

## Chapter II

### Sensory ecology of pollination:

#### The distribution of floral colours and sugar rewards in the bee's natural environment

**Abstract** Flowers advertise themselves using colours that are learned by the pollinators as signals for profitable food sources. If competition for pollinators is a selective factor in the evolution of flower colours, it may be identifiable by the way in which both colour signals and reward properties diverge or converge within a plant community. We therefore measured the floral colours and corresponding rewards of co-flowering plants as well as their floral abundance, functional flower shape and visitation rates by pollinators in four natural high- and lowland habitats in Europe. Flower spectra were grouped by their shape and their chromatic properties were quantified based on the Receptor-Noise-Limited Model of colour vision in honeybees, since bees form the main pollinator group. Rewards were quantified by maximally available sugar crop per flower and reward rate per plant species taking into account their relative abundance. We investigated how the determined colour-reward distributions may be related to foraging strategies of pollinators and whether they match innate colour preferences of bees, as it has been proposed that some colours may correlate with higher rewards for pollinators. Our main findings were:

- 1) Flowers of highly rewarding plant species are often visited by Apoidea. They are often blue-violet (with or without ultraviolet (UV)) and less often yellow (with or without UV) or white in colour. However, they rarely matched the found innate colour preferences of bees.
- 2) The degree of accessibility of the nectar was clearly correlated with the reward strength. Flowers with open access to the nectar were the best rewarding and had mainly non-Apoidea visitors. Plant species with hidden nectar were usually less rewarding and had fewer, mainly Apoidea, visitors.
- 3) Plant species with significantly larger single flower displays presented various colours, usually had no particularly high reward and only few Apoidea visitors.
- 4) Flowers of highly rewarding species were not particularly different in colour to less rewarding species' flowers.
- 5) we could detect no consistent in- or decrease in reward in relation to in- or decreasing chromatic distance between flower colours. Thus, reward distributions were not clustered with respect to chromatic properties of the plant species. The complexity of reward estimation in pollinators and different strategies to achieve efficient pollination are discussed.

### **Introduction**

Sprengel and Darwin were the first and most prominent researchers pointing out many examples of floral traits which have coevolved with their corresponding pollinators' morphological and physiological traits (for reviews see: Faegri & van der Pijl 1979, Jones and Little 1983, Paulus 1988, Cohen and Shmida 1993, Dafni *et al.* 1997, Chittka *et al.* 2001, Waser and Ollerton 2006). One of the most striking traits is the floral colouration, which evolved as an advertisement for pollinators. Although flowers of different species can have a variety of colours, plants have not evolved an infinite number of flower colours within the perception range of pollinators (Vorobyev and Menzel 1999). On the one hand, limitations in pigment synthesis and/or light straying cellular structures are constrained by biochemical pathways and developmental processes in plants. Yet, interesting questions remain: What are the limiting costs and benefits for flowering plants to evolve such a diversity of colours? And how important is competition between co-flowering plants for pollinators, in the evolution of flower colours? In an ongoing discussion on the intensity of influence pollinators have had and continue to have on flower evolution, Chittka and Waser outlined that pollinators possess broad preferences and thus do not push an evolutionary process that leads to floral characters (Waser *et al.* 1996, Chittka *et al.* 1999). Contrarily, Schemske and Bradshaw (1999) showed in their work about petal carotenoid pigments and nectar volume in *Mimulus*, that there is a likelihood for the adaptive divergence of flower colouration through pollinator-mediated selection. Also, Gumbert and Kunze (2001) detected a flower mimicry system in orchids, in which the similarity to rewarding plants determines the reproductive success of a food deceptive orchid and Gigord *et al.* (2002) in their experimental study with bumblebees claim evidence that pollinators could select for floral mimicry in rewardless orchids. Following up this dispute, we intended to look at the distribution of flower colours and reward properties in simultaneously flowering species, that are exposed to the same pollinator community, in order to assess relationships between floral colour signals as they are perceived by pollinators, and the corresponding reward properties which drive the pollinator's foraging strategies. More specifically, we asked whether the recorded colour-reward distributions feature patterns, in which highly rewarding plant species display different colourations than low rewarding species, or in which plant species with very similar colours are similarly rewarding. As far as different strategies of flowering plants were to be identifiable, we were also interested in understanding the impact of such strategies on the foraging decisions of pollinators. We

focused on bees, because they form the main pollinator group for European plants, and their foraging behaviour and visual perception has been studied in detail (von Frisch 1965, Heinrich 1975, Menzel and Backhaus 1991, Peitsch *et al.* 1992, Goulson 1999). Bees possess an excellent trichromatic colour vision which is based on three types of photoreceptors, which are sensitive in the short (S) or ultra-violet (UV), medium (M) or blue and long (L) or green part of the wavelength spectrum. Bees' photoreceptors are well-adjusted for the discrimination of their natural coloured targets, the flowers (Chittka *et al.* 1994, Vorobyev and Menzel 1999), although there are many additional colours which can be perceived by the bees (Vorobyev and Menzel 1999). They learn colours of food sources very quickly and may generalise them (Menzel 1967, 1968, Backhaus *et al.* 1987, Giurfa 1991). During foraging bees develop an expectation of the reward based on the signals of the food source (Greggers and Menzel 1993). Thus, the interesting question to be addressed in our survey of colour-reward distributions is: how reliable must a particular flower colour be to provide a good reward from the point of view of the pollinator? The present data are particularly valuable because the investigation sites were selected considering the occurrence of authentic plant communities and undisturbed plant-pollinator interactions.

## **Material and Methods**

### *Investigation sites*

Grassland was chosen as the general vegetation form for the investigation sites, since the concentration of herbaceous flowering plants is very high compared to other forms of vegetation, and the spectrum of life-forms is comparable (Bosch *et al.* 1997, Heß 2001, Stang *et al.* 2006). Furthermore, the proportion of shade caused by trees is negligible, so that the illumination of the entire investigation area is homogeneous.

We chose weakly to medium anthropogenic influenced meadows, defined as a meadow with an established tradition (~ 1-5 centuries) of use as hay-meadows and/or rare usage as pasture and one artificially founded, but near-natural meadow in the Botanical Garden. Traditional meadows were the Pockhorner Meadows in the Hohe Tauern National Park (HTNP)/Carinthia Province, Austria (47°03'30'' N, 12°47'54'' E, 1978 msm, section of ~3 ha; Kreisch 2001) and the Lower Havelaue in Brandenburg Province, Germany (52°43'00'' N, 12°15'24'' E, 25 msn, section of ~20 ha; Burkart 1998). The meadow with rare usage as pasture was the Obnassfeld in the core zone of the HTNP/Salzburg Province, Austria (47°07'20'' N,

12°49'15'' E, 2343 msm, section of ~ 20 ha; Menzel and Ziegler 2001). The meadow in the Botanical Garden was located in Berlin, Germany (52°27'07'' N, 13°18'21'' E, 35 msn, section of ~ 4 ha; Graf and Rohner 1984).

The whole Pockhorner Meadows, an area of around 100 ha, are subalpine mountain hay meadows on the southern slopes of the Alps in the buffer zone of the HTNP, inclined up to 35°. After centuries of extensive cultivation by bi-annual mowing, the Eastalpine Panicked Fescue Meadow (*Hypochoerido uniflorae-Festucetum paniculatae*) and alpine grasslands with *Sesleria albicans* and *Nardus stricta* (*Campanulo scheuchzeri-Festucetum noricae*, *Trifolio nivalis-Seslerietum albicanti*) are the dominating plant communities (Kreisch 2001, Menzel and Ziegler 2001, Abl 2003).

The Obernassfeld is a high alpine meadow. It inclines slightly towards the East and is bordered by a scarp, the *Piffkarschneid*, with cattle stairs and marmot caves (Menzel and Ziegler 2001). Investigations were made on the lower areas in the plant communities *Seslerio-Semperviretum* (Schiechtl and Stern 1985) and *Arabidion coeruleae* (Menzel and Ziegler 2001).

The Lower Havelaue near Gülpe (~225 ha) originated from a glacier reservoir. It was never completely covered with forests after the ice age, but featured open areas without anthropomorphic influence. The investigated areas were populated by a mosaic of the plant communities *Diantho-Armerietum*, *Arrhenatheretalia*-community and *Molinio-Arrhenatheretea*-community, *Cnidio-Deschampsietum*, *Phalaridetum*, *Ranunculo-Alopecuretum* (Burkart 1998).

The meadow selected at the Botanical Garden is an *Salvio-Arrhenateretum* with transition to *Diantho-Armerietum* (Graf and Rohner 1984) and is an area undergoing dynamic population changes influenced by the near vicinity of different plant species. Due to sowing of plant species, plant communities are not completely authentic, and it might be possible that the proportion of species with large flowers is exceptionally high.

Measurements were performed from 22.06.-07.04.2002 on the Obernassfeld (henceforth: OB site), from 02.06.-18.06.2003 in the Botanical Garden (BG site), from the 14.07.-24.07.2003 on the Pockhorner Meadows (PM site) and from 24.04.-30.04.2004 in the Lower Havelaue (LH site).

*Investigated plant species*

We quantified the colours and abundance of all flowering species in our investigation sites (see App. 1 for species list; Germany: N = 62, Austria: N = 84; Adler *et al.* 2005, Rothmaler 2005). In ten random chosen squares of 1 m<sup>2</sup> per investigation site, all open flowers were counted and their abundance proportions calculated for each species. We categorised species according to the abundance of their open flowers during our investigations to obtain a snapshot of the site as perceived by the pollinator. The groups consisted of species with very rare flowers (< 1 %), rare flowers (< 5 %), intermediate flowers (< 10 %), common flowers (10-25 %) and very common flowers (> 25 % of the total number of flowers). In the following, we refer to these categorisations when discussing e.g. rare or common flowers in the context of other flower properties.

The full cycle of measurements, including pollinator visitation rates, nectar quantity and quality and spectral reflectance of the flowers petals were completed in parallel for 11-18 plant species per investigation site (total N = 60, App. 1). These species were selected according to the following criteria: i) species exploited by bees (*Apoidea*), ii) indicator species for the respective vegetation form, and iii) other species with a high abundance (common and very common flowers) as described above (Kugler 1970, Heß 1990, 2001). Measurements took place randomly during 6-16 consecutive days to reduce the influence of temperature, humidity and other weather conditions on the nectar quality and quantity as well as on the behaviour of the pollinators (von Frisch 1965, Corbet 1990, Corbet *et al.* 1993).

*Pollinator visitation*

To determine the spectrum of visitors and the frequency of flower visits for each plant species we counted all insects landing on flowers to extract nectar. Visitors were subdivided into the following categories: *Apis*, *Bombus*, other *Apoidea*, other Hymenoptera, Coleoptera, Diptera and Lepidoptera. Single flowers were selected and observed for 30 min (single flower observations, henceforth: SFO). Several flowers of the same species were observed at randomly selected locations and times of day to include consistently minimum and maximum activity of the pollinators (Núñez 1977a, Neumayer and Paulus 1999). Additionally to the SFO, we performed activity walks (henceforth: AW). By traversing the whole observation site while observing all plant species once a day for 30 min, all flower visits were counted. The route was chosen such that all plant species were observed with the same frequency. Plant

species were categorised into five groups: plants with very rarely visited flowers (< 1 %), with rarely visited flowers (< 5 %), with intermediately visited flowers (< 10 %), with commonly visited flowers (10-25 %) and with very commonly visited flowers (> 25 % of total visits).

