

# The adaptedness of the floral phenotype in a relict endemic, hawkmoth-pollinated violet. 1. Reproductive correlates of floral variation

CARLOS M. HERRERA

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

Received 31 July 1989, accepted for publication 10 November 1989

This paper examines the relationship between quantitative variation in floral morphology (sizes of petals, spur and peduncle) and maternal reproductive success (seed production) in *Viola cazorlensis* (Violaceae), a narrowly endemic violet of south-eastern Spain pollinated by day-flying hawkmoths (Sphingidae). This plant is characterized by broad intraspecific variation in size and proportions of floral parts. Floral morphology does not influence significantly the probability of fruit set. Among flowers setting fruit, spur length and size of petals have no significant effect on seed production, but capsules from long- and short-peduncled flowers contain significantly more seeds than capsules from flowers with intermediate peduncles. Individual plants differ significantly in average floral characteristics. Plants with comparatively long and short peduncles tend to produce more seeds than those with intermediate ones, even after accounting statistically for individual differences in flower production. These findings are interpreted as evidence of disruptive selection on peduncle length during the study season. Floral variability in this species may be explained by the combined action of disruptive selection on peduncle length (the character most variable among individuals) and little, if any, stabilizing selection on spur length and size of petals.

**KEY WORDS:**—Endemism – disruptive selection – floral morphology – pollination – quantitative floral variation – Violaceae.

## CONTENTS

Introduction . . . . .	263
Study area and methods . . . . .	264
Results . . . . .	266
Pollination and seed production . . . . .	266
Fruit set by individual flowers . . . . .	267
Seed set of individual flowers . . . . .	267
Individual variation and reproductive success . . . . .	270
Discussion . . . . .	271
Acknowledgements . . . . .	273
References . . . . .	273

## INTRODUCTION

It is universally accepted that the floral morphology of animal-pollinated angiosperms has evolved in close connection with the activity of their pollinating agents (Darwin, 1877; Leppik, 1957; Stebbins, 1970; Faegri & van der Pijl, 1971). Nevertheless, studies directly examining the adaptive significance of floral traits and their influence on plant reproductive success are comparatively scarce (see Waser, 1983 for review). Testing the adaptiveness of floral traits requires the

existence of some variation in the trait, either occurring naturally or induced by experimental manipulation (Waser, 1983). Intraspecific variation in floral traits occurring naturally in time or space may be used to ask how variation alters plant reproductive success. Using this approach, recent studies have often demonstrated the influence of flower colour, scent, and morphology on aspects directly relevant to plant fitness such as pollinator foraging behaviour, seed production, and outcrossing rate. Most of these investigations, however, have dealt with discontinuously varying floral traits in polymorphic species (e.g. colour, scent; Kay, 1976; Hannan, 1981; Waser & Price, 1981; McDade, 1983; Galen, 1985; Schoen & Clegg, 1985; Stanton, Snow & Handel, 1986; Epperson & Clegg, 1987; Ernst, 1987; Galen, Zimmer & Newport, 1987; Stanton, 1987; Abbott & Irwin, 1988; Galen & Newport, 1988), while relatively few studies have examined the significance of continuously varying characters such as morphometric traits. These investigations have often shown that variation in the size of flowers or floral parts translates into differences in seed production and outcrossing rate (e.g. Beare & Perkins, 1982; Harder *et al.*, 1985; Thomson & Stratton, 1985; Galen & Newport, 1987; Nilsson, 1988).

This paper examines the relationship between quantitative variation in floral morphology and maternal reproductive success in *Viola cazortensis* (Violaceae), a relict endemic of south-eastern Spain. This species is characterized by broad continuous variation in the size and proportions of floral parts (Herrera, 1988), thus providing an excellent opportunity to investigate the influence of continuously varying floral traits on reproductive success under natural conditions. Two questions are addressed here. (1) Does the morphology of an individual flower influence its probability of setting fruit or the number of seeds eventually set? (2) Do differences between individual plants in floral morphology translate into differential maternal reproductive success in terms of number of seeds produced?

*Viola cazortensis* is a perennial, suffruticose violet endemic to two limestone mountain ranges in south-eastern Spain (Melchior & Cuatrecasas, 1935). This species, along with two closely related taxa occurring in the Balkan Peninsula (*V. delphinantha* and *V. kosaninii*), constitute the small section *Delphiniopsis* of the genus *Viola* (Tutin *et al.*, 1964–1980), an old and apparently primitive lineage originating in the Pliocene (Valentine, 1962; Quézel, Gamisans & Gruber, 1980). Flowers of *V. cazortensis* have pinkish-purple corollas, and are characterized by a long spur (up to 37 mm in length, mean = 25.1 mm; Herrera, 1988), the longest of all European species in its genus. The main pollinators are day-flying hawkmoths (Lepidoptera, Sphingidae; Herrera, 1988, unpublished observations), an unusual feature for the genus *Viola* (Beattie, 1974).

