The dynamics of light adaptation in Ascalaphus
(Libelloides macaronius; Neuroptera)

Dinamika svetlobne adaptacije pri metuljčnici
(Libelloides macaronius; Neuroptera)

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Abstract. The owl-fly or Ascalaphus (*Libelloides macaronius; Neuroptera*) is an insect with a UV-sensitive superposition eye. Although optical superposition is mainly a feature of dusk/dark active animals, this is a predator living and hunting in bright sunlight. In such conditions the process of light adaptation is believed to be very important, yet it has so far only been partially explored in the owl-fly. Here we present physiological evidence for the migration of the screening pigment, which functions as a light control mechanism. The process of light adaptation was studied optically by dynamic imaging and optical reflection spectroscopy of the eye-glow. We established that the eye-glow is reduced uniformly upon illumination and that its diameter doesn't get smaller, which is indicative of pigment migration in the primary pigment cells. The change in spectral absorbance of the dorso-frontal eye is very similar to the absorbance spectrum of the primary pigment cell screening pigment. We found that the change in the light screening due to adaptation is rather small – no more than 10 fold for a 10000 fold change in light intensity. We also found that the rate of adaptation is light-sensitive. We propose that a significant part of this light sensitivity is due to indirect heating of the eye and to the very steep temperature dependency of the rate of adaptation between 30 and 35°C.

Keywords: Ascalaphus, *Libelloides macaronius*, owl-fly, light adaptation, pigment migration, eye glow, light intensity dependence, temperature dependence

Izvleček. Metuljčnica ali Ascalaphus (*Libelloides macaronius; Neuroptera*) je žival, ki ima superpozicijske oči občutljive na UV. Čeprav je optična superpozicija večinoma lastnost živali, ki so aktivne v mraku oz. temi, je to plenilec, ki živi in lovi ob močni sončni svetlobi. V takšnih pogojih naj bi bil proces svetlobne adaptacije zelo pomemben, vendar pa je do sedaj le delno raziskan pri metuljčnici. Tu predstavljamo fiziološke dokaze za premik zaščitnega pigmenta, ki uravnava količino svetlobe, ki pride v oko. Proces svetlobne adaptacije smo proučevali optično z dinamičnim slikanjem in optično refleksijsko spektroskopijo očesnega sija. Ugotovili smo, da se ob osvetlitvi očesni sij zmanjša enakomerno in se njegov premer ne spremeni, kar je značilno za premik pigmenta v primarnih pigmentnih celicah. Tudi sprememba spektralne absorbance dorzo-frontalnega očesa je zelo podobna spektru pigmenta primarnih pigmentnih celic. Ugotovili smo, da je sprememba zastiranja svetlobe na račun adaptacije precej majhna – nič več kot 10- kratna za 10000-kratno spremembo svetlobne jakosti. Poleg tega smo ugotovili, da je tudi stopnja ali hitrost adaptacije občutljiva na svetlobo. Predlagamo, da sta razlog za znaten del te svetlobne občutljivosti posredno segrevanje očesa in zelo strma temperaturna odvisnost hitrosti adaptacije med 30 in 35°C.

Ključne besede: Ascalaphus, *Libelloides macaronius*, metuljčnica, svetlobna adaptacija, premik pigmenta, očesni sij, svetlobna občutljivost, temperaturna odvisnost.

Introduction

The owlfly or Ascalaphus (*Libelloides macaronius*) is a Neuropteran found mainly in warm, sunny grass habitats. The genus *Libelloides*, comprises many species which can be found all along the Southern Europe and into central Asia. (A large portion of its species, including the one under study here, was formerly a part of the genus *Ascalaphus.*) In Slovenia, the species *Libelloides macaronius* is found mainly in the Carst meadows. The adult animals can be seen there from the end of May till mid July. In the past the animal has been the focus of intense scientific interest mainly due to the fact that it is one of only a handful of animal species with almost exclusively UV-sensitive eyes with a UV-absorbing visual pigment (Gogala et al. 1970, Hamdorf et al. 1971, Schwemer et al. 1971, Hamdorf & Gogala 1973, Schneider et al. 1978, Nilsson et al. 1992, Gribakin et al. 1995, Stušek & Hamdorf 1999, Bentrop et al. 2001). A photon absorbed by this UV rhodopsin converts it into a thermostable metarhodopsin state that absorbs maximally in the blue wavelength range. Consequently, prolonged monochromatic light illumination results in a rhodopsin to metarhodopsin ratio that strongly depends on the light wavelength. UV light causes a high metarhodopsin content, and blue light results in a virtually pure rhodopsin content (Schwemer et al. 1971).

