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Experiments on Wavelength Specific Behavior of *Pieris brassicae* L. During Drumming and Egg-Laying*

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Summary. Female cabbage white butterflies, under constant laboratory conditions exhibit wavelength specific behavior in choosing sites for egg-laying and drumming.

In preliminary spontaneous choice experiments with colored PVC-sheets (Table 1, results) butterflies gave somewhat different preference order than with monochromatic lights of different wavelength but equal relative quantum flux (Table 2A, B). These differences probably depended on the relative number of quanta and the spectral composition of the light reflected by the sheets.

White light is ignored by the butterflies during both drumming and egg-laying, up to intensities 60 times that of the monochromatic light stimulus.

The functional categories "egg-laying" and "drumming" are both associated with the spectral region from 497 nm to ca. 585 nm (Fig. 10). The functional category "feeding reaction" is associated with regions adjoining that for "egg-laying and drumming" on both the shorter-wavelength side (< 497 nm) and the longer-wavelength side (> 578 nm).

Wavelength specific behavior and the significance of odor substances in the chain of stimuli that lead up to egg-laying are discussed.

Introduction

That lepidopterans exhibit wavelength specific behavior has been demonstrated in a number of publications dealing with the role of color stimuli in feeding behavior (Knoll 1922; Ilse 1928, 1956, 1965; Crane 1955; Swihart et al. 1970; Swihart 1971; Miyakawa 1976). In these experiments colored papers were displayed and the wavelength specific behavior of the animal was determined by observing the color on which it landed and unrolled its proboscis (feeding reaction). *P. brassicae* was found by Ilse (1928) to give the feeding reaction in response to yellow, blue and violet papers, but not to green and blue-green papers. In 1937 Ilse observed "drumming" by female *P. brassicae* on green surfaces. This behavior – a rapidly repeated up-and-down movement of the first pair of thoracic legs – provides a criterion for testing wavelength specific behavior in the green part of the spectrum.

In the experiments described here, the drumming and egg-laying responses were used to analyze the wavelength specific behavior of *P. brassicae* in the yellow-green, green and blue-green regions, and to establish the functional categories correlated with this part of the spectrum. Because the physical characteristics of the colored papers used by previous authors to study lepidopteran wavelength specific behavior have been inadequately specified, a new methodological approach is introduced here.

Materials and Methods

Preliminary Experiments with Colored Strips

The experiments were done in a climate-controlled room under constant external conditions. The temperature was 23–25 °C. Illumination was provided by eight 40-W "daylight" fluorescent tubes (Truelite, Duro-Test, New Jersey).

Three strips of colored PVC sheet (green, yellow-green, and yellow; Plastiflex, Overath), each 272 cm^2 in area, were glued to a rectangular plexiglass stand. To elicit egg-laying, they were sprayed with sinigrin solution (0.03 g/400 ml distilled water). The number of eggs deposited was recorded after 18–20 h. All experiments were begun at the same time of day, to exclude the possible influence of circadian rhythmicity.

* Respectfully dedicated to Prof. H. Autrum on the occasion of his 75th birthday

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The reflectance of the sheets was measured with the spectrophotometer PM Q II (Zeiss) with reflectance attachment RA 2, and graphed. To obtain an estimate of the spectral composition of the light reflected from the sheets in the experiment, the measured reflectance curves were corrected at 10-nm intervals by reference to the emission spectrum of the fluorescent light. The corrected reflectance curves were used to calculate the relative numbers of quanta reflected by the sheets, as follows. The wavelength $\lambda = 600$ nm was assigned the number 1 and the wavelength $\lambda = 300$ nm, the number 0.5. To obtain the relative number of quanta reflected at any wavelength, one multiplies the number given for the wavelength (between or above 300 and 600 nm) on this scale by the corresponding reflectance reading. By adding these results, again calculated at intervals of 10 nm, we obtained the following relative numbers of quanta (RNQ) for the sheets used:

Table 1

	λ max	RNQ	Range of wavelengths
Green	510–515 nm	329	(between 390 and 610 nm)
Yellow-green	540–550 nm	1,643	(between 350 and 750 nm)
Yellow	570–610 nm	1,578	(between 450 and 750 nm)

Experiments with Monochromatic Light

Further experiments were done with monochromatic lights of equal relative quantum flux (apparatus in Fig. 1).

