115

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research report

SELECTION ON COMPLEXITY OF COROLLA OUTLINE IN A HAWKMOTH-POLLINATED VIOLET

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Abstract

Moth- and butterfly-pollinated flowers often have deeply dissected corollas, and this pattern has been related to a preference by these pollinators for dissection of floral outlines. This note examines the relationship between complexity of corolla outline and fecundity in the hawkmoth-pollinated violet *Viola cazorlensis* (Violaceae). The complexity of the corolla contour, measured using the fractal dimension of the outline, differed significantly among plants and was directly related to absolute fruit production and proportional fruit set. The results support the notion that moths discriminate among plants in favour of those with more deeply dissected corollas.

Introduction

It has long been accepted that the diversity of floral features exhibited by animal-pollinated angiosperms is largely a consequence of the contrasting selective pressures exerted by a morphologically and behaviourally diverse array of pollinating agents (Grant, 1949; Leppik, 1957; Stebbins, 1970; Faegri and van der Pijl, 1971). Intraspecific correlations between plant fecundity and floral traits documented by recent studies have generally supported this view.

Most of these studies have investigated the reproductive consequences of variation in either discontinuously varying (e.g. colour, scent; Kay, 1976; Hannan, 1981; Galen *et al.*., 1987; Stanton, 1987; Galen and Newport, 1988) or metric (e.g. Nilsson, 1988; Stanton and Preston, 1988; Galen, 1989; Herrera, 1990a; Johnston, 1991) floral characters.

Nevertheless, even though the idea that floral shape has played a significant role in floral evolution is implicit in all classical treatments of floral morphology (Leppik, 1957; Stebbins, 1970; Faegri and van der Pijl, 1971), the relationship between plant reproductive success and flower shape has been examined much less frequently. The scarcity of empirical studies examining intraspecific variation of flower form in relation to reproductive success must be attributed to the same practical complexities involved in the quantification of form that have historically hindered the development of objective morphometric methods (Rohlf, 1990; Bookstein, 1992). New quantitative tools developed in recent years for the analysis of form (e.g. Rohlf, 1990; Rohlf and Bookstein, 1990; Reyment, 1991; Bookstein, 1992) are of immediate application to many plant structures, yet they have largely remained in the realm of animal investigations, and have received little attention in plant studies (but see, e.g., White and Prentice, 1988; White *et al.*, 1988; Lönn and Prentice, 1990; Ray, 1990; Herrera, 1993).

This note examines the relationship between complexity of corolla outline and reproductive success in *Viola cazorlensis* Gand. (Violaceae), a hawkmoth-pollinated violet. Moth- and butterfly-pollinated flowers often have deeply dissected corolla outlines, and this pattern has been related to a greater sensitivity and/or preference of lepidopterans for dissection of outlines (Vogel, 1954; Proctor and Yeo, 1973; Faegri and van der Pijl, 1971). To my knowledge, however, no empirical study has previously examined this hypothesis on a quantitative basis.

I address here the following question: Does the variability in complexity of corolla outline exhibited by plants of *V. cazorlensis* translate into differential reproductive success? Reproductive success will be assessed by examining fruit set and fruit production of individual plants, and complexity of corolla contours will be estimated by computing the fractal dimension of the outlines. In addition to answering the biological question raised above, a further aim of this contribution is to bring to the attention of researchers in plant reproductive biology a novel morphometric tool that, despite its simplicity, may provide useful quantitative descriptions of flower shape.

Study plant and methods

Results reported in this note were obtained during a long-term investigation of the ecology and reproductive biology of *Viola cazorlensis* in the Sierra de Cazorla (Jaén province, southeastern Spain), most of which has been reported elsewhere (Herrera, 1988, 1989, 1990a,b, 1993). The plant is a perennial, suffruticose violet, endemic to a geographically restricted mountain area in southeastern Spain (Gómez-Campo, 1987).

Flowers are produced singly on peduncles arising from the axils of the leaves. Regardless of variation in the inclination of the substrate, petal blades always fall in a nearly vertical plane, and flowers lack a 'landing platform' for pollinators (drawings and photographs of flowers are shown in Herrera, 1990a, 1993). Flowers are scentless (to the human nose), have pinkish-purple corollas, and are characterized by a distinctive long (mean length ± *SD* = 25.0 ± 3.9 mm, range 8-42 mm, $N = 1089$) and thin spur. Nectar accumulates at the tip of the spur, and can only be reached by long-tongued insects. Pollination is virtually restricted to a single insect species, the day-flying hawkmoth *Macroglossum stellatarum* (Lepidoptera: Sphingidae). Although flowers are self-compatible, fruit set in the absence of pollinators (i.e. within exclosures in the field) is negligible in comparison to flowers exposed to natural pollination (outside exclosures) (Herrera, 1990a, 1993).

