The Journal of Experimental Biology 205, 201–210 (2002)
Printed in Great Britain © The Company of Biologists Limited 2002
JEB3637

Light-induced and circadian changes in the compound eye of the haematophagous bug *Triatoma infestans* (Hemiptera: Reduviidae)

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Accepted 2 November 2001

Summary

We analysed dynamic changes in the ommatidial structure of the compound eyes of *Triatoma infestans*. This nocturnal insect possesses open-rhabdom eyes, in which a ring of six rhabdomeres from retinula cells 1–6 (R1–6) surrounds a central pair of rhabdomeres from retinula cells 7 and 8 (R7–8). Screening pigments are located in all the photoreceptors and in the primary (PPC) and secondary (SPC) pigment cells. During the day, pigments within R1–6 and the PPCs form a small 'pupil' above the rhabdom and pigments within R7–8 are clustered around the central rhabdomere, allowing light to reach only the central rhabdomere. At night, the 'pupil' widens, and pigments inside R7–8 concentrate in the proximal region of the cells, allowing light to reach the peripheral rhabdomeres. In addition, the distance between the cornea and the rhabdom decreases. These rhythmic changes adapt the sensitivity of the eye by controlling the amount of light reaching and travelling within the rhabdom. Furthermore, the rhythm persists under conditions of constant darkness (DD), i.e. it is controlled by an endogenous oscillator. Remarkably, there are differences in pigment movements between the retinula cells of a single ommatidium. The migration of pigments in R1–6 is regulated by a circadian input, while that in R7–8 is regulated by both direct light and circadian inputs. The rhythm vanishes under constant-light conditions (LL). In this species, the circadian rhythm of photonegative behaviour persists in both DD and LL conditions, suggesting that these two rhythms, in retinal morphology and visual behaviour, may be generated by different circadian oscillators.

Key words: light/dark adaptation, compound eye, vision, circadian rhythm, Triatominae, insect, haematophagous bug, *Triatoma infestans.*

Introduction

Animals active in a broad range of light intensities need mechanisms to adjust the sensitivity of their eyes to the level of ambient illumination. In arthropods, visual adaptation involves two main mechanisms: (i) changes in the transduction gain in the photoreceptors cells and (ii) modulation of the amount of light absorbed by the photoreceptors (Nordström and Warrant, 2000). In the latter mechanism, the most important processes are the movements of screening pigments within pigment and retinula cells and morphological changes in the dioptric apparatus and/or in the rhabdom structure (for a review, see Autrum, 1981). Some of these mechanisms are activated directly by changes in light intensity, but others are under endogenous control (Barlow et al., 1989; Meyer-Rochow, 1999). The adaptive value of these endogenous mechanisms is to adjust the visual sensitivity in anticipation of changes in ambient light intensity.

Circadian (i.e. endogenous) rhythms have been described in the retina of several arthropod species (Barlow et al., 1989; Bennett, 1983; Chen et al., 1999; Colwell and Page, 1989; Fleissner and Fleissner, 1977; Horridge et al., 1981; Koehler and Fleissner, 1978; Menzi, 1987; Page and Larimer, 1975; Wills et al., 1985). These rhythms regulate visual sensitivity and can be recorded as circadian variations in the amplitude of the sustained negative component of the electroretinogram (ERG), which arises from the depolarisation of retinula cells (Colwell and Page, 1989; Chen et al., 1999). In nocturnal insects, the amplitude of this component is higher during the subjective night than during the subjective day (Bennett, 1983; Wills et al., 1985; Colwell and Page, 1989).

In several arthropod species with superposition eyes, circadian migrations of screening pigments within secondary pigment cells (SPCs) increase the visual sensitivity during the night, although other mechanisms also contribute (Aréchiga and Rodríguez-Sosa, 1997; Barlow et al., 1989; Bennett, 1983; Fleissner and Fleissner, 1977; Warrant and McIntyre, 1990). In the case of apposition eyes, daily changes in ommatidial structure have been extensively described (for a review, see Autrum, 1981), but in most cases their persistence under

constant conditions has not been examined. Where such studies have been made, few if any changes continued on a circadian basis or they continued with a reduced amplitude compared with the amplitude of the daily rhythm [cockroaches (Ferrell and Reitcheck, 1993); locust and mantis (Horridge et al., 1981); ants (Menzi, 1987)]. In the lateral (apposition) eyes of the horseshoe crab *Limulus polyphemus*, a clock located in the brain mediates circadian movements of screening pigments located in the retinula cells and in the distal pigment cells as well as longitudinal movements of the rhabdom. These structural changes, together with additional variations in retinal physiology (e.g. changes in gain and noise), can increase visual sensitivity as much as 100 000-fold at night, but at the expense of a decrease in spatial resolution (Barlow et al., 1989). All these examples refer to eyes with a fused rhabdom, in which screening pigments generally migrate radially within retinula cells (Stavenga, 1979; Autrum, 1981). In open-rhabdom apposition eyes without neural superposition, pigments within primary pigment cells (PPCs) usually form a variable pigment 'pupil' in front of the rhabdom tip (Nilsson, 1989). In the diurnal beetle *Tenebrio molitor* (Wada and Schneider, 1968) and in tipulid flies (Ro and Nilsson, 1994), this pupil opens and closes with a circadian rhythm. Recently, Hariyama et al. (2001) described an endogenous variation in visual sensitivity and the associated morphological changes in the open-rhabdom eye of the isopod *Ligia exotica*.

Triatoma infestans, a nocturnal haematophagous bug of the family Reduviidae, possesses apposition compound eyes with open rhabdoms, in which a ring of six rhabdomeres from retinula cells 1–6 (R1–6) surrounds a central pair of rhabdomeres from retinula cells 7 and 8 (R7–8). Although superposition eyes would be the most efficient design for a nocturnal insect exposed to low light intensities (Nilsson, 1989; Warrant and McIntyre, 1990), the visual system of these bugs is extremely sensitive to light (Reisenman et al., 1998; Reisenman, 2000). Furthermore, this species exhibits a circadian rhythm of negative reaction to light with a maximum at night (actual or subjective) (Reisenman et al., 1998). Given that a behavioural rhythm of visual sensitivity exists in *T. infestans*, we investigated whether the compound eyes of this species express circadian changes in retinal anatomy. To do this, we analysed the ommatidial structure of the eyes when the insect was subjected to light/dark cycles (LD) and under conditions of constant darkness (DD) and constant light (LL).

Materials and methods

Experimental procedure

Fourth-instar larvae of *Triatoma infestans*, reared in a laboratory colony at 28 °C and fed weekly on chicken blood, were used throughout. Bugs were maintained in light/dark cycles (L:D 12 h:12 h, 140 μW cm^{-2}) at a constant temperature (25 °C) for 5 days. The compound eyes were then fixed (i) at the fourth hour of the photophase under white illumination (140 μW cm^{-2}) and (ii) at the second hour of the scotophase. To prevent any interference from light, preparations were performed under far red light illumination (λ>695 nm; light filtered through an RG695 filter; Schott, Germany). These two times of the day were chosen because these are when *T. infestans* displays minimal (fourth hour of the photophase) and maximal (second hour of the scotophase) photonegative sensitivity (Reisenman et al., 1998). Illumination during the light phase of the LD cycle was provided by a fluorescent lamp (Osram Dulux EL E27, 7W/41-827). Light intensity was measured with a radiometer (SEL 033 sensor module, IL 1400 radiometer; International Light, Newburyport, MA, USA).

