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SPECTRAL SENSITIVITY OF PHOTORECEPTORS AND COLOUR VISION IN THE SOLITARY BEE, *OSMIA RUFA*

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Summary

The spectral sensitivity of single photoreceptors of *Osmia rufa* was determined by a fast voltage-clamp technique. Three receptor types were found whose spectral sensitivity functions followed a rhodopsin-like photopigment absorption function with λ_{max} values at 348 nm (ultraviolet receptor), 436 nm (blue receptor) and 572 nm (green receptor). The λ_{max} of the green receptor in *Osmia rufa* is shifted to much longer wavelengths compared with other insect species. Discrimination of colour signals was tested after training a bee at the entrance to its nest. The colour signals were filter discs (70 mm in diameter) with a hole (10 mm in diameter) in the centre and the bees quickly learned to use the coloured disc as a marker of the nest entrance. Tests were dual forced-choice tests with two coloured discs closely positioned next to each other. 94 different tests were each repeated 5–15 times and were performed after training to 12 different colour signals.

A photoreceptor model was used to calculate the loci of the colour signals in a three-dimensional colour space and in a chromaticity diagram. The perceptual distance between the colour loci was calculated as line elements (minimum number of just noticeable difference, jnd-steps), which were based on the noise properties of the photoreceptors. The discrimination determined by the behavioural tests correlated very well with the jnd-steps. The correlation was better for the line elements in the colour plane than in the colour space. *Osmia rufa* was compared with the honeybee *Apis mellifera* and the stingless bee *Melipona quadrifasciata*. There is no difference in colour selection between *Osmia* and *Apis*, whereas *Melipona* discriminates less well in the violet–blue region. The model calculation was used to compare the chromaticity diagrams and the spectral discrimination functions of the three species. It is concluded that the receptor model used in this study predicts the discrimination behaviour of the three bee species very well. Therefore, comparative studies on colour vision in flower-visiting insects may be based on spectral measurements of the photoreceptors, and in many cases this reduces the extent of laborious behavioural studies.

Key words: spectral sensitivity, colour vision, photoreceptors, colour space, spectral discrimination.

Introduction

Colour vision studies in insects have so far been limited to a few species, and only the honeybee's colour vision system has been analysed in any detail at receptor, interneurone and behavioural levels (see Menzel, 1979, 1985*a*). This provokes the question of whether other flower-visiting insect species are equipped with a similar colour vision system or whether species-specific adaptations lead to different colour vision systems. Flower-visiting hymenopterans are of particular interest with regard to this question since they span a large evolutionary scale (from primitive wasps to apoide bees), live in very different habitats (from dense tropical rain-forest to alpine regions and northern tundras), and apply different strategies in searching for food (ranging from oligolectic specialists to polylectic generalists) (Kevan & Baker, 1983). We report in another paper that the stingless bee *Melipona quadrifasciata* has a trichromatic colour vision quite similar to that of the honeybee but that the ultraviolet and blue receptors are shifted to longer wavelengths. Other social hymenopterans such as bumblebees and wasps are equipped with the same spectral receptor types as the honeybee (Fietz, Hertel & Menzel, 1986; R. Menzel, A. Fietz, D. Peitsch & D. Ventura, unpublished observations) and discriminate colours as well as the honeybee (Beier & Menzel, 1972; R. Menzel & D. Ventura, unpublished observations).

We report here that the solitary bee *Osmia rufa* discriminates colours even better than the honeybee, and is equipped with a trichromatic colour vision system similar to that of *Apis mellifera*, four species of *Bombus* (*B. terrestris*, *B. lapidarius*, *B. monticola*, *B. jonellus*) and two wasp species (*Paravespula germanica*, *P. vulgaris*). Model calculations based on the properties of the receptors are used to define a quantitative measure of the discrimination between a pair of colour signals (Backhaus & Menzel, 1987). This measure – the just noticeable different steps (jnd) – correlates very well with the behavioural results from colour discrimination tests.

Materials and methods

Recordings

Conventional techniques were used to record intracellularly from photoreceptors in the compound eye of *Osmia*. The spectral sensitivity $S(\lambda)$ was determined by a voltage-clamp technique, which enabled the establishment of a $S(\lambda)$-function within 16 s and a spectral resolution of 4 nm (Menzel *et al.* 1986). A grid monochromator was used to scan the spectrum from 300 to 700 nm (and *vice versa*) and a circular neutral-density wedge was used to adjust the light flux, with the help of a feedback system, in such a way that the response of the cell was clamped to a preselected receptor potential. The receptor potential was clamped to 3–6 mV above resting potential (about −40 mV) in most of our recordings. A computer calculated and displayed on-line the $S(\lambda)$-function from the settings of the neutral-density wedge for 100 wavelengths between 300 and 700 nm.

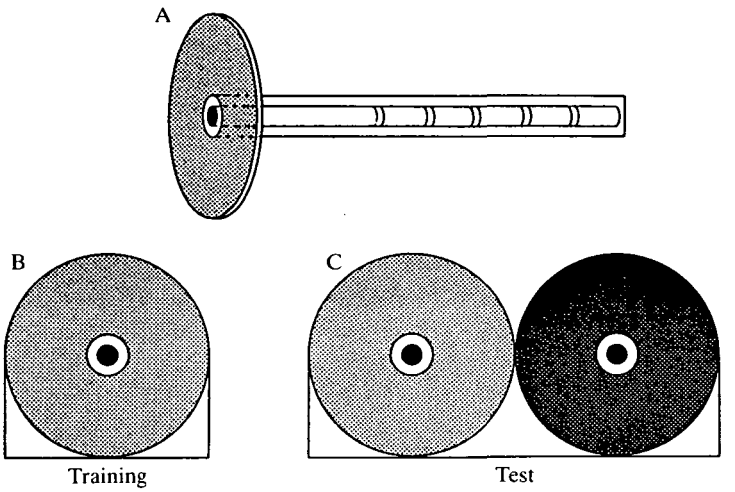

Fig. 1. Experimental arrangement for the training of *Osmia rufa* to colour signals. (A) Side view of the bamboo stem which houses the brood cells. The bee reaches the cells through the entrance hole which is surrounded by a coloured disc (70 mm diameter). (B) During training the bee views the coloured disc on its approach to the nest and learns quickly to orient itself with respect to the colour signal. (C) In the test situation two coloured discs are presented equally distant from the former position of the training disc, which is now removed together with the nest site. One of the two alternative colour discs resembles the training colour. The position of the two discs is changed between tests.