### *Nectar properties*

Subsequently to the SFO, we measured nectar quality and quantity of the same individuals. The flowers were enclosed with gauze nets at 5 pm for 24 hours to obtain a full nectar crop. Flowers of species like *Erodium cicutarium*, *Potentilla erecta* and *Taraxacum officinale*, that opened only for one day, were enclosed as buds that were about to flower, so that observations and nectar/spectral measurements took place at different individuals. After taking the gauze net off, the flowers were picked and taken into the laboratory. They were hermetically sealed in plastic and cooled until measurements proceeded, so that they were protected from dehydration and oxidation, which could have led to changes of the sugar concentration of the nectar and to changes of the colour of the petals (Corbet 2003). We used micro capillaries of appropriate sizes (0.2-5 µl, Microcap, Drummond Scientific Co., USA) to measure nectar volume (Corbet 2003). The sugar concentration of extracted nectar was measured by means of a refractometer (0-80 % Brix scale, Optech, Canada). Nectar volumes smaller than 1 µl had to be diluted into a defined volume of distilled water, because the refractometer was only constructed for larger volumes. The original concentration was recalculated afterwards (Corbet 2003).

The maximum (max.) sugar crop per day per flower (in mg) was calculated using the measured values of nectar volume and sugar concentration of the nectar (Kearns and Inoue 1993). We did not differentiate between sucrose, glucose and fructose, because the differences in the refractometer reading between sucrose and hexose sugars are trivial in relation to the variance in concentration usually found in European species (Corbet 2003). Complementary to max sugar crop, reward rate of a species was defined as the mean of max. sugar crop (mg) multiplied by the relative abundance (%) of the flower species (App. 1).

### *Flower colours*

To determine the colour of flowers, we measured the spectral reflectances of their petals by means of a photospectrometer (SD 2000, Ocean Optics Inc., USA). For the calculation of the

colour loci one first needs to determine the quantum catches ( $Q_i$ ) of the three photoreceptor types for short (S), medium (M) and long (L) wavelength in the honeybee eye (Wyzcecki and Stiles 1982).

$$Q_i = \int_{300}^{700} I(\lambda)R(\lambda)S_i(\lambda)d\lambda \quad (1)$$

Three parameters are important: the illumination spectrum ( $I$ ), the reflectance spectrum ( $R$ ) of the flower petals and the spectral sensitivity function ( $S_i$ ) of the three photoreceptor types ( $i = S, M, L$ ; Menzel and Backhaus 1991).  $\lambda$  indicates the wavelength. For the lowland illumination we took the daylight function D65 (Wyzcecki and Stiles 1982). Illumination in the Alps was measured during the experiments at one hour intervals and compared to the daylight function D65. Application of either of the spectral curves did not lead to any significant changes of our results with respect to the similarity and discriminability of colours by bees. For accurate comparison, we used D65 for all investigation sites. Spectral range and peaks of sensitivity of the receptor types vary among the different pollinators (Menzel 1979, Peitsch *et al.* 1992, Lunau and Maier 1995). We focused our analyses on the honeybee, because bees form the main group of pollinators, and because the honeybee's colour vision system is among the best understood systems in insects (Menzel 1979).

The average colour loci of the flowers of one species were calculated in the honeybee colour space using the Receptor-Noise-Limited (RNL) Model of honeybee colour vision (Vorobyev *et al.* 2001). It quantifies the distances between the flowers' colour loci and the background. The background is represented by leaf green, which was measured for the majority of the species and universalised into a mean spectral curve. The division of the quantum catches of the photoreceptors that are activated by the flower petals ( $Q_{fi}$ ) and the background ( $Q_{bi}$ ), respectively, results in the receptor specific contrast ( $q_i$ ) that was calculated for every species.

$$q_i = \frac{Q_{fi}}{Q_{bi}} \quad (2)$$

The chromatic and achromatic distances between average flower colours of different species ( $\Delta S$ ) were computed with the RNL-Model using the programm *QCalc*:

$$\Delta S = \sqrt{\frac{\omega_S^2(\Delta f_L - \Delta f_M)^2 + \omega_M^2(\Delta f_L - \Delta f_S)^2 + \omega_L^2(\Delta f_S - \Delta f_M)^2}{(\omega_S\omega_M)^2 + (\omega_S\omega_L)^2 + (\omega_M\omega_L)^2}} \quad (3)$$

$\omega_i$  stands for the standard deviation of the receptor noise in the photoreceptor types  $i$ ,  $f_i = \ln(q_i)$  is the receptor signal and  $\Delta f_i$  describes the deviation of the receptor signals between two flower colours (Vorobyev *et al.* 1998). The values for  $\omega_i$  derive from electrophysiological

recordings in single photoreceptor cells. For honeybees they are  $\Delta S = 0.13$ ,  $\Delta M = 0.06$  and  $\Delta L = 0.12$  (Peitsch 1988).

The colour loci of the flowers can be displayed in a 2-dimensional colour diagram, in which the receptor signals  $f_i$  are assigned to the coordinates (Vorobyev *et al.* 2001). Thereby X and Y are defined as follows:

$$X = A(f_L - f_M) \quad \text{and} \quad Y = B(f_S - (af_L + bf_M)), \quad (4)$$

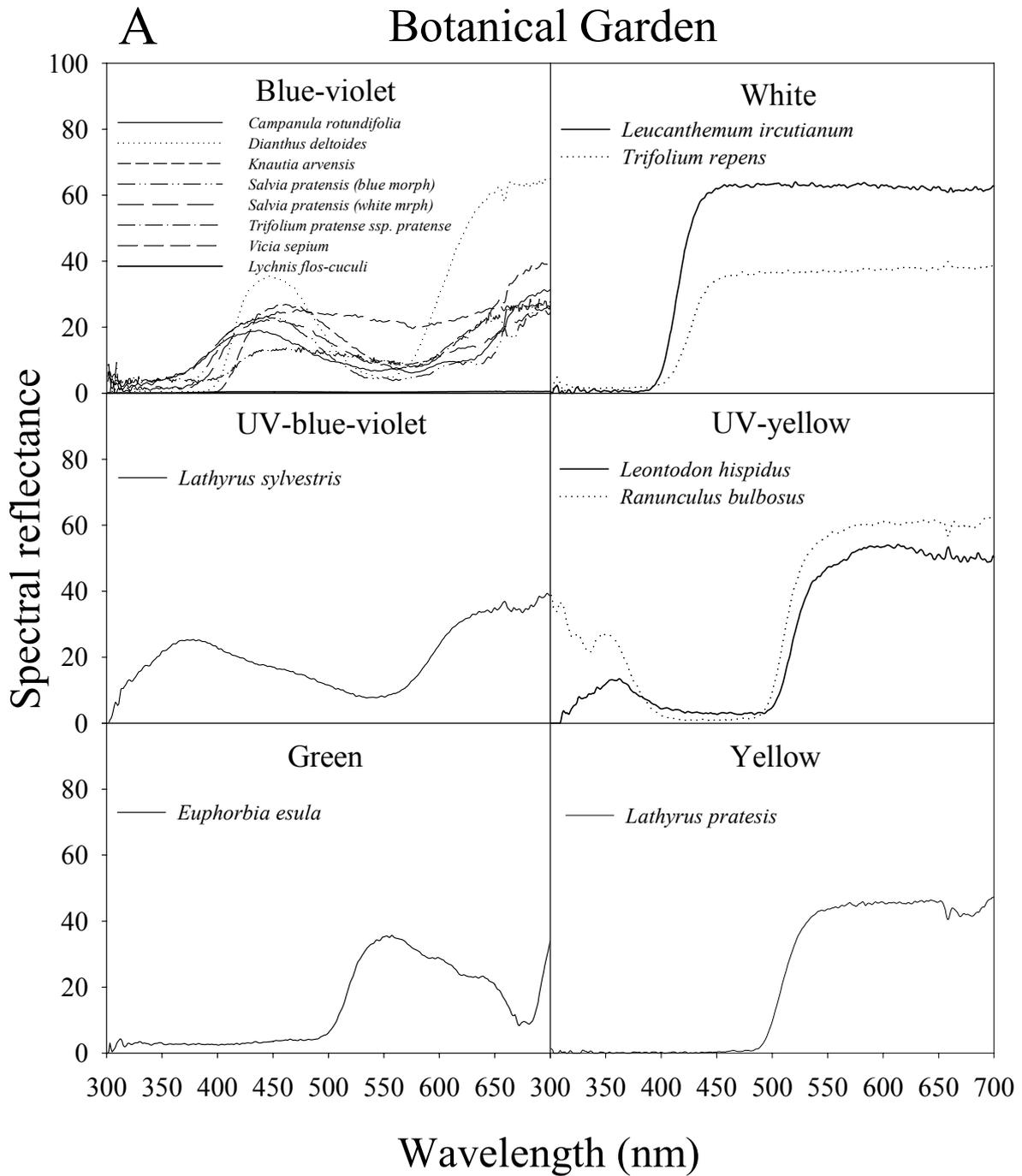
where

$$A = \sqrt{\frac{1}{\omega_M^2 + \omega_L^2}}, \quad B = \sqrt{\frac{\omega_M^2 + \omega_L^2}{(\omega_S \omega_M)^2 + (\omega_S \omega_L)^2 + (\omega_M \omega_L)^2}}, \quad (5, 6)$$

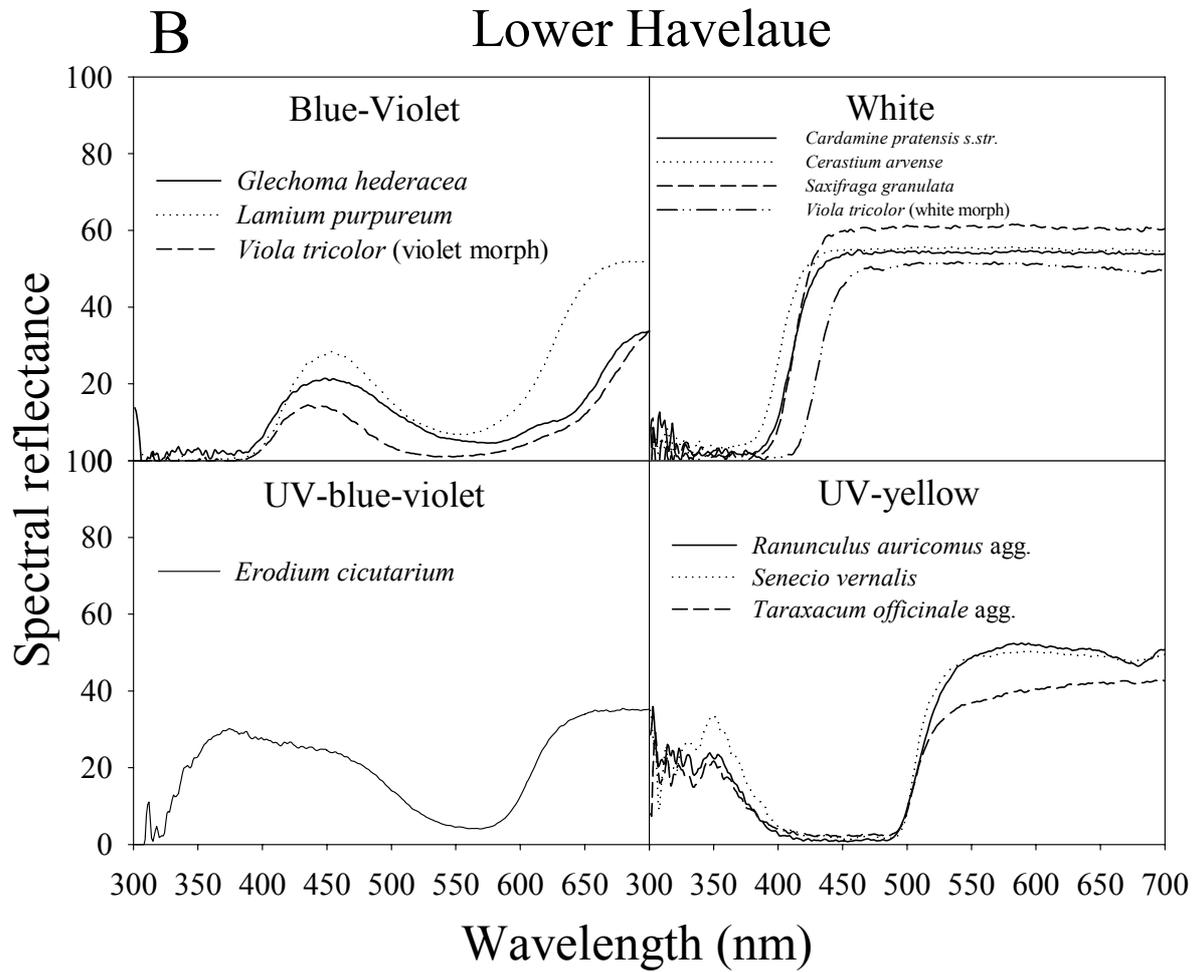
$$a = \frac{\omega_M^2}{\omega_M^2 + \omega_L^2} \quad \text{and} \quad b = \frac{\omega_L^2}{\omega_M^2 + \omega_L^2}. \quad (7, 8)$$

