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Spectral sensitivity of single photoreceptors and color vision in the stingless bee, *Melipona quadrifasciata*

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Summary. 1. The spectral sensitivities of the photoreceptors in the compound eye of the stingless bee, *Melipona quadrifasciata* (Hymenoptera, Apoidea), was determined by the spectral scanning method. Three spectral receptor types were found with λ_{max} at 356 nm, 424 nm, and 532 nm (Fig. 1). Intracellular markings confirmed one morphological type of green receptor (svf 1) and one type of UV receptor (lvf 1) whose axon morphology resembles that of the corresponding spectral receptor types in the honeybee, *Apis mellifera* (Fig. 2).

2. Training experiments with a large number of color signals were performed at the hive entrance and the feeding place under natural daylight conditions (Figs. 4–6). The tests were either dual (2 alternative color signals) choice tests or multiple (12 simultaneously presented alternative color signals) choice tests. *Melipona* discriminates colors very well in both behavioral contexts, but discrimination is generally better at the feeding place (Fig. 7). A comparison with *Apis* shows that *Melipona* discriminates colors in the bluish green better than *Apis*, and that *Apis* discriminates all other colors better.

3. The spectral properties of the receptor types were used to construct a color space in which all the color signals tested in the behavioral experiments are represented at particular loci (Fig. 3). A receptor model of color vision as proposed by Backhaus and Menzel (1987) for the honeybee is used to calculate the perceptual distance between the colors corresponding to the loci of the color stimuli. This model interprets the perceptual distance between two color stimuli as the number of just noticeable difference steps in the corresponding receptor voltage signals. The predicted distances are highly correlated with the discrimination values of the behavioral tests (Fig. 12).

Key words: Spectral sensitivities – Photoreceptors – Color vision – Stingless bee – *Melipona quadrifasciata*

Introduction

Many animal species see the world in color. However, the range of the light spectrum seen and the parts of the spectrum that are particularly highly resolved may differ widely from one species to another. There are good reasons to assume that the spectral properties of the photoreceptors, and thus the characteristics of the color vision system, are adapted to the particular demands of visual orientation of each species (see e.g. Lythgoe 1979). What are the co-evolutionary rules behind the adaptations between the spectral properties of the receptors, the chromatic light climate of the habitat, and the spectral properties of those objects which are important for the species' visual orientation? We consider this question for a certain ecotype of insects, flower-visiting hymenopterans. Flower-visiting insects have evolved their color vision in a co-evolutionary fashion with the coloration of flowers. Flying hymenopteran insects (bees, wasps) are the most abundant and most effective pollinators. Honeybees, which have been studied extensively (Autrum and von Zwehl 1964; Daumer 1956; von Frisch 1965; von Helversen 1972; Menzel 1967, 1979; Menzel and Blakers 1976; Menzel et al. 1986) possess a trichromatic color vision system with UV ($\lambda_{max}=344$ nm), blue ($\lambda_{max}=436$ nm), and green ($\lambda_{max}=556$ nm) receptors. Are all flower-visiting hymenopterans provided with the same set of spectral receptor types and the same color vision system? Are there species-specific adaptations to the particular flowers they serve and/or to the particular chromatic light climate they are exposed to during their activities?

Here we report the results of behavioral and electrophysiological experiments with a social stingless bee (*Melipona quadrifasciata*) which forages predominantly in the dense rain forest of South America. The genus *Melipona* is systematically related to *Apis* (both belong to the subfamily Apinae) but ecologically quite different. Both bee genera collect nectar and pollen from many

different flowers and live in hollow trees in large colonies. *Apis* forages predominantly in the open or in sparsely covered woods, whereas *Melipona* collects food from flowering trees in the rain forest (Kleinert-Giovanni and Imperatriz-Fonseca 1987). Thus, *Melipona* has to identify colored objects in a chromatic light climate dominated by the filtered or reflected green light of the foliage, whereas *Apis* is exposed to light of higher short-wave components (Menzel, unpubl.). This investigation shows that there are differences in the spectral sensitivity curves of their photoreceptors. The UV receptor is moved to longer wavelengths in *Melipona* (λ_{max} = 356 nm; *Apis:* λ_{max} = 344 nm), and the blue receptor to shorter wavelengths (λ_{max} = 424 nm; *Apis:* λ_{max} = 436 nm). Wavelength discrimination is optimal in the violet and bluish-green region, as one would expect from the λ_{max} positions of the photoreceptors (von Helversen 1972). However, color discrimination depends not only on the sensational differences but also on the evaluation according to behavioral context. This is demonstrated by the selective reduction of discrimination between violet colors at the hive entrance but not at the feeding place. Our analysis documents aspects of the power and limitations of psychophysical studies on color vision in a flower-visiting social insect. Quantitative predictions from a receptor model of color vision and comparative studies on several species are used as keys for a better understanding of the evolutionary adaptations of color vision in flower-visiting insects.

Methods

Electrophysiology: Workers of *Melipona quadrifasciata* were collected at the hive entrance and glued to a stage without narcosis or cooling. A small hole (about 4 facet lenses in diameter) was cut in the region of the eye furthest away from the region selected for recording. Microelectrodes (70–200 MΩ) filled with 2.5 *M* KCl or 5% Lucifer yellow were advanced slowly through the retina perpendicular to the visual axes of the ommatidia. The exit pupil of a flexible, UV-transmitting light guide (3 mm diameter, 1.2° visual angle) was positioned carefully on the axis of the recorded cell using a perimeter device. Criteria for the acceptance of an intracellular recording were: resting potential >40 mV, maximal light response >20 mV, resting potential stable longer than 20 min. Intracellular responses were amplified with a WPI high impedance preamplifier (mod. 750). Recordings were done in the dark when the eye had been dark adapted for at least 20 min. Intracellular markings with Lucifer yellow of some spectrally identified photoreceptors were performed. These identifications were made with the traditional flash method. The tissue was fixed with 4% formaldehyde, embedded in a 70% agar solution and cut in 80–100 µm thick sections with a homemade vibratome.

The spectral sensitivity was determined with the spectral scanning method (Menzel et al. 1986). The photoreceptor response to light presented at one end of the spectrum (300 or 700 nm) was clamped to a preselected DC potential level (2–10 mV) by adjusting the light intensity. A spectral scan from 300 to 700 or from 700 to 300 nm produced by a grid monochromator (Bausch & Lomb) was then presented at 4 nm steps over 18 s. The clamped level was maintained by automatic adjustment of the light intensity through a circular neutral density wedge (Melles Griot, 2.5 log units), controlled by a voltage comparator and a lab computer. All cells were tested with spectral scans in both directions. The light flux was measured with a calibrated photomultiplier and a Tektronix Radiometer J 16 (probes 6502 and 6504).

Behavioral experiments: The bees were trained to color signals either at the hive entrance or at a feeding place. The same training and test arrangements were used in previous work with *Apis* (Menzel and Lieke 1983). Colonies of *M. quadrifasciata* were brought to the laboratory from the forest near São Paulo, Brazil. The bees had access to the hive through a plastic tube which connected the hive with the outside air by a hole in the wall. The entrance hole (10 mm diameter) was surrounded by a colored disc (70 mm diameter). The bees learned quickly to use the color of the disc as a marker of the hive entrance. In the test situation, the hive entrance was covered with cardboard of the same grey color as the background, and two colored discs were exposed to the approaching bee 50 cm to the right and left of the covered entrance hole. The choice behavior of returning forager bees was counted during a period of several minutes. A choice was recorded when a searching bee landed on one of the colored discs. The test situation was terminated when the trained color signal received 40 choices, and then repeated several times with the same pair of color marks whose positions were systematically changed.

At the feeding place the bees were trained in a dual or multiple choice situation. The dual choice training resembles that described above for the hive entrance. In the multiple choice situation one of 12 color signals was the training signal, and the others were unrewarded alternatives. The 12 colored discs (70 mm ∅) were symmetrically arranged on a round board (2 m) on a vertical test plate. The round board was rotated at short intervals (between visits), and the 12 colored discs were rearranged frequently. Only one bee was trained at a time, and the choices were counted as described above. All behavioral experiments were performed with the vertical test plates facing south (away from the sun), thus being illuminated by the diffuse sky.

The colored discs were of either colored cardboard or color filters on ground aluminium discs (Menzel and Lieke 1983). The latter included ultraviolet colors, which cannot be produced with pigment coloration. The filters used were 1-mm thick Schott filters (UG 1, UG 11, UG 3, UG 5, BG 3, BG 12, BG 25, BG 24, BG 28, BG 27, BG 23, BG 18, VG 9, VG 6, GG 495, OG 530, OG 550) on aluminium discs or combinations of filters with colored cardboard (see Table 1). The color signals are explained in the Results.

Results

Spectral receptor types

The resting potential (−40 to −60 mV), the depolarizing light response (maximum responses up to 70 mV, average around 40 mV), and the phasic-tonic time course of the graded light response resemble the typical receptor characteristics of fast insect photoreceptors (see for example Laughlin 1981). Discrete potential fluctuations can be seen at low light intensity, but these were not analyzed in detail.

