Running title: Oxidative stress and screening pigments in insect eyes

OXIDATIVE STRESS, PHOTODAMAGE AND THE ROLE OF SCREENING PIGMENTS IN INSECT EYES

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ABSTRACT

Using triatomine bugs (Hemiptera: Reduvidae) red-eyed mutants, we tested the hypothesis of an alternative function of insect screening pigments against oxidative stress. To tests our hypothesis, we studied the morphological and physiological changes associated with the mutation. We found that wild-type eyes possess great amount of brown and red screening pigment inside the primary and secondary pigment cells as well as in the retinular cells. Red-eyed mutants, however, have only some scarce red granules inside the pigmentary cells. We then compared red-eyed mutants and wild-types visual sensitivity by measuring photonegative responses of insects reared in light/dark cycles (LD 12:12) or constant darkness (DD). Finally, we analyzed both the impact of oxidative stress associated with blood ingestion and photodamage of UV light on the eye retina. We found that red-eyed mutants reared in DD conditions were the most sensitive to the light intensities tested. Retinae of LD reared mutants were gradually damaged over the life cycle while for DD reared insects retinae were conserved intact. No retinal damages were observed on non-fed mutants exposed to UV lights for two weeks while insects fed on blood prior to UV exposition showed clear signs of retinal damage. Wild-type insects exposed to UV-light showed a marked increase in the amount and density of screening pigments.

INTRODUCTION

Mutations in eye color have been identified in several insect species and have been used as a marker in genetic studies or to follow different experimental populations. For instance, differences in eye color have been used to quantify the reproductive success of competing individuals by comparing the proportion of mutants to wild type in the offspring (Pires et al. 2002).

Mutants are also used as model systems for studies on the functional organization of visual systems. Indeed, eye color is given by screening pigments inside animal eyes, and, although in some species they may play a role in camouflage or in the protection against UV light photodamage, these pigments are mostly related to the visual function (Stavenga, 1989). Therefore, any modification of their nature or distribution, particularly their absence, may have important consequences on visual performance. In vertebrates and crustacean it has been suggested that screening pigments could also protect cells from the photo-oxidative damage derived from lipid peroxidation (Sakina et al., 1987; Ostrovsky et al., 1987; Dontsov et al., 1984; Dontsov et al, 1999). Such a function has, however, never been tested in the compound eyes of insects.

For different species mutations can differently affect parts the screening pigment synthesis pathway and consequences on visual sensitivity might differ. Despite the frequent use of eye color mutants in experiments, the morphological, physiological and behavioral consequences of such mutations remain unknown for most insects. The physiological and behavioral consequences of eye-color mutations have been studied in flies and honeybees and alterations in visual sensitivity were described (e.g. Hotta and Benzer, 1969; Gribakin and Chesnokova, 1982). Among hemimetabolous insects, such mutations have been reported in several species of triatomine bugs (Hemiptera: Reduviidae), red-eyed mutants being the most frequent. Red-eyed mutant bugs are a particularly interesting system model for studying the visual function for multiple reasons. First, these bugs are highly sensitive to light due to their nocturnal habits (Reisenman et al., 1998). Triatomine have a particular visual system, i.e. organisation of compound eyes and complexity of the ocellar system (Reisenman et al., 2002; Insausti and Lazzari, 2002). Additionally, they are hemimetabolous insects, making it possible to experiment on all stages throughout

life (6 to 12 months). Finally, they are obligatory haematophagous, throughout their entire life, which expose them to the oxidative stress derived from the heme group of the ingested blood (Vincent, 1989; Graça-Souza et al, 2006).

The goal of this study is to shed some light onto the role of screening pigments present in the insect eyes. Using two species of triatomine bugs, we investigated: 1) the morphological changes in the compound eyes and ocelli, associated to the red-eye mutation; 2) the visual sensitivity of mutants by comparison to wild-type insects; 3) the effect of light and blood ingestion on retinal cells; 4) the role of screening pigments.

