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Behavioral experiments on the visual processing of color stimuli in *Pieris brassicae* L. (Lepidoptera)*

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Summary. In spontaneous-choice experiments on the butterfly *Pieris brassicae* L. (Pieridae), spectral-effectiveness and spectral-sensitivity functions were obtained for various behaviors.

Pilot experiments with colored PVC films, for which the relative number of reflected quanta with regard to the given illumination had been calculated, showed that the feeding response is distinctly intensity-dependent (Fig. 4). The animals are also capable of color discrimination independent of this intensity discrimination; *P. brassicae* prefers blue to other colors (e.g., orange, red and purple) with higher relative quantum numbers (Fig. 3) and distinguishes golden yellow and red from gray shades as well as from black and white (Fig. 5a, b).

The results of subsequent spontaneous-choice experiments, using as stimuli monochromatic lights with known quantum flux, indicate that the various visually controlled functional categories of behavior can be assigned to the following spectral regions (Figs. 6, 8): 1. The open-space reaction corresponds to the UV and violet region, ca. 320–420 nm; 2. The feeding reaction corresponds to the blue region, ca. 420–500 nm, and the orange-red region, ca. 590–610 nm; 3. Egg-laying and drumming correspond to the green-yellow region, ca. 520–580 or 590 nm, respectively. The intensity dependence of the individual responses is again apparent in these experiments with monochromatic light stimuli (Figs. 7, 11, 12a).

Even at very high intensities and when the content of the relevant wavelength is high, white light is practically ineffective for the feeding reaction (Fig. 9), drumming and egg-laying (cf. Results), regardless of its UV content. The open-space reaction, however, can be elicited by white light according to its UV content (Fig. 12b). *P. brassicae* cannot be trained to give a feeding response to monochromatic light stimuli (Fig. 10).

Experiments with mixtures of wavelengths have shown that the combination of the two maxima in the spectral sensitivity curve for the feeding reaction ($\lambda = 600$ plus 447 nm) is just as effective as $\lambda = 447$ nm alone (Fig. 13, left). Moreover, the mixture producing the hypothetical '*Pieris* purple' ($\lambda = 600$ plus 370 nm) is no more or less effective in eliciting the feeding and open-space reactions than the more effective component for each of these reactions when presented alone (Fig. 13, right). With the mixture of $\lambda = 600$ plus 558 nm, both the feeding reaction and drumming are distinctly reduced (Fig. 13, middle). This mixed color, unlike the other two mixtures tested, has a color quality different from that of the component colors.

That the behavior of *P. brassicae* is exclusively wavelength-specific can thus be ruled out. There are indications that wavelength-specific behavior and color vision are both present.

Introduction

Since von Frisch's training experiments, published in 1914, showed that the bee is capable of color vision, members of a great variety of insect orders have been examined for this ability. In such tests, color vision has usually been considered to be demonstrated only when training is effective in eliciting a response regardless of a color's intensity. According to Menzel (1979), this is necessary to rule out wavelength-specific behavior, which differs from true color vision in that, in the former case, the

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Abbreviation: RNQ relative number of quanta

* This publication is dedicated to Professor Dr. Dr.h.c. H. Autrum on the occasion of his 80th birthday

signals from various visual receptors are processed separately. If these receptors differ in wavelength specificity, different wavelengths can elicit different behavior although no colors are actually seen.

Attempts to train butterflies have previously been only partially successful, in a few species (Ilse 1928; S.L. Swihart 1970, 1972a; C.A. Swihart 1971). The behavioral studies of butterflies published so far are rather unsatisfactory, for these and other methodological reasons. Although these problems were also pointed out by Burkhardt (1983), he nevertheless stated that color vision had been demonstrated by Ilse (1928), Magnus (1953) and C.A. Swihart (1971) as well as by Swihart and Swihart (1970). Similar statements were made by Wehner (1981), who regarded the spontaneous-choice experiments of Ilse (1928, 1937, 1941) and Crane (1955) on butterflies as demonstrating color contrast and not only brightness contrast. Butterfly color vision thus remains controversial, and evidently requires further study.

Here we describe behavioral experiments in which the processing of color stimuli is examined under reproducible, extensively standardized conditions, with large statistical samples, and with stimuli having accurately quantified physical characteristics.

Pieris brassicae L. was allowed to choose between targets of different colors on which to perform various kinds of behavior; the colors were produced in two ways, first by pigmented films (the relative quantum level of which was measured precisely) and then, in more extensive experiments, by monochromatic light of known quantum flux. No tests of this kind on lepidopterans have previously been published.

Because of the difficulty of training *P. brassicae*, described by Ilse (1928) and confirmed in the present study, the spontaneous-choice paradigm was chosen here.

The results of these experiments were analyzed with regard to the wavelength regions in which *P. brassicae* is sensitive and the extent to which wavelength-specific behavior and color vision can be distinguished in this species.

Materials and methods

Animals. Large cabbage white butterflies (*Pieris brassicae* L.) were raised in cages in a climate-controlled room in the Zoological Institute, at 23–25 °C and 55–65% relative humidity. Light was provided by eight fluorescent tubes ('Truelite Powertwist', 40 W, from the firm Duro-Test, New Jersey, USA) with emission spectrum closely approximating that of sunlight. The automatically controlled light phase lasted from 0600 to 2200 h. About 300 animals, on average, were used in each experiment.

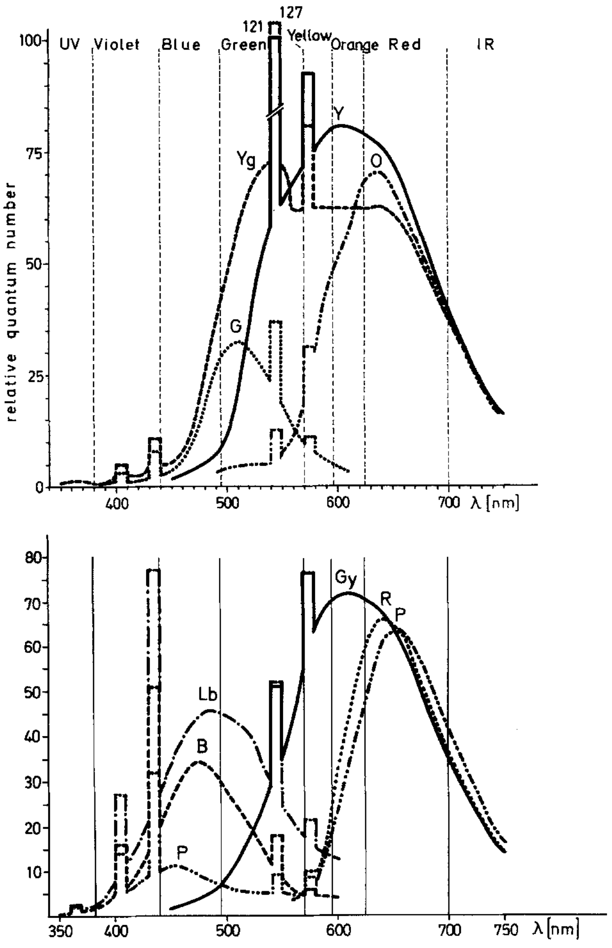

Fig. 1. Relative quanta reflected by the colored PVC films as a function of wavelength. Above: green (*G*); yellow-green (*Yg*); yellow (*Y*); orange (*O*). Below: purple (*P*); blue (*B*); light blue (*Lb*); golden-yellow (*Gy*); red (*R*). Ordinate: relative quantum number. Abscissa: wavelength [nm]

Behavioral criteria. Four different behavior patterns were defined by the following criteria, so that the observer could reproducibly categorize and record the spontaneous responses of the butterflies to various color or light stimuli. First is the behavior called 'feeding reaction' in accordance with Ilse (1928); the butterflies fly to the color stimulus, land on it and touch it with unrolled proboscis. In the second, called 'drumming' (Ilse 1937), the first thoracopod pair is moved rapidly up and down. This behavior serves to test the chemical properties of the colored substrate on which the animal has landed. The third, 'egg-laying', is performed after drumming if the substrate contains a mustard-oil glycoside. For the fourth, the name 'open-space reaction' was proposed in accordance with some considerations by Mazokhin-Porshnyakov (1969); the criterion for this is that the animals fly vigorously to the light source and make rapid, apparently unsystematic back-and-forth movements on it, without unrolling the proboscis or drumming.

