

The adaptedness of the floral phenotype in a relict endemic, hawkmoth-pollinated violet. 2. Patterns of variation among disjunct populations

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This study examines patterns of variation in quantitative floral traits among 18 disjunct populations of *Viola cazortensis* (Violaceae), a relict endemic violet of south-eastern Spain. At all sites, the species is almost exclusively pollinated by a single species of day-flying hawkmoth. Differences between populations were significant for all traits examined, and population means exhibit a broad range of variation. When all characters are considered together, each population displays a unique combination of characters. Despite interpopulation differences in character means, local populations retain most of the variability of the entire species. Floral traits do not vary in unison among flowers, and at least four different subsets of independently varying traits are identifiable. Floral similarity between populations of *V. cazortensis* was largely unrelated to geographic proximity, as revealed by analyses at both large and small geographic scales. The geographic pattern of floral variation among populations represents a random patchwork, with unique combinations of character means occurring randomly across the study region. Marked population differences in quantitative floral traits in spite of spatial constancy in the identity of pollinators, a disintegrated floral phenotype, and prevalingly random geographic variation among populations, suggest low adaptedness of the floral phenotype of *V. cazortensis* to its current hawkmoth pollinators.

KEY WORDS:—Endemism – floral morphology – geographic variation – pollination – quantitative floral variation – subdivided populations –Violaceae.

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INTRODUCTION

Diversification in floral morphology runs parallel to the radiation of angiosperms, and this effect is particularly pronounced in predominantly

animal-pollinated lineages. Among these, floral characters contribute to species separation much more often than among wind- and water-pollinated taxa (Grant, 1949). This fact, among others, lends support to the universally accepted notion that, among animal-pollinated plants, floral morphology has evolved in close connection with the activity of their pollinating agents (Leppik, 1957; Stebbins, 1970; Faegri & van der Pijl, 1971).

Studies directly examining the adaptive significance of floral traits are, however, comparatively scarce (see Waser, 1983 for review). One possible approach to test the adaptiveness of floral traits is to ask if individual variation translates into differential reproductive success (Harding, 1970; Waser & Price, 1981; Thomson & Stratton, 1985; Stanton, Snow & Handel, 1986; Stanton & Preston, 1988; Nilsson, 1988; Galen & Newport, 1988; Herrera, 1990b). On the other hand, where significant floral variation occurs between populations, a further approach would consist of examining whether this variation correlates with changes in the identity of pollinators. This approach has been less commonly undertaken (but see e.g. Miller, 1981; Armbruster, 1985), although studies of geographic variation in floral traits are relatively numerous (Mogford, 1974; Ritland & Jain, 1984; Crawford & Jones, 1988; Philbrick & Bogle, 1988). Regardless of the approach chosen, most investigations on floral variation have dealt with discontinuously varying floral traits in polymorphic species (e.g. colour, scent; Kay, 1976; Hannan, 1981; Galen, 1985; Schoen & Clegg, 1985; Galen, Zimmer & Newport, 1987; Abbott & Irwin, 1988; Galen & Newport, 1988), while relatively few studies have examined the significance of continuously varying characters (see references in Herrera, 1990b). This contrasts with the proportional occurrence in nature of these two types of intraspecific floral variation, as polymorphisms are comparatively scarce in relation to quantitative variation.

The present study examines patterns of variation in quantitative floral traits among disjunct populations of *Viola cazortensis* (Violaceae), a perennial, suffruticose violet endemic to two adjacent limestone mountain ranges in south-eastern Spain (Melchior & Cuatrecasas, 1935). This species is characterized by broad continuous variation in the size and proportions of floral parts (Herrera, 1988). It occurs as small, discrete populations, almost invariably associated with scattered rocky outcrops, cliffs, and pockets of sandy soils originating from heavily weathered limestone on mountain ridges (Herrera, 1988, 1990a). Populations comprise from a few dozen to a few thousand plants, and are usually separated by several kilometres of unsuitable habitat (various types of coniferous and mixed forest). The flowers have pinkish-purple corollas, and are characterized by a long and thin spur (length up to 37 mm, mean = 25.1 mm; Herrera, 1988), the longest of all European species in its genus. A single species of long-tongued, day-flying hawkmoth (*Macroglossum stellatarum*, Sphingidae) is its almost exclusive pollinator at all populations studied to date (Herrera, 1988, 1990b, unpublished observations). Hawkmoth pollination is an unusual feature for the genus *Viola* (Beattie, 1974) and, in the case of *V. cazortensis*, this pollination system is coupled with an extremely narrow range of pollinator taxa (virtually a single species).

In the face of this uniformity of pollinators and thus, presumably, of potential selective pressures on floral morphology, the present study addresses the following questions. (1) Are disjunct populations of *V. cazortensis* homogeneous in

floral morphology? (2) If they are not how is variance in floral morphology partitioned within and between populations? (3) Do floral traits vary independently or are they organized into co-ordinately varying subsets? (4) Is there any distinct geographic pattern of local differentiation in floral morphology? Answers to these questions are pertinent to the more general one, (5) to what degree may the broad range of floral morphologies found in this species be explained in the context of its relationship with pollinators?