Another interesting feature of the owlfly is its superposition eye, usually found in night and dusk active species; yet it is a predator, hunting in bright sunlight. This is especially interesting since the common view is that superposition eyes have low spatial acuity as a trade-off for good light-gathering properties (Warrant & McIntyre 1996). The unique light-sensing properties have provoked speculations on how the owlfly adapts to changes in light intensity in order to optimize its visual acuity. Stušek and Hamdorf (1999) reported the presence of light controlling mechanism, enacted by migrating screening pigment. The spectral sensitivity of this so-called pupil mechanism was high in the UV and exhibited a small hump at about 450 nm. The spectral sensitivity was similar to that of the pupil mechanism of *Deilephila* (Nilsson et al. 1992), but it appeared to be different from the spectral sensitivity of the Ascalaphus photoreceptors (Hamdorf et al. 1971, Schwemer et al. 1971, Hamdorf & Gogala 1973). The latter finding, in combination with findings from cut eye preparations, implied that the sensors for the pigment migration are not located in the photoreceptor cells, or at least their rhabdomeric parts (Nilsson et al. 1992). Neither the location nor the nature of the sensor for the screening pigment migration have been clarified yet. What is also unclear is whether there is any bulk movement of the screening pigment in the secondary pigment cells. Morphological studies on dark and light adapted specimens of Ascalaphus do not favour the possibility of massive screening pigment migration in the secondary cells (Drašlar 1997, Wolfrum et al. 2003, Drašlar & Wolfrum 2005b, Drašlar & Wolfrum 2005a).

In the present study we have measured the absorption spectra of the various screening pigments of the Ascalaphus eye, as well the absorption spectra of the pupil mechanism, in order to establish which pigment is actually moving during light adaptation. We have also investigated the type of dimming of the eye, so as to reveal the migration either of the pigment in the primary or in the secondary pigment cells. Furthermore, we have measured the light and temperature sensitivities of the screening pigment migration. We conclude that the main screening pigment movement occurs in the primary pigment cells and that the difference absorption spectra of the Ascalaphus eye in situ closely matches the absorption spectra of the pigment in the primary pigment cells.

Material and Methods

The investigated owlflies were caught in the Carst meadows near the town of Komen in Slovenia in late June, early July of 2005–2007. Up till the use in experiments they were kept at 20–25 °C and fed blowflies (*Calliphora vicina*, mutant chalky) daily. Prior to the experiments, the animals were immobilized using standard Eppendorf centrifuge tubes with obliquely cut bottoms, through which the

heads of the otherwise intact animals were pushed. The heads were additionally immobilized using a mix of Krönig's wax (a bee wax and colophony mix) and thermal conductive paste. The tubes with the animals thus immobilized were mounted with their dorsal eyes facing vertically onto microscope slides using Blu Tack® (Bostik™, UK).

The light adaptation process in the eyes of Ascalaphus was monitored optically with a modified Leitz™ (Germany) Orthoplan microscope, using epi-illumination provided by either a 900 W or a 150 W Xe arc lamp. The intensity of the wide band, white light beam was adjusted using a series of quartz neutral density filters. The microscope

objective used was ×4, 0.14 NA, Plan Fl, Leitz™ (Germany). A quartz coverslip, positioned before the objective at 45° to the incident light axis, reflected the incident light beam onto the preparation. The light reflected from the preparation was collected, after passing through the objective and the same coverslip, by a 200 μm diameter optical fibre (Ocean optics™, USA), using a quartz collimator lens. In order to reject any light reflected from the surface of the cornea as well as from the lenses inside the microscope (specular reflection), we used a combination of a polarizer in the illumination pathway and an analyzer in the emission pathway (positioned just before the collimator lens). Essentially the setup was the same as published previously (Zupančič 2003). This arrangement reduced the effective light intensity considerably, however other components used for light production and detection were efficient enough to more than compensate for the losses. The light collected was then fed into a miniature CCD spectrophotometer (USB2000®, Ocean Optics™, USA), which integrated the signal of the 2048 spectral channels, covering the wavelength range 200 and 800 nm, for 50 ms. During the next integration cycle the spectra were transferred to the PC allowing a seamless dynamic spectral acquisition. The raw spectra were analysed offline. First transmittance (relative to a piece of MgO_2 as a 100% diffusive reflection standard) and then absorbance spectra were calculated. In all the experiments dark adapted animals were used. The animals were kept mounted onto a microscope slide in a dark drawer for >1 h prior to use. To assess the changes in absorbance, attributable to screening pigment movements, difference absorbance spectra were calculated by subtracting the initial (dark adapted) absorbance spectrum from all the other absorbance spectra. To increase the signal to noise ratio of the recordings, a PCA (principal component analysis) step was used to extract only the time-spectral components that changed significantly during the experiment (Zupančič 2003). In the present work 7 components were found to be sufficient to account for >99% of all the changes. All the other (non-significant) components were discarded. This procedure significantly increased the resolution and allowed precision measurements over a wide light intensity range.

In one series of experiments the process of the adaptation was also dynamically imaged. For this we used white light illumination on the same setup. The imaging was done using a Coolpix 4500® (Nikon™, Japan) digital camera mounted onto the camera port of the microscope. We used the camera's rapid time-lapse recording mode whereby a 640x480 color image was recorded every second for up to 100 s. The »color temperature« of the Xe arc light source was adjusted on the camera using the same MgO_2 block procedure as described for transmittance measurements. In this way the contributions of the red, green and blue channel were equalized with respect to the colour composition of the source. In a single series of experiments we also assessed the temperature sensitivity of the light adaptation process. For these experiments the animals were mounted on a copper block using a specially made copper yoke and the same thermal conductive paste/Krönig's wax mix. The copper block was temperature controlled by a Peltier element. The temperature of the animal was measured using a 0.1 mm diameter thermocouple probe inserted into the animal's thorax.

