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The evolutionary adaptation of flower colours and the insect pollinators' colour vision

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Summary. The evolutionary tuning between floral colouration and the colour vision of flower-visiting Hymenoptera is quantified by evaluating the informational transfer from the signalling flower to the perceiving pollinator. The analysis of 180 spectral reflection spectra of angiosperm blossoms reveals that sharp steps occur precisely at those wavelengths where the pollinators are most sensitive to spectral differences. Straightforward model calculations determine the optimal set of 3 spectral photoreceptor types for discrimination of floral colour signals on the basis of perceptual difference values. The results show good agreement with the sets of photoreceptors characterized electrophysiologically in 40 species of Hymenoptera.

Key words: Colour vision – Flower colours – Evolution – Hymenoptera – Pollination ecology

Introduction

Many insects such as social and solitary bees are highly dependent on the pollen and nectar diet offered by flowers. Since flowers are unreliable and scattered food sources, the insects should be equipped with learning capacities and sensory systems that favour an efficient foraging strategy. All Hymenoptera so far tested have the ability to learn colours as a stimulus associated with a reward (von Frisch 1914, 1967; Mazokhin-Porshniakov 1962; Menzel 1979; Dukas and Real 1991; Menzel and Backhaus 1991; Chittka et al. 1992). This ability allows them to tell known from unknown flowers and, amongst the familiar ones, to discriminate profitable food sources and inefficient ones. An essential prerequisite for this capacity is a colour vision system that allows for optimal discrimination between flowers of different species.

Many angiosperm plant species compete with one another for animals as pollen vectors (Darwin 1876; Kevan 1978; Feinsinger 1983; Rathke 1983; Waser 1983, 1986). For pollen to be effectively transferred, the plants generally "have an interest" that an individual pollinator visits con-specific flowers *exclusively*. The learning capacities of Hymenoptera offer a great opportunity in this regard. Making use of this, the plant does not have to restrict the pollinator type by means of morphological adaptations, which is may be an evolutionary deadlock and can also be rather insecure if it makes the plant exclusively dependent on one pollinator species. Instead, the flower has the possibility to "advertise" its reward by means of a species-specific label, which has two major consequences: 1. They potentially address a large spectrum of pollinator *species*. 2. *Individual* visitors that have experienced this particular flower as rewarding will have a high tendency to visit flowers of the same species more frequently, thus favouring an effective pollination. Hence, the flower signals must not only be well detectable, but also easy to distinguish from those of competing species (Daumer 1956; von Frisch 1914, 1967; Menzel 1967; Kevan 1978; Waser 1986).

The relationship between floral colours and the pollinators' colour vision may thus be regarded as a signal-receiver system whose components are likely to be evolutionarily adapted to each other (Menzel and Backhaus 1991) so as to allow optimal discrimination of flowers. Such processes of evolutionary tuning take place within the scope of certain physical and biological constraints (Lythgoe 1972, 1979; Lythgoe and Partridge 1989; Goldsmith 1991). On the signal side, the limits are set by the possibilities of obtaining flower colours through combinations of the available pigments and surface structures. With regard to the "receivers", the development is constrained by the optical design of compound eyes, the absorption properties of the photopigments, the mechanisms of signal transduction and light adaptation, and the neural processes evaluating the receptor of signals (Snyder et al. 1973; Menzel 1979; Laughlin 1981; Burkhardt 1983; Stavenga 1989).

The spectral reflection functions of angiosperm blossoms can be easily measured. The neural code underlying

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colour discrimination has recently been identified for the honeybee (Backhaus 1991). The concept was successfully extended to several other Hymenoptera, these including social and solitary bees as well social and solitary wasps (Chittka et al. 1992; Chittka and Lunau, 1992). A standard measure of perceptual colour difference (colour hexagon distance) was devoloped (Chittka 1992) and found applicable to all the 10 species investigated.

This model of colour perception allows the assignment of numerical values to perceived colour differences between any two colour stimuli under a given spectral illumination. These values may be calculated for any trichromatic colour vision system with known receptor spectral sensitivities. We search for the set of spectral photoreceptor types which yields an optimum of discrimination between natural flower colours in an insect's system of colour perception.

Materials and methods

Spectral measurements of flowers. The spectral reflection functions of floral petals were measured from 300 to 700 nm by means of a flash photometer (resolution 1 nm). The white standard was a freshly pressed pellet of dry $BaSO_4$. The circular flash bulb illuminated the probe (⌀ 10 mm) under an angle of 45°, and a light guide transmitting the light to the monochromator collected the reflected light under an angle of 0°. If the structures to be measured were smaller than ⌀ 10 mm, many petals were arranged like fish scales, such that only the identically coloured parts were exposed to the photometer (see Menzel and Shmida, in press, for details).

We selected the Israeli flora as a study case, because the interactions between angiosperm plants and their pollinators have been particularly well studied there (see Menzel and Shmida, in press, for review). As practically everywhere else, Hymenoptera have been found to be by far the most important flower visitors in Israel (Dukas and Shmida, in press).

It is important to note that only approximately 10 out of more than 1200 Israeli species of Hymenoptera are specialists (oligolectic) that appear to have an innately fixed preference to forage only on a restricted number of plant species (Shmida, personal communication). All others are generalists in the sense that individuals of the same species are found to visit several plant species. As mentioned above, in a flora dominated by generalists with learning capacities, there is a high selective pressure for plants to use their blossoms for species-specific labelling, i.e. to differ from each other with regard to the perception of the pollinators.

For all the following considerations, we evaluated a sample of 180 spectral measurements from plants that are known to be most predominantly visited by Hymenoptera.

