

Discrimination by pollen-collecting bumblebees among differentially rewarding flowers of an alpine wildflower, *Campanula rotundifolia* (Campanulaceae)

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We studied bumblebees (*Bombus wurflenii* and *B. sichelii*) that were collecting pollen from the flowers of *Campanula rotundifolia* in an alpine meadow in Switzerland. Flower size varied greatly among individual plants, while pollen and nectar availability varied among the flowers in association with their sexual phase. Compared to random samples, bumblebees significantly favoured female phase flowers on one day, but favoured male phase flowers on the following day and this change coincided with a decline in overall pollen availability. The bumblebees' behaviour is consistent with a simple economic interpretation: initially, bumblebees favoured female phase flowers, many of which offered both pollen and nectar, but as pollen became less abundant, bumblebees increasingly favoured the relatively pollen-rich, nectarless male phase flowers, which implies that pollen was the more valued resource. Like previous studies, we showed that bumblebees are capable of responding to pollen availability, apparently by using visual assessment of pollen before landing on a flower as the basis for discriminatory foraging. Unlike previous studies, we found no evidence for discrimination among flowers on the basis of size, which was uncorrelated with pollen availability.

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The nature of pollinator behaviour has been intensively studied and we have learned that nectar foragers such as bumblebees can discriminate among flowers of plant species (Heinrich 1976) or even among conspecific floral variants (Galen 1989). Nectaring bumblebees are able to select the floral variants that offer larger amounts of nectar, apparently by associating the potential value of the reward with a visual cue (Bell et al. 1984, Cresswell and Galen 1991) and many such instances are explicable by reference to simple economic models that derive diets offering the maximum rate of nectar uptake (reviewed in

Stephens and Krebs 1986). However, bee-pollinated plants often provide pollen rewards and, despite earlier scepticism (Hodges and Miller 1981, Haynes and Mesler 1984), recent studies have demonstrated that bumblebees detect differences in pollen availability as they harvest the pollen and consequently adjust the amount of time spent on a flower or inflorescence (Buchmann and Cane 1989, Harder 1990). As yet, no published study has shown pre-alighting (remote) discrimination among floral variants on the basis of differential pollen rewards. Here, we document remote discrimination among floral

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Table 1. The descriptive measurements and the indices of variation in all flowers measured, [CV = coefficient of variation (SD/mean)], and the loading of the floral character variables on the first two principal components, PC1 and PC2. 'n.a.' means not applicable (see text).

| Number | Description | Units | Mean | CV | PC1 | PC2 |
|--------|--|---------|------|------|--------|--------|
| 1 | height of flower above ground | cm | 28.3 | 0.23 | 0.048 | -0.355 |
| 2 | ranked height on stem (1 = lowest) | integer | 1.3 | 0.40 | -0.137 | -0.052 |
| 3 | nodding angle subtended between longitudinal floral axis and ground (0 = mouth of flower down) | degrees | 83.3 | 0.49 | -0.203 | -0.089 |
| 4 | distance across flower mouth | mm | 23.7 | 0.20 | -0.137 | -0.447 |
| 5 | internal diameter of corolla tube (widest point) | mm | 16.2 | 0.18 | -0.102 | -0.431 |
| 6 | length along corolla tooth | mm | 9.6 | 0.23 | -0.185 | -0.328 |
| 7 | distance from base of flower to base of tooth | mm | 13.0 | 0.17 | -0.206 | -0.354 |
| 8 | length of style | mm | 16.0 | 0.14 | 0.082 | -0.424 |
| 9 | phase (0 = male, 1 = female) | integer | 0.9 | 0.41 | -0.416 | 0.144 |
| 10 | amount of pollen presented on style (0-3) | integer | 1.1 | 1.07 | 0.317 | -0.144 |
| 11 | number of open nectaries (0-5) | integer | 1.9 | 1.04 | -0.297 | 0.005 |
| 12 | number of stigmatic lobes reflexed (0-5) | integer | 2.6 | 0.47 | -0.386 | 0.071 |
| 13 | number of stigmatic lobes bearing pollen (0-5) | integer | 1.2 | 1.16 | -0.409 | 0.107 |
| 14 | amount of pollen on stigma (0-3) | integer | 0.8 | 1.25 | -0.383 | 0.087 |
| 15 | number of open flowers on inflorescence | integer | 1.8 | 0.51 | n.a. | n.a. |

variants by pollen-collecting bumblebees and attempt to explain their behaviour as the product of simple economic decision-making.