MATERIALS AND METHODS

Experimental animals

Larvae and adults of red-eye mutants and wild type of *Triatoma infestans* and *Rhodnius prolixus* were used throughout their life cycle. The insects were reared in a laboratory colony at 26°C and fed weekly on heparin-treated sheep blood, through an artificial feeder (Núñez and Lazzari, 1990).

Provided that response to light, structure of compound eyes and ocelli, as well as pigment migration have been well characterized in *T. infestans* (Reisenman et al., 1998; Reisenman et al., 2002; Insausti and Lazzari, 2002; Lazzari et al., 1998; Lazzari et al., 2012), this species was chosen for studying the modification of eye structure associated to the red-eye mutation, the visual sensitivity of mutants and the impact of light exposition on the retina and visual performance.

R. prolixus is a classical model for the study of oxidative stress associated to blood ingestion (Graça-Souza et al, 2006; Caiaffa et al, 2010; Stiebler et al, 2010). For this reason, we used this species for analyzing the response of mutants and wild-type bugs to the exposition of photo and heme oxidative stress.

The wild type insects were maintained under 12:12 hours light/dark cycles along their whole life.

Red-eye mutants were maintained during their whole life (i.e. since eggs) either in permanent darkness or exposed to light/dark cycles (L:D 12 h:12 h, 140 $mW.cm^{-2}$).

Eye morphology

Larvae and adults of *T. infestans* and *R. prolixus* were analyzed. Light microscopy was performed on the insect heads following the procedure described previously (Reisenman et al., 2002). In brief, the posterior half of the head (containing the compound eyes and the ocelli) was fixed for 3·hours in a mixture of 2.5% glutaraldehyde and 2.0% paraformaldehyde in phosphate buffer (pH·7.3) with glucose and $CaCl_2$ added. After dehydration, they were embedded via propylene oxide in Durcupan ACM (Electron Microscopy Sciences no. 14040). Blocks were serially sectioned at 5·µm using glass knives mounted on a microtome. The sections were stained on a hot plate with 1% methylene blue and mounted on a slide with DPX (Electron Microscopy Sciences no. 13510).

Visual sensitivity

As an experimental paradigm for behavioral tests, we measured the photonegative response of both groups of mutants and of wild *T. infestans* (Reisenman et al., 1998).

The phototactic behavior of bugs was measured in a rectangular arena as done by Lazzari et al (1998). The arena (25 cm length x 5.5 cm width x 2.2 cm high), had filter paper for substrate and was covered with a rectangular piece of glass. Half of the arena was kept in the dark by means of a black cardboard fixed to the glass cover and the other half remained uncovered. The uncovered half was illuminated with an halogen (= white light source) (OSRAM 41860 WF, 12V/20W) located in an aluminum cylinder (diameter: 8 cm, height: 17 cm) that rested on top of a diffusing glass, 60 cm above the arena. The lamp was located above the right or left end of the arena in order to accentuate differences between dark and illuminated halves.

Neutral density filters (Melles Griot fused silica filters) were interposed to obtain a light intensity on the illuminated side of the arena of either 0.6 or 6 $\mu W/cm^2$. Light intensity was measured with a radiometer (SEL 033 sensor module, IL 1400 radiometer; International Light, Newburyport, MA, USA, provided with a photometric filter).

All of the experiments were done at 25 °C, and each bug was used in only one trial. Spatial asymmetries were avoided by interchanging areas between trials.

For each trial, a single bug was released. The insect was first placed within a small dark bowl in the dark end of the area. After 40 seconds of rest, the bowl was carefully inverted and the trial started. Each trial lasted five minutes. The behavioral variable quantified was *Permanence time in darkness* (%): total time (measured in seconds) spent in the dark half of the arena, expressed as percentage of the total time of the trial. Controls were tested in total darkness and permanence time was computed considering the side in which the insect was released (right or left). Experiments in total darkness were monitored with the aid of a night vision system provided with an IR illumination source (900nm), which light is not perceived by bugs (Reisenman et al., 1998).

