

# Color Constancy in the Honeybee

Annette Werner, Randolf Menzel, and Christian Wehrhahn<sup>1</sup>

Institut für Tierphysiologie, D 1000 Berlin 33, and <sup>1</sup>Max-Planck-Institut für biologische Kybernetik, 7400 Tübingen, FRG

**A multicolored display was illuminated by 3 bands of wavelengths corresponding to the maxima of the spectral sensitivities of the 3 types of photoreceptors found in the bee retina. The intensity of each band could be varied individually. The light fluxes emitted by the colored areas of the multicolored display were determined quantitatively. Free-flying honeybees were trained with sugar solution to choose one of the colored areas. The illumination was then changed in such a way that the light fluxes formerly emitted by the training area were now measured on another area. When the trained bees were tested under those conditions, they still chose the training area. The relative positions of the colored areas were changed in order to exclude learning of position. It is concluded that color vision in bees is, in a certain range, independent of the spectral content of the illumination. Model calculations show that the behavior observed in bees is consistent with the retinex theory (Land, 1977), i.e., an algorithm using long-range interactions is required to explain color constancy.**

An important issue in color vision is that the color of objects can be identified in spite of dramatic changes in the spectral content of the illumination. Such changes induce large variations in the spectral energies reflected from surfaces. The human visual system is able to cope with this situation. For a recent demonstration of this well-known color constancy, see Land (1977). Color constancy was also investigated in the visual system of the goldfish (Ingle, 1985). The aim of this paper is to investigate color constancy in an invertebrate, the honeybee (*Apis mellifera carnica*), whose color vision is well-documented. Bees discriminate between flowers by their colors (von Frisch, 1914; von Helversen, 1972). Color contrast effects have been demonstrated for blue and green (Neumeyer, 1980, 1981). The bee visual system is trichromatic with UV, blue, and green receptors (Autrum and von Zwehl, 1964; Menzel and Blakers, 1976).

Earlier experiments indicated that bees are capable of color constancy (Neumeyer, 1981). However, they were carried out without testing the influence of a change in the UV content of the illumination. In order to control the spectral content of the illumination used, the experiments were carried out in a room shielded from daylight. A surface having different colors (A, B, C, etc.) was illuminated with light in the 3 bands of wavelengths

corresponding to the maxima of the spectral sensitivities of the 3 types of photoreceptors found in the retina of worker bees (for review, see Menzel, 1985). For practical reasons we used colored glass plates as colored areas and illuminated them from behind (see Materials and Methods). Therefore, the word “emitted” is used in our experiments where “reflected” is used in experiments with surface colors. It was possible to train a bee to prefer a certain color, A, and to measure the energy emitted in the 3 wavebands for this color. We then changed the relative intensity levels of the 3 wavebands illuminating the surface. This was done in such a way that another square with color B (different from A) now emits exactly those energies measured before on color A. If the color of square A perceived by the bee is the result of the spectral content of the energies emitted by this square, we would expect that a worker bee trained on A would now choose B. If, however, the color discriminating system in bees is independent of the spectral content of the illumination, we would expect that a bee trained on A would still choose this square. As we shall see, this latter result is exactly what was found.

## Materials and Methods

**Stimulus.** Single, free-flying bees (*Apis mellifera*) from the laboratory's beehive were trained to visit the experimental setup, which consisted of a vertical surface composed of 25 square fields. Twelve of these fields were black and 13 were glass plates of different spectral transmittances serving as colored stimuli (Fig. 1). Pilot experiments had shown that a surface consisting of colored fields only was interpreted by the bees as one single large flower. Therefore, all the colored fields were separated from one another by black areas in a pattern similar to a multicolored checkerboard. The experimental setup was situated in a room shielded from daylight, whose walls were covered with black velvet. The display was homogeneously illuminated from behind by 3 lamps having 3 different bands of spectral wavelengths (“short”:  $\lambda_1 = 350$  nm; “middle”:  $\lambda_2 = 440$  nm; “long”:  $\lambda_3 = 540$  nm) corresponding to the 3 spectral types of photoreceptors found in the retina of worker bees (Autrum and von Zwehl, 1964; Menzel and Blakers, 1976). The light fluxes in the 3 wavebands could be varied in order to satisfy the conditions outlined in the introduction. The measurements were made with a calibrated radiation meter (IL 700 with detector PM 270 D from International Light Corp.).