Results

A cross-section of a single Ascalaphus ommatidium is shown in Fig. 1 (reproduced from Stušek & Hamdorf 1999). Light entering the ommatidium passes first the corneal lens and the crystalline

cone. Along the cone there are primary pigment cells (ppc), flanked by the distal part of the secondary pigment cells (spc). The pigment in the secondary pigment cells is divided in distal (dpg) and proximal (ppg) pigment granules. The distal pigment granules are situated at the level of the primary pigment cells while the proximal pigment granules are situated at the level of the rhabdom. Between the distal and the proximal part of the ommatidium there is a large clear zone (cz). Long processes of the photoreceptor cells extend from the rhabdomeric part through the clear zone all the way to the crystalline cone and primary pigment cells. The rhabdom has a six petal flower-shaped cross-section, created by the fusion of the photoreceptors' rhabdomeres. In the distal part, at the level of the rhabdom, the ommatidium is entirely enclosed by tracheae that have a crescent shape in cross-section.
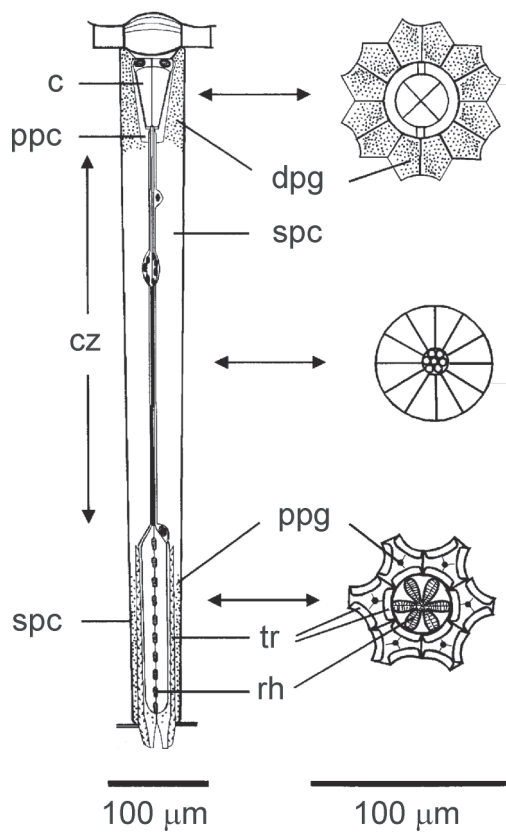

Figure 1: Schematic drawing of an Ascalaphus ommatidium (adapted from STUŠEK & HAMDORF 1999). The three cross-sections at the levels indicated by the double-headed arrows are shown on the right. Crystalline cone (c), clear zone (cz), distal pigment granules – brown pigment (dpg), proximal pigment granules – brown pigment (ppg), primary pigment cell – yellow pigment (ppc), secondary pigment cell (spc), rhabdom (rh), tracheole (tr).
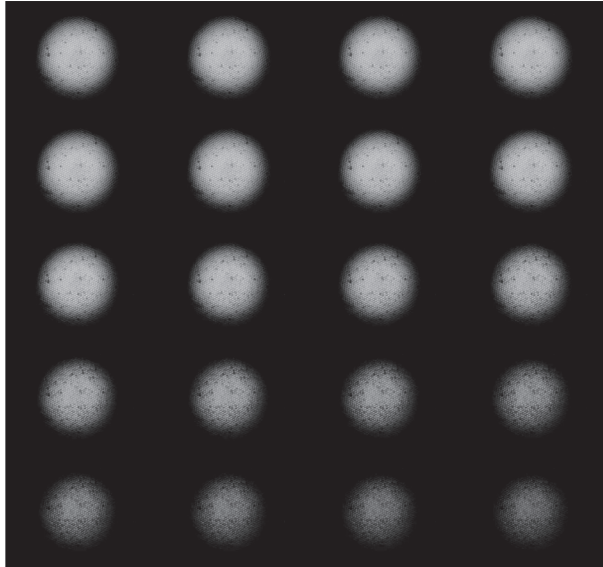
Figure 2: The process of adaptation imaged as a series of eye-glow images. The montage of images shows the transition from a dark-adapted eye with a bright eye-glow (top left) through intermediary states to the light-adapted state with reduced eye glow (bottom right). The time interval between the successive images (from left to right) is 4 s. The eye was illuminated with white light from a Xe arc light source.