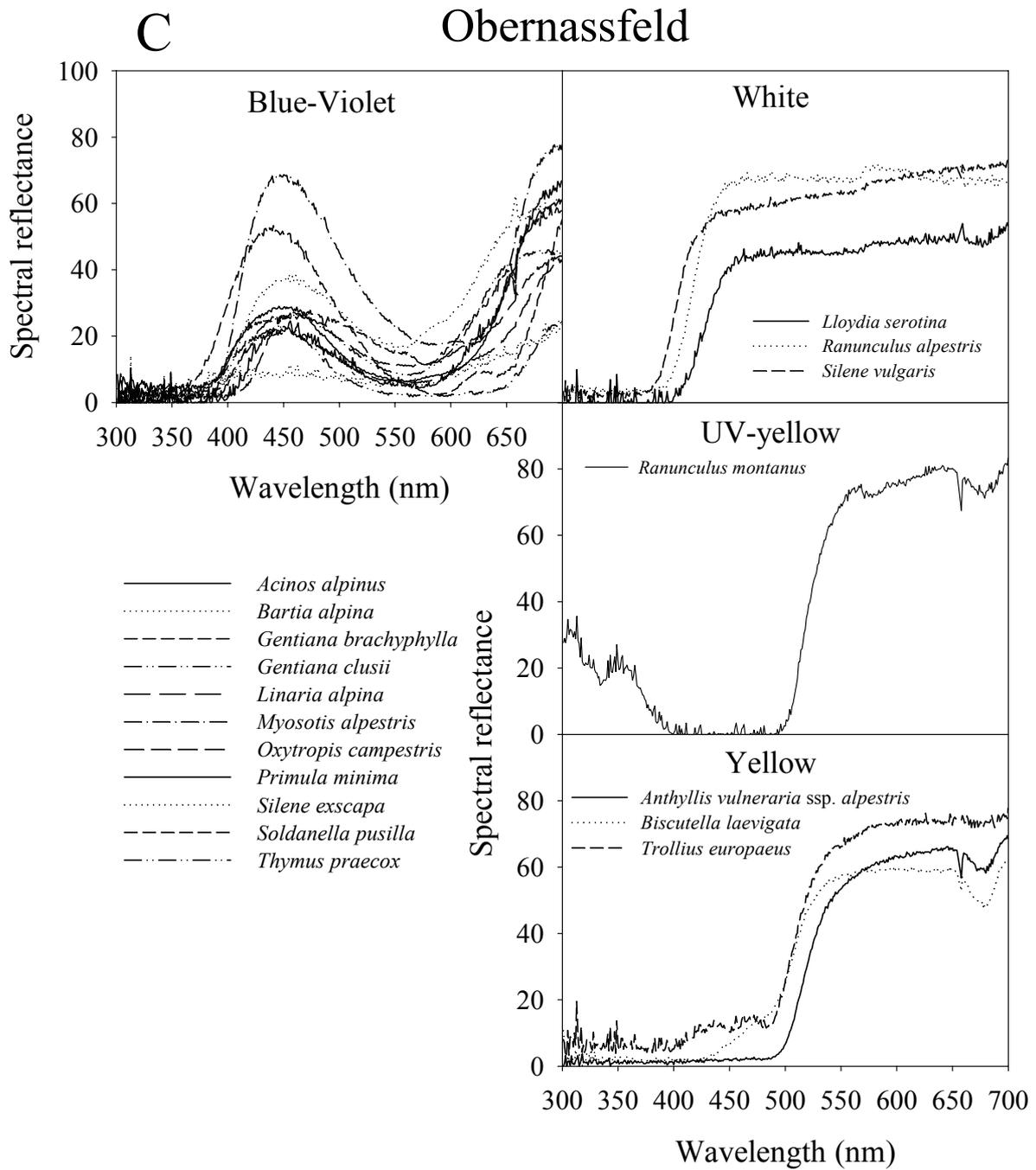
We used human colour names and UV for illustrative purposes, although not accurately applicable to bee vision (Daumer 1956, Backhaus and Menzel 1987). When colours were specified in the text, e.g. blue-violet or UV-yellow, they were geared to the colour spectra, which we categorised into six groups of occurring flower colours: blue-violet, UV-blue-violet, white, yellow, UV-yellow and green (Fig. 1A-D).

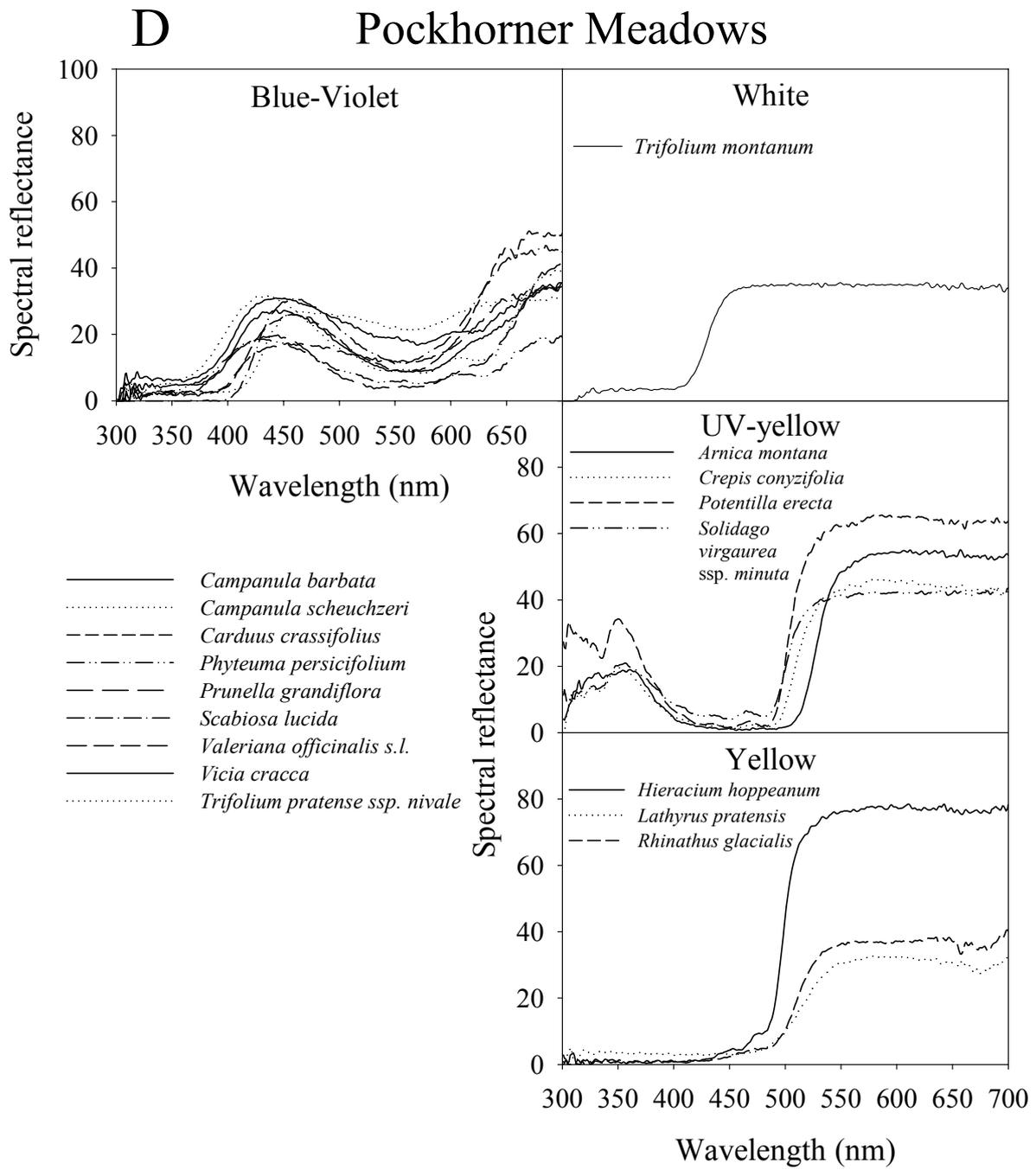
We approached the question whether colour generalisation would be adaptive for honeybees within context of specific reward properties found in their natural habitats. The RNL Model postulates that colours whose loci are separated by less than 2.3 RNL units, cannot be discriminated by honeybees (Vorobyev *et al.* 2001). Plant species with displays of such colours were grouped as species displaying flowers with indistinguishable colours. The other three groups contained colours whose loci were separated by up to 3.5, up to 5 and greater RNL values, respectively. If bees profit from exact colour discrimination, mean max. sugar crop or reward rate should be higher in the groups of flower colours with chromatic distances of less than 2.3. If bees profit from colour generalisation, mean max. sugar crop or reward rate should be higher in the groups of flower colours with chromatic distances of up to 3.5 and up to 5.



**Fig. 1** Colour classifications of flower colours according to their reflectance spectra, separately for the sites **A** Botanical Garden, **B** Lower Havelaue, **C** Obernassfeld and **D** Pockhorner Meadows.







### *Functional shape*

All measured species were divided into three groups of functional shapes, i.e. the degree of ease with which the flower visitor is able to access the nectar. The first group contained species with flowers that required only a minor effort by the pollinator, to obtain the open, easily accessible nectar. Flowers in the second group required a medium effort, e.g. crawling into the flower to obtain the hidden nectar or robbing the nectar. And the third group required a high effort of the pollinator, which had to move parts of the flower to obtain the hidden nectar. Species with flowers whose nectar was not obtainable by bees, e.g. because the nectar was hidden too deeply, were not included in the analysis. This was only the case for *Gentiana brachyphylla*. *Dianthus deltoides* also seems to be psychophil, but since we observed *Apis* and other Apoidea as nectar feeding flower visitors, we concluded that the nectar level must have been high enough for them to obtain a reward (see App. 1 for categorisation).