STUDY AREA AND METHODS

During May-June 1988, floral characteristics were examined in 18 populations of *Viola cazorlensis* from the Sierra de Cazorla, Jaén province, south-eastern Spain (see Appendix for location and elevation of populations). Populations studied ranged in size from a few dozen to a few thousand plants, and were distributed over an area of approximately 16 × 30 km, at elevations between 850–1860 m above sea level. Although some small populations may have been missed, I am confident that most populations of the species occurring in the study area were found and their flowers sampled.

Thirty flowers were collected from each population, taking a single flower from each of 30 randomly chosen plants. Flowers were held in sealed glass containers and kept in a refrigerator until measured, usually within 5 h of collection. Measurements taken (Fig. 1) included the length of the peduncle, spur (straightened, E in Fig. 1), and the upper, middle and lower petal blades ('anterior', 'lateral' and 'posterior' petals, respectively, in Beattie's (1969)

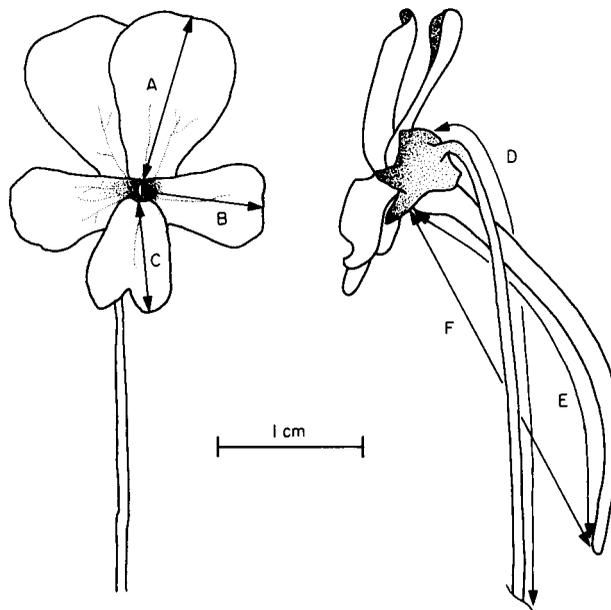


Figure 1. Front and side views of a *Viola cazorlensis* flower, to illustrate some of the measurements used. A, B and C, Upper, lateral and lower petal length, respectively. D, Peduncle length. E, Spur length (straightened). F, Spur chord length. An index of spur curvature was obtained by combining measures E and F (see text). In natural conditions, the spur is visible in front view, but it is omitted here for simplicity.

terminology). The distance between the tip and the base of the spur (chord length, F in Fig. 1) was also measured, and used in conjunction with spur length to obtain an index of spur curvature by means of the expression $(E-F)/E$. This index, that ranges from 0 (straight spur) to 1 (circular spur, with tip touching the base), was transformed with arcsin for all analyses.

Viola cazortensis flowers have two long filiform nectaries originating from the posterior staminal filaments and extending back into the spur. The length of the longest of these nectaries was also measured. For each flower, the ovary was examined under a dissecting microscope and the number of ovules counted.

Analytical methods used are described below.

RESULTS

Variation among populations

Populations differ significantly in the mean values of all floral traits examined, as revealed by univariate analyses of variance (Table 1). The within-population component represents 63.2–94.5% of the total estimated variance of individual characters in the pooled sample for all populations. Peduncle length was the trait proportionately exhibiting most variation between populations (36.8% of total variance), and ovule number the least (5.5%).

There are significant differences between populations in the position of their centroids in the multivariate morphospace defined by floral traits (Wilks' $\lambda = 0.288$, $F(136,3483) = 4.77$, $P < < 0.0001$; multivariate analysis of variance). The contribution of floral traits to population differences in the multivariate morphospace was assessed using multiple discriminant analysis. Only the first three of the eight canonical axes provided significant discrimination, and summarized 86.3% of the differences between groups (individual contributions of the first three axes were 55.7%, 17.4% and 13.2%). The variance attributable to the five remaining canonical axes (4 through 8) was not significant ($\chi^2 = 61.0$, d.f. = 52, $P = 0.18$; Bartlett's test).

Standardized canonical discriminant function coefficients on axes 1–3 are shown in Table 2. The unlike signs of coefficients on the same axis denote that differences between populations depend more on 'shape' than 'size' relationships (Pimentel, 1979). In particular, the contrasting signs of the coefficients for the three petal dimensions on the same axis indicate that variation in the degree of symmetry of the corolla is important to discriminate between populations.

The distribution of the centroids of populations in the space defined by the three canonical axes is shown in Fig. 2. There is no apparent clustering or arrangement of populations along simple morphological gradients. Most possibilities of morphological variation hypothetically possible along the three canonical axes actually occur in the set of populations studied.

Covariation of characters

In this section, character correlation matrices are first tested for homogeneity among populations. Then, factor analysis of the pooled character correlation matrix is used to identify independent, orthogonal trends of variation that may be interpreted as covarying subsets of floral characters.

