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The spectral input systems of hymenopteran insects and their receptor-based colour vision

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Summary. Spectral sensitivity functions S(λ) of single photoreceptor cells in 43 different hymenopteran species were measured intracellularly with the fast spectral scan method. The distribution of maximal sensitivity values (λ_{max}) shows 3 major peaks at 340 nm, 430 nm and 535 nm and a small peak at 600 nm. Predictions about the colour vision systems of the different hymenopteran species are derived from the spectral sensitivities by application of a receptor model of colour vision and a model of two colour opponent channels. Most of the species have a trichromatic colour vision system. Although the S(λ) functions are quite similar, the predicted colour discriminability curves differ in their relative height of best discriminability in the UV-blue or blue-green area of the spectrum, indicating that relatively small differences in the S(λ) functions may have considerable effects on colour discriminability. Four of the hymenopteran insects tested contain an additional R-receptor with maximal sensitivity around 600 nm. The R-receptor of the solitary bee *Callonychium petuniae* is based on a pigment (P596) with a long λ_{max}, whereas in the sawfly *Tenthredo campestris* the G-receptor appears to act as filter to a pigment (P570), shifting its λ_{max} value to a longer wavelength and narrowing its bandwidth. Evolutionary and life history constraints (e.g. phylogenetic relatedness, social or solitary life, general or specialized feeding behaviour) appear to have no effect on the S(λ) functions. The only effect is found in UV receptors, for which λ_{max} values at longer wavelengths are found in bees flying predominantly within the forest.

Key words: Photoreceptors – Spectral sensitivity – Colour vision – Hymenopterans

Introduction

The different spectral sensitivities of the photoreceptors provide the neural colour coding system with the necessary information for colour discrimination. Therefore, a comparative study of colour vision in any group of animals requires the exact determination of the spectral properties of the photoreceptors as an initial step in the analysis of colour vision (Lythgoe 1972; Menzel and Backhaus 1989a, b). For the honeybee, a receptor model of colour vision has been constructed on the basis of the spectral input functions which predicts quantitatively the colour discrimination in behaviour (Backhaus et al. 1987; Backhaus and Menzel 1987). It appears to be possible to extrapolate to closely related insect species and to make predictions about their colour vision systems by an analysis of their spectral receptor types. The model calculations of the receptor-based colour vision system in the honeybee show that the accurate determination of spectral sensitivity functions is a crucial step in the analysis. Such measurements of single photoreceptors are possible with the help of intracellular recording techniques in conjunction with a fast spectral scan method (Menzel et al. 1986).

Electrophysiological measurements are advantageous in comparison with photopigment studies, because the actual signal of the receptor to the neural colour coding system is the receptor potential, which is a non-linear function of the spectral absorption properties of the photopigments depending on several additional parameters. Parameters like screening pigments, waveguide effects, filtering by additional photopigments, antennal pigments and electrical interactions between adjacent receptors are known to alter the spectral signal from the photoreceptors (Menzel 1979; Kirschfeld 1986; Snyder et al. 1973). However, imperfections of the intracellular recording techniques curtail the results to a certain extent and make corrections necessary before the data can be used for an estimation of the receptor-based colour vision.

The aim of this study is to collect the material for a comparative study of the peripheral components of the colour vision systems of ecologically and systematically closely related insect species. All species analysed here are flying hymenopterans. Most of them visit flowers, many

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for the purpose of providing their larvae with food. Others visit flowers only for their own nutrition, and a few specimens (e.g. the drone honeybee) never visit flowers at all. We thus search for generalities and peculiarities in the spectral properties of the photoreceptors which might be interpreted as evolutionary or ecological adaptations of their colour vision systems to flower detection under natural light conditions.

Methods

Conventional techniques are used to record intracellularly from photoreceptors of different species. The spectral sensitivity function $S(\lambda)$ is determined by a voltage-clamp technique, which allows the establishment of a $S(\lambda)$ function within 16 s at a spectral resolution of 4 nm (Menzel et al. 1986). A grid monochromator is used to scan the spectrum between 300 and 700 nm. A circular neutral-density wedge automatically adjusts the light flux to each wavelength (in 4 nm steps) so that the response of the receptor cell is clamped to a preselected receptor potential. The potential is usually clamped to 3–8 mV above the resting potential. Therefore, only the tonic component of the receptor potential contributes to the response, and the cell becomes slightly light-adapted during the scan. A computer calculates and displays the $S(\lambda)$ function on-line. A more detailed description of the method and an evaluation of its advantages over the usual flash method is given by Menzel et al. (1986).

The preparation is dark adapted for at least 20 min before spectral measurements are taken, and strong illumination of the photoreceptor is avoided. Certain criteria are applied in order to judge the quality of the intracellular recording, such as long-term stability of the dark resting potential and the fact that strong light induces potentials larger than 15 mV depolarisation. However, the photoreceptors of different species behave quite differently in recording experiments and thus require adjustments of these criteria. More details are given in the Results. The illuminating light is calibrated with a radiation meter IL760 from International Light (see also Menzel et al. 1986).

The specimens are collected in the field. In the case of solitary hymenopterans the number of specimens is often limited, as they are difficult to find. Since every species requires certain procedural optimizations, the number of successfully recorded cells is sometimes quite small, and occasionally only certain classes of spectral receptor types could be recorded.

Results

Spectral receptor types

For the 43 hymenopteran species investigated, the frequency distribution of the λ_{max} values of the spectral sensitivities (Fig. 1a) indicated 3 major and an additional minor peak, each corresponding to a distinct receptor type. The eyes of most of the species contain 3 spectral receptor types which are the UV-receptor, blue receptor and green receptor. Most UV-receptors have their sensitivity maxima around 340 nm, the blue receptors (B-receptor) around 430 nm and the green receptors (G-receptor) around 535 nm. The full set of these 3 receptor types was recorded in 26 of 43 species. In 7 species, only 2 types were recorded (mostly the B- and G-receptors) and in 7 species, only one type, the G-receptor. The UV-receptor was most difficult to record from. This is the reason why UV-receptors are not described in 15 of the total of 43 species, since it would make sense for these species also to possess a UV-receptor. This is supported by the existence of secondary sensitivity peaks of B- or G-receptors in the UV (see below). The eyes of 4 species contain an additional 4th receptor type, a red receptor with λ_{max} around 600 nm. These 4 species belong to the tenthrenid wasps (*Tenthredo campestris* and *T. scrophulariae*), the xiphydrid wasps (*Xiphydria*

Fig. 1a. Frequency distribution of λ_{max} values of photoreceptors of the hymenopteran species. All species were investigated with the spectral scan method. The values were arranged in 10 nm sections. Each number indicates one species: 1 = *Tenthredo campestris*, 2 = *Tenthredo scrophulariae*, 3 = *Tenthredo* sp., 4 = *Xiphydria camelus*, 5 = *Ucerus gigas*, 6 = *Ichneumon* sp., 7 = *Ichneumon stramentarius*, 8 = *Polistes gallicus*, 9 = *Paravespula vulgaris*, 10 = *Paravespula germanica*, 11 = *Vespa crabro*, 12 = *Vespa crabro* (male), 13 = *Dolichovespula norwegica*, 14 = *Philanthus triangulum* (male), 15 = *Cerceris rybynensis*, 16 = *Cerceris rybynensis* (male), 17 = *Colletes fulgidus*, 18 = *Lasioglossum malachurum*, 19 = *Lasioglossum albipes*, 20 = *Oxea flavescens* (recorded in Brazil), 21 = *Andrena florea*, 22 = *Callonychium petuniae*, 23 = *Osmia rufa*, 24 = *Chelostoma florisomne*, 25 = *Anthophora acervorum*, 26 = *Melecta punctata*, 27 = *Xylocopa brasilianorum* (recorded in Brazil), 28 = *Proxylocopa* sp., 29 = *Schwarziana* sp., 30 = *Nomada alboguttata*, 31 = *Melipona quadrifasciata* (recorded in Brazil), 32 = *Melipona marginata* (recorded in Brazil), 33 = *Trigona spinipes* (recorded in Brazil), 34 = *Lestrimelitta limao* (recorded in Brazil), 35 = *Apis mellifera*, 36 = *Apis mellifera* (drone), 37 = *Bombus lapidarius*, 38 = *Bombus terrestris*, 39 = *Bombus jonellus*, 40 = *Bombus monticula*, 41 = *Bombus mori*, 42 = *Bombus manicatum*, 43 = *Anthidium manicatum*. We distinguish between species living in the tropical forest *(arrow)* and those living in open spaces *(small triangle)*. Small and large bodied species are shown in *different shades*. Oligolectic species are plotted in a *circle*. Wasps are outlined with a *thick line*, and predatory ones are marked with a *large dot*, whereas wasps which lay their eggs on leaves are marked with a *star*. The photoreceptor types are grouped into 3 distinct large and an additional smaller class of maximal spectral sensitivity. The λ_{max} of 57% of the recorded UV-receptors is around 340 nm, while 60% of the blue receptors are most sensitive around 430 nm. Most green receptors show maximal sensitivity at 535 nm. **b.** The distribution of λ_{max} values of the recorded photoreceptors of other authors in other insect species as shown in Fig. 15.2 of Menzel and Backhaus (1989a). Each number corresponds to the same number in that drawing, in which the species, the different methods and references were described.

Orthoptera:	UV: 7, 50, 51
	blue: 73, 74, 112, 116
	green: 145, 147, 165, 179
Hemiptera:	UV: 1, 14, 48, 52, 61, 63
	blue: 67, 68, 75, 122
	green: 148, 159, 212, 216
Odonata:	UV: 4, 19, 35, 43, 44, 45, 46
	blue: 64, 66, 72, 91, 104
	green: 133, 134, 141, 142, 143, 144, 146, 151, 152, 177
	red: 232
Blattoptera:	UV: 47
	green: 153
Diptera:	UV: 5, 6, 16, 21, 22, 37
	blue: 93, 94, 107, 109, 111, 115, 125, 126, 130
	green: 131, 135, 136, 137, 140, 160, 182, 241
Lepidoptera:	UV: 8, 13, 20, 38, 39, 53, 54, 55, 56, 60, 62
	blue: 65, 95, 96, 106, 113, 114, 123, 127, 128, 129
	green: 157, 161, 162, 163, 164, 180, 181, 199, 211, 215, 217
	red: 200, 221, 222, 223, 224, 225, 226, 227, 229, 230, 231

camelus) and the solitary andrenid bees (*Callonychium petuniae*).