#### STUDY AREA AND METHODS

The study was conducted on a population of *V. cazortensis* growing at 1290 m elevation in La Cruz de Quique, Sierra de Cazorla (Jaén province, south-eastern Spain). Plants occur there on loose sandy soils, rock crevices and cliffs (Herrera, 1990). The vegetation of the area is open pine (*Pinus nigra* and *P. pinaster*) forest with a sparse understorey of *Juniperus phoenicea* treelets and a xerophytic shrub layer dominated by *Echinopartum boissieri*, *Rosmarinus officinalis* and *Fumana ericoides*.

In April 1988, 75 plants of *V. cazortensis* were marked before the start of flowering in the population. Only 45 of these eventually flowered, and the study reported here is based on these plants, which were continuously monitored throughout the flowering period (3 May–19 June). Every 2–3 days (individual flowers last 8–15 days) all newly opened flowers on marked plants were measured (see below) and individually marked with small numbered tags attached to the base of the peduncle. The development of all marked flowers was subsequently followed until the end of the reproductive period (fruit maturation and seed dispersal). Mature capsules were collected prior to dehiscence, examined under a dissecting microscope, and the number of seeds counted. Furthermore, the number of initial ovules that had failed to develop into seeds could be accurately counted for most of the capsules. By adding this figure to the number of seeds, it was possible to know *a posteriori* the number of ovules of the flower that yielded the capsule. Ovules failing to produce mature seeds were further separated into those that failed at initiating development and those that did but were aborted at some intermediate stage before maturation. The final data set consisted, for each individual flower, of information on its morphology and its reproductive outcome (successful or unsuccessful) and, for successful flowers, the number of ovules and number of seeds set.

Measurements taken on flowers are shown in Fig. 1. Flowers of *V. cazortensis* are produced singly on 25–95-mm-long peduncles arising from the axil of leaves. Regardless of variation in the inclination of the substrate (from horizontal soil to vertical cliffs), petal blades always fall in a nearly vertical plane (Fig. 1). For this reason, the ‘anterior’, ‘lateral’ and ‘posterior’ petals in Beattie’s (1969) terminology are designated here as ‘lower’, ‘middle’ and ‘upper’ petals,

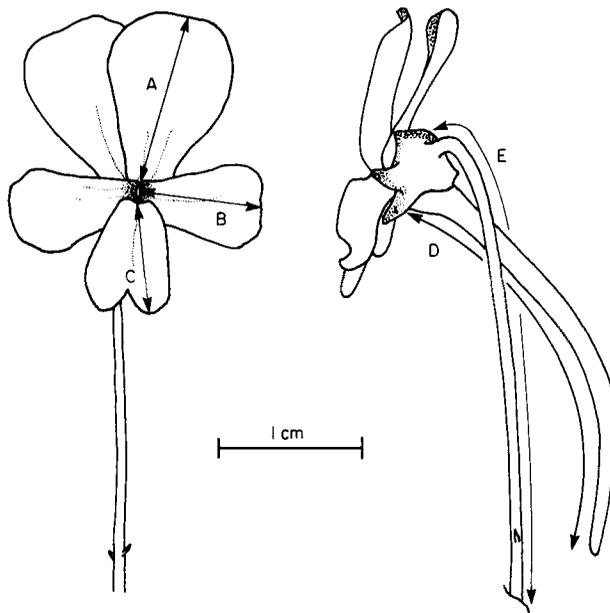


Figure 1. Front and lateral views of a *Viola cazortensis* flower, with indication of the measurements used. A, B and C, Upper, lateral and lower petal length, respectively. D, Spur length. E, Peduncle length. In natural conditions, the spur is visible in front view, but it is omitted here for simplicity.

respectively. Maximum length of petal blades ('petal length' hereafter) were measured for upper, middle and lower petals. Measurements of the spur and the peduncle were taken after gently straightening these structures ('peduncle length' and 'spur length' hereafter).

Data on floral morphology and maternal reproductive success are analysed below both on a per flower basis and grouped by individual plants. For individual plants, the total number of flowers and seeds produced was determined from information on marked flowers and seed counts. At the study population, plants of *V. cazortensis* grow on three distinct substrate types (sandy soil, small cliffs, and crevices in nearly horizontal rocks). Plants from different substrates differ in some aspects of their reproductive ecology (Herrera, 1990). Marked plants used for this study were thus assigned to one of the three substrate types, and the effect of this factor on reproductive success will be considered in some of the analyses below.

In addition to the data on flower morphology and reproductive success providing the basis for this paper, other aspects of the reproductive biology of *V. cazortensis* were studied at the same population in 1987 and 1988 (Herrera, 1988, 1990, unpublished observations). Relevant data on pollination and reproductive biology are summarized here.