TABLE 1. Average values (sd in parentheses) of floral traits for the 18 populations of *Viola cazortensis* studied. Values for spur curvature were obtained by back-transforming mean and sd figures for arcsin-transformed data (see text). Range and CV, range and coefficient of variation of population means. S_A and S_W , among-locality and within-locality variance estimates expressed as percent of total variance estimate. F , F -ratio statistic from univariate analyses of variance. $N = 30$ flowers for each site

Population	Peduncle length (mm)	Spur length (mm)	Spur curvature (%)	Upper petal length (mm)	Middle petal length (mm)	Lower petal length (mm)	Nectary length (mm)	Number of ovules
1	38.7 (9.0)	24.5 (4.5)	7.8 (4.1)	11.2 (2.0)	9.4 (1.4)	7.6 (1.2)	17.8 (3.4)	27.5 (4.8)
2	43.8 (9.2)	26.1 (3.8)	1.9 (1.9)	12.8 (1.7)	10.2 (1.6)	8.4 (1.5)	15.7 (3.3)	21.6 (6.2)
3	52.4 (10.6)	29.6 (5.1)	4.0 (1.3)	13.3 (1.7)	10.7 (1.5)	8.7 (1.5)	13.4 (5.8)	26.9 (6.0)
4	30.0 (5.9)	24.1 (3.4)	3.3 (1.7)	10.8 (1.3)	8.9 (1.1)	7.5 (1.2)	17.3 (2.8)	27.7 (6.8)
5	39.3 (12.3)	23.9 (4.3)	8.0 (2.1)	11.2 (1.3)	9.3 (1.1)	7.8 (1.1)	17.5 (3.4)	26.4 (6.0)
6	46.0 (10.1)	26.0 (3.5)	7.5 (3.4)	11.6 (1.6)	9.5 (1.2)	7.9 (1.0)	16.9 (3.7)	27.6 (5.3)
7	43.2 (10.0)	25.2 (3.9)	3.7 (0.8)	12.0 (1.5)	9.8 (1.3)	7.8 (1.1)	16.6 (4.4)	25.4 (7.7)
8	54.4 (12.9)	27.6 (5.0)	4.2 (1.8)	12.4 (1.7)	10.1 (1.2)	8.3 (1.3)	19.7 (4.3)	26.5 (8.2)
9	58.7 (11.1)	27.1 (4.2)	10.3 (6.8)	12.6 (1.5)	10.0 (1.3)	8.4 (1.2)	15.4 (3.6)	25.8 (7.3)
10	40.4 (10.4)	28.0 (4.9)	7.8 (2.6)	12.1 (1.7)	9.5 (1.2)	7.9 (1.2)	17.6 (2.7)	26.9 (5.7)
11	45.6 (10.3)	25.4 (3.7)	4.5 (1.3)	11.2 (1.4)	9.2 (1.0)	7.5 (0.8)	17.8 (3.7)	25.3 (7.7)
12	36.5 (9.2)	24.3 (3.7)	3.8 (2.3)	11.3 (1.6)	9.5 (1.1)	8.0 (1.2)	16.5 (3.7)	27.8 (6.1)
13	39.0 (6.7)	23.7 (3.3)	4.7 (1.7)	11.1 (1.5)	9.1 (1.4)	7.6 (1.2)	17.4 (2.1)	27.6 (6.2)
14	32.5 (7.8)	23.2 (4.0)	7.5 (2.5)	10.6 (1.8)	9.3 (1.3)	8.0 (1.1)	16.6 (3.4)	28.4 (6.2)
15	38.4 (7.7)	24.6 (3.8)	2.5 (1.4)	11.5 (1.6)	9.6 (1.3)	8.0 (1.2)	17.9 (2.9)	26.7 (5.0)
16	43.5 (8.4)	26.0 (4.8)	11.0 (5.5)	11.8 (2.2)	9.6 (1.7)	8.1 (1.5)	17.0 (4.7)	27.9 (5.8)
17	44.2 (8.0)	27.0 (3.8)	2.1 (1.9)	12.1 (1.4)	9.7 (1.3)	7.7 (1.2)	17.7 (3.3)	25.3 (7.3)
18	47.6 (9.4)	25.5 (3.1)	4.1 (2.1)	11.7 (1.5)	9.3 (1.0)	7.7 (1.0)	17.5 (2.2)	27.7 (7.4)
Range	30.0-58.7	23.2-29.6	1.9-11.0	10.6-13.3	8.9-10.7	7.5-8.7	13.4-19.7	21.6-28.4
CV (%)	16.9	6.6	26.6	6.3	4.6	4.1	7.6	5.9
S_A (%)	36.8	14.7	13.6	17.1	10.4	6.9	11.2	5.5
S_W (%)	63.2	85.3	86.4	82.9	89.6	93.1	88.8	94.5
F	17.1***	4.8***	4.6***	5.7***	3.1***	2.0**	3.1***	1.8*

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

TABLE 2. Standardized canonical discriminant function coefficients of original floral variables on the three significant canonical axes

Variable	Canonical axis		
	1	2	3
Peduncle length	0.820	0.651	-0.259
Spur length	0.252	-0.560	0.479
Spur curvature	0.122	0.482	0.604
Upper petal length	0.823	-0.820	-0.542
Middle petal length	-0.461	-0.009	0.270
Lower petal length	-0.309	0.408	0.462
Nectary length	-0.461	0.519	-0.858
Number of ovules	-0.231	0.170	0.259

(a) *Comparison of character correlation matrices.* Separate character correlation matrices were computed for each of the populations studied. The null hypothesis that populations are similar in the structure of character correlation matrices was tested using the method described in detail by Riska (1985).