Arrangement for experiments with colored films. Spontaneous choice behavior during the feeding reaction was studied with Plexiglas feeding stands (Scherer 1979), each of which was hollowed out in 16 places to form bowls for honey-water. Around the edge of each bowl were attached self-adhesive stars of PVC film (Plastiflex, Overath) in nine different colors (Fig. 1) form-

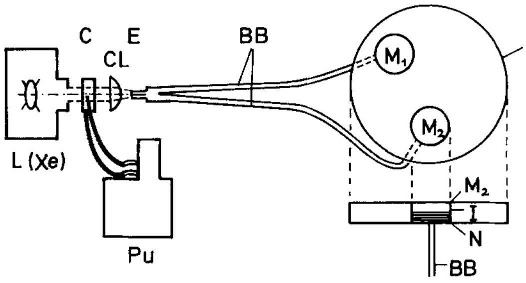

Fig. 2. Experimental apparatus for monochromatic lights: *BB* glass fiber-optic bundle; *C* cooling cuvette; *CL* quartz plano-convex lens; *E* entrance end of fiber-optic bundle; *I* interference filter; *L(Xe)* XBO 900; $M_{1,2}$ Plexiglas matt disks, *N* neutral gray filters; *P* experimental plate; *Pu* roll-round pump

ing various arrangements. During the experiments every bowl was filled with the same quantity of honey-water at the same concentration, so that the influence of odor was exactly the same for all colors.

For each of the nine films the amount of light reflected at different wavelengths was measured approximately and plotted in terms of the relative quantum number (Fig. 1; see Kolb and Scherer 1982), so that the quantum levels of the variously colored stars could be taken into account when evaluating the results of the experiments. The experiments were subdivided as follows: series = set of five to ten trials; trial = set of at least 50 visits; visit = single decision. The terms 'experiment' and 'test' are used in a more general sense, and usually include some series of trials. As a rule, about 1000 visits were recorded per series. The individual trials lasted 40–80 min on average; they were carried out between 0830 and 1200 h in the climate-controlled room with illumination intensity 400 μW/cm². From the original data (visits to test targets as percentage of total visits in each trial) the mean and standard deviation were calculated for each series and normalized with respect to the maximum in those series. The standard deviation $s=\sqrt{s^2}$ (s^2 = variance) was calculated by the formula

$$s^2=1/(n\text{-}1)\cdot[\Sigma x^2\text{-}1/n\cdot(\Sigma x)^2]$$

where n is the number of trials, x is the percentage of visits per trial, and Σ denotes summation.

Arrangement for experiments with monochromatic light stimuli. Flicker-free light stimuli were provided by an Osram Xenon high-pressure lamp (XBO, 900 W) in combination with a rectifier REXA 50 ROZ (Schoeffel). For wavelengths >400 nm the light was passed through a heat filter (a cuvette through which water flowed continuously; Fig. 2C) and focussed by a quartz plano-convex lens (CL) onto a two-branched glass fiber-optic bundle (BB). The branches illuminated two Plexiglas matt disks (M_1 and M_2) 5 cm in diameter, set into a round, gray plastic plate (P: diameter 15 cm, thickness 1 cm) so that they could be easily exchanged. Narrow-band interference filters (I), with half-height widths between 7 and 15 nm, and neutral density filters (N) were placed in the beam between the exit ends of the fiber-optics and the matt disks.

For experiments in the UV range M_1 was replaced by a quartz matt disk and illuminated with a quartz fiber-optic bundle. The selected interference filter was placed in a water-cooled cuvette (cf. Raschke 1967) so that it was in the beam of the Xenon lamp. M_2 was illuminated by a special halogen light source by way of the glassfiber optic.

A further rearrangement enabled the mixing of two spectral colors. M_1 was illuminated simultaneously by the quartz-fiber optic bundle and the glass-fiber optic. Their ends were mounted below M_1 so that the emerging light cones exactly covered the matt disk. Both fiber optics received light from sources in water-cooled cuvettes with built-in interference filters. M_2 was illuminated by a second beam from the Xenon lamp by way of a second glass-fiber optic. In this case the white light from the Xenon lamp could also be used directly as a stimulus. Because the glass fibers were nearly opaque to UV light, the emerging white light had only a small UV component, but white light with a high UV content could be provided by exchanging the glass-fiber optic and the quartz-fiber optic. In analogy with Daumer's (1963) 'white without UV' (zinc white) and 'white with UV' (lead white), the two kinds of white light used here are called 'white (UV−)' and 'white (UV+)'. White (UV−), at 5000 μW/cm², contained $\lambda=370$ nm at 0.5 μW/cm², $\lambda=396$ nm at 13.3 μW/cm², and $\lambda=447$ nm at 90 μW/cm². For white (UV+) at 5000 μW/cm² the corresponding values are 21.7, 86 and 115 μW/cm².

The quantum flux at each wavelength was equalized on the basis of light measurements with an optometer (United Detector Technology, Type 40 X) with specially calibrated detector. The measurements were made directly at the surface of the matt disks. The stimuli had the geometrical form of a circular illuminated plane. The lights were scattered by the matt disks to such an extent that there were no differences in hue or intensity at any angle of view.

In each of the spontaneous-choice trials performed in the laboratory the butterflies could choose between two matt disks, one of which was illuminated by a constant comparison light and the other with the test light. When egg-laying was to be elicited, it was necessary to spray the experimental plate with sinigrin solution (ca. 0.03 g/400 ml distilled water). After 4–5 h the eggs laid on it were counted, a 120° sector of the plate being assigned to each matt disk. A sector of 120° was chosen because the test plate was constructed in such a way that a third matt disk could be inserted. Therefore with two stimuli, each represented only a third of the plate, and this had to be considered in order to obtain an accurate count of the eggs related to each stimulus. This form of behavior was studied in series of four trials with a given stimulus; in each such series, ca. 400–1000 eggs were laid.

For other forms of behavior (the feeding, drumming and open-space reactions), records were kept of the animals' choices in series of from four to ten trials, with 50 visits per trial. The duration of a single trial was 10 to 100 min, depending on the number of animals, their condition (e.g., state of hunger), the temperature of the room and the attractiveness of the wavelengths being tested. To ensure that the spontaneous choices at all wavelengths were equally subject to whatever local influences were present, the two matt disks were reversed to the mirror-image arrangement halfway through each series of trials. The experiments were carried out between 1000 and 1800 h under constant conditions, at a temperature of 24–29 °C. The intensity of the lighting from above (Philips TL fluorescent tube, 40W/33) was 23 μW/cm² at the level of the experimental plate (cf. Kolb and Scherer 1982). In tests of the open-space reaction the illumination above the apparatus was lowered to 1–2 μW/cm² by darkening the cage in which the butterflies had been put into the laboratory, because the white room lighting acted as a strong positive phototactic stimulus toward which the butterflies were diverted. A change in the state of adaptation of the animals, otherwise kept constant in all experiments, had to be accepted in order to avoid this problem.