Twenty wild-type or mutant individuals were tested by group. A two-way ANOVA was performed (normality and homocedasticity tests passed) in order to test the effect of light-intensity and the experimental group on the results.

The effect of blood ingestion and UV-exposition

To analyze whether both types of oxidative pressure, i.e. light and heme, could have interactive effects, we compared the eyes of fed and unfed, wild-type and mutants exposed to UV light. This light was chosen because it imposed a stronger photo-oxidative stress than white light (Meyer-Rochow, 1994; Meyer-Rochow et al, 2002). Groups of fifth-instar larvae of *R. prolixus* were exposed to 31 $\mu W.cm^{-2}$ UV-light (Hanau Fluotest Typ 5301) or kept in darkness for two or four weeks. The intensity of UV light was chosen to be within the visual sensitivity of bugs, which range from 0.05 $\mu W.cm^{-2}$ to 115 $\mu W.cm^{-2}$ (Reisenman et al., 1998).

The analysis of the structure and condition of the eyes was conducted by means of histological preparations and light microscopy as described in the "*Eye morphology"* section above.

RESULTS

Eye morphology

On gross external inspection, the eye colour of wild individuals of both species analysed is dark brown, whereas the eyes of mutant insects are bright red (Fig. 1A-D). Compound eyes of wild individuals *T. infestans* and *R. prolixus* have been described by Reisenman et al. (2002) and by Müller (1970), respectively. These

bugs posses apposition compound eyes with open rhabdoms, in which a ring of six rhabdomeres (rh 1-6) from retinula cells 1–6 surrounds a central pair of rhabdomeres (rh 7-8) from retinula cells 7 and 8. The crystalline cone is surrounded by two primary pigment cells (PPC). Twenty-four secondary pigment cells (SPC) enclose each ommatidium. Dark granules of 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' (Figs. 2A, 3A, 4A, B, 7A, C). In the ocelli, large amount of pigment granules are found inside the retinal cells and in the outer ring of pigmentary cells (Insausti et al., 2002) (Fig. 5A).

When we studied mutant compound eyes, we observed a lack of screening pigments inside the retinular cells for both species. For *T. infestans*, we found red pigment granules inside the primary and secondary pigment cells. However, the amount of pigment found in the primary pigment cells was lower than for the wild bugs (Figs. 2B, 3B, 4C, D). We found similar results for *R. prolixus* secondary pigment cells but we found no pigment at all in the primary pigment cells for this species (Fig. 7C, D).

The analysis of postembryonic development of the eyes of *T. infestans* mutants kept under LD conditions showed intact retinular cells in first instar larvae (Fig. 2B). As the individuals grew, progressive cellular damage was observed. Consequently, damage was particularly severe in adults (Figs. 3C, 4E, F).

For mutants reared in complete darkness, no damage of the cellular structure was observed for larvae or adults (Figs. 3B, 4C, D).

Examination of the ocelli of red-eyes mutants (Fig. 5B) revealed the absence of pigment granules inside both, pigmentary and retinal cells. No cellular damage was observed in the ocellar retina for both treatments (light/dark cycle or kept in the darkness).

Visual performance

Figure 6 depicts the sensitivity to light of wild-type and mutant *T. infestans*, as revealed by the intensity of the photonegative response. Four groups were compared: 1) light-adapted wild-type bugs (i.e. dispersed screening pigments); 2) dark-adapted wild-type bugs (i.e. retracted screening pigments); 3) red-eye mutants

reared in DD (i.e. no screening pigments, intact retina) and 4) red-eye mutants reared in LD (i.e. no screening pigments, damaged retina). All groups reacted to light at both light intensities tested. The intensity of the response to light varied across groups as follows: mutant bugs reared under constant darkness > dark-adapted normal bugs > mutants reared in LD > light-adapted normal bugs.