Modelling of photoreceptor spectral sensitivity functions. We will proceed to extract an optimal photoreceptor set from the flower spectra by systematically shifting the spectral sensitivity curves along the wavelength scale. For this purpose, templates of photoreceptor spectral sensitivity curves are modelled according to Maximov (1988). This procedure is appropriate because of the structure of the fused rhabdom in Hymenoptera. The mutual filtering effects of the different visual pigments (rhodopsins) packed together in one light guiding structure results in spectral sensitivity functions of the single receptors which are very close to the spectral absorbance of a thin layer (Snyder et al. 1973). Consequently, the spectral sensitivity functions measured intracellularly with electrophysiological techniques correspond closely to the spectral absorbance of the respective photopigments (Menzel et al. 1986) although small deviations are found at scrutinized inspection (Gribakin 1988; Stavenga and Schwemer 1984). In this study, we do not attempt to test whether and how strong these deviations affect the perceptual measures of colour vision. A discussion with regard to this point can be found in Menzel and Backhaus (1991), Peitsch et al. (1992). Since the spectral sensitivity functions follow a rhodopsin template function (Maximov 1988) closely, it is appropriate to characterize each function by its λ_{max} value. The long wavelength photopigment templates ($\lambda_{max} > 500$ nm) had to be corrected manually in the short wavelength part, because they deviate systematically from all measured spectral sensitivity functions in the uv ($\lambda < 400$ nm). The correction was performed such that it gave the best fit to a large number of spectral sensitivity measurements of photoreceptors in 40 species of Hymenoptera (Peitsch et al. 1992).

Calculation of photoreceptor excitations. The procedure for calculating the graduate potential in a photoreceptor with a known spectral sensitivity function $S(\lambda)$ stimulated by a given stimulus reflection curve $I(\lambda)$ which is illuminated by a light with the spectral composition $D(\lambda)$ is reviewed in detail by Backhaus and Menzel (1987), Chittka et al. (1992). The relative quantum flux P is defined by

$$P = R \int_{300}^{700} I(\lambda)\, S(\lambda)\, D(\lambda)\, d\lambda. \quad (1)$$

The coefficient R is adjusted such that it will yield a half maximal excitation (E in Eq. 2) in the photoreceptor when it is stimulated by the light reflected from the adaptation background (Laughlin 1981), following a von Kries (1905) type coefficient law.

We assume the receptors to be adapted to a background reflection function averaged from the reflections of various leaves and anorganic materials (sand and stones) found in close proximity of the plants of which the measurements were taken (Fig. 1, dashed line). The spectral illumation curve used in all the model calculations is the normfunction D65 (clear sky, Fig. 1, solid line).

The non-linear transfer function relating the receptor voltage signal (excitation E) with the quantum flux P follows Eq. 2 (Lipetz 1971; Backhaus and Menzel 1987, for reviews):

$$E = P^n/(P^n + 1). \quad (2)$$

The exponent n depends on the species in question and slightly on the adaptation state (see Backhaus and Menzel 1987; Chittka et al. 1992, for details).

As mentioned above, the adaptation process is assumed to adjust the sensitivity such that the excitation in each receptor will be half maximal for the light reflected from the background (Laughlin 1981). This regulation may not hold under the extreme low light

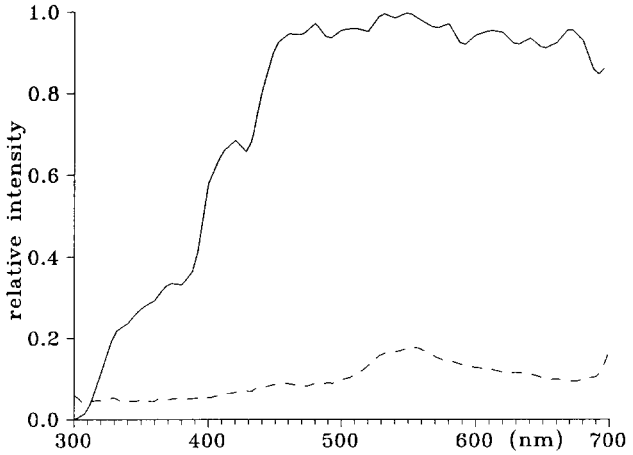


Fig. 1. The *dashed line* marks the spectral reflection of the background to which the photoreceptors are assumed to be adapted in the model calculations. This curve is averaged from the reflection functions of several leaves, sand and stones found in close vicinity of the flowers measured. The *solid curve* corresponds to the spectral composition of the daylight normfunction D65

conditions that receptors with $\lambda_{max} < 340$ nm would be exposed to. Following this rule, and given our standard background and the standard illumination function, a receptor at $\lambda_{max} = 300$ nm would be 84 times more sensitive than a green-receptor at $\lambda_{max} = 550$ nm (corresponding to values of $R = 0.2106$ for $\lambda_{max} = 300$ nm and $R = 0.0025$ for $\lambda_{max} = 550$ nm in Eq. 1). Accordingly, for the λ_{max} between 300 and 360 nm, the relations are the following: ($\lambda_{max} = 310$ nm: 51 times more sensitive than the mentioned green receptor; $\lambda_{max} = 320$ nm: 35*; $\lambda_{max} = 330$ nm: 26*; $\lambda_{max} = 340$ nm: 20*; $\lambda_{max} = 350$ nm: 16*). These values are at odds with the results of behavioural investigations of the honeybee's spectral sensitivity (von Helversen 1972). These experiments showed that the uv-receptor cannot be more than 16.5 times more sensitive than the green-receptor at a background light that contains practically no ultraviolet.

We thus introduce this limit as an additional constraint for the adaptation of the S-receptor. This means, that the coefficient R (in Eq. 1) of all the receptors with $\lambda_{max} < 350$ nm is clamped to a value that equals 16.5 times the adaptation coefficient R for a green receptor with $\lambda_{max} = 550$ nm. In all other receptors, R is calculated according to Laughlin (1981).

Results

Spectral reflection functions of angiosperm blossoms

The floral reflection curves were analyzed to see if there is any underlying pattern to the ways in which the floral colouring components are combined to form the reflection spectra.

The most vivid colours are those that produce the greatest differences between the signals in the different receptor colour types. These vivid colours can only be generated by spectra that change rapidly as the wavelength shifts from the spectral band dominated by one receptor to the band dominated by the next. Thus, to generate colours that stand out from the background and from competitors flowers must use combinations of pigments that generate sharp steps in the spectra, preferably at the boundaries between receptors.

