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Inter- and intra-floral heterogeneity of nectar production in *Helleborus foetidus* L. (Ranunculaceae)

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Three southern Spanish populations of *Helleborus foetidus* L. (Ranunculaceae) were sampled for nectar content in the absence of nectarivorous flower visitors. Nectar volume was measured in individual nectaries of flowers at the same stage in the anthesis cycle. Total nectar content per flower was extremely variable between plants within populations and between flowers within plants, but much less so between populations. Average sugar content per flower was roughly similar in the three populations sampled. High variances in nectar abundance occurred also among nectaries within the same flower. Heterogeneity in pollinator rewards thus occurs simultaneously at several spatial scales, namely between nectaries, between flowers and between plants. This implies a strong component of uncertainty to foraging pollinators, which may therefore tend to avoid *Helleborus* flowers.

KEY WORDS:—*Helleborus foetidus* – nectaries – nectar production – Ranunculaceae – Spain.

CONTENTS

INTRODUCTION

Differential feeding value of individual flowers to pollinators may substantially affect foraging behaviour of pollinating agents and hence potentially gene flow (Feinsinger, 1978; Levin, 1979; Real, 1981a; Waddington, 1981; Zimmerman, 1982). Despite their profound potential significance, studies aimed at documenting patterns of interfloral variation in pollinator rewards are remarkably scarce, as stressed by Real (1981a) (but see Heinrich, 1975; Feinsinger, 1978; Pleasants & Zimmerman, 1979; Brink & deWet, 1980). The existence of conspicuous intrafloral variation in nectar abundance is shown here, revealing that nectar rewards may be distributed heterogeneously within flowers when several separate nectaries occur.

Helleborus foetidus is a winter-blooming woodland perennial herb with

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pendulous-flowered, terminal inflorescences (Proctor & Yeo, 1973; Corbet *et al.*, 1979a). Several tubular, petal-derived nectaries (Percival, 1965) are radially inserted on the floral axis. The bases of contiguous nectaries never make contact, being usually 1.5–3 mm apart, and their apertures (about 1 × 2 mm) are always separated by more than 4 mm. They thus represent discrete nectar sources for pollinators. Flowers are markedly protogynous (Percival, 1965; Proctor & Yeo, 1973; and personal observations) and, in the populations we have studied, nectaries secrete nectar from the start of the female phase, wilting and falling by the middle of the male period. Long-tongued insects, mainly honeybees and bumblebees, are the principal flower visitors, and presumably pollinators, of *H. foetidus* (Proctor & Yeo, 1973).

STUDY SITES AND METHODS

Field work was carried out during 9–12 February 1982 in the Sierra del Pozo mountains, Serranía de Cazorla-Segura, Jaén province, southern Spain. *Helleborus foetidus* is common there above 1100 m growing on relatively disturbed sites with rocky soils, such as old gaps in pinewoods, margins of rocky streams, and trail edges. Reliance on these microhabitats results in a distinct patchy distribution of the species.

Three populations (A, B & C) of *H. foetidus* were sampled, located at elevations of 1300 m, 1200 m and 1160 m respectively. Two of the populations were on rocky soils near the margins of temporary streams, the other (population A) was close to an old house settlement. Populations were between 950 and 1900 m apart.

Six or seven plants of similar size, as spaced as possible, were arbitrarily chosen at each site. Five or six flowers were collected from every plant from different inflorescences and kept in closed plastic bags until examination (always within 1 h of collection). Our restrictive conditions for flower selection (see below) precluded the acquisition of larger flower samples per plant. Collections were done at about the same time of day (13.00–15.00 hours GMT) at the three sites on consecutive days. Weather was similarly cool and cloudy (but rainless) on the three collection dates. Particular care was taken to collect all flowers at exactly the same stage in the anthesis cycle, namely the transition between the female and male phases. At this stage the outermost stamens begin to elongate and some of them may dehisce, while styles still stand well separated from anthers. Usually only one flower was at this particular stage in any one inflorescence.

Sepals were removed from each flower collected to expose the nectaries, and each of the latter was carefully cut along its concave inner side. No nectar was lost during this manipulation, which was necessary to extract all the nectar present. The number of nectaries in each flower was recorded, and the amount of nectar was measured separately for every nectary with calibrated micropipettes. Sugar concentration in nectar was measured with a temperature-compensated hand refractometer. Nectar from several nectaries had to be pooled to obtain sufficient quantity for refractometer reading.