The effect of blood ingestion

As for *T. infestans*, the red-eye of *R. prolixus* mutants unfed and kept in DD exhibits an ommatidial structure that only differs from wild-type insects by the absence of dark screening pigments (Fig. 7A-D).

For insects that were exposed two weeks to UV-light, the influence of blood feeding became evident. While non-fed insects did not exhibit any retinal damage when compared to DD-reared insects, mutants that were blood fed before UV exposition showed clear signs of retinal damage (Fig. 8 A, B). Feeding also affected wild-type insects. After two weeks of exposition to UV, unfed wild-type insects did not exhibit any noticeable change but, insects that were fed showed an increase in the relative amount of screening pigments (Figs. 8C, D). Finally, after a month of exposition to UV, for the red-eye bugs photodamage was evident, as shown in Fig. 9A. In wild-type insects however only a marked increase in the amount and density of screening pigments was observed (Fig. 9B).

DISCUSSION

The nature of pigments

The screening pigments present inside the pigment and retinula cells determine the color of insect eyes. These pigments belong to two chemical classes: ommochromes and pteridines (Langer, 1975). Pteridines are found in smaller amounts, they can be white, yellow and red (Langer, 1975). The nature of the ommochromes present in the secondary pigment cells may vary with the insect species. Some insects only have xanthommatin, while in others a mixture of xanthommatin and ommins is present. Xanthommatin can be present in an oxidized form (yellow-brown) or in a reduced form (red) (Langer, 1960; Linzen, 1967). The pigment granules containing ommins were described in the receptors cells and the

primary pigment cells (Langer, 1975). The eye-color mutants lack or have low amounts of one or more of these screening pigments (Summers et al., 1982).

Among triatomines, red-eye is the most frequent mutation. In wild-type *R. prolixus*, the presence of both ommins and xanthommatin has been reported (Butenandt et al., 1960; Linzen, 1974). In *T. infestans*, Moraes et al. (2005) concluded that xanthommatin is the only ommochrome present in both, wild and red-eyes insects. These authors excluded the presence of pteridines in the eyes of *T. infestans* and did not report the presence of ommins. They suggested that the red color of mutant eyes was caused by a smaller amount of xanthommatin compared to wild-type eyes. Our morphological analysis revealed that in *R. prolixus* and *T. infestans* the retinular cells of red-eyes mutants completely lacked pigments and pigment cells contained only red pigment granules. The amount of pigments inside the primary pigment cells of *T. infestans* mutants is lower than for wild-type bugs. We thus concluded that for the eyes of *T. infestans*, as previously reported for *R. prolixus*, two kinds of ommochromes are present. Ommins must be the dark pigment found inside the retinula cell and red xanthommatin the one present in the secondary pigment cells. In wild-type insect, a mixture of both must be present in the primary pigment cell. This way, if red-eye mutants lack dark ommins, xanthommatin will be the red pigment present in secondary cells and primary pigment cells. Since the ocelli of mutant insects lack screening pigments both inside retinal and pigmentary cells, we can state that the only pigment present in the wild-type ocelli should be ommins. A biochemical analysis is necessary to confirm this hypothesis, but it seems the most parsimonious one according to the available evidences.

The visual performance of mutants

The present work revealed that mutation does not abolish visual input, but affects light sensitivity threshold. Mutants reared in DD were more sensitive to light than wild-type bugs, probably because they lack most of the eye screening pigments and have an intact retina (more photons can stimulate the photoreceptors). When reared in LD, light exposition damaged some of the photoreceptors located in the medial regions of the eye (those present along the whole development of bugs). It should be noted that because new ommatidia are added at the eye periphery at each molt (Settembrini, 1984), photodamage is more marked in the old medial ommatidia than for the peripheral ones (not shown). This allows bugs to remain relatively

sensitive to light along their life despite the degradation of the oldest ommatidia. Provided that the number of functional photoreceptors is reduced upon exposition to light, the sensitivity of LD-reared mutant bugs is nevertheless lower than for the insects reared in DD.