S(λ)-functions (Fig. 1 a) were obtained by the spectral scan method (see Methods) on 15 cells. Five photoreceptors were maximally sensitive in the UV, 6 in the blue, and 9 in the green. The number of spectral scans obtained in each cell varied depending on how long we could keep recording from it. The data presented are from a total of 21 spectral scans for the UV photoreceptors, 51 for the blue, and 20 for the green. The maxima of the S(λ)-functions are at 356, 424, and 532 nm, respectively, for the UV, blue, and green photoreceptors. A comparison with the theoretical absorption functions of the corresponding λ_{max} as calculated by MacNichol's (1986) formulas indicates a very good match in the blue

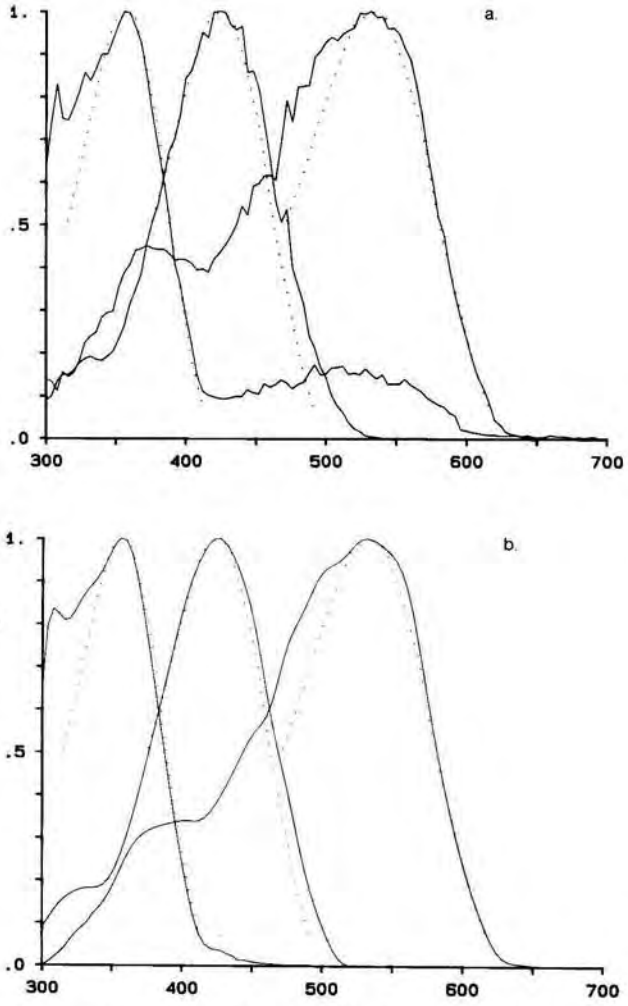


Fig. 1. a Average spectral sensitivity functions of the 3 receptor types in the compound eye of *Melipona quadrifasciata* as determined by the spectral scan method. The UV receptor (λ_{max} = 356 nm) was recorded in five cells, which were measured by 21 spectral scans, the blue-receptor (λ_{max} = 424 nm) in six cells with 51 scans, and the green receptor (λ_{max} = 532 nm) in nine cells with 20 scans. Dotted lines give the spectral absorbance functions with similar λ_{max} as determined by the MacNichol (1986) formulas. **b** Corrected S(λ)-functions. Arguments for correction and procedure described in the text. Dotted lines give the absorbance functions. These corrected functions are used for the receptor model (Fig. 3)

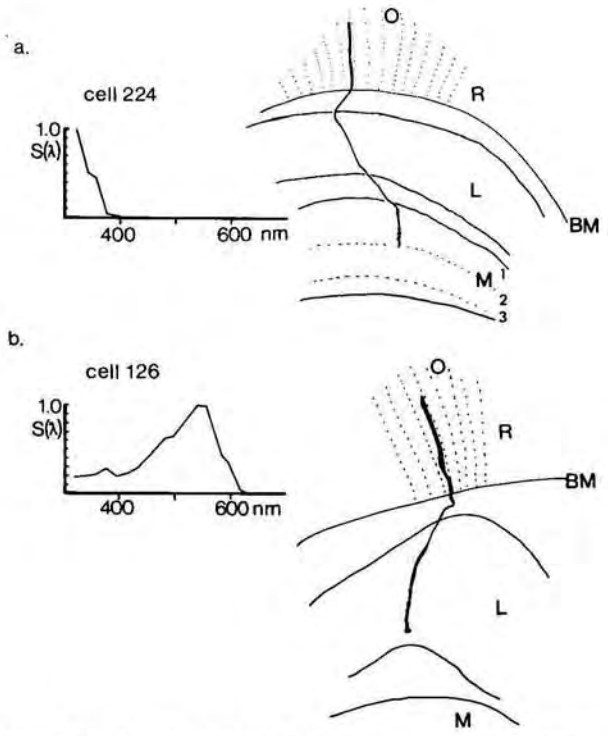


Fig. 2a, b. Two examples of intracellularly marked photoreceptors and the corresponding S(λ) of the marked cell. **A** Cell no. 224 is a UV receptor whose axon penetrates the lamina (*L*) and projects to the distal layer *1* of the medulla (*M*). **b** Cell no. 126 is a green receptor with a fat axon which projects to the proximal layer of the lamina in a fork-like structure. *O* ommatidia, *R* retina, *BM* basement membrane

receptor and broader functions in the UV and green receptors. The higher sensitivity of the UV receptor at wavelengths shorter than 330 nm is a highly significant effect, which may result from absorption at additional pigments, but nothing is known so far. The higher sensitivity of the UV receptor at wavelengths longer than 410 nm and of the green receptor at wavelengths shorter than 410 nm indicates positive electrical coupling between UV and green receptors and may well be a recording artefact. Arguments for this interpretation were presented in detail in Menzel et al. (1986). In short, these spectral side sensitivities vary from cell to cell; they can be selectively adapted, and they are in general lower for particularly good recordings as judged by the height of the light response and the stability of the baseline. Since we interpret the source for the spectral side sensitivity as being the other receptor type (in case of the UV receptor, it is the green receptor, and vice versa) and the cause of the electrical coupling as a recording artefact, we corrected the spectral sensitivity functions as shown in Fig. 1 b. The corrections for the UV-receptor lead to less than 1% sensitivity above 470 nm due to the assumption that the green receptor is not coupled to the UV receptor, and those for the green receptor lead to a slightly reduced sensitivity below 390 nm based on the assumption that the UV receptor is not coupled to the green receptor. No other assumptions were made for the corrections (e.g. sensitivity of the UV receptor below 340 nm, and half bandwidth of the green receptor). The corrected functions are used as spectral input functions for the receptor model of color vision (see below).

Intracellular markings with Lucifer yellow were combined with a rough determination of the S(λ) by the flash method. They revealed single cell markings only for green receptors (Fig. 2b), but multiple markings for other receptor types. The single marked green receptor projects with a fat axon into the proximal layer of the lamina. The branches of the axon resemble those of the green receptors of *Apis* (Menzel and Blakers 1976); however, the branches are shorter and blunt in *Melipona*. UV and blue cells were not marked separately. Markings

including long axons, which project through the lamina into the distal layers of the medulla, always correspond to spectral sensitivities with either a single peak in the UV (e.g. Fig. 2a) or with very high sensitivity in the UV. In the case of cell no. 224 (Fig. 2a), two identical markings appeared in two separate lamina cartridges and medulla columns (4–5 cartridge columns apart, Fig. 2a shows only one cell). The reason for this kind of double marking is unknown, since no other UV cell was recorded before or after dye injection.

We conclude that long visual fibres come from UV receptors as is the case in honeybees (Menzel and Blakers 1976), dragonflies (Meinertzhagen et al. 1983), and flies (Hardie 1979). Blue receptors have not yet been identified.

The receptor model of color vision in Melipona

According to Cornsweet (1970), Rushton (1972), and others, the 3 spectral receptor types can be interpreted as a special set of primary color codes which define an orthogonal 3-dimensional space. The 3 orthonormal basis vectors of the color space correspond to the primaries or tri-stimulus values of unit amount in the calculations of lower color metrics. Each color stimulus is defined by a vector that results from an additive mixture of the tri-stimulus values. The color vectors are elements of a linear Euclidean space. If the length of the vector (brightness) is not important, a color is unequivocally defined by 3 linear dependent values – the normalized tri-stimulus values or the chromaticity coordinates in a chromaticity diagram (Fig. 3). UV, B, and G in capital letters (see Table 1) will be used to refer to the tri-stimulus values in vector space and small letters (uv, b, and g) after transformation in two dimensions. UV, B, and G are normalized such that each value is of unity length for an achromatic object (e.g. the white standard $BaSO_4$) illuminated by natural daylight (spectral radiation of the D65 norm function; see Backhaus and Menzel 1987 and Backhaus et al. 1987 for more details). Figure 3 gives the chromaticity diagram for the corrected sensitivity functions of the 3 photoreceptors (from Fig. 1b). The line of the loci for spectral lights from 300 to 650 nm indicates the corresponding chromaticity diagrams within which all other color stimuli are located. The chromaticity diagram spans most of the potential area. This would not have been the case if the uncorrected $S(\lambda)$-functions had been used. The chromaticity diagram would be compressed in the bluish-green due to the spectral side sensitivity of the UV receptor. A priori any color vision system should be superior if color stimuli are separated as much as possible, and it is thus likely that the corrected input functions are the more appropriate functions. Since color mixing experiments have not yet been carried out with *Melipona*, the adequacy of the chromaticity diagram has not yet been tested. However, the data presented below indicate that color vision in *Apis* and *Melipona* does not differ in many respects. In *Apis* the results of color mixing experiments are very well predicted by the receptor model of color