First, every pairwise correlation between characters was independently tested for among-locality differences using a χ^2 test derived from a weighted sum-of-squares of Hotelling's ζ^* transformation (Sokal & Rohlf, 1981). Out of a total of 28 correlation coefficients, only the one corresponding to the correlation between spur length and nectary length differed significantly between populations ($\chi^2 = 28.66$, d.f. = 17, $P = 0.038$). Nevertheless, after applying Bonferroni's correction to account for the fact that 28 simultaneous tests were performed, the critical value for $\alpha = 0.05$ would become $\alpha = 0.05/28 = 0.002$, hence the null hypothesis of homogeneity of correlation coefficients could not be rejected even for the single significant test obtained.

To determine whether correlation matrices differed significantly among localities, a jackknife procedure was used to test if the ratio of the summed χ^2

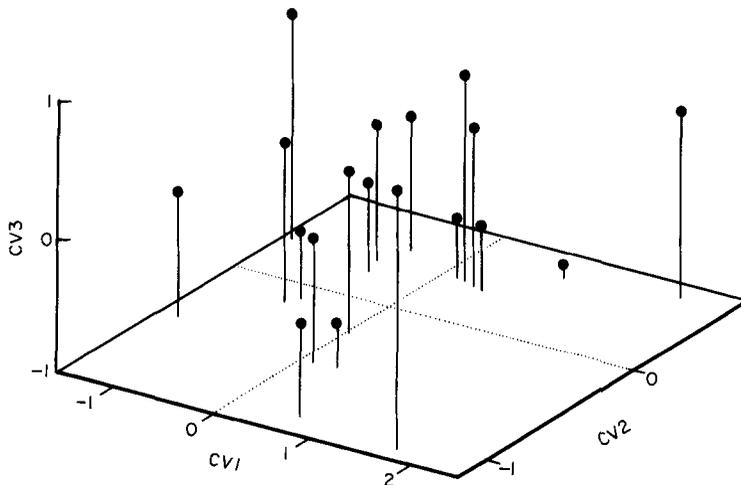


Figure 2. Distribution of population centroids in the space defined by the three canonical axes (CV1–CV3) obtained from discriminant analysis (Table 2).

values to the summed degrees of freedom of the individual tests above departs significantly from a expected F -ratio of unity. The log of the F -ratio was jackknifed, and the mean and variance of the pseudovalues obtained were used to perform a one-tailed t -test of the null hypothesis that the logarithm of F is zero or less against the alternative hypothesis that the logarithm of F is larger than zero. A logarithm of F significantly larger than zero indicates significant heterogeneity of the correlation matrices. The logarithm of F -ratio obtained for the set of 18 matrices was -0.036 , with a standard error (resulting from the jackknifing procedure) of 0.045 . The observed value does not differ significantly from zero ($t=0.803$, $P=0.216$), thus the null hypothesis that character correlation matrices are similar for all populations is not rejected. In spite of the significant differences in the means of floral characters shown above, populations do not differ with regard to the pattern of covariation of these traits between flowers.

(b) *Covarying sets of floral characters.* Canonical axes derived from the multiple discriminant analysis (Table 2) are not orthogonal (Pimentel, 1979), thus they cannot be used to examine the existence of mutually uncorrelated, internally correlated (covarying) character sets. Factor analysis of the character correlation matrix (across flowers) will be used here to this end. As correlation matrices have been found not to differ between populations, the pooled correlation matrix, obtained by combining data from all populations into a single sample, was factored using SAS procedure FACTOR (SAS Institute, 1985) with Varimax rotation. Although only the first two factors resulting from the analysis have associated eigenvalues > 1 (a frequent rule of thumb for determining the number of factors to be retained; Srivastava & Carter, 1983; Legendre & Legendre, 1983), four factors were retained, as eigenvalues for factors 3 and 4 closely approach unity (0.952 and 0.932, respectively). This 4-factor model was tested by means of the likelihood ratio test (Srivastava & Carter, 1983), and found to be acceptable (H_0 : 4 factors are sufficient, *vs.* H_A : more factors are needed, $\chi^2=1.48$, d.f.=2, $P=0.48$; H_0 : 3 factors are sufficient *vs.* H_A : more factors are needed, $\chi^2=13.0$, d.f.=7, $P=0.07$).