Calculation of the spectral sensitivities. The functions describing spectral sensitivity were calculated on the basis of the characteristic curves for intensity dependence of the various behavioral reactions. Because the characteristics for the different reactions only approximately paralleled one another (see, e.g., Figs. 7, 11, 12a), the univariance principle (Rushton 1972) was not entirely applicable to any response magnitude. In choosing the response level on which to base the spectral-sensitivity functions, we therefore used the criterion of subjectively equal brightness. That is, it can be assumed that when a test wavelength and the constant-intensity comparison wavelength are chosen with equal frequency (each 50%), the two appear equally bright to the animals; therefore any test wavelengths eliciting 50% response with the same comparison wavelength will appear equally bright. Hence we used the intensity-dependence functions to find those quantum fluxes at which the animals visited the variable and constant wavelength equally often, by drawing a line parallel to the abscissa at the 50% response level and reading off the desired values at the points (equivalence points: Schümperli 1973) where this line intersected the individual curves (see, e.g.,Fig. 7). From the reciprocals of these quantum fluxes we obtained the functions of spectral sensitivity. For the points representing the constant comparison wavelengths, we took their (constant) quantum flux in the corresponding experiments. If the constant wavelength could have been compared with a light of the same wavelength (i.e., constant = variable wavelength), this is the quantum flux for which 50% choice frequency would have been expected.

Results

Feeding reaction

Pilot experiments with colored PVC, the characteristics of which are given inFig. 1 in terms of relative quantum number vs. wavelength, revealed the connection between the feeding reaction and the integral relative number of quanta (RNQ) of the film (see Kolb and Scherer 1982). In these trials a total of 6394 visits were recorded; the results are plotted in Fig. 3. There was a significant preference for the yellow film (RNQ 1578); the other responses were normalized with respect to this maximum (=100%). Golden yellow (RNQ 1208) followed with 50.6%, and blue (RNQ 456) with 26.6%. Other colors were visited less often than blue despite their higher RNQ – red (RNQ 719) with 15.2%, orange (RNQ 905) with 12.7%, and purple (RNQ 890) with 4.1%. Green (RNQ 329; 0.9%) elicited almost no feeding reaction.

Four shades of blue and four of yellow (blue and yellow films covered with translucent films of different neutral density) were then tested, to learn the extent to which the tendency to visit these colors depended on their intensity (Fig. 4; total of 1589 visits). To explore this question further, golden yellow was presented together with a sequence consisting of black, four shades of gray and white (Fig. 5a). The RNQ of golden yellow (1209) was well below that of white (2693) and the lightest shade of gray (2129). If the decision were based mainly on brightness, white and the lightest gray shade should be preferred and golden yellow should not be discriminated from the most similar gray (RNQ 1026). In fact, however, golden yellow was clearly picked out from the rest (852 visits).

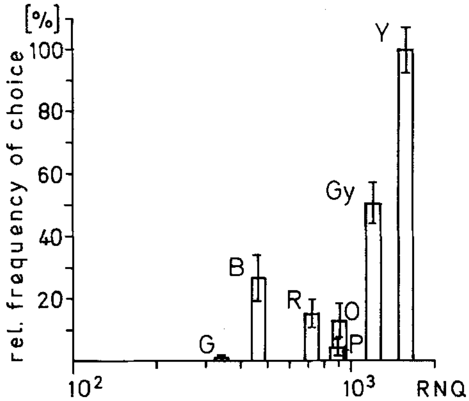

Fig. 3. Feeding reaction to colored PVC as a function of the relative quantum number of the film. From left to right: green (*G*); blue (*B*); red (*R*); purple (*P*); orange (*O*); golden-yellow (*Gy*); yellow (*Y*). Ordinate: relative frequency of choice [%], normalized to yellow (=100%). Means with standard deviation. Abscissa: relative number of quanta (RNQ)

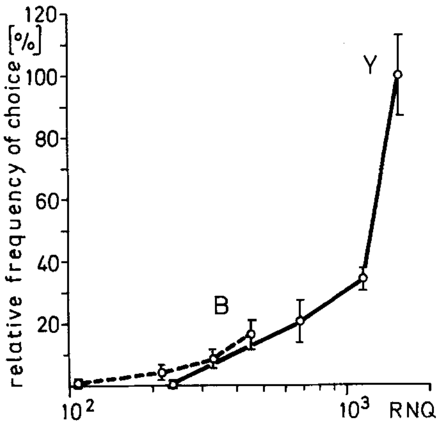

Fig. 4. Intensity dependence of feeding reaction to blue (*B*) and yellow (*Y*). Ordinate: relative frequency of choice [%], normalized to yellow (=100%). Abscissa: relative number of quanta (RNQ)

Red was also tested against black, gray and white (Fig. 5b). This color was interesting inasmuch as bees confuse it with black and dark gray (von Frisch 1914). Although white (RNQ 2693) and three gray shades (RNQ 2129, 1026, 858) had higher relative quantum numbers than the red, the latter was clearly preferred (a total of 640 visits).

The *spectral effectiveness* of monochromatic lights (with equal quantum flux) in eliciting the feeding reaction was tested with 19 wavelengths between 352 and 615 nm: 352, 370, 396, 411, 417, 427, 447, 469, 483, 496, 517, 524, 542, 558, 582, 588, 600, 605 and 615 nm. The constant comparison wavelength was $\lambda = 447$ nm, with a quantum flux of 3×10^{13} quanta $\times \mathrm{s}^{-1} \times \mathrm{cm}^{-2}$. The other

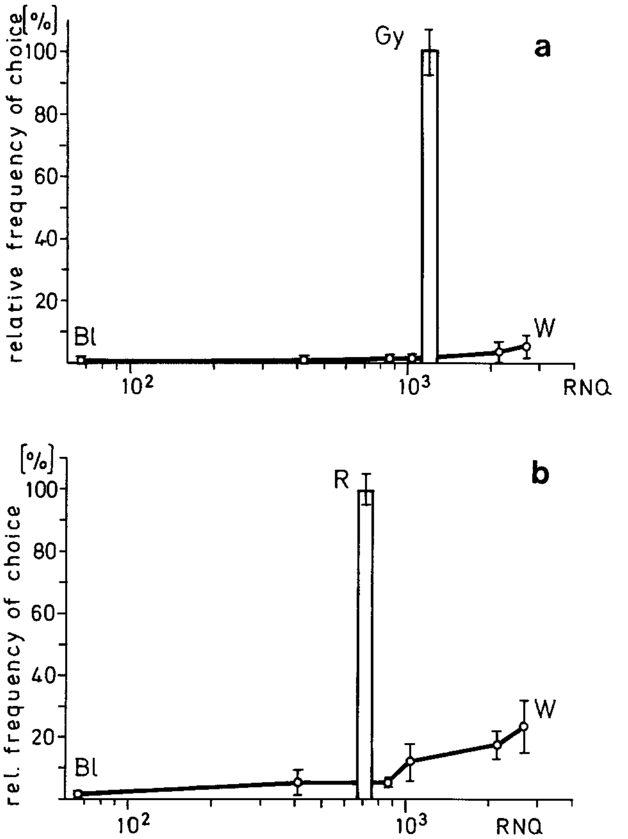

Fig. 5. Feeding reaction to **a** golden-yellow (*Gy*) and **b** red (*R*) as compared with black (*Bl*), white (*W*) and shades of gray. Ordinate: relative frequency of choice [%], normalized to golden-yellow and red, respectively (=100%). Means with standard deviation. Abscissa: relative number of quanta (RNQ)

wavelengths were adjusted to this level. Each wavelength was presented together with $\lambda = 447$ nm (apparatus shown in Fig. 2). In a total of 9000 recorded visits, the spectral effectiveness function (Fig. 6FR) was found to have a main peak at $\lambda = 447$ nm (=100%), a secondary peak at $\lambda = 600$ nm (44.1% relative frequency of choice) and a minor rise to 2.7% at $\lambda = 370$ nm, not a significant departure from the flat tail of the curve.