To test whether daily changes in ommatidial structure are under endogenous control, insects were maintained in LD cycles (L: 140 μW cm^{-2}) for 5 days, and a group of bugs was then kept in constant darkness (DD), while another group was kept in constant light LL (L: 140 μW cm^{-2}). After three 'subjective' days, the eyes of half of the bugs from each experimental group (LL and DD) were fixed at the fourth hour of the subjective day and the remaining half at the second hour of the subjective night. The free-running periods used for estimating the subjective day and night of the bugs were approximately 23 h 50 min for bugs kept in DD conditions and approximately 26 h 40 min for bugs kept in LL conditions (Lazzari, 1992). This experimental protocol is identical to that used by Reisenman et al. (1998) to study the circadian rhythm of photonegative sensitivity in this bug.

To study the effect of light intensity on the ommatidial structure, the procedure described above was repeated under a lower light intensity, i.e. animals were maintained in LD cycles (L: 8 μW cm^{-2}) and then transferred to LL conditions (L: 8 μW cm^{-2}). The eyes were fixed during the light and dark phases of the LD cycle or during the subjective night and day of the bugs (LL conditions).

The age of the insects at the time when the eyes were fixed ranged between 12 and 18 days post-ecdysis.

Tissue preparations

Light microscopy was performed on eyes processed following the technique described by Ribi (1987). The posterior half of the head (containing the eyes) was fixed for 3 h in a mixture of 2.5 % glutaraldehyde and 2.0 % paraformaldehyde in phosphate buffer (pH 7.3), with added sucrose and $CaCl_2$. The heads were then rinsed five times over a 20 min period in phosphate buffer and post-fixed with buffered 1 % osmium tetroxide for 1–2 h. After dehydration through a series of alcohol dilutions, they were embedded *via* propylene oxide in Durcupan. Blocks were serially sectioned at 2 or 5 μm using glass knives mounted in a microtome. The sections were stained on a hot plate with 1 % Methylene Blue and examined under a light microscope.

Results

Changes in ommatidial structure under light/dark cycles

Fig. 1 shows a schematic representation of the light- and dark-adapted ommatidia of *T. infestans*. The photosensitive

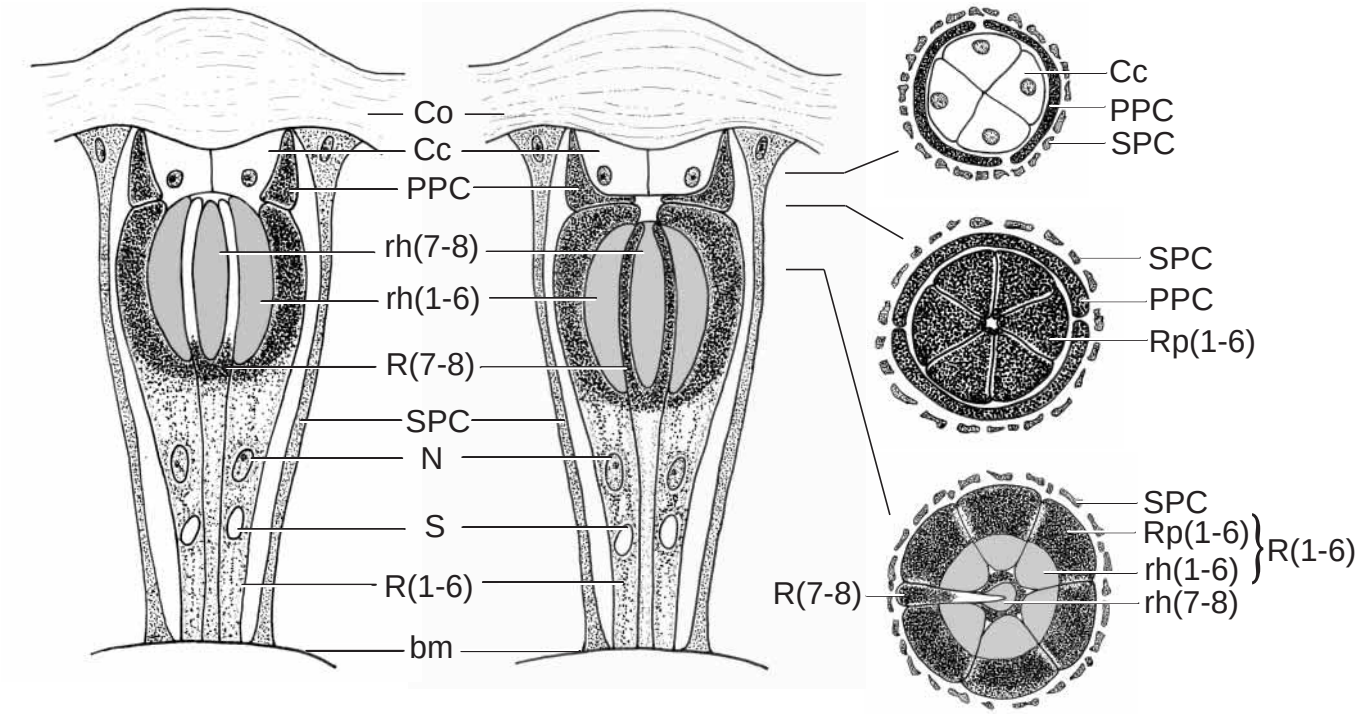

Fig. 1. Schematic representation of a dark- (left) and a light- (right) adapted ommatidium of *Triatoma infestans*. Transverse sections at different levels of the light-adapted ommatidium are also shown on the far right. bm, basal membrane; Co, cornea; Cc, crystalline cone; N, nucleus of the retinula cell; PPC, primary pigment cell; S, sphaeroid; SPC, secondary pigment cell; R, retinula cell; rh, rhabdomere; Rp, retinal pigment.

portion of the ommatidia is composed of a peripheral ring of six rhabdomeres from retinula cells 1–6 (R1–6) and a central pair of rhabdomeres from retinula cells 7 and 8 (R7–8). The crystalline cone is surrounded by two primary pigment cells (PPCs). Twenty-four secondary pigment cells (SPCs) enclose each ommatidium. Screening pigments are located not only in the pigment cells, but also inside all retinula cells. The rhabdomeres and most of the screening pigments are restricted to the distal half of the retinula cells; the proximal half is occupied by the nucleus, some pigments and clear globular structures, termed 'sphaeroids' (Müller, 1970).

During the day, screening pigments within R1–6 and the PPCs form a narrow 'pupil' or aperture, less than 4 μm in diameter, above the distal tip of the central rhabdomere. This pupil is formed mainly by pigments inside R1–6, which are mostly packed above the rhabdom; pigments in the PPCs are homogeneously distributed (Fig. 2A,B). Thus, during the day, light reaches the central rhabdomere, but the peripheral rhabdomeres are shielded from incident light. In addition, pigments inside the central pair of photoreceptors R7–8 are densely packed around the length of the central rhabdomere (Fig. 2A,B). This disposition of pigment granules in the central photoreceptors prevents light travelling within the central rhabdomere to reach the outer rhabdomeres. Thus, as a consequence of both pupil and pigment shielding, light can only reach the central rhabdomere during the day. The rhabdom distal tip lies 12±2 μm (mean ± S.E.M., *N*=7) from the crystalline cone.

At night, pigments inside R1–6 and within the PPCs occupy a lateral position, allowing incident light to reach both the central and the peripheral rhabdomeres (Fig. 2C,D). In the dark-adapted eye, the 'pupil' is nearly six times wider than during the day (24±1 μm, i.e. a 36-fold increase; mean ± S.E.M., *N*=7). Screening pigments within R7–8 move along the longitudinal axis of the cells and concentrate proximally; the cytoplasm is free of pigments around the central rhabdomere. In addition, the rhabdom moves distally so that its distal end is located immediately below the crystalline cone (Fig. 2C), in the place that was occupied by screening pigments during the day (Fig. 2A). Such a movement of the rhabdom towards the crystalline cone diminishes the effective focal length (Land et al., 1999). This widening of the ommatidial aperture and shortening of the focal length increase photon capture at night and, therefore, visual sensitivity (Nilsson, 1989; Warrant and McIntyre, 1993).