A comparison of the λ_{max} values of all 43 hymenopteran species investigated (Fig. 1a) with those of other insect species (Fig. 1b, published earlier by different authors, for references see legend) shows a clear separation of the λ_{max} values into 3 major (UV-, B- and G-receptor types) and an additional small group (R-receptor) in the hymenopteran species, whereas in the other insect species there are 4 main peaks around 350 nm, 450 nm, 510 nm and 615 nm, and two smaller peaks around 390 nm and 560 nm. However, this difference has to be interpreted with care, since different methods, for example intracellular recordings with the flash method, ERG, microspectrophotometry, spectrophotometry of extracted pigment(s) or of the pupillary response, have been applied

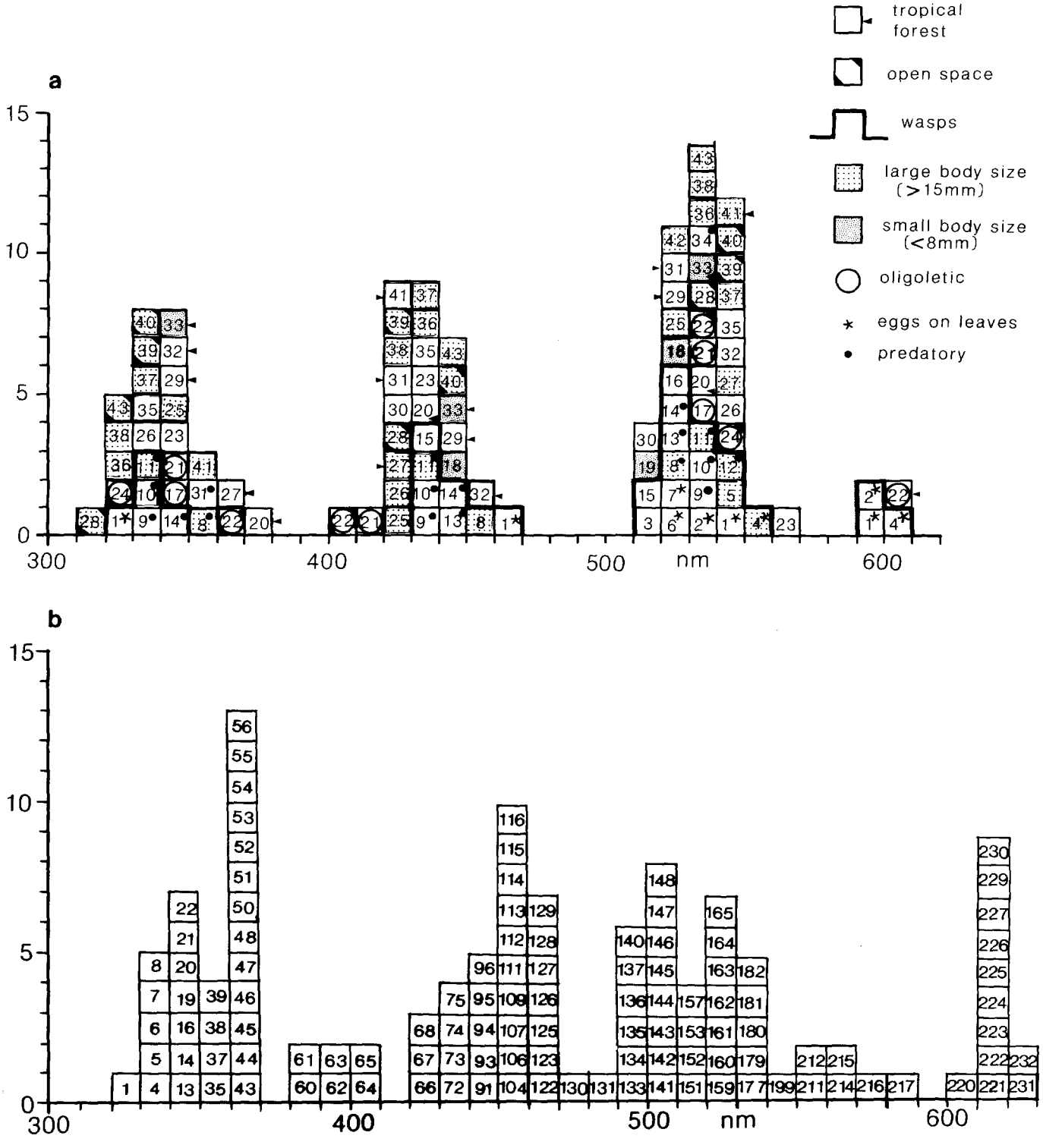


Table 1. Parameters of spectral sensitivity functions of photoreceptors in 43 measured hymenopteran species: position of maximal sensitivity λ_{max}, the halfband widths of most of the $S(\lambda)$ functions and the wavelength distance between the λ_{max} of the UV-blue receptor, blue-green receptor and green-red receptor. The species are arranged according to phylogenetic aspects. All of the recorded animals are females, except the ones marked with an asterisk

	UV	Blue	Green	Red	UV-B	B-G	G-R
Symphyta							
Tenthredinidae							
Tenthredo campestris	328/59	464/79	540/108	596/69	136	76	69
Tenthredo scrophulariae			532/120	592/78			60
Tenthredo sp.			516/104				
Xiphydriidae							
Xiphydria camelus			556/80	604/84			48
Siricidae							
Urocerus gigas			542				
Apocrita							
Ichneumoidae							
Ichneumon sp.			524/112				
Ichneumon stramentarius			524/112				
Vespidae							
Polistes gallicus	352/72	452/89	528/118		100	76	
Paravespula vulgaris	336/72	432/92	536/118		96	104	
Paravespula germanica	336/75	432/102	544/105		96	112	
Vespa crabro	336/66	436/77	536/131		100	100	
*Vespa crabro**			542				
Dolichovespula norwegica		448/88	524/116			76	
Sphecidae							
*Philanthus triangulum**	344/69	444/77	524/131		100	80	
Cerceris rybynensis		436/110	516/110			80	
*Cerceris rybynensis**			528/116				
Apoidea							
Colletidae							
Colletes fulgidus	340/68		532/128				
Halictidae							
Lasioglossum malachurum		442/76	528/128			86	
Lasioglossum albipes			516/110				
Oxeidae							
Oxea flavescens	370	435	536		65	101	
Andrenidae							
Andrena florea	340/69	412/(150)	536/115		72	124	
Callonychium petuniae	360/89	404/89	536/131	600/154	44	132	64
Megachilidae							
Osmia rufa	344/52	432/98	560/102		88	128	
Chelostoma florisomne	324/56		548/128				
Anthophoridae							
Anthophora acervorum	348/72	428/85	528/118		80	100	
Melecta punctata	336/62	428/89	540/128		92	112	
Xylocopa brasilianorum	360/72	428/82	544/118		68	116	
Proxylocopa sp.	312/62	424/102	532/131		112	108	
Nomada alboguttata		428/84	512/110			84	
Schwarziana sp.	343/82	440/(146)	528/131		97	88	
Meliponidae							
Melipona quadrifasciata	356	428	528		72		100
Melipona marginata	340	450	540		110	90	
Trigona spinipes	340/79	440/95	536/105		100	96	
Lestrimelitta limao			536				

Table 2 (continued)

	UV	Blue	Green	Red	UV-B	B-G	G-R
Apidae							
Apis mellifera							
worker bee	344/66	436/88	544/118		92	108	
drone bee*	328/82	436/95	532/115		108	96	
Bombus lapidarius	332/66	432/95	544/105		100	112	
Bombus terrestris	328/72	428/85	536/115		100	108	
Bombus jonellus	336/56	432/89	544/102		96	112	
Bombus monticula	336/82	440/105	544/115		104	104	
Bombus mori	352/82	428/85	548/118		76	120	
Bombus hypnorum			524/120				
Anthidium manicatum	324/66	440/(138)	532/125		116	92	

to determine the spectral sensitivities in these other insect species (see legend of Fig. 1b), whereas the fast spectral scan method was used exlusively in the case of the 43 hymenopteran species. It is still quite obvious that the overall distribution of the UV-receptors is shifted to longer wavelengths in non-hymenopteran species, λ_{max} values around 390 nm and 490 nm are a rare exception or do not exist in hymenopterans, and red receptors are much more common in butterflies. (A closer inspection of this distribution is presented in Menzel and Backhaus 1989a).

One might argue that the distribution of the λ_{max} values along the wavelength scale reflects a statistical scatter of the spectral measurements rather than an inherent and reliable value for each animal species. This question can be examined by collecting several S(λ) functions from individual cells and comparing them with measurements from the same spectral type in the same eye or with those from different eyes of the same or different species. Therefore, inter- and intraspecific variance can be analyzed. The distributions around the mean for the 4 receptor types of all of our measurements are for the UV-receptor: 340 ± 12 nm (28 species, $n = 55$ measured cells), for the B-receptor: 434 ± 11 nm (30 species, $n = 77$ measured cells), for the G-receptor: 534 ± 10 nm (43 species, $n = 522$ measured cells), and for the R-receptor: 597 ± 5 nm (4 species, $n = 8$ measured cells).

The distributions of the λ_{max} values after repeated measurements of the same receptor cell or the same spectral receptor type within one eye are similar at the 5% level (*t*-test). In addition, the same spectral receptor type in different eyes of one species does not vary significantly with respect to the λ_{max} values. Therefore, we pooled all measurements from the same receptor type of one species and examined the statistical distributions of λ_{max} values. Take, for example, the G-receptor of the hornet, *Vespa crabro*; 68 cells were recorded with 198 spectral runs, and the distribution around the mean was 532 ± 6 nm. Another example is the drone bee; the values for the normal pigmented eye are: UV-receptor 323 ± 11 nm (17 runs), B-receptor 440 ± 9 nm (12 runs) and G-receptor 526 ± 8 nm (25 runs). All other species show the same trend towards smaller variance in intraspecific distributions than in interspecific distributions, although in some species this is not as clear, particularly in cases in which fewer cells were recorded or in which the recordings were less stable.

Another statistically valuable parameter is the halfband width of the S(λ) function. The average halfband width for all spectral measurements is 70 ± 9 nm for the UV-receptors, 90 ± 8 or 96 ± 19 nm for the B-receptor excluding or including the very broad S(λ) functions of the two species *Andrena* and *Anthidium* (see Table 1 and Figs. 3a, 4e), 116 ± 10 nm for the G-receptor and 96 ± 33 nm for the R-receptor. The same mean values and variances were found for the intraspecific comparison. The expected halfband widths on the basis of respective rhodopsin absorbance functions (Maximov 1988) are 76 nm for a λ_{max} of 340 nm, 100 nm for a λ_{max} of 440 nm, 110 nm for a λ_{max} of 540 nm and 118 nm for a λ_{max} of 600 nm. The values for the UV-, B- and G-receptors lie well within the expected range for relatively unaltered rhodopsin absorbance functions. However, this does not exclude the necessity to examine each receptor type separately and each species for any deviation of the S(λ) from the absorbance function of a photopigment in solution in an attempt to analyse additional factors influencing the spectral properties of the photoreceptors (Gribakin 1988; see below). The halfband widths of the R-receptors are smaller and have to be examined more closely (see below).

Taking together the results of the inter- and intraspecific variance of λ_{max} values and of the halfband width, our analysis supports the conclusion that species-specific differences rather than inaccuracies of the method are responsible for the positioning of rhodopsin-like spectral sensitivity functions along the wavelength scale. Therefore, we ask next what parameters may be responsible for the species-specific positioning of the S(λ) functions.

