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Temperature dependence of photoreception in the owlfly *Libelloides macaronius* (Insecta: Neuroptera: Ascalaphidae)

Temperaturna odvisnost fotorecepcije pri metuljčnici *Libelloides macaronius* (Insecta: Neuroptera: Ascalaphidae)

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Abstract. The owlfly *Libelloides macaronius* (Insecta: Neuroptera: Ascalaphidae) is an airborne predator, hunting for flying insects during the daytime in the summer. Its success relies entirely on the quality of image supplied by the bipartite superposition eyes, sensitive almost exclusively to UV. The speed of visual performance in the owlfly was investigated at temperatures between 10 °C and 45 °C by means of electroretinography and intracellular recordings. The laboratory experiments were supplemented with activity and temperature measurements in the field. All parameters of the electrical response of the eye were dramatically affected by temperature. At body temperatures below 26 °C the owlfly remains landed, and the photoresponse becomes sluggish. Vision speeds up monotonically with the temperature, exhibiting large values of Q_{10} (up to 10), and a clear optimum at 40 °C. The flicker fusion frequency reaches a maximum of 230 Hz at 40 °C, and the receptor potential latency attains a minimal value of 4 ms at 38 °C. Compared to the values measured in *Calliphora* (flicker fusion frequency > 400 Hz, latency < 3 ms), the speed of vision in the owlfly is almost half of what is possible to encounter in an insect eye. The physiological working range of *Libelloides* eyes is above 30°C, indicating that the animal is optimised to high body temperatures, resulting from the heat-generating muscle work while hunting in a hot habitat.

Keywords: *Libelloides macaronius*, owl-fly, *Ascalaphus*, photoreceptor, ERG, flicker fusion frequency, latency, temperature dependence.

Izvleček. Metuljčnica (*Libelloides macaronius*, Insecta: Neuroptera) je plenilec, ki v poletnih dneh med letom lovi leteče žuželke. Njegov uspeh je v celoti odvisen od kakovosti slike, kakršno zajemajo njegove dvodelne oči superpozicijskega tipa, občutljive skorajda izključno na UV svetlobo. Hitrost gledanja metuljčnice smo raziskali pri temperaturah med 10 °C in 45 °C s pomočjo elektroretinografije in znotrajceličnih posnetkov iz retinula celic. Laboratorijske poskuse smo dopolnili s poljskimi meritvami aktivnosti in temperature. Vsi parametri električnega odgovora očesa so izkazovali izredno temperaturno odvisnost. Pri temperaturah telesa pod 26 °C metuljčnica ni aktivna, odgovor oči pa postane zelo upočasnjen. Hitrost gledanja monotono narašča s temperaturo, vse do optimuma pri 40 °C. Pri tem izkazuje zelo visoke vrednosti Q_{10}, vse do 10. Fuzijska frekvenca znaša 230 Hz pri 40 °C, latenca receptorskega potenciala pa najmanj 4 ms pri 38 °C. V primerjavi z vrednostmi, izmerjenimi pri muhi *Calliphora* (fuzijska frekvenca > 400 Hz, latenca < 3 ms), je hitrost gledanja pri metuljčnici skoraj prepolovljena glede na hitrosti, ki jih lahko srečamo v očesu žuželke. Fiziološko delovno območje oči metuljčnice sega nad 30° C, kar nakazuje, da je žival prilagojena na visoke telesne temperature, ki so posledica toplote iz dela letalnih mišic v vročem habitatu.

Ključne besede: *Libelloides macaronius*, metuljčnica, *Ascalaphus*, fotoreceptor, ERG, fuzijska frekvenca, latenca, temperaturna odvisnost.