Both at night and during the day, pigments inside the SPCs are dispersed along the longitudinal axis of the cells, isolating the ommatidia from each other (Fig. 2).

Changes in ommatidial structure under constant conditions

To study whether the changes in ommatidial structure are under endogenous control, the possible influence of the state of adaptation of the eyes to the actual light conditions must be excluded. For this, bugs were transferred from LD cycles to either LL or DD conditions. If changes in ommatidial structure were under circadian control, then eyes from animals maintained in constant conditions and fixed during the subjective day or night phase would be expected to display

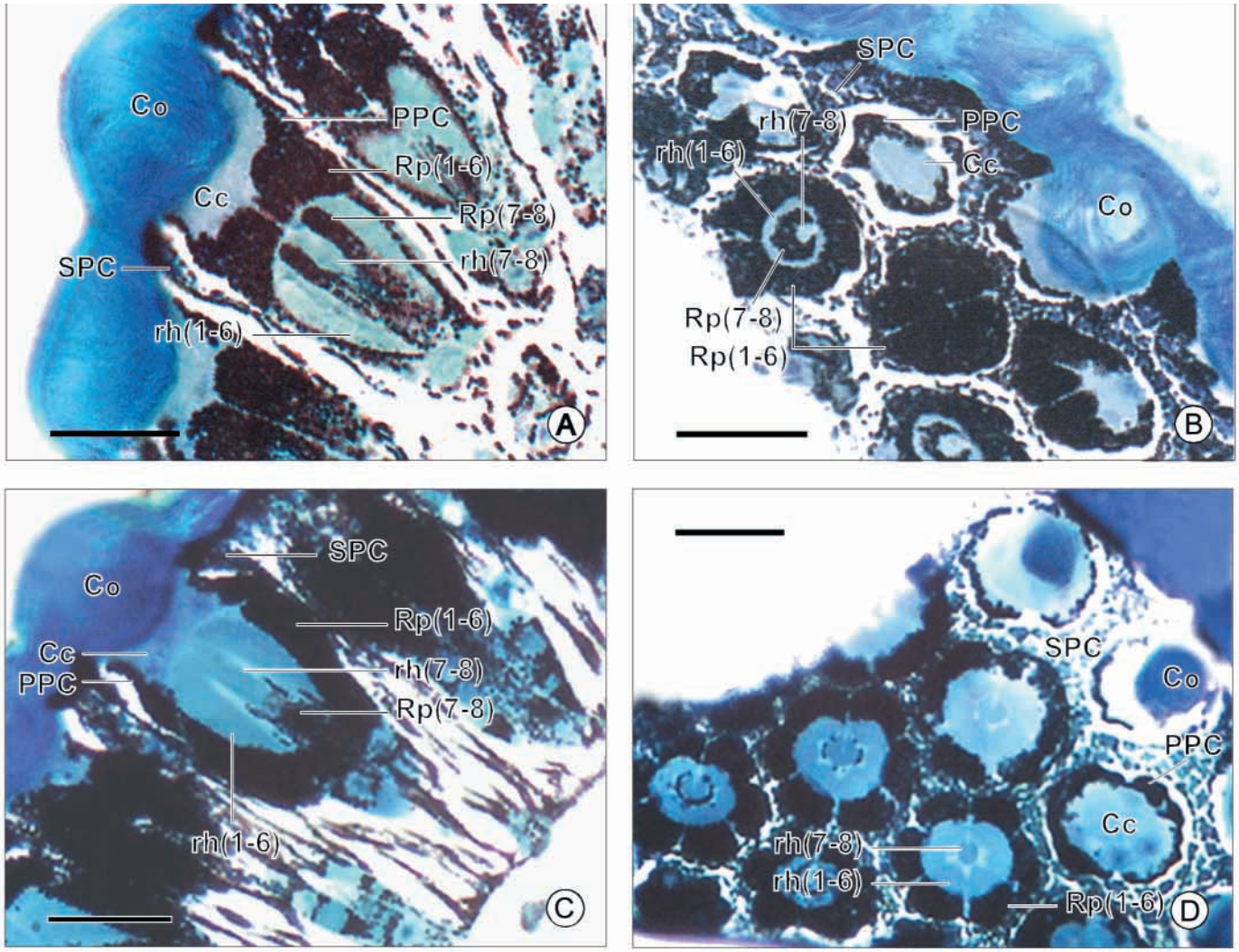

Fig. 2. Longitudinal (left) and transverse (right) sections of eyes of *Triatoma infestans* kept in LD conditions (140 μW cm^{-2}:0 μW cm^{-2}) and fixed during the day (A,B) and during the night (C,D). Co, cornea; Cc, crystalline cone; PPC, primary pigment cell; SPC, secondary pigment cell; rh, rhabdomere; Rp, retinal pigment. Scale bars: 25 μm (A,B), 30 μm (C), 50 μm (D).

similar variations in their structure to those of eyes from animals maintained in LD and fixed during the photophase and scotophase of the animal.

Fig. 3 shows the structure of the ommatidia of insects maintained in DD conditions. During the subjective day, pigments inside R1–6 and the PPCs form a small aperture above the rhabdom (Fig. 3A,B), just as they do during the actual day (Fig. 2A,B). At night, the aperture widens and the rhabdom locates just below the crystalline cone (Fig. 3C,D), as observed during the actual night (Fig. 2C,D). A circadian oscillator therefore regulates both the migration of the pigments forming the 'pupil' and the movements of the rhabdom.

During the subjective day, the pigment granules in R7–8 surround only the proximal half of the central rhabdomere; the distal region of R7–8 remains free of pigments (compare Fig. 3A with Fig. 2A). In contrast, during the subjective night, the pigments are concentrated in the proximal region of R7–8, as they are during the actual night (compare Fig. 3C with Fig. 2C). Thus, the fully dark-adapted eye appears only during the subjective night. These results show that the migration of pigments in R7–8 is regulated by both direct light and circadian inputs.

Fig. 4 shows the structure of the ommatidia of insects maintained under LL conditions (L: 140 μW cm^{-2}). At both subjective daytimes, the ommatidia were in the light-adapted state: the pupil aperture remained small, the screening pigments within R7–8 completely surrounded the central rhabdomere and a dense cluster of pigments separated the crystalline cone from the rhabdom.

The effects of light intensity in ommatidial structure

Many circadian rhythms are not expressed under LL conditions. This effect can be observed even at very low light intensities, particularly in nocturnal animals (Aschoff, 1981). To examine this possibility, we repeated the experiments under a lower light intensity (8 μW cm^{-2}).

