SPECTRAL CHARACTERISTICS AND REGIONALIZATION OF THE EYES OF DIPTERA, ESPECIALLY TABANIDAE

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Keywords: fly eye, spectral sensitivity, vision, interference colors, horsefly

Summary

The physical origin of the color patterns in the eyes of Tabanidae is well understood: a stack of layers in the facet lenses acts as an interference reflectance filter (Bernard & Miller 1968). This causes a reduced transmittance in specific wavelength bands. Because a facet lens focuses light into the underlying rhabdomeres, where the visual pigment molecules can absorb the incident light, the spectrally affected transmittance presumably causes a modified spectral sensitivity of the photoreceptors. We have investigated this conjecture by measuring reflectance spectra from the golden-greenish shining facets of the blinding breeze fly *Chrysops relictus*. By combining the associated transmittance spectrum with various visual pigment spectra, we conclude that noticeable shifts in photoreceptor sensitivity spectra can only occur with visual pigments that have absorption spectra peaking around 540 nm, i.e. in the green, in accordance with ERG measurements of the related horsefly *Haematopota pluvialis* (Kirschfeld 1986).

INTRODUCTION

Image processing by the eyes of higher Diptera starts at the level of the retina. The spatial aspects of the eye of the fruitfly, housefly and blowfly are already clear for a few decades, due to combined optical and anatomical investigations (reviews Kirschfeld 1973; Hardie 1985). The open rhabdom organization combined with the wiring of the photoreceptor axons has resulted in the so-called neural superposition eye. The eight photoreceptor cells are divided into two classes, i.e. R1-6 and R7,8. The photoreceptors of the R1-6 class all have the same spectral sensitivity, determined by a unique rhodopsin that has an absorption maximum at about 490 nm. The spectral characteristics of the photoreceptors R7 and R8 are non-unique and even are sex-dependent (Hardie 1985). Recently the four additional rhodopsins expressed in these cells have been identified (Salcedo et al. 1999).

All visual pigment molecules consist of a protein part, the opsin, to which a chromophore is linked. The chromophore of vertebrate rhodopsins is retinal, but fly rhodopsins utilize 3-hydroxy-retinal. The absorption spectrum of most visual pigments has a main, so-called α-band in the visible wavelength range and a small β-band in the ultraviolet. Generally, the sensitivity spectrum of a photoreceptor cell is assumed to be more or less identical to the absorption spectrum of the visual pigment. However, electrophysiological recordings have demonstrated that these spectra can differ substantially. For instance, in the cyclorrphan flies, the R1-6 cells, in addition to a main band peaking at ca 490 nm, have a strongly enhanced sensitivity band in the UV, due to a sensitizing pigment, 3-hydroxy-retinol. Furthermore, the sensitivity spectra of R7 and R8 cells are distinctly affected by mutual spectral filtering effects of the visual pigments as well as by photostable pigments in the rhabdomeres (Hardie 1985).

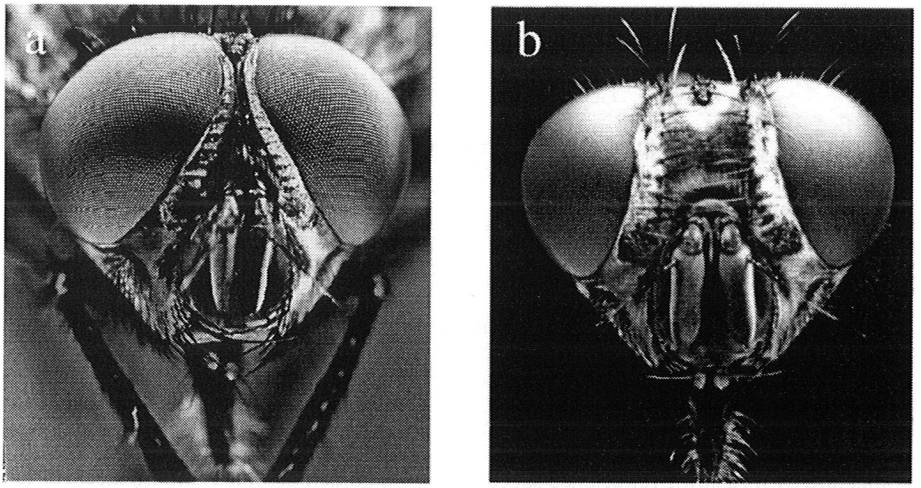

Fig. 1 Sexual dimorphism of the eyes of the blowfly Calliphora vicina. *The male* (**a**) *eye is larger and extended in the dorsal region compared to the female* (**b**)