Although the database is not yet very large, it is worth analysing the role of the phylogenetic or ecological relationship between the species. Figure 1a includes several labels which mark the respective λ_{max} values of the corresponding species according to a few criteria which may have an effect on the S(λ) functions. The phylogenetic grouping obviously has no effect. Wasps (separated from the bees by the darker line) show no differences in λ_{max} distribution from the bee families. Also, no difference is seen between the bee families. A few wasp species which select leaves for egg positioning were studied (marked

with* in Fig. 1a). These species differ in λ_{max} distribution in no way from those which rear their offspring in nests in the ground (e.g. sand wasps) or in hollow trees (e.g. social wasps). The same applies to wasp species which are rarely seen on flowers and are predominantly predatory (marked with a large dot).

Next, we examined whether bees of different body sizes may differ in respect to their spectral receptor types. Bees with large bodies and a long proboscis are known to visit a different spectrum of flowers for food provision than small-sized bees with short proboscis (Menzel and Shmida, unpublished). Figure 1a again shows that no difference exists between these two groups of bees. Most bee species which we have studied collect nectar and pollen from many plant species (polylectic), but a few bee species on our list are known to be specialized to one or a very few plant orders for nectar and/or pollen collection (oligolectic). For example, *Andrena florea* forages only on *Bryonia alba* and *B. dioica* with green-white flowers, and *Chelostoma florisomne* visits only the yellow flowers of *Ranunculus* for pollen, but several other plant species for nectar. The oligolectic species are marked by a circle in Fig. 1a. It is quite obvious that their spectral receptor types are well within the distribution of all the polylectic species.

The last parameter we inspected is related to the predominant light conditions in which these hymenopterans perform their foraging flights. We distinguish between two groups, tropical bees which fly predominantly in the dense tropical forest, and those paleartic species which fly under particularly bright light conditions (e.g. at a higher altitude like the alpine bumblebee species *Bombus monticula* and *B. jonellus* or in the open space of Mediterranean habitats, e.g. *Proxylocopa,* or northern habitats, e.g. *Chelostoma* and *Anthidium*). The first group should be exposed to chromatic light conditions, which are characterized by a relatively low intensity, particularly of short-wave radiation; the latter group should be exposed to relatively higher intensity, also of short-wave radiation. We find an indication that the UV-receptors of the first group (dense tropical forest) tend to be shifted to longer wavelengths, whereas those of the latter group (open space) have λ_{max} at certain shorter wavelengths. The B- and G-receptors do not differ between these two ecological groups. It is interesting to note that the honeybee drone also has a UV-receptor with a very short λ_{max} of 328 nm, whereas the λ_{max} of the UV-receptor of the female honeybee is at 344 nm. Honeybee drones and foragers differ in many respects, but the major difference lies in their preference for the light conditions under which they fly. Drones fly only in bright light, and identify the queen bee against the light of the sky with their UV- and B-receptors (van Praagh et al. 1980). It thus appears that the only factor reliably influencing the positioning of the S(λ) function of one of the 3 receptor types – the UV-receptor – is the ambient light condition of the species' habitat. It appears that other behavioural or phylogenetic differences have no effect. With respect to the ambient light conditions, the question of whether the differences in absolute light flux or the spectral differences form the more important parameter remains open.

The S(λ) as input functions to receptor-based models of colour vision

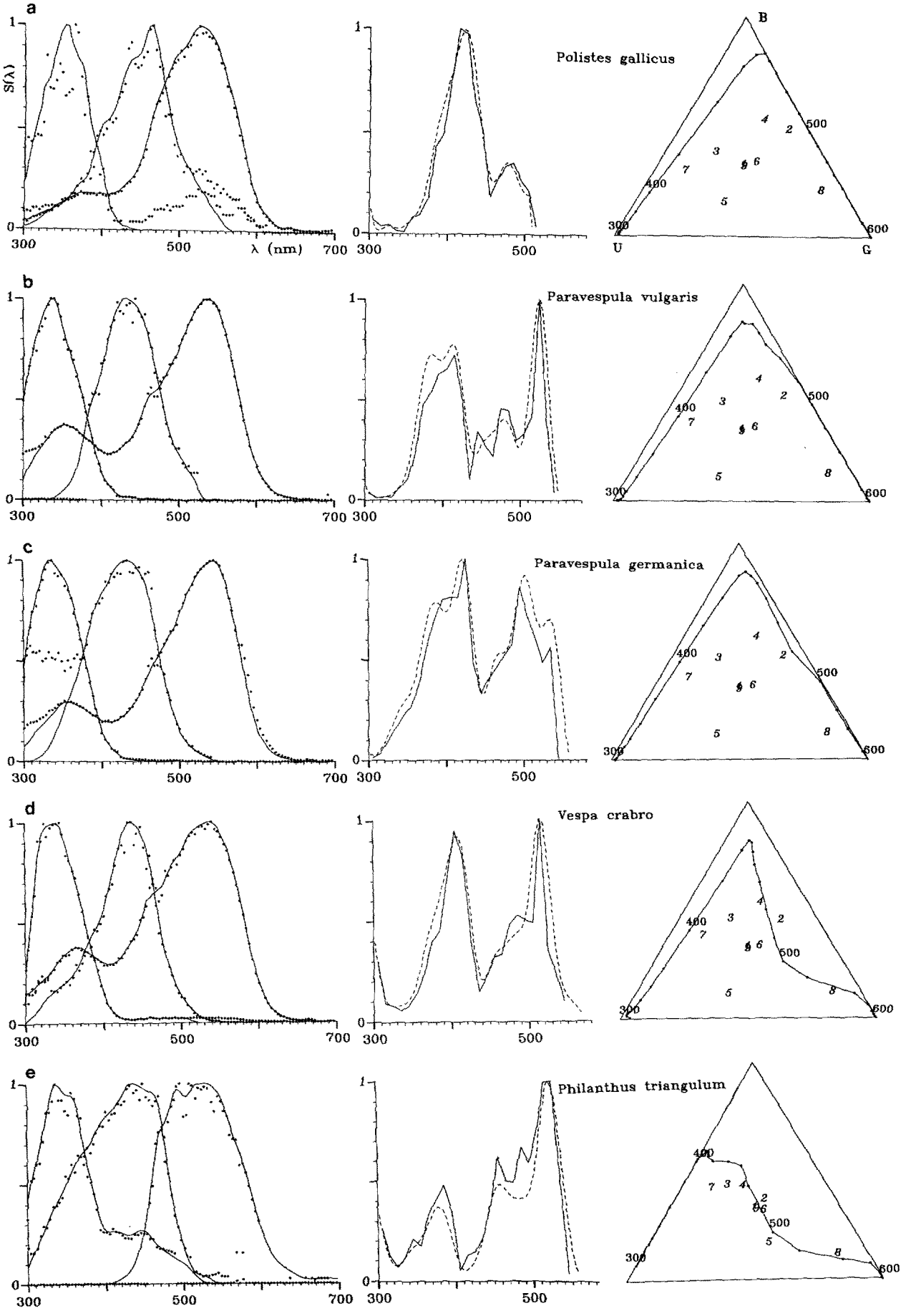
Any receptor-based model of colour vision demands accurately determined spectral sensitivity functions. It has been shown for the honeybee that even relatively small differences in the S(λ) functions are of importance to the outcome of model calculations and thus for the predictive power of such an approach (Menzel and Backhaus 1989b). Sufficiently accurate data have only recently become obtainable by means of fast and highly resolving spectral measurements. These measurements are based on the determination of the light-evoked intracellular receptor potential and thus have the advantage of measuring the actual signal to the nervous system and not just the spectral absorption by the extracted photopigments. However, intracellular recordings are subject to a range of technical imperfections which cause distortions of the S(λ) function. It is, therefore, necessary to correct such distortions before the S(λ) function can be used for further calculations. Figures 2–7 give the results for those species for which a whole set of spectral receptor types were recorded. The data points which result from averaging the actual measurements are presented together with the S(λ) functions (smooth lines) as they were used for further evaluations of the receptor-based

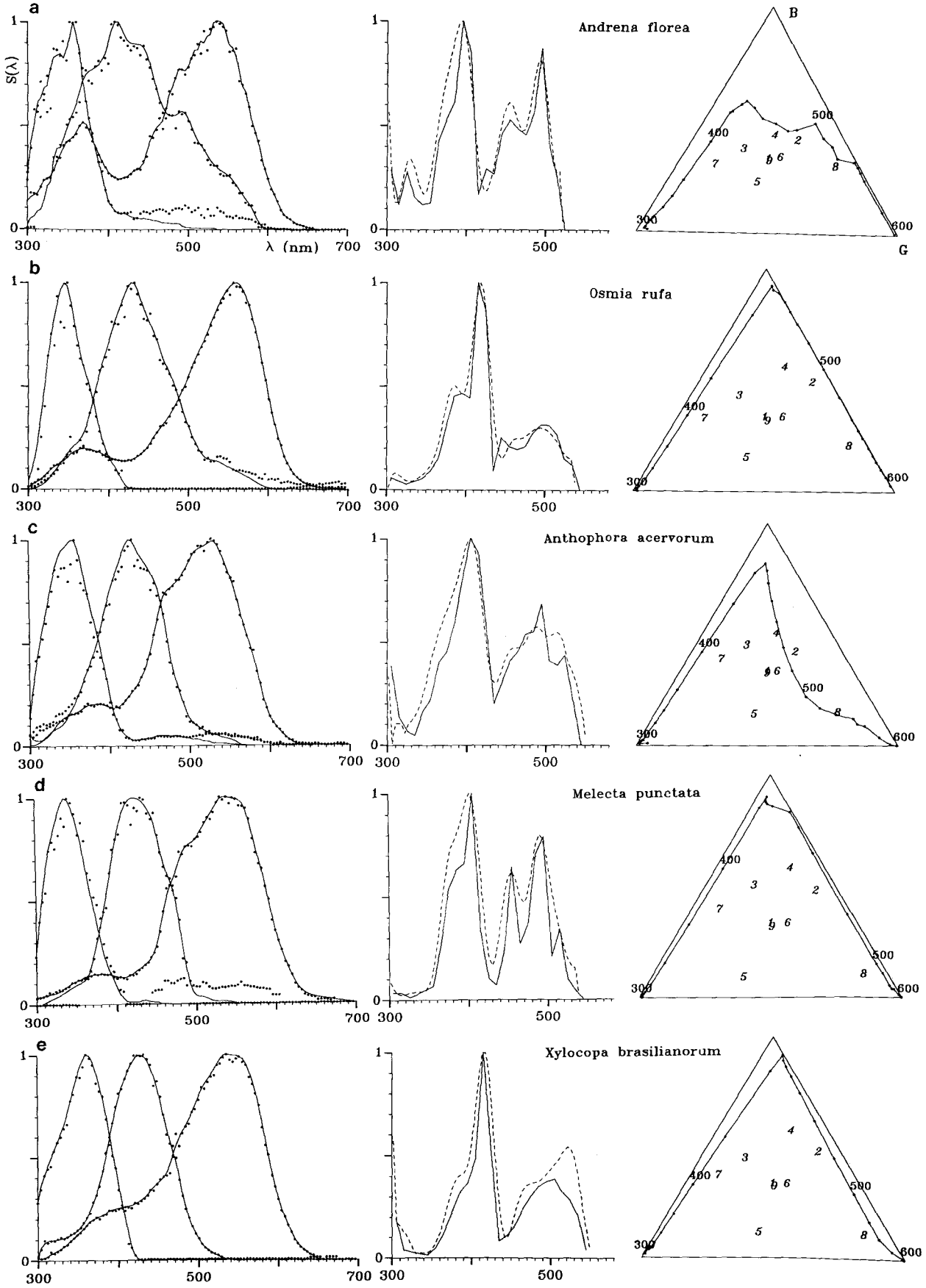
Fig. 2a–e. Spectral sensitivity functions of 3 photoreceptor cell types and trichromatic colour vision systems in 4 different social wasps (**a–d**) and 1 digger wasp (**e**). The left panel shows the averaged and normalized S(λ) functions *(dots)* together with the corrected and smoothed functions *(line)* which were used in the model calculations (see text). Two models of colour discrimination are shown in the middle panel, one based on the just noticeable difference steps in the receptor *(dashed line)* and the other derived from normalized spectral antagonistic subsystems. The functions are cut around 550 nm because only one receptor is sensitive at longer wavelengths. They are normalized to their maximum which represents the best discriminability in relative units. The chromaticity diagrams in the right panel represent the loci of equally bright colours and the spectral line which is divided into 10 nm steps *(dots)*. The numbers 1–9 indicate colour loci of different flowers and leaves (see Fig. 9). Although the S(λ) functions of these wasps are quite similar, the calculated colour discriminabilities differ. *Paravespula vulgaris, Vespa crabro* and *Philanthus* show the best colour discriminability around 500 nm, whereas the other wasps are better in the UV-blue part of the spectrum around 410 nm