Introduction

The owlfly *Libelloides macaronius* (Insecta: Neuroptera: Ascalaphidae), in the visual physiology literature named also as *Ascalaphus,* is a daytime airborne predator famous due to the exclusive UV sensitivity of the dorsofrontal (DF) part of its bipartite eyes (Gogala and Michieli, 1965; Stušek et al., 2000; Kral, 2002). It inhabits warm, uncultivated meadows and steppes of the Pontomediterranean region (Aspöck et al., 2001). As an adult, it only lives during the two months around the summer solstice. Even then, the owlfly is actively hunting other insects only on warm, if possible also clear days. The visual system of owlfly should match the demands of a fast flying hunter, supplying the brain with a sharp, contrasted image, with as little motion blur as possible. Insect photoreceptors are one of the fastest known sensory systems, achieving response latencies less than 3 ms, and flicker fusion frequencies higher than 400 Hz (Tatler et al., 2000). In this study, we investigated the performance of owlfly photoreceptors in terms of speed of their response to illumination. Our field measurements revealed that the animal stays rather warm during the periods of activity, suggesting that the laboratory experiments should be conducted at elevated temperatures, and not at room temperature.

Therefore, we examined the speed of the photoresponse by means of electroretinography and intracellular measurements at a range of temperatures which an animal encounters in nature, from 10 °C in the night, to 45 °C in the bright sun.

Materials and methods

Adults of the owlfly species, *Libelloides macaronius*, predominantly females, were caught in the Slovenian part of the Karst near Komen in June and July in the years 2005–2007. Subsequently, they were kept at 24 °C and fed liver and blowflies regularly. For all laboratory experiments, the animals were tethered to a copper yoke and immobilized with a mixture of bee wax, colophony and thermal conductive paste. The temperature was maintained constant with a Peltier element below the yoke, and simultaneously monitored with a K-type thermocouple, inserted into the opposite eye. The animals used in intracellular measurements had their mouth parts removed in order to eliminate the mouth musculature movements. Electroretinograms were recorded with borosilicate glass electrodes with resistance in the kΩ range, filled with insect saline. The reference electrode was an Ag/AgCl wire inserted into the head capsule. Intracellular recordings were obtained with borosilicate glass microelectrodes with resistances between 100 and 150 MΩ, filled with 3 M KCl. The electrode was inserted through a triangular hole in the cornea, sealed with Vaseline. Successful cell penetrations were possible between the clear zone and the rhabdom layer, ca. 500 μm below the cornea in the dorsofrontal (DF) eye. A successful impalement of the cell was characterized by a sudden drop to the resting membrane potential (at least –60 mV) and by a vigorous, directionally sensitive depolarisation upon UV illumination. The reference electrode was an Ag/AgCl wire inserted into the same eye.

Light stimulation was provided with UV LED sources (wavelengths 350, 360 and 390 nm, supplied by Roithner Lasertechnik GmbH, Austria) mounted on a ball joint support. The LED positioning was done by optimising the eye response while moving the source into different directions. The driving current was adjusted so that flashes elicited submaximal electrical responses. Blue background illumination was utilized to prevent excessive metarhodopsin accumulation.

Field measurements of animal activity were conducted on a meadow near Komen, Slovenia, during the nine days of July of 2005 and 2006. The animal activity was estimated by means of a wire

frame (100×20 cm). The frame was viewed from a viewpoint 80 cm away, forming with it a triangle with approximately equal sides. The end of the meadow was a tree line 36 m away. We estimated the activity by counting the number of animals appearing within the space delimited by the frame and the tree line during 1 min period every 15 min from 6 am till 8 pm. At the same time we also measured the temperature of the air at 200 cm height, as well as the temperature of an immobilized owlfly facing the sun. The animal was tethered to a grass stalk 20 cm above the ground. Measurements of the body temperature in the field and the eye temperature in the laboratory were made with small 0.1 mm diameter K-type thermocouples, inserted into the thorax.