Wild-type bugs also vary in their sensitivity to light as a consequence of the movement of screening pigments (Reisenman et al., 2002). They are less sensitive during the photophase than during the scotophase (Reisenman et al., 1998). When compared to mutants, the strong limitation of photon arrival to the rhabdom exerted by screening pigments in light-adapted normal eyes (i.e. tested during the photophase) make these bugs the less sensitive group of insects. Dark-adapted bugs (i.e. tested during their scotophase), as expected, were more sensitive than light-adapted ones, but not as sensitive as mutants reared in DD.

It should be noted that the daily adaptation of the compound eye of wild bugs involves two processes, the migration of screening pigments and the axial displacement of the rhabdom (Reisenman et al. 2002). In red-eye mutants neither process takes place and, as a consequence, no change in their condition occurs between the scotophase and the photophase. Two conclusions can be drawn from this observation. First, no adaptation occurs in the red-eye and, second, the axial migration of the rhabdom is not driven by its own movement, but by the displacement from screening pigments present into the retinular cells.

This is the first time that the effects of a mutation on the visual system of hemimetaboulous insects are being tested. So far, most work has been done on holometabolous insects and thus research is restricted to the adult stage only. Working with hemimetabolous insects offers the possibility to evaluate long-term effects across developmental instars. Furthermore, most of these studies are purely descriptive and not manipulative. Despite these differences, however, our results are consistent with the results found for holometaboulous insects. Compound eye of worker honeybees with snow and laranja mutations (an alteration of tryptophan metabolism) had an increased sensitivity to light, which was explained by the absence of any screening pigment (Gribakin and Chesnokova, 1982). Similarly, in the blowfly *Calliphora erythrocephala*, an important increase in sensitivity was evinced in mutants lacking screening pigments when compared to normal flies (Streck, 1972). This is logical as in the eye of flies screening pigments optically isolate adjacent ommatidia.

In the case of *Drosophila*, several studies on the effect of mutations on visual performance have been published. Visual performance was measured by means of electroretinogram and phototactic behavior (Broda and Willmund, 1981; Pak, 1995; Belušič, 2011). Provided the existing diversity of mutations and the fact that in many cases previous exposition to light was not controlled, it is difficult to discriminate the relative importance of different parameters, such as lack of pigment, retinal damage, aging, etc.

The photo-protector role of the screening pigments

The main function assigned to screening pigments in the compound eyes of insects is related to vision, i.e. screen out stray light, control the incident light flux at the photoreceptors, and their angular acceptance (Stavenga, 1989). On the other hand, in vitro studies revealed that melanin in vertebrates and ommochromes in invertebrates fulfill the same function as effective inhibitors of free radical induced by lipid peroxidation, protecting eye structures against UV or bright light-induced damage (Ostrovsky et al., 1987; Sakina et al., 1987; Meyer-Rochow et al., 2002). In vivo studies of deep-water living crustacean have shown that the exposition to illumination ranging from moderate to high produced retinal damage, revealing the deleterious effect of light (Nilsson and Lindström, 1983). As underlined by Boulton et al. (2001) *"The retina represents a paradox, in that, while light and oxygen are essential for vision, these conditions also favour the formation of reactive oxygen species leading to photochemical damage to the retina."* Screening pigments protect retinal integrity in two ways, first physically, by reducing the light intensity reaching the photoreceptors and chemically as an antioxidant. However until now this last function has never been investigated for the screening pigments of the compound eyes of insects.

In the present work we demonstrated the protective role of the screening pigments in the eyes of triatomine bugs and most likely insects in general. This assertion is supported by the fact that red-eye mutant bugs, which lack screening pigments within the retinula cells, presented a highly degraded retina and severe reduction of visual performance when exposed to light. This degradation became more severe as the insects aged, i.e. with the cumulative time of light exposure. Ocelli exhibited no damage, probably due to the fact that their exposition to light is shorter given their imaginal character.