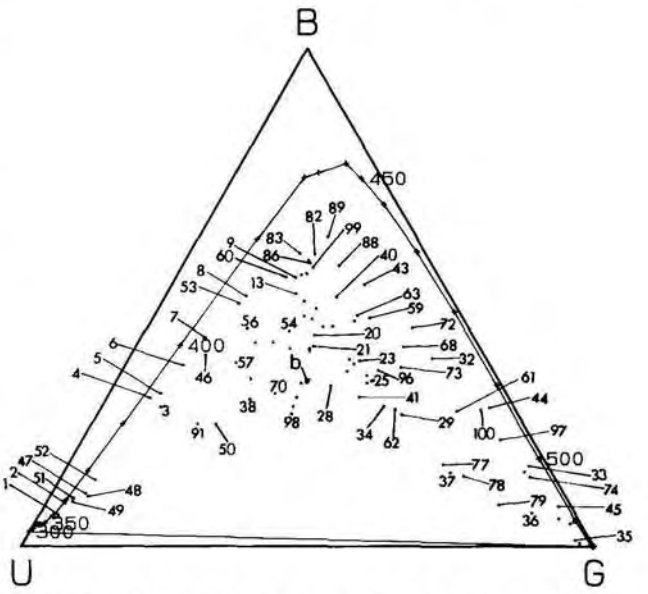


Fig. 3. Receptor model of color vision in *Melipona* as described in the text. The chromaticity diagram is calculated on the basis of the corrected $S(\lambda)$-functions (Fig. 1b). The color loci of the 100 stimuli used in this study are indicated by *dots*. *Numbers* refer to Table 1, which lists all the stimuli used. *b* background

vision if the spectral side sensitivity of the UV receptor is very low (in the range of 1% in the bluish-green; Menzel and Backhaus 1989).

The chromaticity diagram has its value also in an appropriate graphical representation of color signals in the corresponding loci (see Fig. 3). For such a graph it is important to define the adaptation of the receptors and correct the model calculation accordingly (see Backhaus and Menzel 1987, for detail). In the case of Fig. 3, which shows the loci of all the color stimuli used in the behavioral experiments, the adaptation is defined by the spectral distribution of average daylight (D65-function) and the spectral reflection of the background used in the behavioral experiments.

Color discrimination: qualitative effects

Color learning at the feeding place and the hive entrance. *M. quadrifasciata* is equipped with the necessary spectral input system for trichromatic color vision. Does the bee use it in the expected way? In order to test color discrimination, bees were trained to color signals at the hive entrance and at a feeding place under natural light conditions.

Foragers learn quickly to use a colored disc as a marker of the hive entrance or of a pool of sucrose solution.

Color learning at the feeding place does not markedly differ between *Melipona* and *Apis*, although particular care has to be drawn to odor marks in *Melipona*. These stingless bees are also not so consistent: They often work only for a few hours and then do not return to the feeder for another couple of hours. They are also much more prepared to give up foraging if they are not work-

Table 1. List of color stimuli used in the behavioral experiments. The number (left column) identifies the stimulus and is used throughout the text and the figures. The abbreviations for glass filters of the Schott Company are used if filters are presented on top of a ground aluminium plate. If the filters lie on top of a pigmented paper, the abbreviations of these papers are given together with the filter. The number 2 behind the filter code indicates thickness of the filter (2 mm). All filters without an additional number are 1 mm thick. The next 3 columns are the UV, B, and G values as defined in the text, and the last column lists the brightness value: H = UV + B + G

Nr.	Object	UV	B	G	H	Nr.	Object	UV	B	G	H
0	Background	1.000	1.000	1.000	1.000	50	UG11 + B1	0.731	0.337	0.297	0.455
1	UG11	1.617	0.114	0.062	0.598	51	UG5	2.496	0.286	0.117	0.966
2	UG1	1.464	0.146	0.056	0.555	52	UG5 + B1	0.456	0.077	0.036	0.189
3	UG3	2.039	0.935	0.340	1.105	53	BG25 + B5	1.128	1.480	0.409	1.006
4	BG3	2.688	1.292	0.326	1.435	54	BG25 + B1	0.545	0.751	0.438	0.578
5	BG24	3.154	1.626	0.472	1.751	55	BG24 + B3	1.059	0.950	0.258	0.756
6	BG25	1.381	0.944	0.263	0.863	56	BG24 + B5	2.154	2.458	0.976	1.863
7	BG12	1.606	1.426	0.394	1.142	57	BG24 + GR4	1.525	1.281	0.662	1.156
8	BG25 + B3	0.986	1.406	0.392	0.928	58	BG24 + G5	1.866	1.651	0.442	1.320
9	BG28/2	0.249	0.574	0.227	0.350	59	BG28 + GR1	0.104	0.297	0.243	0.215
10	V2	2.315	2.678	1.538	2.177	60	BG28 + V1	0.439	0.896	0.316	0.550
11	V1	1.507	1.603	0.799	1.303	61	BG18 + GR1	0.118	0.313	0.712	0.381
12	V3	2.824	3.396	2.311	2.844	62	BG18 + GR2	0.506	0.670	1.241	0.806
13	BG28	0.719	1.381	0.611	0.904	63	BG18 + B4	0.636	1.644	1.246	1.176
14	BG28 + V3	0.830	1.798	0.695	1.107	64	BG23 + GR3	0.889	1.337	1.781	1.335
15	BG28 + GR3	0.715	0.953	0.733	0.800	65	BG23 + BV2	0.829	1.311	1.339	1.160
17	B1	0.609	1.162	0.581	0.784	66	G4	1.500	1.422	1.403	1.441
18	B2	1.114	1.880	1.063	1.352	67	G5	2.324	2.369	2.365	2.353
19	B3	1.355	2.354	1.433	1.714	68	W	3.153	9.581	11.157	7.964
20	B4	2.830	4.368	3.082	3.427	69	B1	0.609	1.162	0.581	0.784
21	B5	2.707	3.770	2.907	3.128	70	G3	0.815	0.624	0.585	0.674
22	BG27	0.365	0.867	0.675	0.636	71	VG6 + Y2	0.043	0.140	0.740	0.307
23	BG18	0.918	1.541	1.664	1.374	72	VG6 + B5	0.251	1.131	1.187	0.857
24	BG23	1.593	2.093	1.639	1.775	73	VG6 + G3	0.061	0.139	0.186	0.129
25	BV1	0.644	0.991	1.325	0.987	74	VG9 + W	0.057	0.194	1.123	0.458
26	BV2	1.085	1.665	2.082	1.611	75	VG9 + G3	0.445	0.328	0.386	0.386
27	BV3	2.330	3.235	3.600	3.055	76	VG9 + B1	0.360	0.294	0.323	0.326
28	BV5	1.481	1.613	1.882	1.659	77	GG495 + GR1	0.285	0.261	1.029	0.525
29	GR1	0.534	0.699	1.394	0.876	78	GG495 + GR3	0.442	0.401	1.979	0.941
30	GR2	0.697	0.548	0.376	0.541	79	GG495 + G3	0.039	0.026	0.247	0.104
31	GR3	1.450	1.815	3.150	2.138	80	GG495 + G5	0.037	0.065	1.037	0.380
32	VG6	0.181	0.733	1.021	0.645	81	GG495 + W	0.056	0.149	2.950	1.052
33	VG9	0.022	0.111	0.547	0.227	82	BG282 + W	0.438	1.332	0.498	0.756
34	GG495	0.019	0.092	1.718	0.610	83	BG28/2 + V1	0.181	0.490	0.160	0.277
35	OG550	0.016	0.008	0.588	0.204	84	BG28/2 + G5	0.226	0.620	0.232	0.359
36	OG590	0.011	0.010	0.128	0.050	85	BG28/2 + G3	0.064	0.154	0.063	0.094
37	Y2	0.732	0.616	2.793	1.380	86	BG28/2 + G4	0.143	0.388	0.150	0.227
38	BG24 + Y2	0.989	0.651	0.549	0.730	87	BG28/2 + B4	0.462	0.901	0.518	0.627
39	OG530	0.018	0.007	0.840	0.288	88	BG28/2 GR3	0.121	0.421	0.203	0.248
40	BG28 + GR4	0.393	0.996	0.591	0.660	89	BG282 + B1	0.086	0.350	0.126	0.187
41	BG23 + GR2	0.633	0.737	1.072	0.814	90	BG232	0.907	1.585	1.095	1.196
42	BG23 + BV1	0.478	0.749	0.808	0.678	91	UG1/2 + AL	0.942	0.411	0.308	0.554
43	BG27 + B4	0.451	1.732	1.101	1.094	92	BG23/2 + GR2	0.421	0.601	0.799	0.607
44	VG6 + GR3	0.107	0.485	1.168	0.587	95	BG23/2 + G5	0.668	1.263	0.913	0.948
45	GG495 + B4	0.019	0.071	0.771	0.287	96	BG23/2 + GR3	0.578	1.025	1.290	0.964
46	BG37	2.587	2.050	0.695	1.777	97	VG6 + GR2	0.065	0.247	0.835	0.382
47	UG11 + G5	0.182	0.023	0.013	0.073	98	GG495 + B1	0.441	0.300	0.378	0.373
48	UG11 + G3	0.333	0.041	0.027	0.134	99	BG28 + B4	0.627	1.682	0.688	0.999
49	UG11 + W	0.674	0.071	0.035	0.260	100	VG6/2	0.028	0.177	0.427	0.210

ing in a group, but rather as an individual bee. Often, the selected experimental bee returns to the feeder only if the comrades which were arrested in a cage during a test procedure are released again. Furthermore, the recruitment behavior is less reliable and effective in *Melipona*, because we had to eliminate all odor marks, and odor marks are essential guide posts for newly recruited hive mates (Lindauer and Kerr 1958).