The light source was a 900-W xenon high-pressure lamp (XBO, Osram). The light was passed through a cooling cuvette and various glass heat filters, and then collected in a fiber-optic bundle by a convex quartz lens. Each of the three branches of the light-guide illuminated a ground disk 3 cm in diameter, the ground disks were mounted in a plastic plate (Fig. 1, P; 15 cm diameter, 1 cm thick) in such a way that they could easily be exchanged. Interference and neutral gray filters were inserted into the light beam between the output ends of the light guides and the ground disks. The plate bearing the disks was turned back and forth over a 90° arc by an electric motor, the rotation in each direction taking ca. 130 s. This motion was designed to rule out positional cues. The light measurements were done with the optometer 40X (United Detector Technology), equipped with a specially calibrated detector. All the experiments were done with 200–500 marked butterflies between 11 a.m. to 6 p.m. under constant conditions in the laboratory; the temperature was 25–28 °C. The room was lit by a 40-W fluorescent lamp (Philips) such that the intensity at the level of the rotating experimental plate was 23 $\mu W/cm^2$. The reflection of this light by the ground disks could be neglected because of wavelengths between 497 and 554 nm the flux of this reflected light at a height of 30 cm above the plate was less than 0.6% of the intensity of the light emitted by the monchromatically illuminated ground disk themselves.

The transmission maxima of the interference filters used in this apparatus were 467, 497, 522, 542, 554, 578, and 594 nm. In order for the plate to elicit egg-laying, it was necessary to spray it with sinigrin solution; after this procedure, regardless of the nature of the surface, eggs were deposited in the region of the ground disks. Experiments on drumming could be done without sinigrin.

Data Analysis

Responses were evaluated in terms of the number of visits in which drumming was associated with each of the ground disks

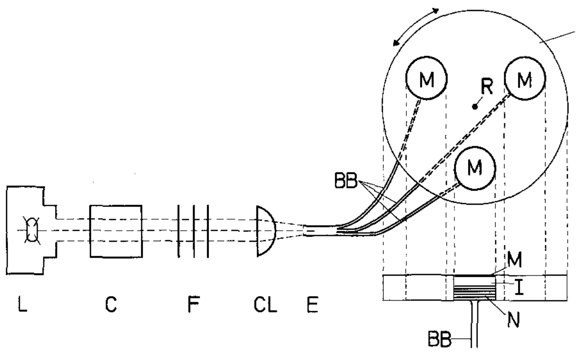

Fig. 1. Diagram of the experimental apparatus: *L* XBO 900; *C* cooling cuvette; *F* heat filters; *CL* convex lens; *E* entrance end of fiber-optic bundle; *BB* branches of bundle; *M* ground disk; *R* center of rotation of experimental plate; *I* interference filter; *N* neutral gray filters; *P* experimental plate

(duration 15–40 min per experiment) or the number of eggs deposited after 4–5 h. The area associated with each ground disk was considered to be the 120° sector of the landing plate within which it was located. To identify repeated visits by the same butterfly during the experiments on drumming, the number of each visitor was recorded; each series of experiments comprised 10 experiments, with 50 visits each. In the egg-laying experiments, 1,000–2,000 eggs were deposited in each series of 5–10 experiments. The significance of the observed differences was evaluated by the chi-square test.

Results

Preliminary Experiments with PVC Strips

Three experiments in which the effect of colored PVC on egg-laying was tested gave the following results:

Green	5,312 eggs
Yellow-green	351 eggs
Yellow	14 eggs.

In spite of the fact that the green sheet showed the lowest relative number of quanta (Table 1) it released most of the registered egg-laying.

Experiments with Interference Filters

a) Egg-Laying. The preference order indicated by the above results was further examined with the apparatus illustrated in Fig. 1. When the three monochromatic stimuli $\lambda = 578$, 542 and 522 nm are presented with equal relative quantum flux, $\lambda = 542$ nm is preferred (Fig. 2a). Of 2,420 eggs, 1,177 were laid in the sector $\lambda = 542$ nm, 979 in $\lambda = 522$ nm, and 264 in $\lambda = 578$ nm.

The wavelength $\lambda = 542$ nm was also preferred when tested in combination with other colors (Figs. 2b–4). Taken together, the four series of experiments gave the following preference order:

Table 2A

1.	542 nm	(49.3 ± 14.1%)
2.	522 nm	(35.3 ± 13.9%)
3.	554 nm	(35.0 ± 12.2%)
4.	578 nm	(9.7 ± 3.2%)
5.	497 nm	(6.0 ± 6.0%)

The spectral efficiency curve for egg-laying (Fig. 10) was constructed from these data. (Calculated from a total of 14,157 deposited eggs).