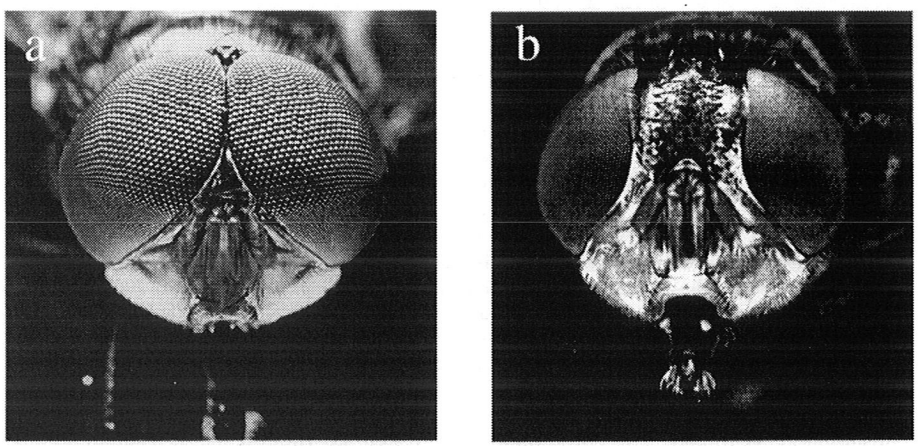

Fig. 2 Sexual dimorphism of the blowfly Chrysomia megacephala. *In this species the male* (**a**) *eye is dorsally enormous compared to the female* (**b**). *Furthermore, the male eye features a distinct dorso-ventral difference; see Van Hateren et al. (1989)*

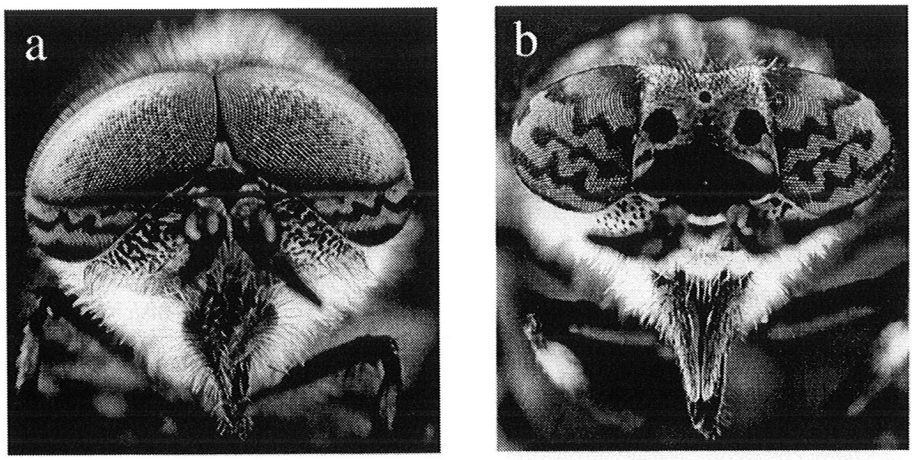

Fig. 3 Sexual dimorphism of the eyes of the tabanid Haematopota pluvialis. *The male* (**a**) *eye is clearly divided in a dorsal and a ventral part. The corneal facet lenses dorsally are colorless on a whitish underground. Ventrally the facet lenses show wavy bands of colors, due to interference effects in the facet lenses, very similar as occurs throughout the eye of the female* (**b**)

In principle, another filtering effect can occur when the dioptric apparatus has some spectral selectivity. For, the facet lens focuses light into the rhabdomeres and, like in sunglasses, spectral filtering might occur at this level. In all investigated Cyclorrhapha, the corneal facet lenses are quite colorless, i.e. they are very transparent throughout the visible spectrum, including the ultraviolet. However, in many brachyceran species the facet lenses display distinct colors. As already reported by Friza (1929), outstanding examples of beautifully colored facets are found in the eyes of horseflies (Tabanidae) and long-legged flies (Dolichopodidae). Bernard and Miller (1968) demonstrated that the physical origin of the colors is a stack of layers in the most distal part of the facet lenses. Because the refractive indices of the layers alternate between a higher and a lower value, the stack acts as an interference reflectance filter. This results in spectral reflectance bands, at wavelengths about four times the optical thickness of the layers.