Therefore, the most important characteristic of a flower spectrum is a sharp step. Correspondingly, we

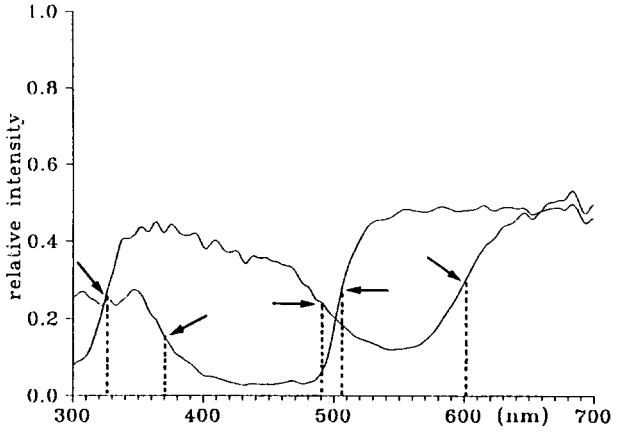


Fig. 2. Two examples for typical spectral reflection functions of blossoms are depicted. The *arrows* denote the 50%-values of the steep slopes in the curves. The wavelength positions of these sharp steps were manually determined by going systematically through all the 180 floral reflections

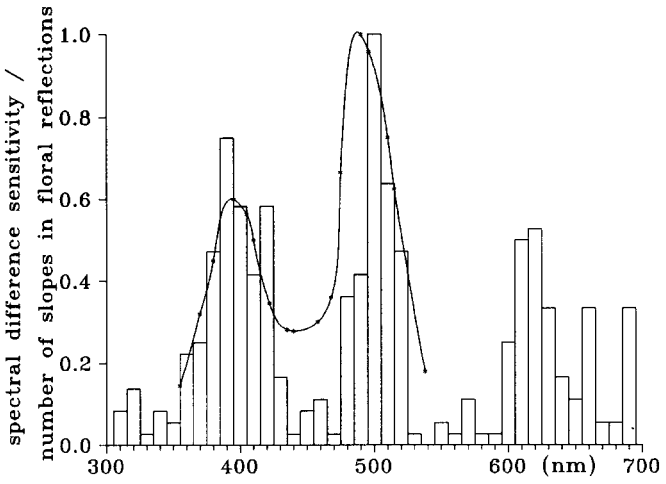


Fig. 3. The columns denote the number of the 50%-values of the distinct slopes in the spectral reflections of the flowers at the respective wavelengths. The histogram shows 3 evident accumulations of such sharp steps. Two of these clusters (around 400 and 500 nm) can be very well evaluated by hymenopteran trichromats, which are all maximally sensitive to spectral differences around 400 and 500 nm (Menzel and Backhaus 1991; Peitsch et al. 1992) as exemplified by the inverse $\Delta\lambda/\lambda$-function of the honeybee (von Helversen 1972, *solid line*). The curve and the columns are normalized to a maximum of unity. The highest column corresponds to a total of 36 slopes

determined the positions of the slopes as a function of the wavelength. In order to characterize a prominent slope in the reflection function by *one* wavelength value, we chose to assign the 50% – value of the slope to its respective wavelength. The wavelength position of the 50% – values was determined by eyesight, because the large variety of curve shapes made an automatic procedure less reliable. We proceeded as follows: the intensity values of the neighbouring maximum and minimum (or the adjacent plateaus, respectively) were determined and the wavelength was read at which the reflection function crosses the 50%-intensity value between the two adjacent extremes (maxima, minima or plateaus) of the slope (Fig. 2).

It is obvious that the sharp steps in the spectra are not randomly distributed (Fig. 3). Three frequency peaks occur at regular intervals (around 400, 500 and 600 nm) through the spectrum. In a first approximation, a colour vision system would be able to discriminate these signals optimally if its spectral discrimination ability were maximal in those parts of the spectrum where the spectral differences occur most frequently. This aspect will be scrutinized in the subsequent section.

Spectral receptor types of flower-visiting Hymenoptera

All available spectral information is first filtered and decomposed by a small number of photoreceptor colour types, consequently their spectral sensitivities primarily determine an animal's ability to discriminate colours. If flower colour and/or hymenopteran colour vision have evolved to maximize the distinctiveness of flowers, then

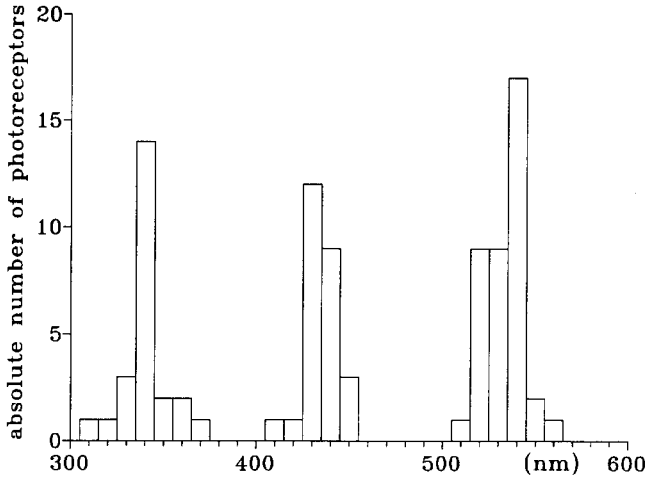


Fig. 4. The histogram plots the frequency of occurrence of λ_{max} of photoreceptors of hymenopteran trichromats against the wavelength scale. The columns give the absolute number of λ_{max} of the electrophysiologically characterized types of photoreceptors in 40 trichromatic Hymenopteran species (Peitsch et al. 1992) rounded to the closest 10 nm step. The diagram has 3 clear peaks at λ_{max}=330–350 nm, λ_{max}=430–450 nm and λ_{max}=520–540 nm

the photoreceptor spectral sensitivities should be matched to floral reflection spectra so as to optimize discrimination. This means that the floral reflection spectra should contain the information on the optimal set of spectral receptor types. Before we extract this information, we summarize the experimental data on photoreceptor colour types in Hymenoptera (see Peitsch et al. 1992).