In order to monitor the butterflies' behavior in choosing between colored stimuli in a narrow region of the spectrum and white-light stimuli, in some experiments two disks were illuminated with green light ($\lambda = 542$ and 554 nm) and one with white light (Fig. 4). The intensity of the green stimuli with equal relative quantum flux was constant in all trials, 10 $\mu W/cm^2$ for $\lambda = 542$ nm and 11 $\mu W/cm^2$ for $\lambda = 554$ nm. The intensity of the white light was increased twice by a factor of ten (from 6 to 60 and 600 $\mu W/cm^2$). Regardless of its intensity, this white stimulus was almost completely ignored – even though in the third trial (Fig. 4c) it was more than ten times as intense as the colored stimuli. This result shows that the decisive criterion is the wavelength. There can be no doubt that the butterflies exhibit wavelength specific behavior.

The influence of intensity of the two green stimuli $\lambda = 542$ nm and 554 nm was also studied (Fig. 5). Their equalized relative quantum flux used in the experiments shown in Fig. 2b was adequate to the 100% level of relative intensity in Fig. 5. The $\lambda = 522$ nm stimulus was kept constant at this level. The resulting curves indicate that in both cases egg-laying is reduced (respectively increased) as intensity decreases (respectively increases).

b) Drumming. Drumming was studied with the same experimental apparatus as egg-laying (Figs. 6, 7a, 8). The preference order found for drumming differed somewhat from that for egg-laying:

Table 2B

1.	554 nm	(59.3 ± 6.6%)
2.	542 nm	(49.5 ± 14.9%)
3.	578 nm	(29.6 ± 6.4%)
4.	522 nm	(16.7 ± 11.6%)
5.	497 nm	(2.8 ± 2.1%)