Fig. 3a–e. S (λ) functions of photoreceptors recorded in one solitary bee which lives in the temperate zone of Brazil (**e**) and in 4 solitary bees (**a–d**) living in paleartic regions. The corresponding models of colour vision systems and colour diagrams are also presented. All of these bees have a colour vision system which shows better discrimination of UV-blue colours than blue-green ones

Fig. 4a–e. Spectral sensitivity functions and the model calculation of 3 solitary bees (**a, b, e**) and two tropical, social bees. Because of a limiting number of animals and some difficulties during the recordings, the S(λ) functions of *Proxylocopa, Schwarziana* and *Anthidium* fluctuate more than in other species. The halfband widths of these functions are much broader than the theoretical one of a corresponding photopigment, especially in blue receptors

Fig. 5a–e. The colour vision systems and spectral sensitivities of 5 species of bumblebees. With the exception of *Bombus monticula,* these species discriminate colours best at around 400 nm. The second peak in the blue-green varies in relative height


a
S(λ)
λ (nm)
Polistes gallicus
B
U
G
b
Paravespula vulgaris
c
Paravespula germanica
d
Vespa crabro
e
Philanthus triangulum


a
b
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S(λ)
λ (nm)
Andrena florea
Osmia rufa
Anthophora acervorum
Melecta punctata
Xylocopa brasilianorum
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300
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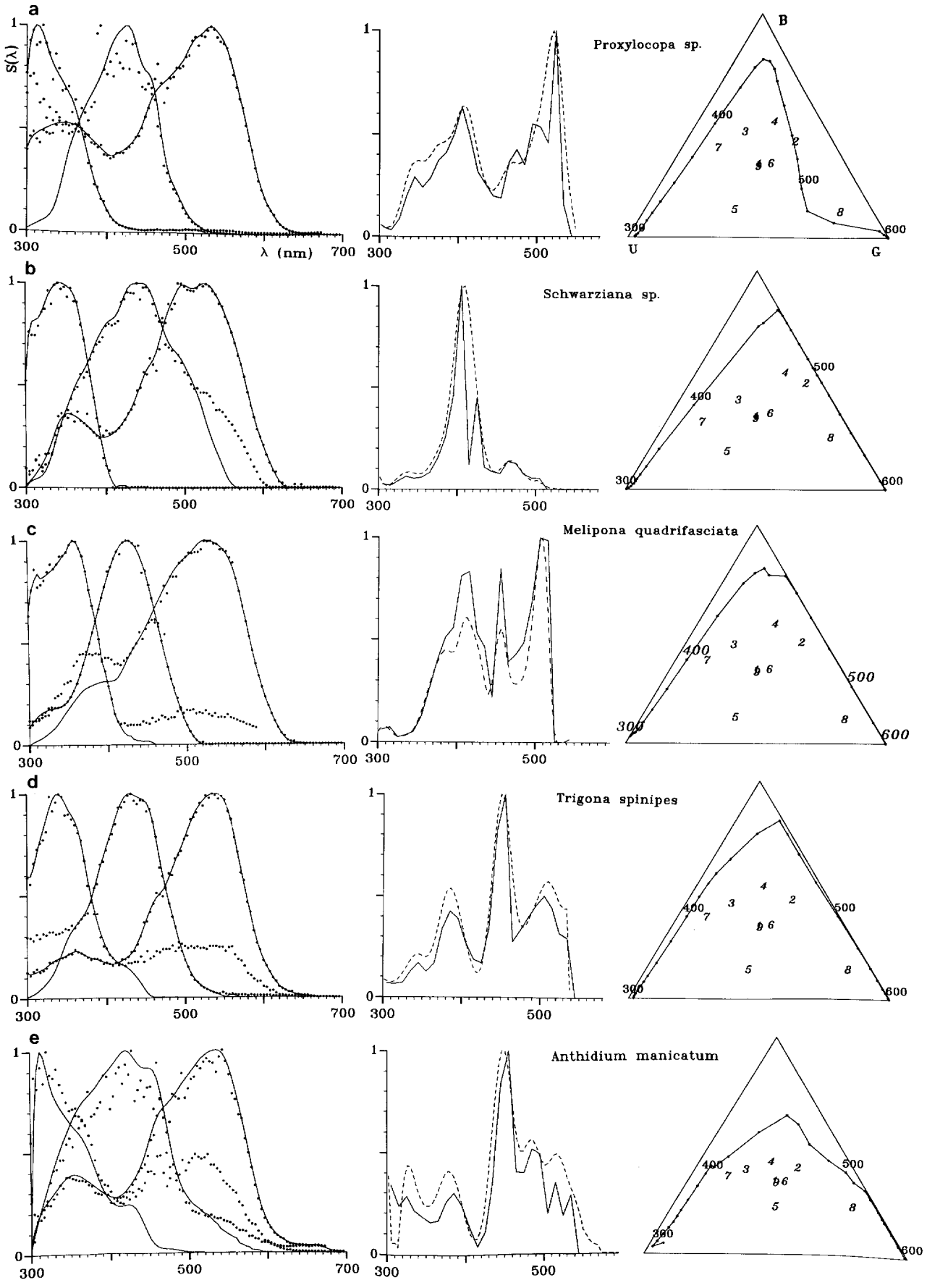
700

Fig. 3


a
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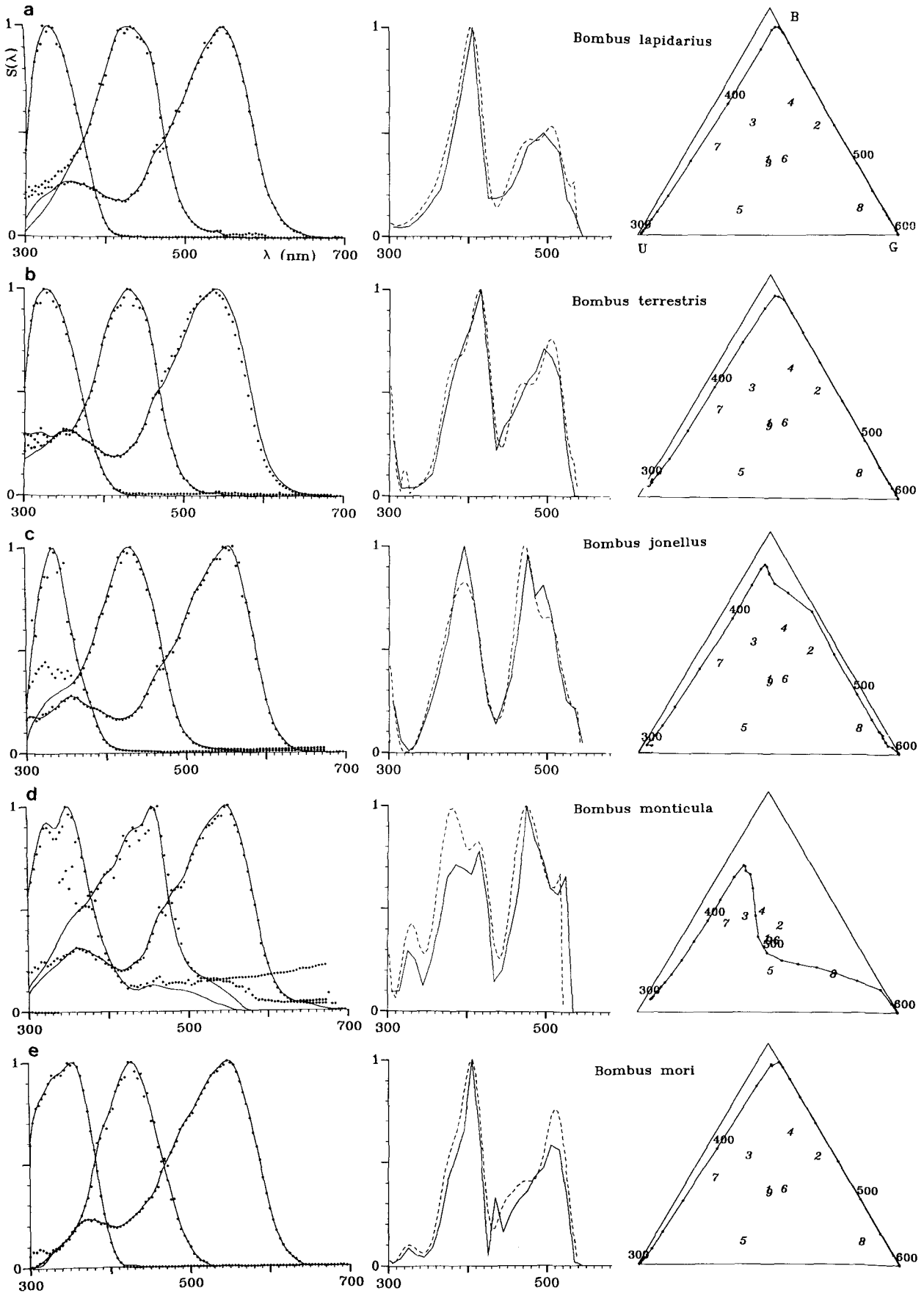
S(λ)
λ (nm)
Proxylocopa sp.
Schwarziana sp.
Melipona quadrifasciata
Trigona spinipes
Anthidium manicatum
B
U
G

Fig. 4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S(λ)
λ (nm)
Bombus lapidarius
Bombus terrestris
Bombus jonellus
Bombus monticula
Bombus mori
B
U
G