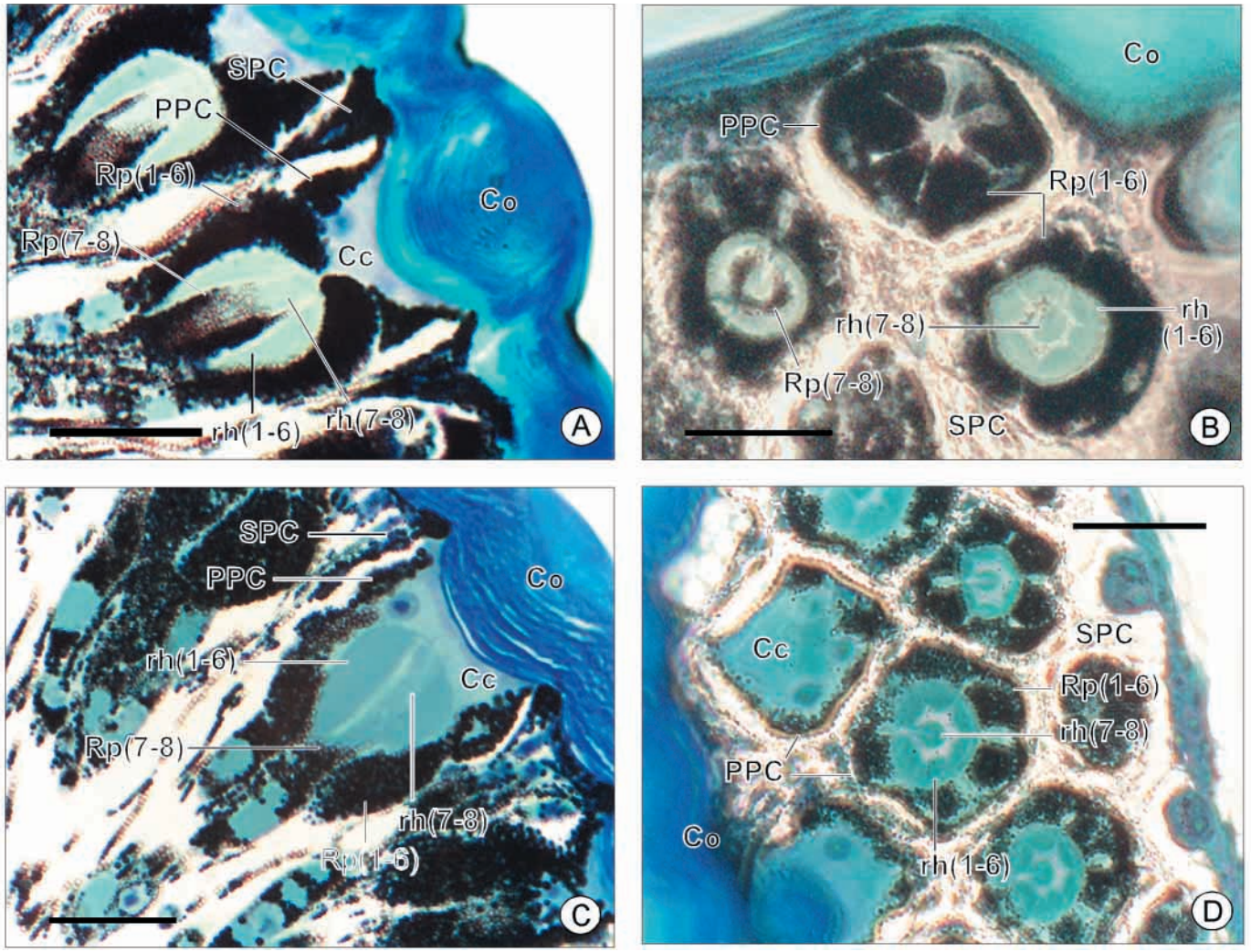

Fig. 3. Longitudinal (left) and transverse (right) sections of eyes of *Triatoma infestans* kept in DD conditions and fixed during the subjective day (A,B) and during the subjective night (C,D). Co, cornea; Cc, crystalline cone; PPC, primary pigment cell; SPC, secondary pigment cell; rh, rhabdomere; Rp, retinal pigment. Scale bars, 25 μm.

Fig. 5A,B shows longitudinal sections of ommatidia from insects entrained to LD cycles and fixed during the photophase and the scotophase. As expected, the size of the pigment aperture, the position of the pigments within R7–8 and that of the rhabdom changed on a daily basis (Fig. 5A,B). However, as observed at the higher light intensity, the rhythm was not expressed under LL conditions: during both subjective night and subjective day, the pigments were in the light-adapted state (compare Fig. 5C,D with Fig. 4).

Discussion

Daily changes in the ommatidia of Triatoma infestans

T. infestans possesses apposition compound eyes with open rhabdoms, in which a ring of rhabdomeres from R1–6 surrounds a central pair of rhabdomeres from R7–8. Pigment cells and all retinula cells, including the central ones, contain dense pigment granules. The position of pigments changes between day and night. During the day, pigments within R1–6 and the PPCs form a narrow ‘pupil’ in front of the distal tip of the central rhabdomere; pigments within R7–8 are compacted around the central rhabdomere (Fig. 2A,B). At night, the pupil opens, and the pigments inside R7–8 move to a proximal location; the rhabdom moves distally to a position immediately below the crystalline cone (Fig. 2C,D). During the day, light can therefore only reach the central rhabdomere, while at night light can also reach the peripheral rhabdomeres. These daily variations adjust the sensitivity of the eye to the environmental light conditions by controlling the ommatidial aperture and, hence, the light flux reaching the photoreceptors. The change in size of the aperture during the night may improve light sensitivity, but at the expense of a decrease in spatial resolution (Land et al., 1999; Nilsson, 1989; Nilsson and Ro, 1994).

The presence of a variable pigment aperture in front of the rhabdom has been described in other insects with open-rhabdom eyes that lack neural superposition (Lüdtke, 1953; Nilsson and Ro, 1994; Ro and Nilsson, 1993, 1994, 1995;