Experiments on *intensity dependence* of the feeding reaction were carried out with 12 wavelengths (Fig. 7). Here the constant comparison wavelength was $\lambda = 496$ nm (3×10^{13} quanta $\times\, s^{-1} \times cm^{-2}$), because only a relatively unattractive wavelength allows enough latitude for intensity gradations of other wavelengths. Even so, this choice put upper limits on some of the wavelengths that are relatively ineffective in eliciting the feeding reaction. For instance, given the transmittance of the interference filter and the emission spectrum of the Xenon lamp, the lights at $\lambda = 396$ and 582 nm could not be made intense enough to attract visits at a rate equal to or higher than that for $\lambda = 496$ nm. For these two wavelengths, the curve was extrapolated to the 50% response level. The 12 functions plotted in Fig. 7 are based on a total of 29250 visits.

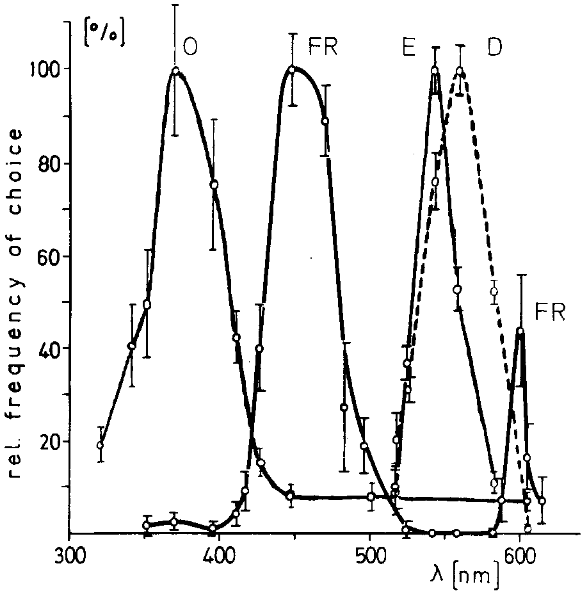

Fig. 6. Spectral effectiveness functions for the open-space reaction (*O*), the feeding reaction (*FR*), egg-laying (*E*) and drumming (*D*). Ordinate: relative frequency of choice [%], normalized to the maximally visited wavelength (=100%). Abscissa: wavelength [nm]

The characteristics in Fig. 7 were used to calculate the function for *spectral sensitivity* of the feeding reaction (FR in Fig. 8). The two maxima of spectral effectiveness were confirmed precisely: $\lambda_{max1} = 447$ nm (63 quanta$^{-1} \times s \times cm^2 \times 10^{-14}$) and $\lambda_{max2} = 600$ nm (4 quanta$^{-1} \times s \times cm^2 \times 10^{-14}$). Again there is a minor rise at $\lambda = 370$ nm (1 quantum$^{-1} \times s \times cm^2 \times 10^{-14}$).

In tests of the feeding reaction to *white light* as compared with $\lambda = 447$ nm, white (UV −) was raised from 500 through 5000 to 50000 $\mu W/cm^2$ while $\lambda = 447$ nm was presented at a constant intensity of 41 $\mu W/cm^2$. The latter corresponded to only half the standard quantum flux of 3×10^{13} quanta $\times\, s^{-1} \times cm^{-2}$, so as to make any effects of the white light easier to discern. Despite its high intensities, the white light attracted only a small fraction of the total visits: $6.4 \pm 3.2\%$ at 500 $\mu W/cm^2$, $5.6 \pm 4.6\%$ at 5000 $\mu W/cm^2$, and $17.2 \pm 6.6\%$ at 50000 $\mu W/cm^2$ (Fig. 9, solid line; 750 visits). The contribution of $\lambda = 447$ nm to the white stimuli (measured with the 447-nm filter in the beam of the white lights) was 9, 90 and 900 $\mu W/cm^2$, respectively, so that at the two highest white-light intensities this component exceeded the monochromatic comparison stimulus by factors of two and twenty.

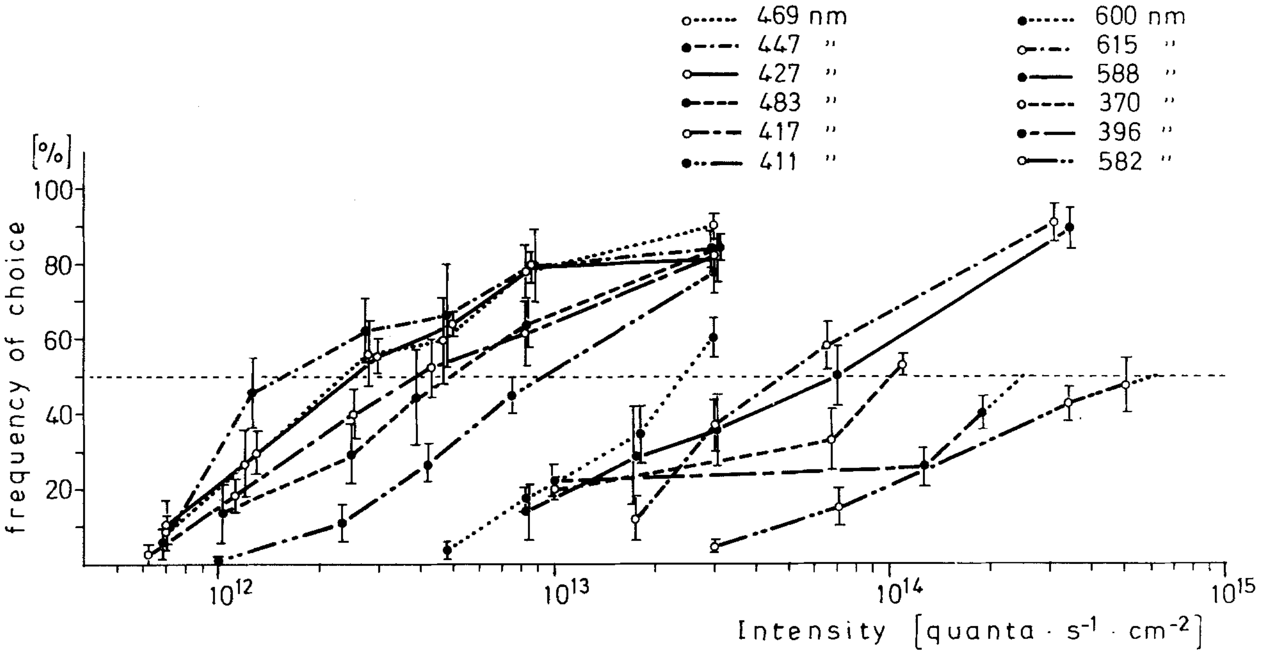

Fig. 7. Intensity dependence of feeding reaction at twelve wavelengths, $\lambda = 496$ nm constant at 3×10^{13} quanta $\times s^{-1} \times cm^{-2}$. Ordinate: frequency of choice [% of total visits]. Means with standard deviation. Abscissa: intensity [quanta $\times s^{-1} \times cm^{-2}$]