Hymenopteran trichromats possess very similar sets of uv, blue and green receptors with peaks clustering in the spectral regions 330–350 nm, 430–450 nm and 520–540 nm respectively (Fig. 4). A colour vision system is most sensitive to changes in wavelength in those regions of the spectrum where two spectral sensitivity functions overlap such that they have steep slopes in opposite directions. In those parts of the spectrum, small differences on the wavelength scale will cause maximal differences of reverse sign in two photoreceptor excitations and thus a large perceptual colour distance in any system that evaluates the receptor signals antagonistically in a colour opponent system. Consequently, the spectral discrimination function has similar characteristics in all the species from whose receptors were recorded: the spectral difference sensitivity is maximal at around 400 and 500 nm (Menzel and Backhaus 1991; Peitsch et al. 1992). As a representative example, the spectral discrimination function (inverse $\Delta\lambda/\lambda$-curve) of the honeybee (von Helversen 1972) is compared with the spectral distribution of the sharp steps in the spectra (Fig. 3). The peaks of both functions match very well. Hence, the investigated trichromatic systems are excellently adjusted to the task of evaluating the measured flower spectra. The flowers, in turn, concentrate their prominent spectral differences where the pollinators are most sensitive to them (i.e. around 400 and 500 nm).

A further accumulation of slopes in the floral reflection spectra around 600 nm can not be exploited for discrimination by animals without red-receptors. It thus appears that pollinators with tetrachromatic systems (such as beetles and butterflies and very few species of Hymenoptera) that possess such receptors might be able to extract more information from the flower spectra.

Precisely how well are photoreceptors tuned to code floral colours?

A measuring instrument may be regarded as optimal if it renders as many values differing from each other as possible for the sample of analyzed objects. A spectral receptor is badly designed for colour discrimination if it generates identical signals to all coloured objects. The receptor is well designed if the set of all coloured objects produces the widest possible range of different signals. We modelled photoreceptor responses to our set of 180 floral reflection spectra to see which particular spectral sensitivities produced the greatest ranges of signals.

A single spectral sensitivity curve modelled as given by the Maximov (1988) template function is shifted along the wavelength scale from $\lambda_{max}=300$ to $\lambda_{max}=600$ nm in 10 nm steps. For every position of the receptor sensitivity maximum we calculated the set of 180 receptor potentials produced by our set of 180 flower signals. The range encompassed by this set of signals was then taken to be their standard deviation.

The result is displayed in Fig. 4; one finds 3 optima at 340, 430 and 550 nm and thus a good agreement with the λ_{max} positions of natural hymenopteran photoreceptors.

In order to make sure that these results were not by some means inherent to our model calculations, but

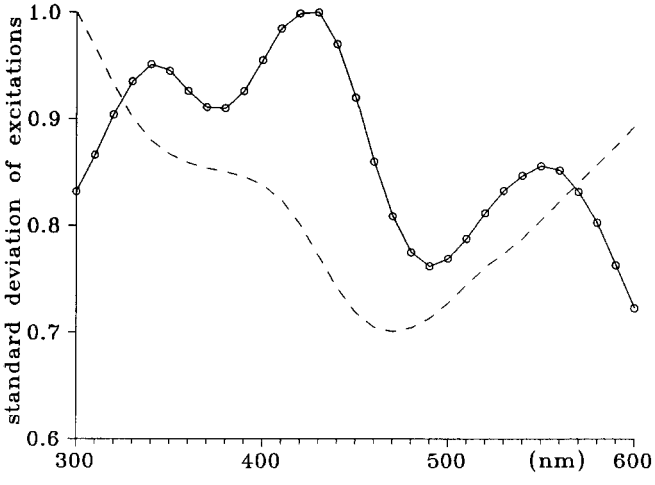


Fig. 5. One Maximov receptor template is shifted from $\lambda_{max}=300$ to 600 nm (abscissa). For each position, the receptor excitations for all colour signals and the standard deviation of these values are calculated. The latter serves as a measure for how well the receptor signals assigned to the objects will be spread along the receptor's dynamic range (ordinate). Three optima appear in sections of the spectrum where the λ_{max} of natural photoreceptors actually occur. The dashed line shows the result of the same procedure with a sample of 200 randomly picked colour filters from the Schott Co., and cardboards

rather a consequence of stimuli characteristics, we proceeded to use a sample of 200 spectral measurements of biologically irrelevant objects (coloured glass filters, Schott Co., Mainz, and coloured cardboards) as the input to the same mathematical procedure (Fig. 5, dashed line). In this case, no optimum comparable to any receptors of Hymenoptera is reached.

Summarizing the results of the last two sections, we may conclude that the receptors are well placed in the spectrum to allow for optimal evaluation of *steps* in the floral spectra (between the λ_{max} of the receptors) *and* to render maximal differences in *absolute* values of photoreceptor excitations for our sample of flower signals.

In order to see how these features of the receptor level are integrated on the level of colour perception, we will now proceed to "look into the brain" of the insect, to see if there is a relationship between perception and ecology. We will quantitatively predict how different the insects will perceive the flowers from each other, depending on which receptors serve as input to the neural colour coding system.

Optimizing colour discrimination on the level of the insects' perception

Receptors are the interfaces between the environment and the brain, consequently their design must be considered in terms of both the range of sensory signals and the computational capabilities of the nervous system.

In trichromatic bees and wasps, colour is coded by means of two colour opponent mechanisms, i. e. the receptor signals feed antagonistically into two different neural processes (Backhaus 1991; Chittka et al. 1992; Chittka and Lunau 1992). The perceptual colour difference between two stimuli can be adequately predicted by their distance in a standardized colour opponent space, the colour hexagon (Chittka 1992).

We will now systematically shift all three photoreceptors along the wavelength scale and calculate the perceptual differences between all flower colours for every set of 3 receptor colour types. If the sum of all these perceived differences is small, we may consider the receptor set as poorly designed for colour discrimination. A combination of receptors is optimal if it renders a maximal spread of stimuli in a perceptual colour space, i.e. the differences between colours are maximal.

