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Differential control of light–dark adaptation in the ocelli and compound eyes of *Triatoma infestans*

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ABSTRACT

The adaptation to light of compound eyes in insects has been extensively documented and their adaptive role is well understood. Much less attention has been paid, however, to the control of ocelli sensitivity, a study which could help us to understand the functional role of these simple eyes. We analyzed the dynamic changes in the distribution of screening pigments which occur in the ocelli of the haematophagous bug, *Triatoma infestans*, when the insects are subjected either to light/dark cycles (LD), to constant darkness (DD) or constant light (LL). We then compared these changes with those occurring in the compound eyes of the same individuals and found that, while compound eyes are subject to the control of an endogenous circadian clock, the adaptation of the ocelli is entirely dependent on environmental illumination. In addition, we have observed that environmental temperature is not involved in the control of screening pigments in either ocelli or compound eyes as a direct stimulus, nor as a *Zeitgeber*. The existence of a differential control in the components of the dual visual system represents an adaptive advantage in the adjustment of visual sensitivity in insects exposed to quick changes in lighting conditions in their natural habitat. We discuss the implications of our findings with regards to the biology of triatomines and with respect to the general understanding the functional role of insect ocelli.

1. Introduction

The insect visual system is composed of both simple and compound eyes. The ocelli are the simple eyes of adult insects. They usually number 2 or 3 and coexist with compound eyes. Although ocelli are not present in all insect species, they are present in at least some members of most orders.

The functional role of ocelli is not consistent across species. From a behavioral point of view, 2 categories have been recognized (Mizunami, 1995a) depending on their relationship with the compound eyes. On the one hand, they can modulate behaviors mediated by the compound eyes, such as positive phototaxis in *Drosophila*, *Calliphora* and *Locusta* and synchronization of the circadian system in crickets, among others (see reviews by Goodman, 1970; Goodman, 1981; Mizunami, 1995a,b). On the other hand, they are directly involved in some visual responses that complement those of the compound eyes such as object fixation in *Musca* (Wehrhahn, 1984), orientation by means of the sky-polarization pattern in bumblebees and *Cataglyphis,* the control of the flight course in locusts and dragonflies (reviewed by Mizunami, 1995a) as well as negative phototaxis in triatomines (Lazzari et al., 1998).

Up to now, most of the data on the structure and function of ocelli were obtained using insects considered to be "good" flyers such as bees, wasps and dragonflies (Cornwell, 1955; Wilson, 1978; Stange and Howard, 1979; Stange, 1981; Rowell and Pearson, 1983; Schuppe and Hengstenberg, 1993; Warrant et al., 2006). One reason for this bias is the fact that ocelli are usually associated with flight-control. This is not the case, however, with *T. infestans*, whose ocellar system has a high degree of complexity, despite the fact that they are not good flyers (Insausti and Lazzari, 1996, 2002). Triatomines have 2 well-developped ocelli located behind their compound eyes, looking dorso-laterally and frontally (Insausti and Lazzari, 2002) (Fig. 1). These nocturnal bugs are mainly walking insects which only perform dispersing flights under particular conditions (Lehane and Schofield, 1982). Up to the present, the only function established for the *T. infestans* ocelli is related to negative phototactic behavior. In fact, both their ocelli and compound eyes are capable of independently mediating the phototactic response (Lazzari et al., 1998). In other words, the input from their ocelli controls negative phototaxis by itself, i.e., not through the modulation of compound eyes sensitivity.

T. infestans are haematophagous bugs, vectors of Chagas disease. They are highly-adapted to domestic environments and their activity is split in two temporal windows. One occurs at dusk

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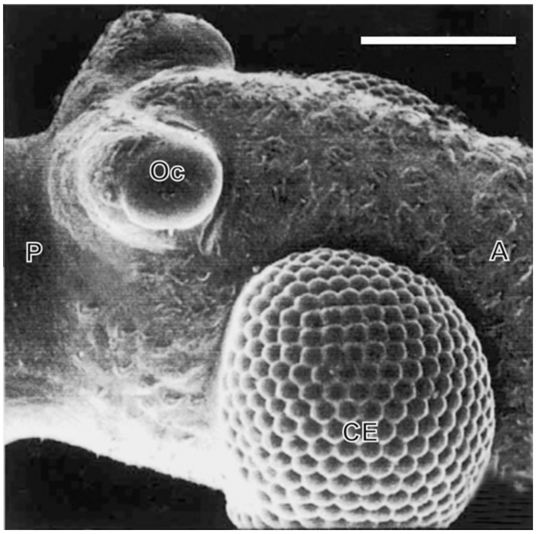

Fig. 1. Head of *Triatoma infestans* showing the position of ocelli (Oc) and compound eyes (CE). A, anterior; P, posterior. Scale bar, 300 μm.

and another at dawn, thus exposing them to a broad range of light intensities and their eye sensitivity needs to adjust accordingly. In arthropods, one of the main mechanisms for accomplishing this is the movement of screening pigments within pigmentary and retinal cells in order to modulate the amount of light reaching their photoreceptors (Autrum, 1981; Nordström and Warrant, 2000).