The potential insect pollinators of *H. foetidus* were almost totally absent during the study period. Despite special attention, in the course of a 1-week stay in the field only two foraging bumblebee queens were observed in the study area. Honeybees were never seen. We did not observe any insect actually foraging at

H. foetidus blossoms. The patterns of intra- and interfloral variation in nectar abundance shown below should be attributed entirely to plant-related features, and not to the eventual result of pollinator foraging activity.

RESULTS

Interfloral heterogeneity: A majority of flowers examined had five nectaries (60.9%), the observed range being 3–8 nectaries/flower. There is substantial intra- and interpopulation variation in this magnitude (Fig. 1). Observed ranges were 3–6, 5–7 and 3–8 nectaries/flower for populations A, B and C, respectively. Interpopulation variation in nectary numbers (all plants combined) is statistically significant (Fig. 1). Within sites, variation between plants in the size of the nectary complement is also significant at the three populations.

The total amount of nectar present per flower varies widely within and between plants, and also, although much less markedly, between sites (Fig. 2). The mean volume of nectar present per flower (all plants combined) was similar in A and C (7.1 ± 0.9 μl and 6.9 ± 0.8 μl, respectively; mean ($\bar{x}$) ± 1 s.e.), and

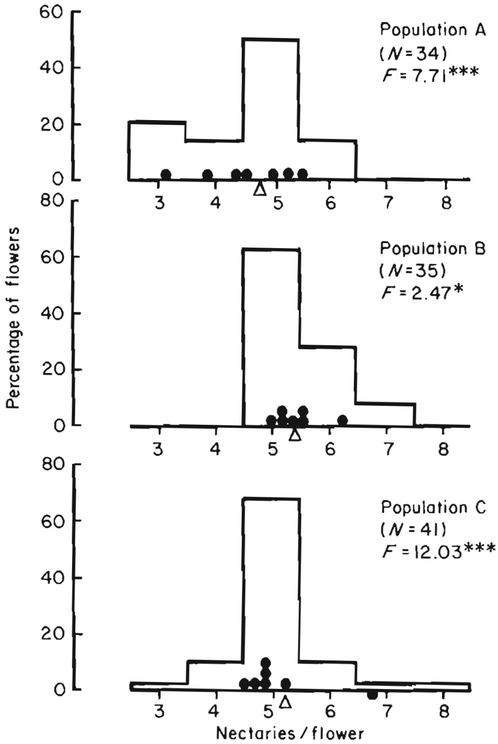

Figure 1. Between-site variation in the number of nectaries per flower. Flowers were collected from 6, 7 and 7 plants at populations A, B and C, respectively (N = number of flowers examined at each site). Dots on the horizontal axis denote within-plant means for nectary number, and the triangles mark population means. Difference between sites is statistically significant ($F = 9.88$, d.f. = 2, 107, $P < 0.001$). F-values presented for each population refer to interplant variation in nectary number per flower; at the three sites plants statistically differ with regard to this variable. There is therefore substantial intra- and interpopulation heterogeneity in the nectary complement of individual *Helleborus foetidus* flowers. *, $P < 0.05$; ***, $P < 0.001$.