When the dark-adapted dorsal eye of Ascalaphus is observed with a microscope, in a set up as described in the Methods section, an eye glow is clearly visible. The glow is most likely light reflected in the proximal part of the eye, where the tracheae cause total reflection on the water/air interface. When the eye is illuminated by white light for 80 s and photographed with a digital camera, a gradual dimming of the eye is observed, as shown in the photo montage on Fig. 2. Analysis of the photographs yields that the dimming represents a reduction of the amplitude of the reflectance profile, rather than a reduction of its width (Fig. 3). Such a dimming is consistent with an adaptation mechanism involving the primary pigment cells rather than pigment migration in the secondary pigment cells. The latter would produce a reduction in the eye-glow image diameter (WARRANT & MCINTYRE 1996). By comparing the three channels of the camera (red, green and blue), we can see that the change is fastest in the blue, followed by that in the green and red channels. This result reflects the spectral characteristics of the screening pigment involved. To analyze these spectral characteristics in more detail, we used a spectroscopic analysis of the reflected light. In order to determine whether the primary or the secondary cells were the source of the decrease in the eye glow, we calculated the absorbance difference spectrum – the difference between the absorbance spectra of the dark adapted and the light adapted state of the eye (Fig. 4a). We compared the difference spectrum to the absorbance spectra of the pigment isolated from the primary and the secondary pigment cells (SCHNEIDER et al. 1978). The difference spectrum resembles the absorbance spectrum of the pigment from the primary pigment cells, but rather differs from the spectrum of the secondary pigment cells – a result, which also supports the idea of an adaptation mechanism involving the primary pigment cells (ppc).
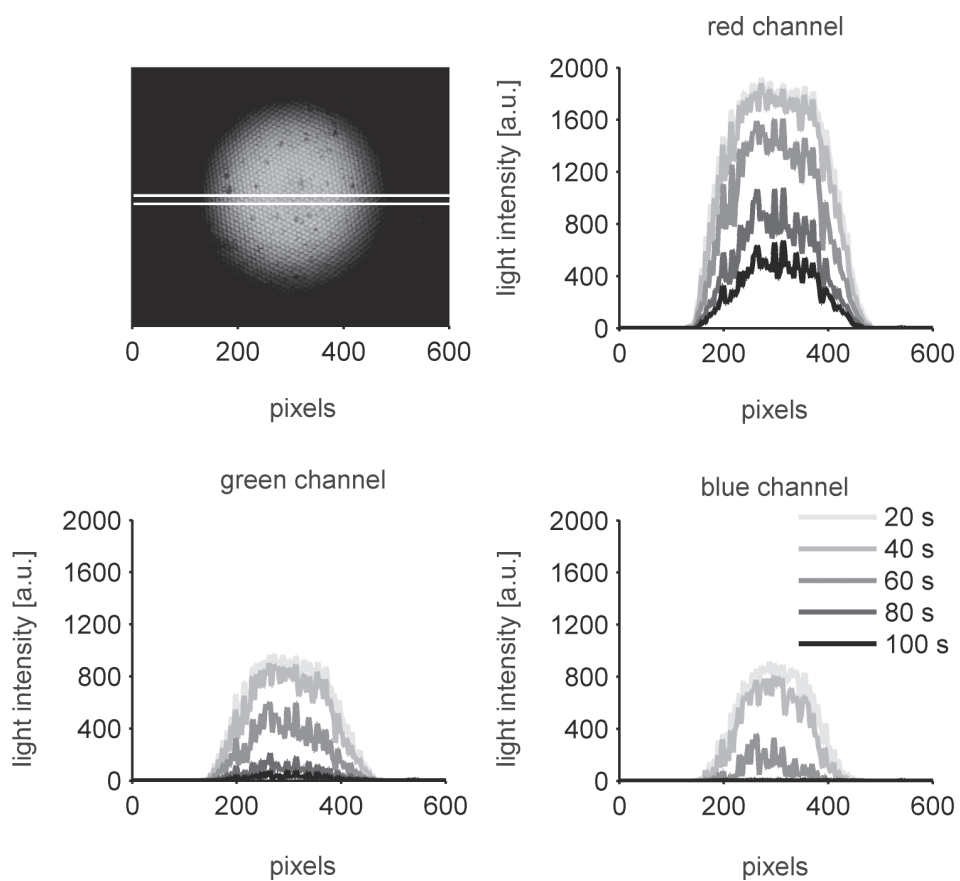
Figure 3: The intensity profiles of the eye glow. The image of the eye glow is shown in the top left panel. This is
          the first image from the montage shown on Fig. 2. The two parallel horizontal lines indicate the region
          from which the intensity profiles were obtained. The intensity profiles of the three channels (red, green
          and blue) are shown on the other three panels. The units shown are arbitrary intensity units of pixels
          from the image. The profiles are shown in different shades of gray – palest for 20 s after the start of
          illumination and black for 100 s after the start of illumination.
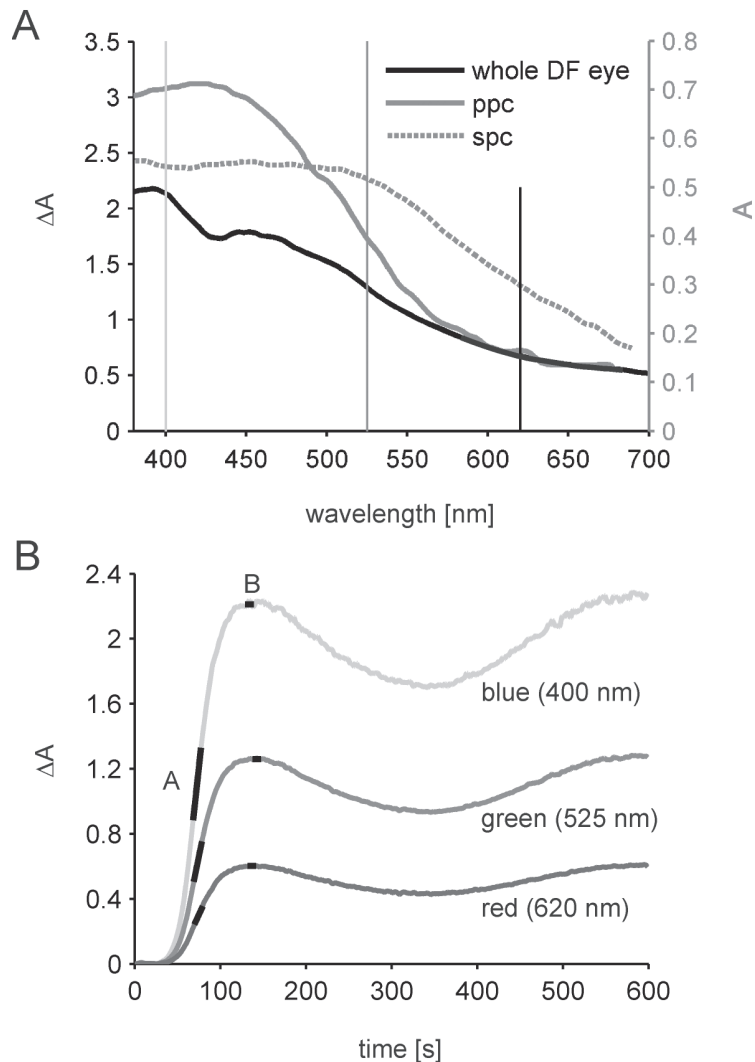
Figure 4: The light absorption of the Ascalaphus eye. **A:** The comparison of the absorbance spectra. Published absorbance spectra (Schneider et al. 1978) of the two screening pigment types, from the primary pigment cells (ppc; solid line) and the secondary pigment cells (spc; dashed line) are shown in gray. The axis for these two spectra is on the right hand side displayed in gray. The measured difference absorbance spectrum of the whole dorso-frontal (DF) eye is shown in black with the appropriate axis, also in black, shown on the left hand side. Note the values of the absorbance units for the change in absorbance of the whole DF eye, which are >1 for wavelengths shorter than 550 nm. The published and the measured spectra were equalized to values at 650 nm. The three vertical lines indicate the wavelengths that were used for further calculations (400, 525 and 620 nm). **B:** The time course of the change in the eye glow. The time courses obtained at the three wavelengths mentioned above are shown together. The two regions used for further analysis are indicated: A – the maximal slope of the change, calculated as the average of the slope between 40 and 60% of the maximal change, and B – indicates the area, the average of which was taken as the maximal change in absorption.