In the first approach, 3 model calculations were performed by varying only one receptor, and keeping two receptors constant at the wavelength positions at which they occur most frequently (340, 430 and 540 nm) in Hymenoptera.

1. The short-wave (S-) receptor was shifted from 300 to 400 nm (clamped: the medium-wave (M-) and the long-wave (L-) receptor at 430 and 540 nm). 10 nm steps were used.

2. M-receptor shifted from 400 to 500 nm (clamped: S- and L-receptor at 340 nm, 540 nm)

3. L-receptor shifted from 500 to 600 nm (clamped: S- and M-receptor at 340 nm, 430 nm).

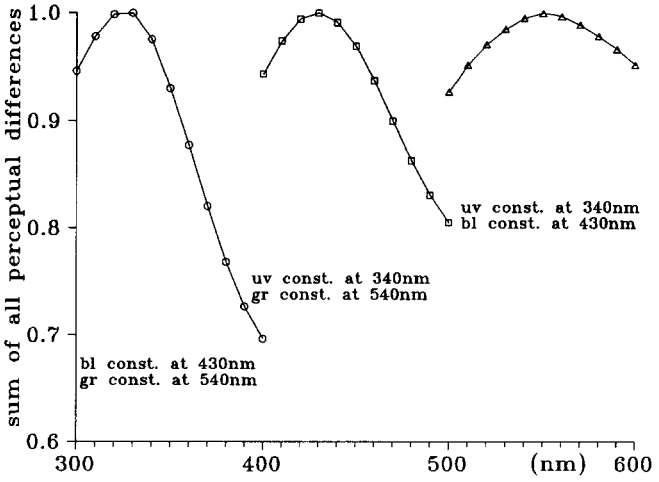


Fig. 6. For determination of the optimal receptors with regard to the criterion of maximal perceptual distances between all floral colours, two receptors were kept constant at the λ_{max} where they are most frequently found in Hymenoptera (λ_{max} = 340, 430, 540 nm, see Fig. 3), and the third one was varied in 10 nm steps from 300 to 400 nm, from 400 to 500 nm or from 500 to 600 nm. For all combinations all the perceived colour differences from every colour signal to every other signal were calculated, summed up, and plotted in the diagram. The resulting optima (λ_{max} = 330, 430 and 550 nm) show good concurrence with the receptor sets that have been described by means of electrophysiological methods

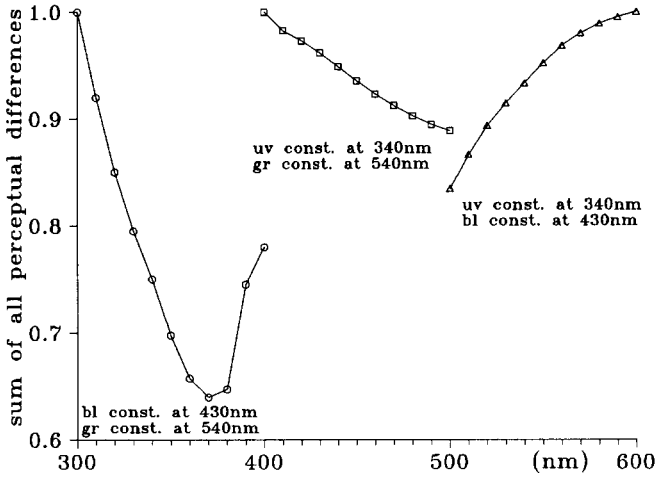


Fig. 7. The same procedure as in Fig. 6, but with a different set of stimuli used as input to the model calculations. The displayed results are obtained for a set of 200 coloured cardboards and glass filters. For none of the 3 variations an optimum is found, indicating that the results in Fig. 6 are indeed based on the spectral characteristics of the flower colours rather than being a consequence inherent to the model procedures

For every receptor combination in the variations, the perceptual differences from every flower colour to all other flower colours are calculated. The measure of the spread in the perceptual colour space is taken to be the sum of all these differences.

The results are depicted in Fig. 6. The optima of these variations (S-receptor: 330 nm; M-receptor: 430 nm; L-receptor: 550 nm) match very well with the distribu-

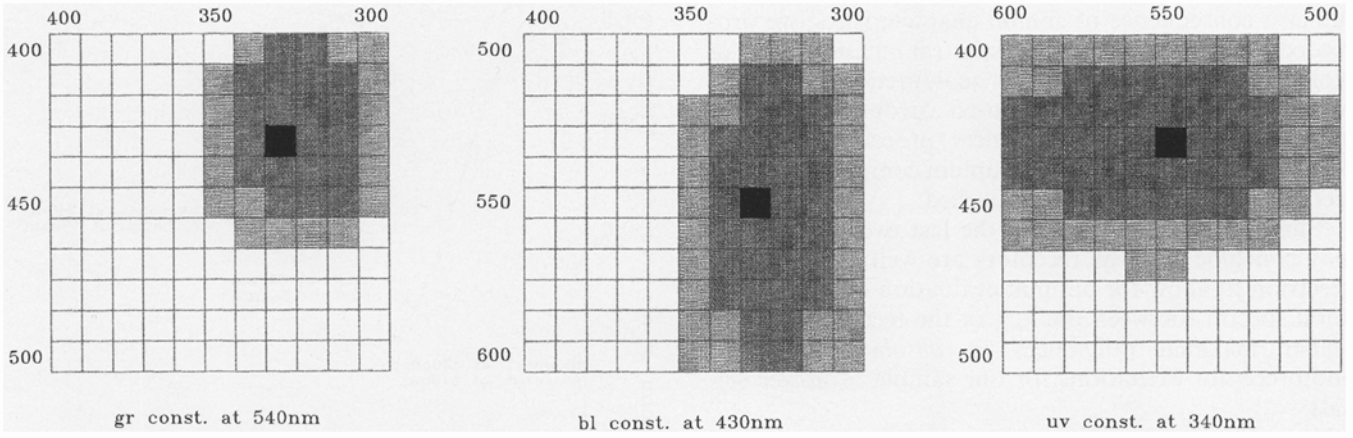
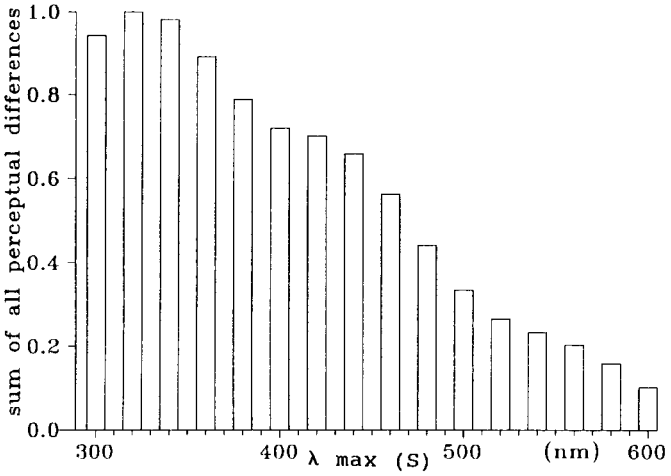