Methods

We studied individuals of *Bombus wurflenii* Radoszkowski and *B. sichelii* Radoszkowski foraging for pollen and nectar from the flowers of *Campanula rotundifolia* L. in an alpine meadow near Bourg St Pierre, Switzerland (45°47'N, 7°13'E). As in many Campanulaceae (Faegri and van der Pijl 1979, Richardson and Stephenson 1989), the anthers of *C. rotundifolia* shed pollen onto the style where it adheres. Pollen is presented when a flower opens, but the stigmatic lobes do not reflex for 2-3 d so that the flower is initially functionally male and then either cosexual or functionally female for a further 2-3 d, depending on whether the pollen is completely removed. The broad bases of the anthers are contiguous, but as the stigma opens they retract to create up to 5 openings to a nectiferous tissue. An inflorescence typically holds about two open flowers and the quality of the floral reward changes with their sexual phases, with male phase flowers offering pollen and female phase flowers offering variable amounts of nectar and pollen.

Our design was to detect floral preferences of bumblebees by comparing the kinds of flowers selected by bees with a sample selected at random. For the random sample, we measured all the flowers on each of about 30 randomly located inflorescences (for details of measurements, see below). By scanning the entire study area, we located bees that were foraging for pollen and measured each flower that they probed on up to 5 successively visited inflorescences. We repeated the sampling procedure on each of two consecutive days, 28 and 29 June, 1992. All randomly selected plants or quadrats were

located by using random numbers as x-y coordinates on a 1 × 1 m grid extending over the 30 × 20 m study area, which was located in the centre of the meadow where the inflorescences were dense (\bar{x} = 18.4 stems/m², SE = 4.02, n = 30 random 0.25 m² quadrats).

Floral morphometry and statistical analysis

For each flower we recorded 14 descriptors of its shape, location, sexual phase and reward levels and the number of open flowers on the supporting inflorescence (Table 1). The amounts of pollen present on the style and on the stigma were scored on a 4 point integer scale (0-3). To calibrate the scales, we collected 13 flowers from each category and preserved them in microcentrifuge vials containing 70% ethanol. To count pollen on the style, the style was macerated in the collection vial using a glass rod and, after centrifugation, the ethanol solution was removed and replaced with 0.25 ml of 0.7 M mannitol solution containing acetocarmine dye. The pellet of pollen and debris was resuspended in the mannitol solution and pollen grains were counted in 8 subsamples placed in a haemocytometer. We analysed the variation among the field-scored categories by ANOVA. Because many pollen grains had adhered to the non-receptive back of the stigma, we were unable to count pollen on the stigmatic surface in typical microscope preparations. Instead, we used fluorescence microscopy to examine pollen tubes in the style (Martin 1958). Styles were scored into one of four categories depending on the amount of fluorescence observed (there were usually too many pollen tubes to distinguish them individually). We compared our field scores of stigmatic pollen with the score of the intensity of pollen tube fluorescence by correlation analysis.

To compare statistically the bee-selected with the randomly-selected flowers, we randomly chose one inflorescence from the data collected from each bumblebee and then randomly chose one flower from those probed

Table 2. Analysis of variance in PC1 scores (sexual phase) of floral characters among bumblebee-chosen and randomly selected samples. ** indicates $p < 0.005$.

| Source | df | MS | F | p |
|-----------|----|-------|------|---------|
| Treatment | 3 | 17.15 | 5.31 | 0.002** |
| Error | 90 | 3.23 | | |

and compared these with one randomly chosen flower from each randomly selected inflorescence (this procedure eliminates pseudo-replication). The 14 descriptors of these flowers (characters 1–14; Table 1) were subjected to principal component analysis (PCA) and we discarded components with eigenvectors less than 1 (Frey and Pimental 1978). The PCA scores of each sample were then compared by ANOVA and significant heterogeneity among means was further analysed by Student-Newman-Keuls (S-N-K) tests (Snedecor and Cochran 1968). Our tests are conservative because the random sample probably contained flowers that might have been chosen by a bumblebee. Therefore, the bumblebee-selected sample must be quite different from the random sample to return a statistically significant comparison. All analyses presented here except the S-N-K tests were performed using MINITAB (Ryan et al. 1985).