**Training and testing of bees.** When a test bee landed on the training color, it was rewarded by a small drop of sugar water. In order to exclude learning of the position of the training color within the other colors of the multicolored display, the positions of all the colors were changed between consecutive visits of the bee or the display was rotated. This also served to avoid possible influences of simultaneous and successive color contrast on the learned color (Neumeyer, 1980). This was continued until the animal had safely learned the training color, i.e., until after about 10 visits, with a total of 50 rewarded trials. Thereafter the bee was tested for discrimination of the trained color without reward. Test and training sessions were alternated. During the 3 min of every test, the choice behavior of the animals, such as tipping or landing on the color plates, was monitored with the help of a video recorder and a protocol was spoken on a tape recorder. The position of the colored fields was also changed from test to test. Since the multicolored display presented to the bee also contained colored areas that were relatively

Received Mar. 3, 1987; revised July 16, 1987; accepted July 16, 1987.

This work was supported by DFG Grant Me 365/12-2. We thank Bill Harris, Christa Neumeyer and the (unknown) referees for comments on the manuscript and Inge Geiss for typing it.

Correspondence should be addressed to Annette Werner, Institut für Tierphysiologie, Königen-Luise-Strasse 28-30, 1000 Berlin 33, FRG.

Copyright © 1988 Society for Neuroscience 0270-6474/88/010156-04\$02.00/0

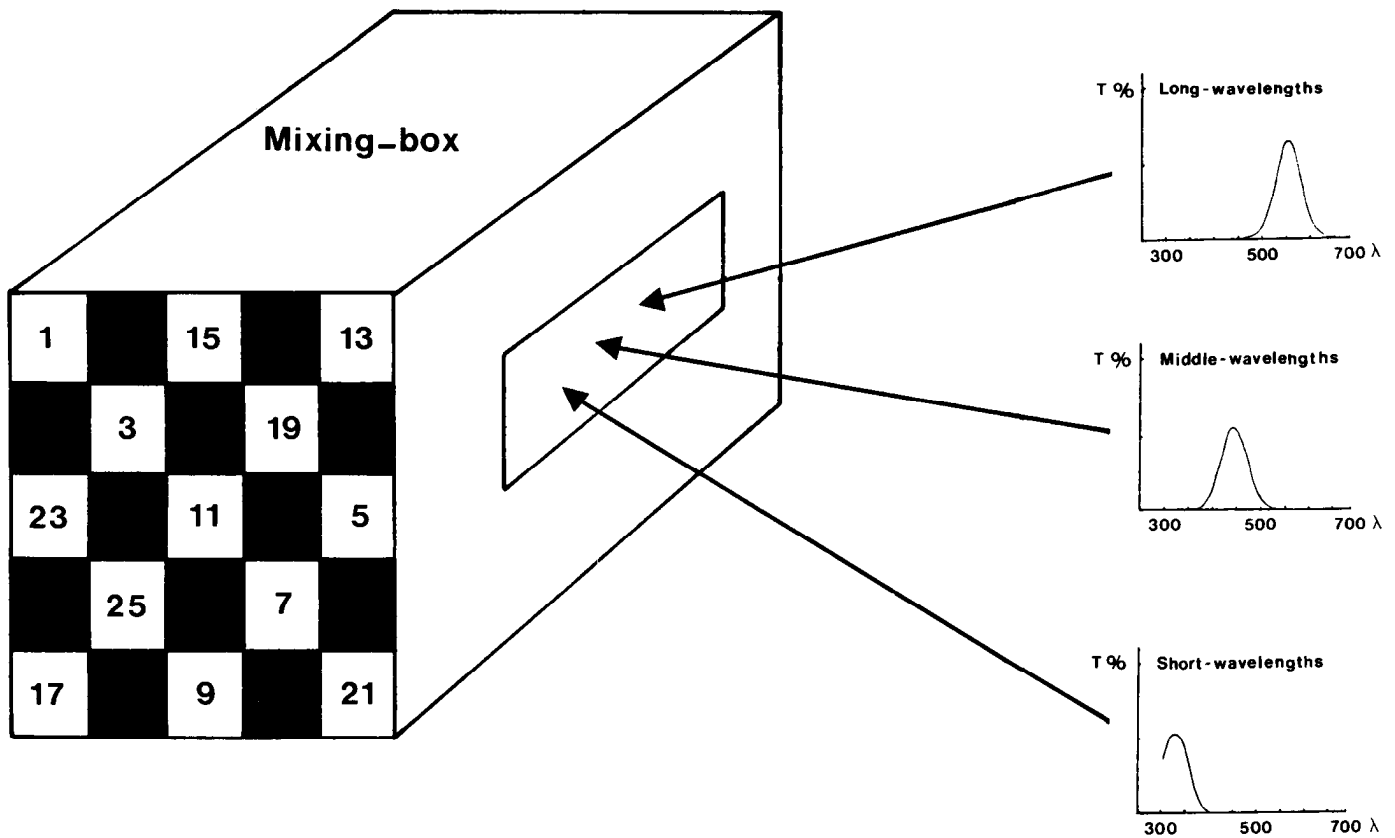