### *Flower display size*

Display sizes and spectral properties of all available flowering species in the investigation site Lower Havelaue were measured from 7.-14.5.2005 (App. 1). 30 individuals per species were randomly collected and taken into the laboratory. The size was measured using a sliding calliper. We noted the diameter of circular flowers (e.g. *Ranunculus auricomus*) and length and width of zygomorphic flowers (e.g. *Viola tricolor*). Only the coloured parts of the flower were included into the measurements, e.g. only the lower lip of *Glechoma hederacea*. Inflorescences, such as basket flower types (*sensu* Kugler 1970), were regarded as one functional display unit if the distance between their single flowers was smaller than their size. In that sense, blossoms and flowers were treated as equivalents when comparing them on the basis of reward properties.

### *Statistics*

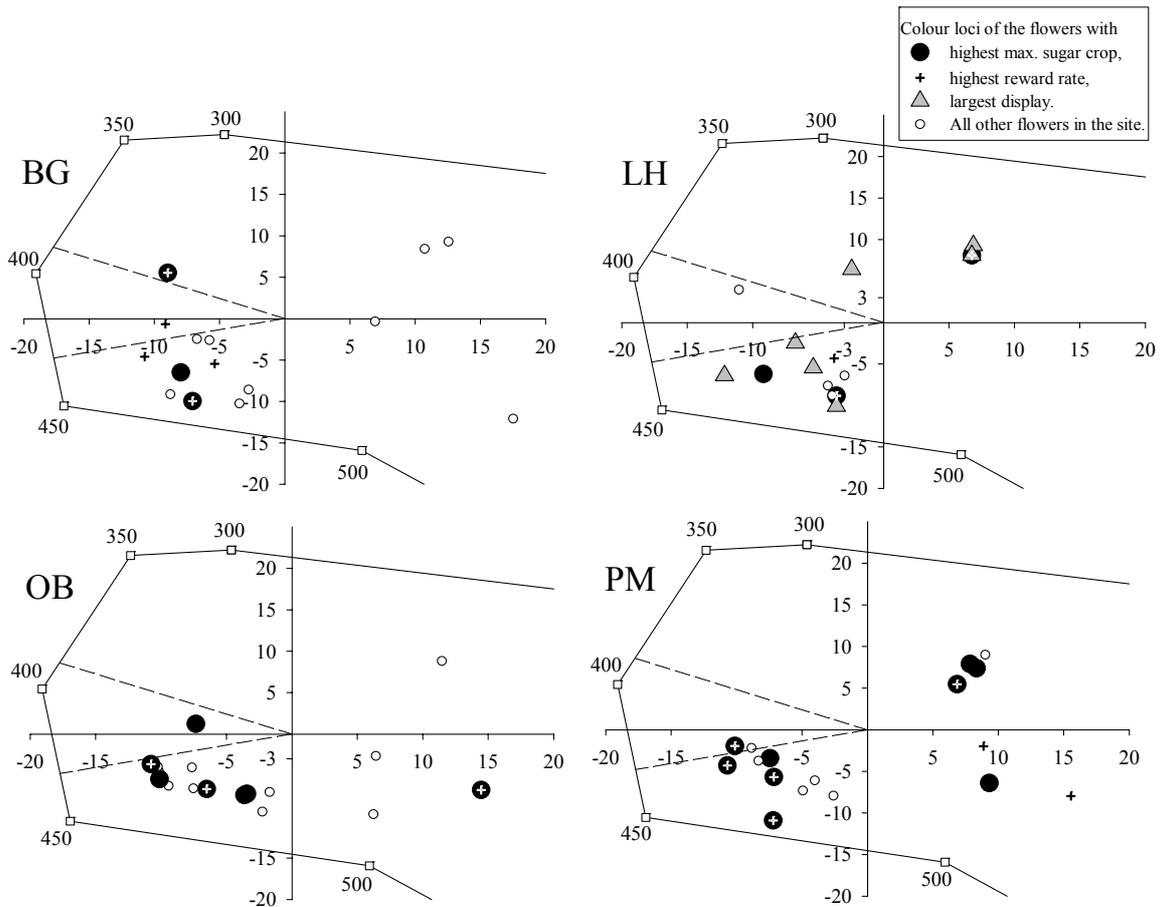
We tested for normality and homogeneity of variances with the Kolmogorov-Smirnov Test and the Levene's Test, respectively. One Way ANOVA (post-hoc Test: Holm-Sidak Method) and the Kruskal-Wallis Test (post-hoc Test: Dunn's Method) were used to compare different species of one investigation site regarding their max. nectar crop, sugar concentration of the nectar, max. sugar crop and reward rate as well as abundance and display size (Zar 1999).

## Results

### *Floral reward and display properties*

We measured max. nectar crop, as nectar accumulated in the flower during 24 hours, and the sugar concentration of the nectar. These values were used to determine the max. sugar crop of the flower. Max. nectar crop and max. sugar crop correlated positively ( $R = 0.99609$ ,  $P < 0.0001$ ). Thus, species with a significantly higher max. nectar crop usually also presented a significantly higher max. sugar crop than most of the other species is the same site, and *vice-versa* (Fig. 2, App. 1). A significantly higher sugar concentration of the nectar produced by some plant species did not reliably lead to a relatively higher max. sugar crop compared to the magnitudes of max. nectar volume. Therefore, we concentrated our analyses on max. sugar crop.

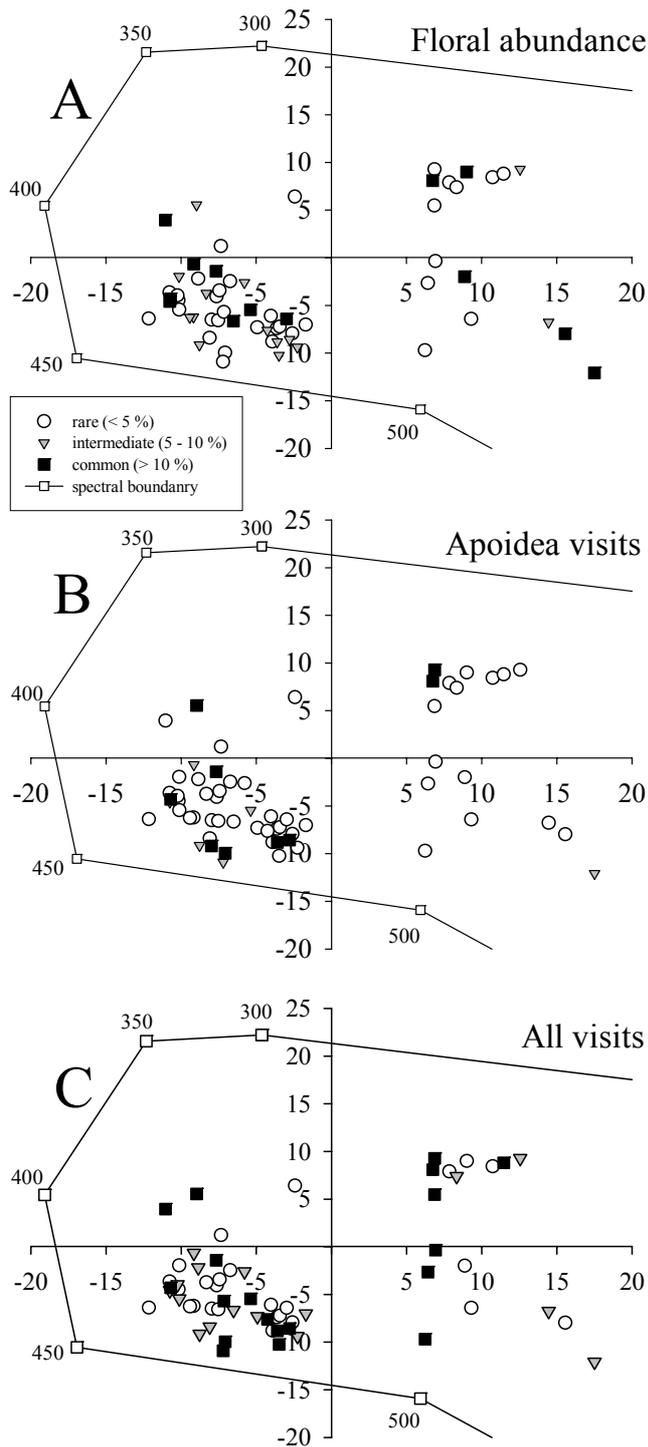
In each investigation site we found a number of species presenting flowers with a significantly higher max. sugar crop than most of the other species (Fig. 2, BG: 20 %, LH: 27,3 %, OB: 38,9 %, PM: 52,9 % of all measured species;  $P < 0.05$ , Kruskal-Wallis Test). These flowers' colour loci appeared in different areas of the honeybee colour space and were thus perceived as different colours by the bees. In the BG site, these were *Lathyrus sylvestris* (UV-blue-violet in human terms), *Knautia arvensis* and the whitish morph of *Salvia pratensis*, both showing blue-violet colours. *Taraxacum officinale* in the LH site with by far the largest amount of max. sugar, possessed UV-yellow flowers, whereas *Glechoma hederacea* and *Saxifraga granulata* presented blue-violet and white flowers, respectively. In the OB site, *Bartsia alpina*, *Gentiana brachyphylla* and *Gentiana clusii*, *Oxytropis campestris* and *Silene exscapa* (all blue-violet), *Silene vulgaris* (white) and *Anthyllis vulneraria* (yellow), had a higher max. sugar crop. Finally in the PM site, *Campanula barbata*, *Campanula scheuchzeri*, *Carduus crassifolius*, *Prunella grandiflora* and *Scabiosa lucida* (all blue-violet), *Hieracium hoppeanum* (yellow), *Arnica montana*, *Crepis conyzifolia* and *Solidago virgaurea* (all UV-yellow) were the species with a higher max. sugar crop. The listed species were phylogenetically widely dispersed, i.e. they belong to a number of different plant families (App. 1).



**Fig. 2** Colour loci of all measured species, according to the investigation sites (see App. 1 for species list). From the relative quantum catch of each photoreceptor the chromatic coordinates and distances to background have been calculated for each flower according to the RNL-Model of honeybee colour vision (Vorobyev et al. 2001). The unity distance corresponds to one standard deviation of noise. The colour locus of the background (leaf green) is per definition at the cross-point of the axes of the colour diagram. The spectral boundaries of the bee colour space are indicated by the line with open squares. The following properties were measured: max. sugar crop (mg), reward rate (the product of max. sugar crop and abundance) and display size ( $\text{mm}^2$ ). Some species combined two or three of these properties, symbolised by a cross or a triangle on a black circle. Colours of the best rewarding or largest flowers occurred in various areas of the colour space and were rarely located in the approximate area preferred by inexperienced bees under laboratory conditions, indicated by the dashed line (Giurfa *et al.* 1995).

The abundance of flowers might play an important role when pollinators assess the profitability of a plant species. Since bees are to a large extent flower constant (Grant 1950, Goulson 1999) more abundant flowers might provide, in total, a higher reward to the visitor than less abundant species. Plant species in our investigation sites varied widely in flower

abundance, but did not show any statistical differences (Fig. 3A, App. 1;  $P > 0.05$ , Kruskal-Wallis Test). However, combining the abundance with the max. sugar crop, a measure defined here as reward rate, revealed a new ranking of species with respect to their potential for reward (Fig. 2). In the BG site, the number of species with a high reward rate was larger than the number of species with a high max. sugar crop. Additionally to *Knautia arvensis* and *Lathyrus sylvestris*, now *Campanula rotundifolia*, *Trifolium pratense* (all blue-violet) and the blue-violet morph of *Salvia pratensis* became most rewarding. In the LH site, *Lamium purpureum* (blue-violet) instead of *Glechoma hederacea* became more rewarding than the other species. In the OB site, the number of more rewarding species decreased leaving *Anthyllis vulneraria* (yellow), *Gentiana brachyphylla* and *Silene exscapa* (both blue-violet). In the PM site, the number of more rewarding species also decreased and partially changed to *Campanula scheuchzeri*, *Carduus crassifolius*, *Prunella grandiflora* and *Scabiosa lucida* (all blue-violet), *Lathyrus pratensis* and *Rhinanthus glacialis* (both yellow) and *Solidago virgaurea* (UV-yellow).