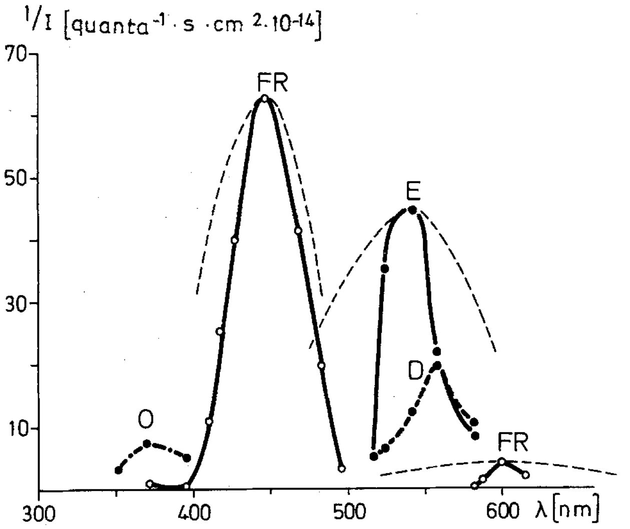

Fig. 8. Spectral sensitivity functions for the open-space reaction (*O*), the feeding reaction (*FR*), egg-laying (*E*) and drumming (*D*). Thin broken curves: absorption curves of hypothetical rhodopsins, from Dartnall (1953). Ordinate: reciprocal quantum flux 1/I [quanta$^{-1} \times s \times cm^2 \times 10^{-14}$]. Abscissa: wavelength [nm]

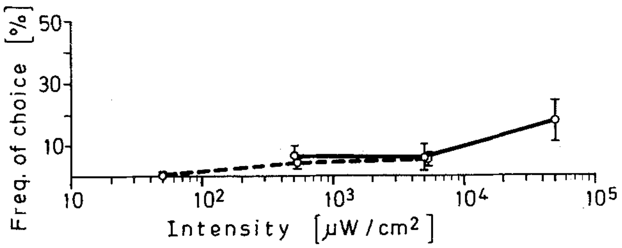

Fig. 9. Intensity dependence of feeding reaction to white light. Continuous line: white (UV−), $\lambda = 447$ nm constant at 41 µW/cm²; dashed line: white (UV+), $\lambda = 447$ nm constant at 82 µW/cm². Ordinate: frequency of choice [% of total visits]. Means with standard deviation. Abscissa: intensity of white light [µW/cm²]

Similar results were obtained in experiments with white (UV+) at the intensities 50, 500 and 5000 µW/cm². Again the constant comparison light was $\lambda = 447$ nm, in this case at 82 µW/cm², corresponding to the standard 3×10^{13} quanta $\times s^{-1} \times cm^{-2}$. Here, again, the visits to the white light were far fewer than those to the comparison stimulus: 0.6±1% at 50 µW/cm², 4±1.5% at 500 µW/cm², and 5.2±2% at 5000 µW/cm² (Fig. 9, dashed). Each value is based on 500 visits. The contribution of $\lambda = 447$ nm to the white light was 1.2, 11.5 and 115 µW/cm², so that this component was more intense than the monochromatic comparison light at the highest white-light intensity.

Training experiments involving the feeding reaction were carried out as follows. For about an hour a matt disk illuminated with $\lambda = 600$ nm (3×10^{13} quanta $\times s^{-1} \times cm^{-2}$) was presented together with food. During this time 100 visits were recorded. Then the test stimulus was uncovered, a light with $\lambda = 447$ nm and a quantum flux equal to that of the 600-nm training light, and the matt disk with food above the training light was ex-

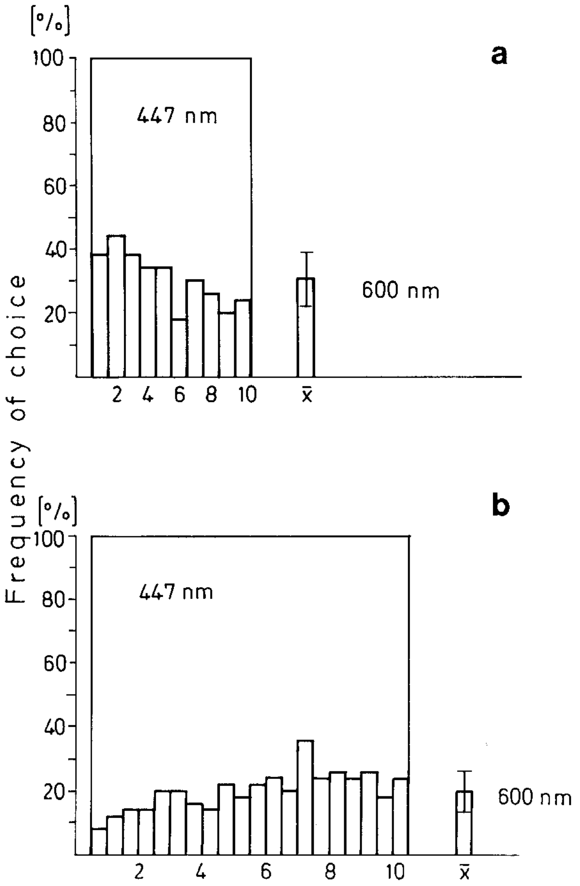

Fig. 10a, b. Training experiment with feeding reaction to $\lambda = 600$ nm in comparison with $\lambda = 447$ nm. **a** Spontaneous choice; **b** choice after training session. Ordinate: frequency of choice [% of total visits]. Below (histogram): $\lambda = 600$ nm; above (white box): $\lambda = 447$ nm, together = 100%. Abscissa: individual experiments, in the order in which they were done (numbers below graph). $\bar{x}$ mean of all experiments, with standard deviation

changed for a matt disk without food. The animals' choices were observed in two consecutive trials with 50 visits each. The whole procedure (beginning with the training and ending with the two consecutive trials) was repeated ten times, so that a total of 2000 visits were counted. The training period did not raise the proportion of visits to the disk illuminated with $\lambda = 600$ nm (20 ± 6.2%) above that observed in spontaneous-choice trials (30.6 ± 8.4%); indeed, there was a slight but not significant reduction in the frequency of visits (Fig. 10).

The butterflies were numbered on their wings with a felt pen, so that a record could be kept of the individuals that visited the training wavelength, both during training and in the subsequent trials. The individuals' behavior conformed to the overall results; of the butterflies that visited $\lambda = 600$ nm in the test, only 30% on average had also visited $\lambda = 600$ nm during the training period. That is, 70% of the visits to this disk after training were spontaneous choices. This result, again, reveals no effect of training.