The problem of examining the differential reproductive success of flowers or plants differing in quantitative floral characters is methodologically analogous to comparing the differential success of phenotypes in studies of natural selection. Furthermore, some morphometric traits examined here are correlated between themselves. For these reasons, analytical methods applied to the study of natural selection on correlated characters based on multiple regression (Lande & Arnold, 1983; Endler, 1986; Schluter & Smith, 1986; Schluter, 1988; Lechowicz & Blais, 1988), will be used. The effect on reproductive success of a qualitative variable (type of substrate) in combination with quantitative ones (floral traits, flower production) will be tested using general linear models. Most statistical analyses were performed using procedure MGLH in SYSTAT (Wilkinson, 1986).

## RESULTS

### *Pollination and seed production*

The nectar of *V. cazortensis* flowers accumulates at the distal portion of the long spur and thus can be reached only by insects with long proboscides. The only floral visitors recorded during 32 h of observations over two flowering seasons were two species of day-flying hawkmoths (*Macroglossum stellatarum*, 19 observations; *Hemaris tityus*, 1 observation) and two undetermined species of bombyliid flies (1 observation each) (Herrera, 1988, unpublished observations). Average proboscis length ( $\pm 1$  SD) for *M. stellatarum* and *H. tityus* are  $26.4 \pm 2.0$  mm ( $N=19$ ) and  $16.9 \pm 0.5$  mm ( $N=4$ ), respectively, and fall within the range of spur lengths found in the study area (Herrera, 1988). No proboscis measurements are available for the flies, but the longest-tongued local bombyliids have proboscides shorter than 10 mm (Herrera, unpublished observations), thus these flies are probably unable to reach the nectar of *V. cazortensis* flowers.

Although some flowers produce fruits in absence of pollinators, the activity of these significantly increases fruit set. Flowers under pollinator exclusions exhibited significantly reduced fruit set relative to control flowers (flowers under exclusions: 14.3%,  $N=322$ ; naturally pollinated flowers: 43.2%,  $N=1082$ ;  $\chi^2=89.2$ ,  $P<0.0001$ ; data for two years combined). Preliminary evidence suggests that fruit production may be pollinator-limited in some years, as annual variation in fruit set is related to changes in local hawkmoth abundance (Herrera, unpublished observations).

#### *Fruit set by individual flowers*

Individual flowers were classified as either 'successful' or 'unsuccessful', depending on whether they produced a mature capsule or not. Flowers destroyed by invertebrate (mainly *Fabriciana adippe* caterpillars, Nymphalidae) and vertebrate (mainly wild goats, *Capra pyrenaica*) herbivores during anthesis (5.5% of the original flower sample), and flowers for which developing capsules were eaten by pyralid moth larvae (*Heliothela wulfeniana*) prior to maturation (8.5% of the original sample), were excluded from the analyses. The final sample used consisted of 68 unsuccessful and 165 successful flowers from 45 plants.

Successful and unsuccessful flowers did not differ in either average values or variability of floral characters. Overall differences between successful and unsuccessful flowers in mean values of morphological characters were tested with multivariate analysis of variance. There was no significant difference between successful and unsuccessful flowers in overall morphology (Wilks' lambda = 0.981,  $F(5,225)=0.855$ ,  $P=0.512$ ). Separate univariate testing of characters likewise yielded no significant results (Table 1). Levene's test (Van Valen, 1978) was used to test for differences in variability between successful and unsuccessful flowers. None of the tests was significant (Table 1).

#### *Seed set of individual flowers*

For successful flowers, I examined whether the number of seeds in a mature capsule bears some relationship to the morphology of the flower that originated it. A multiple regression was used to test simultaneously for the effect of floral characters (independent variables) on the number of seeds eventually produced

TABLE 1. Mean characteristics (standard deviation in parentheses) for successful (producing a mature capsule) and unsuccessful (not producing it) flowers of *Viola cazorlensis*, all plants combined. All measurements are in mm

	<i>N</i>	Peduncle length	Spur length	Upper petal length	Middle petal length	Lower petal length
Successful flowers	165	41.2 (9.3)	23.5 (3.5)	11.8 (1.8)	9.6 (1.4)	7.6 (1.2)
Unsuccessful flowers	68	40.6 (10.7)	24.0 (4.6)	12.1 (1.9)	9.7 (1.7)	7.6 (1.0)
Difference in means: <sup>a</sup>						
<i>F</i>		0.21 n.s.	0.86 n.s.	1.42 n.s.	0.13 n.s.	0.003 n.s.
Difference in variability: <sup>b</sup>						
<i>F</i>		0.96 n.s.	1.81 n.s.	0.10 n.s.	0.48 n.s.	0.93 n.s.