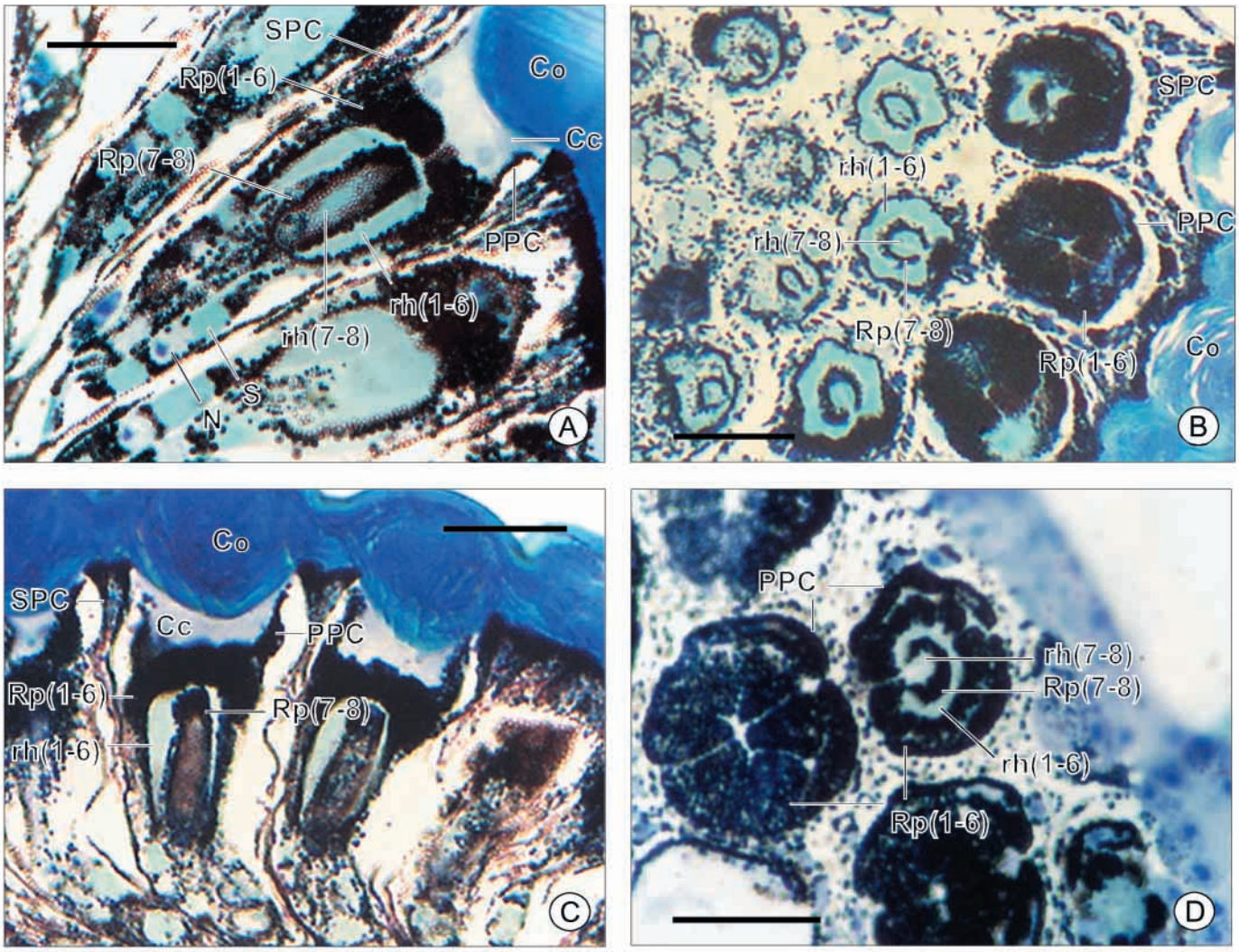

Fig. 4. Longitudinal (left) and transverse (right) sections of eyes of *Triatoma infestans* kept in LL conditions and fixed during the subjective day (A,B) and during the subjective night (C,D). Co, cornea; Cc, crystalline cone; N, nucleus of the retinula cell; PPC, primary pigment cell; S, sphaeroid; SPC, secondary pigment cell; rh, rhabdomere; Rp, retinal pigment. Scale bars: 25 μm (A), 30 μm (B,C), 20 μm (D).

Wada and Schneider, 1968; Walcott, 1971; Williams, 1980). In all such cases, the pigment aperture is invariably formed by the PPCs alone. In *T. infestans*, however, the 'pupil' is formed mainly by screening pigments within R1–6, although pigments within the PPCs also contribute (Fig. 2A,B). In addition, during the day, pigments within R7–8 isolate the central rhabdomeres from the peripheral ring, a feature that has not been described in this kind of eye. Therefore, in *T. infestans*, diurnal vision relies exclusively on the R7–8 subsystem.

The open-rhabdom eye arrangement without neural superposition is characteristic of insects, such as *T. infestans*, that possess a dominantly crepuscular or nocturnal lifestyle (e.g. crane flies, cave beetles, backswimmers). As a result of the presence of a pigmentary pupil, this kind of eye would have two types of image channels, one with low sensitivity and high resolution (the central rhabdomeres), and one with high sensitivity and poor resolution (the peripheral rhabdomeres) (Ioannides and Horridge, 1975; Nilsson, 1989). However, in some species, the peripheral retinula cells are involved in neural pooling in the lamina during the night, a system that maximizes spatial resolution at low light intensities (Nilsson and Ro, 1994). It has been proposed that this nocturnal mechanism led directly to the evolution of the neural superposition eye of higher Diptera (Nilsson and Ro, 1994). In addition, the open-rhabdom arrangement has the potential to feed information into parallel spatial channels, each optimised for a particular light intensity. This is particularly important for animals that are exposed to rapid and extensive changes in light intensity at dawn and dusk, which is the case for *T. infestans*.

As mentioned above, only the R7–8 subsystem is functional during the day in *T. infestans*. In other insects with open-rhabdom eyes, the R1–6 and R7–8 subsystems contain visual pigments with different absorbance spectra (Hardie, 1979; Schwind et al., 1984). If this were also the case in *T. infestans*, its visual system would be monochromatic during the day. At night, both subsystems are exposed, and the eye will therefore

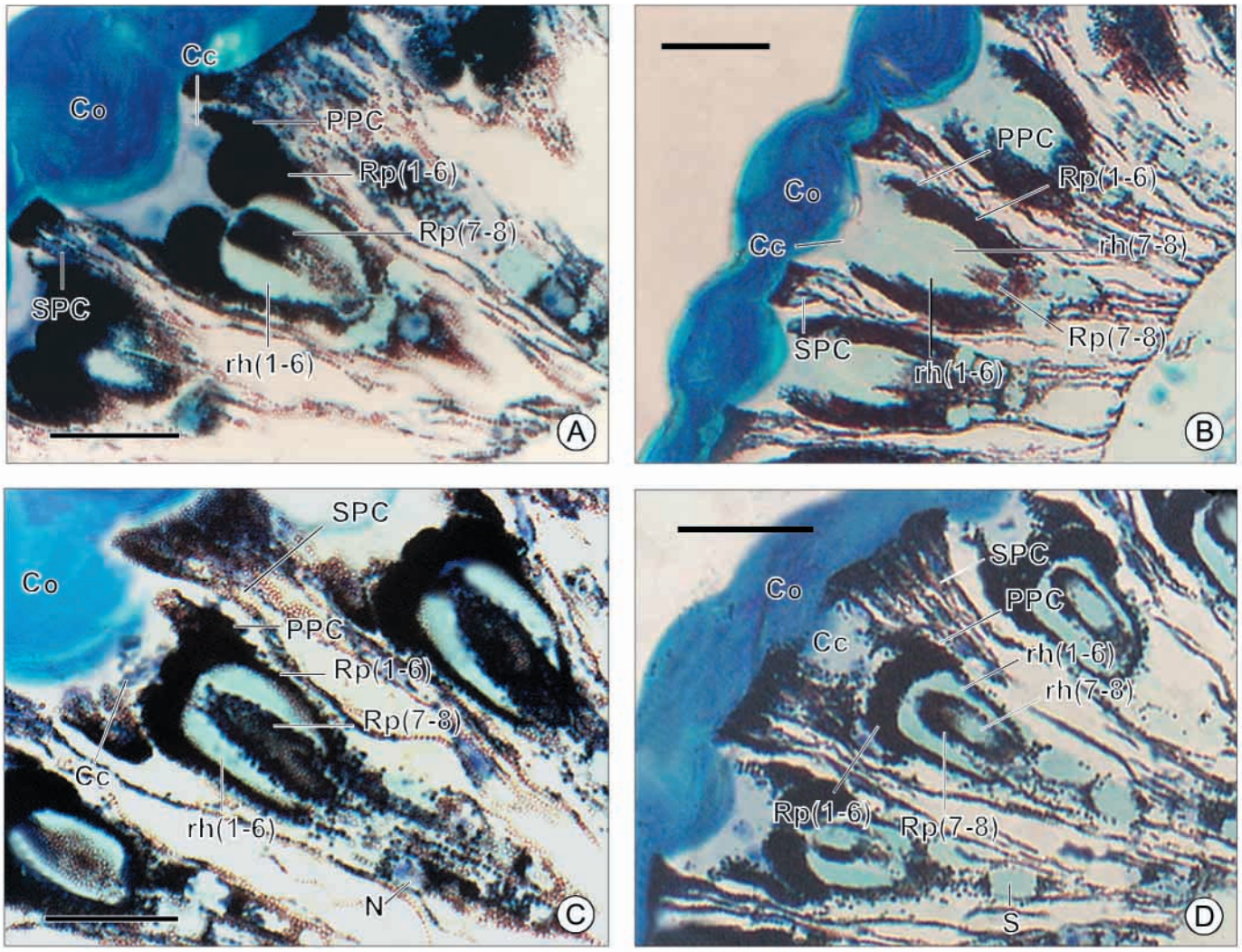

Fig. 5. (A,B) Longitudinal sections of eyes of *Triatoma infestans* kept in LD conditions (8 μW cm^{-2}:0 μW cm^{-2}) and fixed during the day (A) and during the night (B). (C,D) Longitudinal sections of eyes of insects kept in LL conditions (8 μW cm^{-2}) and fixed during the subjective day (C) and during the subjective night (D). Co, cornea; Cc, crystalline cone; N, nucleus of the retinula cell; PPC, primary pigment cell; S, sphaeroid; SPC, secondary pigment cell; rh, rhabdomere; Rp, retinal pigment. Scale bars: 25 μm (A,C), 30 μm (B,D).

be sensitive to a broader range of wavelengths of light. This functional division of the retina into a day/night duplex with different spectral sensitivities has also been suggested for the backswimmer *Notonecta glauca* (Ro and Nilsson, 1995).