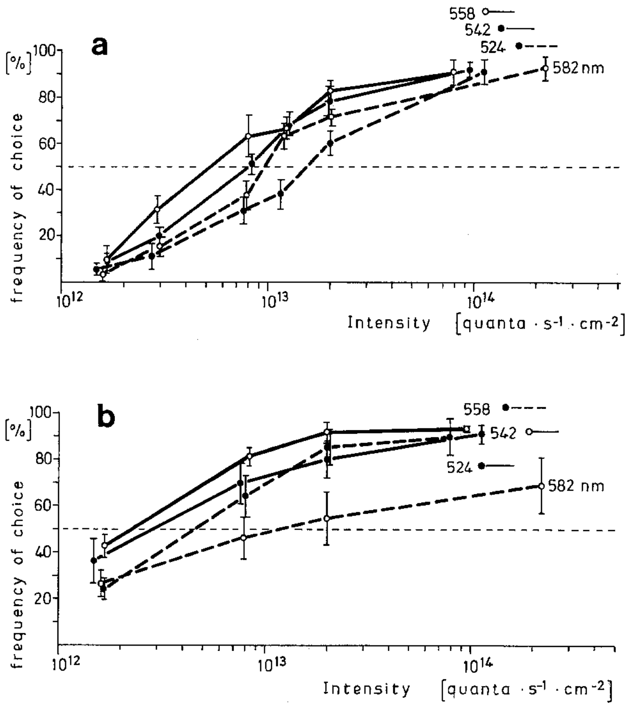

Fig. 11. Intensity dependence of **a** drumming and **b** egg-laying at four wavelengths. $\lambda = 517$ nm constant at 2×10^{13} quanta $\times$ $s^{-1} \times cm^{-2}$. Ordinate: frequency of choice [% of total visits]. Means with standard deviation. Abscissa: intensity [quanta $\times$ $s^{-1} \times cm^{-2}$]

Attempts to train the animals to $\lambda = 417$, 469, 496 and 588 nm in single trials (100 training and 100 test visits each) were also unsuccessful.

Drumming

The *spectral effectiveness* of stimuli for drumming was studied with $\lambda = 517$, 524, 542, 558, 582 and 605 nm. From observations during the tests on the feeding reaction it was known that there was no drumming in any other wavelength region. The constant comparison wavelength was $\lambda = 517$ nm, with quantum flux 2×10^{13} quanta $\times s^{-1} \times cm^{-2}$, to which the other wavelengths were adjusted. Evaluation of 2300 visits revealed a maximum at $\lambda = 558$ nm; the normalized spectral-effectiveness function is shown in Fig. 6 (D).

The *intensity characteristics* were measured with $\lambda = 517$ nm as the constant comparison wavelength (2×10^{13} quanta $\times s^{-1} \times cm^{-2}$), for $\lambda = 524$, 542, 558 and 582 nm (Fig. 11a). Each curve is based on 3000 visits.

As in the case of the feeding reaction, the spectral sensitivity function for drumming was calculated on the basis of the 50% response level of the intensity characteristics (Fig. 8, D). Its maximum, like that of the spectral effectiveness function, is at $\lambda = 558$ nm.

The effect of *white (UV+)* was investigated in the same way as for the feeding reaction. White light from the Xenon lamp at 50, 500 and 5000 µW/cm^2, with $\lambda = 558$ nm as the comparison stimulus (constant 40 µW/cm^2, corresponding to the standard level of 2×10^{13} quanta $\times$ s^{-1} $\times$ cm^{-2}) elicited no drumming. Of 200 visits with each intensity, none was to white; all 600 visits were elicited by $\lambda = 558$ nm. The contribution of $\lambda =$ 558 nm to the white-light stimuli was 1.9, 18.6 and 186 µW/cm^2.

Egg-laying

Most of the above wavelengths were tested for egg-laying: $\lambda = 517$, 524, 542, 558 and 582 nm. Since there was no drumming in any other wavelength region (see above), egg-laying also did not take place in any of these. Again the constant comparison wavelength was $\lambda = 517$ nm (2×10^{13} quanta $\times$ s^{-1} $\times$ cm^{-2}). The *spectral effectiveness* function for egg-laying (Fig. 6, E) was significantly different in shape from that for drumming. Whereas the maximum for drumming was $\lambda = 558$ nm, that for egg-laying was at $\lambda = 542$ nm. A total of 2401 eggs were laid in this experiment.

Intensity characteristics were measured for $\lambda =$ 524, 542, 558 and 582 nm, with $\lambda = 517$ nm as constant comparison wavelength (Fig. 11b). In these series of trials a total of 10023 eggs were laid.

The *spectral sensitivity* function is shown in Fig. 8 (E). There are distinct differences between this and the corresponding function for drumming. The maximum, like that of the spectral effectiveness function for egg-laying, is at $\lambda = 542$ nm, to the left of that for drumming. It is also distinctly higher (45 quanta^{-1} $\times$ s $\times$ cm^2 $\times$ 10^{-14}) than that for drumming (19 quanta^{-1} $\times$ s $\times$ cm^2 $\times$ 10^{-14}).

White (UV+), which was ineffective for drumming, was also not selected for egg-laying, although the highest of the three white intensities 50, 500 and 5000 µW/cm^2 had a large component at the comparison wavelength $\lambda = 542$ nm (its contribution to the three white stimuli was 1.7, 16.7 and 166.7 µW/cm^2, as compared with the constant 36 µW/cm^2, or 2×10^{13} quanta $\times$ s^{-1} $\times$ cm^{-2}, of the comparison stimulus). Of the 2813 eggs deposited in tests of the three intensities, only six were on the white disk.

Open-space reaction

The open-space reaction was tested with the following ten wavelengths: 321, 342, 352, 370, 396, 411, 427, 447, 501 and 605 nm. The constant comparison wavelength was $\lambda = 396$ nm, with a quantum flux of 2×10^{13} quanta $\times$ s^{-1} $\times$ cm^{-2}. Evaluation of 3000 visits produced a *spectral effectiveness* function with a maximum at $\lambda = 370$ nm (Fig. 6, O).

The *intensity dependence* was first studied with a constant comparison wavelength of $\lambda = 427$ nm (2×10^{13} quanta $\times$ s^{-1} $\times$ cm^{-2}). But because this wavelength attracted very few visits, after one curve for $\lambda = 370$ nm had been obtained the comparison wavelength was changed to $\lambda = 396$ nm (2×10^{13} quanta $\times$ s^{-1} $\times$ cm^{-2}) and measurements were made for $\lambda = 370$ and 352 nm (Fig. 12a; 8000 visits).

On the basis of the 50% response level of the above two curves (Fig. 12a) and the standard quantum flux for $\lambda = 396$ nm (2×10^{13} quanta $\times$ s^{-1} $\times$ cm^{-2}), a curve approximating the *spectral sensitivity* function was constructed (Fig. 8, O). Although there are so few points, it is evident that the maximum coincides with that for spectral effectiveness, $\lambda = 370$ nm.

Whereas the effectiveness of *white (UV+)* on the feeding reaction, drumming and egg-laying was tested only against monochromatic light, for the open-space reaction it was compared first with $\lambda =$ 396 nm and then directly with white (UV−). In the first case the white (UV+) light had the intensities 50, 500 and 5000 µW/cm^2 and that at $\lambda = 396$ had a constant 43 µW/cm^2 (corresponding to the standard level in the preceding tests, 2×10^{13} quanta $\times$ s^{-1} $\times$ cm^{-2}). The contribution of this wavelength to the white lights was 0.9, 8.6 and 86 µW/cm^2. A total of 1500 visits were recorded. The solid curve in Fig. 12b shows that white (UV+), though it attracts no feeding, drumming or egg-laying, is very effective in eliciting the open-space reaction. At only 50 µW/cm^2 it attracted one-third of the total number of visits (32.4 ± 7.4%). At the higher intensities even more visits were attracted by white (UV+) than by $\lambda =$ 396 nm: 59.2 ± 9.4% for 500 µW/cm^2 and 77.4 ± 8.4% for 5000 µW/cm^2.