In addition, the hematophagous condition of triatomine insects makes them particularly interesting. Hematophagous insects ingest large amounts of blood every meal, the digestion of which produces high concentration of heme. Free heme is a powerful generator of reactive oxygen species, which damage biological systems through oxidation of lipids, proteins and DNA (Vincent, 1989). Thus, the eyes of haematophagous insects are exposed to two type of oxidative stress: the light they receive and the blood that the insect ingests. Screening pigments seem to be able to reduce this stress by means of two mechanisms, first by reducing the amount of light reaching the retinular cells and, second, acting as antioxidant agents. In the present work we demonstrated that screening pigments play a fundamental role in visual cells protection against oxidative processes caused by blood feeding and photo-oxidative stress. This was not only evident in mutants lacking these pigments, but also in wild-type bugs as highlighted by the increase in screening pigments quantity observed in the ommatidia of insects exposed to stress factors (Figs. 8C and 9B). This increase occurred within a single instar, revealing that ommochrome synthesis is independent of molting and is directly induced by stress in order to maintain the retinular integrity.

In conclusion, the eyes of blood-sucking insects are particularly exposed to a double oxidative stress, which is reduced by the presence and active synthesis of screening pigments. In the case of triatomines, which are able to obtain blood-meals reaching more than 10 times the own body weight at each feeding event, we can speculate than they strong photophobic behavior helps to reduce oxidative stress to which they are submitted.

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FIGURE CAPTIONS

Figure 1. Head of *Triatoma infestans* (A, B) and *Rhodnius prolixus* (C, D) in lateral view. **A, C:** wild type. **B, D:** red-eye mutant. The right ocellus (short arrow) and compound eye (long arrow) are shown. Scale bars: 300 µm.

Figure 2. Light micrographs of longitudinal sections through the compound eyes of the recently hatched first larval stage of *Triatoma infestans.* **A:** wild type. **B:** red-eye mutant. C, cornea; Cc, crystalline cone; PPC, primary pigment cell; rh, rhabdomere; Rp, retinal pigment; SPC, secondary pigment cell; 1-8 indicate the retinula cell number. Scale bars: 50 µm.

Figure 3. Light micrographs of longitudinal sections through the compound eyes of the fourth larval stage of *Triatoma infestans.* **A:** wild type. **B:** red-eye mutant reared in DD conditions, showing the intact structure of the retina. **C:** red-eye mutant reared in LD conditions. The partial damage of the retinal structure is evident already. C, cornea; Cc, crystalline cone; PPC, primary pigment cell; rh, rhabdomere; Rp, retinal pigment; SPC, secondary pigment cell; 1-8 indicate the retinula cell number. Scale bars: 30 µm.

Figure 4. Light micrographs of sections through the compound eyes of adult stage of *Triatoma infestans.* **A:** wild type, longitudinal section. **B:** wild type, cross section. **C:** longitudinal section of red-eye mutant reared in DD conditions showing the intact structure of the retina. **D:** cross section of red-eye of the same individual in C. **E:** longitudinal section of red-eye mutant reared in LD conditions. **F:** idem E, cross section. Note in E and F the significant damage of the retina. C, cornea; Cc, crystalline cone; PPC, primary pigment cell; rh, rhabdomere; Rp, retinal pigment; SPC, secondary pigment cell; 1-8 indicate the retinula cell number. Scale bars: 30 µm.

Figure 5. Light micrographs of sections through the ocellus of *Triatoma infestans* recently emerged to adult stage, reared in LD conditions. **A:** wild type. **B:** red-eye mutant. As a new structure, the ocellus shows no damage, as compared with the retina of the compound eye of the same individual (Fig. 4E, F). Scale bars: 100 µm.