Results

The visual performance of the two parts of the owlfly eye, the dorsofrontal (DF) and the ventrolateral (VL) part, was evaluated electrophysiologically at a range of temperatures. First, responses to single flashes of UV light were recorded as electroretinograms (ERG) with extracellular electrodes from the dorsofrontal (DF) eye of ten animals. Representative ERGs obtained in a single eye between 11 °C and 41 °C are shown in Fig. 1a. The depolarisation of photoreceptors at the beginning of the response elicits a fast, corneal negative electrical change, which is then followed by a sustained plateau phase, often showing an oscillatory component. The length of the plateau roughly matches the duration of the stimulus. The return of the potential to the baseline after the stimulus depends on the repolarisation of photoreceptors. Efficient coding of the stimulus (UV LED flash, bold bar above) by the electrical response is enabled only at the higher temperatures. The temperature of the eye dramatically influences all phases of the ERG: the delay, the speed of the depolarisation phase, the length of the plateau, and especially the speed of the repolarisation phase. However, a precise measurement of these ERG parameters is not possible due to gradual transitions between the phases. Thus, the speed of the photoresponse was quantified by measuring the time to half maximal depolarisation (t_{50d}) and the time at which the response decays to a half of the peak (t_{50r}). Both parameters are progressively shortened with the increasing temperature (Fig. 1b), attaining minimal values ($t_{50d} < 10$ ms, $t_{50r} < 100$ ms) above 35 °C. The temperature quotient (the Q_{10}) of both parameters approach 1 above 35 °C, indicating an optimum, but both rise sharply below 25 °C (Fig. 1c). The bottleneck limiting the speed of photoresponse seems to be the repolarization phase since it exhibits a higher Q_{10} even at slightly suboptimal temperatures. ERG traces from Fig. 1a were analysed also in terms of the rate of the depolarisation phase (Fig. 1d). The temperature dependence of this parameter indicates an optimum between 28 °C and 35 °C.

Dorsofrontal and ventrolateral eyes of five animals were subjected to electroretinographic examination of the flicker fusion frequency (FFF) utilizing a UV LED source on a cardan arm, flickering at a range of frequencies between 20 and 250 Hz in 10 Hz steps. A sequence of raw ERGs obtained in a DF eye is shown in Fig. 2a. At the high frequencies of flickering, the response to single flashes gradually fuses to a flat line. The frequency range of this transition is obviously affected by the temperature of the eye. The FFF rises monotonically with the temperature between 20 °C and 40 °C, and reaches a maximum of 230 Hz at the highest experimental temperature, 45 °C (Fig. 2b). At 36 °C and 41 °C, an oscillation of biological, but non-visual origin is superimposed at the ERG traces.Fig. 2b indicates a slight, but not significant difference between the two parts of the eye in the range between 20 °C and 30 °C, the VL part performing slightly faster under the given conditions.

The ERG experiments were supplemented with a few successful intracellular recordings from photoreceptors in the DF eye. The initial phases of responses of a single photoreceptor cell to UV flash at three different temperatures are shown in Fig. 3a. The latency of the receptor potential progressively shortens with increasing temperature, approaching 4 ms at 40 °C (Fig. 3b). The time to peak of the response is approximately 10 ms at the highest temperature.