<sup>a</sup>Univariate analysis of variance; <sup>b</sup>Levene's test for absolute variation (Van Valen, 1978); n.s., not significant.

TABLE 2. Effect of floral traits on the number of seeds set by individual flowers (only flowers setting fruit included, all plants combined). *b*, Standard partial regression coefficient; *t*, Student's *t*; *P*, significance of partial regression coefficient (multiple regression) or effect (general linear model); *F*, *F*-ratio statistic. Only variables with significant regression coefficients ( $P < 0.05$ ) were included in the linear model.  $N = 165$  flowers for the multiple regression,  $N = 130$  flowers for the linear model (number of ovules not available for all flowers)

Independent variable/ Effect	Multiple regression			General linear model	
	<i>b</i>	<i>t</i>	<i>P</i>	<i>F</i>	<i>P</i>
Peduncle length	-2.12	3.69	< 0.001	5.86	0.01
(Peduncle length) <sup>2</sup>	1.91	3.35	0.001	5.32	0.02
Spur length	0.59	1.05	0.29	—	—
(Spur length) <sup>2</sup>	-0.57	1.02	0.31	—	—
Upper petal length	-1.36	0.99	0.32	—	—
(Upper petal length) <sup>2</sup>	1.55	1.17	0.24	—	—
Middle petal length	2.58	1.75	0.10	—	—
(Middle petal length) <sup>2</sup>	-2.42	1.70	0.10	—	—
Lower petal length	-1.69	1.47	0.14	—	—
(Lower petal length) <sup>2</sup>	1.52	1.33	0.18	—	—
Number of ovules	—	—	—	19.72	< 0.001
Substrate type	—	—	—	1.94	0.15

per successful flower (dependent variable). To account for possible non-linear relationships between floral traits and the number of seeds eventually produced, squared morphometric variables were also included among independent variables.

Partial regression coefficients for both the linear and quadratic terms are significant for peduncle length (Table 2), thus denoting a non-linear relationship with the dependent variable. No further morphometric trait exhibits significant regression coefficients. The number of ovules varies among flowers, thus the possibility exists that the significant relation found between peduncle length and number of seeds set per capsule may be due to an indirect effect of ovule number. Substrate type may likewise be a confounding factor. This was examined by fitting a general linear model to the data, using the number of seeds per capsule as the dependent variable, and substrate type, number of initial ovules (determined *a posteriori* for 78% of successful flowers), and peduncle length and its square, as independent variables (Table 2). The effect of type of substrate on number of seeds per capsule is not significant, but there is a highly significant effect of the initial number of ovules on the number of seeds set per capsule. After accounting statistically for this effect, however, the effect of peduncle length (both linear and quadratic terms) on seeds set per capsule is still significant. It may thus be concluded that indirect effects of ovule number or substrate type are not responsible for the observed effect of peduncle length on number of seeds set per capsule.

The fitted quadratic regression curve for the relationship between number of seeds per capsule and peduncle length is shown in Fig. 2A. Flowers with intermediate peduncles tended to produce fewer seeds than flowers with either relatively long or short peduncles. Non-parametric regression was used to corroborate this pattern. A cubic spline was fitted to the data using the

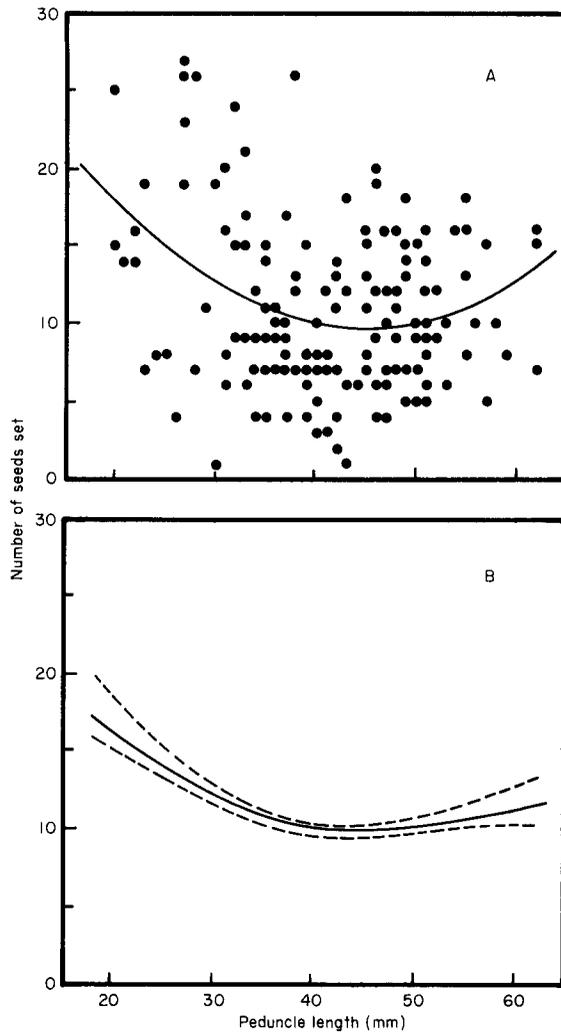


Figure 2. Variation in number of seeds set per capsule with length of the floral peduncle. A, Observed values (dots) and least squares-fitted quadratic regression line (Table 2). B, Non-parametric regression line fitted to the same data using a generalized cross-validation method (continuous line). Dashed curves indicate  $\pm 1$  SE of predicted values from 100 bootstrap regression replicates. See text for further details.

