stability, pollination efficiency, and experimental manipulation of the corolla phenotype in *Nemophila menziesii* (Hydrophyllaceae). *Am. J. Bot.* **81**: 1397–1402.
- Andersson, S. 1997. Genetic constraints on phenotypic evolution in *Nigella* (Ranunculaceae). *Biol. J. Linn. Soc.* **62**: 519–532.
- Andersson, S. & Widén, B. 1993. Pollinator-mediated selection on floral traits in a synthetic population of *Senecio integrifolius* (Asteraceae). *Oikos* **66**: 72–79.
- Armbruster, W.S. 1985. Patterns of character divergence and the evolution of reproductive ecotypes of *Dalechampia scandens* (Euphorbiaceae). *Evolution* **39**: 733–752.
- Armbruster, W.S. 1988. Multilevel comparative analysis of the morphology, function, and evolution of *Dalechampia* blossoms. *Ecology* **69**: 1746–1761.
- Armbruster, W.S. 1991. Multilevel analysis of morphometric data from natural plant populations: insights into ontogenetic, genetic, and selective correlations in *Dalechampia scandens*. *Evolution* **45**: 1229–1244.
- Armbruster, W.S., di Stilio, V.S., Tuxill, J.D., Flores, T.C. & Velásquez Runk, J.L. 1999. Covariance and decoupling of floral and vegetative traits in nine Neotropical plants: a re-evaluation of Berg's correlation–pleiades concept. *Am. J. Bot.* **86**: 39–55.
- Armbruster, W.S. & Schwaegerle, K.E. 1996. Causes of covariation of phenotypic traits among populations. *J. Evol. Biol.* **9**: 261–276.
- Berg, R.L. 1959. A general evolutionary principle underlying the origin of developmental homeostasis. *Am. Nat.* **93**: 103–105.
- Berg, R.L. 1960. The ecological significance of correlation pleiades. *Evolution* **14**: 171–180.
- Björklund, M. & Merilä, J. 1993. Morphological differentiation in *Carduelis* finches: adaptive vs. constraint models. *J. Evol. Biol.* **6**: 359–373.
- Campbell, D.R. 1996. Evolution of floral traits in a hermaphroditic plant: field measurements of heritabilities and genetic correlations. *Evolution* **50**: 1442–1453.

- Campbell, D.R., Waser, N.M. & Price, M.V. 1996. Mechanisms of hummingbird-mediated selection for flower width in *Ipomopsis aggregata*. *Ecology* **77**: 1463–1472.
- Campbell, D.R., Waser, N.M., Price, M.V., Lynch, E.A. & Mitchell, R.J. 1991. Components of phenotypic selection: pollen export and flower corolla width in *Ipomopsis aggregata*. *Evolution* **45**: 1458–1467.
- Cheverud, J.M. 1988. A comparison of genetic and phenotypic correlations. *Evolution* **42**: 958–968.
- Cheverud, J.M., Wagner, G.P. & Dow, M.M. 1989. Methods for the comparative analysis of variation patterns. *Syst. Zool.* **38**: 201–213.
- Conner, J.K., Davis, R. & Rush, S. 1995. The effect of wild radish floral morphology on pollination efficiency by four taxa of pollinators. *Oecologia* **104**: 234–245.
- Conner, J.K. & Sterling, A. 1995. Testing hypotheses of functional relationships: a comparative survey of correlation patterns among floral traits in five insect-pollinated plants. *Am. J. Bot.* **82**: 1399–1406.
- Conner, J.K. & Sterling, A. 1996. Selection for independence of floral and vegetative traits: evidence from correlation patterns in five species. *Can. J. Bot.* **74**: 642–644.
- Conner, J. & Via, S. 1993. Patterns of phenotypic and genetic correlations among morphological and life-history traits in wild radish, *Raphanus raphanistrum*. *Evolution* **47**: 704–711.
- Cresswell, J.E. 1998. Stabilizing selection and the structural variability of flowers within species. *Ann. Bot.* **81**: 463–473.
- Darwin, C. 1862. *On the Various Contrivances by Which British and Foreign Orchids are Fertilised by Insects*. Murray, London.