Rotated factor loadings of floral characters on the four factors (Table 3) reveal that petal size (upper, middle and lower petal length; factor 1), spur and nectary length (factor 2), number of ovules (factor 3), and spur curvature (factor 4) tend to vary independently, each character contributing high loadings on single factors. Peduncle length exhibits roughly similar loadings on factors 1 and 2. These results indicate that the eight floral characters examined vary across flowers along four independent (orthogonal) trends that represent uncorrelated subsets of floral characters. Two of these subsets contain single characters each (spur curvature and number of ovules), while the other two are made up of covarying attributes.

Spatial patterns of variation

Variation is studied here in a multivariate context, using factor scores obtained from the factor analysis reported above. Scores on factors 1–4 were obtained for all individual flowers examined, and average figures obtained for

each population. These mean values have been used to morphologically characterize populations in a space of reduced dimensionality.

(a) *Broad-scale patterns.* This section examines whether floral characters, as summarized by population means of factor scores, exhibit some pattern of broad-scale geographic variation over the area sampled. General linear models (procedure MGLH in SYSTAT; Wilkinson, 1986) were used. Average factor scores on factors 1–4 (Table 3) were the dependent variables, and geographic coordinates (original X–Y data plus second order powers and cross-products) were the independent ones. This procedure is conceptually analogous to canonical trend surface analysis (Wartenberg, 1985), which is based on canonical correlations between the sets of phenetic and geographic variables. It has the advantageous difference, however, that separate testing may be performed on subsets of independent variables. This allows separation of purely geographic effects from other confounding influences when both types of variables are included in the analysis. The populations of *V. cazorlensis* studied occur over a broad range of elevations, and the region has a rugged topography, which may lead to spurious correlations between elevation and geographic coordinates. Elevational effects could thus confound purely geographic patterns of variation. To account for this possibility, elevation was added to the set of independent variables, and its influence on floral characters analysed simultaneously to that of geographic (X–Y) variables.

There is a significant relationship between elevation and mean population scores on factors 1 and 3 (Table 4). Average scores on factor 1 decrease, and on factor 3 increase, with increasing elevation (Fig. 3). Regardless of geographic location, therefore, populations growing at higher elevations tend to have flowers with smaller petals, shorter peduncles, and greater number of ovules than populations growing at lower elevations. No significant elevational gradients exist in the variation of factor 2 (nectary and spur length) and factor 4 (spur curvature).

The influence of geographic location on floral characters was assessed by simultaneously testing the effect on factor scores (dependent variables) of all

TABLE 3. Results of the factor analysis of the correlation matrix for floral characters, all populations combined (rotated factor loadings). Loadings with absolute values ≤ 0.150 have been omitted to improve presentation

Variable	Factor			
	1	2	3	4
Middle petal length	0.944	—	—	—
Upper petal length	0.922	0.160	—	—
Lower petal length	0.911	—	—	—
Peduncle length	0.527	0.353	–0.186	—
Nectary length	—	0.884	0.165	—
Spur length	0.475	0.735	—	—
Number of ovules	—	—	0.956	—
Spur curvature	—	—	—	0.996
Eigenvector	3.514	1.234	0.953	0.914
Variance explained (%)	43.9	15.4	11.9	11.4

TABLE 4. Significance of the effects of elevation and geographical location (X-Y coordinates plus their squares and cross-product) on mean population scores for factors 1-4, as determined by simultaneous testing in a general linear model. See Table 3 for meaning of factors

Dependent variable	Significance of effect			
	Elevation (d.f. = 1,11)		Geographical location (d.f. = 5,11)	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Factor 1	26.32	0.0004	2.45	0.10
Factor 2	1.16	0.31	0.33	0.89
Factor 3	53.42	<0.0001	8.77	0.001
Factor 4	0.06	0.81	0.28	0.91

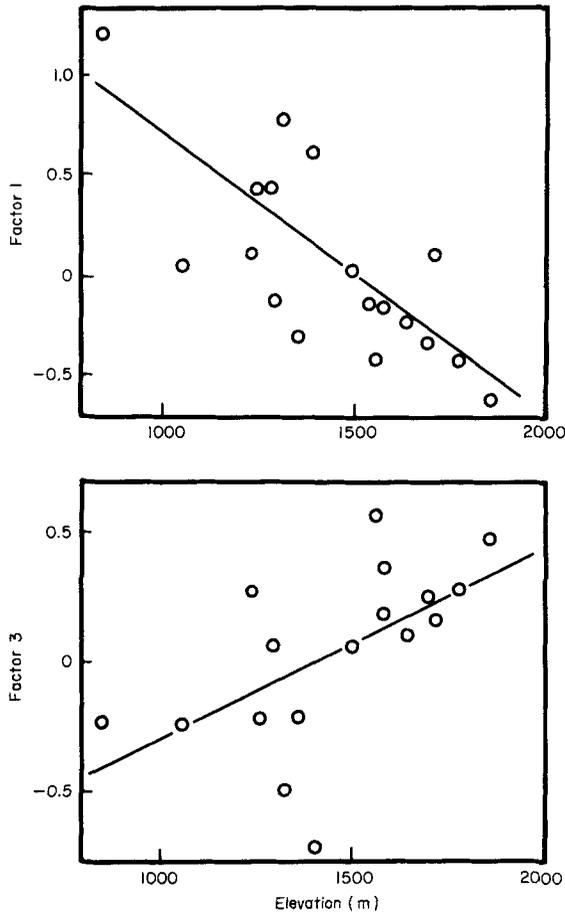


Figure 3. Variation with elevation of average population scores on factors 1 and 3 obtained from the correlation matrix for floral characters, all populations combined (Table 3). Shown are also least-squares fitted regression lines.