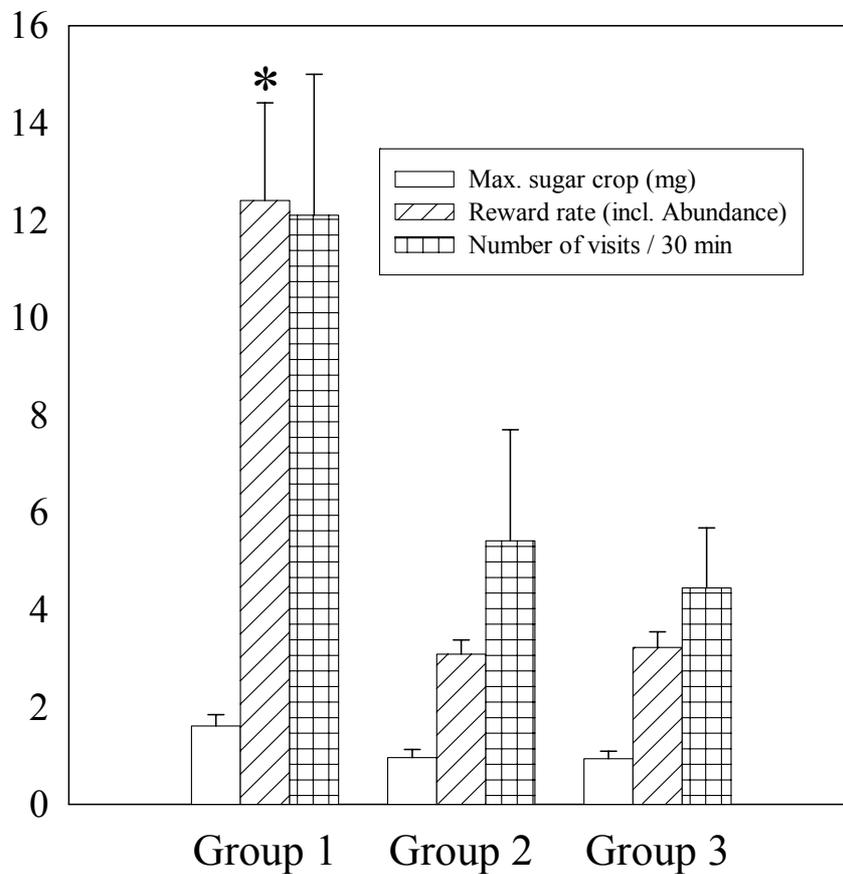


**Fig. 3** Colourloci of all measured species according **A** to their floral abundance (percentage of open flowers of the total number of open flowers), **B** to the frequency of bee visits (percentage of visits of the total number of visits) and **C** to the frequency of all pollinator visits. **A** Colours of plant species with common flowers (> 10 % of the flowers) appeared in various areas of the honeybee colour space. Also, visits of **B** bees and **C** all pollinators commonly (> 10 % of visits) occurred on flowers with colourloci of various different colours. The spectral boundaries of the bee colour space are indicated by the line with open squares.

To summarise, the percentage of plant species with a significantly higher max. sugar crop (value range:  $0,37 \pm 0,04$  mg (white morph of *Salvia pratensis*; BG site) -  $18,90 \pm 6,17$  mg (*Carduus crassifolius*, PM site); App. 1) that appeared in the UV-blue-violet area of the honeybee colour space, was between 30 and 60 % (BG: 33,3 %, LH: 33,3 %, OB: 57,1 %, PM: 55,6 %). Between 0 and 45 % of them presented UV-yellow, yellow or green colours (BG: 0 %, LH: 33,3 %, OB: 14,3 %, PM: 44,4 %) and between 0 and 70 % presented UV-less white colours (BG: 66,7 %, LH: 33,3 %, OB: 28,6 %, PM: 0 %). The percentage of plant species with a significantly higher reward rate (value range:  $2,25 \pm 0,84$  mg sugar\*abundance (*Campanula rotundifolia*; BG site) -  $108,71 \pm 21,86$  mg (*Silene excapa*, OB site)) that appeared in the UV-blue-violet part of bee colour space was slightly enhanced to be between 30 and 100 % (BG: 100 %, LH: 33,3 %, OB: 66,7 %, PM: 57,1 %). Between 0 and 45 % of the species presented UV-yellow, yellow or green colours (BG: 0 %, LH: 33,2 %, OB: 33,3 %, PM: 42,9 %) and between 0 and 35 % presented UV-less white colours (BG: 0 %, LH: 33,2 %, OB: 0 %, PM: 0 %; Fig. 1A-D).

All species whose nectar was measured were divided into three groups of functional flower shapes (App. 1) in order to relate the measured reward to the accessibility of the nectar and thus to the relative costs of handling the flowers. Plant species with flowers that had easily accessible nectar provided the highest mean value for the max. sugar crop (Fig. 4; group 1:  $1,61 \pm 0,23$  mg sugar  $\pm$  SE, N = 27), whereas the groups with intermediately accessible nectar (group 2:  $0,96 \pm 0,17$  mg, N = 24) and complicatedly accessible nectar (group 3:  $0,94 \pm 0,15$  mg, N = 9) provided similar max. sugar crops. However, differences were not statistically different (P = 0,059; Kruskal-Wallis Test). The difference is stronger when comparing the mean reward rates of plant species assigned to these groups: group 1 -  $12,41 \pm 2,01$  mg sugar\*abundance; group 2 -  $3,09 \pm 0,29$  mg; group 3 -  $3,22 \pm 0,321$  mg (Fig. 4). In this case, the mean reward rate of group 1 was significantly higher (P < 0,001; Kruskal-Wallis Test, Dunn's Method). Plant species with flowers in this group were also the most visited species in the corresponding site (excluding OB site):  $12,1 \pm 2,9$  % (mean percentage of visits of the total number of visits per 30 min  $\pm$  SE). Flowers with intermediately accessible nectar received  $5,4 \pm 2,3$  % and flowers with complicatedly accessible nectar received  $4,4 \pm 1,2$  % mean visits (Fig. 4). Differences were again not statistically different (P = 0,080; Kruskal-Wallis Test, Dunn's Method). Further, 75 % of the species in group 1 (n = 5 (BG); 7 (LH); 9 (PM)) were mainly visited by non-Apoidea, whereas only 25 % were mainly visited by Apoidea (App. 2). No visitors were observed at flowers of *Cardamine pratensis*. Species with intermediately or complicatedly accessible were all

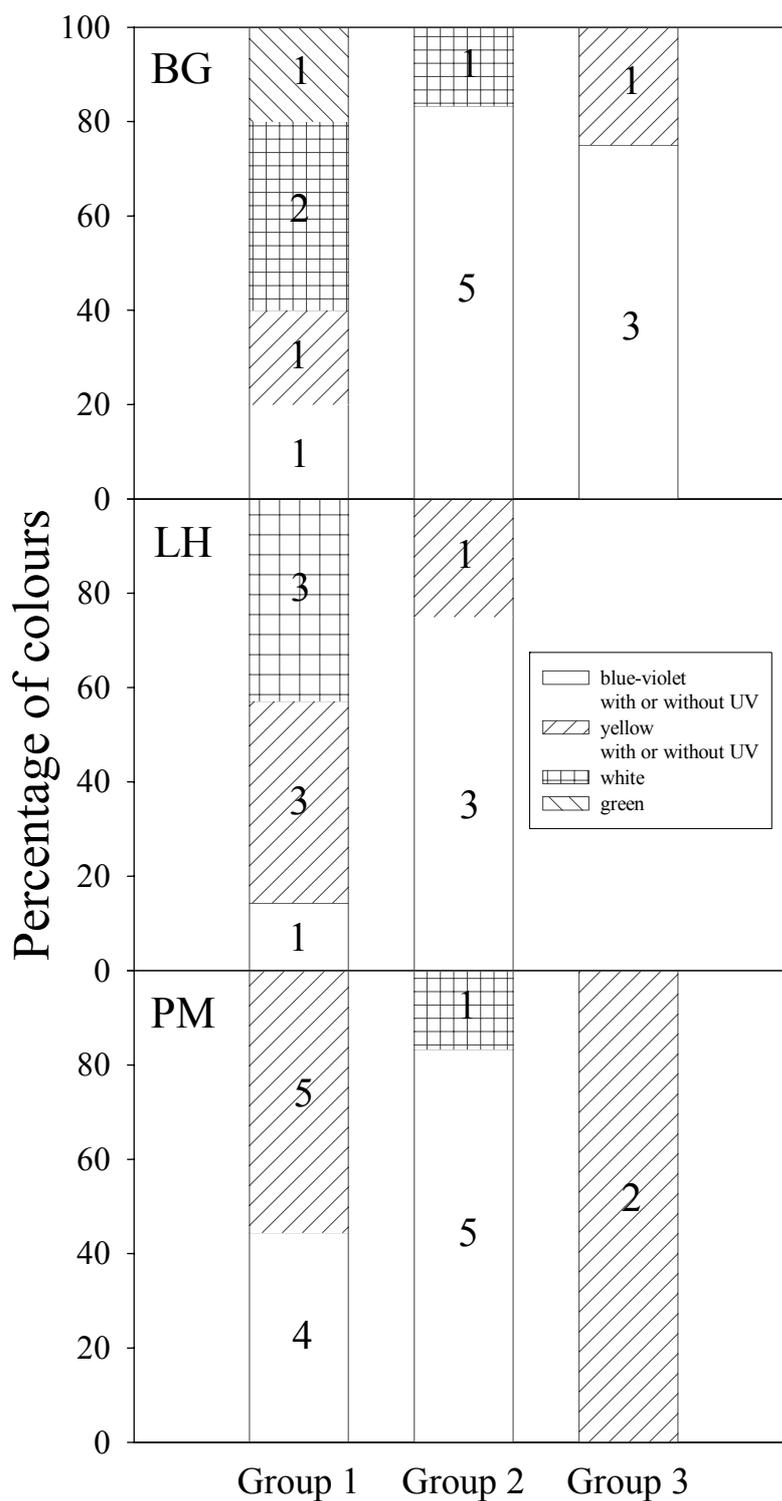
mainly visited by Apoidea (group 2: n = 3 (BG); 2 (PM); group 3: n = 6 (BG); 3 (LH); 6 (PM)). No visitors were observed at flowers of *Glechoma hederacea* and *Viola tricolor*. Hence, the group of plant species that have flowers with easily accessible nectar had a high mean max. sugar crop, highest mean reward rate and the most visitors, that were mostly non-Apoidea. The groups of plant species with intermediatly or complicatedly accessible nectar had a lower mean max. sugar crop, a lower reward rate and less visitors, that were mostly Apoidea.



**Fig. 4** The mean max. sugar crop (mg), mean reward rate (mg sugar\*abundance) and mean number of visitors per 30 min according to the three types of functional flower shapes. Plant species presenting flowers with easily accessible nectar (group 1, N = 21) offer a significantly higher reward rate than species with hidden nectar (group 2, N = 16 and group 3, N = 6) ( $P < 0,001$ ; Kruskal-Wallis Test, Dunn's Method). Although not significant, max. sugar crop and visitation rate are also higher in this group ( $P = 0.059$  and  $P = 0.080$ , respectively).