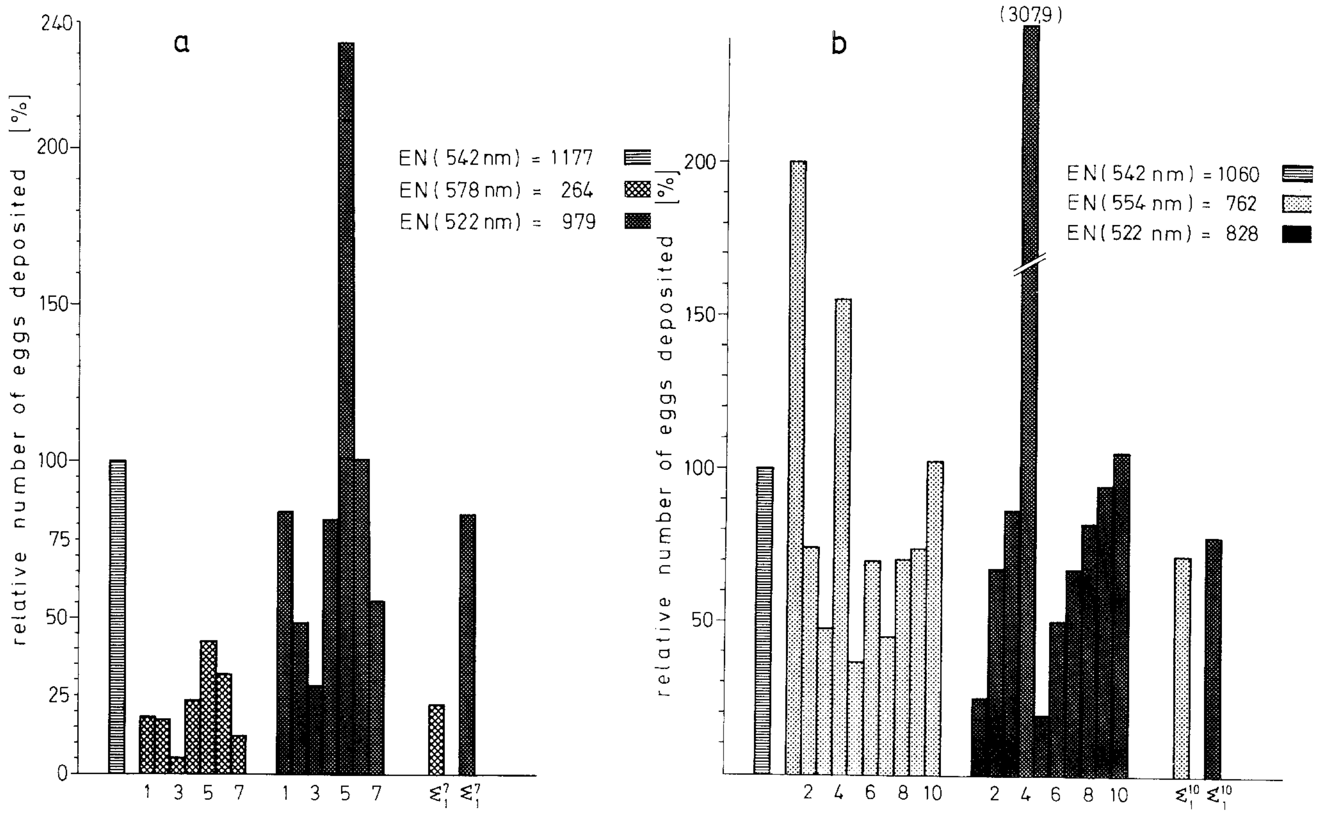

Fig. 2a, b. Spontaneous-choice experiments (egg-laying) using interference filters. Ordinate: relative number of eggs deposited, normalized (i.e., the maximal number deposited in the sector of the preferred wavelength was set equal to 100%); abscissa: number of individual experiments. EN: total number of eggs deposited at each wavelength. Σ: normalization of the absolute sums (≙ EN) obtained by addition of the numbers in the individual experiments. **a** Filters with $\lambda = 542$, 578 and 522 nm. The results are summarized in the value Σ_1^7 on the right, the ratio of EN ($\lambda = 578$ nm) and EN ($\lambda = 522$ nm) to EN ($\lambda = 542$ nm). **b** Filters with $\lambda = 542$, 554 and 522 nm

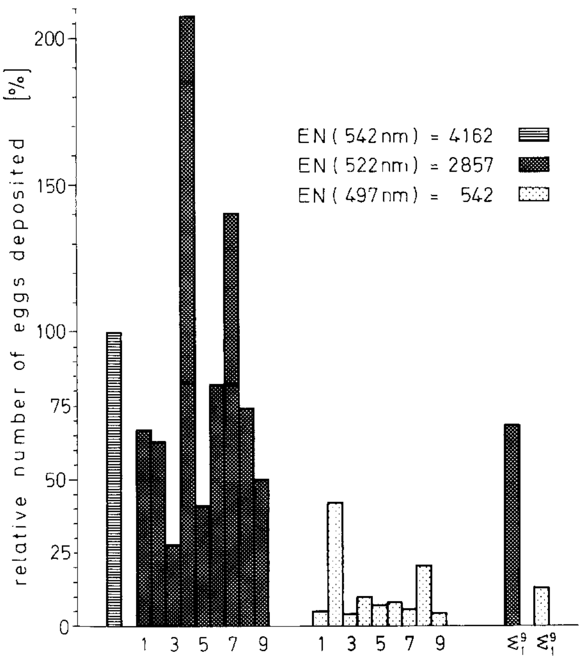

Fig. 3. Spontaneous-choice experiments (egg-laying) using interference filters: $\lambda = 542$, 522 and 497 nm

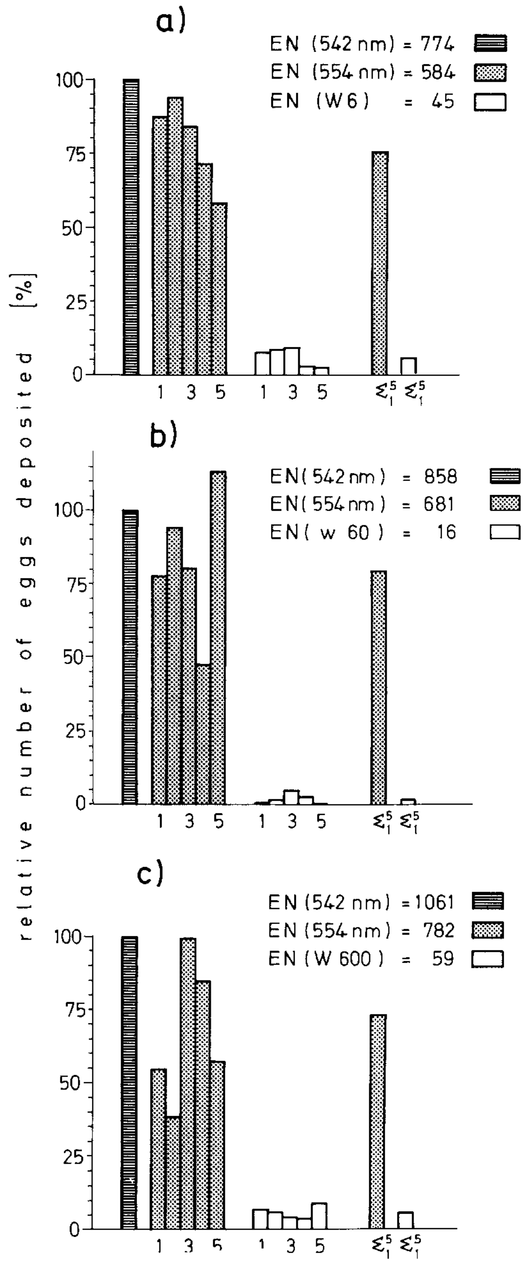

Fig. 4a–c. Spontaneous-choice experiments (egg-laying) using interference filters: $\lambda = 542$ and 554 nm together with white light at 6, 60 and 600 $\mu W/cm^2$ (a total of 4860 eggs deposited)

This preference order is based on a total of 2000 visits, with the stimuli in various combinations; the resulting efficiency curve is shown in Fig. 10.