- Dow, M.M. & Cheverud, J.M. 1985. Comparison of distance matrices in studies of population structure and genetic microdifferentiation: quadratic assignment. *Am. J. Phys. Anthropol.* **68**: 367–374.
- Efron, B. & Tibshirani, R.J. 1993. *An Introduction to the Bootstrap*. Chapman & Hall, New York.
- Endler, J.A. 1986. *Natural Selection in the Wild*. Princeton University Press, Princeton.
- Endler, J.A. 1995. Multiple-trait coevolution and environmental gradients in guppies. *Trends Ecol. Evol.* **10**: 22–29.
- Falconer, D.S. 1989. *Introduction to Quantitative Genetics*, 3rd edn. Longman, Harlow, UK.
- Fenster, C.B. 1991. Selection on floral morphology by hummingbirds. *Biotropica* **23**: 98–101.
- Flury, B. 1988. *Common Principal Components and Related Multivariate Models*. Wiley, New York, NY.
- Galen, C. 1996. The evolution of floral form: insights from an alpine wildflower, *Polemonium viscosum* (Polemoniaceae). In: *Floral Biology. Studies on Floral Evolution in Animal-Pollinated Plants* (D.G. Lloyd & S.C.H. Barrett, eds), pp. 273–291. Chapman & Hall, New York.
- Gómez, J.M. 2000. Phenotypic selection and response to selection in *Lobularia maritima*: importance of direct and correlational components of natural selection. *J. Evol. Biol.* **13**: 689–699.
- Grant, V. 1975. *The Genetics of Flowering Plants*. Columbia University Press, New York.
- Hansen, T.F., Armbruster, W.S. & Antonsen, L. 2000. Comparative analysis of character displacement and spatial adaptations as illustrated by the evolution of *Dalechampia* blossoms. *Am. Nat.* **156**: S17–S34.
- Harding, J., Huang, H., Byrne, T. & Huang, N. 1990. Quantitative analysis of correlations among flower traits in *Gerbera hybrida* Compositae. 1. Genetic and environmental correlations. *Theor. Appl. Genet.* **80**: 552–558.
- Herrera, C.M. 1987. Components of pollinator 'quality': comparative analysis of a diverse insect assemblage. *Oikos* **50**: 79–90.
- Herrera, C.M. 1988. Variation in mutualisms: the spatio-temporal mosaic of an insect pollinator assemblage. *Biol. J. Linn. Soc.* **35**: 95–125.
- Herrera, C.M. 1993. Selection on floral morphology and environmental determinants of fecundity in a hawk moth-pollinated violet. *Ecol. Monogr.* **63**: 251–275.
- Herrera, C.M. 1996. Floral traits and plant adaptation to insect pollinators: a devil's advocate approach. In: *Floral Biology. Studies on Floral Evolution in Animal-Pollinated Plants* (D.G. Lloyd & S.C.H. Barrett, eds), pp. 65–87. Chapman & Hall, New York.
- Herrera, C.M. 2001. Deconstructing a floral phenotype: do pollinators select for corolla integration in *Lavandula latifolia*? *J. Evol. Biol.* **14**: 574–584.
- Herrera, C.M., Sánchez-Lafuente, A., Medrano, M., Guitián, J., Cerdá, X. & Rey, P.J. 2001. Geographical variation in autonomous self-pollination levels unrelated to pollinator service in *Helleborus foetidus* (Ranunculaceae). *Am. J. Bot.* **88**: 1025–1032.
- Herrera, C.M. & Soriguer, R.C. 1983. Intra- and inter-floral heterogeneity of nectar production in *Helleborus foetidus* L. (Ranunculaceae). *Bot. J. Linn. Soc.* **86**: 253–260.
- Heywood, V.H. 1962. The flora of the Sierra de Cazorla, S.E. Spain. I. *Feddes Rep.* **64**: 28–73.
- Johnson, S.D. 1994. Evidence for Batesian mimicry in a butterfly-pollinated orchid. *Biol. J. Linn. Soc.* **53**: 91–104.