Fig. 5

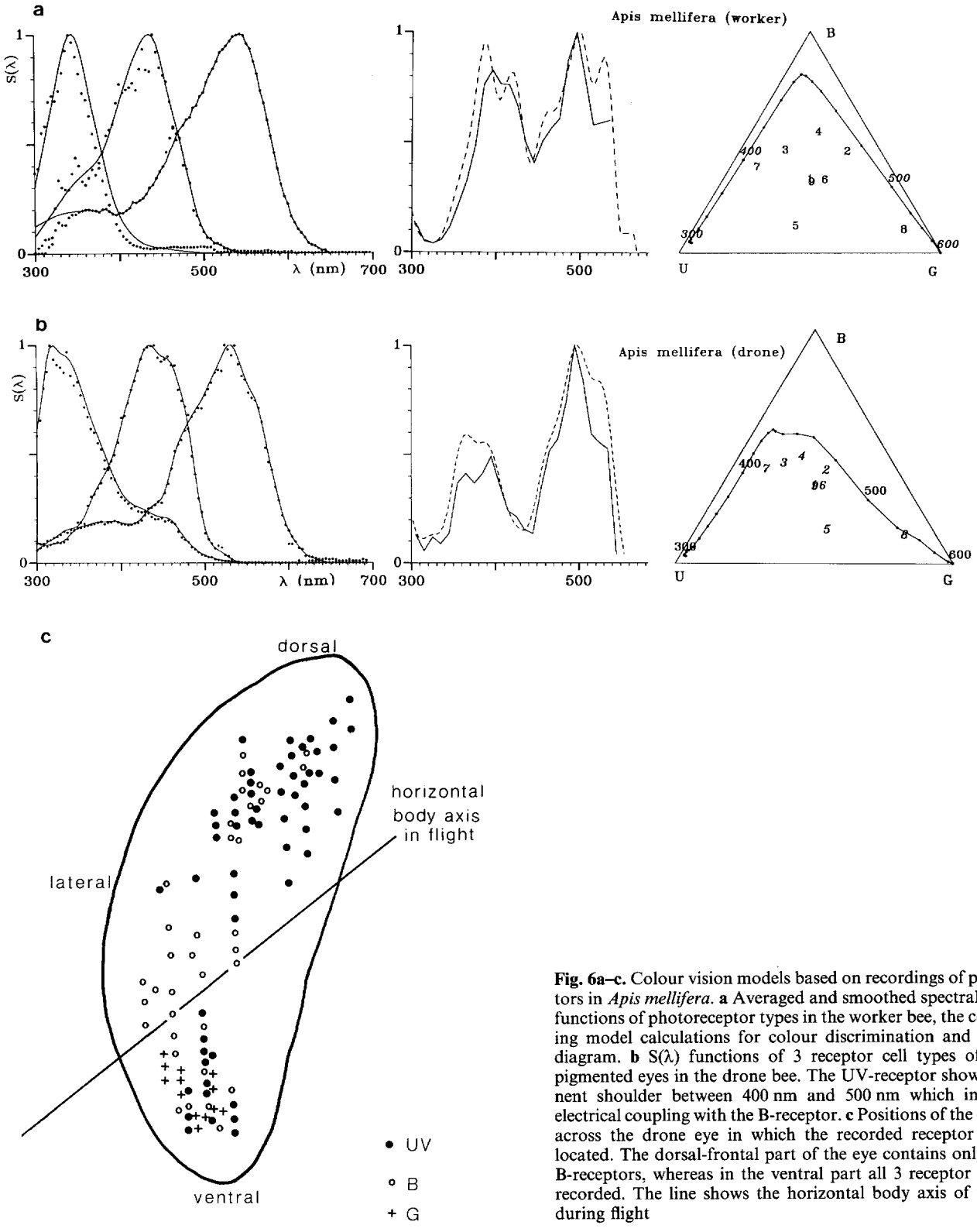

Fig. 6a–c. Colour vision models based on recordings of photoreceptors in *Apis mellifera*. **a** Averaged and smoothed spectral sensitivity functions of photoreceptor types in the worker bee, the corresponding model calculations for colour discrimination and the colour diagram. **b** S(λ) functions of 3 receptor cell types of normally pigmented eyes in the drone bee. The UV-receptor shows a prominent shoulder between 400 nm and 500 nm which indicates an electrical coupling with the B-receptor. **c** Positions of the ommatidia across the drone eye in which the recorded receptor cells were located. The dorsal-frontal part of the eye contains only UV- and B-receptors, whereas in the ventral part all 3 receptor types were recorded. The line shows the horizontal body axis of the animal during flight

model of colour vision. No corrections, or only very small ones, were necessary in the worker and drone bee, *Apis mellifera* (Fig. 6), the bumble bees *Bombus terrestris* and *B. mori* (Fig. 5), the solitary bees *Xylocopa brasilianorum* and *Osmia rufa* (Fig. 3), and the wasps *Philanthus triangulum*, *Vespa crabro* (Fig. 2) and *Tenthredo campestris* (Fig. 7a). In all these cases, the recordings were of a particularly high quality, and many cells were collected. The small corrections necessary are explained below.

The spectral measurements of the G-receptors usually

need no corrections, because the recordings from this class were relatively easy in all species and are frequent, stable and long-lasting. Therefore, many cells were recorded, often with many spectral runs per cell. Only one kind of correction was applied in a few cases, namely a slight reduction of the sensitivity at short wavelengths (below 340 nm; see for example *Bombus terrestris, B. mori, B. monticola, B. lapidarius* in Fig. 5, *Schwarziana* in Fig. 4b and *Anthophora* in Fig. 3c). The reason for this correction is that otherwise the spectral loci of wavelengths below 340 nm would not lie consecutively along the spectral line in the chromaticity diagram, and the spectral line would show loops and other irregularities. The necessary corrections are indeed quite small and lie within the accuracy of the method. Spectral measurements below 330 nm are less accurate than those at longer wavelengths due to the lower output of the xenon arc used for stimulation and the decreasing sensitivity of the receptors at these short wavelengths.

B-receptors are usually much harder to record from. As a result, they were only found in 30 of the 43 hymenopteran species, and a few measurements are affected by experimental artefacts. S(λ) functions, which follow closely the absorbance function of the corresponding rhodopsin pigment (Maximov 1988), are considered to be less affected by the recording technique, and therefore, no corrections were applied. Such functions were found in 3 *Bombus* species (*B. terrestris, B. mori, B. lapidarius,* Fig. 5) and in *Xylocopa, Osmia, Melecta* (Fig. 3), *Philanthus, Vespa crabro* (Fig. 2), *Tenthredo* (Fig. 7). Also, no correction was made to the S(λ) of the B-receptor in the drone bee, although the S(λ) function is significantly broader than the corresponding absorbance function. A large number of stable recordings of B-receptors were collected from the drone bee, and therefore, it is very likely that this average function corresponds very closely to the undisturbed S(λ) function (see below).

Considerable corrections were necessary in several species from which only a few relatively unstable recordings could be collected. (See Figs. 2c, 4a, 4d, 5c for corrections at shorter wavelengths and Figs. 4b, 4e, 5d for corrections at longer wavelengths). Since the sensitivity around the main peak was consistently well documented, corrections were necessary only at wavelengths at which the increased sensitivities indicated an artificial coupling to other spectral receptor types due to the limited quality of the recording.

The procedure for correction is as follows. From the unchanged S(λ) functions we calculate chromaticity coordinates for spectral lights and draw the spectral line in the chromaticity diagram [see Backhaus and Menzel

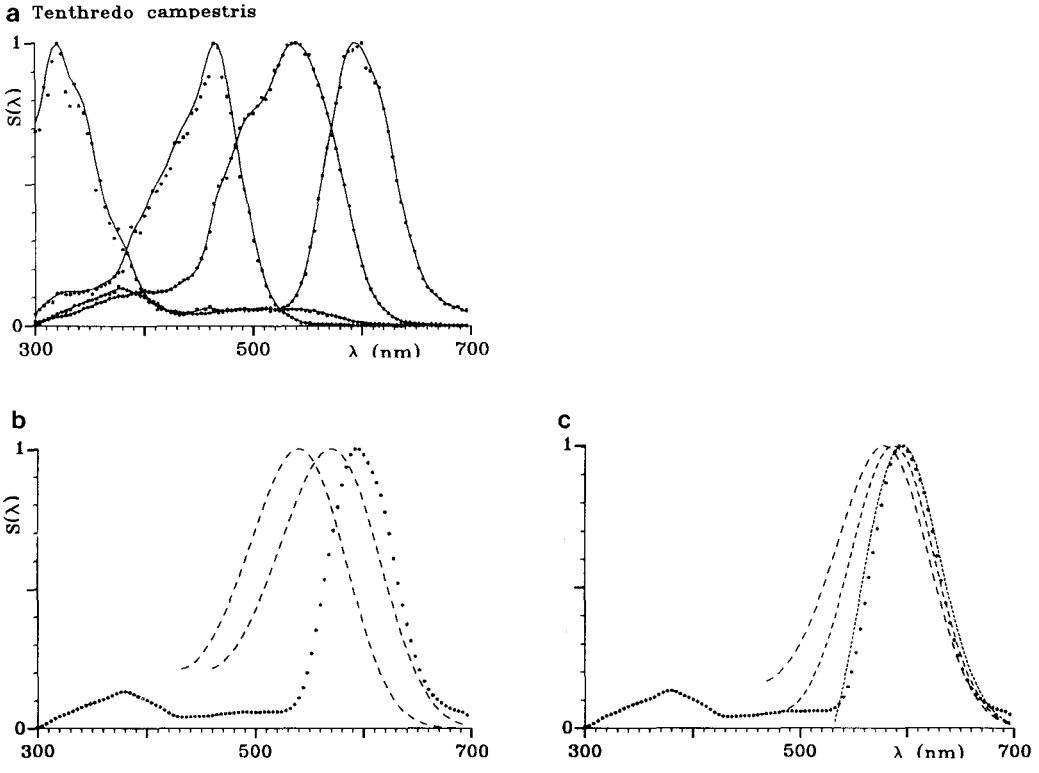

Fig. 7a–c. First postulation of a possible tetravariant input system in the wasp *Tenthredo campestris*. **a** This primitive wasp possesses 4 different photoreceptor types which are most sensitive at 328 nm, 464 nm, 540 nm and 592 nm. **b** Because of the narrow halfband width of the S(λ) function of the R-receptor, which is in contrast to the theoretical one of a corresponding photopigment, we attempted to explain this deviation of the spectral sensitivity curve. The G-receptor may screen a basic photopigment of the R-receptor which is maximally sensitive at 570 nm. The Maximov function of this pigment fitted the longwave part of the measured curve best if the Maximov function was not normalized to 1 but to a higher relative absorbance value. The absorbance functions worked out for filtering effects of 25%, 50% and 75% by the G-receptor are shown in **c**. The best compromise between the recorded spectral sensitivity function and the calculated curves is reached for a screening effect of 75% by the G-receptor

(1987) for detalis, see also below]. Then we correct the S(λ) functions as much as necessary to produce a continuous succession of wavelength positions along the spectral line, avoiding loops and sharp angles. If a particular average S(λ) function is well supported by stable recordings, no corrections are made. In any case, the corrections were limited as much as possible, based on the criterion that only a continuous spectral line in the chromaticity diagram resulted from S(λ) functions as close as possible to the actual recorded ones. The argument for keeping the S(λ) functions as close as possible to the recorded ones is that we have no additional observation which would justify any further changes. This means, for example, that the S(λ) of *Anthidium* (Fig. 4e) and *Andrena* (Fig. 3a) are very broad and thus deviate considerably from the corresponding pigment absorbance function or the template functions of B-receptors as found in the *Bombus* species, *Apis* and *Osmia* and other uncorrected functions.