Training at the hive entrance is easier and more effective in *Melipona* than in *Apis*, because the entrance hole is guarded by only one or two bees which sit inside the connecting tube and thus do not interfere from outside with the choice behavior of the approaching bees. Figure 4 shows a reversal learning curve in a dual choice situation at the hive entrance. The bees were first trained for several days to enter the hive through a bluish-green

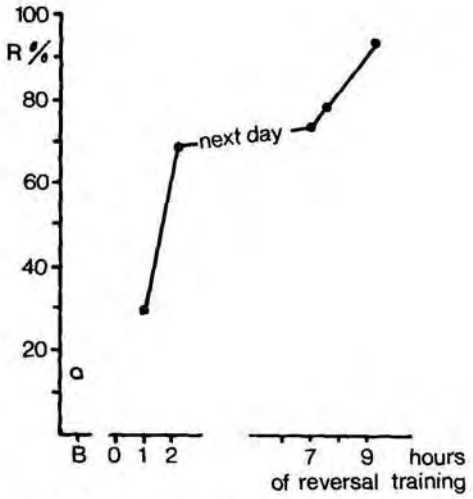


Fig. 4. Reversal learning of *Melipona quadrifasciata* at the hive entrance. Bees were first trained for several days to color signal no. 23. In a dual choice test the trained color signal was preferred by 85% (open circle at *B*: choice of the alternative color signal no. 51 to 25%). At time zero the color signal no. 51 marked the entrance. The former trained color signal no. 23 was used to test learning of signal no. 51 during the following 1, 2, 7, 7.5, and 9 h. *Ordinate* plots choice behavior in % choice for the color signal no. 51

disc (filter BG 18, no. 23 in Table 1). At time zero the former alternative UV disc (filter UG 5, color signal no. 51) marked the entrance to the hive, and the bluish-green disc was closed. After only 2 h, the UV disc was preferred, and a day later it was chosen at a 90% preference level relative to the former entrance marker. This experiment demonstrates that *Melipona* foragers learn to reverse a trained preference for color signals at the hive entrance within several hours. At the feeding place learning might be faster, but this was not studied in more detail. All discrimination tests reported below were performed after the asymptotic learning plateau had been reached.

Comparison between dual and multiple choice tests. The results of the dual choice tests are given in Fig. 5 (hive entrance) and Fig. 6 (feeding place) and those of the multiple choice tests at the feeding place in Fig. 7. The numbers on the choice columns refer to the color signals of Table 1 and are partially depicted as loci of color stimuli in the chromaticity diagram of Fig. 3. It is immediately obvious that *Melipona* discriminates the colors very well in both behavioral contexts. The columns in Figs. 5–7 are arranged in such a way that those for similar colors (as judged by the choice frequency) are represented in adjacent columns, and those for less similar colors are separated by other columns. It is also obvious that discrimination improves with the distance of the two loci of the respective pairs of color stimuli (compare Figs. 5–7 with Fig. 3). Before the quantitative aspects of this relationship are analyzed on the background of a receptor model of color vision, a few behavioral observations should be described.

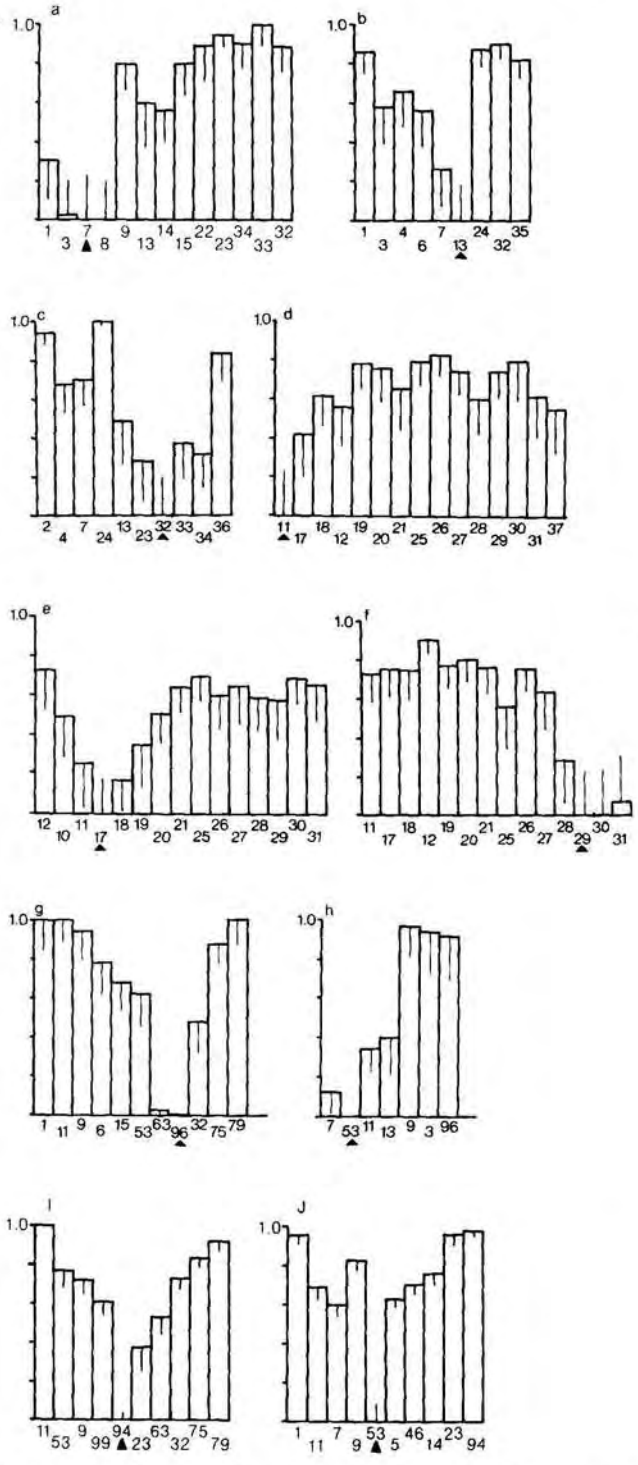


Fig. 5a–h. Ten series of experiments (**a–h**) in which different color signals were trained at the hive entrance and choice performance was tested in a dual choice test. Numbers at the columns indicate color signals (see Table 1). The trained color signals are marked with a black triangle under the number. Ordinate gives the proportion $N^+/(N^+ + N^-)$ where N^+ is the number of correct choices, N^- the number of incorrect choices. N in the following table is $N^+ + N^-$. **a:** no. 4, N = 7278; **b:** no. 13, N = 2493; **c:** no. 32, N = 641; **d:** no. 11, N = 5846; **e:** no. 17, N = 5161; **f:** no. 29, N = 6790; **g:** no. 96, N = 1961; **h:** no. 53, N = 1361; **i:** no. 94, N = 1961; **j:** no. 53, N = 1361. Scatter bars (standard deviation) calculated from repetitions of same test situation (more than 10 times in each case)

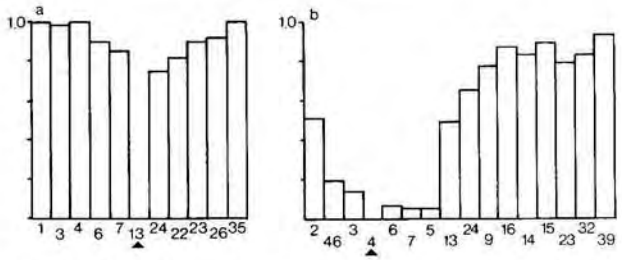


Fig. 6a, b. Two examples of dual choice tests at the feeding place. **a** Trained color signal no. 13, total number of choices, $N=2207$. **b** Trained color signal no. 4, total number of choices, $N=4040$. Each column gives the proportional response to the trained color signal when the color signal indicated by its number at the base of the column is the alternative in the test. Each test situation was repeated more than 10 times. After training to no. 13 all color signals are discriminated significantly ($P \leq 0.01$). After training to no. 4, color signals no. 46, 3, 6, 7, and 5 are not significantly discriminated, but all other color signals are ($P \leq 0.01$, χ^2-test)

Comparison between feeding place and hive entrance experiments. Comparison of the same color pairs in Figs. 5b and 6a shows that the bees discriminate better when they search for food reward than when they are on their way back into the hive. Figure 8 gives examples for several trained color signals in the dual choice test situations. The same color signals were tested at the feeding place and the hive entrance with exactly the same training and test arrangements. Choice values are higher for all pairs of color signals at the feeding place, particularly if violet-blue colors have to be discriminated (compare symbols and numbers in Fig. 8). For example, when trained to color signal no. 13, discrimination from color signals no. 3, 4, 6, and 7 (UV to violet signals) is much better at the feeding place than at the hive entrance.

Comparison between multiple and dual choice tests. Comparison between the results of the dual and the multiple choice tests shows that the response values are highly correlated, with slightly higher values for the multiple choice situation (Fig. 9). For a better comparison, choice behavior is expressed in the same way in both test situations, namely by calculating the percentage of correct choices separately for each pair of trained and alternative color signals. The close correspondence between the two situations is particularly interesting, because it indicates that choice behavior may be relatively independent of the training and test conditions and predominantly influenced by the perceptual differences between the color signals.