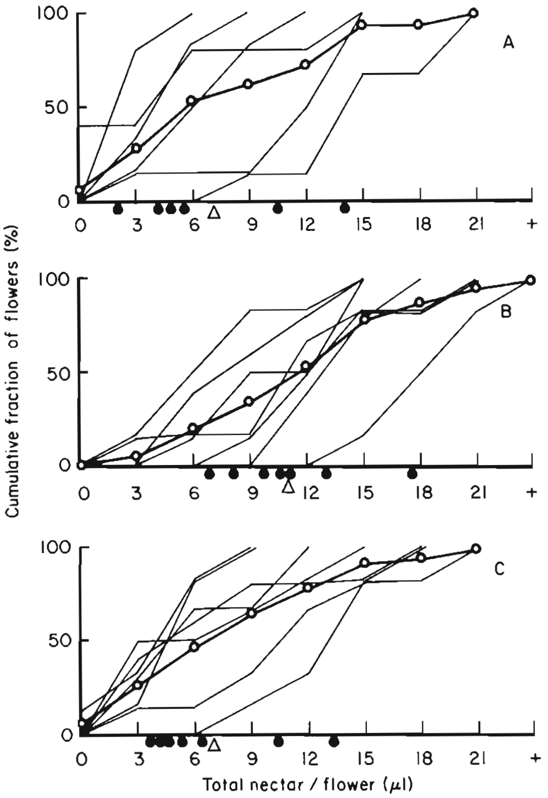

Figure 2. Cumulative frequency distributions of total nectar content per flower at the three study populations of *Helleborus foetidus*. Curves for individual plants (thin lines) and for the population as a whole (all plants combined, thick lines) are plotted for the three sites. Dots on the horizontal axis mark plant means, and triangles denote population means (all plants combined).

significantly higher in B (11.0±0.8 µl). On the other hand, sugar concentration of nectar (on a w/w basis) was found to be similar in populations A and C (55.9±0.35% and 50.3±1.06%, respectively), and significantly lower in population B (34.6±0.79%). As a result, the average weight of sugar present per flower, computed after transforming w/w concentrations to w/v percentages (Weast & Astle, 1980: D-282), was remarkably constant among populations (5.0, 4.2 and 4.4 mg; A, B and C, respectively).

Between-plant differences in nectar volume per flower are significant ($P < 0.01$ or better) at the three sites ($F = 7.24$, 4.02 and 3.85; A, B and C, respectively). At sites A and C, nectar content of individual flowers varies between 0 and 20 µl, and at site B between 3 and 22 µl (all plants combined). Within-plant means oscillate between 2 and 14, 7 and 13, and 4 and 13 µl/flower (A, B and C, respectively). Nectar content of flowers on the same plant encompasses a broad range; the difference between flowers with the greatest and smallest amounts is often in the order of 10–15 µl (Fig. 2). The slope and shape of most within-plant and all within-site cumulative frequency distributions of total nectar content are indicative of high variance and extremely platykurtic distributions of nectar abundance across flowers, both within and between plants.

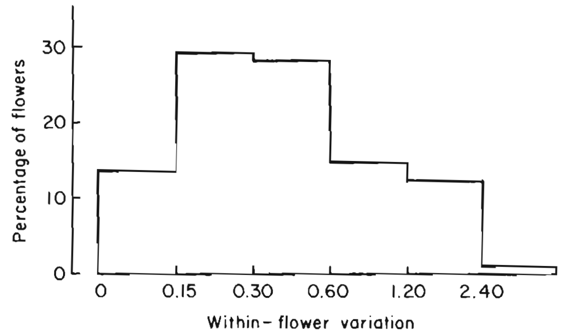

Figure 3. Frequency distribution of within-flower variation in nectar content, plants from the three populations combined (see text). Intrafloral heterogeneity in nectar abundance was estimated with the coefficient of variation of nectar volume per nectary (C.V. = S.D./$\bar{x}$).

Intrafloral heterogeneity: Intrafloral variance in nectar rewards was evaluated with the coefficient of variation of nectar content (volume) per nectary (C.V. = S.D./$\bar{x}$). Within-population comparisons did not reveal any significant difference among plants in the levels of intrafloral heterogeneity in nectar abundance ($P = 0.22$, 0.74 and 0.13; populations A, B and C, respectively), and data from all plants within a population were combined. Interpopulation comparisons similarly failed to show significant heterogeneity among populations in levels of intrafloral heterogeneity ($P = 0.24$). C.V. values from all sites have therefore been fused into a single sample and its frequency distribution is plotted in Fig. 3.

Fifty-seven per cent of flowers examined had a C.V. >0.30, and 13% had a C.V. > 1.20, indicating that substantial variability in nectar content occurs among nectaries of individual flowers. In addition to the common occurrence of high levels of interfloral heterogeneity, Fig. 3 also reveals the existence of a broad range of C.V. values (0.08 -2.45). This indicates an extreme unpredictability in the levels of within-flower (among nectaries) variation of nectar abundance.

We attempted to relate nectar content of individual nectaries to their position in the flower. Contiguous nectaries often tended to be relatively more similar in nectar content than those more distant on the circular array, but this was not a rule for all plants, nor even for different flowers on the same plant, and results of the analysis were inconclusive at this respect.