When the two green stimuli $\lambda = 554$ nm (11 $\mu W/cm^2$) and $\lambda = 542$ nm (10 $\mu W/cm^2$) were compared with white light (6, 60 and 600 $\mu W/cm^2$), the white light – as in the case of egg-laying – was ignored (Fig. 8). The intensity-dependence of drumming was examined by the same procedure as for egg-laying (Fig. 5a, b).

By marking the butterflies with numbers we were able to determine how many of the individual females engaged in drumming. For example, with the combination $\lambda = 522$, 542, and 578 nm (Fig. 6a), about 10% of the females tested were responsible for all of the 50 visits in an experiment.

Of these, an average of 80% visited the plate once or twice, whereas 20% were counted three or more times. Repeated visits by individuals were thus relatively rare.

Egg-laying, however, was measured by a method that did not allow determination of individual performance. In some cases individual variation may account for the marked discrepancies in some of the experiments on egg-laying (Fig. 2). The following temporal relation was found between drumming and egg-laying: a female either began laying eggs within a few seconds after drumming, or she flew away.

The efficiency curves for drumming and for egg-laying lie chiefly in the spectral region between 497 and 578 nm (Fig. 10). This situation raises the question whether wavelengths in the spectral regions <497 nm and >578 nm are discerned. As Fig. 9 shows, they are. The butterflies respond to wavelengths in these regions by unrolling the proboscis and exploring the substrate with it (feeding reaction). In the short-wavelength region the switch between drumming/egg-laying and the feeding reaction occurs at 497 nm (Fig. 7); at this wavelength hardly any egg-laying or drumming is

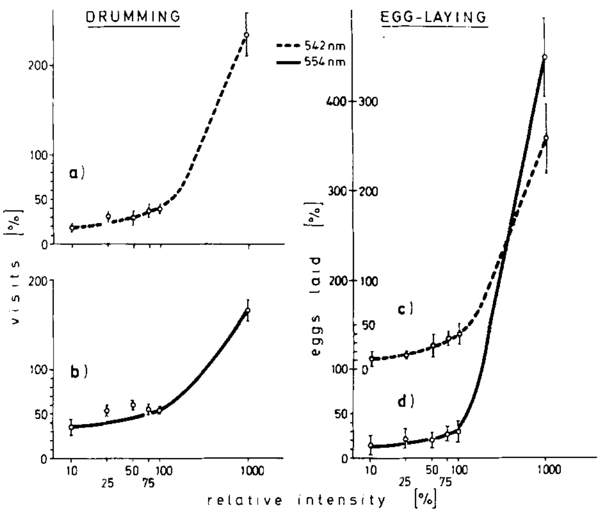

Fig. 5. Intensity dependence of drumming (a and b) and egg-laying (c and d) at $\lambda = 542$ and 554 nm. Comparison wavelength: $\lambda = 522$ nm (intensity constant). Ordinate (a and b): visits, in percent of total. Data for each intensity level are derived from a series of experiments comprising a total of 500 visits to the 3 wavelengths together; $n = 10$ (a total of 3,000 visits registered). Ordinate (c and d): number of eggs laid, in percent of total. Data for each intensity level are derived from a series of experiments in which a total of 1,000–2,000 eggs were deposited on the 3 wavelengths together; $n = 10$ and 5, respectively. Abscissa: relative intensity (%). (A total of 12,199 eggs deposited)