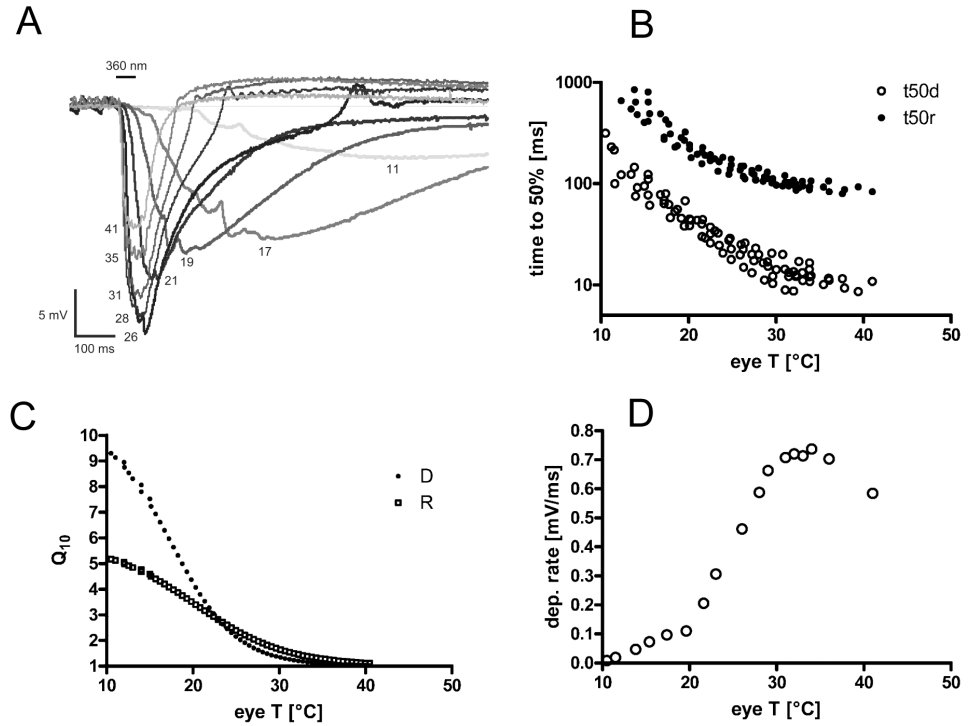

Figure 1: Electroretinograms of flash responses of the DF eye.

A: Overlay of ERGs from the DF eye at the temperatures indicated next to the traces. **B:** Parameters of the ERG in Fig. 1a. t_{50d}, time between the onset of light and the half maximal depolarising phase; t_{50r}, time between the end of illumination and the return of the trace to half maximal depolarising phase. **C:** Temperature quotients (Q_{10}) of the depolarising phase (D) and the repolarising phase (R) of the ERG. Note that the Q_{10} values approach unity above 30 °C, indicating an optimum in this range. Below 25 °C, both Q_{10} exceed 2. **D:** Rates of the depolarising phase of the ERG in Fig. 1a. Highest rates are achieved above 30 °C. The lower value at 41 °C is due to the overall reduction of the ERG amplitude.