Comparison of the dual and multiple choice tests (see Figs. 5–7) reveals that an alternative color signal is sometimes more frequently chosen than the trained color signal in multiple choice tests, whereas this was not observed in dual choice tests. However, it appears that all these colors were always very similar to the trained signal in chromaticity (hue and saturation, Fig. 3) but were darker than the trained signal (see Table 1). Such conditions were not tested in any dual choice test. We are thus unable to resolve the question of whether a preferred choice over the trained signal in multiple choice tests is related to signal parameters or depends on the test procedure.

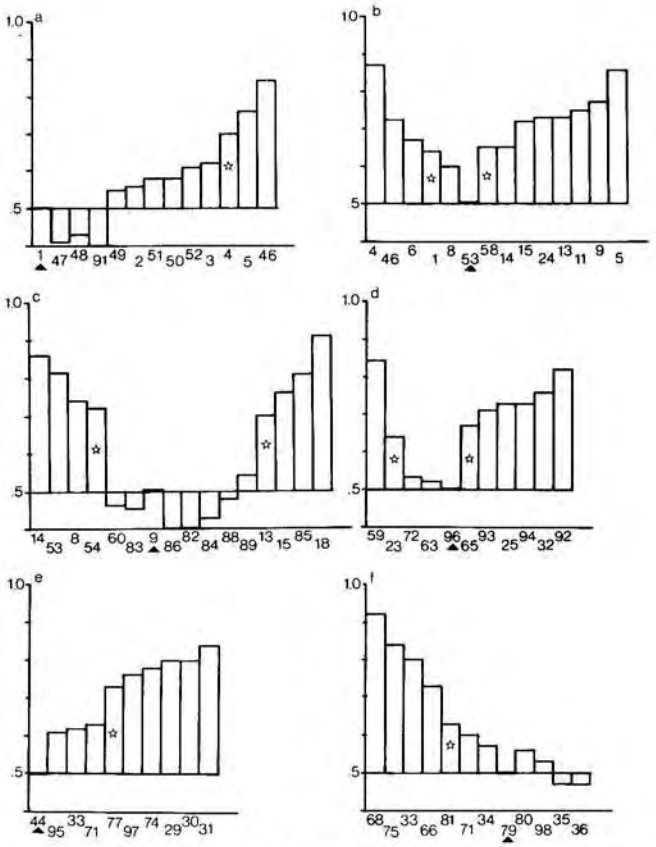


Fig. 7a–f. Discrimination of colors at the feeding place. Bees were trained to the stimulus indicated by the *black* triangle without presentation of any other color signal. In the test situation 12 stimuli (11 plus the trained stimulus) are presented simultaneously (multiple choice test). In each case **a–f** a total of 20–24 stimuli were tested in different combinations of the 12 simultaneously presented stimuli. Those stimuli chosen less than 5% are not depicted in these graphs. The proportional choice level (ordinate) $N^+/(N^+ + N^-)$ is calculated for each combination of trained color signal and one alternative color signal separately. Choice level below 0.5 means that the alternative color signal is chosen more frequently than the trained color signal (see text). **a:** no. 1, $N=102$; **b:** no. 53, $N=169$; **c:** no. 9, $N=160$; **d:** no. 96, $N=95$; **e:** no. 44, $N=171$; **f:** no. 79, $N=311$. The lowest response values which are significantly different ($P \leq 0.01$, χ^2-test) from the 50% choice level are indicated with star

The effect of the training procedure. To test whether the training procedure affects the choice behavior, a bluish-green color signal (no. 96) was trained at the feeding place under 3 different conditions: (1) absolute training: only the color no. 96 was shown during training; (2) differential training vs. color signal no. 9 (deep blue color): in this case no. 9 was presented during training to no. 96 but not rewarded; (3) differential training vs. color signal 100 (deep green color). The choice behavior was tested in multiple choice tests (Fig. 10). Discrimination is better after differential training in four of six cases. These four cases refer to discriminations between

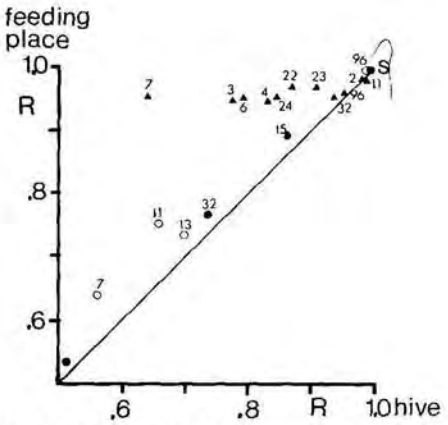


Fig. 8. Comparison of choice behavior at feeding place and at hive entrance. Bees were trained to one color signal in the centre of a large grey background and tested in a dual choice test to the same pairs of color signals. Axes give the proportional choice level at feeding place (ordinate) and at hive entrance (abscissa) ▲ choices after training to color signal no. 13, ● same after training to color signal no. 96, ○ same after training to color signal no. 53. Numbers indicate the alternative color signals in the dual choice test. If the bees would choose the trained color signals equally well in both behavioral contexts data points should scatter around the diagonal line. The choice level is consistently higher at the feeding place

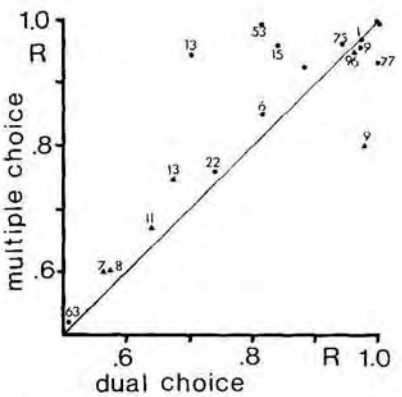


Fig. 9. Comparison of the choice behavior in dual choice (abscissa) and multiple choice (ordinate) tests after training to color signal no. 96 (circles) or color signal no. 53 (triangles) at the feeding place. The choice level is compared for the same pair of color signals. The proportional choice level ($N^+/N^+ + N^-$) is calculated as in Figs. 5 and 7 (see also text). Dual and multiple choice tests give similar results with slightly enhanced choice values in the multiple choice test

close bluish-green colors, whereas in the two cases in which absolute training resulted in discrimination values similar to differential training, the bluish-green trained color had to be discriminated from blue or violet colors. In two of the six cases the two differential training conditions improved the discrimination significantly in the expected direction, namely a better discrimination from test colors which are similar to the non-reinforced alternatives during training (see Fig. 10, cases no. 25 and 9). In the other four cases no such statistically significant

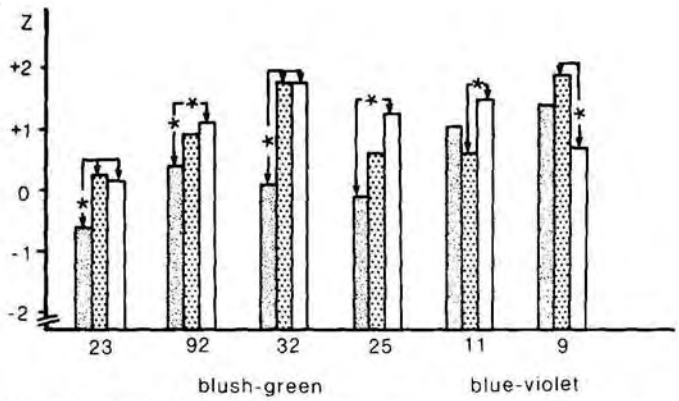


Fig. 10. Effect of training conditions on choice behavior at the feeding place. Bees were trained to color stimulus no. 96 under 3 different conditions: left column (grey), only the trained stimulus is presented during training (absolute training); middle column (dotted), the color stimulus no. 9 is presented during training and not reinforced (no sucrose solution) (differential training vs. color 9); right column (white), color stimulus no. 100 is presented during training and not reinforced (differential training vs. color 100). The test was a multiple choice test with 11 alternatives. The choice behavior to those stimuli not discriminated very well in either of the 3 training conditions are shown here. *Star* indicates statistically significant differences (χ^2-test, $P \leq 0.01$). Ordinate: percentage of correct choices expressed in Z-values (probability transformed percentage scale, $Z=0$ corresponds to 75% correct responses in a dual choice situation)

effect was found. It is concluded that training conditions may indeed have an influence on choice behavior. This will be the case if the unrewarded alternatives presented during training are similar to the trained color and, therefore, are chosen quite frequently. Under these conditions the choice behavior becomes more accurate with respect to the trained color signal, and any other color signals are less frequently chosen, not only those which were presented as alternatives during training.

Comparison between Melipona and Apis. Many dual color discrimination tests at the hive entrance were performed in parallel with the stingless bee, *Melipona,* and the honeybee, *Apis.* This allows a direct comparison of color discrimination in the two species. The experimental arrangement and the pairs of color signals were the same in both series of experiments. The only difference between the tests was the background screen which was painted light grey in the experiments with *Melipona* and white in those with *Apis.*

The result of the comparison is shown in Fig. 11. If the two species discriminate the colors equally well, the data points in Fig. 11 should scatter around the diagonal midline, but this is not the case. The discrimination probabilities for bluish-green color pairs lie above the midline, those of UV, violet and yellow colors lie below the midline, independently of the color signal used for training (either no. 13 or 32). This result means that *Melipona* discriminates colors somewhat better in the bluish-green, and *Apis* better in all other regions.