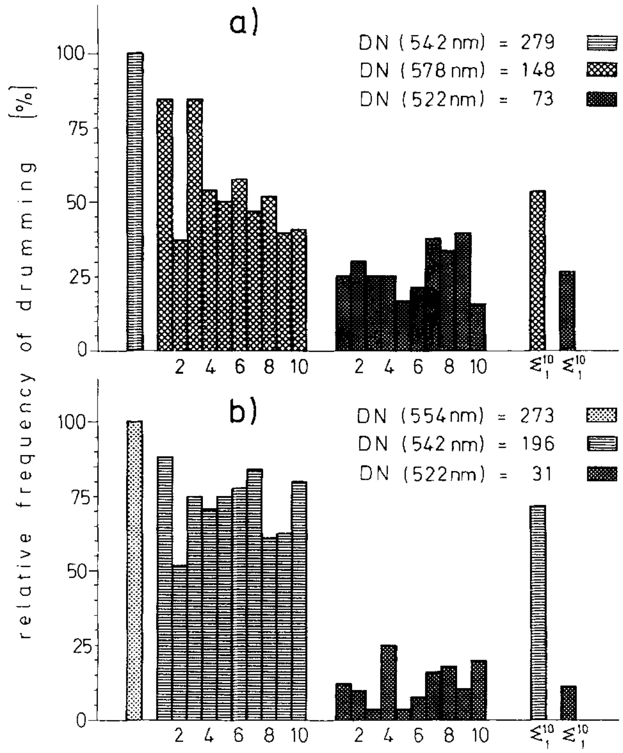

Fig. 6a, b. Spontaneous-choice experiments (drumming) using interference filters. Ordinate: relative frequency of drumming, normalized (i.e., the maximal number of visits to the preferred wavelength was set equal to 100%). Abscissa: the number of individual experiments. DN: total number of visits with drumming to the wavelength indicated. Σ: normalization of the absolute sums (≙ DN) obtained by addition of the numbers in the individual experiments. **a** Filters with $\lambda = 542$, 578 and 522 nm. **b** Filters with $\lambda = 554$, 542 and 522 nm

observed (Figs. 3, 7a), whereas the feeding reaction is elicited considerably more frequently (Fig. 7b). The corresponding boundary in the red-orange region is at ca. 585 nm. Whereas $\lambda = 578$ nm elicits mainly drumming and egg-laying, with very few feeding reactions (Figs. 2a, 6a, 9a), at $\lambda = 594$ nm the only response is the feeding reaction (Fig. 9b).

The Significance of Odor Substances

The following observations bear on the role of odors in egg-laying and drumming. Illuminated ground disks without sinigrin, in a period of 2–3 h, elicit only drumming; egg-laying was never observed. Ground disks sprayed with sinigrin but not illuminated are entirely ignored. Similar results were obtained in the preliminary experiments on egg-laying with colored PVC; PVC strips not sprayed with sinigrin elicited no egg-laying, regardless of their color and intensity.

Discussion

The experiments described here are the first published tests of the behavioral responses of lepidopterans to monochromatic colored stimuli. – Because the quantum flux of these stimuli was equalized, differences in response should reflect wavelength specific preferences rather than intensity discrimination. Moreover, because butterflies from a laboratory colony were available, they could be tested in statistically adequate numbers (200–500 animals). The sex ratio of the animals tested was 1:1, so that in each experiment ca. 100–250 females were present. Only the females responded to colors in the green region ($\lambda = 497$–578 nm), by drumming and laying eggs. Thus the present data give no information as to the ability of male *P. brassicae* to wavelength specific behavior in the green region. However, electrophysiological studies by Gemperlein and his coworkers revealed no differences in spectral sensitivity between males and females (personal communication). It is likely, then, that the two also do not differ in color-discrimination ability.

In contrast to the methods used in the present experiments, Ilse (1937) gave no statistical evaluations of the observed color preferences of *P.*

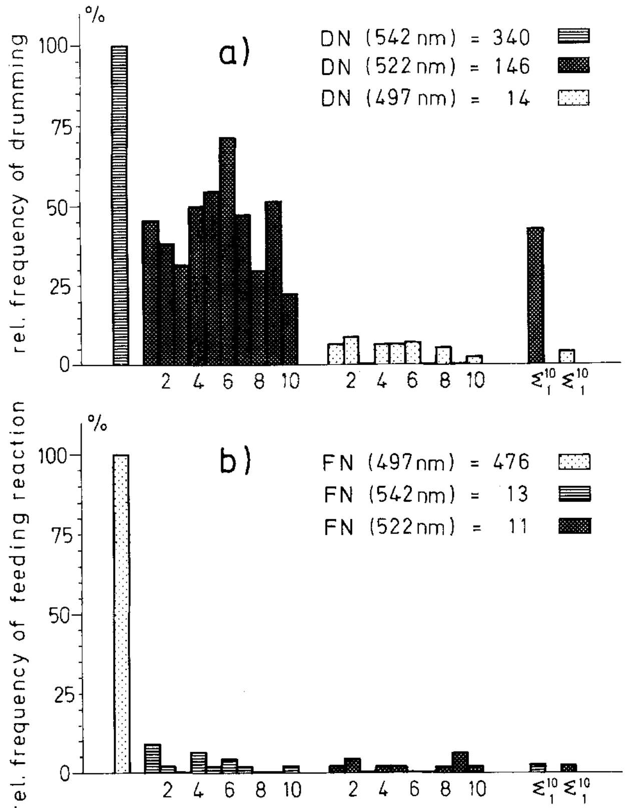

Fig. 7a, b. Spontaneous-choice experiments using interference filters (λ = 542, 522 and 497 nm): drumming **a** and feeding reaction **b**. FN: total number of visits with feeding reaction to the wavelength indicated

brassicae during drumming, nor did she describe the physical characteristics of the Ostwald papers used.