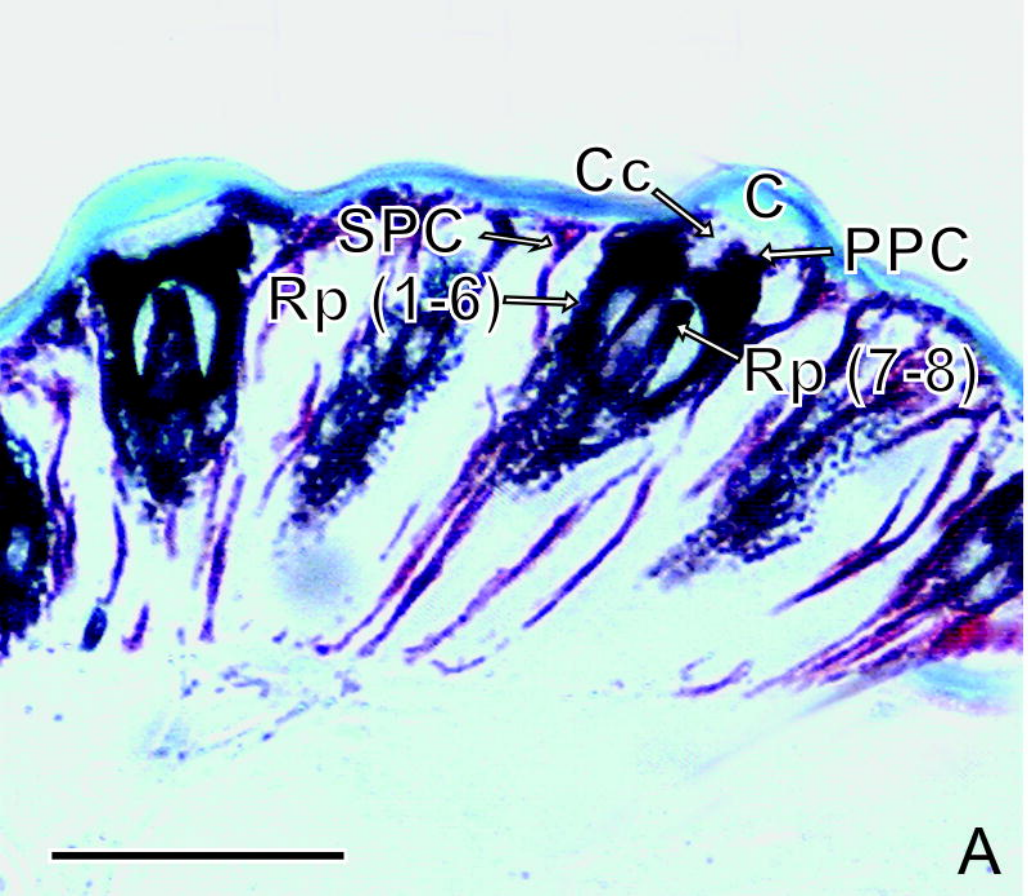
Figure 6. Phototactic sensitivity of wild-type and red-eyes mutants of *Triatoma infestans*. Provided that the migration of screening pigments modulates the sensitivity to light of wild-type bugs, they were tested during the scotophase and during the photophase. Mutants do not experience adaptation, because of the lack of those pigments, but their exposition to light produce retinal photodamage. LD, bugs kept under a light/dark cycle; DD bugs maintained in constant darkness.

Figure 7. Light micrographs of sections through the compound eyes of fifth-instar larvae of *Rhodnius prolixus*. **A:** wild type, longitudinal section. **B:** wild type, cross section. **C:** unfed red-eye mutant reared in permanent darkness (longitudinal section), showing the intact structure of the retina. **D:** idem as C in cross section. C, cornea; Cc, crystalline cone; PPC, primary pigment cell; rh, rhabdomere; Rp, retinal pigment; SPC, secondary pigment cell; 1-8 indicate the retinula cell number. Scale bars: 30 µm.