Figure 1. Schematic drawing of the multicolored checkerboard arrangement and its illumination, as used in the experiments. The numbered squares consist of glasses of different spectral transmittance. The other squares are black. The display was illuminated from behind by light in the 3 indicated bands of wavelengths. Further details are given in the text.

similar to the trained area, a change of choice behavior towards these fields should indicate whether the phenomenon of color constancy is perfect or is limited to a certain range of spectral change.

**Results**

Color constancy was investigated for 4 pairs of violet and blue-green colors. As bees are able to discriminate color in these chromatic regions particularly well, changes in color perception can be observed with high sensitivity. The results of these experiments are shown in Figure 2. The magnitude of double blocks represents the relative choice frequency of trained color and matched color.

*Violet colors (Nos. 3, 17)*

The energy emitted by the fields under training conditions is shown in Table 1 (first 3 columns). In this experiment, light of middle and short wavelength was added in order to match the alternative color, No. 3, to the training color, No. 17 (see second 3 columns of Table 1). Relative choice frequency for these 2 color fields under training illumination is presented by the first double block in Figure 2. The 2 colors are well-discriminated ( $\chi^2$  test;  $p \leq 0.01$ ). The choice behavior under changed illumination is given by the second double block. It can be seen that the bees chose the training and matching colors with the same percentage of correct choices as before, even though the spectral content of the matched color during the matching test was identical to that of the training color during training. It should be emphasized that the spectral content of the light flux

emitted by the training field was simultaneously changed in this situation (Table 1).