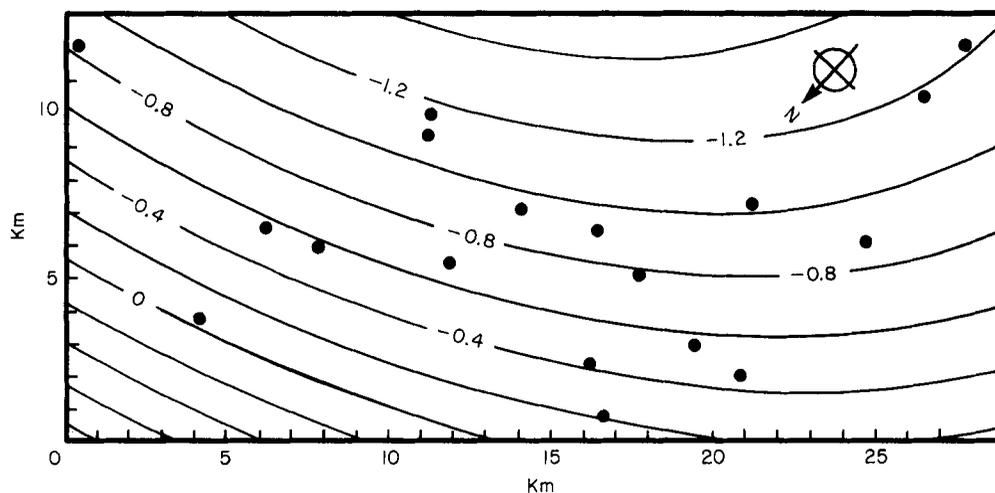


Figure 4. Fitted surface trend for geographical variation in mean population scores on factor 3 (number of ovules) from the correlation matrix for floral characters (Table 3). Dots indicate the location of populations.

geographic variables included in the linear model (X - Y plus squares and cross-product). A significant broad-scale geographic pattern independent of elevation underlies the variation between populations of mean scores on factor 3 (number of ovules; Table 3), but not the variation of the other three factors (Table 4). The fitted surface trend for geographic variation in mean population scores on factor 3 is shown in Fig. 4. After the effect of elevation is accounted for, mean number of ovules per flower tends to decrease steadily from north-west to south-east.

(b) *Small-scale patterns.* Trend surface analysis removes local variations and anomalies, leaving just the overall trends, thus the method is adequate for viewing broad patterns of geographic variation. Small-scale geographic patterning, however, may remain undetected with this technique (Wartenberg, 1985). To determine if small-scale geographic patterning in floral characters exists, spatial autocorrelation was used (Sokal & Oden, 1978a, b). Mean scores for factors 2 and 4 were subjected directly to the analysis, as no significant effect of elevation was found for these factors (Table 4). For factors 1 and 3, residuals obtained after fitting univariate linear regressions using elevation as the single independent variable, were subjected to the autocorrelation analysis instead of the original (and thus elevation-influenced) factor score data. Binary symmetrical weightings were used to weight pairs of populations, and adjacent pairs of localities were identified by two different algorithms (Gabriel and nearest-neighbour methods; Sokal & Oden, 1978a). Computations were performed with the program AUTOCORR written by J. S. Heywood.

Spatial autocorrelation coefficients (Moran's I) are not significantly different from zero for any of the variables considered and with any of the methods used (Table 5). Correlograms (Sokal & Oden, 1978a) were obtained for the four variables using distance classes producing roughly similar sample sizes among classes. None of the correlograms contained spatial autocorrelation coefficients

TABLE 5. The coefficient of spatial autocorrelation (Moran's I), standard deviation (SD), and statistical significance (P), for population mean factor scores for floral characters (Table 3). Results from two methods of pairing populations are presented. For factors 1 and 3, residuals from the regressions on elevation are used instead of original factor score data (see text for rationale)

Variable	Gabriel-connected map			Nearest-neighbour map		
	I	SD	P	I	SD	P
Factor 1 residuals	0.077	0.196	0.50	0.158	0.312	0.50
Factor 2 scores	-0.008	0.196	0.79	0.170	0.312	0.52
Factor 3 residuals	0.113	0.195	0.60	0.480	0.311	0.10
Factor 4 scores	-0.347	0.196	0.16	-0.702	0.312	0.06

significantly different from zero at any distance class, and presentation of this material is omitted here.