Behavioural experiments

Osmia rufa is a solitary bee, which breeds in holes in wood. Fabre (1925) described a method for housing *Osmia* and other solitary bees in stems of bamboo, and this is the method that was used here. *Osmia* builds its nest in such a stem, and provides the brood cells with pollen and nectar by flying in and out for 3–4 weeks in late spring. The bees can be trained to colour marks which surround the entrance to the bamboo stem (Steinmann, 1981). Learning of the colour marks has not yet been studied in detail, but after 1 day of training no improvement of choice behaviour was observed and, therefore, tests were performed after the second day of training. Fig. 1 shows the experimental arrangement. During training the bee had open access to the nest through the centre hole (diameter 10 mm) of the vertically arranged colour mark (diameter 70 mm). The colour mark was a 1 mm thick glass filter fixed on top of cardboard painted with silver paint or on top of coloured cardboard. The filters used were 1 mm thick Schott filters (Schott & Gen. Mainz; see Table 1). Such colour stimuli permitted the testing of colour discrimination in the ultraviolet without self-luminant stimuli (Menzel & Lieke, 1983). During the test, two colour marks were placed side by side and equally distant from the former position of the colour mark. Two empty and fresh bamboo stems were inserted in the centre hole, and the stem with the nest was removed. The bees approached the colour marks, and landings were counted as choices; the

test was completed after 10 landings had been counted. At the next test the same colour pairs were interchanged in position. Special care was taken to remove odour marks on the filters, and only freshly cleaned filters were used during the test. The surrounding background was the wall of a wooden shelf in front of which many bamboo stems were arranged as nests for *Osmia*. Since *Osmia* flies out only on sunny days the bright light conditions were similar for all experiments. The same bee could be used in another series of experiments after it had been trained to the new colour signal for at least 1 day. No differences were found between initial trainings and reversal trainings.

Statistics

The choice behaviour was expressed as the proportion, p, for the trained colour relative to the alternative colour. The significant intervals were determined from the proportions, p, and the total number of choices N as the deviation, s, of the binomial distribution:

$$s = \sqrt{p(1-p)/N}\,.$$

The non-parametric χ^2-test (Kolmogoroff–Smirnoff test) was used to determine the homogeneity of the choices of two series of experiments. Differences were considered significant if the probability of homogeneity was less than 0·05.

Results

Spectral receptor types

The resting potential (−40 to −50 mV), the depolarizing light response (up to 60 mV), and the phasic–tonic time course of the graded light response resembled the typical receptor characteristics of fast insect photoreceptors. Discrete potential fluctuations (quantum bumps) appeared at very low light intensities, but they never exceeded 0·5 mV. The V/logI-function (slope 0·7) was independent of the wavelength of the stimulating light and of the receptor type. The average spectral sensitivity, S(λ), of the three spectral receptor types is given in Fig. 2. S(λ) of the ultraviolet receptor (λ_{max} = 348 nm) is in accordance with the spectral absorption of the ultraviolet photopigment in other insect species (e.g. *Ascalaphus*, Hamdorf, Paulsen & Schwemer, 1973). Sensitivity above 430 nm is very low – less than 1 % between 430 and 620 nm and 1–2 % between 620 and 700 nm. The small increase at long wavelengths may be an artefact resulting from the secondary wavelength bands of the grid monochromator at half the wavelength of the main band for which the ultraviolet band is most sensitive. The blue receptors (λ_{max} = 436 nm) follow the theoretical absorption function of a rhodopsin pigment with the corresponding λ_{max}. The hump around 370 nm probably originates from the β-peak of the photopigment, since a model calculation precisely describes the short-wavelength part of the S(λ) if one assumes an addition of 10 % of a β-absorption at 380 nm to the Dartnall-function of the rhodopsin. The λ_{max} of the β-absorption has been calculated from the green receptors of both *Osmia* and *Apis*. In *Apis*, the broadening of the blue receptor sensitivity at shorter wavelengths can also be

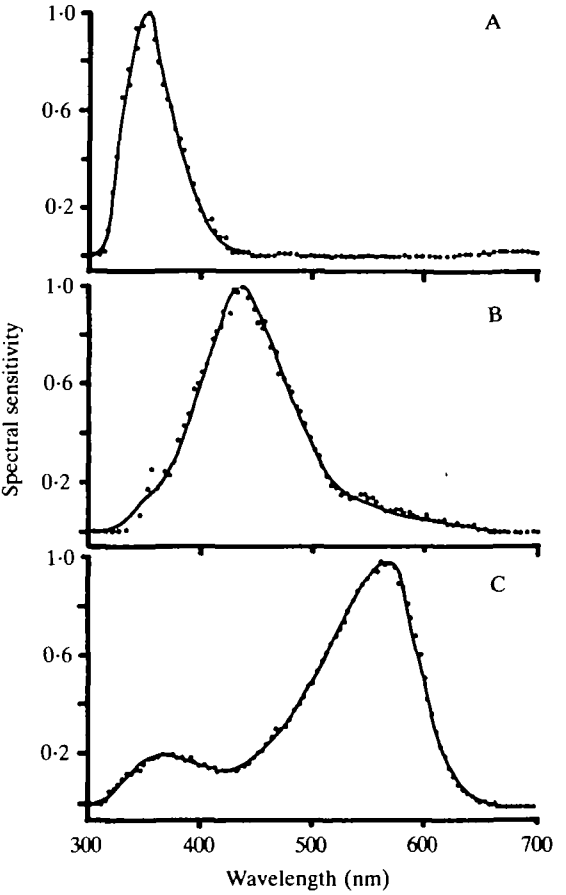

Fig. 2. Average spectral sensitivity functions of the three spectral receptor types in the compound eye of *Osmia rufa*. (A) Ultraviolet receptor, average of three cells (eight spectral runs), $\lambda_{max} = 348$ nm; (B) blue receptor, average of three cells (eight spectral runs), $\lambda_{max} = 436$ nm; (C) green receptor, average of 26 cells (60 spectral runs), $\lambda_{max} = 572$ nm.