There are nevertheless some deviations between the spectra. When the spectrum of the pigment from the primary pigment cells and that of the whole dorso-frontal (DF) eye are equalized at the absorbance at 650 nm, the DF absorbance in the blue is lower (Fig. 4a). Apparently some other spectral effects are involved. A part of the explanation is that the absorption in the short wavelength range is high, and that the concentration / absorbance relationship is no longer linear in the specific preparation – spectrophotometer combination. This of course is most likely only the case when analysing the reflected light, which passes the pigment twice. This light is of very low intensity when it is detected by the spectrophotometer's CCD chip, which allows introduction of errors due to the minute amounts of stray light, the signal-to noise ratio of the detector etc. On the other hand other effects like the light scattering within the preparation, the non-homogeneous distribution of the pigment etc. may also contribute to this non-linearity.

The other spectral changes in the 380-500 nm region are most likely due to changes in absorption properties of the respiratory pigments. The cytoplasm of the photoreceptor cells near the rhabdom is packed with mitochondria. The absorption properties of the respiratory pigments, the cytochromes and the flavoproteins, change with their redox states, which in turn change due to the metabolic load on mitochondria and/or the availability of the O_2. The so-called Soret band region of the cytochromes closely matches the region where there is the biggest departure from the smooth shape of the absorption spectrum of the ppc pigment.

The time course of the reduction in the eye glow is relatively slow at room temperature (approx. $0.02 \, s^{-1}$). The eye is fully light adapted only after 100–200 s (Fig. 4b). Typically there is a slow oscillation present in the reflected light signal with a period longer than 300 s. This oscillation is most clearly observable in the red part of the signal. The % of reduction in absorbance relative to maximal absorbance and time to maximal decrease in absorbance from the start of illumination are $20 \pm 3\%$, 332 ± 42 s (n=2) at maximal light intensity – 2291 W/m^2; $39 \pm 14\%$, 375 ± 47 s (n=2) at 275 W/m^2; 24 ± 6 %, 409 ± 8 s (n=3) at 33 W/m^2; 26 ± 6 % , 421 ± 27 s (n=4) at 4 W/m^2 and $72 \pm 27\%$, 364 ± 55 s (n=4) at 0.5 W/m^2. The blue signal often does not exhibit these oscillations and also becomes very noisy at the highest absorption changes (not shown). All this is due to the fact that the actual measured light signal becomes close to zero when the eye becomes very dark and this dramatically reduces the signal to noise ratio. In addition, as stated before, the total absorption in the UV and blue regions is most likely no longer linearly related to the screening pigment concentration, when the eye is fully light adapted.