DISCUSSION

The populations of *V. cazortensis* sampled in this study display a high degree of distinctiveness in quantitative floral traits. Observed differences were statistically significant for every trait examined and, for most characters, population means exhibit a broad range of variation (e.g. 23.2–29.6 mm for spur length; 30.0–58.7 mm for peduncle length). When all characters are considered together each population displays a unique combination of characters. No studies have been conducted on the genetic basis of floral variation in *V. cazortensis*. Studies on other species, however, have most often found a good correlation between quantitative phenotypic (including reproductive) traits and genetic variation (Cotter & Platt, 1959; Hamrick & Allard, 1975; Ritland & Jain, 1984; Armbruster, 1985; Schwaegerle, Garbutt & Bazzaz, 1986), hence phenotypic differences among *V. cazortensis* populations are most likely indicative of genetic differentiation, as found in other species with subdivided populations (Pamilo, 1983; King, 1987).

Compared to a set of 50 southern Spanish species of perennial plants, *V. cazortensis* ranks first in variability of corolla size (length or width, whichever is largest; Herrera, unpublished data). It has sometimes been argued that endemic plants lack genetic diversity and that this may be one of the reasons for their rarity (Stebbins, 1942; Drury, 1974). Several studies have shown that some endemics lack variability and are genetically depauperate (Gottlieb, 1973; Levin, Ritter & Ellstrand, 1979; Ledig & Conkle, 1983; McClenaghan & Beauchamp, 1986; Waller, O'Malley & Gawler, 1987; Crawford, Stuessy & Silva, 1988; Lesica *et al.*, 1988), and statistically significant trends for geographically restricted species to exhibit lower levels of genetic polymorphism than widespread ones have been documented (Hamrick, Linhart & Mitton, 1979; Karron, 1987; Karron *et al.*, 1988). There are also, however, examples of endemics with considerable genetic or phenotypic variability (Cotter & Platt, 1959; Stebbins, 1980; Primack, 1980; Coates, 1988; Baskin & Baskin, 1988; Karron *et al.*, 1988). In particular, considerable variability in floral traits has been reported for some endemics from rock outcrop plant communities of eastern

United States (Cotter & Platt, 1959; Wyatt, 1984; Baskin & Baskin, 1988), which are ecologically analogous to *V. cazortensis* in the type of habitat and degree of subdivision of their populations.

Despite significant interpopulation differences in the means of floral characters, disjunct populations of *V. cazortensis* appear to retain much of the variability of the entire species. Most of the variance occurs within populations, such that floral differences among individuals within a population are almost as great as differences among individuals of different populations. This result is consistent with other studies that partition total phenotypic or genetic variance into its within- and among-population components (Levin, 1977; Schwaegerle, Garbutt & Bazzaz, 1986; Soltis & Bloom, 1986; McClenaghan & Beauchamp, 1986).

Correlations between characters may arise from shared genetic or developmental determinants, while independent variation is indicative of autonomous genetic control or developmental pathways. Cheverud (1988) has shown that phenotypic correlations generally are reliable indicators of genetic correlations. The homogeneity of *V. cazortensis* populations in the structure of character correlation matrices despite differences in character means would suggest that the general determinants of the floral phenotype in this endemic plant are a species-specific attribute that has not undergone significant differentiation in local populations despite long-term isolation and restricted gene flow (see below).

Floral traits of *V. cazortensis* do not vary in unison among flowers (from different individuals), and at least four different subsets of independently varying traits are identifiable (petals, spur-nectaries, ovule number, and spur curvature). There is thus little integration of the floral phenotype, whose variation is not governed by a single 'size factor' affecting similarly to all floral elements, in contrast to the findings reported by Berg (1960) for a series of insect-pollinated plants. Factor analysis of the correlation matrix for floral characters presented by Wyatt (1984: Table 4) for *Arenaria uniflora*, an ecological analogue of *V. cazortensis*, further reveals the existence of a prevailing 'size factor' and co-ordinated variation of floral parts (Herrera, unpublished observations). The disintegrated floral phenotype of *V. cazortensis* is probably unusual among insect-pollinated plants.

Different subsets of floral traits display contrasting patterns of variation among populations, a result that has been found in other geographic studies of quantitative variation (Schennum & Willey, 1979) and that, in the present case, provides a further proof of the genetic or developmental independence of the various elements of the floral phenotype.

Character subsets represented by factor 1 (size of petals) and factor 3 (ovule number) vary non-randomly among populations. The size of petals decreases, and the number of ovules increases, with increasing elevation. A close correlation between a character and some environmental variable has been traditionally interpreted as evidence for natural selection on the trait (see e.g. Gould & Johnston, 1972; Futuyma, 1979). The correlations of ovule number and size of petals with elevation might be interpreted similarly, but the potential adaptive significance of the variation is not intuitively obvious. In the study area, changes in elevation are associated with variation in temperature, length and timing of the plant growing season, and amount of summer rainfall. Any of these factors,

in isolation or in combination with others, might have selected for ecotypic elevational differentiation of *V. cazortensis* with regard to the floral characters involved, although the possible mechanism of selection is unclear at present. Intraspecific elevational gradients in plant physiology, life history and resource allocation patterns have been often reported (Mooney, 1963; Jolls, 1980; Douglas, 1981; Vickery & Hsu, 1984). As floral characters may be closely correlated with other elements of the phenotype (Ritland & Jain, 1984; Schoen *et al.*, 1984), selection on correlated, non-floral characters could be responsible for observed elevational variation in ovule number and size of petals.