modelled accurately if one assumes the contribution of such a β-absorption. The enhanced sensitivity of the blue receptor at longer wavelengths ($\geq$520 nm) is more difficult to interpret. Since recording from blue receptors is very difficult, we consider this to be an artefact due to electrical coupling to the green receptors. This interpretation is supported by the finding of one blue receptor without any enhanced long-wavelength sensitivity. Since the source of the increased green sensitivity of the blue receptors is unknown, we have used the measured $S(\lambda)$-function (not the theoretical function) in the receptor model (see below). The same procedure is followed for the other two receptor types. The green receptors ($\lambda_{max} = 572$ nm) are easier to record from. $S(\lambda)$ follows a theoretical absorption function of a rhodopsin pigment with the corresponding λ_{max}. The β-sensitivity is very flat with a peak around 380 nm. The main peak spectral sensitivity at 572 nm is

Table 1. *List of all colour signals used in this study*

No.	Filter	U	B	G	H
1	UG 11	4·11	0·89	0·607	5·61
2	UG 3	5·32	6·99	3·50	15·8
3	BG 3	7·11	9·33	2·33	18·8
4	BG 25	3·56	7·07	1·79	12·4
5	BG 12	3·90	10·76	2·57	17·2
6	BG 25 + B 3	8·42	3·31	1·01	5·17
7	BG 28	1·57	11·60	4·46	17·6
8	BG 28 + B 4	4·04	3·49	1·65	5·54
9	BG 24	8·30	12·1	3·72	24·0
10	BG 23	3·78	19·5	15·9	39·1
11	BG 27	0·79	8·26	6·10	15·1
12	BG 23 + Bv 1	0·87	7·41	7·61	15·9
13	BG 28 + Gr 4	0·84	9·03	4·59	14·4
14	BG 23 + Gr 2	0·70	6·90	10·55	18·2
15	BG 27 + B 4	0·89	15·89	8·93	25·7
16	BG 24 + y 2	1·61	2·67	2·14	6·4
17	BG 18	2·11	15·61	18·08	35·8
18	VG 6	0·38	8·16	11·40	19·9
19	VG 6 + Gr 3	0·23	6·90	13·09	20·2
20	VG 9	0·05	2·37	6·53	8·9
21	GG 495	0·04	5·39	25·53	30·9
22	GG 495 + B 4	0·04	2·72	10·21	12·9
23	OG 530	0·05	2·08	15·23	17·4
24	OG 550	0·05	1·55	12·11	13·7

The number (first column) is used in all figures and in the text to assign a particular colour signal.

Column 2 gives the filters and filter-paper combinations. If only a filter is indicated it is combined with a silver-painted cardboard; otherwise the filter lies on top of a coloured paper.

U, B, G are the tristimulus values for each colour signal, and H is a measure of the brightness of the colour signal (H = U+B+G) (see text).

at much longer wavelengths than in the green receptors of the honeybee ($\lambda_{max} = 540$ nm).

Colour discrimination: qualitative aspects

Osmia is equipped with three spectral receptor types – ultraviolet, blue and green. These provide its nervous system with the necessary information required for colour vision. How is this information used? To answer this question we tested colour vision by training single animals at the entrance of their nests. The colour signals used in the training experiments are listed in Table 1. In the following only the running numbers given in Table 1 are used to indicate the filter or filter/cardboard combinations. Table 1 also gives the coordinates of the colour loci of the respective colour signals. The colour loci were calculated by a procedure which follows that of Cornsweet (1970) and Rushton (1972), and which takes the

three spectral receptor types with their particular spectral sensitivity (see Fig. 2) as being a special set of primary colours. These define an orthogonal three-dimensional space, and the three orthonormal basis vectors of the colour space correspond to the tristimulus values of unit amount. Each colour signal is defined by a vector (Rodiek, 1973). The colour vector is obtained by vector addition from the components, i.e. the relative photon fluxes U, B and G absorbed by each of the receptors. These U, B and G values are given in Table 1 together with a measure of the subjective brightness (H), which is assumed to be equal to the sum of the tristimulus values. The vectors obviously depend on the spectral reflection of the filter/cardboard combination and the spectral distribution of the illuminating natural light. These factors are taken into account in our conclusions. (The procedure for the calculation is described in more detail in Menzel, 1985*a*, and Backhaus & Menzel, 1987.) Since the length of the vector is not important in describing the perceived chromaticness (hue and saturation) of a colour, the normalized tristimulus values or chromaticity coordinates in a chromaticity diagram, from which only two are linearly independent, define unequivocally the chromaticness of a colour signal. Chromaticity diagrams of this kind have been successfully used to describe quantitatively the colour vision of honeybees at the level of both lower and higher colour metrics (Backhaus & Menzel, 1987). In our model calculation, the tristimulus values U, B and G are normalized in such a way that each value is of unit length for an achromatic object ($BaSO_4$), which is illuminated by natural daylight (spectral radiation of the international D 65 norm function). Fig. 3 gives the chromaticity diagram together with the loci of the colour signals listed in Table 1.

Osmia quickly learned to find the entrance hole of its nest with the help of a coloured disc, which surrounded the hole. The training started when an *Osmia* female flew regularly in and out of a bamboo stem, indicating that this particular stem had been selected for breeding and that brood cells were being built. The first visual mark at the entrance was a small ring painted with silver paint. This ring was exchanged for a larger disc, and after the individual had adapted to this disc, a silver disc was presented with the final diameter (70 mm) of the coloured discs. This silver disc was finally exchanged for one of the coloured discs, the trained colour signal. Tests could be undertaken a few hours later, since the animal learned the colour signals very quickly. In most of the experiments the same bee was trained to a second or third colour signal when the tests with the first trained colour had terminated. However, in this case the bee viewed the silver disc for 2 days before learning the new signal. The choice behaviour of a bee for the trained colour was independent of a previously trained colour or colours.