generalized cross-validation method described in detail by Schluter (1988). As this method does not depend on *a priori* assumptions about the underlying mathematical function, it provides a more reliable assessment of the shape of the relationship between dependent and independent variables than least square-based curve fitting (Schluter, 1988). To estimate the sampling variability of the regression line obtained, 100 bootstrap regressions were generated, and the standard errors for the predicted values are shown in Fig. 2B along with the cubic spline fitted to the data. Results confirm that flowers with intermediate peduncles tend to produce fewer seeds than flowers with comparatively longer and shorter peduncles.

For each capsule, the number of original ovules failing to initiate development, and of those aborted between initiation of development and seed maturation, were expressed as proportions of the original number of ovules, arcsin-transformed, and used as two dependent variables in a general linear model having peduncle length and its square as independent variables. The effect of peduncle length and its square was not significant ( $F(2,127)=1.42$ ,  $P=0.25$ ) on the proportion of ovules that aborted after initiating development, and significant ( $F(2,127)=3.35$ ,  $P=0.038$ ) on the proportion of ovules that did not initiate development. Flowers with intermediate peduncles tended to have a greater proportion of ovules failing at initiating development than flowers with either longer or shorter peduncles.

#### *Individual variation and reproductive success*

Average values for floral traits were computed for all plants that produced at least three flowers, including both successful and unsuccessful ones. Ranges and coefficients of variation of individual means denote broad variation between plants in floral characteristics (Table 3). There is significant heterogeneity between individuals in the means of all the traits examined, as revealed by univariate analyses of variance (Table 3). There is also significant overall heterogeneity between plants when all characters are simultaneously tested by multivariate analysis of variance (Wilks' lambda=0.0042,  $F(119,616)=6.82$ ,  $P=0.0001$ ).

The total number of seeds produced by each plant (log-transformed) was used as a measure of its maternal reproductive success (RS) in the study season. Individual plants differed broadly in the number of seeds produced, and the frequency distribution of this magnitude was strongly skewed to the right. Most plants produced fewer than 30 seeds, while a few individuals produced more than 150 seeds.

The influence of floral morphology on RS was examined using this magnitude (log-transformed) as the dependent variable in a general linear model. Peduncle length and its square were included among independent variables, as this was the single morphometric trait exhibiting a significant effect on seed production per flower (preliminary analyses including the other floral traits did not produce any significant result). Substrate type and total number of flowers produced by individuals (log-transformed) were also included among independent variables. Results are summarized in Table 4.

TABLE 3. Summary of interindividual differences in floral characters ( $N=45$  plants). All measurements are in mm

	Peduncle length	Spur length	Upper petal length	Middle petal length	Lower petal length
Individual means:					
Average (SD)	41.4 (8.8)	24.9 (3.3)	11.7 (1.2)	9.5 (1.0)	7.5 (0.9)
CV (%)	21.3	13.1	10.6	10.5	11.5
Range	23.0–59.0	19.4–34.3	8.0–14.8	6.5–12.0	6.0–10.5
Difference of means:					
<i>F</i>	38.97**	7.56**	6.35**	4.52**	1.93*

\* $P<0.05$ ; \*\* $P<0.0001$ .

TABLE 4. Significance of the effect of substrate type, number of flowers produced and peduncle length, on the total number of seeds (log-transformed) produced by individual plants ( $N=41$ ), using this latter variable as the dependent one in a general linear model

Effect	<i>F</i>	<i>P</i>
Substrate type	4.20	0.02
Number of flowers <sup>a</sup>	31.85	< 0.0001
Peduncle length	3.83	0.06
(Peduncle length) <sup>2</sup>	3.29	0.07

<sup>a</sup>Log-transformed.

The effects of substrate type and number of flowers produced were significant, and those of peduncle length and its square marginally significant. An analysis of the multiple  $R^2$  values for the linear model reveals that, quantitatively, the influence of peduncle length and substrate type on RS is relatively unimportant in comparison to the influence of the number of flowers. Using the number of flowers as the single independent variable in the model yields an  $R^2=0.463$ . Including substrate type as a further independent variable adds 0.103 to  $R^2$ , and the addition of peduncle length and its square increases it by a further 0.052 up to a value of  $R^2=0.618$ . Differences between individuals in number of seeds produced, therefore, are mostly due to differences in flower production, although type of substrate and floral morphology (peduncle length) also contribute to individual differences in realized fecundity.