Circadian and non-circadian changes in the ommatidial structure of Triatoma infestans

Our results show that the daily migration of the screening pigments forming the pupil and the movements of the rhabdom are controlled by a circadian oscillator. In bugs maintained under DD conditions, the pigments within R1–6 and the PPCs form a narrow aperture in front of the rhabdom during the subjective day, while the pigments become displaced to a lateral position, widening the ommatidial aperture, during the subjective night (Fig. 3). In contrast, during the subjective day, the pigments within R7–8 are in an intermediate position between the light- and the dark-adapted states (Fig. 3A). Therefore, the migration of pigment granules in R7–8 is regulated both by direct light and by circadian inputs. If the movements of pigments within R7–8 were controlled by a circadian clock only, the pigments should have been found completely flanking the central rhabdomere, as was observed during the photophase in insects maintained in LD conditions. In the same way, if changes were under exogenous control only, the pigments should have been found compacted in a proximal position, as in the dark-adapted eye. Only during the subjective night were the pigments found in the fully dark-adapted state (Fig. 3C), indicating that, during the day, light is needed to move the pigments distally within R7–8. None of these changes was observed under LL conditions, at either high (140 μW cm^{-2}) or low (8 μW cm^{-2}) light intensity: the pigments stayed in the light-adapted state at both daytimes (Figs 4, 5C,D).

Circadian changes in retinal anatomy have been described

in arthropods (for a review, see Meyer-Rochow, 1999). These changes include, among others, pigment migrations within the SPCs (Aréchiga and Rodríguez Sosa, 1997; Bennett, 1993; Fleissner and Fleissner, 1977; Warrant and McIntyre, 1993) or PPCs (Barlow et al., 1980; Ro and Nilsson, 1994; Wada and Schneider, 1968). In the eyes of *T. infestans*, as in other apposition compound eyes, pigments inside the SPCs do not move upon light/dark adaptation. Circadian movements of screening pigments within retinula cells have been described in the horseshoe crab *Limulus polyphemus* (Barlow et al., 1980) and in the isopod *Ligia exotica* (Hariyama et al., 2001). As in *T. infestans*, these movements affect the light flux to the rhabdom by controlling the ommatidial aperture. In flies, a circadian rhythm in the number of pigment granules in the lamina terminals of R1–6 has been described (Pyza and Meinertzhagen, 1997). Although this rhythm may reflect a circadian redistribution of granules within the soma of the photoreceptors, the evidence for this is indirect. Radial movements of pigments within the fly's R1–6 were observed under LD conditions (Stavenga, 1979) but, unfortunately, the actual distribution of granules within the soma of retinula cells has not been analysed under constant conditions. Thus, our results constitute direct evidence for circadian movements of pigments within retinula cells in insect compound eyes. The present study also demonstrates differences in the control of pigment movements between retinula cells of a single ommatidium since the migration of pigments within R1–6 is regulated by a circadian clock while that in R7–8 is regulated both by direct light and by circadian inputs. The adaptive value of such endogenous control resides in its capacity to adjust the visual sensitivity in advance of changes in light intensity. Furthermore, the presence of an exogenous mechanism is also advantageous because it can modify the visual sensitivity in response to sudden and unpredictable changes in light intensity. This kind of dual control, by endogenous and exogenous mechanisms, has been described in other species, but in different cellular types within the ommatidia (Meyer-Rochow, 1999). For instance, in *Tenebrio molitor*, the movements of screening pigments inside the PPCs are controlled by a circadian oscillator, while the length of the crystalline cone changes in direct response to light, irrespective of the time of day (Wada and Schneider, 1967).

The rhythm damps out under LL conditions

The circadian rhythms in the ommatidial structure of *T. infestans* do not persist in LL conditions. This effect of light has been described repeatedly for other insect circadian rhythms (Saunders, 1982) and also in triatomines (Lazzari, 1991; Schilman, 1998). We therefore tested whether the rhythm still damps out at a relatively low light intensity ($8\,\mu W\,cm^{-2}$) to which bugs are sensitive, as demonstrated in behavioural experiments (Reisenman et al., 1998). This light intensity was sufficient to entrain the rhythm (Fig. 5A,B), but under LL conditions the rhythm vanished again (Fig. 5C,D). It has yet be determined whether this is due to a direct effect on the photoreceptors or to a 'masking' effect of light on the clock controlling the rhythm (Aschoff, 1960).

Direct effects of illumination on the mechanisms of visual adaptation have been described before (Autrum, 1981). For instance, irrespective of the time of the day, light causes the formation of a long crystalline cone tract in several species of insect (Meyer-Rochow, 1999) and the breakdown of microvilli and the movement of pigments in *Limulus polyphemus* (Barlow et al., 1980; Chamberlain and Barlow, 1979; Kier and Chamberlain, 1990). Nevertheless, some direct responses to light are certainly advantageous; for instance, the movements of screening pigments induced by light avoid excessive conversion of rhodopsin to its inactive state (metarhodopsin) (Järemo Jonson et al., 1998; Schwemer, 1989; Stavenga, 1989). In the case of *T. infestans*, the direct effect of light on the ommatidial structure would also have a protective effect. This hypothesis is supported by the fact that red-eye mutants of *T. infestans* that lack screening pigments within the retinula cells show a highly degraded retina and a severe reduction in visual performance (Reisenman, 2000; Reisenman et al., 2000).

Considering the dual role of the light as a *Zeitgeber* and as the stimulus causing the movements of pigments, it would be interesting to analyse whether the rhythm can be entrained by other environmental cycles. In particular, it has been shown that, in the absence of changes in light intensity, temperature cycles can synchronise the rhythm of pigment migrations in the eyes of moths, butterflies, flies and several species of crustacean (Nordström and Warrant, 2000). This could also be the case in *T. infestans* because temperature cycles are able to entrain other circadian rhythms in this species (Lazzari, 1992).

Oscillators controlling visual rhythms in insects

In a traditional view of the circadian systems, a central master oscillator located in the brain regulates all rhythmic functions in the insect body, either directly or *via* a system of 'slave' pacemakers (Giebultowicz, 1999). However, recent evidence suggests that the insect circadian system consist of many peripheral clocks, some of which are brain-independent. For instance, completely autonomous oscillators have been described in the lateral neurons of the brain (Ewer et al., 1992), the Malpighian tubules (Giebultowicz and Hege, 1997; Hege et al., 1997), the prothoracic glands (Emery et al., 1997) and, interestingly, in retinula cells R1–6 of *Drosophila melanogaster* (Cheng and Hardin, 1998).