Correlates of pollen production

To discover whether pollen production was correlated with other aspects of floral form, we located 26 stems that had a flower in bud and a companion flower in female phase. We preserved the bud in ethanol and recorded the descriptors of flower size (characters 4–9, Table 1) on the companion. We subsequently estimated the amount of pollen in each bud as described above and regressed this on the first principal component of the flower size descriptors of the companion. To validate the use of paired flowers and to demonstrate the similarity of flowers within inflorescences, a pair of flowers was chosen at random from each of the randomly selected inflorescences and the size descriptors of these flowers were reduced by PCA. The scores on the first principal component of each pair comprised a bivariate datum and a least-squares relationship was sought by regression; perfect floral similarity within an inflorescence would be indicated when all of the data lay on a line with slope of 1 passing through the origin.

Quantifying nectar rewards

To confirm the presence of nectar in the flowers, we used a filter paper wick (McKenna and Thomson 1988) to probe each of 30 flowers that had 5 openings between the anther bases. Each wick was mounted on an insect pin and air-dried for storage. To redissolve the sugars, each wick was placed in a vial with 1 ml of distilled water and heated in a boiling water bath for 10 min. Total carbohydrate content of each solution was estimated using the anthrone colorimetric technique (Roe 1955). Because the

Table 3. Means of PC1 scores (sexual phase) of floral characters among bumblebee-chosen and randomly selected samples. Differing letters indicate significant differences between means by S-N-K test. Standard error and sample size in parentheses.

| | Day 1 | Day 2 |
|-----------|---------------------|---------------------|
| Bumblebes | -1.11 b (0.265, 12) | 0.87 a (0.552, 19) |
| Random | 0.51 a (0.321, 33) | -0.67 b (0.280, 30) |

sugar content of the samples is estimated by comparison to a sucrose standard curve, we report all assays in units of μg sucrose equivalents (μgSeq).

Results

The population was highly variable in all aspects of floral form, but particularly in characters relating to reward availability (pollen on style, number of nectaries) and plant reproductive success (pollen on stigma) (Table 1).

After the PCA, the first 5 principal components were retained and they explained 26, 18, 11, 8 and 8 percent of the total variation respectively (total = 71%). However, we consider in detail only the first two components (Table 1) because they alone have a straight-forward biological interpretation: PC1 reflects variation associated with sexual phase (characters 9–14 load most heavily) while PC2 reflects variation in flower size (characters 4–8 load most heavily).

Data were obtained from visits by 31 bumblebees. Most bumblebees were individuals of *Bombus wurflenii* (day 1: 10 out of the 12 bees observed; day 2: 15 out of 19) and the remainder were of *B. sichelii*. All individuals were workers except two queens of *B. wurflenii*. We pooled the data from both species because we are interested in the response to the flowers of the bumblebee guild, rather than each individual species.

Floral phase, as indicated by scores of PC1, varied significantly among the four sample groups (ANOVA, $P < 0.001$, Table 2). On day 1, bumblebees chose flowers that were significantly more female (negative PC1 scores) than random samples ($P < 0.05$, S-N-K test, Table 3). Bumblebees' choices differed significantly between days and on day 2, when there were more female flowers

Table 4. Contingency table analysis of the frequencies of exclusively female phase flowers (pollen score = 0) in randomly selected samples on each of two days. Expected values in parentheses. $\chi^2 = 8.29$, $P < 0.05$.

| | Pollen score | |
|-------|--------------|-----------|
| | 0 | ≥ 1 |
| Day 1 | 8 (13.6) | 25 (19.4) |
| Day 2 | 18 (12.4) | 12 (17.6) |

Table 5. Quantification of field scores of the amount of pollen present on a flower's style by comparison with the number of pollen grains counted in microscope preparations.

| Score | Grains on style | | |
|-------|-----------------|--------|----|
| | \bar{x} | SE | n |
| 0 | 2.575 | 375.3 | 13 |
| 1 | 11.077 | 940.0 | 13 |
| 2 | 25.038 | 2579.4 | 13 |
| 3 | 42.404 | 2696.1 | 13 |

in the population, bumblebees chose flowers that were significantly more male than random samples ($P < 0.05$, S-N-K test, Table 3).

We suspected that the change in bumblebee preferences resulted from a changed resource distribution, particularly the availability of pollen. Indeed, the mean pollen availability in the randomly sampled flowers declined from day 1 (character 10: $\bar{x} = 1.14$, $SE = 0.200$, $n = 33$) to day 2 ($\bar{x} = 0.73$, $SE = 0.191$, $n = 30$). Moreover, the frequency of flowers offering any pollen was significantly dependent on sampling day ($P < 0.005$, chi-squared contingency table test, Table 4). We conclude that bumblebees increasingly favoured male phase flowers as pollen became scarcer.