#### DISCUSSION

The morphology of the flowers of *V. cazortensis* does not influence significantly the probability of fruit set. Among flowers setting fruit, spur length and size of petals have no significant effect on seed production, but capsules produced by comparatively longer and shorter peduncled flowers contain significantly more seeds than capsules from flowers with intermediate peduncles, and this effect is independent of variation in the number of ovules. Flowers with intermediate peduncles tend to have a greater proportion of ovules failing at initiating development than flowers with either longer or shorter peduncles. Individual plants differ significantly in average floral characteristics for all the traits examined. After accounting statistically for differences in flower production, individuals with comparatively long and short peduncles still tend to produce more seeds than those with intermediate ones, although most individual variation in seed production is accounted for by differences in flower production.

Previous investigations have provided both positive (spur length, Nilsson, 1988; corolla size, Galen & Newport, 1987) and negative (corolla size, Stanton & Preston, 1988) evidence on the influence of quantitative variation in floral traits on maternal reproductive success. The present study also provides contrasting results. While corolla size (as estimated by the three petal measures) and spur length have no significant effect on seed production, peduncle length does significantly influence it. These results indicate that negative evidence may sometimes be due to a failure at examining those floral characters actually

influencing reproductive success and that ancillary, apparently secondary extrafloral structures such as the peduncle may have unanticipated reproductive consequences in some species. In the case of the hawkmoth-pollinated *V. cazorlensis*, the finding that variation in spur length did not influence seed production is particularly striking. In this long-spurred plant, one would have predicted a significant influence of spur length on maternal reproductive success, as documented by Nilsson (1988) for long-spurred, hawkmoth-pollinated orchids. Variation in spur length may not always translate into differential reproductive output, as found here, and Darwin's (1877:165–166) model for the evolution of orchids with deep corolla tubes (tested and verified by Nilsson, 1988) may not apply universally to all species with deep tubular corollas (see also Nilsson, 1978; Miller, 1981).

This study has examined the influence of floral morphology on a single aspect of the maternal component of plant fitness, but floral variation may have reproductive consequences other than differences in seed production. *Viola cazorlensis* flowers are hermaphrodite, thus the possibility that variation in spur length and size of petals, even without influencing seed production, is consequential in terms of differential male fitness cannot be ruled out (Schoen & Clegg, 1985; Stanton, Snow & Handel, 1986). Furthermore, outcrossing rate may also be affected by floral variation (Rick, Holle & Thorp, 1978; Thomson & Stratton, 1985; Abbott & Irwin, 1988). The results of this study should thus be properly interpreted only in relation to the single component of maternal fitness examined here. Seed production, however, is certainly a most important component of fitness in this species (*V. cazorlensis* does not propagate vegetatively), and it seems reasonable to assume that seed production of individual plants will be correlated with fitness. On this assumption, the relationship found here between peduncle length and seed production points to the existence of disruptive natural selection on peduncle length during the study season. For a given number of flowers and ovules, plants with comparatively long and short peduncles tend to produce more seeds, and hence presumably have greater fitness, all else being equal, than plants with intermediate peduncles.

The reasons for the reproductive advantage of plants with long and short peduncles remain unclear at present. These may be related to differential foraging by pollinators on plants differing in average peduncle length. If pollinators limit seed production, as it seems is often the case in the study population (Herrera, unpublished observations), preferential visitation by pollinators of long- and short-peduncled flowers would result in differential seed set as a consequence of differential pollination. The significant effect of peduncle length on the proportion of ovules initiating development (and thus presumably fertilized) is consistent with this explanation. Pollinators were scarce and infrequently observed (an average of 0.6 individuals  $\text{h}^{-1}$  for sphingid moths in 1987–1988), and no field data are available to test this possibility. Alternatively, the relationship between peduncle length and maternal reproductive success could be a consequence of peduncle length being correlated with some other trait unaccounted for here, yet influencing seed production (directly or through influencing pollinator foraging). Nectar availability to pollinators was examined at the study population in 1987 and 1988 as part of other investigations (Herrera, unpublished observations). No correlation was found between peduncle length and nectar volume (standing crop) of individual flowers

( $r=0.006$ ,  $N=58$ ), thus it is unlikely that differential nectar availability in flowers of varying peduncle length influenced pollinator foraging and seed production. Other correlates of peduncle length potentially influencing seed production may include the genetic constitution of plants, that could influence compatibility relationships, and their physiological traits, that could influence reproductive output via differential nutrient acquisition efficiency. Further studies are needed to evaluate these possibilities.