In flies, a new group of circadian rhythms has been found in the first visual neuropil, the lamina. The lamina of flies exhibits circadian changes in the number of synapses between photoreceptor terminals and their first-order interneurons (Pyza and Meinertzhagen, 1993), in the vertical migration of screening pigments within terminals of R1–6 (Pyza and Meinertzhagen, 1997) and in the diameter of the monopolar neurons L1 and L2 (Pyza and Meinertzhagen, 1995). Changes in the size of L1 and L2 are driven by three inputs: by a circadian clock located in the brain, which differs from the clock controlling the locomotor activity rhythm of the fly, and by oscillators located in the retina and in the epithelial glial

cells of the lamina (Pyza, 2001). In the cockroach, however, rhythms in the amplitude of the ERG and in locomotor activity are controlled by the same pacemaker, which is composed of two mutually coupled oscillators located in the optic lobes (Page, 1990; Wills et al., 1985).

Several circadian rhythms in behaviour and physiological processes are under endogenous control in *T. infestans* (e.g. locomotor activity, negative phototaxis, egg hatching, ecdysis, thermopreference) but, unfortunately, the anatomical location of the oscillators controlling these rhythms has not yet been studied. In this species, the circadian rhythms of locomotor activity (Lazzari, 1992) and of negative phototaxis (Reisenman et al., 1998) persist under both DD and LL conditions, while the circadian rhythms in retinal morphology described here are maintained under DD but not under LL conditions. This difference indicates that these rhythms may be generated by different circadian systems. In blowflies, different circadian oscillators control the rhythm of locomotor activity and the cyclic changes in the size of the monopolar neurons in the lamina (Pyza and Cymborowski, 2001). Moreover, *Drosophila melanogaster* photoreceptors R1–6 contain an autonomous circadian clock (Cheng and Hardin, 1998). If a similar situation were to exist in *T. infestans*, the rhythms in retinal morphology would be controlled by a retinal oscillator, while the behavioural rhythms would be controlled by one or more different circadian clocks, probably located in the brain. The behavioural and morphological rhythms would be independent, although synchronized by light or some other *Zeitgeber*. These results do not contradict previous models of the relationship between circadian timing systems (e.g. Page, 1990), but add new elements that may help our understanding of the temporal organisation of the life of this particular insect.

The authors are deeply indebted to Jochen Zeil for critically reading the manuscript and to two anonymous referees for their improvements and valuable suggestions. The authors acknowledge the staff members of the Laboratory of Insect Physiology for many fruitful discussions. This work received financial support from the University of Buenos Aires, CONICET (Argentina) and the WHO/UNDP/World Bank Special Programme for Research and Training in Tropical Diseases (TDR).

References

Aréchiga, H. and Rodríguez-Sosa, L. (1997). Coupling of environmental and endogenous factors in the control of rhythmic behaviour in decapod crustaceans. *J. Mar. Biol. Ass. UK* **77**, 17–29.

Aschoff, J. (1960). Exogenous and endogenous components in circadian rhythms. *Cold Spring Harb. Symp. Quant. Biol.* **25**, 11–28.

Aschoff, J. (1981). Free running and entrained circadian rhythms. In *Biological Rhythms, Handbook of Behavioral Neurobiology*, vol. IV (ed. J. Aschoff), pp. 81–93. New York: Plenum Press.

Autrum, H. (1981). Light and dark adaptation in invertebrates. In *Comparative Physiology and Evolution of Vision in Invertebrates C, Invertebrate Visual Centers and Behaviour II*, chapter 1 (ed. H. Autrum), pp. 1–91. Berlin: Springer-Verlag.

Barlow, R. B., Chamberlain, S. C. and Lehman, H. K. (1989). Circadian rhythms in invertebrate retina. In *Facets of Vision*, chapter 13 (ed. D. G. Stavenga and D. G. Hardie), pp. 257–280. Berlin, Heidelberg, New York: Springer-Verlag.

Barlow, R. B., Chamberlain, S. C. and Levinson, J. Z. (1980). *Limulus* brain modulates the structure and function of the lateral eyes. *Science* **210**, 1037–1039.

Bennett, R. R. (1983). Circadian rhythm of visual sensitivity in *Manduca sexta* and its development from an ultradian rhythm. *J. Comp. Physiol.* **150**, 165–174.

Chamberlain, S. C. and Barlow, R. B., Jr (1979). Light and efferent activity control rhabdom turnover in *Limulus* photoreceptors. *Science* **206**, 361–363.

Chen, B., Meinertzhagen, I. A. and Shaw, S. R. (1999). Circadian rhythms in light-evoked responses of the fly's compound eye, and the effects of neuromodulators 5-HT and the peptide PDF. *J. Comp. Physiol. A* **185**, 393–404.

Cheng, Y. and Hardin, P. E. (1998). *Drosophila* photoreceptors contain an autonomous circadian oscillator that can function without period mRNA cycling. *J. Neurosci.* **18**, 741–750.

Colwell, C. S. and Page, T. L. (1989). The electroretinogram of the cockroach *Leucophaea maderae*. *Comp. Biochem. Physiol.* **92A**, 117–123.

Emery, I. F., Noveral, J. M., Jamison, C. F. and Siwicki, K. K. (1997). Rhythms of *Drosophila period* gene expression in culture. *Proc. Natl. Acad. Sci. USA* **94**, 4092–4096.

Ewer, J., Frisch, B., Hamblen-Coyle, M. J., Rosbash, M. and Hall, J. C. (1992). Expression of the *period* clock gene within different cell types in the brain of *Drosophila* adults and mosaic analysis of these cells' influence on circadian behavioural rhythms. *J. Neurosci.* **12**, 3321–3349.

Ferrell, B. R. and Reitcheck, B. G. (1993). Circadian changes in cockroach ommatidial structure. *J. Comp. Physiol. A* **173**, 549–555.

Fleissner, G. and Fleissner, G. (1977). The optic nerve mediates the circadian pigment migration in the median eyes of the scorpion. *Comp. Biochem. Physiol.* **61A**, 69–71.

Giebultowicz, J. M. (1999). Insect circadian clocks: is it all in their heads? *J. Insect Physiol.* **45**, 791–800.

Giebultowicz, J. M. and Hege, D. M. (1997). Circadian clock in Malpighian tubules. *Nature* **386**, 664.

Hardie, R. C. (1979). Electrophysiological analysis of fly retina. I. Comparative properties of R1–6 and R7 and R8. *J. Comp. Physiol.* **129**, 19–33.

Hariyama, T., Meyer-Rochow, V. B., Kawauchi, T., Takaku, Y. and Tsukahara, Y. (2001). Diurnal changes in retinula cell sensitivities and receptive fields (two-dimensional angular sensitivity functions) in the apposition eyes of *Ligia exotica* (Crustacea, Isopoda). *J. Exp. Biol.* **204**, 239–248.

Hege, D. M., Stafnewsky, R., Hall, J. C. and Giebultowicz, J. M. (1997). Rhythmic expression of a PER-reporter in the Malpighian tubules of decapited *Drosophila*: evidence for a brain-independent circadian clock. *J. Biol. Rhythms* **12**, 300–308.

Horridge, G. A., Duniec, J. and Marcelja, L. (1981). A 24-hour cycle in single locust and mantis photoreceptors. *J. Exp. Biol.* **91**, 307–322.

Ionannides, A. C. and Horridge, G. A. (1975). The organization of visual fields in the hemipteran acone eye. *Proc. R. Soc. Lond. B* **190**, 373–391.

Järemo Jonson, A. C., Land, M. F., Osorio, D. C. and Nilsson, D.-E. (1998). Relationships between pupil working range and habitat luminance in flies and butterflies. *J. Comp. Physiol. A* **182**, 1–9.

Kier, C. K. and Chamberlain, S. C. (1990). Dual controls for screening pigment movement in photoreceptors of the *Limulus* lateral eye: circadian efferent input and light. *Visual Neurosci.* **4**, 237–255.

Koehler, W. and Fleissner, G. (1978). Internal desynchronisation of bilaterally organised circadian oscillators in the visual system of insects. *Nature* **274**, 708–710.