Fig. 8. In each of 3 variations, one receptor is kept constant at a wavelength position of Hymenopteran S-, M- or L-receptors. The two others are varied in 10 nm steps. The wavelength range of the respective variation is given by the values next to the chart. For every combination of 3 photoreceptor colour types, all perceptual differences between all flower colours were determined and their sum was calculated. Each field in the grey scale is assigned to a combination of 3 photoreceptors; the shade denotes the magnitude of the sum of perceptual differences. The black field marks the maximal sum. The dark grey area comprises such combinations that yield a sum which is up to 5% lower than this maximum (10% for the light grey area). All 3 optima are assigned to a combination of photoreceptors with $\lambda_{max} = 330$, 430 and 550 nm



λ max (S)	λ max (M)	λ max(L)
300	440	560
320	440	560
340	440	560
360	440	560
380	440	560
400	440	560
420	560	640
440	560	640
460	560	640
480	560	640
500	560	640
520	560	640
540	560	640
560	580	640
580	620	640
600	620	640

Fig. 9. All 3 receptor templates are varied freely in 20 nm steps from 300 to 640 nm. At every given wavelength position of the S-receptor, the positions of the M- and L-receptors are shifted between the λ_{max} of the S-receptor and 640 nm. All possible combinations of three photoreceptor colour types are included in this model calculation. For every set of photoreceptors, the sum of all occurring perceptual differences between the floral colour loci is calculated. For each position of the S-receptor, the combination with M- and L-receptors is determined which yields the maximal sum of perceptual differences. This maximal sum is denoted for every λ_{max} position of the S-receptor by a column in the chart. The wavelength positions of the S-, M- and L-receptors assigned to these columns are listed in a table on the right side of the chart. The absolute optimum occurs at 320, 440 and 560 nm

tion of the intracellularly measured hymenopteran photoreceptors.

Again, we controlled our results by performing the same model calculations with objects that should have no evolutionary relevance to the bees, i.e. coloured glass filters and cardboards (see above) (Fig. 7). No optimum is found for any of the 3 variation procedures.

We then proceeded to increase the number of independently varied receptors in our model computations. In the optimization procedures 4, 5 and 6, only one receptor will be held constant at the wavelength position of most frequently occurring hymenopteran photoreceptors, and the two others will be systematically varied.

4. The M-receptor was shifted from 400 to 500 nm in conjunction with the L-receptor being shifted from 500 to 600 nm. The S-receptor was held constant at 340 nm. 10 nm steps were used.

5. The S-receptor was varied from 300 to 400 nm in combination with the L-receptor being shifted from 500 to 600 nm. The M-receptor was clamped at 430 nm.

6. The S-receptor was shifted from 300 to 400 nm in combination with the M-receptor being shifted from 400 to 500 nm. The L-receptor was fixed at 540 nm.

The results of the variations 4–6 are depicted in Fig. 8, using 3 grey-scale charts. The black field marks the maximal sum of perceptual distances for each variation procedure. In order to give an impression about what other combinations of receptors might also be taken into

consideration, we mark the combinations that yield a sum of perceptual distances up to 5% below the maximum (dark grey) and up to 10% (light grey). Again, the optimal combinations of photoreceptor colour types (330 nm, 430 nm, 550 nm) correspond closely to the receptor sets that most Hymenoptera possess (Fig. 4). A combination of 340, 430 and 540 nm (corresponding to the most frequent photoreceptors in these insects) falls within the dark grey area in all the 3 diagrams, i.e. it yields a sum of perceptual distances which is less than 5% lower than the absolute maximum marked by the black fields.

In a final model calculation, all 3 receptors were shifted freely in 20 nm steps from 300 to 640 nm. In this procedure, the whole range of wavelength positions is covered in which λ_{max} of insect photoreceptors have ever been reported (Menzel and Backhaus 1991, Fig. 14.2). All possible combinations of 3 receptors within this range are tested.

We proceeded as follows: the S-receptor was clamped at a given value (starting with 300 nm), and the M-receptor was varied over the whole range *between* the S-receptor and a given position of the L-receptor, whilst the L-receptor was varied over the whole range *above* the M-receptor and up to 640 nm. Then the S-receptor was set to the next value (320 nm), and so forth. To make the procedure clear, consider two examples:

1. The S-receptor is held constant at 300 nm. The M-receptor is varied from 320 to 620 nm and the L-receptor from 340 to 640 nm. The M-receptor can not reach any wavelength position larger than the one of the L-receptor; consequently, if the M-receptor is positioned at 600 nm, the L-receptor will only be varied from 620 to 640 nm.
2. The S-receptor is clamped at 560 nm. In this case, the M-receptor is varied from 580 to 620 nm and the L-receptor from 600 to 640 nm.

The absolute optimum (maximal sum of all perceptual distances) appears at $\lambda_{max} = 320$, 440 and 560 nm (Fig. 9). Thus, we find some deviation in the S- and L-receptor from the ones described in Hymenoptera (where they cluster around 340 and 540 nm) but, given a step size of 20 nm, the differences in wavelength are equivalent to only one step of the model calculations. The combination of 340, 430 and 540 renders a sum of perceptual distances which is only very little lower (0.97 if the maximum equals unity).

