SPECTRAL CHARACTERISTICS AND REGIONALIZATION OF THE EYE OF THE SATYRID *BICYCLUS ANYNANA*

Doekele G. Stavenga[1], Takahiko Hariyama[2] & Kentaro Arikawa[3,4]

[1] Department of Neurobiophysics, University of Groningen, NL-9747 AG Groningen, the Netherlands
[2] Graduate School of Information Sciences, Tohoku University, Sendai 980-77, Japan
[3] Graduate School of Integrated Science, Yokohama City University, and
[4] PRESTO, Japan Science and Technology Corporation, Kanazawa-ku, Yokohama, Japan

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Summary
The ommatidial characteristics of the eyes of many insects are non-uniform (Stavenga, 1992). The retinal heterogeneity has been investigated in the satyrid *Bicyclus anynana* by *in vivo* microspectrophotometry of the eye shine from individual ommatidia. The ommatidial reflectances fall into two classes. The reflectance of the first, yellow-green (YG) class has a main band peaking at 570 nm (width ca 50 nm) and a subsidiary band in the violet; the second, red (R) class has a broad band from 580 to 680 nm. All ommatidia in the dorsal part of the eye belong to the YG-class. The ommatidia of the ventral part of the eye belong to either the YG- or the R-class. The density of the R-class is highest in the downward-looking area. The eye shine is maximal in the dark-adapted eye, but it rapidly decreases upon illumination with bright light, due to migration of pigment granules towards the light guiding rhabdom. The time constant of this pupil mechanism is in the order of a few seconds. The reflectance also exhibits distinct spectral changes due to changes in the content of the visual pigments in the rhabdom. This phenomenon allows studying the decay and renewal of visual pigment in vivo.

INTRODUCTION

Anatomical investigations of insect eyes suggest that the building block, the ommatidium, has a unique structure. It has been recognized in numerous cases that insect eyes can exhibit a strong regionalization of their compound eyes, often dependent on the sex (rev Stavenga 1992). A usual assumption is that the ommatidia at least locally have a uniform composition, i.e., the characteristics of the anatomically corresponding photoreceptor cells are taken to be repetitive. Recent optical, electrophysiological and molecular biological studies have however provided accumulating evidence that the ommatidial characteristics are often locally heterogeneous (flies: Hardie 1985, Salcedo et al. 1999; butterflies: Bandai et al. 1992; Arikawa & Stavenga 1997; Arikawa et al. 1999a,b). Interestingly, for the limited cases where strong local heterogeneity is firmly established, the emerging picture is that the fate of the photoreceptor cells is certainly not determined at random. Rather, the heterogeneity consists of a mixture of a limited number of types of ommatidia. In the case of the fruitfly *Drosophila* there exist two classes of ommatidia (Salcedo et al. 1999), and in the Japanese yellow swallowtail butterfly *Papilio xuthus* at least three classes can be distinguished (Kitamoto et al. 2000). Within each class, all the characteristics of anatomically corresponding photoreceptors appear to be the same.

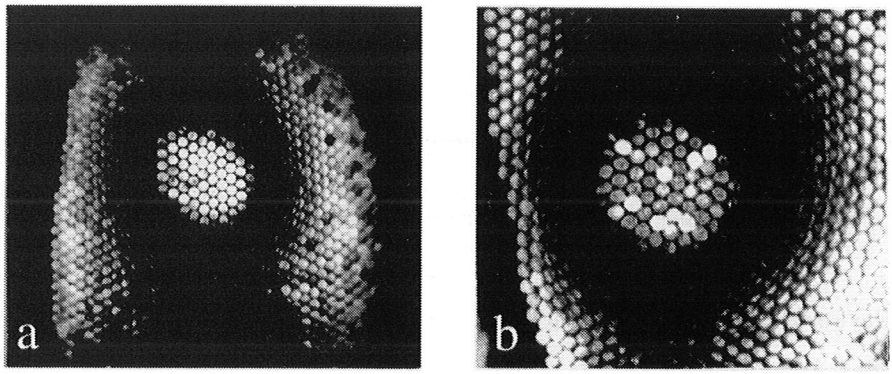

*Fig. 1Photographs of the eye shine made with an incident-light microscope. In the dorsal eye half (**a**), the color of all shining facets is yellow-green. In the ventral eye half (**b**), most of the facets are yellow-green, but a number of randomly interspersed facets are bright red (the bright facets in **b**)*

The sensitivity spectra of the photoreceptors are largely determined by the absorption spectra of the visual pigments. However, distinct modifications can occur due to filtering effects. These changes can be due to mutual filtering by the different visual pigments within one and the same visual waveguide, the rhabdom. Also, absorbing pigments within or close to the rhabdom can execute a filtering effect. As most butterfly species (but not papilionids, Miller 1979) have a reflecting tapetum proximal to the rhabdom, light reflected at the tapetum can add to the tuning of the photoreceptor sensitivity spectra (Stavenga 1979).