Land, M. F., Gibson, G., Horwood, J. and Zeil, J. (1999). Fundamental differences in the optical structure of the eyes of nocturnal and diurnal mosquitoes. *J. Comp. Physiol. A* **185**, 91–103.

Lazzari, C. R. (1991). Circadian rhythm of egg hatching in *Triatoma infestans* (Hemiptera: Reduviidae). *J. Med. Entomol.* **28**, 740–741.

Lazzari, C. R. (1992). Circadian organisation of locomotion activity in the haematophagous bug *Triatoma infestans*. *J. Insect Physiol.* **38**, 895–903.

Lüdtke, H. (1953). Retinomotorik und Adaptationsvorgänge im Auge des Rückenschwimmers (*Notonecta glauca* L.). *Z. Vergl. Physiol.* **35**, 129–152.

Menzi, U. (1987). Visual adaptation in nocturnal and diurnal ants. *J. Comp. Physiol. A* **160**, 11–21.

Meyer-Rochow, V. B. (1999). Photoreceptors and photo-environments. Compound eye: circadian rhythmicity, illumination and obscurity. In *Atlas*

of Arthropod Sensory Receptors, chapter III-2 (ed. E. Eguchi and Y. Tominaga), pp. 97–124. Tokyo: Springer-Verlag.

Nilsson, D.-E. (1989). Optics and evolution of compound eyes. In *Facets of Vision*, chapter 3 (ed. D. G. Stavenga and R. C. Hardie), pp. 30–73. Berlin, Heidelberg, New York: Springer-Verlag.

Nilsson, D.-E. and Ro, A.-I. (1994). Did neural pooling for night vision lead to the evolution of neural superposition eyes? *J. Comp. Physiol. A* **175**, 289–302.

Müller, von J. (1970). Feinbau und Dunkelanpassung der Komplexaugen von *Rhodnius prolixus* (Stal). *Zool. Jb. Physiol.* **75**, 111–133.

Nordström, P. and Warrant, E. J. (2000). Temperature-induced pupil movements in insect superposition eyes. *J. Exp. Biol.* **203**, 685–692.

Page, T. L. (1990). Circadian rhythms of locomotor activity in cockroach nymphs: free running and entrainment. *J. Biol. Rhythms* **5**, 273–289.

Page, T. L. and Larimer, J. L. (1975). Neural control of circadian rhythmicity in the crayfish. II. The ERG amplitude rhythm. *J. Comp Physiol.* **97**, 81–96.

Pyza, E. (2001). Cellular circadian rhythms in the fly's visual system. In *Insect Timing: Circadian Rhythmicity and Seasonality* (ed. D. L. Delinger, J. Giebultowicz and D. S. Saunders). Amsterdam: Elsevier Science.

Pyza, E. and Cymborowski, B. (2001). Circadian rhythms in behaviour and in the visual system of the blow fly, *Calliphora vicina*. *J. Insect Physiol.* **47**, 897–904.

Pyza, E. and Meinertzhagen, I. A. (1993). Daily and circadian rhythms of synaptic frequency in the first visual neuropile of the housefly's (*Musca domestica*) optic lobe. *Proc. R. Soc. Lond. B* **254**, 97–105.

Pyza, E. and Meinertzhagen, I. A. (1995). Monopolar cell axons in the first optic neuropil of the housefly, *Musca domestica* L., undergo daily fluctuations in diameter that have a circadian basis. *J. Neurosci.* **15**, 407–418.

Pyza, E. and Meinertzhagen, I. A. (1997). Circadian rhythms in screening pigment and invaginating organelles in photoreceptor terminals of the housefly's first optic neuropile. *J. Neurobiol.* **32**, 517–529.

Reisenman, C. E. (2000). Fisiologia del sistema visual de la vinchuca *Triatoma infestans*: un enfoque comportamental. PhD thesis, University of Buenos Aires, Argentina. 211pp.

Reisenman, C. E., Insausti, T. C. and Lazzari, C. R. (2000). Morphological and functional characterization of red-eye mutants of *Triatoma infestans*. In *Abstracts of the XVth International Congress for Tropical Medicine and Malaria* (ed. J. M. Cordevez), p. 83. Columbia: Universidad de Los Andes.

Reisenman, C. E., Lazzari, C. R. and Giurfa, M. (1998). Circadian control of photonegative sensitivity in the haematophagous bug *Triatoma infestans*. *J. Comp. Physiol. A* **183**, 533–541.

Ribi, W. A. (1987). *A Handbook in Biological Electron Microscopy* (ed. W. A. Ribi), pp. 106. Switzerland: Ribi, W.

Ro, A.-I. and Nilsson, D.-E. (1993). The circadian pupil rhythm in *Tenebrio molitor*, studied noninvasively. *Naturwissenschaften* **80**, 186–189.

Ro, A.-I. and Nilsson, D.-E. (1994). Circadian and light-dependent control of the pupil mechanism in tipulid flies. *J. Insect Physiol.* **40**, 883–891.

Ro, A.-I. and Nilsson, D.-E. (1995). Pupil adjustments in the eye of the common backswimmer. *J. Exp. Biol.* **198**, 71–77.

Saunders, D. S. (1982). *Insect Clocks*, second edition. Oxford: Pergamon Press. 408pp.

Schilman, P. E. (1998). Factores que afectan la reproducción de las vinchucas: aspectos fisiológicos y comportamentales. PhD thesis, University of Buenos Aires, Argentina. 126pp.

Schwemer, J. (1989). Visual pigments of compound eyes – structure, photochemistry and regeneration. In *Facets of Vision* (ed. D. G. Stavenga and R. C. Hardie), pp. 112–133. Berlin, Heidelberg, New York: Springer-Verlag.

Schwind, R., Schlecht, P. and Langer, H. (1984). Microspectrophotometric characterization and localization of three visual pigments in the compound eye of *Notonecta glauca* L. (Heteroptera). *J. Comp. Physiol. A* **154**, 341–346.

Stavenga, D. G. (1979). Pigments in the compound eye. In *Comparative Physiology and Evolution of Vision in Invertebrates*, A, *Invertebrate Photoreceptors* (ed. H. Autrum), pp. 357–439. Berlin, Heidelberg, New York: Springer-Verlag.

Stavenga, D. G. (1989). Pigments in compound eyes. In *Facets of Vision* (ed. D. G. Stavenga and R. C. Hardie), pp. 152–172. Berlin, Heidelberg, New York: Springer-Verlag.

Wada, S. and Schneider, G. (1967). Eine Pupillenreaktion im Ommatidium von *Tenebrio molitor*. *Naturwissenschaften* **54**, 542.

Wada, S. and Schneider, G. (1968). Circadianer Rhythmus der Pupillenweite im Ommatidium von *Tenebrio molitor*. *Z. Vergl. Physiol.* **58**, 395–397.

Walcott, B. (1971). Unit studies on receptor movement in the retina of *Lethocerus* (Belostomatidae, Hemiptera). *Z. Vergl. Physiol.* **74**, 17–25.

Warrant, E. and McIntyre, P. D. (1990). Screening pigment, aperture and sensitivity in the dung beetle superposition eye. *J. Comp. Physiol. A* **167**, 805–815.

Warrant, E. J. and McIntyre, P. D. (1993). Arthropod eye design and the physical limits to spatial resolving power. *Prog. Neurobiol.* **40**, 413–461.

Williams, D. S. (1980). Organisation of the compound eye of a tipulid fly during the day and night. *Zoomorphologie* **95**, 85–104.

Wills, S. A., Page, T. L. and Colwell, C. S. (1985). Circadian rhythms in the electroretinogram of the cockroach. *J. Biol. Rhythms* **1**, 25–37.