No significant heterogeneity among the samples was found among the scores on the remaining principal components (ANOVA, $P > 0.05$). In particular, there was no significant heterogeneity in scores of flower size (PC2) (ANOVA, $P > 0.6$).

Regression analysis of the PC1 scores of size measures of paired flowers (F1, F2) demonstrated a significant similarity within an inflorescence ($F1 = 0.33 \times F2 + 0.29$, $r^2 = 0.21$, $P < 0.005$). Of the 26 buds in which we measured pollen and took morphometric data on a companion flower, two contained no pollen and were by far the smallest in the sample (mean distance across flower mouth = 16 mm; mean of random samples = 23.7, $SE = 0.60$, $n = 63$). Excluding these male-sterile flowers, there was no significant relationship between the floral descriptors and pollen production. PCA performed on the morphometric data of the remaining 24 samples yielded a single component (PC1) with eigenvalue greater than 1 that accounted for 55% of the total variance and that was highly correlated with all floral size characters ($r < -0.37$). Regression analysis found no significant correlation between PC1 and pollen production ($P > 0.2$). Male-sterile plants were rare so that, in general, while pollen production is highly variable among individuals, potential pollen reward is not indicated by flower size.

The categorical field scores of the amount of pollen adhering to the style consistently represented differences in the number of grains counted in microscope preparations (ANOVA, $P < 0.001$, Table 5). Field scores of pollen on the stigma were highly correlated with scores of the

intensity of pollen tube fluorescence in the style ($r = 0.65$, $P < 0.001$, $n = 36$).

The wicks that were inserted into floral nectaries yielded significant amounts of nectar sugars ($\bar{x} = 82.5$ μgSEq , $SE = 9.97$, $n = 30$) compared to control wicks ($\bar{x} = 14.1$, $SE = 0.47$, $n = 4$). This amount of sugar represents approximately 0.1 μl of a typical nectar, which contains 40% sugar by weight.

Discussion

Previous studies have shown that nectaring bumblebees discriminate among flowers that vary in their sexual phase (Thomson et al. 1982, Bell et al. 1984, Klinkhamer and de Jong 1990). Our study shows that pollen-collecting bumblebees can exercise similar preferences. The salient feature of our study system is that the amount of available pollen, which is deposited on the style, can be visually estimated by ourselves and, apparently, by the bumblebees before they alight. Thus, the bumblebees must be able to quantify pollen rewards (cf. Hodges and Miller 1981) and associate the potential value of the pollen reward with a visual cue.

The bumblebees' behaviour is consistent with a simple economic interpretation: initially, bumblebees favoured female phase flowers, many of which offered both pollen and nectar, but as pollen became less abundant, probably because of the harvesting activities of numerous bees, bumblebees increasingly favoured the relatively pollen-rich, nectarless male-phase flowers, which implies that pollen was the more valued resource. This contrasts with Thomson's (1989) finding that bumblebees preferred nectar-rich over pollen-rich flowers except when displaced by aggressive wasps. Presumably, the differences in the bumblebees' hierarchy of preferences reflected differences in the needs of their colonies. We cannot compare the bumblebees' behaviour to optimal economic decisions because no models exist that combine pollen and nectar, two qualitatively different resources, into a single, fitness-related currency. Nevertheless, we have shown that pollen-collectors, like nectaring bumblebees (e.g. Heinrich et al. 1977, Real 1981), can respond to changing resource availability by showing labile preferences among floral variants.

Initially, we chose to study *Campanula rotundifolia* because of the unusually high variability among individuals in floral form, particularly in flower size. Because pollinators are the sexual agents of the plants that they visit, their behaviour is a major force in shaping the evolution of floral form (Bell 1985, Stanton et al. 1986) and, in particular, discriminatory foraging by floral visitors among intraspecific variants can result in pollinator-mediated natural selection on components of floral form such as flower size (Galen 1989). However, we found that bumblebee preferences were unrelated to flower size, probably because this was unrelated to pollen availabil-

ity. Instead, bumblebees discriminated among flowers on the basis of the amount of pollen presented, which is largely a function of visitation rate and, therefore, environmentally determined to a corresponding extent. Thus, in this system, the potential for pollinator-mediated selection on floral form may be limited except through the mechanics of pollen transfer during a floral visit, which remain uninvestigated. Consequently, the existence of the floral variation in this population remains unexplained.