The colored facets are arranged in patterns that are highly species and sex specific. The biological function of the phenomenon is quite uncertain, however. Here we present a preliminary study, investigating the possible effects of the colored facets as spectral filters. We specifically treat the case of the blinding breeze fly, *Chrysops relictus*.

MATERIAL AND METHODS

For a comparative survey, photographs were taken with a macrophotography setup from the eyes of various fly species. Blowflies *Calliphora vicina* and *Chrysomia megacephala* were taken from a laboratory culture.

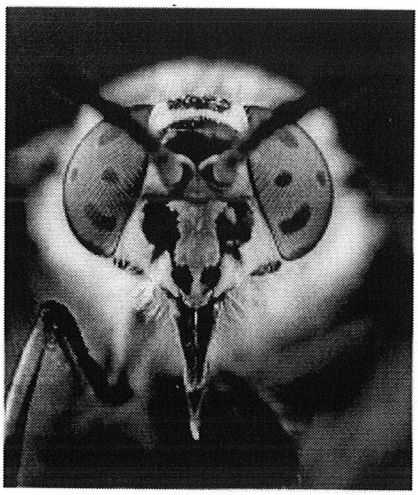

Fig. 4 The blinding breeze fly, Chrysops relictus, *has brightly greenish coloured eyes, due to interference filters in the corneal facet lenses. The filters are absent in a number of dark areas, which are dark-reddish due to the pigmentation in the screening pigment cells.*

Reflectance spectra of single facet lenses were measured from fully intact horseflies, *Chrysops relictus*. A fly was first immobilized with wax and then mounted at the stage of a microscope, equipped with an Oriel diode array spectrophotometer.

To investigate the reflectance vs. the transmittance characteristics of the corneal facet lenses, eyes were first dissected. The retinal tissue then was largely removed with fine forceps and subsequently fully cleaned in an ultrasonic bath.

RESULTS

The eyes of many fly species have a distinct sexual dimorphism. The eyes of male blowflies *Calliphora vicina* (Fig. 1a) and *Chrysomia megacephala* (Fig. 2a) are substantially larger than their female companions (Figs 1b and 2b). The eyes of these blowflies, male and females alike, are more or less dark-red-brown, due to the screening pigments located in the pigment cells in the retina, that is covered by the array of facet lenses. The acuity of the enlarged dorsal eyes of males is high, and therefore their main function is most likely to detect a potential, female partner; for, the behavior of males is to chase females from below, which thus appear as a dark, contrastful spot against the skies.

A functional interpretation for partner recognition can be advocated also for the enormous dorsal eyes of male *Haematopota pluvialis* (Fig. 3a). Quite remarkably, in strong contrast to the dorsal eyes, which are rather whitish, due to scattering structures in the retina, the small ventral eyes of male *Haematopota pluvialis* display colored, wavy bands, very alike those occurring throughout the eyes of the female (Fig. 3b). The colors, clearly due to interference effects in layered structures in the facet lenses, are quite prominent, although their function is not yet firmly established.

A striking case for investigating spectral filtering by corneal interference effects is the horsefly *Chrysops relictus*, because Lunau and Knüttel (1995) reported pronounced modulations in the transmittance spectrum for the corneal facet lenses. A general golden green luster, interspersed with dark-red-brown patches (Fig. 4) marks the eyes of this fly. Whereas the green sheen clearly is due to interference reflection, the dark patches are due to reflectionless facet lenses, through which the dark-red screening pigments are visible. This assertion can be immediately demonstrated by observing a cleaned cornea in

reflection (Fig. 5a) and in transmission (Fig. 5b). Where the cornea brightly reflects it is in transmission darker, and where it is in reflection dark the cornea is clearer in transmission. Clearly, transmittance is reduced in the reflecting facets, and transmittance is high in non-reflecting facets.