Flower colours were differently distributed within the groups of functional flower shapes (Fig. 5). Plant species of group 1 presented blue-violet colours (including UV-less combinations and those with UV) as well as yellow colours (also including combinations with or without UV) in all analysed investigation sites. Additionally, white colours were displayed in two sites and a green colour in one site. Plant species that belonged to group 2 and 3 displayed mainly blue-violet colours (with and without UV) and to a usually minor degree also white and yellow (without UV) colours (see also Fig. 1A-D).

Besides the functional flower shape, we recorded the size of the floral display for several species at the LH site to investigate which colours are represented most strongly (Fig. 2). Sizes ranged from  $0.2 \pm 0.001 \text{ cm}^2$  (*Medicago lupulina*, *Myosotis* spec.) to  $9,69 \pm 0,373 \text{ cm}^2$  (*Taraxacum officinale*, App. 1), where displays of *Taraxacum officinale* (UV-yellow) were exceptionally large. Its mean display size was more than double the size of every other species' flower ( $P < 0.05$ , Kruskal-Wallis-Test). Additionally, it had the highest max. sugar crop and reward rate in the site, and we observed a high number of Apoidea visitors. Six other species presented a significantly larger display than most of the other species: *Lychnis flos-cuculi*, *Trifolium pratense* (both blue-violet), the blue-violet morph of *Viola tricolor*, *Anthemis rutherfordica* (white), *Ranunculus auricomus* and *Senecio vernalis* (both UV-yellow;  $P < 0.05$ , Kruskal-Wallis-Test). These species did not belong to the species with the highest max. sugar crop or reward rate (App. 1; *Anthemis rutherfordica* was not measured due to its small nectar volume and complicated nectar accessibility.), and only on *Senecio vernalis* we observed a higher number of Apoidea visitors (App. 2).



**Fig. 5** The distribution of flower colours, according to functional flower shape and investigation site. Plant species with open nectar (group 1) present a greater variety of colours than species with hidden nectar (group 2 and 3). Species are grouped according to their flowers' colour spectra as depicted in Fig. 1. N = number of species.

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*Pollinator visitation and reward properties*

Visitor activity was not distributed equally over all observed plant species (Fig. 3B, C, App. 2). During SFO in the BG site, *Euphorbia esula* (24,1 %, mainly Hymenoptera), *Knautia arvensis* (23,1 %, mainly *Apis*) and *Lathyrus sylvestris* (13,0 %, mainly *Apis* and other Apoidea) were the most visited plant species among all those observed. During activity walks (AW), again *Knautia arvensis* (16,6 %, mainly *Bombus* and other Apoidea) and *Euphorbia esula* (10,5 %, mainly Coleoptera), but also *Trifolium repens* (14,0 %), *Trifolium pratense* (10,5 %, both mainly *Bombus*) and *Leucanthemum ircutianum* (10,5 %, mainly other Apoidea) were the most visited species. During SFO in the LH site, *Lamium purpureum* (38,3 %, only *Bombus*) and *Saxifraga granulata* (34,5 %, mainly other Apoidea) had most visitors. During AW, again *Saxifraga granulata* (21,5 %, only Diptera) and in addition *Taraxacum officinale* (26,9 %, mainly other Apoidea) and *Cerastium arvense* (43,0 %, only Diptera) had most visitors. In the OB site, we counted the most pollinators on *Ranunculus montanus* (25,2 %, only Diptera), *Biscutella laevigata* (13,5 %, only Diptera) during SFO. During AW, we counted the most visitors (as above) on *Ranunculus montanus* (66,9 %, mainly Diptera) and further on *Trollius europaeus* (19,1 %, mainly Diptera). And in the PM site, visitor activity was highest on *Carduus crassifolius* (16,9 %), *Prunella grandiflora* (11,1 %, both mainly *Bombus*) *Scabiosa lucida* (13,8 %, mainly Lepidoptera), *Leontodon hispidus* (11,9 %), *Solidago virgaurea* (11,2 %, both mainly Diptera) during SFO. During AW, visitor activity was highest only on *Scabiosa lucida* (75,8 %, mainly Lepidoptera) and *Crepis conyzifolia* (8,9 %, mainly Lepidoptera and Diptera).

Similarly, visitor activity was not equally distributed over all visitor categories. The main pollinator group in the BG site was the group of Apoidea (SFO/AW: 70,2/60,2 %; *Apis* 31,7/12,8 %, *Bombus* 10,5/23,4 %, other Apoidea 28,0/24,0 %). The observed non-Apoidea were mostly either other Hymenoptera (15,6 %, SFO) or Diptera (13,0 %, AW). In the LH site, the representation of the pollinator groups was very different in the SFO and the AW. During SFO, Apoidea were observed to 72,8 % (*Bombus* 38,3 %, other Apoidea 34,5 %) and non-Apoidea to 27,2 %. During the AW, the situation was reversed: 23,7 % Apoidea and 76,3 % non-Apoidea (Diptera 73,1 %). Interestingly, we observed no *Apis* at all in the LH site. Nor did we observe any *Apis*, and only very few *Bombus* (9,6 % SFO/4,3 % AW) and other Apoidea (0,0 % SFO/0,3 % AW) in the OB site. Here, the majority of the pollinators were non-Apoidea (SFO/AW: 90,4/95,4 %; Diptera 86,1/94,4 %). In the PM site, we found very few but at least some *Apis* (SFO/AW: 0,3/1,2 %). During SFO, Apoidea were

comparatively frequent and were about equally represented as non-Apoidea (46,6 %/53,4 %). During the AW, non-Apoidea outweighed the Apoidea (81,0 %/19,0 %). More specifically, during SFO we observed mainly *Bombus* (45,7 %) and Diptera (29,0 %), whereas during the AW, we observed mainly Lepidoptera visits (68,1 %).

The bottom line is: observations during single flower observations and activity walks did not always lead to the same results. Their combination provides a more comprehensive picture of the abundance of the occurring flower visitors which is as follows: The proportions of our seven visitor categories were different in the four investigation sites. *Bombus* occurred in all sites, *Apis* and other Apoidea did not occur in the LH and the OB site. We did not use the visitor observations in the latter site to derive our conclusions, since during the experiments flower visits occurred much less frequently than usual and lacked the typical presence of Apoidea (Kreisch, personal observations, Neumayer and Paulus 1999). We explain this phenomenon with exceptionally cold and rainy weather and late spring onset as compared to weather observations of Neumayer and Paulus (1999). After excluding the OB site, the group of Apoidea was the main group of flower visitors (App. 2).

If we compare which flowers were visited most frequently, it turns out that flowers with a high reward rate, i.e. the product of max. sugar crop and floral abundance of a plant species, were often visited very frequently by Apoidea, but also by Diptera and Lepidoptera. To be specific, this occurred in *Knautia arvensis*, *Lathyrus sylvestris* and *Trifolium pratense* in the BG site (Fig. 2 and 3B, C, App. 2). They were mostly visited by *Bombus*, *Apis* or other Apoidea. In the LH site, *Bombus*, other Apoidea and Diptera were very frequently observed on *Lamium purpureum*, *Saxifraga granulata* and *Taraxacum officinale*, species with the highest reward rate in that site. In the PM site, *Carduus crassifolius*, *Scabiosa lucida*, *Solidago virgaurea* and *Prunella grandiflora* had both a high reward rate and a high frequency of visits. Here, main visitors were *Bombus*, Lepidoptera and Diptera. 54,5 % of these species had easily accessible nectar, 36,4 % had intermediate and 9,1 % of them had complicatedly accessible nectar (Fig. 4, App. 1).

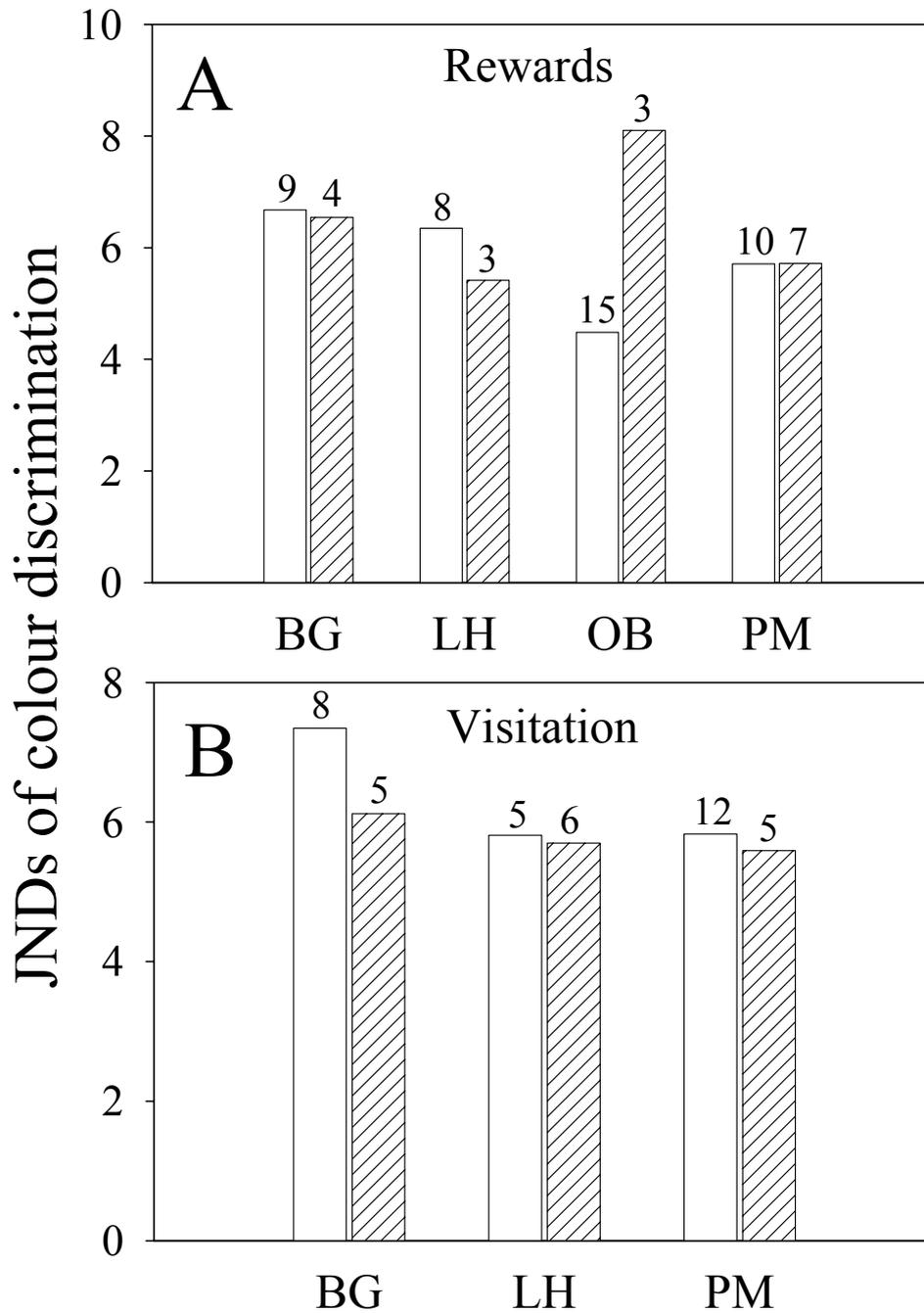
#### *Flower colours and reward properties*