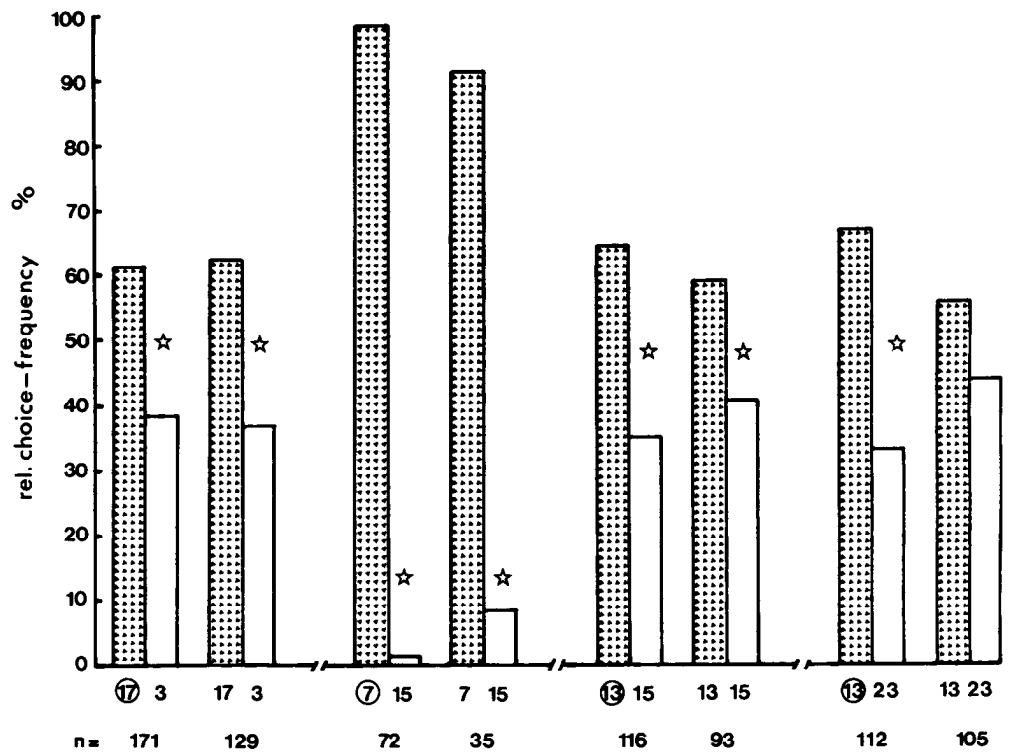
*Blue-green colors (Nos. 7, 13, 15, 23)*

Three color matches were carried out for blue-green colors. In all cases, light of middle and/or short wavelength had to be added. The third and fourth double blocks in Figure 2 show the

Table 1. Relative energy ( $\mu\text{W}/\text{cm}^2$ ) of UV, blue, and green light transmitted through the different filters during the 2 illuminations

Filter no.	Training illumination			Matching illumination		
	UV	Blue	Green	UV	Blue	Green
23	12	97	182	13	168	165
15	25	112	199	29	192	178
1	42	104	57	47	180	50
5	32	141	370	39	244	333
19	104	110	258	117	178	327
25	99	72	82	113	102	68
27	94	133	470	105	217	428
7	28	128	450	34	221	407
11	11	87	88	13	152	78
21	129	146	111	142	218	97
3	99	49	106	114	118	95
17	114	118	94	130	185	85
29	101	107	512	120	153	469

Figure 2. Relative choice frequency of bees towards 3 different training colors (shaded blocks) and the colors that were matched to the training colors by changing the spectral content of the illumination (empty blocks). The trained color is indicated by a circle around the corresponding number. The colors were in the bluish green and violet region (see Table 1). Number of decisions (n) is given for every test situation. In all cases, distribution of choices between training and matching color remains almost constant during training illumination (first double block of every experiment) and during matching illumination (second double block of every experiment). The columns marked with an asterisk indicate a significant difference in choice frequency ( $p \leq 0.01$ ;  $\chi^2$  test).



results for training color No. 7 and matching color No. 15. In this case, the change in spectral content was greater than that imposed in the preceding experiment. Inspection of Figure 2 shows that the choice frequency changed only marginally.

Changing illumination to match the alternative colors No. 15 and 23, respectively, to training color No. 13 led to similar results. As can be seen in Figure 2, in both cases the bee chose the training color relatively independently of illumination conditions.