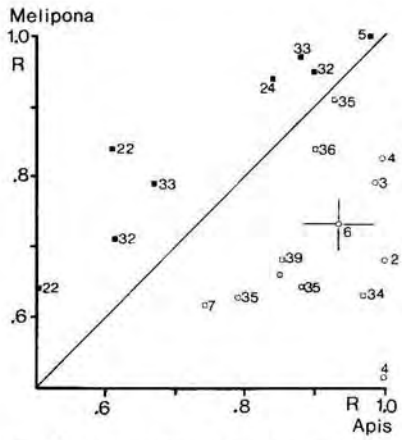


Fig. 11. Comparison of *Apis*'s and *Melipona*'s choice behavior at the hive entrance after training to same color signals and dual choice discrimination tests. Axes as in Fig. 5. Bees were trained to color signals no. 13 or 32. Numbers indicate the alternative color signals (see Table 1). Colors in bluish-green are given in solid squares, those in UV and violet in open circles, and those in yellow in open squares. Standard deviations calculated from repetitions of the same test situations are plotted for one example (alternative color signal no. 6)

Color discrimination and the predictions of the receptor model of color vision

The receptor model of color vision makes predictions about the perception of color stimuli on two levels: (1) color stimuli which occupy the same locus in the color space should appear identical to the animal (identity judgement, lower colorimetry of color matching experiments); (2) color stimuli further apart in the color space should be discriminated better than those close together (higher colorimetry of color discrimination experiments). The behavioral experiments reported in the section above are all of the second kind. Let us ask, therefore, how well the discrimination values obtained from these experiments are predicted by the receptor model.

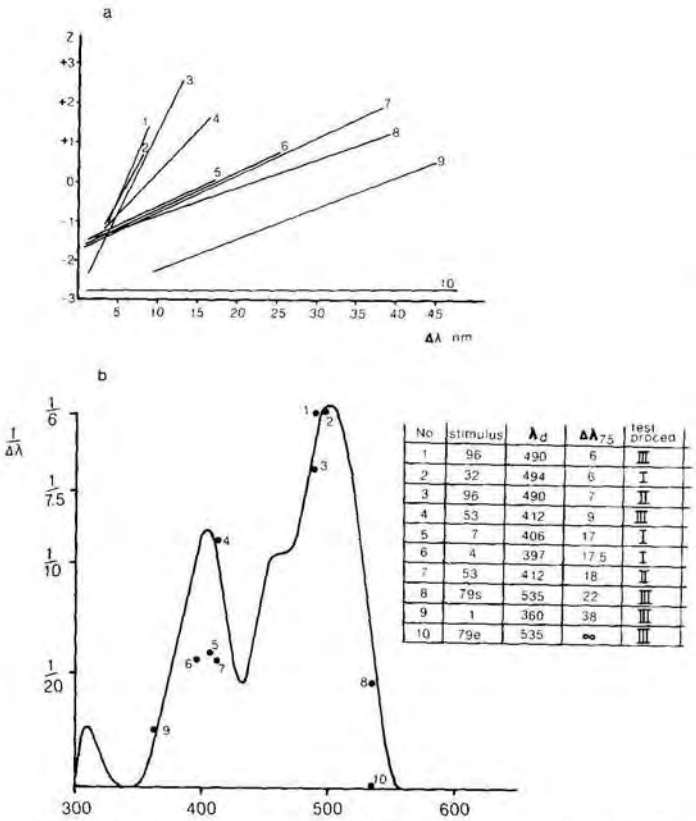
It is well established that the Euclidian distance between the loci of two color stimuli is not an appropriate measure of the perceptual distance between the two color stimuli, because the mixture line between the two stimuli is not the shortest perceptual distance (von Helmholtz 1896; Schrödinger 1920). Instead, a line element has to be established which divides Riemann's non-Euclidean manifold into elementary components and counts the shortest distance as the minimal number of these line elements between the two stimuli in question. Von Helmholtz (1896) was the first to present an analytical expression for a line element which describes the sensations lying between two colors. He postulated that an adequate measure for spacing is the number of perceptually just noticeable difference steps (pjnd steps) between the two colors. The lines with the smallest number of pjnd steps are geodesics in a Riemannian manifold with non-constant curvature. Schrödinger (1920) followed this argument and derived a modified line element as a measure of similarity. For different practical applications, several nonlinear distance functions of the chromaticity coordinates in human color vision have been derived (Richter 1981).

It has been shown by Backhaus and Menzel (1987) that the pjnd-scaling can be related to a scaling of just noticeable difference steps on the receptor level (rjnd) in honeybees. The receptor signal of any photoreceptor is superimposed upon extrinsic and intrinsic noise components, which in honeybees add up to voltage fluctuations of about 0.4% of maximal response to light. The total noise component in each spectral receptor type can be converted back into a fictive fluctuation of effective (absorbed) photons and thus into a fluctuation of the corresponding tri-stimulus value, taking into account the log/lin scale of the intensity/response function and the shift of the intensity/response function during adaptation. For simplicity, it is assumed that a change of a perceptual state cannot be produced by subthreshold summations of the receptor signal. As a consequence, it is necessary that at least one tri-stimulus value changes significantly to cause a significant change in the color perception state (see Backhaus and Menzel 1987 for details).

Two distance measures were used in the study with the honeybees:

1. sD(jnd) (spatial jnd distance) between two stimuli – the sum of rjnd steps caused in the photoreceptors when one signal replaces the other. In this case the bee would make a judgment based on the information about a change of total light flux. With a noise fluctuation of up to 0.4% of the maximal light response 3 photoreceptors can transmit up to 10^3 (a single photoreceptor 333) discrete voltage states with a probability of 99% (3 corresponds to 0.3% of the maximal light response in each receptor if the total noise fluctuation lies below 0.4% of the maximal light response). Thus, a maximum of 10^3 different pjnd steps (brightness values) are possible.

2. A second measure for color spacing is pD(jnd) (plane jnd distance). This measure is based on the assumption that the neural color vision system produces 3 color quantities, u, b, and g, that are independent of changes in total light intensity over a wide intensity range, because the sum of these color quantities is normalized to unity ($u+b+g=1$, see above). Independence of the intensity makes it necessary to compensate changes in one color quantity by changes in the other two color quantities. An approximation procedure is offered in our analysis of the honeybee (Backhaus and Menzel 1987) which finds the shortest line between two colors. This means, according to von Helmholtz' postulate, the procedure finds the color distance pD(jnd) with the smallest number of perceptual jnd steps between two colors based on the assumption that a neural color coding system normalizes the 3 color quantities u, b, and g to unity and thus ignores any intensity differences. This is the simplest model that reflects similarity relations between colors without making any further assumptions about neuronal mechanisms that lead to a color continuum (see Backhaus and Menzel 1987, for further details). The predictions of these receptor models



No	stimulus	λ_d	$\Delta\lambda_{75}$	test procedure
1	96	490	6	III
2	32	494	6	I
3	96	490	7	II
4	53	412	9	III
5	7	406	17	I
6	4	397	17.5	I
7	53	412	18	II
8	79s	535	22	III
9	1	360	38	III
10	79e	535	∞	III

Fig. 12a, b. Comparison of the $\Delta\lambda/\lambda$-function as predicted by the receptor model of color vision with behaviorally determined spectral discrimination. **a** Nine stimuli were selected on the basis of high spectral purity (see Table 1 and corresponding color loci in Fig. 3). These stimuli were trained under three different conditions: I, hive entrance, dual choice tests; II, feeding place, dual choice tests; III, feeding place, multiple choice tests. Alternative stimuli presented in the tests were selected also on the basis of high spectral purity and on the fact that their wavelengths (λ_d) were close to that of the trained color stimulus (λ_d in Table 1). Between two and five alternative signals for each of the nine trained signals were selected on this basis. Figure 12a plots regression lines between the choice behavior (discrimination expressed in Z values, ordinate) and the $\Delta\lambda$ between the dominant wavelengths of the trained and tested stimuli (abscissa). Regression lines are marked with numbers which are explained in Table 1. $\Delta\lambda$ for 75% correct choices ($Z=0$) is determined for each trained color ($\Delta\lambda_{75}$, see Table 1); the reciprocal is calculated to express wavelength discriminability on a sensitivity scale in **b**: sensitivity factors are plotted together with the $\Delta\lambda$-function as predicted by the receptor model. Numbers at the points refer to numbers in Table 1. The maximum (15 jnd's) of the model's discrimination function at 494 nm is normalized for comparison with the discrimination value ($1/\Delta\lambda_{75}=1/6$ nm) of stimulus no. 2

of color vision in *Melipona* were tested by comparing the discrimination values from the experiments described in Results part 3 with the calculated rjnd steps between the stimuli presented in the behavioral tests.