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References

- Bell, G. 1985. On the function of flowers. – *Proc. R. Soc. Lond. B* 224: 233–264.
- , Lefebvre, L., Giraldeau, L.-A. and Weary, D. 1984. Partial preference of insects for the male flowers of an annual herb. – *Oecologia* 64: 287–294.
- Buchmann, S. L. and Cane, J. H. 1989. Bees assess pollen returns while sonicating *Solanum* flowers. – *Oecologia* 81: 289–294.
- Cresswell, J. E. and Galen, C. 1991. Frequency-dependent selection and adaptive surfaces for floral character combinations: the pollination of *Polemonium viscosum*. – *Am. Nat.* 138: 1342–1353.
- Faegri, K. and van der Pijl, L. 1979. The principles of pollination biology (3rd ed.). – Pergamon Press, Oxford.
- Frey, D. F. and Pimentel, R. A. 1978. Principal component analysis and factor analysis. – In: Colgan, P. W. (ed.), *Quantitative ethology*. Wiley, New York, pp. 219–245.
- Galen, C. 1989. Measuring pollinator-mediated selection on morphometric floral traits: bumblebees and the alpine sky pilot, *Polemonium viscosum*. – *Evolution* 43: 882–890.
- Harder, L. D. 1990. Behavioral responses by bumble bees to variation in pollen availability. – *Oecologia* 85: 41–47.
- Haynes, J. and Mesler, M. 1984. Pollen foraging by bumblebees: foraging patterns and efficiency on *Lupinus polyphyllus*. – *Oecologia* 61: 249–253.
- Heinrich, B. 1976. The foraging specializations of individual bumblebees. – *Ecol. Monogr.* 46: 105–128.
- , Mudge, P. and Deringis, P. 1977. A laboratory analysis of flower constancy in foraging bumblebees: *Bombus ternarius* and *B. terricola*. – *Behav. Ecol. Sociobiol.* 2: 247–266.
- Hodges, C. M. and Miller, R. B. 1981. Pollinator flight directionality and the assessment of pollen returns. – *Oecologia* 50: 376–379.
- Klinkhamer, G. L. and de Jong, T. 1990. Effects of plant size, plant density and sex differential nectar reward on pollinator visitation in the protandrous *Echium vulgare* (Borraginaceae). – *Oikos* 57: 399–405.
- Martin, F. W. 1958. Staining and observing pollen tubes in the style by means of fluorescence. – *Stain Tech.* 34: 125–128.
- McKenna, M. A. and Thomson, J. D. 1988. A technique for sampling and measuring small amounts of floral nectar. – *Ecology* 69: 1306–1307.
- Real, L. 1981. Uncertainty and pollinator-plant interactions: the foraging behavior of bees and wasps on artificial flowers. – *Ecology* 62: 20–26.
- Richardson, T. E. and Stephenson, A. G. 1989. Pollen removal and pollen deposition affect the duration of the staminate and pistillate phases in *Campanula rapunculoides*. – *Am. J. Bot.* 76: 532–538.
- Roe, J. H. 1955. The determination of sugar in blood and spinal fluid with anthrone reagent. – *J. Biol. Chem.* 212: 335–343.
- Ryan, B. F., Joiner, B. L. and Ryan, T. A. 1985. *Minitab handbook* (2nd ed.). – PWS Publ., Boston, MA.
- Snedecor, G. W. and Cochran, W. G. 1968. *Statistical methods* (6th ed.). – Iowa State Univ. Press, Ames, IA.
- Stanton, M. L., Snow, A. A. and Handel, S. N. 1986. Floral evolution: attractiveness to pollinators increases male fitness. – *Science* 232: 1625–1627.
- Stephens, D. W. and Krebs, J. R. 1986. *Foraging theory*. – Princeton Univ. Press, Princeton, NJ.
- Thomson, J. D. 1989. Reversal of apparent feeding preferences of bumble bees by aggression from *Vespula* wasps. – *Can. J. Zool.* 67: 2588–2591.
- , Maddison, W. P. and Plowright, R. C. 1982. Behavior of bumble bee pollinators of *Aralia hispida* Vent. (Araliaceae). – *Oecologia* 54: 326–336.