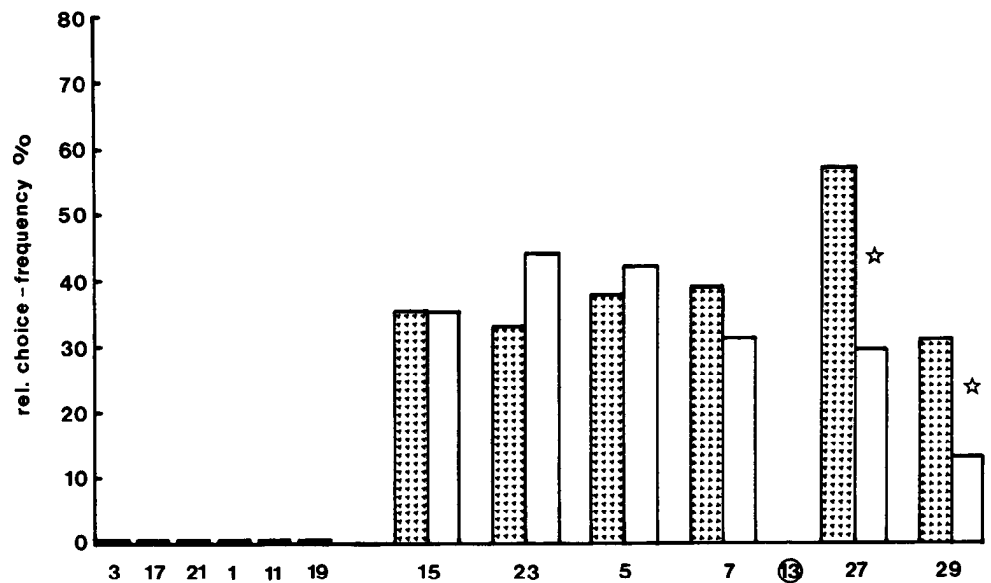
One more point emerges from this experiment. In the case where color 23 (blue) was matched to training color 13 (blue-green), light of "long"-wavelength was added. As a consequence,

the blue colors in the display (Nos. 5, 15, and 23) are slightly more preferred, compared to the training situation. By contrast, the green colors (Nos. 7, 27, 29) were less preferred (Fig. 3). Colors that were not similar (Nos. 1, 3, 11, 17, 19, 21) to the training color were never chosen.

### Discussion

The results of this study strongly suggest that choice behavior and hence color perception in bees is, at least up to the range specified in our experiments, independent of the spectral content of the illumination. The results also confirm the earlier experiments of Neumeier (1981), and are similar to observations in

Figure 3. Choice frequency of bees towards all colors of the display during the experiment in which color 23 (blue) was matched to training color 13 (blue-green). Choice frequency is shown for training illumination (dark bars) and matching illumination (white bars). No bars are shown for the training color (13), as this is the reference color. The colors are arranged according to their hue from left (violet) to right (yellow). For the match, "long"-wavelength light was added. The colors that are more bluish than the training color are chosen more frequently. The colors that are more greenish than the training color are chosen less frequently. The hue of color field 7 seems to be shifted towards green, and becomes even greener than the training color. The columns marked with an asterisk indicate a significant difference in choice frequency ( $p \leq 0.01$ ;  $\chi^2$  test).



man and goldfish (Land, 1977; Ingle, 1985). It would also be interesting to test color constancy in man under conditions similar to those used in the present study. This has not yet been done and will be carried out in a later study by one of us.

As can be seen from the results of Figure 3, the slight but systematic changes in choice behavior under different test illuminations are correlated with the specific changes imposed on their spectral content. It can be concluded that, under the experimental condition described above, the bees' color perception was not *completely* independent of the spectral content of the illumination. However, the number of fields in the multicolored display was only 13. In addition, the checkerboard geometry of the display separated the colors by black fields. The number of colors was small compared with the number of differently colored fields present in the natural surrounding. It is likely that with an increasing number of differently colored areas, color vision becomes increasingly independent of the spectral content of the illumination. In humans, about 100 differently colored areas are required to achieve color constancy (Land, 1977).

According to our observations, the color of a certain field that is perceived by a bee depends not only on the energy flux emitted from the area but also on the energy fluxes emitted from the other colored areas.

It was shown earlier that the perception of color in bees is also dependent on the color of *directly* neighboring fields (Neumeyer, 1980). The effects of direct-neighbor relations between colored areas was reduced by the geometry of the experimental arrangement (checkerboard).