Variability in corolla shape was studied in 1991 on a sample of 230 flowers from 52 individually-marked plants of a single local population ('Cruz de Quique' site; see description in Herrera, 1993). Close-up photographs were taken of individual flowers in the field over the whole flowering period, using a standardized protocol (front view, white cardboard as background, and linear scale and identification label added for reference). Flowers were photographed after all petals had fully expanded to final size (36-48 hours after opening), and well before any petals exhibited the characteristic twisting associated with floral senescence in this species (usually starting 7-10 days after flower opening). I am therefore confident that variations observed in floral outline reflect intrinsic differences between individual flowers and were not due to age-dependent changes in floral morphology.

The corolla contour of each flower was digitized from black-and-white prints using a digitizing tablet, and these digitized outlines were then used for computing the fractal dimension of corolla contours (see below). The total number of flowers and fruits produced by each marked plant during the study season (April-June 1991) was also recorded.

Concepts derived from fractal geometry (Mandelbrot, 1983) have been successfully applied in recent years to a variety of biological issues (e.g. Sugihara and May, 1990; Shorrocks *et al.*, 1991; Green, 1991; Hegde *et al.*, 1991), and potential applications to the analysis of shape were recently suggested by Reyment (1991). Of particular relevance is the concept of fractal dimension D (Mandelbrot, 1983) that may be used as a simple, objective measure of the complexity of an outline (Sugihara and May, 1990; Reyment, 1991). I used the 'dividers method' for computing D. This procedure involves stepping along a curve (corolla outline in this case) with dividers to see how apparent length, $L(\delta)$, changes as the dividers are brought closer together. Using a spectrum of widths of dividers (δ), D is determined as 1.0 minus the slope of the linear regression of $\log L$ versus $\log \delta$ (Sugihara and May, 1990). To account for the possible variance in L due to differences in the starting point, I obtained 10 replicates of D for each flower by randomly choosing different starting points on the curve (Sugihara and May, 1990). The mean D of these replicates was used to characterise the complexity of its floral contour.

The Fractal-D program (Slice, 1989) was used for computations of D, and the SAS package (SAS Institute, 1988) for statistical analyses. The distribution of D values for individual flowers did not depart significantly from normality, and the raw data were used in the analyses. The distributions of the flower and fruit production figures of individual plants were strongly skewed to the right, and these data were log-transformed for the analysis.

Results

Viola cazorlensis flowers exhibit broad variability in both size and shape components (Herrera, 1988, 1990a,b, 1993). This variation translates into differences in the complexity of the corolla outline, and D figures adequately capture this variation (Fig. 1). High D values correspond to deeply dissected corollas with rather narrow and distinct petal lobes, while low D values are associated with floral contours having shallow clefts (Fig. 1).

Mean D (± *SD*) for individual flowers, all plants combined, was 1.327 ± 0.046 ($N = 230$ flowers; Range = 1.140-1.450). Plants differed significantly in the mean fractal dimension of their corolla outlines ($F = 4.99$, df = 51,178, $P << 0.001$), and individual variation accounted for a large proportion of total variance in D ($R^2 = 0.588$). Mean D values for individual plants ranged between 1.140 and 1.385.

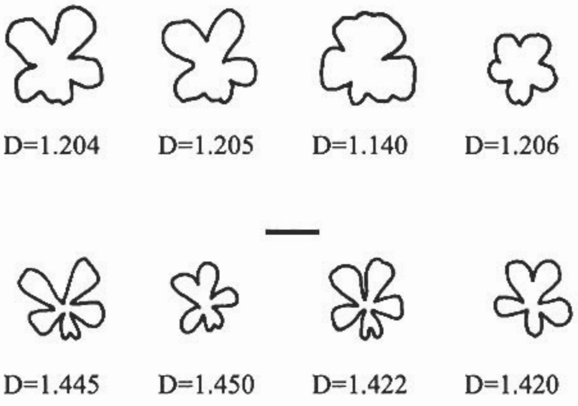

Fig 1. Variability in corolla shape (front view) of *V. cazorlensis* flowers. Shown are the outlines of flowers exhibiting the lowest (top row) and highest (bottom row) values of fractal dimension (D) in the studied sample. Contours were traced directly from photographs. All flowers are not drawn to exactly the same scale (black bar approximately equals 1 cm).