The spectral efficiency curve for drumming should be shifted toward the long-wavelength part of the spectrum (ca. 510 to 580 nm), as compared with that found by Ilse (1937). This shift from blue-green into the yellow-green also applies to the egg-laying responses observed in the present paper (Fig. 10a). The differences between the results obtained with PVC sheets and those with monochromatic lights of equal relative quantum flux probably depended on 1) the relative number of quanta, and 2) the spectral composition of the light reflected by the sheets. Comparison of various reflectance curves (Knoll 1922; Miyakawa 1976; Scherer 1979) shows that in general, yellow and yellow-green papers or PVC sheets have very high reflectance in the long-wavelength part of the spectrum, whereas green papers have lower reflectances and comprise a smaller region of the spectrum. Yellow and yellow-green papers would more likely elicit feeding reactions than drumming, as did the yellow PVC we used and the Hering papers used by Ilse in other experiments (Ilse 1928;

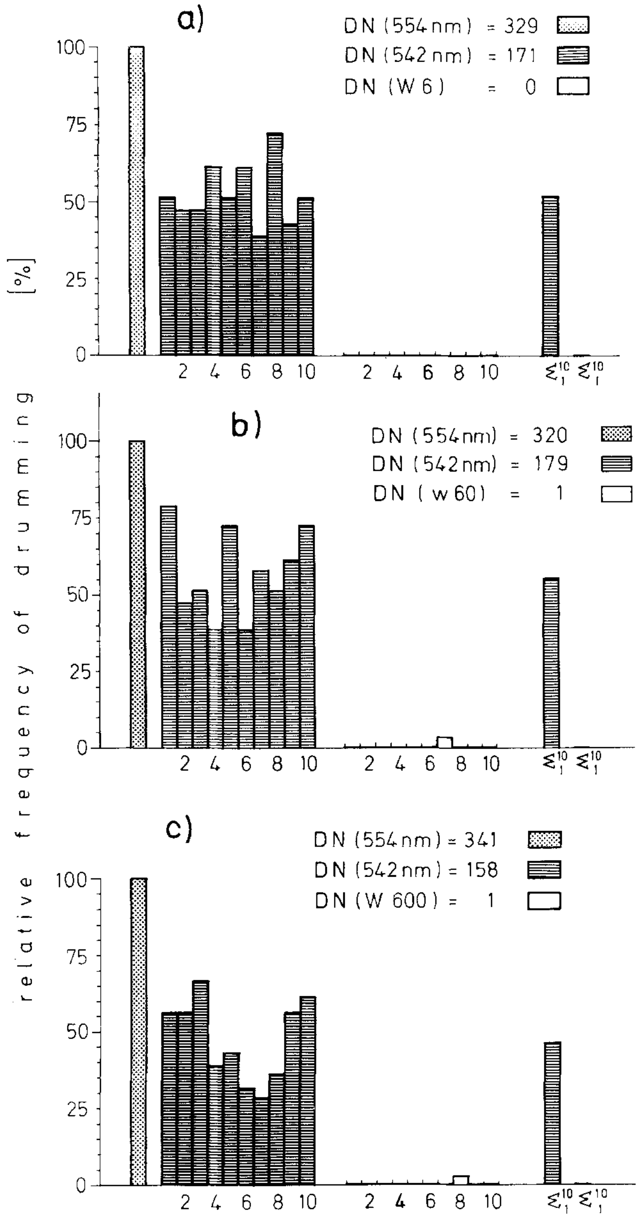

Fig. 8a–c. Spontaneous-choice experiments (drumming) using interference filters: λ = 542 and 554 nm together with white light at 6, 60 and 600 $\mu W/cm^2$; a total of 1,500 visits

Scherer 1979). In contrast to colored-paper stimuli, the monochromatic yellow stimulus (λ = 578 nm) had a relative quantum flux equal to that of the simultaneously presented monochromatic green stimuli (λ = 542 and 522 nm), and it was perhaps for this reason that this yellow elicited drumming and egg-laying but not feeding reaction. The latter observations suggest the hypothesis that at a given (or adequate) quantum level certain wavelength bands stimulate one function and suppress another. The large component of long-wavelength light in yellow papers could act as a disturbing factor with