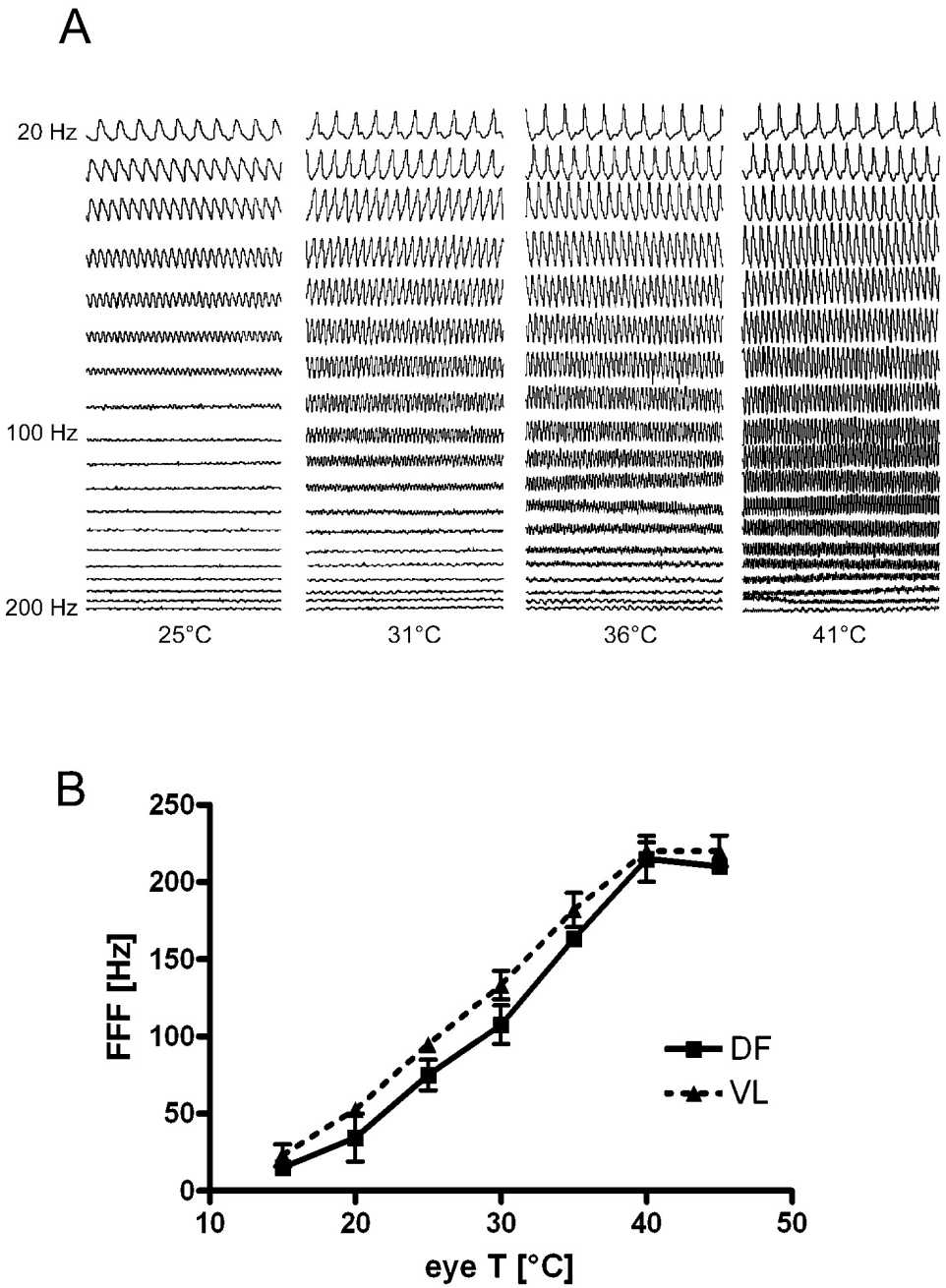

Figure 2: Measurements of the flicker fusion frequency (FFF).
A: Temperature sequence of raw ERGs from a DF eye at the different frequencies of flicker (UV LED, as in Fig. 1a). **B:** FFF of both parts of the eye (DF, dorsofrontal eye, VL, ventrolateral eye) at a range of temperatures. The difference between the two eye parts is not statistically significant.

Discussion

The successful detection of prey during flight in the owlfly *Libelloides macaronius* relies exclusively on its visual performance. Therefore, one should expect to encounter many optimisations in favour of speed and reliability of this animal's visual system. While the not so obvious advantages of the exclusive UV sensitivity and the superposition optics will be treated in a separate article, here we focused on a very basic electrophysiological investigation of owlfly' eyes. To our surprise, we found that a large part of research on owlfly eyes by other authors in the past had been done in suboptimal thermal conditions, e.g. at room temperature. Normally, the animal does not live in a room, but under the bright Mediterranean sun in the peak of the summer. Indeed, our field measurements demonstrated that the owlfly is active at air temperatures well above 20 °C (Fig. 4, air). Besides that, the body temperature plot (Fig. 4, animal) suggests that the animal is active only if the body temperature is higher than 26 °C. The number of animals flying is close to zero at body temperatures below 27 °C. However, the body temperature can be high enough even when the air is colder: (1) due to direct irradiance of the black surface of owlfly by the sun, and (2) due to the active production of heat by means of shivering