A most appropriate test would be a comparison between the calculated and measured $\Delta\lambda/\lambda$-function. Such a function exists for *Apis* (von Helversen 1972), but in *Melipona* only reflecting colors with relatively broad spectral reflection functions were used and no monochromatic stimuli. We nevertheless applied the procedure to calculate $\Delta\lambda$ for a constant level of discrimination (75% correct responses) after a few highly saturated color signals were trained, and discrimination was tested between close colors of equally high saturation (see Fig. 12a). An inspection of the corresponding color loci indicates that these colors were close to the spectral line (see no. 1, 4, 7, 32, 53, 79, and 96 in Fig. 3). Figure 12b gives the result of the comparison with the $\Delta\lambda/\lambda$-function. Most of the data points are in the vicinity of the calculated function. Only two points (no. 5 and 6) are far off, and a third one (no. 10) also needs further inspection. The two points (no. 5 and 6) in the violet come from training experiments at the hive entrance. It has been noted already (Results part 3, Figs. 8 and 11) that violet colors are surprisingly less well discriminated at the hive entrance but very well at the feeding place (see also data point 4 in Fig. 12b). This deviation is, therefore, not related to a sensational capacity but to a context-specific evaluation of the information provided by the visual system. Data points 8 and 10 refer to the discrimination of color signal no. 79 from shorter and longer wavelength stimuli in a dual choice test at the feeding place. *Melipona* did not discriminate no. 79 significantly from any longer wavelength stimuli (see line 10 in Fig. 12a, also Fig. 7f) but distinguished it quite well from stimuli with shorter dominant wavelengths (line 8 in Fig. 12a, also Fig. 7f). The $\Delta\lambda/\lambda$-function of the model calculation is not affected by such asymmetries at the border of color discrimination, because it gives the jnd-steps for 4-nm intervals around the wavelengths.

In summary, the measured $\Delta\lambda$ values follow quite well the predictions of the receptor model. Since the stimuli tested differ considerably also in intensity and thus should have appeared dissimilarly bright to the animal (see H values in Table 1), the good agreement indicates that brightness differences may have little influence on color discrimination in *Melipona*.

The receptor-based line elements can also be calculated for any two stimuli within the color space. In such a case the two measures sD (jnd) and pD (jnd), the spatial and plane jnd distances (see above), are particularly relevant because the stimuli may differ considerably in their intensity component. Figure 13 gives an arbitrarily selected example of the correlation between the response values and the number of pjnd's calculated for each pair of color signals in a hive entrance training to color signal no. 4 (compare Figs. 5 and 3). For the calculation of the rjnd's it was assumed that the receptors are adapted to the background, which is illuminated by natural daylight. There is a high correlation between the response values and distances both in the color plane and in the color space. The correlation coefficient is lower for the latter ($r=0.7861$ vs. $r=0.8426$, $n=16$). More response/distance functions for other experimental conditions and trained color signals are given in Fig. 14.

The following conclusions can be drawn from these results:

1. The distance pD(jnd) in the color plane always correlates better with the discrimination data than the spatial distance sD(jnd), therefore Fig. 14 gives only the

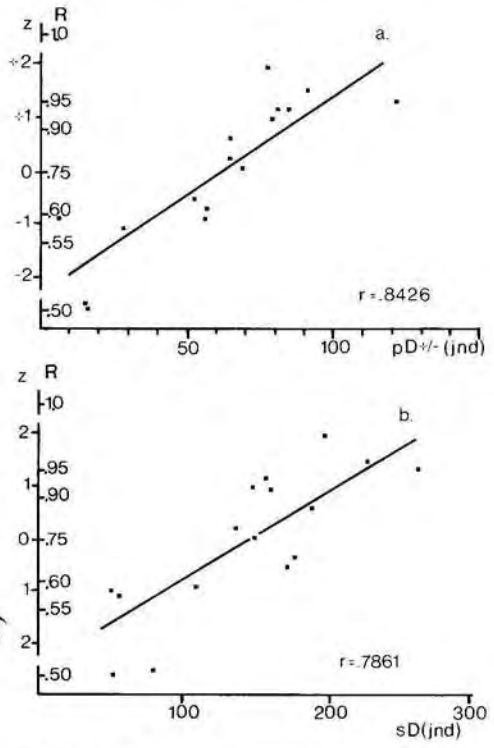


Fig. 13a, b. Response/distance functions for two distance values. **a** pD(jnd), minimal number of just noticeable difference steps between the two colors in the chromaticity plane and **b** sD(ind), the just noticeable difference steps in the color space (see text). Bees were trained to color signal no. 4 at the hive entrance and tested in dual choice tests. The proportional response to the trained color signal is expressed in *Z*-values on the ordinate; *r*, correlation coefficients

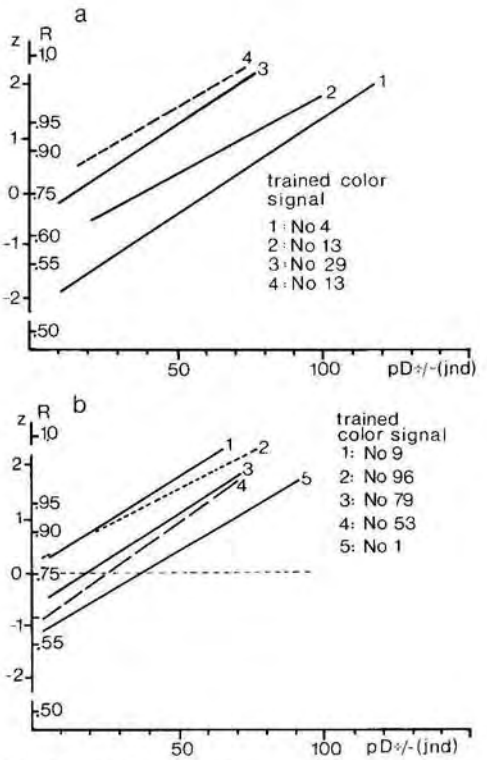


Fig. 14a, b. Response/distance functions for distance value pD and four different series of discrimination tests. **a** Color signals no. 4 (regression line 1), no. 13 (line 2), no. 29 (line 3) were trained and tested at the hive entrance (dual choice tests), and no. 13 (dotted line 4) was trained at the feeding place (dual choice test). Correlation coefficients: line 1, $r=0.84$; line 2, $r=0.89$; line 3, $r=0.67$; line 4, $r=0.91$. **b** All color signals were trained and tested at the feeding place (multiple choice tests). Correlation coefficients: line 1, $r=0.78$; line 2, $r=0.78$; line 3, $r=0.72$; line 4, $r=0.81$; line 5, $r=0.70$

pD(jnd). For example, the corresponding correlation coefficients for one series of experiments (Fig. 14b, training at the feeding place, multiple choice test, see also Fig. 5) are: trained color signal no. 1, $r=0.70$ for pD and $r=0.64$ for sD; no. 53, 0.81 and 0.59; no. 9, 0.78 and 0.24; no. 96, 0.78 and 0.50; no. 79, 0.72 and 0.30. The same applies to the results from the hive entrance training.

2. The regression lines presented in Figs. 13 and 14 were calculated for color pairs which were separated by less than 120 pD(jnd) or 290 sD(jnd). Stimuli separated by more jnd's did not follow the correlation so well and sometimes were even less well discriminated than the stimuli included in this analysis (see Discussion).

3. The steepness of the response/distance function in the color plane is independent of the color signal used for training and of the training and test procedure. However, the functions are displaced along the abscissa pD(jnd). Discrimination of 75% correct choices (pD_{75}) is reached for very small distances ($pD<5$) when bluish-green color signals are used as training stimuli, in both the feeding place and hive entrance situations, thus confirming the observation described above for a smaller number of tests ($\Delta\lambda/\lambda$-function). For example, $pD_{75}=15$ for signal no. 29 and $pD_{75}=5$ for signal no. 96 (see Fig. 14a, hive entrance and Fig. 14b, feeding place). All other colors are better discriminated at the feeding place (compare Fig. 14a, curves 2 and 4: signal no. 13, $pD_{75}=40$ at the hive entrance and $pD_{75}=5$ at the feeding place; Fig. 14a and b: signal no. 4 or 53, $pD_{75}=62$ at the hive entrance and $pD_{75}=26$ at the feeding place). This means that the information from the receptors is used optimally only for color discrimination in the bluish-green. Other color discriminations are subject to color-specific reduction of the available information. This process depends on the behavioral context (hive entrance vs. feeding place).

4. Results from dual choice tests generate response/distance functions with higher correlation coefficients than multiple choice tests. In dual choice tests bees learn an isolated color and successively compare its memory with that of another single color signal, because the color signals are separated by 100 cm in the test. In multiple choice tests bees perform their choices between a set of 12 simultaneously presented color signals which are positioned relatively close to each other (10–20 cm apart). The different degrees of correlation may indicate some influence of the neighboring color signals, which

may reduce the predominant influence of the receptor parameters of color vision, but conclusive evidence is lacking.

5. Deviations from the predictions of a receptor model of color vision may indicate the influence of color preferences and categorical perceptions (generalizations) in the choice behavior of the bees. Color- and context-dependent shifts of the response/distance functions prove the importance of such central nervous weighting processes in addition to the differential receptor signals (see point 3 above). Further evidence for this comes from the observation that certain color signals are even more frequently chosen than the color signal to which the bee is trained (Figs. 5, 7). In these rare cases, training does not overcome the tendency to choose some colors preferentially. We are not yet able to analyze in more detail what makes these colors more attractive to the bees because we have not yet trained the bees to all the color signals used as test signals.