The pretraining to silver paint may have affected choice behaviour in a colour-selective manner. Since we did not use a different pretraining procedure, we could only test the possibility of a colour bias by comparing symmetrical test situations, i.e. tests where the same pairs of colour signals were tested either after training to one colour or training to the other. For example, if colour signal no. 2 is used for training, and the discrimination between no. 2 and no. 7 was tested, the average

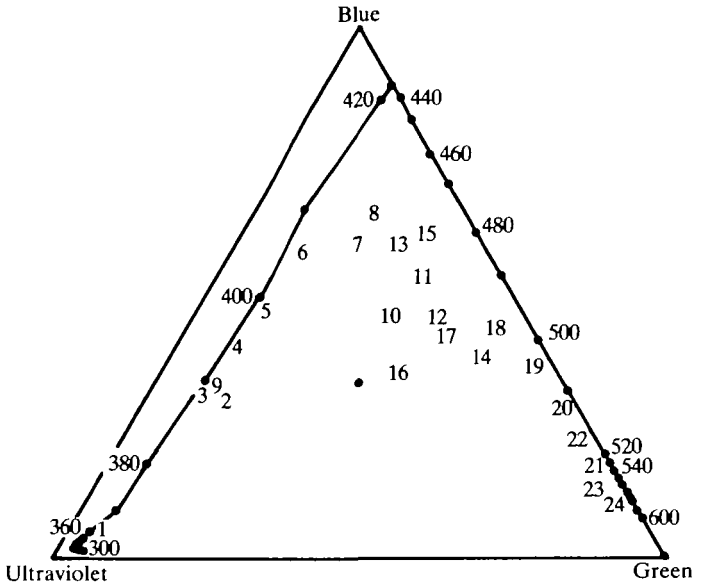

Fig. 3. Chromaticity diagram for *Osmia rufa*. The dots at the periphery give the loci for spectral lights between 300 and 600 nm in 10-nm steps. The dot in the centre marks daylight (see text). The numbers are located at the colour loci of the respective colour signals (see Table 1).

choice proportion p was 0·95 (95 % correct choices). If the same pair of colour signals was tested after training to no. 7, p was 0·83 (0·12 less than in the first case). We found the following rank order in symmetrical tests: 2, 7, 24, 18, 17, 21. Violet (2) and blue (7) colours were chosen more frequently than other training colours. The asymmetry effects were, however, never very strong, and rarely exceeded 15 % differences between symmetrical tests. We conclude, therefore, that colours are chosen with a certain but small bias, which might result from the pretraining to the silver-painted disc (but see Discussion). Compared with the strong learning ability this colour bias is relatively weak.

The results of all the discrimination tests are given in Fig. 4A–L. The training experiments show that *Osmia* discriminates colours very well, and that colour signals further apart in the chromaticity diagram are discriminated better than closer colour signals. As mentioned above, discrimination is only weakly dependent on the colour signal to which the bees have been trained. Fig. 4A–L indicates with a thick top line the average response value of the symmetrical test series, and with a thin or broken top line the response value of the test series in which only one colour signal was trained (the one indicated for the particular set of columns). Symmetrical tests were not carried out for all pairs of colour signals. The difference between symmetrical and non-symmetrical tests was small (see above), showing that *Osmia* learns any of the colour signals as a guide mark for the nest

entrance. Furthermore, *Osmia* does not show a strong tendency to use particular colours (or a colour component such as brightness), which cannot be overcome by training.