The recorded changes in absorption depend on the light intensity (Fig. 5a). They cover 4 log units of light intensity and the changes in the eye glow are from 0.90 ± 0.04 at 620 nm to 2.3 ± 0.1 at 400 nm (mean $\pm$ s.e.m.; n = 2–10). Taking into account the double passage of the light through the pigment cells, this translates into modulation of the incident light in the blue and UV regions of roughly only 1 log unit.

An important parameter of interest for the predatory activities of the animal, is the rate at which the light adaptation takes place. In order to obtain a measure of the rate, which would be independent of the amplitude of the change in eye glow, we divided the maximal slope of the change in the eye-glow by the maximal amplitude of the change. Thus we obtained a value which was independent of the screening pigment concentration and had units of frequency (s^{-1}). It turns out that the dependency of the rate of change on the light intensity is similar to the amplitude and that it covers a similar or a slightly narrower intensity range. At high light intensities the rates get smaller and the dependency also has a distinct plateau in the mid intensity range.

What causes this dependency of the rate on light intensity? One possible explanation is that it is due to the heating of the eye, because of the absorbed light. The light we used did not contain any appreciable amounts of IR, due to a very effective light cooling arrangement, consisting of two cold mirrors arranged into a periscope (Zupančič 2003). This effectively removed any light at wavelengths longer than 700 nm. However any light absorbed will eventually be transformed into heat. Since the screening pigment is an effective light absorbent, the eye will get warmer. Any temperature dependent processes in the eye will therefore be affected by the light intensity.
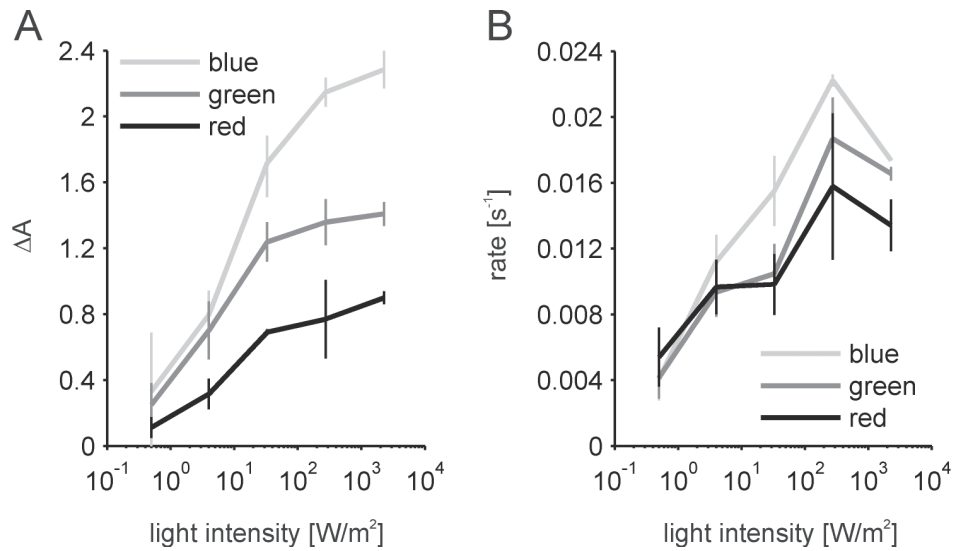
Figure 5: The light-intensity dependence of the changes in absorbance. A: The relationship between the light intensity and the maximal change in absorbance. Measurements were perfomed at 400 nm (blue), 525 nm (green) and 620 nm (red), indicated by the lines with different shades of gray. The error bars indicate s.e.m. intervals. The total white light intensities hitting the eye were: 0.5, 4, 33, 275 and 2291 W/m². **B:** The relationship between the light intensity and the maximal rate of change in absorbance. The rate was calculated by dividing the maximal slope (indicated with A on Fig. 4b) with the maximal amplitude of change (indicated by B on Fig. 4b).
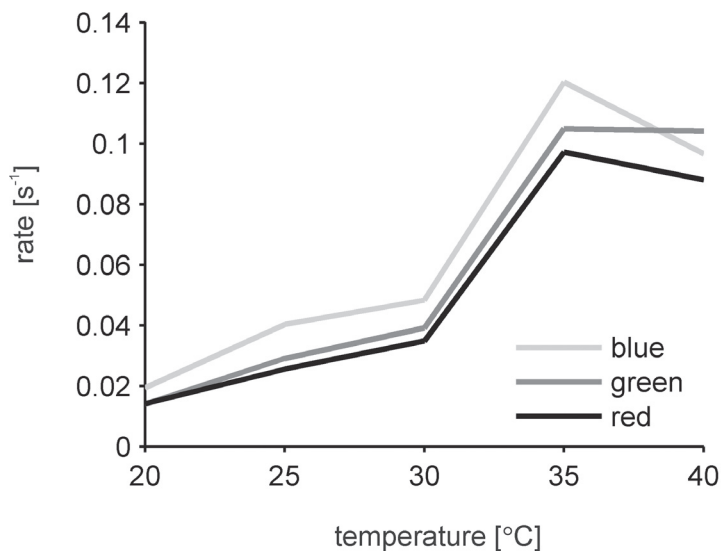

Figure 6: The temperature dependence of the maximal rate of change in absorbance. The same wavelengths were used as indicated in Fig. 4a. There are no error bars shown, because there was only a single experiment done at each temperature.