Discussion

This paper is part of a study which aims at a comparative analysis of color vision of flower-visiting insects. The differential spectral sensitivities of the photoreceptors provide the information necessary for the nervous system to discriminate colors. The receptor spectral sensitivities are relatively easy to measure with the necessary accuracy, but behavioral experiments are often very difficult to perform. It is thus of importance to test, whenever possible, how well color discrimination can be predicted from the receptor properties and which parameters may influence color choice besides the perceptual properties of the stimuli. So far, two species of flower-visiting insects have been analyzed with this concept: the worker of the honeybee, *A. mellifera* (Backhaus et al. 1987; Backhaus and Menzel 1987), and the solitary carpenter bee, *Osmia rufa* (Menzel et al. 1988). Here we add a third species, the tropical stingless bee, *M. quadrifasciata*. The objectives of this paper can be given as the following questions:

1. What are the spectral properties of the receptors, how do they relate to the morphological types of receptors, and are there indications for ecophysiological adaptations?

2. Does *Melipona* learn color signals, and does this depend on the behavioral context?

3. How well is *Melipona*'s color discrimination predicted by a receptor model of color vision which was originally developed for the honeybee?

Let us treat these questions in turn.

Photoreceptors

We found recently that the S(λ)-functions of photoreceptors in insect compound eyes can be measured with high accuracy and spectral resolution if one avoids the traditional flash method and uses a fast spectral scan method (Menzel et al. 1986). The recordings from the *Melipona* eye emphasize this point. Whereas the S(λ)-functions scattered largely with the flash method (see Figs. 1–3 in Menzel et al. 1986), they were much more reliable with the scan method (Fig. 1). However, the average functions of the UV and green receptors are still contaminated by enhanced spectral side sensitivities, which we interpret as artificial coupling effects. The arguments for this interpretation are of two different kinds. 1. Receptor physiology: The highest quality intracellular recordings show the least spectral side sensitivities. Spectral side sensitivity can be selectively adapted, indicating at least two different receptors and excluding the possibility of pigment mixtures. Intracellular markings with Lucifer yellow resulted frequently in double markings of UV and green receptors, those receptor types which display the enhanced sensitivity at the reciprocal spectral maxima. None of these observations, however, excludes the possibility of a functional coupling between the two receptor types. 2. Receptor model of color vision: Receptors with spectral side bands contract the effective space for coding chromatic differences. If a system has evolved to code chromatic differences as effectively as possible, one would not expect receptors with a high spectral side sensitivity from an a priori point of view. This argument is supported by the general finding of our study, namely that the receptor model of color vision based on the corrected S(λ)-functions describes in a quantitative fashion color discrimination in *Melipona* (see below).

The 3 spectral receptor types with λ_{max} of 356, 424, and 532 nm appear to be a special set of receptors adapted to the ecological light conditions experienced by *Melipona* in the dense tropical forest. The λ_{max} of UV receptors of all 19 hymenopteran species we have measured so far (Menzel and Backhaus, in press) are positioned at shorter wavelengths, and those of the blue receptors at longer wavelengths than in *Melipona*. The λ_{max} of the green receptor lies well within that of the other species. The shift of the UV receptor to 356 nm would increase its effectiveness under the conditions of natural light filtered through the leaves. The shift of the blue receptors to shorter wavelengths might be interpreted as a mechanism to improve the contrast of violet-blue objects to the overall green light climate in the forest, but model calculations have not yet been performed.

The few intracellular markings we have collected so far in *Melipona* allow us to conclude that at least one type of green receptor and the UV receptors are morphologically very similar in the two species, *Apis* and *Melipona*. The marked green receptor is the one with the fat axon and forklike projections in the proximal layer of the lamina (svf 1) (Ribi 1975; Menzel and Blakers 1976). The marked UV receptor has a long axon penetrating the lamina and projecting to the distal layers of the medulla (lvf 1) (Ribi 1975; Menzel and Blakers 1976).

Color learning and color discrimination

M. quadrifasciata learns colors at the hive entrance and at the feeding place. Learning at the feeding place is impressively fast, and choice behavior is very accurate and selective. At the hive entrance learning is slower

(see Fig. 4), and choice behavior is not as accurate. Higher discrimination indices were always found at the feeding place (Fig. 8). This might be due to differences either in the motivational state or in the experimental situation, which at the hive entrance involves the whole colony, whereas at the feeding place it is just one marked individual bee. Evidently, a higher degree of experimental control could be achieved in the latter case, and less well trained bees may have been tested at the hive entrance. With reference to the effect of task or motivational state, it should be pointed out that the difference between the respective choice behaviors at the feeding place and at the hive entrance is larger in the blue-violet but not in the fluish-green region, suggesting that colors are used differently to guide choice behavior in the two behavioral contexts. When bees return to the hive, discrimination is selectively reduced in the blue-violet. When they search for food they use all information provided by the receptor input. Context-specific differences of color discrimination cannot result from different receptor signals, because exactly the same training and test procedures were used. *Melipona* bees must, therefore, use the information from the receptors differently in the two situations; in particular, they must ignore information in the blue-violet when they search for the hive entrance. Consequently, a comparison between *Melipona* and *Apis* shows that *Melipona* discriminates bluish-green colors, and *Apis* blue-violet, UV, and green colors, better at the hive entrance (Fig. 11). We know of no other cases of behavioral selective color vision in insects.

It is a general phenomenon in training experiments that discrimination improves if the stimuli to be discriminated are differentially reinforced. This is also the case in *Melipona* (Fig. 10). We avoided contamination of the discrimination values by this effect by presenting in most experiments only the training stimulus during the learning trials, or we changed the nonreinforced stimulus frequently during learning as in the case of dual hive entrance training.

Multiple choice experiments have a great advantage, because they provide much more data in the same time. It is thus of importance to test whether these data are equally reliable as dual choice data. This is confirmed for *Melipona* (Fig. 5–7, 9) as it was for *Apis* (Menzel 1985; Backkaus et al. 1987). The slightly improved choice values are probably due to the distribution of the neutral choices over 12 instead of 2 alternatives. Furthermore, the large number of alternatives makes the choice practically independent of the particular combination of other alternative signals presented in the test. For example, if 24 signals are tested at the feeding place in various combinations of 12 simultaneously presented signals, no dependence of the choice on any one of these signals was found for the set of other simultaneously presented stimuli. We may thus conclude that the training and test procedures used in this study are suitable to uncover the perceptual parameters involved in color discriminations with just one important exception, namely the reduced discrimination of blue-violet stimuli at the hive entrance.

Predictions of the receptor model of color vision

The receptor model of color vision, already tested in *Apis* (Backhaus and Menzel 1987), can be successfully applied to *Melipona* as well. The core of the model is a measure of the similarity of colors based on the assumption that two colors become increasingly dissimilar with increasing numbers of perceptual difference steps. The notion of perceptual just noticeable difference steps (pjnd) was introduced by von Helmholtz (1896) and applied by Schrödinger (1920). The present model employs the calculation of the pjnd's from receptor just noticeable difference steps (rjnd), derived from the noise in the receptors. Laughlin (1981) analyzed the shot noise and intrinsic noise components and found that the total noise is relatively independent of the stimulus intensity in light-adapted photoreceptors. Such an analysis is still lacking in any photoreceptor of hymenopteran insects. We concluded from a rough inspection of the fluctuations around a ramp function of the voltage response that similar conditions apply to the photoreceptors in the honeybee and determined a value of 0.4% of maximum voltage for the largest fluctuations (Backhaus and Menzel 1987). One can convert the voltage fluctuations back into equivalent fluctuations of photons considering the response/log intensity function and the state of light adaptation in photoreceptors and treat the corresponding fluctuations of effective quanta as the total unavoidable noise in the receptor system. This noise produces an uncertainty space around each color locus within the color space. Thus, the rjnd's are represented either as ellipsoid bodies of uncertainties in the color space (spatial rjnd's) or as ellipses in the color plane ignoring the intensity differences of colors (plane rjnd's). Spatial and plane rjnd's correspond to distance measures in the color space (called sD and expressed in spatial rjnd's) or in the color plane (called pD and expressed in plane rjnd's).

If the color discrimination behavior depends only on the perceptual distance as expressed in jnd's, one should find (1) a high correlation between the response values in the discrimination tests and the jnd's between the colors and (2) the same response/rjnd function ('distance function') for all pairs of colors. Indeed, the $\Delta\lambda/\lambda$-function as predicted by this consideration is well supported by the behavioral data (see Fig. 12b, exception hive entrance data in the blue-violet, see above). Also, the distance functions for color signals within the chromaticity diagram (Figs. 13, 14) show a high correlation between the jnd values and the discrimination values. The distance functions in the color plane [discrimination/pD (jnd)] always have a higher correlation coefficient than those in the color space [discrimination/sD(jnd)]. This means that *Melipona* refers more to the chromaticness of color (hue, saturation) independent of intensity to judge the difference between two colors. We arrived at the same conclusion with the $\Delta\lambda$-values, because they match very well with the theoretical function, although the stimuli were not of the same brightness as required for the $\Delta\lambda/\lambda$-function.

In summary, the jnd-based values are as useful a

measure of dissimilarity of color stimuli in the stingless bee, *M. quadrifasciata,* as they are in *A. mellifera* and *O. rufa,* at least for similar standardized training and test conditions. It should be emphasized that our aim in all studies with these three species was to reduce the effect of higher color phenomena such as color induction, color contrast, unique color phenomena, and color preferences by designing the standardized training and test conditions so that the response values depended as little as possible on special procedures applied during the test. It is, therefore, the role of future studies to uncover the existence of higher order color phenomena in these and other insect species and to determine their role in visual orientation.

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