To assess colour similarity from the bee's perceptual point of view, we calculated the chromatic distances between the colour loci of all measured species' flowers in the honeybee colour space (Vorobyev *et al.* 2001). First, we tested whether highly rewarding species or commonly visited species differ strongly in colour from other co-flowering species (Fig. 6A

and B, see also Fig. 2). For each investigation site, we identified the colour spread of the less rewarding species by calculating the mean chromatic distance between them. This value was compared to the mean chromatic distance between low rewarding species and highly rewarding species. The same comparisons were applied to the species, which we identified to be very commonly ( $> 25\%$  of the total number of visits/site; App. 2) and less commonly ( $< 25\%$ ) visited (Fig. 6B). Colours of flowers with highly rewarding or very commonly visited species appeared in all areas of the honeybee colour space. Their loci were usually not strongly separated from those of less rewarding species or less commonly visited species and thus did not tend to be strongly distinguishable from co-flowering plants. This results in a more demanding process of reward estimation for the pollinator, which associates the reward with the floral colouration. Consequently, the question arises: how was the reward distributed between colours at one investigation site, if highly rewarding plants are not conspicuous? The foraging decisions of flower constant pollinators are, among others, usually influenced by the choice and the variability of the colour. Thus, we tested whether species with similar colours are similar in their reward properties (Tab. 1). About 40% of all investigated plant species in our sites displayed colours that were indistinguishable to at least one other species' flower colour, i.e. many flowering plants in our investigation site shared the same colour. More specifically, we found pairs, triplets or quartets with the same colours in three of the sites. Starting from these species we compared median max. sugar crop and reward rate for species with chromatic distances of less than 2.3 RNL units (same colour), up to 3.5 and up to 5 RNL units, respectively, and between colours of all species' flowers. There was no consistent increase or decrease in either max. sugar crop or reward rate in relation to increasing or decreasing chromatic distances within the groups (Tab. 1), indicating a non-clustered distribution of reward values.

**Tab. 1** Median max. sugar crop (mg) and reward rate (the product of max. sugar crop and floral abundance) of the flower species of three investigation sites, categorised into groups of flowers with different chromatic distances ( $\Delta S$ ) in the honeybee colour space (Vorobyev *et al.* 2001).

| Chromatic distances |                    | $\Delta S < 2.3$ | $\Delta S < 3.5$ | $\Delta S < 5$ | All         |
|---------------------|--------------------|------------------|------------------|----------------|-------------|
| LH                  | sugar crop         | 0.20             | 0.20             | 0.15           | 0.16        |
|                     | reward rate        | 0.25             | 0.28             | 0.29           | 0.21        |
| OB                  | sugar crop         | 0.33             | 0.36             | 0.46           | 0.50        |
|                     | reward rate        | 0.41             | 0.51             | 0.52           | 0.45        |
| PM                  | sugar crop         | 0.67             | 0.58             | 0.75           | 0.36        |
|                     | reward rate        | 0.63             | 0.72             | 0.72           | 0.68        |
| All                 | <b>sugar crop</b>  | <b>0.33</b>      | <b>0.36</b>      | <b>0.46</b>    | <b>0.36</b> |
|                     | <b>reward rate</b> | <b>0.41</b>      | <b>0.51</b>      | <b>0.52</b>    | <b>0.45</b> |



**Fig. 6** Mean chromatic distance **A** within the group of species with low reward rates (open bars) and between the group of species with low reward rates and the group of species with high reward rates (hatched bars) and **B** within the group of species with low visitation rates (open bars) and between the group of species with high and the group of species with low visitation rates (hatched bars). One JND (just noticeable differences) corresponds to 2.3 RNL model units (Vorobyev *et al.* 2001); Numbers indicate the N of highly rewarding species (above open bars) and of low rewarding species (above hatched bars).

### **Discussion**

The present study investigated whether floral colouration might be influenced by the selective pressure of insect flower visitation. In our approach to understanding this interrelation we focused on i) the analysis of flower colour distributions within highly rewarding bee pollinated plant species in relation to the colour distributions of less rewarding species to find out if differences are perceived by bees and ii) the analysis of reward properties of flowers with very similar or very different colours to determine whether reward distribution in the site is clustered which would allow the bees to select adaptive choice strategies based on different degrees of colour generalisation.

#### *Reward and profitability*

The most challenging task in interpreting data of reward properties in natural plant communities is to determine what a high reward is for a pollinator and how it can be measured appropriately. Reward estimation in pollinators is widely studied and reveals complex relationships between biological and physical spatio-temporal parameters. The main reward parameter of the extracted nectar is its energetical value for the flower visitor. Honeybees are able to discriminate sucrose concentrations (Haupt 2004) and they react to changes in the nectar flow rate, i.e. they are able to estimate the amount of sugar imbibed over time (Núñez 1970, Varju and Nuñez 1991, Klinkhamer *et al.* 2001). The amount of sugar that can be gained during one flower visit can be highly variable in the natural environment. Firstly, different plant species or individuals of one species can secrete different volumes of nectar at particular rates, depending on the available resources (Sakai 1993) and with particular sugar concentrations whereas the volume is limited by the flower's size and/or shape (for reviews see: Kugler 1970, Faegri and van der Pijl 1979). Secondly, the frequency of visits of other pollinators can also strongly influence the flower's current reward. Honeybees are very good learners and are able to switch to highly rewarding or highly reliable species very quickly (Menzel 1967, 1969, Núñez 1970). As an increasing number of pollinators specialises on a single species, the current reward decreases, which leads to a redistribution of pollinators to other flowering species which have in turn become more highly rewarding on a subjective relative scale of available nectar sources (Heinrich 1979). The pollinator needs to find the best food sources in a given situation in order to obtain a

positive cost-benefit energy balance. Reward evaluation is therefore a process that involves repeated sampling to assess the variability of the reward at a particular food source type and to estimate the mean reward value. The latter ability has been found to be meaningful for bees in estimating reward differences and consequently selecting food sources on the basis of their relative profitability (Fülöp and Menzel 2000, Keasar *et al.* 2002). Núñez also demonstrated that honeybees react to changes in the flow rate of the nectar in artificial (1966, 1970, see also Greggers and Menzel 1993) and in natural conditions (1977a, see also Klinkhamer *et al.* 2001), proving that they are able to estimate profitability by means of reward comparisons between different plant species. Heinrich (1979, 1983) described how bumblebees “major” and “minor”, i.e. they adjust their visitation rates, when given a choice between flower species of different profitabilities.

Estimation of plant species' profitabilities has been approached in several studies. Some of them derived their data from qualitative observations (Giurfa *et al.* 1995), whereas others implemented quantitative measurements by determining the secretion rates or standing crops of nectar of selective plant species (Núñez 1977b, Comba *et al.* 1999a, 1999b, Corbet 2003). Measurements of the highly variable standing crop require a relatively large sample size to assess reliable results, which is not convenient for the measurement of many species in parallel. Chittka *et al.* (2004) quantified nectar production rates as sugar crop produced three hours after emptying the flower, extrapolated to a daily reward for pollinators. Instead, we used the nectar volume, accumulated during 24 hours to represent the maximal reward a pollinator could obtain at any particular visit. Measurements were performed in parallel at all investigated plant species of one site and repeatedly over several days. They were combined with observations of flower visitors. Thereby we assumed that the studied plants have similar nectar flow rates, as has been shown for most native European flowering plants (Comba *et al.* 1999a, 1999b, Corbet *et al.* 2001). Visitation rates of pollinators indicate which flowers are able to satisfy a higher or lower number of visitors successfully. Therefore, we recorded visitation rates of all occurring pollinator groups and estimated reward potentials of plants from their visitation frequency.

We compared the plant species' profitabilities on the basis of the sugar reward that a flower visitor could obtain at most, i.e. the max. sugar crop of the flower. Thus, we assessed the relative relationships between the reward potentials of different flower species. As a result, we determined a number of plant species with a significantly higher max. sugar crop than most of the other species in their investigation site (Fig. 2). However, max. sugar crop on its own may not be sufficient to make a statement about the current reward that is obtained by a single

pollinator. Plant species with a relatively lower max. sugar crop may still be profitable by producing a high number of flowers or being abundant. Honeybees and bumblebees and even a number of wild bees, which are known to be flower constant, might prefer to visit a highly abundant plant species than a rare species (for reviews see: von Frisch 1927, Grant 1950, Goulson 1999). To some extent, we were able to observe this behaviour in our investigation sites. Two of the common species ( $\geq 10\%$  of open flowers of all available open flowers in the site) were also commonly visited species ( $\geq 10\%$  of flower visits of the total number of flower visits in the site) and visited by bees: *Lamium purpureum* (LH site) and *Prunella grandiflora* (PM site; Fig. 3). However, other species commonly visited by bees were intermediately ( $< 10\%$ ) abundant species (*Saxifraga granulata* and *Taraxum officinale* agg, LH site; *Trifolium repens*, BG site) or rare ( $< 5\%$ ) species (*Knautia arvensis*, BG site; *Senecio vernalis*, LH site; *Carduus crassifolius* and *Scabiosa lucida*, PM site; Fig. 3). Except *Senecio vernalis* and *Trifolium repens*, all these species belonged to the groups with significantly higher max. sugar crop and/or reward rate (Fig. 3).

Given that large differences in abundance may also influence the reward potential of a flower species, we defined reward rate as being the mean max. sugar crop multiplied by mean abundance. Reward rate distributions within one investigation site were statistically different in relation to max. sugar crop distributions. Estimating current reward from that perspective, six species in total were now more rewarding, ten species became less rewarding and twelve species did not change significantly compared to the distribution of max. sugar crop (App. 1). To summarise: Since the profitability of a plant species is a relative phenomenon with spatio-temporal dynamics and thus potentially different in every habitat, we analysed the colour distribution of flowers in relation to the different parameters separately. Our aim was to compare rewards and colours, because bee pollinators (honeybees and bumblebees) usually start foraging by sampling the available resources broadly. In the process they learn to associate certain flower features with the respective reward, and they develop flower constancy as well as specific but relatively risk indifferent expectations, e.g. of the colour-reward pairings (Fülöp and Menzel 2000, Heinrich 1979, 2001). The acquired memory for the floral features in combination with the reward properties can influence the bees' choices and their degree of constancy (Greggers and Menzel 1993).

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*The relation between flower colours and reward properties*

Flowering plant species require efficient pollination to assure reproduction, population growth and spread. Two strategies to obtain efficient pollination are sequential flowering and divergence in appearance (Heinrich 1974). Sequentially flowering species may have convergent or similar flowers, because they cannot be confused by pollinators. Common species that flower simultaneously may have divergent or dissimilar flower types, e.g. they may present flowers with different colours. Contrarily, rare plant species may also have similar flowers to profit from pollinators which are attracted through the more common co-flowering species presenting a similar colour (Benitez-Vieyra *et al.* 2006). Similar mechanisms of mimicking a magnet plant were also shown to be successful when flowers are food deceptive (Johnson *et al.* 2003a), because they still receive occasional visits (Gumbert and Kunze 2001, Johnson *et al.* 2003b, for a review see Dafni 1984). When two visually different flower types are equally predictable in profitability, Marden and Waddington (1981) could show that bumblebees specialise on one of them, but only when flowers were placed equidistantly from each other. When flowers were spaced unequally, bees most often chose to visit the closest flower, as it would be predicted by optimal foraging (Waddington and Holden 1979). For the same reason bees will rather specialise to visit a flower with low profitability when it is abundant. Hence, flowers have to be more rewarding the lower their abundance is (Waser 1983). In that case, it would be useful to diverge strongly in signal from sympatric species. Gumbert *et al.* (1999) investigated those two possibilities and found out that rare plant species (< 10 specimens/site) show a tendency to diverge in colour from sympatric plant species. Colouration in co-flowering plants in some habitats was not random.