In order to assess the contribution of the temperature to the rate of eye glow reduction, we performed a series of experiments at different temperatures (Fig. 6). The experiments were done at a light intensity that produced the highest change rates at room temperature (280 W/m^2). The relationship is quite interesting since its slope changes dramatically above 30 °C and then even becomes negative above 35 °C. In any case the shape of the relationship bears a close resemblance to the relationship between the light intensity and the rate of eye glow reduction. It has to be noted, however, that the actual rates are very different. At room temperature and 280 W/m^2 the rates are between 0.015 and 0.02 s^{-1}, while the maximal rates at 35 °C are 0.1 and 0.13 s^{-1}, which is 6.5 times faster. This indicates that although temperature may play an important role it is most likely not the sole cause of the light dependency of the rate of the eye glow reduction.

Discussion

The principal aim of the presented work was to test the hypothesis that the movement of the pigment in the primary pigment cells rather than the secondary pigment cells is the cause of the light adaptation of the eyes of Ascalaphus. Adaptation can be conveniently monitored by measuring the eye-glow, which is a characteristic of all superposition eyes. There are four pupil mechanisms that were described so far (Warrant & McIntyre 1996):
1.   the longitudinal (secondary) pigment migration mechanism
2.   the cone (primary) pigment migration mechanism,
3.   the dual pigment migration mechanism and
4.   the collar pigment migration mechanism.

The fourth mechanism is present only in crayfish, so that for an insect with superposition eyes only a primary, a secondary or a combination of both mechanisms exists. In Ascalaphus there is good morphological evidence that there is no massive movement of the distal pigment granules in the secondary pigment cells (Drašlar 1997, Wolfrum et al. 2003, Drašlar & Wolfrum 2005b, Drašlar & Wolfrum 2005a). So far however, the physiological research of the pupil mechanism did not either confirm or reject these findings (Nilsson et al. 1992, Stušek & Hamdorf 1999). Even more, the mechanism of the eye-glow reduction in Ascalaphus was for a long time somewhat of a mystery, since there did not seem to be any major structural differences between the dark and the light adapted states of the dorso-frontal eye. In recent years more detailed structural studies however showed that there are small differences in the shape and position of the primary pigment cells and the processes of the photoreceptor cells. It was found that the shape of distal tips of photoreceptor cells, touching the crystal cone, changes from a bulb in the dark adapted state to a very fine tether in the light adapted state. This change is accompanied with the change in the shape and position of the primary pigment cells containing the screening pigment (Drašlar 1997, Wolfrum et al. 2003, Drašlar & Wolfrum 2005b, Drašlar & Wolfrum 2005a). In the present work we made several physiological observations that support the adaptation mechanism involving primary but not secondary pigment cells.

The shape and the diameter of the eye-glow image

The most compelling evidence for the ppc-based mechanism is given by eye glow imaging. In the eyes with the cone (primary pigment cell) mechanism, like in the eyes of the dung beetle (*Copris elphenor*), the eye glow is uniformly reduced throughout the entire pupil upon continuous white light illumination (Warrant & McIntyre 1996). In the eyes with a longitudinal secondary cell mechanism, like in the Bogong moth (*Agrotis infusa*), the eye-glow diameter gets smaller, due to substantial pigment migration of the secondary pigment cells (Warrant & McIntyre 1996). In our experiments we firmly established that in the dorso-frontal eye of the Ascalaphus the entire eye-glow is reduced.

There is no reduction in the diameter of the eye-glow, which is highly indicative of the primary pigment cell mechanism.

The spectral characteristics of the changes in the eye-glow

The spectra of the pigments contained in the primary and the secondary pigment cells are quite different (SCHNEIDER et al. 1978). The highest absorption of the pigment contained in the primary pigment cells is in the UV and blue regions. From there absorption falls off sharply towards the longer wavelengths, giving it a yellow appearance. In comparison the absorption curve of the pigment in the secondary pigment cells is somewhat flatter, giving it a reasonably high absorption also in the green and yellow regions and a brown appearance. In our work we tried to establish, which pigment is responsible for the reduction of the eye-glow during bright light adaptation. The absorbance difference spectrum calculated as the difference between the light and the dark adapted states should give this answer. The measured difference absorbance spectrum does not match the spectrum of the pigment in the secondary pigment cells while it does match to a considerable degree the spectrum of the pigment in the primary pigment cells. However there are also some differences. The most conspicuous is the general shape of the curve. The absorption curve of the isolated pigment has a ratio of the peak at 430 nm to the minimum at 650 nm of roughly 7:1. The spectra we recorded had a ratio of 4:1 at most. Also there is a finer structure of the absorption curve in the near UV and blue regions present in our preparation than in the one recorded for the isolated pigment. The reason for the flatter curve is in our opinion mainly the high light absorption of the eye, especially when measured from the eye-glow. In this case the light has to pass the screening pigment twice, which brings the absorption values in the light adapted eye well into the nonlinear part of the concentration/absorbance relationship, where the Lambert-Beer law no longer entirely applies. This nonlinearity is caused on one hand by problems related to the light detection – the presence of minute amounts of stray light, the signal to noise ratio of the detector in relation to the measured signal, the effects of broadband illumination, and on the other hand on the interaction of the light with the tissue – light scattering on the cell structures, non-homogeneous deposition of the pigment etc. As for the fine structure of the UV/blue peak, we believe that this is actually due to the changes in the absorption of the cytochormes contained in the mitochondria of the photoreceptor cells. The biggest absorption changes, which occur due to the changes in the oxidation state of the haems in the cytochromes, are in the so called Soret band, between 390 and 450 nm. The change in the redox state of the cytochromes, or rather their haems, occurs either due to the change in the P_{O2} within the tissue (ZUPANČIČ 2003) or due to the activation of mitochondria (STAVENGA 1995, JENKO 2007, ZUPANČIČ & PEROVŠEK 2007). Both can occur during intense illumination of the eyes of Ascalaphus while the density of mitochondria is high enough for these changes to contribute significantly to the total absorption changes of the dorso-frontal eye. To summarize, the measurements of the changes in absorption also support the primary pigment cell mechanism.