Discussion

The ecological background

The concept of the flower colours being addressed to their pollinators rather than to our esthetic perception has first been discerningly pointed out by Sprengel (1793). An entirely new aspect was added to the theory of this relationship when it was discovered that the bee's colour vision differs from ours and that flowers contain ultraviolet signals invisible to us (review von Frisch 1967). The notion of an evolutionary relationship between flower signals and the insects' perception has since been consolidated by many authors (Daumer 1956; Kevan 1978; Feinsinger 1983; Menzel 1985; Menzel and Backhaus 1991), but a *quantitative* evolutionary explanation of the components of bee colour vision has never been attempted.

In fact, the first explanation of the wavelength positions of natural photoreceptors for coding of ecologically relevant objects with known spectral reflections was achieved in a quite different context. In a pioneering study, Lythgoe and Partridge (1989) searched for the optimal dichromatic pigment combination for the discrimination of leaves and forest litter. Their results showed good concurrence with the natural photoreceptors of some forest-dwelling vertebrates such as grey squirrels, tree shrews, dichromatic phenotypes of squirrel monkeys, and frogs.

The relationship of the pollinators' colour vision and the world of flower colours is so particularly interesting because its adjustment is of mutual benefit. This coloured world has developed its variety exclusively with respect to the perception of the pollinators, for whom the colours have a vital relevance as food source markers.

The present investigation is fundamentally based on two assumptions: a) the pollinator' s fitness is increased if it can distinguish flowers from each other and b) the plant's fitness is increased if it appears distinct from competitors. Why is this so? This question has been alluded in the introduction, but needs some further explanation.

Why should pollinators discriminate flowers? The efficiency in foraging is closely linked to Darwinian fitness (Pyke et al. 1977), i.e. maximation of the number of viable offspring. In order to understand why flower discrimination is important for effective foraging, we must first understand some basics of how generalists with learning capacities collect food.

The distribution of floral food resources is subject to permanent changes. Consequently, an animal with a fixed preference for a particular food source is clearly at disadvantage. An efficient forager frequently has to update its information about profitable food sources. The learning abilities of Hymenoptera greatly favour a quick adjustment with respect to this task. This implies, however, that these insects must potentially be able to learn the features of *any* flower colour and to *discriminate* it from the ones of all others.

A comprehensive knowledge of all food sources in any habitat which is rich in flowering plants could only be achieved at the expense of neglecting foraging activities to a large degree.

Consequently, individual insects show a tendency to restrict their visits to one or few rewarding plant species out of the multitude of alternatively flowering plant species. This behaviour is referred to as flower constancy (Darwin 1876; Plateau 1901; Clements and Long 1923; Grant 1950; Free 1966; Heinrich 1979; Waser 1986).

Such a strategy may be understood as analog to hu-

man "shopping behaviour" in a supermarket: once we have discovered a product the cost/benefit relation of which makes it worth buying, we may save a lot of time and energy if we *always* buy the same product, instead of comparing it with all other products on the market every time we go shopping. Since, however, the offerings in the market may change, we also have to invest a certain minimal expenditure to inspect whether the choice is still close to optimal. In scientific terms, the degree of flower constancy is closely linked to the problem of optimizing the relation of foraging on known profitable food sources and the acquisition of information about new such sources (Heinrich 1979; Kamil and Roitblat 1985; Stephens and Krebs 1986; Selten and Shmida 1988).

The success of this kind of foraging behaviour is critically dependent on a sensory equipment which is tuned such as to allow to distinguish one flower species from another, and an important component of this equipment is colour vision.

Generally, Hymenoptera are the most abundant and most effective pollinators of flowers (Sprengel 1793; Proctor 1973; Faegri and van der Pijl 1978; Kevan and Baker 1983). Correspondingly, these insects have the strongest selective influences on the community of animal-pollinated plants, at least in a general sense.

Why should flowers differ from each other? The flower constancy of Hymenoptera is of great importance for an effective plant pollination. The degree to which a plant species can accumulate visits of one individual pollinator depends critically on how different it appears from sympatric flowers. In colour discrimination tests with numerous Hymenoptera, it was shown that the frequency of choice of a given colour signal is related to the similarity of this given signal to a colour which they have experienced as rewarding (Backhaus et al. 1987; Chittka et al. 1992).

From the perspective of the plant, "mistakes" of the pollinators (visits to similar flowers) have to be avoided for several reasons: a) the insect loses pollen on foreign plants; b) it "wastes" time on different blossoms, that, from the plant's perspective, should be spent foraging on con-specifics; c) estraneous pollen may clog the stigma or disrupt parts of the female reproductive organs (e.g. Waser 1986). Mimicry strategies as reported by Nilsson (1983) and Koehler and Davenport (1983) are an infrequent component of the pollination market, because once the imitating plant rises above the threshold of being quantitatively negligible, the "model" species is under selective pressure to change its signal.

The evolutionary development of flower signals is thus subject to a very direct selection process and one should expect every change in floral features to have immediate consequences on the plant's fitness.

Colouration is one of the strategies that flowers employ in order to appear most distinguished from sympatric species.

Why color as a parameter? Several features characterize a flowering plant and can thus be used for species-specific labelling and competition against sympatric species. These include the site of the plant, flower height, flowering time during the day, blossom size, shape, colour pattern, plane of symmetry and odour. We do not consider these parameters as less relevant than colour; in fact it has to be expected that all such criteria influence each other in a multilateral fashion. For example, flowers that can be easily recognized by odour would not be under selective pressure to be discriminated by colour.

However, colour vision may still be the most important means for the localisation of a flower at further distance, because odor might not reach as far and, given the relatively poor spatial resolution of the insect eye, floral shape or pattern cannot be distinguished from afar.

Most importantly, there is one good practical reason not to take these additional criteria into account: colour is the only parameter that allows the assignment of numerical values to its subjective qualities in the perception of the pollinator.

This argument may appear rather operational. However, it is fully justified by the results. The characteristics of the signal-receiver system under consideration can be most satisfactorily explained by assuming that colour is the only medium between the two sides. This means, in a general sense, that other parameters than colour serve as *additional* identification cues but do not render superfluous the necessity of colour discrimination.