*Viola cazorlensis* exhibits broad intraspecific variation in quantitative floral traits (Herrera, 1988, and present study). Compared to a set of 50 southern Spanish species of perennial plants, it ranked first in variability of corolla size (length or width, whichever is largest; Herrera, unpublished observations). Several hypotheses have been suggested to explain the high level of floral variability in this endemic plant (Herrera, 1988). Underlying all these is the notion that, in the recent history of the species, floral morphology has been subjected to weak stabilizing natural selection. The negative evidence found here for size of petals and spur length lends support to the idea that this species has very variable flowers because floral variation has no substantial influence on individual differences in fecundity, and there is thus little opportunity for evolutionary change. The results for peduncle length, on the other hand, suggest that there is a potential for evolutionary change in this trait if its variation has some genetic basis. In this case, the disruptive nature of the selection on this character would tend to enhance variability. Interestingly, among the floral traits examined here, peduncle length is the one with the greatest interindividual variability ( $CV=21.3\%$ , as compared to  $CV=10.5-13.1\%$  for the four remaining traits; Table 3). It may then be concluded that, if the results presented here are representative of the prevailing conditions faced by *V. cazorlensis* populations in the recent history of the species, its marked floral variability may be attributed to the juxtaposition of disruptive selection on peduncle length (the most variable character) and little, if any, stabilizing selection on spur length and size of petals.

#### ACKNOWLEDGEMENTS

I thank Dolph Schluter for kindly providing the computer program for non-parametric regression, and Manolo Carrión for assistance with the field work. The Agencia de Medio Ambiente authorized my work in Cazorla and provided invaluable facilities in the study area. This research was supported by the Consejo Superior de Investigaciones Científicas, Programa de Financiación Basal 1988, and DGICYT grant PB87-0452.

#### REFERENCES

- ABBOTT, R. J. & IRWIN, J. A., 1988. Pollinator movements and the polymorphism for outcrossing rate at the ray floret locus in groundsel, *Senecio vulgaris* L. *Heredity*, *60*: 295-298.
- BEARE, M. H. & PERKINS, W. E., 1982. Effects of variation in floral morphology on pollination mechanisms in *Asclepias tuberosa* L., butterflyweed (Asclepiadaceae). *American Journal of Botany*, *69*: 579-584.
- BEATTIE, A. J., 1969. The floral biology of three species of *Viola*. *New Phytologist*, *68*: 1187-1201.
- BEATTIE, A. J., 1974. Floral evolution in *Viola*. *Annals of the Missouri Botanical Garden*, *61*: 781-793.
- DARWIN, C., 1877. *The Various Contrivances by which Orchids are Fertilised by Insects*. 2nd Edn, reprinted 1984, Chicago: University of Chicago Press.
- ENDLER, J. A., 1986. *Natural Selection in the Wild*. Princeton: Princeton University Press.
- EPPELSON, B. K. & CLEGG, M. T., 1987. Frequency-dependent variation for outcrossing rate among flower-color morphs of *Ipomoea purpurea*. *Evolution*, *41*: 1302-1311.