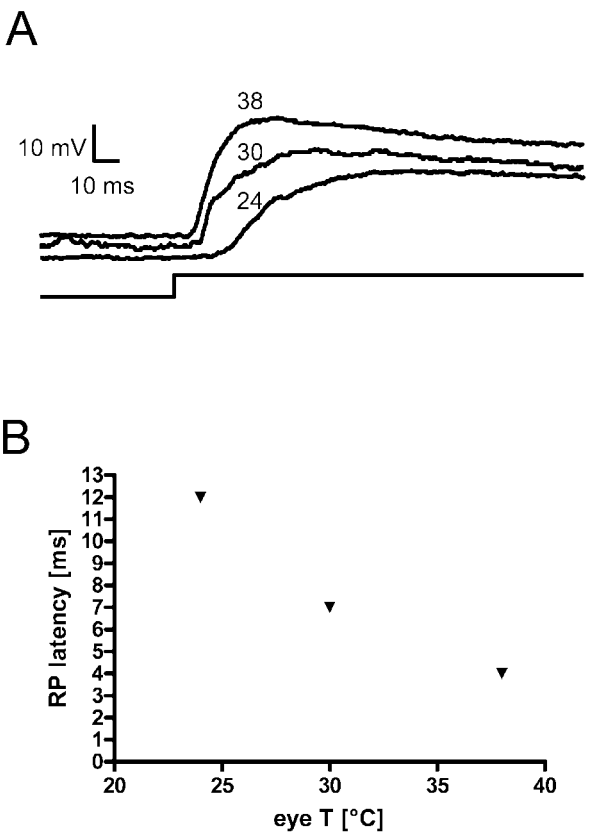

Figure 3: Intracellular recordings from retinula cells in the DF eye.
A: Intracellular recordings of the receptor potential of a photoreceptor cell from the DF eye at three different temperatures. The lower bar indicates the stimulus (UV LED, 390 nm).
B: Latency of the receptor potential at the three temperatures. At 38 °C, the latency approaches 4 ms.

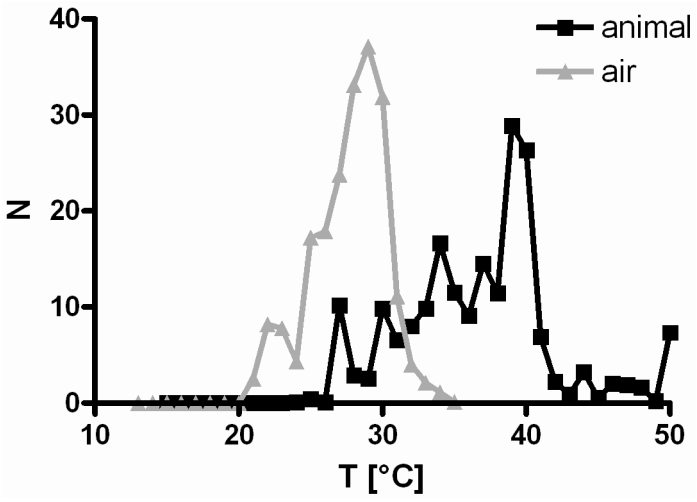

Figure 4: Field measurements of activity and temperature. Average number of animals flying in the field per minute observation time, plotted as a function of body temperature (black line) and as a function of air temperature (grey line).

of the thoracic muscles (Pirih and Belušič, unpublished recordings with a thermal imaging camera). Moreover, the animal's body temperature during the periods of active flight frequently exceeds 40 °C and can rise above 45 °C (Stušek, personal communication), probably due to the combined effect of solar irradiation and the extensive production of heat by the flight muscles. Therefore, it was no surprise to find out that *Libelloides* visual system showed signs of "hot optimisations". Another aspect of our findings is that a body temperature below 27 °C seems to be restrictive, as the animal stays landed when its body is not sufficiently warm.

The task of the owlfly*'* visual system is to pursue small targets in three dimensions. In order to accomplish this, the motion blur must be minimized by the high speed of the photoresponse. Oscillations of the ERG in Fig. 1a are probably an epiphenomenon, indicative of a sensory system possessing a mechanism for boosting the high frequencies of the response (van Hateren, 1987). Slower insect visual systems generally do not exhibit an oscillating ERG (Ruck, 1961). The highly efficient, spatially organised G-protein coupled cascade of the insect phototransduction process (Hardie, 2001) allows for the photoreceptor's extremely short response times, less than 3 ms, as has been measured e.g in the fly *Calliphora* (Tatler et al., 2000). Owlfly's receptor potential latency approaches this value only at 38 °C (4 ms, Fig. 3), while in *Calliphora*, the latency stays below 4 ms within the whole temperature range examined: 19 °C–34 °C (Tatler et al., 2000). The Q_{10} of *Calliphora* in that temperature range is 1.9 for the photoreceptor corner frequency (Tatler et al., 2000), while in *Libelloides* the Q_{10} of the speed of the ERG is much higher (Fig. 1c). This suggests that the owlfly can be considered as a stenothermic animal, adapted to a relatively narrow range of high body temperatures. Temperatures below 20 °C are outside any physiological range of its visual system and therefore lead to extreme values of Q_{10} (Fig. 1c).