The same procedure was applied to the UV-receptors. No changes were necessary for the *Bombus* species *B. terrestris, B. mori, B. lapidarius* (Fig. 5), and for the solitary bees *Xylocopa* (Fig. 3e), *Proxylocopa* and *Schwarziana* (Fig. 4a, b). An additional problem appears regarding the sensitivity of the UV-receptors at longer wavelengths (> 430 nm). The sensitivity may be zero or in the range of less than 5% in good recordings. Examples for zero sensitivity above 430 nm are *Bombus terrestris, B. mori, B. lapidarius* (Fig. 5), *Xylocopa* (Fig. 3e), *Schwarziana* (Fig. 4b), *Osmia* (Fig. 3b) and *Paravespula germanica* (Fig. 2c). Examples for sensitivities up to 5% at longer wavelengths are *B. jonellus* (Fig. 5c), *Proxylocopa* (Fig. 4a) and *Tenthredo* (Fig. 7). Are the side sensitivities of up to 5% at wavelengths above 430 nm intrinsic signals of the UV-receptor, or do they indicate recording artefacts? It has not been possible, so far, to answer this question on the basis of purely physiological arguments (Menzel et al. 1986). In the honeybee, the behavioural spectral discrimination function is better predicted by a receptor model of colour vision if the UV-receptor's longwave sensitivity is not zero (Menzel and Backhaus 1989a). This argues in favour of the functional significance of the extended sensitivity of the UV-receptor at longer wavelengths, but it is no proof. Since there are no arguments, we kept the longwave sensitivities of the UV-receptors as they were recorded and we corrected the S(λ) function only according to the argument as applied above, namely, that the spectral line in the chromaticity diagram should be continuous and free of loops. In the cases in which the low quality of the recording indicated an artificial coupling (e.g. *Anthidium, Andrena, Bombus monticula, Anthophora, Paravespula vulgaris, Polistes*), the sensitivity was set to zero for wavelengths above 460 nm, and the S(λ) function between 410 nm and 450 nm was kept as close as possible to the recorded function.

Photopigment absorption and S(λ) function

G-receptors. Although the S(λ) functions of the worker bee receptors follow the spectral absorbance functions as predicted by the Maximov (1988) formula or the Ebrey–Honig (1977) and Dartnall (1953) nomograms for the respective λ_{max} values quite closely, a closer inspection reveals significant deviations of the corresponding curves from each other. In the case of the G-receptor, the discrepancies between pigment function and S(λ) function have been traced to the screening pigments as determined in eyes lacking these pigments (Gribakin 1988). The basic photopigment appears to be a rhodopsin with maximal absorption at 526 nm (P526). This value is close to that found by Langer et al. (1982) using microspectrophotometric (MSP) methods (λ_{max} values at about 520 nm). The shift towards longer λ_{max} values in normally pigmented eyes (532–544 nm, Table 1) results from the higher optical density of the screening pigments at shorter wavelengths (< 500 nm) than at longer wavelengths. Therefore, the shift should be more prominent for extended light sources and for higher intensities. Experimental series in which an extended light source was used caused a shift of λ_{max} to a longer wavelength (548 nm) and in addition caused an extreme asymmetry of the S(λ) function defined as the ratio of the shortwave limit of the halfband width λ_1 minus λ_{max} and λ_{max} minus the longwave limit λ_2 ($A = (\lambda_1 - \lambda_{max})/(\lambda_{max} - \lambda_2)$; see Gribakin 1988). The asymmetry value of an extended light is 2.0, whereas a point light source (angle of extension 0.5°) revealed λ_{max} values from 532 nm to 540 nm and asymmetry values of 1.46–1.84.

It was reported earlier (Menzel et al. 1986) that G-receptors may show a sensitivity shoulder of varying height at wavelengths around 470 nm. The larger variance of λ_1 corroborates this observation and documents that the effect of screening pigments around 470 nm may vary with respect to the stimulus conditions. Our results are therefore consistent with Gribakin's (1988) analysis and indicate a marked effect of an unknown screening pigment on the S(λ) of the G-receptor.

It is as yet unknown whether the shift in λ_{max}, the position of λ_1 and the increase of the halfband width influence the spectral discrimination function of the honeybee in the blue-green region.

The G-receptor functions in other species look similar. The asymmetry is well expressed and ranges from 1.0 *(Bombus lapidarius)* to 3.0 *(Osmia rufa)*. A sensitivity shoulder around 460–480 nm is obvious in nearly all species, and λ_1 varies much more between each spectral run than λ_2 does. It is thus conceivable that the same longwave photopigment with λ_{max} close to 526 nm (P526 of Gribakin 1988) may be present in most hymenopteran species. The actual S(λ) function may be shaped by screening pigments, and this effect may be quite different in various species (see Fig. 1a).

UV- and B-receptors. The B- and UV-receptors have not yet been analysed in such detail. The actual S(λ) functions vary much more between and within species than those of the G-receptors. The effect of screening pigments is unknown, because absorption spectra including the full UV range have yet to be performed.

In the drone honeybee and several other species (*Paravespula vulgaris, Osmia rufa, Trigona spinipes* and

Bombus lapidarius), the halfband width of the B-receptor is very close or only slightly larger than the expected 92 nm according to the Maximov formula. The asymmetry values are also quite close to the theoretical 1.19 of the Maximov function (e.g. for *Osmia rufa* 1.13, *Melecta punctata* 1.1). The UV-receptors with the clearest functions have halfband widths similar to the theoretical value of 78 nm (according to the Maximov formula) or smaller (worker bee: 66 nm, asymmetry value A = 1.1. *Bombus lapidarius:* 66 nm, A = 1.16, *B. terristris:* 72 nm, A = 1.12, *B. jonellus:* 56 nm, A = 1.32, *Osmia rufa:* 52 nm, A = 1.0, *Melecta punctata:* 62 nm, A = 1.46, *Paravespula vulgaris:* 72 nm, A = 1.34, *Paravespula germanica:* 75 nm, A = 1.24 and *Vespa crabro:* 66 nm, A = 0.75). Since the asymmetry values are quite close to the theoretical one of 1.14, but the λ_{max} of the UV-receptors differ considerably, we conclude that different UV photopigments rather than screening pigments are responsible for the different S(λ) functions. Nothing can be said about the other UV-receptors, whose spectral runs gave less clear results.

The eye of the drone bee is an interesting study case. It contains predominantly UV- and B-receptors, which are known to be partially electrically coupled (Shaw 1969). As Fig. 6b shows, the particular S(λ) functions can best be explained by assuming that electrical coupling leads to an increase in sensitivity of the UV-receptor at wavelengths above 400 nm and of the B-receptor, below 370 nm. The coupling factor of 50% applied in this model calculation for the coupling of the UV- and B-receptor lies well within the range of that reported by Shaw (1969). It is still not certain whether the coupling is an intrinsic property of the receptor cells or whether it might be caused or enhanced artificially through the recording electrode. Over the years we observed a close relationship between the quality of the recording, the experience of the experimenter and the strength of the coupling as judged by the sideband sensitivities. However, even the best recordings by an experienced experimenter result in at least an enhanced sensitivity of the UV-receptor at wavelengths above 440 nm, indicating a functional coupling of the UV- and the B-receptors.

We examined the question of whether the 3 spectral receptor types in the drone bee are restricted to certain regions of the eye. Autrum and von Zwehl (1964) noticed that G-receptors were only recorded in the extreme ventral part of the eye and UV-receptors, predominantly in the dorsal part. In addition, measurements of the fluorescence of the deep pseudopupil indicate only short-wavelength visual pigments in the dorsal part and a green rhodopsin/blue metarhodopsin visual pigment system in the ventral part similar to that in the worker bee (Menzel et al. 1991). Figure 6c shows the location of the ommatidia across the eye from which the recordings came. The ommatidia were roughly localized by using the perimeter device for positioning the stimulating point light source. The upper half of the eye contains UV- and B-receptors. The middle part of the eye may have only B-receptors, but the number of recordings from this area is more limited than in the other parts of the eye. The lower third of the eye contains all 3 spectral receptor types. It is this part of the eye which is used by the flying drone bee to view objects in front and below, because in flight the body is tilted about 45° upwards (see the line marking the body axis in Fig. 6c; van Praagh et al. 1980, and own observation during the approaching flight to the hive entrance).

R-receptor. Four species were found to contain red receptors (R-receptor) (Figs. 1a, 7, 8). The S(λ) function in the solitary bee *Callonychium petuniae* (λ_{max} = 596 nm, λ_1 = 502 nm, λ_2 = 656 nm) has a halfband width which is somewhat larger (154 nm) than the 120 nm of the theoretical absorption function of a photopigment with λ_{max} = 600 nm, whereas the S(λ) functions of the two species of Tenthredinidae and the one of Xiphydriidae are much narrower (*Tenthredo campestris:* λ_{max} = 596 nm, λ_1 = 562 nm, λ_2 = 634 nm, halfband width: 69 nm; *T. scophulariae:* λ_{max} = 592 nm, λ_1 = 554 nm, λ_2 = 638 nm, halfband width: 78 nm; *Xiphydria camelus:* λ_{max} = 604 nm, λ_1 = 564 nm, λ_2 = 648 nm, halfband width: 84 nm). It is therefore most likely that the R-receptor in *Callonychium* is based on an unscreened photopigment of maximal absorption around 596 nm, whereas those of the wasps may be shaped by additional screening effects. In *Tenthredo campestris* we looked for any coloured pigment granules in plastic sections following Ribi's (1978) procedure but did not find anything comparable to his results on the digger wasp *Sphex cognatus.* Since the brownish, dark screening pigment in the pigment cells has a flat spectral transmission function around 600 nm, we examined the question of whether the photopigments of the other receptors, particularly of the G-receptor, may act as filters to a longer-wave length pigment, thus shifting its λ_{max} to longer wavelengths and narrowing its bandwidth. The low sensitivity of the R-receptor at wavelengths below 500 nm is in favour of this possibility. Assuming that the longwave part of the S(λ) of the R-receptor is not affected

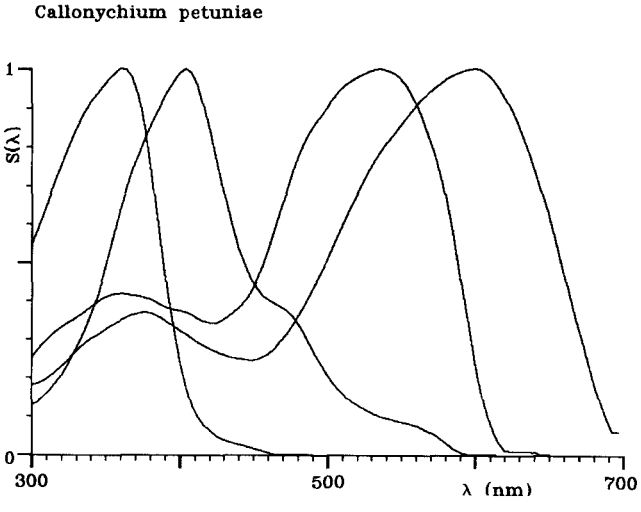

Fig. 8. Second postulation for a tetravariant colour vision system. The spectral sensitivity functions of UV-, B-, G- and R-receptors of the solitary bee *Callonychium petuniae.* The recordings and analysis were done in Brazil on a different computer system, so only the smoothed curves are available. The S(λ) function of the R-receptor is fitted very well by a Maximov function of a pigment P600

by such a screening, we first determined the potential basic pigment by fitting a Maximov function to the longwave part of the S(λ) and reached a λ_{max} of 570 nm (Fig. 7b). Next we calculated the absorbance function for a filtering effect of 25%–75% by the G-receptor ($\lambda_{max} = 540$ nm). As Fig. 7c shows, a close approximation between the recorded S(λ) function and the model calculation is reached for filter effects through the G-receptor in the range of 75%. It appears likely, therefore, that two different ways are used to produce an R-receptor in hymenopteran species, a specialized longwave photopigment P596, as in the case of the solitary bee *Callonychium petuniae*, and a screening of P570 pigment by the pigment P540 of the G-receptor, as found in primitive wasps.