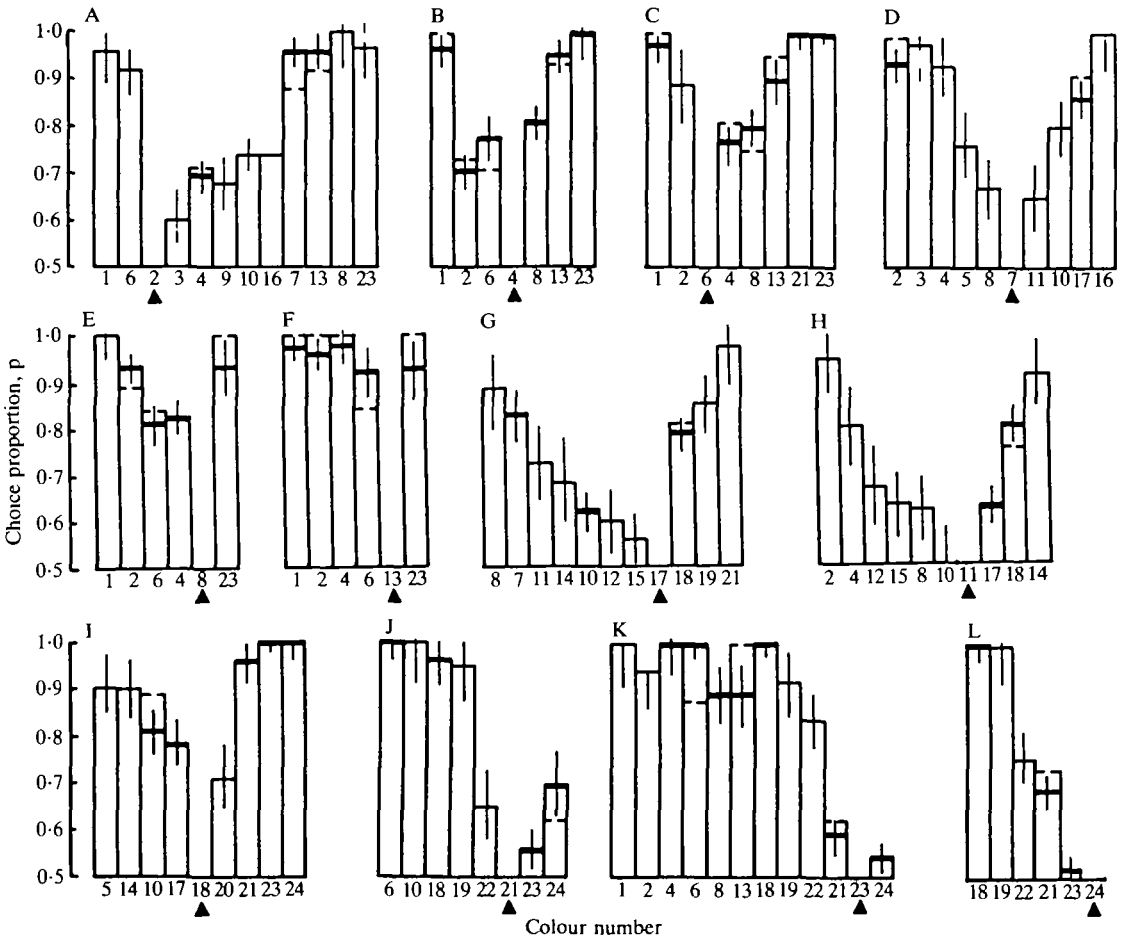

Fig. 4. (A–L). Discrimination tests with *Osmia rufa*. The columns indicate the choice proportion, p, for the trained colour signal (marked with a black triangle). 1·0 represents 100 % choice of the colour signal, 0·5 (bottom line) an equal choice of the two colour signals in the dual forced-choice test. The number of the alternative colour signal marks each column (see Table 1). (A) Trained no. 2 (UG 3), $N = 714$; (B) trained no. 4 (BG 25), $N = 416$; (C) trained no. 6 (BG 25 + B 3), $N = 361$; (D) trained no. 7 (BG 28), $N = 667$; (E) trained no. 8 (BG 28 + B 4), $N = 345$; (F) trained no. 13 (BG 28 + Gr 4), $N = 301$; (G) trained no. 17 (BG 18), $N = 992$; (H) trained no. 11 (BG 27), $N = 359$; (I) trained no. 18 (VG 6), $N = 396$; (J) trained no. 21 (GG 495), $N = 217$; (K) trained no. 23 (OG 530), $N = 814$; (L) trained no. 24 (OG 550), $N = 400$. The thick top line gives the average of all tests including the symmetrical tests, the thin or broken top line gives the average of only those tests that are indicated for the particular column. The scatter bars give the significance intervals ± s as described in Materials and Methods.

Comparison of colour discrimination of Osmia *with* Apis *and* Melipona

Since two other hymenopteran species have been tested with very similar experimental arrangements (*Apis mellifera* and *Melipona quadrifasciata*), a direct comparison between these species could be made in terms of their colour discrimination for identical pairs of colour stimuli. The differences between the colour discrimination tests of the three species were as follows. (1) In experiments with *Apis* and *Melipona* the colour discs appeared on an evenly painted (white or grey), large background screen and were 100 cm apart from each other, whereas in *Osmia* the colour discs were very close and appeared on a structured and darker background. (2) Most of our data for *Apis* come from training experiments at the feeding place, and this data will be used for the comparison made here. We have shown that *Apis* also discriminates colour stimuli very well at the hive entrance, and discrimination in the two behavioural contexts (feeding place, hive entrance) is similar, but that more accurate choices are found at the feeding place (Menzel, 1986). (3) *Melipona* was tested at the hive entrance and at the feeding place. We shall use the data from the hive entrance training experiments here, since the data were collected under the same behavioural conditions (i.e. homing) as the tests with *Osmia*. (4) The criterion for a choice in the experiments with *Osmia* and *Melipona* is a landing on the coloured disc. In the experiments with *Apis*, an approach towards the disc at the feeding place is counted as a choice.

The choice proportions p for identical pairs of colour stimuli are plotted for *Osmia* and *Apis* in Fig. 5. The values scatter around a symmetry line, and this indicates that both species have similar colour discrimination. In contrast, *Melipona* differs considerably from *Osmia* in its colour discrimination (Fig. 6). Most data lie below the symmetry line indicating a better colour discrimination in *Osmia*. Closer inspection of the data shows that *Osmia* is also much better at discriminating between colour signals in the ultraviolet, violet and blue regions, whereas *Melipona* is at least equally as good as *Osmia* at discriminating bluish-green colour signals. We have shown elsewhere that the lesser ability of *Melipona* to discriminate colour signals in the ultraviolet, violet and blue depends on the behavioural context. For example, *Melipona* discriminates ultraviolet, violet and blue signals better at the feeding place than at the hive entrance. It is not surprising, therefore, that *Osmia* (as well as *Apis*) discriminates in the ultraviolet, violet and blue better than *Melipona*, if *Melipona*'s performance at the hive entrance is used as a basis for comparison.

Discrimination of colour signals and perceptual distance

We have recently derived a model calculation in which the perceptual distance of colour signals can be determined from the spectral properties and the intrinsic noise of the photoreceptors (Backhaus & Menzel, 1987). The model assumes that the just noticeable difference steps at the receptor level (rjnd) constitute a measure of the perceptual just noticeable difference steps (pjnd) and, therefore, the perceptual distance between two colour signals. These assumptions are valid

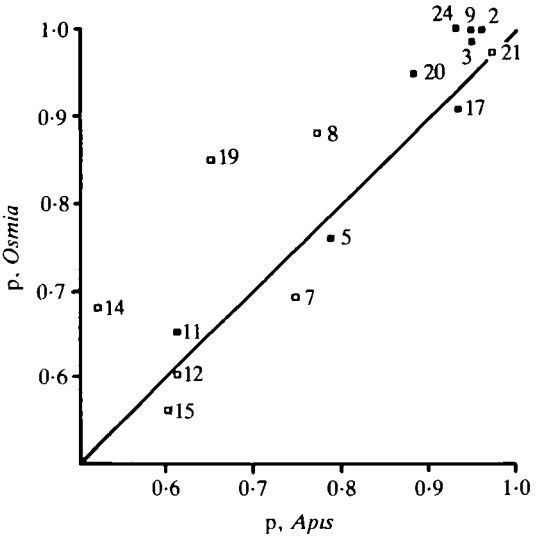

Fig. 5. Comparison of colour discrimination between *Apis* (abscissa) and *Osmia* (ordinate). The axes give the proportion of correct choice (choice proportion p) in a dual-choice test (see Fig. 4). The respective discrimination values for identical pairs of colours are indicated. (■) after training to colour no. 7; (□) after training to colour no. 17. The numbers besides the symbols give the alternative colour of the particular test (see Table 1).