The receptor potential (RP) latency in a warm owlfly (4 ms) is approximately 1 ms longer than the RP latency in *Calliphora.* This explains in part the fact that the owlfly's FFF never exceeds 230 Hz and remains much lower even at high temperatures. In *Calliphora*, FFF above 200 Hz can be measured at room temperature (Autrum, 1950, Ruck, 1961), and values above 400 Hz can be encountered at higher temperatures (Tatler et al., 2000). However, the relatively low FFF of owlfly cannot be attributed to a putative slow activation mechanism in the photoreceptors, since the response latency

is only 30% longer to the shortest values ever measured in *Calliphora*. It is likely that the limiting factor could be the large size of the photoreceptor cells of owlfly. These cells are, not including the axon, over 600 µm long in the DF part of the eye (Ast, 1920). Their large dimensions might result in slow electrical characteristics of the cell membrane. The time to peak of the RP in Fig. 3a exceeds 10 ms at 38 °C, while in *Calliphora*, it is reported to be less than 5 ms (Tatler et al., 2000). Another bottleneck limiting the speed of the response could be the processes underlying the repolarisation. The repolarisation phase is very temperature sensitive, since it exhibits larger Q_{10} values already slightly below the temperature optimum (Fig. 1c).

The visual system of the owlfly faces similar, if not higher demands as the eyes of other actively flying insects, such as *Calliphora*. However, important parameters of the speed of owlfly's photoresponse, such as depolarisation, repolarisation, FFF, are practically halved if compared to the maximal values measured among the insects. The latency of the receptor potential suggests that this could not be only due to the phototransduction cascade. The imperfections are most likely compensated with some other features of the eye design. The bipartite eye could certainly represent an advantage, since it includes the "foveal" dorsofrontal part with small interommatidial angles (Ast, 1920) and a putative higher optical resolution. Another feature is a putative excellent signal to noise ratio of the photoreceptors. Namely, the receptor potential in Fig. 3a does not become noisy when the light is on, e.g. there is no detectable photon shot noise in the response. Taking into account the high operating temperatures of the eye, this is a remarkable achievement, since the thermal noise can represent a serious problem in the visual system. Obviously, the eye of owlfly is well adapted to operate at high temperatures. Furthermore, even the UV vision could be considered as a special aspect of the "hot optimisation". Spontaneous thermal photoisomerisations of rhodopsin can represent a significant source of noise in a photoreceptor cell, and they do occur more frequently in rhodopsin molecules sensitive to long wavelenghts of light, which have relatively low activation energy (Ala-Laurila et al., 2004). The UV sensitive rhodopsin has the higest possible activation energy for a visual pigment molecule. This makes a spontaneous thermal photoisomerisation less possible, what is strongly in favor of a better signal to noise ratio in a hot animal. Taking into account the lifestyle of *Libelloides macaronius* – active flying under the strongest summer sun, with extensive flight musculature generating a lot of heat – the adaptation to a hot environment seems a necessity. This heat adaptation comes at a price, however, as the performance of the owlfly eye is rather poor at "normal" or "room" temperatures in European climates.

Conclusions

Our experiments demonstrate that

(1) the temperature of the eye of *Libelloides macaronius* dramatically affects all parameters of the speed of the photoresponse, with Q_{10} reaching values as large as 9;
(2) the photoresponse exhibits a clear temperature optimum around 40 °C, and becomes sluggish below 27 °C. This also seems to be the restrictive body temperature, since the animals do not fly if their bodies do not achieve at least 27 °C;
(3) the flicker fusion frequency reaches 230 Hz at 40 °C. This is a rather low value compared to 200 Hz (room temperature) or 400 Hz (34 °C) in *Calliphora*.
(4) the receptor potential latency is 4 ms at 38 °C, which is at least 1 ms longer than the shortest reported photoreceptor latency (3 ms, *Calliphora*).

In brief, *Libelloides macaronius* turns out to be adapted to a narrow range of relatively high body temperatures above 30 °C, which enables optimised photoreception. Yet, even when warm, the speed of its vison is largely outperformed by insects like the flies (*Calliphora*).

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