In the comparison with white (UV−) the intensities of white (UV+) were 500 and 5000 µW/cm^2. The constant intensity of the white (UV−) was 5000 µW/cm^2 (contribution of $\lambda = 396$ nm: 13.3 µW/cm^2). The results (Fig. 12b), dashed line) were nearly identical to those of the comparison with $\lambda = 396$ nm; the white lights with large UV

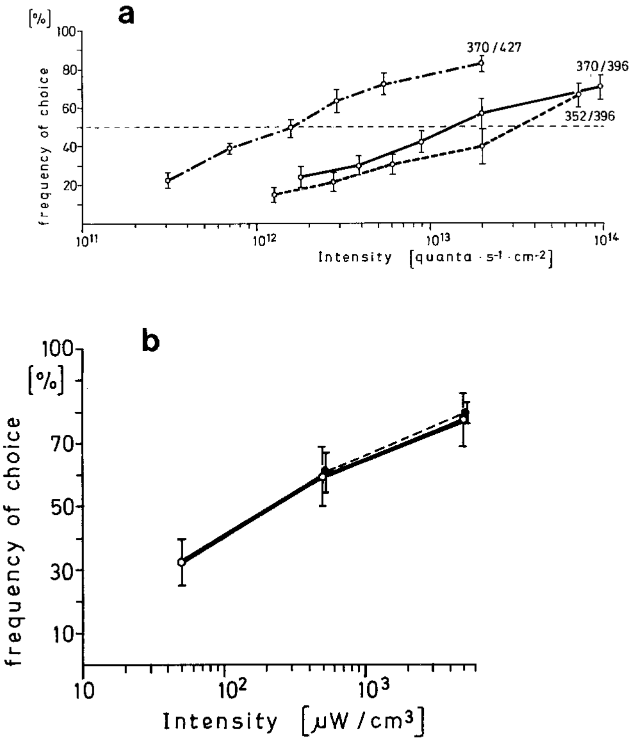

Fig. 12. Intensity dependence of open-space reaction **a** to $\lambda=370$ and 352 nm, compared with $\lambda=427$ and 396 nm constant at 2×10^{13} quanta $\times\,\mathrm{s}^{-1}\times\mathrm{cm}^{-2}$; **b** to white (UV+) compared with $\lambda=396$ nm, constant at 43 μW/cm² (continuous curve) and with white (UV−), constant at 5000 μW/cm² (dashed curve). Ordinate: frequency of choice [% of total visits]. Means with standard deviation. Abscissa: intensity [quanta $\times\,\mathrm{s}^{-1}\times\mathrm{cm}^{-2}$] or [μW/cm²]

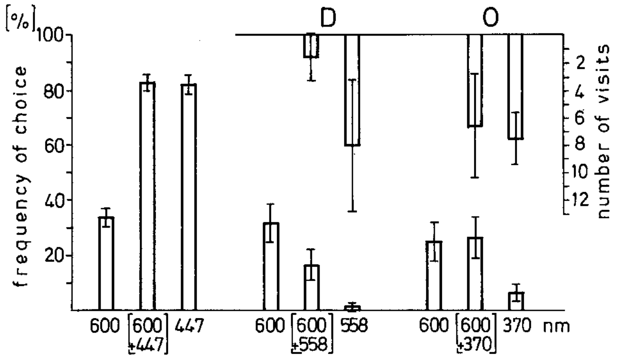

Fig. 13. Effects of mixtures of wavelengths. Below: feeding reaction to mixtures of $\lambda=600$ nm with $\lambda=447$, 558 and 370 nm (3×10^{13} quanta $\times\,\mathrm{s}^{-1}\times\mathrm{cm}^{-2}$) and to the single wavelengths (1.5×10^{13} quanta $\times\,\mathrm{s}^{-1}\times\mathrm{cm}^{-2}$), compared with $\lambda=427$ constant at 3×10^{13} quanta $\times\,\mathrm{s}^{-1}\times\mathrm{cm}^{-2}$. Above: drumming (*D*) and open-space (*O*) reactions to the mixtures or single wavelengths shown on the abscissa. Ordinate: frequency of choice [% of total visits] or absolute number of visits. Means with standard deviation. Abscissa: wavelengths or mixtures of wavelengths

component were clearly preferred (60.2±6% and 79.6±3.6%; 1000 visits).

Mixtures of wavelengths

Among the wavelength mixtures tested, the purple colors were particularly interesting; these are colors not present in the spectrum but produced by mixing the colors at the two ends of the spectrum effective in eliciting an animal's behavior. One of the questions to be answered was whether there exists a '*Pieris* purple', in analogy with the bee purple (a mixture of $\lambda=360$ and 588 nm) found by Daumer (1956). Another was whether the preference of *P. brassicae* for 'human purple' (violet plus red) found by Ilse (1928), but not evident in our pilot experiments with PVC film, would be confirmed with light mixtures. Finally, we expected the results to provide evidence as to whether different receptors interact in the processing of visual stimuli.

Three different mixtures of monochromatic light were presented: 600 + 447 nm ('purple', comparable to the purple of Ilse 1928), 600 + 558 nm and 600 + 370 nm (hypothetical *Pieris* purple). The first color is a mixture of the two maxima for the feeding reaction. The second and third are formed by mixing the secondary maximum for the feeding reaction with the maxima for drumming and the open-space reaction, respectively. In all tests the constant comparison wavelength was $\lambda=427$ nm (3×10^{13} quanta $\times\,\mathrm{s}^{-1}\times\mathrm{cm}^{-2}$, the standard quantum flux in the experiments on spectral effectiveness). The two lights to be mixed were each adjusted to 1.5×10^{13} quanta $\times\,\mathrm{s}^{-1}\times\mathrm{cm}^{-2}$, so that the mixture would have the same quantum flux as the comparison stimulus. The effectiveness of the component lights was tested separately as well as in the mixture.

The mixture of the two maxima for the feeding reaction, $\lambda=447$ plus 600 nm, elicited visits just as often as did the blue component $\lambda=447$ nm alone, although the quantum flux of the mixture was twice as great (Fig. 13, left).

A similar result was obtained with the mixture $\lambda=600$ plus 370 nm (Fig. 13, right). The mixture attracted just as many feeding-reaction visits as $\lambda=600$ nm alone. Occasionally, however, it elicited the open-space reaction. The records of such responses, kept simultaneously, indicate that the mixture of these two wavelengths elicited almost the same proportion of visits for the open-space reaction as did the wavelength maximally effective for that reaction ($\lambda=370$ nm) alone (Fig. 13, upper right: O). Because the number of such visits was smaller than those for the feeding reaction, and not normalizable (600 + 370 nm: average 6.6±3.8

visits per trial, 66 total; 370 nm: average 7.5 ± 1.9 visits per trial, 30 total), the ordinate is scaled in terms of absolute number of visits for the open-space reaction, rather than percentage as for the feeding reaction. This mixture has no color quality of its own ('*Pieris* purple') for *P. brassicae*.

The effect of the mixture $\lambda = 600$ plus 558 nm was quite different. For both reactions – feeding and drumming – the mixed light elicited significantly fewer visits than did the appropriate component alone, but distinctly more than the inappropriate component alone (Fig. 13, middle). The results for drumming are represented by the upper bars in the middle of Fig. 13 (D; 600 + 558 nm: average 1.6 ± 1.7 visits per trial, 16 total; 558 nm: average 8 ± 4.8 visits per trial, 32 total), with ordinate scale as described above for the open-space reaction. In this case, the mixed light apparently has a different color quality for *P. brassicae* than either of the component lights alone.