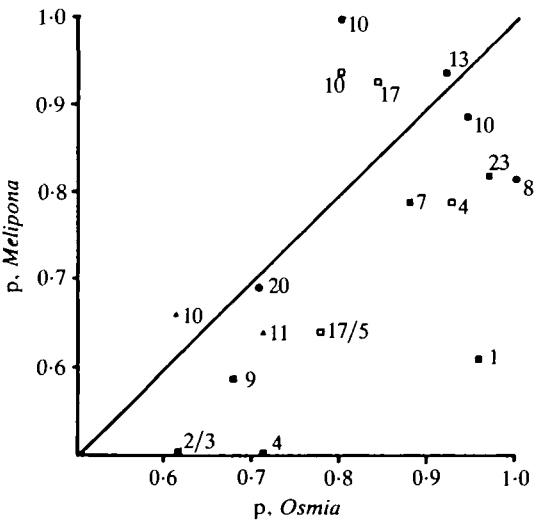

Fig. 6. Comparison of colour discrimination between *Osmia* (abscissa) and *Melipona* (ordinate). The respective p values for identical pairs of colours are indicated. (■) after training to colour no. 3; (□) after training to colour no. 7; (▲) after training to no. 17, and (●) after training to no. 18. The numbers beside the symbols indicate the alternative colour signal of the particular test.

for the honeybee *Apis mellifera* (Backhaus & Menzel, 1987) and for the stingless bee *Melipona quadrifasciata*. As in the model calculations with *Apis* and *Melipona* we can determine the rjnd-steps in the colour space or in the colour plane from the spectral sensitivity functions (Fig. 2), if we assume that the photoreceptors in the eye of *Osmia* resemble the intrinsic noise components of *Apis* (0·4 % of the maximum voltage response). The number of pjnd-steps derived from the colour space is the minimal number of jnd-steps at the receptor level between two colour stimuli and is referred to as the spatial distance sD(jnd). Ignoring the components that result from intensity differences of the two colour signals, and referring only to pjnd-steps at the colour plane, we call the distance value pD(jnd). Both sD(jnd) and pD(jnd) are calculated by a procedure which is described in detail in Backhaus & Menzel (1987). Essentially, a computer program is used to calculate the smallest number of pjnd-steps between two colour loci (in the colour space or in the colour plane). A pjnd is defined by the criterion that a variance in the effective quantal flux has to be significantly larger than the intrinsic noise in at least one of the three spectral receptor types.

An example of the discrimination/distance functions for pD(jnd) is given in Fig. 7A. The choice proportions p are linearized by probability transformation into z-values, z(p) (see Backhaus, Menzel & Kreissl, 1987, p. 297 formula 2); z(p) = 0 corresponds to a proportion of 0·75 correct choices. There is a high

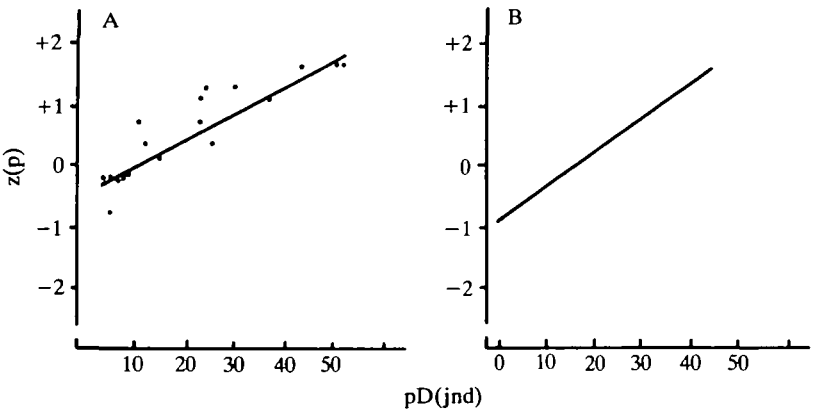

Fig. 7. (A) Discrimination – distance function. The correlation between the distance values pD(jnd) and the respective choice behaviour in dual forced-choice tests is calculated with a regression line. The choice proportion p is expressed in the corresponding z-value and plotted on the ordinate (see text). The distance between the colour loci of the two colour signals shown in the respective discrimination tests is given as pD(jnd) on the abscissa. Data are included in the correlation analysis which fulfil the following criteria: $z(p) \leq +1{\cdot}3$ (95 % correct choices), $pD(jnd) \leq 55$. The following three test series are pooled in this figure: training to nos. 2, 4 and 6 (compare Fig. 4A–C). The correlation coefficient $r = 0{\cdot}892$. (B) Average discrimination–distance function for all data which fulfil the above criteria. $r = 0{\cdot}851$, slope = 0·09. Data are included for seven different trained colour signals with 31 tests and 3800 choices.

correlation between the distance value pD(jnd) and the choice behaviour. The correlation coefficients r for different series of experiments are given in Table 2. The distances in the colour plane, pD(jnd), correlate much better with the choice behaviour than those in the colour space, sD(jnd). This means that subjective brightness differences between the colour stimuli are less important for colour discrimination and may be completely ignored (compare H values in Table 1). An analysis of the correlation between the distance values and the choice behaviour reveals that large distances correlate less well with the choice behaviour than shorter distances. Distances larger than 55 pD(jnd)-steps or 90 sD(jnd) are, therefore, not included in Fig. 7 and Table 2.