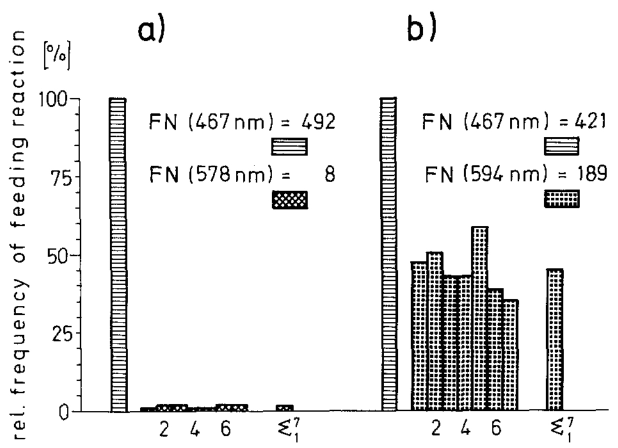

Fig. 9a, b. Spontaneous-choice experiments (feeding reaction) using interference filters. **a** $\lambda = 467$ and 578 nm. **b** $\lambda = 467$ and 594 nm

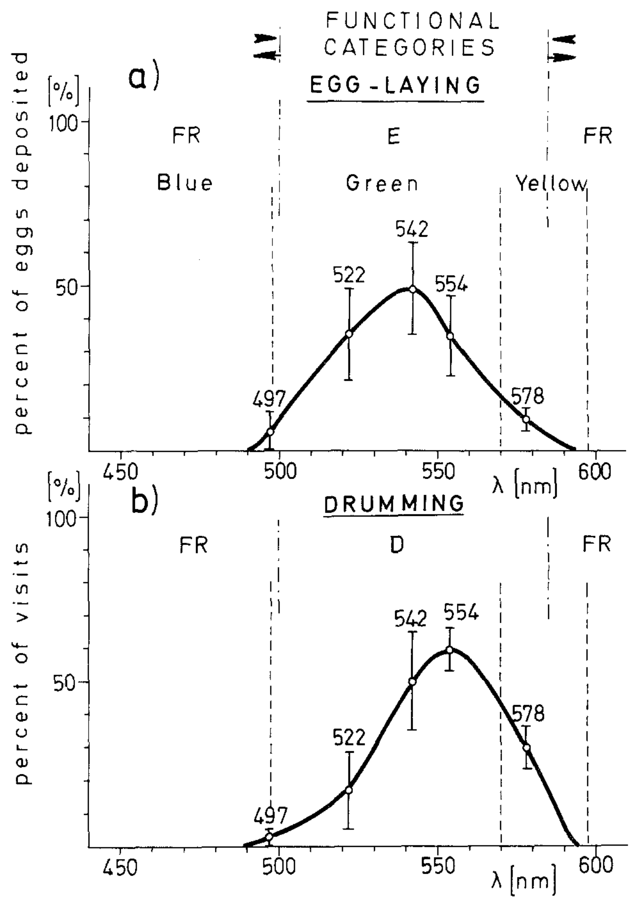

Fig. 10a, b. Spectral efficiency for egg-laying and drumming. Ordinates: percent of total eggs deposited **a** and of total visits **b**. Abscissa: wavelength. Data are derived from all the experiments in which the relevant wavelength was used. Functional categories: *FR* feeding reaction; *E* egg-laying; *D* drumming

regard to egg-laying and drumming while enhancing the feeding reaction.

In general it can be postulated that functional categories exist in butterflies that are correlated with certain wavelength bands. But in many cases other (e.g., chemical) stimuli are required in addition to elicit these functions; the stimulus chain leading to egg-laying by *P. brassicae* is an example. This chain is formed by two key stimuli: first a green substrate must be available to elicit drumming, and then the chemical stimulus of a mustard-oil glycoside elicits egg-laying. Evidently the numerous B hairs on the 5th tarsomeres of the first thoracic legs are responsible for the reception of the glycoside; according to Chun and Schoonhoven (1973) female *P. brassicae* have many more such hairs than males. Indications of this stimulus sequence, which is manifest in our experiments, can be found in the publications of Ilse (1937), Terofal (1965) and Chun and Schoonhoven (1973).

The present results demonstrate that there is a functional category associated with the green region of the spectrum, and establish the boundaries between this region and those associated with other functions. Moreover, the necessity for certain stimulus sequences is revealed. It remains to be determined (i) whether this stimulus chain is a requirement for lepidopterans in general, (ii) what functional categories exist, and (iii) the wavelength bands with which they are associated. The answers to these questions will provide clues to the processing of the wavelengths detected by the receptors in the butterfly retina.

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