- ERNST, W. H. O., 1987. Scarcity of flower colour polymorphism in field populations of *Digitalis purpurea* L. *Flora (Jena)*, 179: 231–239.
- FAEGRI, K. & VAN DER PIJL, L., 1971. *The Principles of Pollination Ecology*. 3rd Edn. Oxford: Pergamon Press.
- GALEN, C., 1985. Regulation of seed-set in *Polemonium viscosum*: floral scents, pollination, and resources. *Ecology*, 66: 792–797.
- GALEN, C. & NEWPORT, M. E. A., 1987. Bumble bee behavior and selection on flower size in the sky pilot, *Polemonium viscosum*. *Oecologia (Berlin)*, 74: 20–23.
- GALEN, C., ZIMMER, K. A. & NEWPORT, M. E. A., 1987. Pollination in floral scent morphs of *Polemonium viscosum*: a mechanism for disruptive selection on flower size. *Evolution*, 41: 599–606.
- GALEN, C. & NEWPORT, M. E. A., 1988. Pollination quality, seed set, and flower traits in *Polemonium viscosum*: complementary effects of variation in flower scent and size. *American Journal of Botany*, 75: 900–905.
- HANNAN, G. L., 1981. Flower color polymorphism and pollination biology of *Platystemon californicus* Benth. (Papaveraceae). *American Journal of Botany*, 68: 233–243.
- HARDER, L. D., THOMSON, J. D., CRUZAN, M. B. & UNNASCH, R. S., 1985. Sexual reproduction and variation in floral morphology in an ephemeral vernal lily, *Erythronium americanum*. *Oecologia (Berlin)*, 67: 286–291.
- HERRERA, C. M., 1988. Biología y ecología de *Viola cazorlensis*. I. Variabilidad de caracteres florales. *Anales del Jardín Botánico de Madrid*, 45: 233–246.
- HERRERA, C. M., 1990. Biología y ecología de *Viola cazorlensis*. II. Uso de sustratos, reproducción, y consumo por los herbívoros. *Anales del Jardín Botánico de Madrid*, 46: in press.
- KAY, Q. O. N., 1976. Preferential pollination of yellow-flowered morphs of *Raphanus raphanistrum* by *Pieris* and *Eristalis* spp. *Nature*, 261: 230–232.
- LANDE, R. & ARNOLD, S. J., 1983. The measurement of selection on correlated characters. *Evolution*, 37: 1210–1226.
- LECHOWICZ, M. J. & BLAIS, P. A., 1988. Assessing the contributions of multiple interacting traits to plant reproductive success: environmental dependence. *Journal of Evolutionary Biology*, 1: 255–273.
- LEPPIK, E. E., 1957. Evolutionary relationship between entomophilous plants and anthophilous insects. *Evolution*, 11: 466–481.
- McDADE, L. A., 1983. Long-tailed hermit hummingbird visits to inflorescence color morphs of *Heliconia irrasa*. *Condor*, 85: 360–364.
- MELCHIOR, H. & CUATRECASAS, J., 1935. La *Viola cazorlensis*, su distribución, sistemática y biología. *Cavanillesia*, 7: 133–148.
- MILLER, R. B., 1981. Hawkmoths and the geographic patterns of floral variation in *Aquilegia caerulea*. *Evolution*, 35: 763–774.
- NILSSON, L. A., 1978. Pollination ecology and adaptation in *Platanthera chlorantha* (Orchidaceae). *Botaniska Notiser*, 131: 35–51.
- NILSSON, L. A., 1988. The evolution of flowers with deep corolla tubes. *Nature*, 334: 147–149.
- QUÉZEL, P., GAMISANS, J. & GRUBER, M., 1980. Biogéographie et mise en place des flores méditerranéennes. *Naturalia Monspeliensis, Hors Série*: 41–51.
- RICK, C. M., HOLLE, M. & THORP, R. W., 1978. Rates of cross-pollination in *Lycopersicon pimpinellifolium*: impact of genetic variation in floral characters. *Plant Systematics and Evolution*, 129: 31–44.
- SCHLUTER, D., 1988. Estimating the form of natural selection on a quantitative trait. *Evolution*, 42: 849–861.
- SCHLUTER, D. & SMITH, J. N. M., 1986. Natural selection on beak and body size in the song sparrow. *Evolution*, 40: 221–231.
- SCHOEN, D. J. & CLEGG, M. T., 1985. The influence of flower color on outcrossing rate and male reproductive success in *Ipomoea purpurea*. *Evolution*, 39: 1249–1249.
- STANTON, M. L., 1987. Reproductive biology of petal color variants in wild populations of *Raphanus sativus*: I. Pollinator response to color morphs. *American Journal of Botany*, 74: 178–187.
- STANTON, M. L., SNOW, A. A. & HANDEL, S. N., 1986. Floral evolution: attractiveness to pollinators increases male fitness. *Science*, 232: 1625–1627.
- STANTON, M. L. & PRESTON, R. E., 1988. Ecological consequences and phenotypic correlates of petal size variation in wild radish, *Raphanus sativus* (Brassicaceae). *American Journal of Botany*, 75: 528–539.
- STEBBINS, G. L., 1970. Adaptive radiation in angiosperms. I. Pollination mechanisms. *Annual Review of Ecology and Systematics*, 1: 307–326.
- THOMSON, J. D. & STRATTON, D. A., 1985. Floral morphology and cross-pollination in *Erythronium grandiflorum* (Liliaceae). *American Journal of Botany*, 72: 433–437.
- TUTIN, T. G., HEYWOOD, V. H., BURGESS, N. A., MOORE, D. M., VALENTINE, D. H., WALTERS, S. M. & WEBB, D. A. (Eds), 1964–1980. *Flora Europaea*. Cambridge: Cambridge University Press.
- VALENTINE, D. H., 1962. Variation and evolution in the genus *Viola*. *Preslia*, 34: 190–206.
- VAN VALEN, L., 1978. The statistics of variation. *Evolutionary Theory*, 4: 33–43.
- WASER, N. M., 1983. The adaptive nature of floral traits: ideas and evidence. In L. Real (Ed.), *Pollination Biology*: 241–285. Orlando: Academic Press.
- WASER, N. M. & PRICE, M. V., 1981. Pollinator choice and stabilizing selection for flower color in *Delphinium nelsonii*. *Evolution*, 35: 376–390.
- WILKINSON, L., 1986. *STATISTAT: The System for Statistics*. Evanston: SYSTAT Inc.