Stimulated by the considerable insight gained from the Papilionidae, we have started to investigate the properties of the reflecting tapetum in butterfly eyes and its ocular heterogeneity in the perspective of its role in color vision. We here present a first analysis of the satyrid *Bicyclus anynana.*

MATERIAL AND METHODS

Satyrid butterflies, *Bicyclus anynana*, were obtained as pupae from a laboratory culture maintained in the Institute of Evolutionary and Ecological Sciences in the University of Leiden. After emergence, the butterflies were supplied with water and fruit (mostly bananas). The experiments were performed on a specimen immobilized by wax and fixed to a flexible holder. The butterfly was then mounted at the stage of an epi-illumination (incident-light) microscope, equipped with a NPL10, 0.22 (Leitz) or an Olympus 10x objective. Photographs were taken from the tapetal eyeshine from various areas of the eye (Fig. 1). Tapetal reflection due to broadband, white light was measured with an Oriel diode array spectrophotometer attached to the microscope (Figs 2, 3). Alternatively, monochromatic light emerging from a monochromator and reflected by the tapetum was measured with a photomultiplier (Fig. 4).

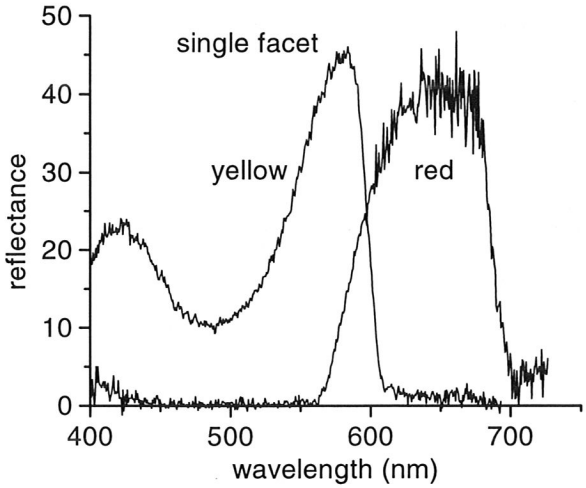

Fig. 2 Reflectance spectra measured from single facet lenses. The spectra fall in two classes, a yellow-green (YG) and a red class (R). The YG reflectance spectrum is broad, extending into the ultraviolet, very minor above 600 nm and peaking at around 580 nm; the R reflectance spectrum is a restricted band around 650 nm, and is negligible below 550 nm.

RESULTS

Observing the eye of a diurnal butterfly with an epi-illumination microscope reveals the usually brightly-colored eye shine emerging from a restricted number of facets. The aperture of the microscope objective determines this number of shining facets. Consequently, the area of the eye shine changes when the eye, i.e. the animal, is rotated. In the satyrid *Bicyclus anynana*, the shine in the dorsal eye region is yellow-green in all facets (Fig. 1a), but ventrally a fraction of the shining facets radiate reddish light (Fig.1b). There exists a rather clear transition area between the dorsal eye half, where the red facets are fully absent, and the ventral eye half, where the red facets make up a small, but prominent fraction of the facets. The red facets (bright in Fig. 1b) are randomly distributed, although the ratio of the red and the yellow-green facets is not at all constant. The ratio is highest (ca 20 %) in the downward looking eye area.

Visual inspection hence indicates that the ommatidia can be divided into two classes on the basis of their reflectance: a yellow-green (YG) and a red (R) class. This was verified by measuring the reflectance spectra from single facets with a microspectrophotometer (Fig. 2). The yellow-green facets have a broad reflectance spectrum, extending into the ultraviolet, but becoming very minor above 600 nm. The reflectance maximum is around 580 nm. The red facets reflect strongly around 650 nm, but reflectance is negligible below 550 nm. There is a slight variation between the reflectance spectra from different ommatidia, but the two classes are clearly very distinct.

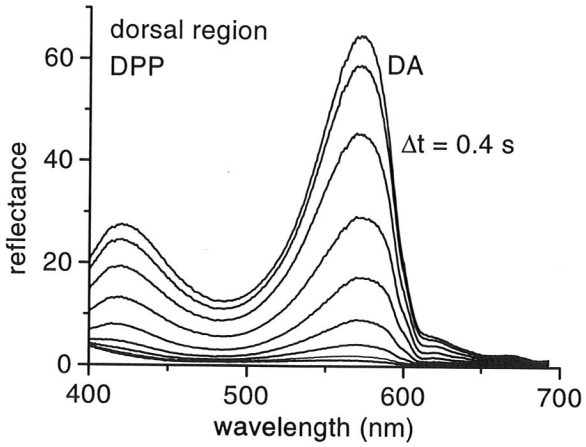

Fig. 3Changes in reflectance during light adaptation, due to the action of the pupil mechanism. The reflectance was measured in the dorsal eye region of B.anynana *(at room temperature) from the deep pseudopupil (DPP); this is the superimposed image of the individual rhabdoms in adjacent ommatidia created at the level of the center of curvature of the eye (Stavenga 1979). The reflectance is high in the dark adapted state (DA, upper curve), but upon illumination with bright light the reflectance decreases in the time course of a few seconds to a very small value (interval between the subsequent curves* $\Delta t = 0.4$ *s)*