- Johnson, S.D. 1997. Pollination ecotypes of *Satyrium hallackii* (Orchidaceae) in South Africa. *Bot. J. Linn. Soc.* **123**: 225–235.
- Johnson, S.D. & Steiner, K.E. 1997. Long-tongued fly pollination and evolution of floral spur length in the *Disa dracornis* complex (Orchidaceae). *Evolution* **51**: 45–53.
- Klinkhamer, P.G.L. & van der Veen-van Wijk, C.A.M. 1999. Genetic variation in floral traits of *Echium vulgare*. *Oikos* **85**: 515–522.
- Kohn, L.A.P. & Atchley, W.R. 1988. How similar are genetic correlation structures? Data from mice and rats. *Evolution* **42**: 467–481.
- Krebs, C.J. 1989. *Ecological Methodology*. Harper & Row, New York.
- Lande, R. 1980. The genetic covariance between characters maintained by pleiotropic mutations. *Genetics* **94**: 203–215.
- Lande, R. & Arnold, S.J. 1983. The measurement of selection on correlated characters. *Evolution* **37**: 1210–1226.
- Maad, J. 2000. Phenotypic selection in hawkmoth-pollinated *Platanthera bifolia*: targets and fitness surfaces. *Evolution* **54**: 112–123.
- Mathew, B. 1989. *Hellebores*. Alpine Garden Society, St John's Woking, UK.
- Mathsoft. 1999. *S-Plus 2000 Guide to Statistics*, Vol. 2. MathSoft, Seattle, WA.
- Merilä, J. & Björklund, M. 1999. Population divergence and morphometric integration in the greenfinch (*Carduelis chloris*) – evolution against the trajectory of least resistance? *J. Evol. Biol.* **12**: 103–112.
- Mitchell, R.J. & Shaw, R.G. 1993. Heritability of floral traits for the perennial wild flower *Penstemon centranthifolius* (Scrophulariaceae): clones and crosses. *Heredity* **71**: 185–192.

- Møller, A.P. & Eriksson, M. 1995. Pollinator preference for symmetrical flowers and sexual selection in plants. *Oikos* **73**: 15–22.
- Nilsson, L.A., Jonsson, L., Rason, L. & Randrianjohany, E. 1985. Monophily and pollination mechanisms in *Angraecum arachnites* Schltr. (Orchidaceae) in a guild of long-tongued hawkmoths (Sphingidae) in Madagascar. *Biol. J. Linn. Soc.* **26**: 1–19.
- O'Connell, L.M. & Johnston, M.O. 1998. Male and female pollination success in a deceptive orchid, a selection study. *Ecology* **79**: 1246–1260.
- Phillips, P.C. & Arnold, S.J. 1989. Visualizing multivariate selection. *Evolution* **43**: 1209–1222.
- Phillips, P.C. & Arnold, S.J. 1999. Hierarchical comparison of genetic variance-covariance matrices. I. Using the Flury hierarchy. *Evolution* **53**: 1506–1515.
- Pigliucci, M., Paoletti, C., Fineschi, S. & Malvolti, M.E. 1991. Phenotypic integration in chestnut (*Castanea sativa*): leaves versus fruits. *Bot. Gaz.* **152**: 514–521.
- Proctor, M. & Yeo, P. 1973. *The Pollination of Flowers*. Collins, London.
- Rice, G. & Strangman, E. 1993. *The Gardener's Guide to Growing Hellebores*. Timber Press, Portland, OR.
- Riska, B. 1985. Group size factors and geographic variation on morphometric correlation. *Evolution* **39**: 792–803.
- Ro, K.E., Keener, C.S. & McPherson, B.A. 1997. Molecular phylogenetic study of the Ranunculaceae: utility of the nuclear 26S ribosomal DNA in inferring intrafamilial relationships. *Mol. Phylogenet. Evol.* **8**: 117–127.
- Robertson, J.L. & Wyatt, R. 1990. Evidence for pollination ecotypes in the yellow-fringed orchid, *Platanthera ciliaris*. *Evolution* **44**: 121–133.