Four spectral receptor types provide the nervous system with the potential to code colours in a tetrachromatic colour vision system. The question, of whether the tetravariant input is actually integrated into a tetravariant colour vision system is studied in a separate paper, in which the necessary tests for tetrachromatic colour vision systems are developed (Peitsch et al., in press).

Models of trichromatic colour vision

Model calculations of colour vision make predictions about colour coding strategies based on the spectral properties of the receptor types and straightforward assumptions concerning the neuronal mechanisms of spectral integration. Two levels of model calculation have to be distinguished: (1) that of just noticeable differences in photoreceptors and (2) that of neuronal coding in spectral antagonistic neural systems.

At the level of lower colorimetry, the effective quantal fluxes in the spectral receptor types determine 3 independent vectors which define a sectional plane of the colour space (the chromaticity diagram). Reasonable assumptions about adaptation mechanisms in the receptors were made. The graphical representation of chromaticism (hue and saturation) is independent of the intensity of a coloured stimulus (Backhaus and Menzel 1987; Menzel and Backhaus 1989b). The triangular chromaticity diagram (see Figs. 2–6) predicts which colour stimuli appear indiscriminable to the animal at a given intensity level. Such stimuli occupy the same locus in the chromaticity diagram. Furthermore, the chromaticity diagram describes quantitatively Grassmann's mixture rules. However, it does not allow one to read the perceptual discriminability between two separate colour stimuli, because of the non-linearity involved in the neural coding of the effective quantal fluxes absorbed by the spectral receptor types. A measure of perceptual discriminability can still be derived from such a receptor model of lower colorimetry, assuming that the intrinsic voltage noise of the receptors is the limiting factor for perceptual discrimination and thus defines the smallest step of spectral discrimination (Backhaus and Menzel 1987). The concept of receptor-based, just noticeable difference steps (jnd-steps) has been successfully applied to colour discrimination of the honeybee. A programme especially optimized for the purpose of species comparison (courtesy of W. Backhaus) is used here to make predictions about spectral discrimination functions of the hymenopteran species studied with intracellular recording techniques (Figs. 2–6). These spectral discrimination functions indicate that all hymenopteran species are most sensitive to spectral changes around 400 nm and 490 nm. In addition, it is quite obvious that the discrimination functions differ considerably in the relative height of their peaks in these two spectral regions.

The second basic concept for predicting the subjective colour difference of an animal is to go a step beyond the receptor level and to take into account the evaluation of the receptor signals in a neuronal colour coding system. Backhaus (1991) has shown that colour discrimination in honeybees can be explained by assuming that the receptors feed into two colour opponent channels (COC) with different weighting factors. The perceptual colour difference between two stimuli is defined as the sum of the excitation differences in the two spectrally antagonistic mechanisms. Chittka et al. (1990) have applied the COC model to hymenopterans by varying the parameters. The weighting factors of these colour opponent mechanisms have been found not to be the same in all species. Thus, Chittka et al. (1990) introduced a standard colour difference measure with normalized weighting factors. The predictions of this model were applied successfully to approximate the perceptual colour difference in 9 different hymenopteran trichromates as derived from their colour discrimination performance in behavioural tests. Seven of these species are investigated in the present work (*Apis mellifera* worker and drone, *Osmia rufa, Melipona quadrifasciata, Vespa crabro, Paravespula vulgaris* and *P. germanica*) (Chittka et al. 1990). Hence, it seems reasonable to extrapolate to other species with similar receptor sets and to assume that they possess a colour coding system with similar characteristics.

Accordingly, we calculated the spectral discrimination functions in 4 nm steps (Figs. 2–6) as predicted by this normalized form of opponent colour coding. A comparison of the two curves presented in Figs. 2–6, middle panel, shows that the jnd-concept applied to the lower colorimetry and the opponent colour coding model arrive at very similar predictions of spectral discrimination in these hymenopteran species.

Discussion

The spectral receptor types provide the nervous system with information on the chromatic differences in objects. Most natural objects are characterized by broad spectral reflection functions (see Fig. 9). Therefore, only a small number of relatively broadband spectral input functions are necessary for the nervous system to extract the relevant information (Barlow 1982, see also discussion in Buchsbaum and Gottschalk 1983). The photopigments (rhodopsin, xanthopsin) are specially suited for this purpose, because their optimal absorption can be shifted along the wavelength scale by altering the protein moiety (Wald and Brown 1965; Vogt 1989; Smith and Goldsmith 1990), and their Fourier components in the fre-

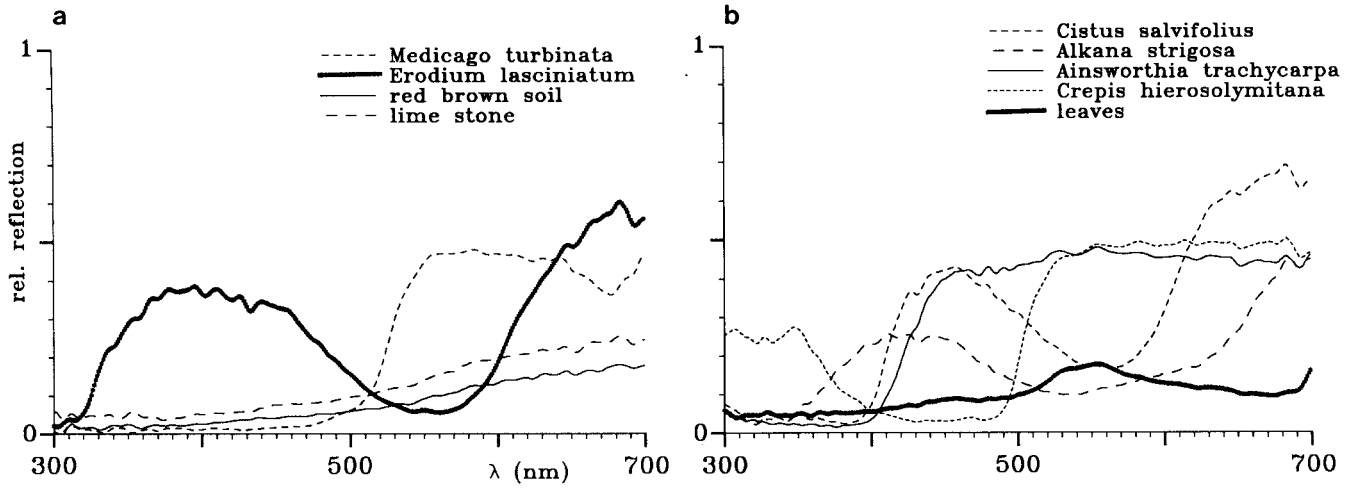

Fig. 9. Relative reflection of natural objects which may be relevant as marks during the flight of the hymenopterans investigated. The reflection curves were used to calculate their colour loci in the spectral diagram for each of the different species. Leaves (number 6 in the spectral diagrams in Figs. 2–6), red-brown soil (number 9) and limestone (number 1) represent reflection of the background, whereas *Ainsworthia trachycarpa* (number 2), *Alkana strigosa* (number 3), *Crepis hierosolymitana* (number 4), *Cistus salvifolius* (number 5), *Erodium lasciniatum* (number 7) and *Medicago turbinata* (number 8) are examples of flowers of varying colour which may be visited by the hymenopteran species

quency domain match those of natural objects well (Peitsch and Menzel, unpubl.). According to Shannon's (Shannon and Weaver 1949) sampling theorem, sampling units with Gaussian characteristics should be separated by the halfband width of the units. In the hymenopteran species studied here with intracellular recording techniques, we find a near-perfect match for most sets of trivariant spectral inputs between the position of the respective λ_{max} and the halfband width of the receptors.

Exceptions to this rule are the UV-receptors of the drone bee and the R-receptors of the tenthredinid wasps. In the first case, electrical coupling and filtering by the screening pigments change the effective spectral sensitivity function considerably by shifting λ_{max} to shorter wavelengths. Thus, increasing the separation between UV- and B-receptors enhances the sensitivity outside the main spectral sensitivity peak. The drone bee is able to discriminate colours at the hive entrance (Menzel et al. 1988), but the main purpose of the UV- and B-receptors appears to be the detection of a small dark object (the queen bee) against the bright sky. Small fluctuations of the receptor response induced by the queen bee at great distances would be best resolved if the effective quantal flux in the photoreceptors were high (Kirschfeld 1974), a goal achieved by shifting $S(\lambda)$ of the UV-receptor to a shorter wavelength and enhancing the sensitivity in both the UV- and B-receptors by mutual electrical coupling.

The R-receptors of the tenthredinid wasps most likely originate from a P570 pigment which is filtered by the P540 pigment of the G-receptors. As a consequence, the halfband width is reduced to 70–80 nm and λ_{max} shifted to 596 nm. Similar long λ_{max} are reached either with original rhodopsin pigments without screening effects, as in the solitary bee *Callonychium petuniae* and other insects like butterflies (Bernard 1979) and dragonflies (Meinertzhagen et al. 1983), or with filtering effects through pigment granules (Ribi 1978). There are two ways in which tetrachromaticity can be realized: one is to maintain the shape of the spectral sensitivity function which leads to an increase in the spectral range over which wavelength discrimination occurs, and the other is to narrow the bandwidths of the $S(\lambda)$ function to keep the region of wavelength discrimination constant but enhance colour discriminability (Bowmaker 1983). In hymenopteran insects both strategies are found: the solitary bee *Callonychium petuniae* is able to produce an additional rhodopsin pigment (P596) with a long λ_{max} and therefore increases its spectral range, whereas in *Tenthredo campestris* the spectral range is only slightly larger than in trichromatic hymenopterans, because the $S(\lambda)$ function of the R-receptor is much narrower.