One might expect that choice behaviour would simply saturate for large perceptual distances. This is indeed the case for many tests as Fig. 4A–L shows. But there are quite a few exceptions, which are more difficult to understand (e.g. Fig. 4G, no. 8; H, no. 4; I, no. 5; K, nos 2, 8). We have not yet analysed the divergence for large distances, but it is interesting to note that violet and blue colours are more frequently involved in these cases particularly when bluish-green or yellow colours are used for training. One might suspect, therefore, the influence of pretraining in the same sense as has been discussed above (colour bias as a result of pretraining) or an innate bias towards violet or blue colours.

All correlations between discrimination and distance are incorporated in Fig. 7B for pD(jnd) values smaller than 55 jnd and choice proportions of $z(p) \leq \pm 1{\cdot}3$ (≤ 95 % correct choices). The data are best fitted by a regression line ($r = 0{\cdot}851$) with a slope of 0·09. This means that a change of $z(p) = \pm 0{\cdot}5$ around $z(p) = 0$ (response change between 65 and 85 %) is reached by an average distance of 11 pD(jnd).

Spectral discrimination [$\Delta\lambda(\lambda)$-function]

One of the most informative methods of assessing a colour vision system is the function which describes the wavelength-dependency of spectral discrimination. The critical test for such a function requires narrowband or monochromatic light

Table 2. *Correlation coefficients* r *for regression lines calculated for the correlation of the discrimination values expressed in z(p)-values and two distance values*

Colour trained	r [pD(jnd)]	r [sD(jnd)]
6	0·884	0·612
4+2	0·904	0·705
7+8	0·802	0·495
18+17	0·881	0·480
23+24+21	0·823	0·505

r is calculated for five sets of experimental series depending on which signals were trained (first column).

Not all discrimination tests shown in Fig. 4A–L are included in the calculation, but only those fulfilling the following two criteria (1) pD(jnd) ≤ 55 or sD(jnd) ≤ 90, and (2) response value (discrimination) ≤95 % correct responses [z(p) ≤ 1·3] (see legend to Fig. 7 for more details).

of equal subjective brightness (see von Helversen, 1972). We carried out our experiments under natural daylight with reflecting colour signals. However, Fig. 3 clearly shows that several of the colour signals were of such pure chromatic light, that the corresponding colour loci are positioned very close to the corresponding monochromatic lights (e.g. nos 2, 3, 4, 5, 6, 9, 18, 19, 20, 21, 22, 23, 24). The calculated brightness value H (see Table 1) demonstrates that these colour signals are not of equal subjective brightness, but since differences in brightness contribute very little or nothing to the discrimination of these colours (see above), the distances between those two pairs of spectrally pure signals are calculated for those discrimination tests that were carried out with spectrally pure colour signals (three tests for colour 2, one test for colour 4, one test for colour 18, one test for colour 19, three tests for colour 22, two tests for colour 21; choice proportions $p \geq 0{\cdot}95$ correct choices were not included). Regression lines are calculated for the three series of correlations (colours 2, 21, 22) between discrimination [z(p)-value] and $\Delta\lambda$, the wavelength distance between the corresponding monochromatic lights (dominant wavelength) (see inset of Fig. 8; compare number of colour stimulus with Fig. 3). Such functions yield the wavelength distance ($\Delta\lambda$) which produces a discrimination of 75 % correct choices [$z(p) = 0$]. The other results for which only one test exists can also be used by applying the closest response – $\Delta\lambda$-function. The results of these calculations are given in Fig. 8 together with the theoretical function which was calculated using the same procedure as described above for the perceptual distance pD(jnd) in the plane of equally bright spectral lights.

Osmia is able to discriminate best in the violet part of the light spectrum, and to a slightly lesser degree light in the bluish-green region. The five values of spectral discrimination sensitivity are close to the theoretical function. We can conclude, therefore, that our model predicts well *Osmia*'s sensitivity to spectral differences.

Discussion

Like the social honeybee *Apis mellifera*, the solitary bee *Osmia rufa* has a trichromatic input system for colour vision. The spectral sensitivity functions of the ultraviolet and blue receptors are similar for the two species (*Apis*, λ_{max} 336 nm and 423 nm; *Osmia*, λ_{max} 348 nm and 436 nm). The green receptor, however, is more sensitive at longer wavelengths in *Osmia* (*Apis*, λ_{max} 532 nm; *Osmia*, λ_{max} 572 nm). This should have consequences for colour discrimination. A receptor model for colour vision in *Apis* has been successfully tested in various colour discrimination experiments (Backhaus & Menzel, 1987; Backhaus *et al.* 1987), and thus may also be applied to the colour discrimination data in *Osmia*. Such a model provides us with predictions concerning the degree of discrimination of two colour signals, since the calculation of jnd-steps gives us a measure of the subjective difference between pairs of colour signals. Furthermore, the receptor-based model allows the comparison of colour vision among insect species solely on the basis of spectral measurements of single photoreceptors. This is particularly