The relationship between corolla contour complexity (D) and fruit production was examined using regression methods. During the study season, insect and vertebrate herbivores destroyed some fruits from several of the marked plants ($N = 7$) before maturation, and data from these plants are omitted from the analyses. Variation in reproductive success occurring in the remaining set of individuals ($N = 45$) will thus be exclusively due to differences between plants in fruit set.

The simple linear regression of number of fruits ($\log_{10}$-transformed, log*FR*) on mean D of individual plants was statistically significant ($F = 6.69$, df = 1,43, $P = 0.013$). The regression equation ($\log FR = -3.012 + 2.866D$; $R^2 = 0.14$) indicates that fruit production increased significantly with increasing corolla complexity. Plants with the most deeply dissected corolla outlines tended to produce, on average, the largest number of fruits.

Table 1. Results of the regression model relating fruit production of individual *V. cazorlensis* plants ($\log_{10}$-transformed) to flower production ($\log_{10}$-transformed, log *FL*) and mean fractal dimension of corolla outline (D). The model is highly significant ($F = 50.44$, df = 2,42, $P << 0.0001$) and explains a substantial proportion of individual variance in fruit production ($R^2 = 0.71$).

Variable	Coefficient	Standard error	*t*-value	*P*
Intercept	-1.966	0.868	2.266	0.029
Log*FL*	0.693	0.077	9.036	<0.0001
D	1.565	0.669	2.339	0.024

As the number of fruits produced by a plant depends on the number of flowers, another regression was run including this latter attribute ($\log_{10}$-transformed, log*FL*) as a further independent variable.

These results are summarised in Table 1. After accounting for differences between plants in flower production, the regression coefficient for D was again significant, indicating that when the effect of differences in the number of flowers is removed statistically, fruit production is still positively related to complexity of corolla outline. No significant correlation existed between mean D of individual plants and their flower production (log-transformed) ($r = 0.215$, $N = 45$, $P = 0.156$).

Discussion

Significant phenotypic selection (*sensu* Lande and Arnold, 1983; Arnold and Wade, 1984) on complexity of corolla outline occurred in the *V. cazorlensis* population during the study season. Plants differed significantly in the complexity of their corolla outlines, and this magnitude was positively correlated with absolute fecundity (number of fruits produced). When individual variation in flower production was accounted for, plants with deeply dissected corollas still tended to be more fecund than those with less-marked petal lobes. Only individuals free from herbivory were included in the sample, and flower production was unrelated to individual mean D. Hence the fecundity advantage of plants with more deeply dissected corollas was due to their higher proportional fruit set (fruit: flower ratio).

Two major alternative explanations may be advanced in relation to the proximate mechanisms involved in the differential fruit set of plants varying in complexity of corolla outline. The correlation between flower shape and fruit set may be independent of pollination phenomena. If floral morphology and fruit set are both related to a third, unknown characteristic of individual plants, then their correlation would be an indirect, spurious one. No data are available to test this hypothesis. Alternatively, the correlation across plants between fruit set and complexity of corolla outline may arise from differential pollination success due to discrimination by pollinators. The evidence available is consistent with this explanation.

Insect pollinators are known to discriminate between conspecific plants on the basis of metric floral characters (e.g. corolla size, inflorescence height) leading to individual variation in flower visitation rates and pollination success (Galen and Newport, 1987; Stanton and Preston, 1988; Galen, 1989; Young and Stanton, 1990; Stanton *et al.*, 1991). The general scarcity at the study site of *M. stellatarum*, the single hawkmoth pollinator of *V. cazorlensis* (Herrera, 1993) precluded a direct assessment of individual differences between the plants studied in pollinator visitation rates. Indirect evidence however, supports the idea that hawkmoth discrimination among plants is involved in the pattern of differential fecundity documented in this study (see also Herrera, 1993). Results of experimental pollinations have shown that fruit set in *V. cazorlensis* increases with increasing frequency of pollination (Herrera, 1993) and individual differences in fruit set could thus arise from differential attractiveness to pollinators due to differences in complexity of corolla outline.

The ability to remember and discriminate between floral shapes has been demonstrated for visually-orientated insect pollinators (Levin, 1969; Anderson, 1977; Gould, 1985, 1986; Pellmyr, 1988). Some lepidopterans are able to discriminate between different shapes (Rausher, 1978; Levin, 1969) and Knoll (1922) demonstrated that the day-flying, visually-orientated *M. stellatarum* possesses this ability to a high degree. Furthermore, the finding that complexity of corolla outline was directly related to fruit set is consistent with the notion that some butterflies and moths have an innate preference for long, deeply dissected flower outlines (Proctor and Yeo, 1973).

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