Our observations show that plant species with rare flowers (< 5 % of the total number of flowers in the site) did not generally have a high max. sugar crop (App. 1). To be specific, all rare flowers on the LH site provided an intermediate or significantly lower max. sugar crop. In the OB site, only five out of the 14 rare flowers (36 %) had a higher max. sugar crop. Further values were: two out of five (40 %, BG site) and seven out of eleven (64 %, PM site) rare flowers had a higher max. sugar crop. Two species, *Knautia arvensis* (BG site) and *Gentiana brachyphylla* (OB site), compensated a low abundance with a significantly higher max. sugar crop, which resulted in a significantly higher reward rate compared to other species. Other species, e.g. *Gentiana clusii* (OB site) and *Campanula barbata* (PM site), did not compensate their low abundance with a significantly higher max. sugar crop to achieve a high reward rate. But they presented similar colours to other species in the site, thus possibly

enhancing their visitation rate (Benitez-Vieyra *et al.* 2006). In most cases, a high reward rate was accomplished via a high or medium max. sugar crop *and* a high or intermediate abundance.

On the other hand, 46,2 % of the species with very rare flowers (< 1 %) displayed indistinguishable colours to flowers of other more common plant species (Fig. 3A, App. 1). Such groups of species displaying indistinguishable colours but different abundance were found in two of our sites. On the OB site, *Llodya serotina* and *Ranunculus alpestris* both presented white colours, *Gentiana clusii*, *Gentiana brachyphylla*, *Acinos alpinus* and *Linaria alpina* presented blue-violet colours and *Thymus praecox*, *Myosotis alpestris* and *Primula minima* had flowers with blue-violet colours. On the PM site, *Campanula barbata* and *Vicia cracca* were both blue-violet. A similar group having blue-violet colours contained *Phyteuma persicifolium*, *Vicia cracca* and *Campanula scheuchzeri*. A group with UV-yellow colours contained *Arnica montana*, *Crepis conyzifolia* and *Potentilla erecta*. At the time of our investigations and according to our categorisations, there were no very rare flowers in the LH site. And none of the very rare flowers in the BG site displayed indistinguishable colours to other species, but one of them was the whitish morph of *Salvia pratensis*, which most probably had a very similar odour to the blue-violet morph of *Salvia pratensis*. Thus, it seems that rare species in our investigation sites rather evolved flowers with a very similar colouration and did not invest in higher rewards, in order to be efficiently pollinated.

These results are different to those obtained by Gumbert *et al.* (1999), which is most likely due to striking differences between investigation sites. All our sites contained defined and authentic plant communities in protected areas and were further characterised by a long (1-5 centuries) and constant utilisation by strictly timed mowings or grazing animals. Our results are rather in line with the results of Petanidou *et al.* (1995), who showed that visitor frequency of the rare *Gentiana pneumonanthe* was highest in the natural setting of an *Erica tetralix*-patch. No colours were measured in this study, but it supports our hypothesis that the common occurrence of another species can influence the visitation rate of a rare species.

We also tested whether highly rewarding species or commonly visited species have different colours to less rewarding or rarely visited species to find out which of the two possibilities (divergence in appearance or colour similarity) the investigated plants took advantage (Waser 1983, Dafni 1984). Flower colours of our investigation sites are widely distributed within the honeybee colour space (App. 1). Colours of species with a high reward rate or a high visitation rate appear in various areas of the colour space and are usually not located apart from colours of flowers with a lower reward rate or a lower visitation rate (Fig. 2; 3B, C).

Further, chromatic distances between flowers with a low reward rate were not consistently larger or smaller than their chromatic distance to flowers with a high reward rate (Fig. 6A). Hence, pollinators could not rely on large chromatic distance of the flowers of one plant species to another within the patch to ensure a high reward rate. A similar picture arose from the comparison of rarely visited and commonly visited flowers. Flowers with higher visitation rates did not have colours of large chromatic distance to the other flower colours (Fig. 6B).

We could not find an indication for Waser's hypothesis (1983) that rare species generally produce a high reward and strongly diverge in signal from co-flowering species. We rather support the hypothesis that rare species try to be as similar as possible in colour to more common species. Since honeybees are able to generalise colours (Backhaus *et al.* 1987), we were intrigued to find indications that a pollinator might profit from narrower or broader colour generalisation to enhance its energy gain during foraging. However, we could detect no general pattern of increasing or decreasing sugar crop or reward rate with increasing or decreasing chromatic distance of the flower colours (Tab. 1).

#### *The influence of functional flower shape and display size*

The functional shape, which we defined as the degree of ease with which the flower visitor is able to access the nectar, is an important trait of flowers that may influence the pollinator's foraging decisions. Honeybees are able to discriminate (Baumgärtner 1928, Hertz 1933, Gould 1985, Giurfa *et al.* 1996a, Campan and Lehrer 2002, Hempel de Ibarra and Giurfa 2003, for reviews see: Wehner 1981, Dafni *et al.* 1997) and to learn the handling (Heinrich 1979, Chittka 1997) of different floral shapes. The visitors in our sites preferred flowers with easily accessible nectar (group 1), which correlates with the measured higher reward rates as compared to flowers that required a greater effort to obtain the nectar (group 2 and 3; Fig. 4). This result was surprising, because it is commonly believed that e.g. campanulate, bilabiate or papilionate flowers, which are the main contributors to groups 2 and 3, offer on average a higher reward to pollinators (Kugler 1970, Faegri and van der Pijl 1979). On the basis of mean max. sugar crop, we could find no significant difference between the groups, but a tendency of higher max. sugar crop in flowers of group 1. Assuming similar nectar flow rates between groups, a high visitation rate would lead to smaller amounts of current reward for most visitors. Since time intervals between visits are variable, a high max. sugar crop would allow for an accumulation of a larger reward in the flower per time unit than a small max. sugar crop and would thus attract a more diverse group of visitors. Another strategy for

competing plant species could be the limitation of the number and types of visitors by having a functional flower shape with hidden nectar. And indeed, we find that species with hidden nectar produce a lower max. sugar crop but are visited by fewer types of visitors – mostly bees. Additionally, these plant species displayed mainly blue-violet colours with or without UV, whereas the plant species allowing open access to their nectar, i.e. that attract a larger variety of pollinators, displayed a more diverse range of colours (Fig. 5).

In general, the relative distribution of the pollinators on the plants in our investigations sites matched the reward distribution measured by means of max. sugar crop and reward rate, well. Visitation rates may be also further influenced by flower detectability (floral display size) and the flower shape (individual level: Giurfa *et al.* 1996b, Spaethe *et al.* 2001, species level: Grindeland *et al.* 2005, Stang *et al.* 2006, for a review see: Ohashi and Yahara 2001). The larger the floral display, the larger the distance from which it could be detected (Giurfa *et al.* 1996b). Spaethe *et al.* (2001) showed that, in bumblebees, larger target size reduces search time. Moreover, several studies provide evidence that plants with large flowers are visited more often than plants with smaller flowers (Robertson and Macnair 1995, Goulson *et al.* 1998, Stout 2000). Hence, plant species with more easily detectable flowers might be preferred and could thus have evoked a preference in bee pollinators for the most commonly displayed colour by these flowers.

We noticed an effect of display size on visitation rates. At the LH site, we determined six species with a significantly larger and seven species with a significantly smaller display than most of the other flowering species (App. 1). The species with the smallest displays were not visited by Apoidea (App. 2). Relatively heavy bees are often not able to land on such a flower, and their proboscis might be too wide to be introduced into the flower (Stang *et al.* 2006). But to some extent, we did observe bee visits on flowers with the largest displays (App. 2). *Taraxacum officinale* with the highest max. sugar crop, the highest reward rate and the largest display size was also visited with the highest frequency by *Bombus*, other Apoidea, Lepidoptera and Diptera. We did not observe pollinator activity of all other six species with a significantly larger display, but *Senecio vernalis* had rather high visitation rates of other Apoidea and some Diptera, whereas *Ranunculus auricomus* and the blue-violet morph of *Viola tricolor* had very low visitation rates of Diptera and *Bombus*, respectively (App. 2). The colours of the flowers with the largest displays occurred in the UV-yellow and yellow as well as in the UV-blue, blue-violet and white areas of the colour space and showed no particular tendency to display a limited number of colours (Fig. 2).

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*Innate colour preferences of bees*

Giurfa *et al.* (1995) postulated that innate preferences for blue-violet colours would quickly lead the forager to the most profitable food sources. They hypothesised that flower colours are related to the amount of nectar they produce: 80 % of blue-violet flowers offer on average a high reward, whereas only 20 % of the white and 5 % of the yellow flowers offer high rewards. Others would lie in between these extremes. We investigated whether the bees' innate preference for blue-violet colours corresponds to higher reward properties of the flowers presenting these colours. It has been shown that honeybees and bumblebees have innate preferences for blue-violet colours which however are usually quickly overridden (Giurfa *et al.* 1995, Gumbert 2000). These colours are also learned faster by experienced foragers (Menzel 1967). We asked whether these preferences would help young foragers to find the best food sources on the first foraging flight. The first foraging flight is associated with a high risk for the forager. Bees usually leave their hive with an empty stomach, so that they have little energy reserves (von Frisch 1965). They need to find profitable food sources soon, while taking care of landmarks for orientation and being aware of predators.

In our investigation sites, the most profitable food sources in terms of max. sugar crop or reward rate were often blue-violet or UV-blue-violet in colour (50 %/66,7 %, respectively). But they also had yellow or UV-yellow (27,3 %/27,8 %) or white (22,7 %/5,6 %) colours (Fig. 1, 2, App. 1). These colour classifications are estimated on the basis of their reflectance spectra, but to be handled with care, since we do not know exactly how honeybees classify colours, i.e. under which circumstances two distinct colours are rated as belonging to the same or to two different classes. There was an indication that flowers with blue-violet or UV-blue-violet colours are often rewarding. But naïve honeybee foragers that were tested under laboratory conditions did not show preferences for all UV-blue-violet colours, but only for a strongly bounded area (390-430 nm). Within this approximate area, we found only 3 species that were highly rewarding (Fig. 2): *Campanula rotundifolia* (BG site), *Bartsia alpina* (OB site) and *Campanula scheuchzeri* (PM site). Interestingly enough, these flower species were classified as complex-shaped flowers with a less easy access to the nectar. Thus, we suggest that the flower-pollinator-system could be too complex to be reduced to such a generalisation. However, innate colour preferences might be adaptive, because first of all, the blue colour is rare in nature compared to e.g. green, brown or yellow colours. This circumstance, among others, could lead to the establishment of an easily acquirable search pattern which would help the bees to find the food source quickly. The food source, in this case, would not

necessarily have to be highly rewarding in terms of the amount of obtainable sugar. Such frequently visited flowers would however be easily detectable and therefore could be rated above a subjective threshold established during sampling in a spatio-temporally dynamic environment (Heinrich 1979).

### **Acknowledgements**

We thank A.A. Cocucci, A. Sérsic and, A.D. Stevens for thoughtful comments on the experimental design, R. Menzel, M. Vorobyev and D. Osorio for fruitful discussion and to R.R.R. Wertlen for help with programming. We acknowledge A.D. Stevens for kind permission to work in the Botanical Garden of Berlin and G. Brokamp, T. Henning, C. Schneider and T. Teutscher for motivated help. A.M. Wertlen was funded by a doctoral fellowship of the Berlin Program for the Promotion of Equal Opportunities for Women in Science and Higher Education.

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