The time course of the eye-glow reduction upon illumination

The initial phase of the reduction in the eye glow is very similar to the one already published by STUŠEK & HAMDORF (1999). Yet, contrary to their experimental protocol we used continuous white light throughout the experiment. This exposed an interesting feature. In all our preparations the eye glow slowly oscillated. The change in absorbance in the red part of the spectrum, where the oscillation was most easy measured, was around 20–30% with a very long interval of approx. 350 s at room temperature. In our opinion this is an indication that the light sensor, which triggers the screening by the primary pigment cells, is situated behind these cells. According to this view the oscillations are the result of a feedback loop with an inbuilt delay connecting the light sensor and the pigment movement. Such

a loop is bound to produce oscillations in any dynamic control system. This finding is in agreement with previously published findings on the position of the sensor (Nilsson et al. 1992).

The dependence on light intensity

The single most intriguing feature of the light adaptation in Ascalaphus is the fact that the eye glow only changes by 2 log units when the light intensity changes for more than 4 log units. When we consider that in eye glow the light passes the pigment twice, this means that, as far as the adaptation goes, the light intensity modulation by the screening pigment is only about 1 log unit (10 fold) for a 4 log unit (10000 fold) change in intensity. Intriguing as it may be, this fact is also corroborated by electrophysiological evidence (Stušek et al. 2005). This means that the entire light screening mechanism present in Ascalaphus is no more efficient than not very dark sun glasses. There are several possible explanations for this fact and its underlying mechanisms. Their detailed dissection is however far beyond the scope of the present paper.

The rate of eye glow reduction

There is on the other hand also another parameter that changes with light intensity – the rate of light adaptation or eye glow reduction. The rate we discuss here is independent of the amplitude, or it is rather the change in rate beyond the effect of the amplitude – see results. The relationship is similarly steep (or flat) to the relationship between the intensity and the amplitude of the eye-glow reduction (a 5–10 fold increase for a 10000 fold increase in light intensity). It is however much less smooth. The relationship has a kind of plateau in the mid-intensity range and a decrease at very high light intensities. The principal question is why should the rate of adaptation need to change at all with light intensity? Currently we do not have a specific answer to this; we do however speculate that the effect could only be a by-product of the temperature sensitivity of the animal and its visual system. As mentioned in the introduction, Ascalaphus is the animal of the warm open grass meadows of the Mediterranean and it is only active in its adult form in a very warm part of the year. Our personal observations (the accompanying paper by Belušič et al. in this issue) show that it only starts to fly and catch its prey until its body temperature increases to above 30°C and that it stops flying once its body temperature goes above 40°C, which would make it adapted to a narrow set of light and temperature conditions. Taking this into consideration it makes sense for Ascalaphus to have a faster light adaptation at higher temperatures since the animal's activity changes so much and the rate of change of the visual information changes in parallel. With this in mind the relationship between the temperature and the rate of light adaptation makes perfect sense. This could also explain certain similarities between the intensity/rate and temperature/rate relationships (Figs. 5b and 6). We believe that a considerable portion of the relationship between the light intensity and the rate of adaptation is due to thermal effects of the absorbed light on the light screening machinery.

Conclusions

The aim of the present work was to explore in more detail the nature and the dynamics of the light adaptation process in the owl-fly or Ascalaphus (*Libelloides macaronius; Neuroptera*). Our findings were:
1. Pigment migration in the primary pigment cells, which surround the crystalline cones, is the light adaptation mechanism of the eye of the owlfly. This finding is based primarily on the dynamic

imaging of the eye-glow reduction during constant illumination. The eye glow is uniformly reduced rather than its diameter getting smaller, which is entirely consistent with the cone mechanism.

2. The light-dependent difference absorbance spectra of the eye glow are consistent with the absorbance spectrum of the pigment from the primary pigment cells, but not with the spectrum of the secondary pigment cells.

3. Upon illumination the eye adaptation due to the pigment migration is no more than 1 log unit for 4 log unit change in light intensity.

4. The maximal rates of pigment migration are relatively slow at room temperature (0.02 s^{-1}). They are however dependent on light intensity and especially on the temperature of the animal. Our results give some indication that at least part of the dependence of the pigment migration rate on light intensity is indirectly due to heating of the eye at high light intensities.

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