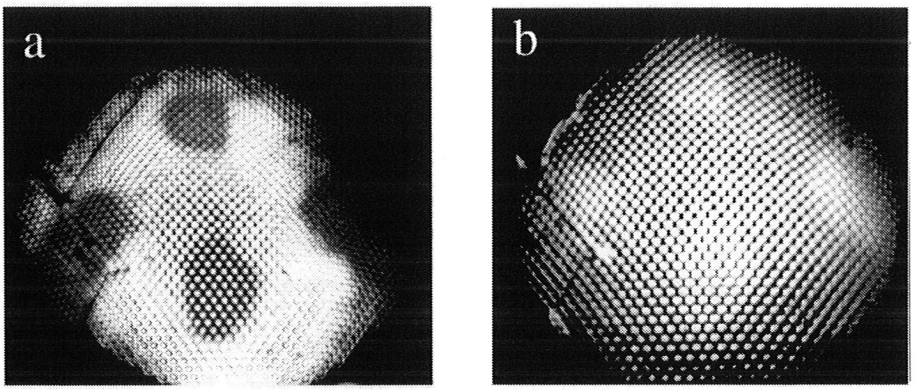

Fig. 5 Reflection (**a**) *and transmission* (**b**) *of a cleaned cornea of* Chrysops relictus. *Facet lenses with high (low) reflectance have low (high) transmittance.*

Reflectance spectra measured from single facets with a microspectrophotometer show distinct bands in the yellow-green, peaking around 585 nm (Fig. 6). It is rather cumbersome to reliably measure the corresponding transmittance spectrum, because of wavelength dependent imaging effects occurring in transmission with the high magnifications necessary. Nevertheless, extreme reductions in transmittance to around 40 – 60% probably result (Fig. 6); see also Lunau and Knüttel (1995). With those values the effect on photoreceptor spectral sensitivity has been calculated.

Visual pigment absorption spectra, $A(\lambda)$, where λ is the wavelength, were modeled using the template of Stavenga et al. (1993). In this template the only variable is the peak wavelength, λ_{max}. In the first step of calculating photoreceptor sensitivity spectra, the spectral transmittance of the facet lens was assumed to be equal to 1, i.e. full transmission at all wavelengths. The only difference of the normalized absorption spectrum, $A_1(\lambda)$, with respect to the visual pigment's absorption spectrum then is a slight broadening due to self-screening, neglecting other optical factors, like waveguide effects. The normalized photoreceptor sensitivity spectrum, $S_1(\lambda)$, then is equal to $A_1(\lambda)$. The second step was to calculate the sensitivity spectrum by incorporating the reduced transmittance spectrum ($T(\lambda)$, Fig. 6), due to interference effects: $T(\lambda)\cdot S_1(\lambda)$; yielding, after normalization, the spectral sensitivity $S_2(\lambda)$; see Fig. 6.

It readily appears that distinctly modified sensitivity spectra can only be obtained when the peak wavelength of the visual pigment, λ_{max}, is around 540 nm. This agrees well with spectral sensitivity measurements by ERG of the related horsefly *Haematopota pluvialis* (Kirschfeld 1986). We conclude that the eye areas with green reflecting and non-reflecting facets have different spectral sensitivities, i.e. $S_2(\lambda)$ and $S_1(\lambda)$, respectively. This difference is possibly utilized in improving the visibility of color contrast of animal patterns.

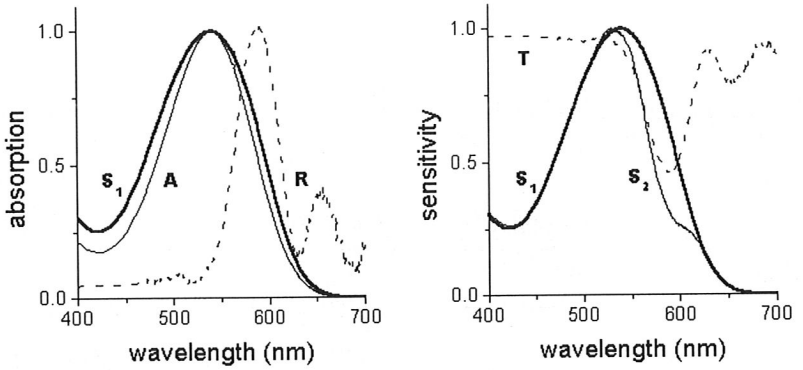

Fig. 6 Modeling the change in the sensitivity spectrum due to the filtering effect of the corneal facet lens in Chrysops relictus. *The reflectance spectrum of the corneal facet* (**R**) *results in a strongly reduced transmittance around 585 nm* (**T**). *The absorption spectrum of a visual pigment peaking at 540 nm* (**A**) *is broadened due to self-screening* ($\mathbf{S}_1$), *but due to the filter a short-wavelength shifted spectral sensitivity* ($\mathbf{S}_2$) *results.*

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