The attempt to correlate λ_{max} values with various parameters (systematic position, body size, social habits, feeding habits and ecological conditions) showed that only light conditions in the habitat have an appreciable effect on the UV-receptors. Species from the dense tropical forest tend to have longer λ_{max} values in the UV-receptors than species flying in open habitats. Only *Callonychium*, whose UV-receptor is maximally sensitive at 360 nm, is found in areas of open space in Brazil. The other species live in the open space of Mediterranean or alpine habitats. It is tempting to speculate that the reduced light flux at short wavelengths in the undergrowth of the forest is a limiting factor for the functioning of the UV-receptors. If this assumption is correct, we would expect to find a shift to longer wavelength UV-receptors, particularly in small insects with small facet lenses, because of the reduced light flux and the lower probability of quantum catch in smaller photoreceptors. So far, no small insects from the tropical forest have been examined.

There is no difference in the λ_{max} distribution of photoreceptors of general and specialized pollinators and

no correlation between the λ_{max} values and the colouration of flowers on which the respective species feed. This result should be taken with care, because all our measurements so far come from medium to large-sized bees and wasps, which do not differ very much with respect to the flowers they visit. Small bees with short proboscis are able to collect nectar and pollen only from flat and open flowers, very much like flies. Such flowers are less frequently coloured blue and UV-blue but more frequently blue-green, green, UV-green and red (Menzel and Shmida, unpublished). It will be interesting to examine the photoreceptors of small bees to see whether they are adapted to the different colouration of their food sources.

The sample of species studied here includes members of the primitive symphytian wasps, which are positioned at the base of the phylogenetic development of hymenopteran insects (Chinery 1984). These tenthredinid wasps are equipped with 4 spectral receptor types. If these 4 spectral inputs are used for tetrachromatic colour vision, one can exclude the possibility that these primitive species were less well equipped with colour vision than the advanced ones like the social Apinidae. In fact, it is even possible that the evolution of the colour vision systems within hymenopterans was towards a simplified trichromatic colour vision, excluding the red region. If this is the case, the question of whether this reduction was a prerequisite for refined colour coding and colour perception mechanisms remains unsolved. It is equally possible that eliminating the red region from colour coding in the bee could be attributed to the co-evolutionary relationship with the angiosperm plants, that is, those flowers which gave up signalling to less specialized pollinators like beetles, primitive wasps and flies and thus excluded competition. The plant may have gained from the greater efficiency of these more highly evolved hymenopterans with their effective learning system.

The exclusion of the red part of the spectrum may also be seen as an adaptation to separate floral signals directed towards bird pollinators. Flowers visited by birds are often brilliant red and thus appear black to a trichromatic hymenopteran colour vision system. Hymenopteran insects with red receptors may use these receptors for purposes other than floral recognition, e.g. the symphytian wasp, which might use them to recognize suitable leaves for egg deposition, or in the case of the solitary bee *Callonychium petuniae*, for the detection of a particular kind of flower such as the red and pink flowers of Petuniae (Cure and Wittmann 1990).

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References

Autrum H, Zwehl V von (1964) Die spektrale Empfindlichkeit einzelner Sehzellen des Bienenauges. Z Vergl Physiol 48:357–384

Backhaus W (1991) Color opponent coding in the visual system of the honey bee. Vision Res 31:1381–1397

Backhaus W, Menzel R (1987) Color distance derived from a receptor model of color vision in the honeybee. Biol Cybern 55:321–331

Backhaus W, Menzel R, Kreißl S (1987) Multidimensional scaling of color similarity in bees. Biol Cybern 56:293–304

Barlow HB (1982) What causes trichromacy? A theoretical analysis using comb-filtered spectra. Vision Res 22:635–643

Bernard GD (1979) Red-absorbing visual pigment of butterflies. Science 203:1125–1127

Bowmaker JK (1983) Trichromatic colour vision: why only three receptor channels. Trends Neurosci 6:43–56

Buchsbaum G, Gottschalk A (1983) Trichromacy, opponent colour coding and optimum colour information transmission in the retina. Proc R Soc Lond B 220:89–113

Chinery M (1984) Insekten Mitteleuropas, 3. Auflage. Paul Parey, Hamburg

Chittka L, Beier W, Hertel H, Steinman E, Menzel R (1990) Opponent color coding as a universal mechanism in hymenopteran insect vision. In: Elsner N, Roth G (eds) Brain – perception – cognition. Georg Thieme, Stuttgart, p 194

Cure IR, Wittmann D (1990) *Callonychium petuniae*, a new panurgine bee species (Apoidea, Andrenidae), oligolectic on *Petunia* (Solanaceae). Stud Neurotrop Fauna Environ 25:153–156

Dartnall HJA (1953) The interpretation of spectral sensitivity curves. Br Med Bull 9:24–30

Ebrey TG, Honig B (1977) New wavelength dependent visual pigments' nomograms. Vision Res 17:147–151

Gribakin FG (1988) Photoreceptor optics of the honeybee and its eye colour mutants: the effect of screening pigments on the long-wave subsystem of colour vision. J Comp Physiol A 164:123–140

Kirschfeld K (1974) The absolute sensitivities of lens and compound eyes. Z Naturforsch 29:592–596

Kirschfeld K (1986) Activation of visual pigment: chromophore structure and function. In: Stieve H (ed) The molecular mechanism of photoreception (Dahlem Konferenzen 1986). Springer, Berlin Heidelberg New York, pp 31–49

Langer H, Schlecht P, Schwemer J (1982) Microspectrophotometric investigation of insect visual pigments. In: Packer L (ed) Methods of enzymology. Biomembranes, vol 81, part H. Academic Press, New York, pp 729–741

Lythgoe JN (1972) The adaptation of visual pigments to the photic environment. In: Dartnall HJ (ed) Photochemistry of vision (Handbook of sensory physiology, vol. VII/1). Springer, Berlin Heidelberg New York, pp 567–624

Maximov VV (1988) An approximation of visual absorption spectra. Sensornye Systemy 2:3–8

Meinertzhagen IA, Menzel R, Kahle G (1983) The identification of spectral receptor types in the retina and lamina of the dragonfly *Sympetrum rubicundulum*. J Comp Physiol 151:295–310

Menzel JG, Wunderer H, Stavenga DG (1991) Functional morphology of the divided compound eye of the honeybee drone (*Apis mellifera*). Tissue Cell 23:525–535

Menzel R (1979) Spectral sensitivity and colour vision in invertebrates. In: Autrum H (ed) Invertebrate photoreceptors (Handbook of sensory physiology, vol. VII/6A). Springer, Berlin Heidelberg New York, pp 503–580

Menzel R, Backhaus W (1989a) Color vision in insects. In: Gouras P (ed) Vision and visual dysfunction, vol. VII. Perception of color. MacMillan Press, Houndsmills, pp 262–293

Menzel R, Backhaus W (1989b) Color vision in honey bee: phenomena and physiological mechanisms. In: Stavenga D, Hardie R (eds) Facets of vision. Springer, Berlin Heidelberg New York, pp 281–297

Menzel R, Ventura DF, Hertel H, Souza JM de, Greggers U (1986) Spectral sensitivity of photoreceptors in insect compound eyes: comparison of species and methods. J Comp Physiol A 158:165–177

Menzel R, Backhaus W, Chittka L, Hoffmann M (1988) Honey bee drones are trichromates. In: Elsner N, Barth FG (eds) Sense organs. Thieme, Stuttgart, p 217

Peitsch D, Backhaus W, Wittmann D, Fix Ventura D, Menzel R (in press) Tetrachromatic colour vision in hymenopterans. Verh Deutsh Zool Ges

Praagh JP van, Ribi WA, Wehrhahn C, Wittmann D (1980) Drone bees fixate the queen with the dorsal frontal part of their compound eyes. J Comp Physiol 136:263–266

Ribi WA (1978) Colour receptors in the eye of the digger wasp, *Sphex cognatus* Smith: evaluation by selective adaptation. Cell Tissue Res 195:471–483

Shannon CE, Weaver W (1949/1963) The mathematical theory of communication. Univ. Illinois Press, Urbana (Ill)

Shaw SR (1969) Interreceptor coupling in ommatidia of drone honeybee and locust compound eye. Vision Res 9:999–1029

Smith WC, Goldsmith TH (1990) Phyletic aspects of the distribution of 3-hydroxyretinal in the class Insecta. J Mol Evol 30:72–84

Snyder AW, Menzel R, Laughlin SB (1973) Structure and function of the fused rhabdom. J Comp Physiol 87:99–135

Vogt K (1989) Distribution of insect visual chromophores: functional and phylogenetic aspects. In: Stavenga D, Hardie R (eds) Facets of vision. Springer, Berlin Heidelberg New York, pp 134–151

Wald G, Brown PK (1965) Human color vision and color blindness. Cold Spring Harbor Symp Quant Biol 30:345–362

Note added in proof. Fig. 1b. The distribution of λ_{max}-values of the recorded photoreceptors in other insect species as shown in Fig. 15.2 of Menzel and Backhaus (1989a). Each number refers to one species and corresponds to the same number in that drawing, in which the different methods and references were described.
Orthoptera: *Gryllus campestris* = 7, 116, 165; *Locusta migratoria* = 50, 51, 73, 74, 112, 145, 147, 179; Hemiptera: *Notonecta glauca* = 48, 67, 122, 159; *Ascalaphus* = 1, 61, 148; *Carabus auratus* = 14, 75; *Photurus lucicrescens* = 52, 68, 212; *Photurus pyralis* = 63, 216; Odonata: *Anax junius* = 43, 141, 144; *Aeschna several species* = 4, 44, 46, 72, 104, 134, 135, 143, 151; *Libellula* several species = 45, 66, 142, 177; *Hemicordula* = 35, 91, 152; *Sympetrum rubicundulum* = 19, 64, 146, 232; Blattoptera: *Periplaneta americana* = 47, 153; Diptera: *Calliphora erythrocephala* = 137, 140; *Drosophila melanogaster* = 135, 136; *Musca domestica* = 5, 6, 49, 126, 131, 133, 137, 140, 214; *Eristalis tenax* = 16, 37, 109, 111, 125, 160; *Eristalis arbustorum* = 94; *Syrphus* sp. = 93; *Allograpta obliqua* = 107; *Toxomerus marginatus* = 115; *Chlorops* sp. = 130; *Simulidae* three species = 21; *Bibio* sp. = 22; *Haematopota white eye* = 182; Lepidoptera: *Heliconius numata* = 62, 123, 199; *Macroglossum stellatorum* = 13; *Papilio aegeus* = 60, 106, 211, 221; *Papilio xuthus* = 54, 65, 129, 163, 220; *Aglais urticae* = 53, 127, 180; *Pieris brassicae* = 55, 56, 113, 114, 215, 231; *Anartia amathea*, *A. fatima* = 222; *Polygonia interrogationis* = 223; *Eurema mexicana* = 224; *Eurema nicippe* = 225; *Phobis senmae* = 226; *Pieris rapae* = 227; *Apodema mormo* = 229; *Everes comyntas* = 230; *Vanessa cardue* = 181; *Anthereae polyphenus* = 8, 128, 164; *Spodoptera exempta* = 39, 129, 157, 217; *Manduca* sp. = 20, 95, 161; *Deilephila elpenor* = 38, 96, 162