There are a number of causes for variations in the reflectance. First, of course, the spectral characteristics of the reflecting tapetum may be not unique, and the same may hold for the waveguide optics, i.e., the dimensions and the refractive index values of the rhabdom and the surrounding medium may vary between the ommatidia. Furthermore, an important factor affecting the waveguiding rhabdom is the pupil mechanism (Stavenga 1979). The photoreceptor cells contain small granules of absorbing pigment that are pulled towards the rhabdom or away from it, depending on the illumination. In the dark-adapted state, the pigment granules are at a distance of the rhabdom, but upon light adaptation the granules accumulate near the rhabdom. There the granules absorb light that propagates along the rhabdom, thus reducing the light flux; effectively similar as a contracting pupil in the human eye affects the illumination of the retinal photoreceptors. Consequently, the reflectance of the butterfly eye is maximal in the dark-adapted state, but upon light adaptation the reflectance reduces due to the effect of the closing pupil (Fig. 3). The time constant of the pupil mechanism is in the order of a few seconds, depending on temperature.

The reduction of the reflectance spectrum is determined by the absorbance spectrum of the pupil. The latter spectrum increases in magnitude during closure of the pupil. The time course of this increase can be calculated by dividing the subsequent reflectance spectra (Fig. 3) by the initial, dark-adapted spectrum. It appears that the pupil absorbance spectrum is in first approximation more or less flat in the wavelength range 400 – 700 nm. In other words, the pupil works as a gray filter wedge, the density of which increases with light adaptation.

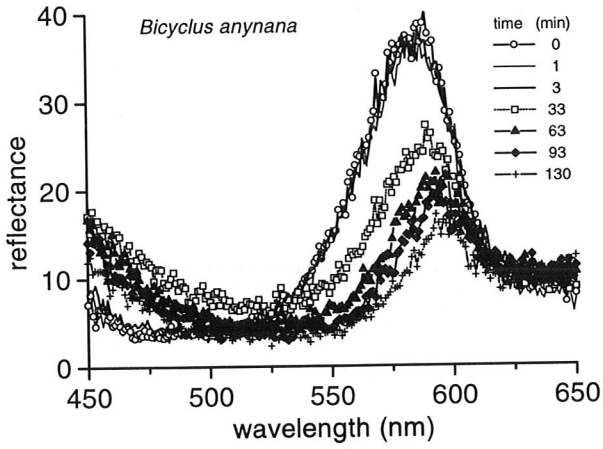

Fig. 4 Changes in the reflectance spectrum due to changes in the visual pigment content, occurring during the process of dark regeneration. The butterfly eye was pre-illuminated with intense white light, resulting in a high reflectance in the yellow-green and a low reflectance in the blue. The high yellow-green reflectance is partly due to degeneration of green absorbing rhodopsin and creation of blue absorbing metarhodopsin. In the dark, in the time course of a few hours, the green-absorbing rhodopsin is regenerated, causing a distinctly lowered reflectance and a peak-shift towards 600 nm.

Other reasons for changes in the reflectance are the visual pigments contained in the rhabdom. The reflected light leaving the eye has traveled twice through the rhabdom length, and hence changes in the visual pigment content show up as changes in the reflectance spectrum. Visual pigment molecules are removed from the photoreceptor membrane after intense illumination, because the created metarhodopsins degenerate. The visual pigments are restored in the dark due to a process called renewal or dark regeneration (Bernard 1983; Schwemer 1984; Bernard & Remington 1991).

This process is studied in the experiment of Fig. 4, performed in the dorsal eye half, where all ommatidia belong to the YG-class, i.e. reflect in the yellow green. The eye was pre-illuminated with intense white light, resulting in a high reflectance in the yellow-green and a low reflectance in the blue. It should be known that the most common visual pigments encountered in butterfly eyes are green absorbing rhodopsins; their metarhodopsin state absorbs in the blue (Stavenga 1992). The initial high yellow-green reflectance in Fig. 4 is partly due to degeneration of such a metarhodopsin. In the dark, in the time course of a few hours, the green-absorbing rhodopsin is regenerated, causing a distinctly lowered reflectance and a peak-shift towards 600 nm.

These results clearly show that visual pigment changes can distinctly modify the magnitude of the reflectance spectra of the YG-ommatidia. However, it also follows from the experiments that the visual pigment densities never reach values yielding negligible reflections in the yellow-green. Yet, in the R-ommatidia the reflectance in the short-wavelength range is certainly extremely low. Presumably, red screening pigment, i.e. pigment strongly absorbing in the short-wavelength range, but becoming transparent in the red causes this. There must nevertheless exist another difference between the two classes of ommatidia, as the tapetal reflectance spectrum in the R-ommatidia extends towards longer wavelengths than the reflectance spectrum in the YG-ommatidia.

Preliminary anatomical work indeed indicates a prominent heterogeneity, especially in the photoreceptor pigmentation.

The existence of only one class of ommatidia vs. two classes ventrally raises the possibility of differences in color vision capacities of the two eye halves. The lower eye half presumably is more involved in recognition of food sources and color vision (Kinoshita et al. 1999). Intriguingly, the visual directions of the ommatidia in the area where the R-class has its highest density seem to point to the tip of the extended proboscis.

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