The Results. The results indicate a very well-tuned relationship between our sample of flower colours and the colour vision systems of their visitors on 3 distinct levels: 1. the sharp steps of the flower spectra occur precisely where the hymenopteran photoreceptor sets can best evaluate such steps; 2. the receptors are placed in the spectrum such that they generate the largest possible range of different excitation values for the flower signals; 3. the receptors are combined so as to maximize the perceived differences between all flowers.

On the first level, however, we find some discrepancy. There are 3 clusters of sharp steps in the flower reflection curves (around 400, 500 and 600 nm), only two of which (at 400 and 500 nm) can be evaluated for colour discrimination by trichromatic Hymenoptera.

This indicates that some of the flowers might parallel address pollinators that possess additional red receptors (such as beetles, butterflies and also very few Hymenoptera; Menzel and Backhaus 1991; Peitsch et al. 1992). These insects might be even better equipped for discrimination of the given sample of flowers. A systematic investigation of this problem is not yet possible, because it is not known how insects with 4 spectral receptors integrate the information from these receptor types into a neural colour coding system. The basic question is the following: does the additional expenditure in neural computation actually yield so much of supplementary information that it increases the fitness of the insect? With regard to this, another interpretation of the sharp steps in the reflection curves around 600 nm may be of relevance.

The slopes around 600 nm belong predominantly to colours which look purple and pink to the human eye.

They reflect both in the blue and the red part of the spectrum. Gottsberger and Gottlieb (1980, 1981) demonstrated that pure blue flower colours are an evolutionary exception. Thus, the easier solution for a plant to occupy the blue niche of a bee's colour space may be to use more widespread pigments such as anthocyanes, which have a blue and a red reflection peak. Following this interpretation, the observed slopes at 600 nm might simply be a necessary adjunct to other characteristics of the flower spectra. If they are inevitably coupled with a blue reflection peak, their evaluation does not yield any additional information and would thus be irrelevant to the pollinators.

With the two last steps of the present investigation, in which photoreceptors are systematically varied across the spectrum, there is little difficulty. The small discrepancies between the modelled optimal photoreceptor sets and the wavelength positions of the natural insect photoreceptors never exceed the size of one wavelength step in the model calculations. Furthermore, this comparison only refers to the *peaks* of the clusters of electrophysiologically characterized photoreceptors. In no case are the modelled optimal photoreceptors outside the periphery of the 3 clusters of the natural receptors of trichromatic Hymenoptera.

Our findings do not exclude the existence of different guilds of plants and pollinators with different strategies of colouration and colour vision systems and multilateral interference amongst those guilds. Once we have established quantitative models of colour vision for pollinators other than Hymenoptera, our methods offer the opportunity to scrutinize such different systems within a similar ecological and evolutionary framework.

Co-evolution or one-sided evolutionary tuning?

In the case of the dichromatic vertebrates discussed by Lythgoe and Partridge (1989, see above) and their discrimination of forest material, this question is obviously easy to answer. The evolutionary interest is uni-directional, consequently the evolutionary tuning must be a one-sided process of tuning the colour vision (the variable side) so as to optimize the discrimination of objects (the invariable side).

In the case of flower-visitors and flowers the interest into a functioning signal-receiver relationship is clearly mutual, but the ambiguity outlined in the headline of this section is somewhat less easy to solve. The match between the two sides in the evolutionary status quo does not necessarily imply that it is based on *mutual* tuning. Certainly, flower colours did not evolve before pollinator colour vision, but it is most likely that Hymenoptera possessed important components of their present colour vision systems prior to the first appearance of a colourful flower.

Their green receptors match well with the prevailing background (green foliage) in most natural bee habitats (Menzel and Backhaus 1991). UV-receptors are already present in numerous lower invertebrates, being particularly suitable for the signal "open space" and specifically connected to behavioural units performed in direct light (Menzel 1979). The blue receptor appears not to be connected to any general behavioural unit, but may have been developed for object detection against the sky. This receptor is often lacking in lower invertebrates and shows the greatest variance in arthropods (Menzel 1979; Stavenga and Schwemer 1984). The roots of invertebrate colour vision may thus reach back to wavelength-selective behaviours controlled by the L- and S-receptors which exist already in lower invertebrates. However, much has still to be learned about the evolutionary steps between such primeval systems and the highly resolving trichromatic colour vision systems of Hymenoptera.

Hence, there are two basic possible interpretations, none of which can be precluded by the present investigation: 1. The development of hymenopteran colour vision was already completed before the plants developed coloured labels. They may have been evolutionarily optimized according to criteria other than flower discrimination 2. The evolutionary adaptation of Hymenoptera to floral food sources required additional fine-tuning of the colour vision.

Of course, this problem is closely linked to the limitations that might result from the molecular biology and biochemistry of visual and floral pigments. It appears that there are no constraints on the wavelength position of insect photoreceptors, because the λ_{max} of insect receptor colour types can be found practically across the whole visual spectrum between 320 and 630 nm (Menzel and Backhaus 1991, Fig. 14.2). Similarly, the majority of plant families have the potential to generate colours that spread in the whole colour space of a bee (Menzel and Shmida, in press).

Conclusion

In the present study, we are applying one of the oldest and most mature branches of experimental psychology, colour science, to a problem in evolutionary biology and behavioural ecology. Our investigation of the evolutionary adaptation in the signal-receiver system of flowers and pollinators takes into account: 1. precise physical measurements of the signals (the floral colours); 2. precise measurements of the receivers (the receptors used to discriminate); 3. exact knowledge of the neural processes underlying the insects' perception of colour. On the basis of this data, we are finally in a position to investigate the evolutionary and ecological aspects of a trichromatic system under specific environmental circumstances. The present results are obtained for one sample of flowers, one standard illumination and one average background. The great potential of the analysis is, however, that it can easily be applied to any object sample under any conditions. This enables us to investigate the evolutionary relationship of other visual systems to their coloured world under their respective ecological conditions.

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