Floral similarity between populations of *V. cazortensis* was unrelated to geographic proximity. No small-scale geographic pattern was detected for any of the four character subsets, and there was only one significant broad-scale geographic pattern (involving ovule number). In general, therefore, the geographic pattern of floral variation in *V. cazortensis* would be best described as a 'random patchwork', with unique combinations of character means occurring randomly across the study region. This finding is consistent with expectations derived from examination of habitat selection and reproductive ecology of this species. Suitable habitats for *V. cazortensis* are essentially scattered islands of rocky substrates in a sea of coniferous or mixed forests. There is probably little gene flow between the disjunct populations that inhabit these islands. Although hawkmoths are strong fliers, pollen transfer over distances in the order of a few kilometres probably occurs rarely (Linhart & Mendenhall, 1977). Furthermore, *V. cazortensis* not only lacks any obvious seed dispersal mechanism (in contrast with other species in its genus, which usually have ballistic and/or myrmecochorous dispersal; Beattie & Lyons, 1975), but its seeds exhibit myxospermy, a trait that reduces secondary dispersal after falling to the ground (Grubert, 1974). Colonization of suitable areas or further immigration of seeds probably occur quite infrequently and always involve a small number of seeds. The most likely agents of dispersal are ungulate mammals, that browse on the capsules (Herrera, 1990a) and may exceptionally pass some seeds unharmed. On the basis of these observations one would predict random local differentiation of populations, as a consequence of genetic drift following infrequent founding of populations by a small number of initial colonizers and subsequent restricted gene flow. The pattern of geographic variation documented here agrees with this expectation.

Variation in all floral characters examined here may potentially translate into differential reproductive success via influences on plant-pollinator interactions. Peduncle length determines the height of flowers over the ground, which may influence pollinator foraging (Waddington, 1979). Spur and nectary length, and spur curvature, determine nectar depth and accessibility. Variations in corolla size (size of petals) may result in differential attractiveness to pollinators, and variation in number of ovules is most likely correlated with changes in the minimum number of pollen grains needed for fruit set. Nevertheless, there is no evidence that variation in these traits is associated with individual differences in reproductive output in *V. cazortensis*. A previous investigation on the relationship between individual variation in floral morphology and reproductive success failed to find evidence of stabilizing or directional selection on quantitative floral traits examined here (except peduncle length, for which some evidence of disruptive selection was found; Herrera, 1990b). The present study, by

documenting marked population differences in quantitative floral traits in spite of spatial constancy in pollinators, a disintegrated floral phenotype, and prevalently random geographic variation in *V. cazorlensis* flowers, further tends to suggest that the floral phenotype of this species probably is not closely adapted to its current pollinating agent, the hawkmoth *Macroglossum stellatarum*. This pollinator apparently does not exert significant selective pressures on the floral morphology of *V. cazorlensis* and, as a consequence, non-adaptive (in relation to pollination) floral variability may persist in this species.

Viola cazorlensis is a relict endemic that originated in the Pliocene (Melchior & Cuatrecasas, 1935; Quézel, Gamisans & Gruber, 1989). This species and its close relatives *V. delphinantha* and *V. kosaninii* from the Balkan Peninsula, comprise the section *Delphiniopsis* of the genus *Viola* (Tutin *et al.*, 1964–1980) and are the survivors of one of the oldest lineages in this genus (Valentine, 1962). The distinctive floral features of this small species group, particularly the very long spur, were thus most likely shaped through interaction with insects only distantly related to their current pollinators. Expectations derived from these historical considerations, predicting a low degree of adaptedness in the relationship between *V. cazorlensis* and its current pollinator, are supported by the findings reported in this paper and in Herrera (1990b).

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APPENDIX

Location (UTM 1-km grid reference) and elevation of the populations of Viola cazorlensis examined 1: Cuerda de los Alcañetes, 30SWG1398, 1700 m. 2: Arenales del Guadalentín, 30SWG1496, 1400 m. 3: Río Borosa, 30SWH1405, 850 m. 4: Pico Cabañas, 30SWG0484, 1860 m. 5: Cabeza del Tejo, 30SWG1095, 1640 m. 6: Cerrada del Pintor, 30SWG0590, 1500 m. 7: Cruz de Quique, 30SWG0494, 1290 m. 8: Cuevas de La Mesa, 30SWG0794, 1260 m. 9: Fuente de Los Chorros, 30SWG1495, 1320 m. 10: Puerto de Los Arenales, 30SWG0498, 1240 m. 11: Cerro Navahondona, 30SWG0289, 1360 m. 12: Puente de Guadahornillos, 30SWG1198, 1580 m. 13: Puerto Lézar, 30SWH2401, 1780 m. 14: Puerto del Tejo, 30SWG0294, 1560 m. 15: Rastrillos de la Víbora, 30SWG0894, 1580 m. 16: Torcal de Linares, 30SWG0483, 1720 m. 17: Vadillo, 30SWG0597, 1060 m. 18: Valdeazores, 30SWH1602, 1300 m.