Discussion

The results of these experiments have demonstrated four behavioral functions of *P. brassicae* – the feeding, drumming, egg-laying and open-space reactions – that are elicited visually, by light in precisely measured wavelength regions. The feeding reaction is clearly the only one with a bimodal spectral-effectiveness curve; its main peak is in the blue, with a secondary peak in the orange-red. Stimuli in the yellow-green region, where the spectral effectiveness curve for the feeding reaction falls to zero, induce the butterflies to lay eggs (more toward green) or engage in drumming (more toward yellow). In these regions the choice percentage for the open-space reaction falls below 10%. There is a strikingly sharp transition from the feeding reaction to egg-laying; within a very narrow range of wavelengths, one behavior gives way to the other.

Ilse mentioned the feeding reaction of *P. brassicae* in 1928 and the drumming behavior in 1937, but not until now have four color-dependent functional categories of behavior been clearly defined in butterflies, with precise documentation of the associated spectral regions (cf. Autrum 1975; Autrum and Thomas 1973; Menzel 1979).

Kolb and Scherer (1982) had already studied the spectral effectiveness of drumming and egg-laying with a somewhat different apparatus. Our reasons for describing similar experiments here are as follows. First, it was necessary to prove with another method the fact that there were really two maxima. To do so, we repeated experiments on spectral effectiveness with only two stimuli presented simultaneously, not three as in 1982. Only these data are strictly comparable with the results of the two-choice experiments on the feeding and open-space reactions. Furthermore, we wanted to obtain a function for the spectral sensitivity of drumming and egg-laying, which has not previously been measured.

The form of the spectral effectiveness and spectral sensitivity functions measured for the four behavioral categories in *Pieris brassicae* suggests a direct correlation with the spectral response functions of this butterfly's receptors.

Can one say, then, that the behavior of *P. brassicae* is directly controlled by the retina? Such a conclusion would be more consistent with wavelength-specific behavior as discussed by Menzel (1979) than with color vision. A comparison between the present behavioral findings and the results of electrophysiological studies on the visual system of the butterfly is of interest in this respect.

The latter data were obtained by Paul (1981), Steiner (1984), Paul et al. (1986) and Steiner et al. (1987) by means of Fourier interferometric stimulation (Gemperlein 1980, 1982) and ERG recording. By this procedure, *P. brassicae* was found to have a tetrachromatic system with maxima at $\lambda =$ 360, 450, 540 and 620 nm in the ventral eye region. The 'nonlinear' analysis of the data (Steiner 1984) shows that the rhodopsins are not located within a single cell. Given the presence of nine visual cells per ommatidium (Kolb 1977, 1978), this result is not unexpected.

To learn whether and in what way these receptors are utilized, behavioral experiments are required. In the following paragraphs, we discuss the bearing of the present results on (a) the correspondences and (b) the differences between behavior and receptor properties.

a. In *P. brassicae* a correspondence is found between the open-space reaction ($\lambda\text{max} = 370$ nm) and the UV receptor ($\lambda\text{max} = 360$ nm), between the feeding reaction ($\lambda\text{max} = 447$ nm) and the blue receptor ($\lambda\text{max} = 450$ nm) and between drumming ($\lambda\text{max} = 558$ nm) and egg-laying ($\lambda\text{max} = 542$ nm) and the green receptor ($\lambda\text{max} = 540$ nm).

Furthermore, white (UV+) is very effective in eliciting the open-space reaction, being preferred to white (UV−) at equal or greater intensities (Fig. 12b). It follows that a UV receptor independent of the rest of the visual system could be present. Behavioral experiments with mixed wavelengths also provide grounds for inferring independently operating receptors. For instance, a mixture of $\lambda = 447$ plus 600 nm (the maxima for the feeding reaction) is visited just as often as $\lambda = 447$ nm alone (Fig. 13).

The results of trials with a light mixture composed of the wavelengths corresponding to the maximum of the open-space reaction ($\lambda = 370$ nm) and to the secondary maximum of the feeding reaction ($\lambda = 600$ nm) appear also to document the independent operation of two receptors (Fig. 13), because both reactions were elicited equally often by the mixture as by each component alone. It follows that the UV and red receptors probably do not collaborate directly – which, again, would be interpretable as evidence of wavelength-specific behavior.

b. However, *P. brassicae* also exhibits differences between behavior and receptor properties that suggest a collaborative operation of receptors.

In the feeding reaction, a single form of behavior is elicited by two different receptors, red and blue. Therefore it seems unlikely that these receptors operate independently of one another, particularly in view of the finding of red-blue-sensitive interneurons in the protocerebrum of *Papilio troilus, Heliconius erato* and *Morpho amathonte* by S.L. Swihart (1970, 1972a, b) and in the optic lobe of *Pieris protodice* by Schümperli (1975). Furthermore, there is only a poor match between the maximum of the red receptor ($\lambda = 620$ nm) and that of the feeding reaction in the orange-red region ($\lambda = 600$ nm; at 620 nm already down).

Drumming ($\lambda\text{max} = 558$ nm) and egg-laying ($\lambda\text{max} = 542$ nm) have separate maxima in the green region. This finding suggests that such a fine discrimination in a relatively narrow spectral region is brought about by operations involving the signals from other receptors.

Whereas the spectral sensitivities of the blue and green receptors of *P. brassicae* are closely matched by the Dartnall curves (Steiner et al. 1987, Figs. 8 and 11), those found for the feeding reaction (in the blue and orange-red region) and for egg-laying (in the green) are narrower than the corresponding Dartnall curves (Fig. 8). This discrepancy may be due to neuronal processes or, conceivably, to lateral electrical inhibition at the receptor level (Shaw 1975; Matić 1983). However, according to Steiner (1984) there is no evidence of lateral inhibition in the ERG of *Pieris brassicae.*

The open-space reaction is also elicited by white (UV−), with no appreciable UV component (Fig. 12b). This result could imply that the open-space reaction depends mainly on the brightness of the light stimuli and/or that the other receptors are to some slight extent involved in eliciting this reaction. The latter possibility is supported by the fact that the spectral effectiveness for the open-space reaction, unlike that for the feeding reaction (Fig. 6), never falls to zero within the spectral region examined. For the feeding reaction (Fig. 9), drumming and egg-laying (cf. Results and Kolb and Scherer 1982), on the other hand, white light (UV+ or UV−) is a poor or ineffective stimulus. These facts offer evidence against independent receptors for these regions, and for a perception of 'white' in which the receptors cooperate so as to prevent the occurrence of feeding behavior, drumming and egg-laying even when the relevant wavelength is present with high intensity.

The experiments with mixed wavelengths also provided evidence of cooperation among receptors. On one hand, the ineffectiveness of the addition of $\lambda = 600$ nm to $\lambda = 447$ nm could indicate inhibition of the red receptor by way of central circuitry. On the other side, a mixture of the wavelengths at the sensitivity maximum for drumming ($\lambda = 558$ nm) and at the secondary maximum for the feeding reaction ($\lambda = 600$ nm) elicited fewer visits in both behavioral contexts than did either component alone (Fig. 13), which makes it plausible that the signals of the green and red receptors influence one another. In case central processing of the signals is involved, such circuitry could be regarded as a prerequisite for color vision.

Kitabatake et al. (1982) suggested, on the basis of the greater phototactic effectiveness of certain wavelength mixtures than of the single components, that the larva of *Bombyx mori* is capable of color vision. Additional experiments with other wavelength mixtures would be helpful in clarifying this question for *P. brassicae.* These, like the experiments with monochromatic light stimuli, should also involve comparisons with species in other families, for a fuller understanding of the processing of colored stimuli by butterflies in general.

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