Circadian (i.e. endogenous) rhythms regulating visual sensitivity have been described in the retina of the compound eyes 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). In *T. infestans*, a circadian clock controls the rhythm of phototactic sensitivity (Reisenman et al. 1998). Changes in their behavioral sensitivity to light are associated with dynamic changes in the distribution of screening pigments within their compound eyes (Reisenman et al., 2002). Concerning the ocelli of *T. infestans*, it is well-known that the amount of light reaching their rhabdom depends on the movement of the screening pigment granules located inside their retinula cells (Insausti et al., 1999). Nevertheless, the mechanism controlling the daily migration of screening pigments is still unknown, not only in triatomines, but in insects in general.

Daily physiological changes rely on a variety of environmental signals which either act directly on certain processes or, if they have a cyclic nature, synchronize the circadian clock (i.e., as a *Zeitgeber*), thus indirectly affecting others. As an example, the cyclic variation of environmental temperature might be used to recognize day and night and, consequently, affect the adaptation of the visual system. It has also been shown that temperature has a direct effect on the adaptation of compound eyes of some nocturnal insects by influencing the migration of screening pigments (Nordström and Warrant, 2000), but it is not known whether or not this can be generalized to other insects and to simple eyes. Triatomine bugs appear to be a good model for the analysis of the effect of the temperature on the adaptation of ocelli, provided both, that they exhibit a high thermal sensitivity and that their ocelli are particularly complex (Lazzari and Núñez, 1989; Insausti and Lazzari, 2002).

The aims of the present work were (1) to study the chronobiological basis of the dynamic changes in the adaptation to light of the ocelli of *T. infestans*; (2) to compare the control of the adaptation of the 2 components of its visual system and (3) to test whether temperature cycles synchronize the daily rhythm of the visual sensitivity of both ocelli and compound eyes.

2. Materials and methods

In order to assess the chronobiological basis of visual adaptation and to compare its influence in ocelli and compound eyes, the distribution of screening pigments within retinal cells was analyzed. Groups of bugs were exposed to various light and temperature regimes (see below) and then processed for histology.

The insects used for the experiments were adults of *Triatoma infestans* (Heteroptera: Reduviidae) (Klug, 1834) at least 20 days after their imaginal ecdysis in order to ensure that the ocelli had completed their development (Insausti and Lazzari, 2000). The insects were reared in the laboratory at 28 °C and fed on chicken. In order to properly compare the visual adaptation of both visual systems, the ocelli and compound eyes of the same individuals were analyzed.

2.1. Adaptation to environmental illumination

The bugs were exposed to light/dark cycles (L:D 12 h:12 h) at a constant temperature (25 °C) for 5 days. They were then killed and their heads were prepared for histology at 2 different times during the day corresponding, respectively, to *T. infestans* minimum and maximum phototactic sensitivity and also to maximum concentration/dispersion of screening pigments in their compound eyes (Reisenman et al., 1998, 2002). In this case, in order to prevent light interference on eyes adapted to darkness, preparations were performed under IR illumination using a night-vision system (i.e., functional darkness for the insects, Reisenman et al., 1998). Illumination during the LD cycle photophase was obtained using a fluorescent light source (Osram Dulux EL E27, 7W/41-827, 140 mW/cm^2). Light-intensity was measured using a radiometer (SEL 033 sensor module, IL 1400 radiometer; International Light, Newburyport, MA, USA).

2.2. Control of pigment migration

To determine whether daily changes in the eyes are subject to endogenous or exogenous control, insects were first kept in LD cycles (L: 140 mW/cm^2, D: 0 mW/cm^2) for 5 days, then one group of bugs ($n = 12$) was transferred to constant darkness (DD: 0 mW/cm^2), while another group ($n = 12$) was kept in constant light (LL: 140 mW/cm^2). After three subjective days, the heads of 6 bugs from each experimental group (LL and DD) were prepared for histology at the 4th hour of the subjective day and the remaining 6 bugs at the 2nd hour of the subjective night. The free-running periods used for estimating the subjective day and night of the bugs were 23 h and 50 min for bugs kept in DD conditions and 26 h and 40 min for bugs kept in LL conditions (Lazzari, 1992) as previously done by Reisenman et al. (2002) in their study of compound eye adaptation. In all cases, the preparations remained under the same light conditions to which they had become adapted during the entire fixation period.

2.3. Temperature cycles and adaptation

Bugs ($n = 10$) were kept in DD conditions and submitted to a high/low temperature cycle (12 h:12 h HT:LT, 27 °C:21 °C) for 7 days. On the eighth day, 2 groups of insects were processed for histology. One-half of the bugs ($n = 5$) were fixed between the 4th and 8th hour of the HT phase and the other half ($n = 5$) between the 2nd and the 4th hour of the LT phase. In all cases, the heads were processed under IR illumination. The selected temperatures