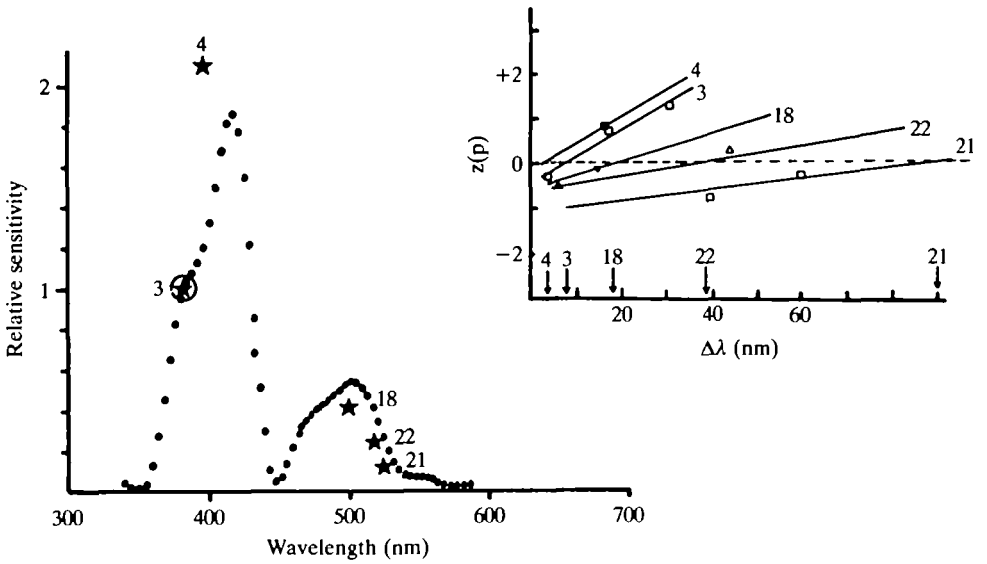

Fig. 8. The wavelength-dependence of spectral discrimination [$\Delta\lambda(\lambda)$-function] is calculated for five different colour signals and compared with the predictions of the receptor model. The five colour signals, which were selected because of their high spectral purity (see Fig. 3) were nos 3, 4, 18, 21 and 22. The inset (upper right) plots the probability-transformed choice proportion z(p) against the spectral separation ($\Delta\lambda$ in nm) between the two colour signals for which the discrimination was determined. From this plot, one can read the $\Delta\lambda$ value at which the choice proportion is 0·75 [75 % correct choices, z(p) = 0]. The arrows indicate the corresponding $\Delta\lambda$ values of the five colour signals. The reciprocals of these values ($1/\Delta\lambda$) are plotted in the main figure as stars. In this graph, $1/\Delta\lambda$ of colour signal 3 ($\Delta\lambda = 8$ nm, $1/\Delta\lambda = 0{\cdot}125$) is set to 1, and the other values are expressed relative to this value. The dots in the graph represent the relative spectral discrimination as predicted by the receptor model. The unit of relative sensitivity corresponds to the reciprocal of 15 pD(jnd) steps.

valuable since electrophysiological data on photoreceptors are relatively easy to collect whereas behavioural discrimination tests are often impossible to perform.

The geometrical distance between loci of colour stimuli in the receptor diagram is not correlated with the dissimilarity of colours. Helmholtz (1896) and Schrödinger (1920) pointed out that a line element has to be established which defines the shortest way between two colour loci. We have developed a model calculation which defines the perceptual line element from the noise components of the photoreceptors (Backhaus & Menzel, 1987). Here we present two independent sets of experiments which support the predictive capability of the jnd-measure for the subjective difference of colour signals. First, we found a high correlation between pD(jnd), the jnd-steps in the colour plane, and the proportion of correct choices as expressed in the z(p)-values (probability-transformed choice proportion) (Fig. 7A,B). It turns out that *Osmia* is less concerned with the brightness differences of colour pairs than differences in chromaticness (hue, saturation).

This is concluded from the better correlation of the behavioural data with the jnd-steps in the colour plane than in the colour space (Table 2). Second, we calculated the $\Delta\lambda(\lambda)$-function for five colour signals and found a good agreement with the corresponding sensitivity values predicted by the receptor model (Fig. 8).

Such a function with many more data points has been established for *Apis* (von Helversen, 1972), and we have recently shown in this species that the line elements along the spectral line of the chromaticity diagram follow exactly the behaviourally measured function (Backhaus & Menzel, 1987). In *Apis*, spectral discrimination is optimal around 400 and 490 nm, 490 nm being somewhat better than 400 nm. *Osmia* differs in that its discrimination is best at 400 nm, and somewhat less in the blue-green (Fig. 8). A comparison between the species should be made with some caution since the test arrangements were quite different. The test lights were further apart for *Apis* than *Osmia*, and the tests were carried out under dim light conditions and without the ultraviolet-reflecting filters that were used in tests with *Osmia* under natural bright light. Nevertheless, the line elements of the receptor model predict the spectral discrimination of both species to a good fit, indicating that spectral discrimination of *Osmia* and *Apis* is relatively independent of the colourless surround and ambient light conditions.

A comparison between species can also be performed without reference to a colour vision model, and without any assumptions of the line elements. This has been carried out (Figs 5, 6) for the three bees *Osmia*, *Apis* and *Melipona*. It should be noted that the colour signals used in the experiments with *Osmia* and *Apis* (nos 7 and 17, see Fig. 3) are in the chromatic range for which one would not expect differences between the two species on the basis of their $\Delta\lambda/\lambda$-function, and this is in fact the case (Fig. 5). In the comparison of *Osmia* and *Melipona*, colour signals in the violet (no. 3) and bluish-green (no. 18) are used. The results clearly show that *Osmia* is considerably better than *Melipona* at discriminating violet colours, and that *Melipona* is better in the blue-green region of the spectrum. Little or no difference was found for the other test colours (nos. 7, 17). Since *Melipona*'s colour vision system is very similar to that of *Apis* we conclude that there are additional factors which reduce the discrimination of *Melipona* in the violet.

Colours are important signals for animals. For an individual, the significance of colour is the result of its own experience and the result of its evolutionary history (Menzel, 1985*b*). One observation – the asymmetry effect – provides additional evidence for the unique perception of colour. When the same pair of colour signals is tested after training an animal either to one or the other colour signal, small but statistically significant (χ^2-test, P between 0·03 and 0·07) differences in the choice behaviour may be found. An analysis of the bias for certain colour signals reveals the following rank order: violet, blue, yellow, bluish-green. What might be the reason for such an order of preference? The animals had to be pretrained to a disc painted silver before training to a particular colour signal started. A generalization process might transfer the pretraining effect selectively to the colour signals. However, the rank order does not depend on any other pretrainings, e.g. a different colour signal. Thus, it is unlikely that the pretraining causes the colour

bias. Another possibility is that *Osmia* has an innate preference for colours which is not completely overcome by learning. It is interesting to note that the same rank order of colours has been observed in colour learning of honeybees when the speed of acquisition is taken as a parameter (Menzel, 1967). In the honeybee this rank order is independent of pretraining and several other parameters, e.g. colour of the alternative signal in the dual-choice tests, age of the bee, time of year, weather conditions etc. The existence of a colour bias, as in the case of *Apis* and *Osmia*, is a strong indication of the perceptual uniqueness of colours.

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