- Roff, D. 1995. The estimation of genetic correlations from phenotypic correlations: a test of Cheverud's conjecture. *Heredity* **74**: 481–490.
- Roff, D. 1996. The evolution of genetic correlations: an analysis of patterns. *Evolution* **50**: 1392–1403.
- Roff, D. 2000. The evolution of the G matrix: selection or drift? *Heredity* **84**: 135–142.
- SAS Institute 1990. *SAS/STAT User's Guide*, Version 6, 4th edn. SAS Institute, Cary, NC.
- Schlichting, C.D. 1989. Phenotypic plasticity in *Phlox*. II. Plasticity of character correlations. *Oecologia* **78**: 496–501.
- Smouse, P.E., Long, J.C. & Sokal, R.R. 1986. Multiple regression and correlation extensions of the Mantel test of matrix correspondence. *Syst. Zool.* **35**: 627–632.
- Sneath, P.H.A. & Sokal, R.R. 1973. *Numerical Taxonomy*. Freeman, San Francisco.
- Soltis, P.S. 1986. Estimates of heritability and correlations of morphometric traits in *Clarkia* (Onagraceae). *Theor. Appl. Genet.* **73**: 88–93.
- Stanton, M. & Young, H.J. 1994. Selecting for floral character associations in wild radish, *Raphanus sativus* L. *J. Evol. Biol.* **7**: 271–285.
- Stebbins, G.L. 1974. *Flowering Plants. Evolution Above the Species Level*. Belknap Press, Cambridge, MA.
- Steppan, S.J. 1997a. Phylogenetic analysis of phenotypic covariance structure. I. Contrasting results from matrix correlation and common principal component analyses. *Evolution* **51**: 571–586.
- Steppan, S.J. 1997b. Phylogenetic analysis of phenotypic covariance structure. II. Reconstructing matrix evolution. *Evolution* **51**: 587–594.
- Tamura, M. 1993. Ranunculaceae. In: *The Families and Genera of Vascular Plants. II. Dicotyledons* (K. Kubitzki, J.G. Rower & V. Bittrich, eds), pp. 563–583. Springer, New York.
- Vesprini, J.L., Nepi, M. & Pacini, E. 1999. Nectary structure, nectar secretion patterns and nectar composition in two *Helleborus* species. *Plant Biol.* **1**: 560–568.
- Wagner, G.P. 1984. On the eigenvalue distribution of genetic and phenotypic dispersion matrices: evidence for a nonrandom organization of quantitative character variation. *J. Math. Biol.* **21**: 77–95.
- Waitt, D.E. & Levin, D.A. 1993. Phenotypic integration and plastic correlations in *Phlox drummondii* (Polemoniaceae). *Am. J. Bot.* **80**: 1224–1233.
- Waitt, D.E. & Levin, D.A. 1998. Genetic and phenotypic correlations in plants: a botanical test of Cheverud's conjecture. *Heredity* **80**: 310–319.
- Waser, N.M., Chittka, L., Price, M.V., Williams, N.M. & Ollerton, J. 1996. Generalization in pollination systems, and why it matters. *Ecology* **77**: 1043–1060.
- Werner, K. & Ebel, F. 1994. Zur Lebensgeschichte der Gattung *Helleborus* L. (Ranunculaceae). *Flora* **189**: 97–130.
- Willis, J.H., Coyne, J.A. & Kirkpatrick, M. 1991. Can one predict the evolution of quantitative characters without genetics? *Evolution* **45**: 441–444.
- Wilson, P. & Thomson, J.D. 1991. Heterogeneity among floral visitors leads to discordance between removal and deposition of pollen. *Ecology* **72**: 1503–1507.
- Wilson, P. & Thomson, J.D. 1996. How do flowers diverge? In: *Floral Biology. Studies on Floral Evolution in Animal-Pollinated Plants* (D.G. Lloyd & S.C.H. Barrett, eds), pp. 88–111. Chapman & Hall, New York.
- Wolfe, L.M. & Krstolic, J.L. 1999. Floral symmetry and its influence on variance in flower size. *Am. Nat.* **154**: 484–488.

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