DISCUSSION

Some degree of intraspecific variability in floral rewards to pollinators seems to be habitual in nature, as judged from the information presented in numerous studies (Heinrich, 1975; Carpenter, 1976; Gill & Wolf, 1977; Feinsinger, 1978; Pleasants & Zimmerman, 1979; Brink & deWet, 1980; Thomson & Plowright, 1980; Brink, 1982). Heterogeneity patterns may result either from the foraging activity of nectarivorous flower visitors and/or from features inherent to the plants themselves (Heinrich, 1975; Feinsinger, 1978; Brink & deWet, 1980; Zimmerman, 1981; Brink, 1982). In the present study, the almost total absence of flower visitors leads to the confident attribution of the observed variation exclusively to plant features. Nectar production rates and sugar concentration often vary with flower age (Pyke, 1978; Bond & Brown, 1979; Gill & Conway, 1979; George, 1980), time of day (Corbet, 1978; Frost & Frost, 1980; Schemske, 1980; Real, 1981b) and weather conditions (Corbet *et al.*, 1979a; Corbet, Unwin & Prŷs-Jones, 1978b). Assuming that all *H. foetidus* flowers sampled for the present study were approximately the same age, and since all collections were done at the same time of day and similar weather, differences in nectar abundance reported above should be considered indicative of differential mean nectar secretion rates over the female period of anthesis.

Results have revealed an extreme variability in nectar production rates, which simultaneously occurs at three different spatial scales: between nectaries within flowers, between flowers within plants, and between plants within populations. Equivalent variation was found at the three populations examined. We have failed to find any published records suggesting similar levels of variability in nectar production rates for other insect-pollinated plants. Considering either the nectary or the flower as the elemental food source unit from the viewpoint of pollinators, results reveal an extreme unpredictability in nectar production within *H. foetidus* populations. Judging from the nectar volume present at any given flower or individual nectary, the chances of rightly predicting nectar content at the nearest nectar source are consistently low. The number of nectaries present per flower, that is the number of elemental nectar sources which an insect forager should sequentially probe, also varies substantially within and between plants. High levels of spatial uncertainty at various size scales thus result from the large variances illustrated in Figs 1–3.

Unfortunately, it is not possible to know to what extent the patterns of variation in nectar abundance shown above would have changed in the presence of pollinators. Nevertheless, it seems reasonable to assume, in the light of previous studies, that although mean nectar volume will certainly decrease, substantial inter- and intrafloral heterogeneity will still persist (Heinrich, 1975; Gill & Wolf, 1977; Hodges & Wolf, 1981), and variability may even be enhanced by pollinators (Feinsinger, 1978; Zimmerman, 1981; Brink, 1982).

The implications of variance in nectar rewards to pollinator foraging behaviour and pollen flow have begun to be recognized. Available information suggests that:

Under some circumstances, variability in nectar rewards may be beneficial in that it promotes between-flower movements of pollen vectors and increases flight length, while simultaneously allowing for some energy saving (Feinsinger, 1978; Real, 1981a).

Unpredictability in nectar rewards is probably unfavourable to pollinators, which incur greater energy expenditures and foraging risks. Bumblebees have been shown to avoid risk during foraging, actively selecting the less variable flowers regardless of the absolute values of average nectar availability (Real, 1981a; Waddington, Allen & Heinrich, 1981).

There is thus clearly conflicting interests in the plant-pollinator mutualistic system with regard to variability in pollinator reward. Extreme variances as those reported here may therefore lead to intense avoidance by pollinators, and this may help to explain the observation reported by Percival (1965: 28) of bees ignoring *Helleborus* blossoms in favour of species producing much smaller rewards. The absence of bumblebees from our Cazorla study area, where *H. foetidus* was the only blooming plant on study dates, could perhaps be explained similarly in terms of an extreme risk avoidance. At nearby habitats at 1150 m elevation, foraging bumblebees are commonly seen throughout the winter visiting the small flowers of *Arbutus unedo* L. (Ericaceae), which provide much smaller, though less variable, amounts of nectar per flower, even during environmental conditions much harsher than those prevailing at the time of this study (C. M. Herrera, personal observation).

We do not know to what extent *H. foetidus* depends on pollinators for seed set in our study area. Although some autogamy is likely to occur, it probably accounts for a small fraction of total seed set (Proctor & Yeo, 1973). Therefore it is not possible to assess the potential negative effects on reproduction derived from the patterns reported above.

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