At the level of the retinex theory, spatial interactions over large distances are required in order to explain the experimental results. However, an explanation on the basis of selective adaptation of the photoreceptors, according to von Kries (von Helmholtz, 1896), cannot be excluded on the basis of the experimental evidence presented here, since bees fly in front of the multicolored display for several minutes and perform choices at various times during the test session. It should be mentioned that the small shifts in the preference of blue and green colors due to the illumination change (see Fig. 2) are not expected in the case of adaptation processes.

A theoretical description of color vision and color constancy is given by the retinex theory (Land, 1986). At the level of the experimental evidence given here, color vision in the honeybee can be described by the retinex theory, with the condition that the spectral distributions of the 3 illuminating lamps are equal to the sensitivities of the 3 receptor classes found in the bee retina. It should be emphasized that the retinex theory gives an algorithm that describes color perception. On the basis of this theory, nothing can be concluded about the physiological mechanisms underlying this task and the different neural mechanisms that may subserve color constancy in bees and man.

An attempt was made to test quantitatively the effect of the retinex algorithm in the experimental situation described above. The quantitative values of the light fluxes of the multicolored display during the training phase, given in Table 1, were determined and the color triplets were computed as in Land (1983, p. 167). These triplets correspond to certain colors perceived by the visual system of bees. Color constancy is expressed when the triplet of a certain area changes very little in spite of substantial changes in the emitted energies. The values of the training phase generated by the model for color 17 were 0.66, 1.43, and 1.31, and those for color 3 were 1.03, 1.54, and 1.68. In the test phase the light fluxes were changed in such a way that the values emitted from field 17 in the training phase were now emitted by field 3. Again, the algorithm proposed by Land (1986) was applied. The triplet generated for color 17 was now 0.70, 1.57, and 1.28, and that for color 3 was 1.07, 1.66, 1.65; these are very similar to those generated for the training phase. Thus, the model describes the choice behavior of the bees in the experiment described in Results. Although color vision in bees has certainly evolved independently from that in humans and may use different neural strategies for color coding, it achieves perceptual tasks of similar complexity.

## References

- Autrum, H., and V. von Zwehl (1964) Spektrale Empfindlichkeit einzelner Sehzellen des Bienenauges. *Z. vergl. Physiol.* 48: 357-384.
- Ingle, D. (1985) The goldfish as a retinex animal. *Science* 227: 651-654.
- Land, E. H. (1977) The retinex theory of color vision. *Sci. Am.* 108-128.
- Land, E. H. (1983) Recent advances in retinex theory and some implications for cortical computations: Color vision and the natural image. *Proc. Natl. Acad. Sci. USA* 80: 5163-5169.
- Land, E. H. (1986) Recent advances in retinex theory. *Vision Res.* 26: 7-21.
- Menzel, R. (1977) Color vision in insects. *Verh. Dtsch. Zool. Ges.* 26-40.
- Menzel, R. (1985) Colour pathways and colour vision in the honeybee. In *Central and peripheral mechanisms in colour vision*, D. Ottoson and S. Zeki, eds., pp. 211-233, Macmillan, London.
- Menzel, R., and M. Blakers (1976) Color receptors in the bee eye—morphology and spectral sensitivity. *J. Comp. Physiol.* 108: 11-33.
- Neumeyer, C. (1980) Simultaneous colour contrast in the honey bee. *J. Comp. Physiol.* 139: 165-176.
- Neumeyer, C. (1981) Chromatic adaptation in the honey bee: Successive color contrast and color constancy. *J. Comp. Physiol.* 144: 543-553.
- von Frisch, K. (1914) Demonstration von Versuchen zum Nachweis des Farbensinnes bei angeblich total farbenblinden Tieren. *Verh. Dtsch. Zool. Ges. Berlin.*
- von Helmholtz, H. (1896) *Handbuch der physiologischen Optik*, Voss, Hamburg.
- von Helversen, O. (1972) The relationship between difference in stimuli and choice frequency in training experiments with the honey bee. In *Information Processing in the Visual System of Arthropods*, R. Wehner, ed., pp. 323-334, Springer-Verlag, Berlin, Heidelberg, New York.