Figure 8. Light micrographs of sections through the compound eyes of fifth-instar larvae of *Rhodnius prolixus* exposed two weeks to UV light. **A:** unfed red-eye mutant**. B:** fed red-eyes mutant. **C:** unfed wild type insect. **D:** feed wild type insect. The structure of the retina of unfed red-eyes mutants (A) as well as that of unfed (C) and fed (D) wild type insects is conserved. On the other hand, the signs of damage in the retina of the fed red-eyes mutants (B) are evident. C, cornea; Cc, crystalline cone; PPC, primary pigment cell; rh, rhabdomere; Rp, retinal pigment; SPC, secondary pigment cell; 1-8 indicate the retinula cell number. Scale bars: 30 µm.

Figure 9. Light micrographs of sections through the compound eyes of unfed fifth-instar larvae of *Rhodnius prolixus* exposed 1 month to UV light. **A:** red-eye mutant showing the damaged retina. **B:** wild type insects, note the large density of screening pigment granules. Scale bars: 30 µm.


A
B
C
D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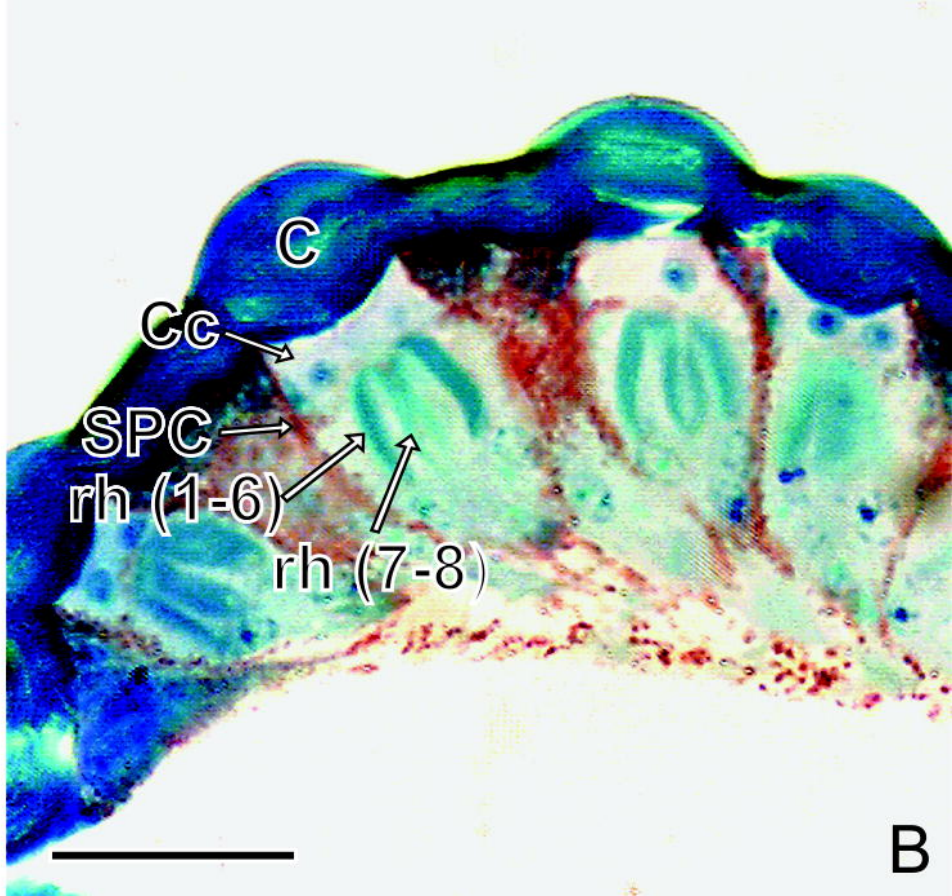
C
SPC
PPC
Rp (1-6)
Rp (7-8)
A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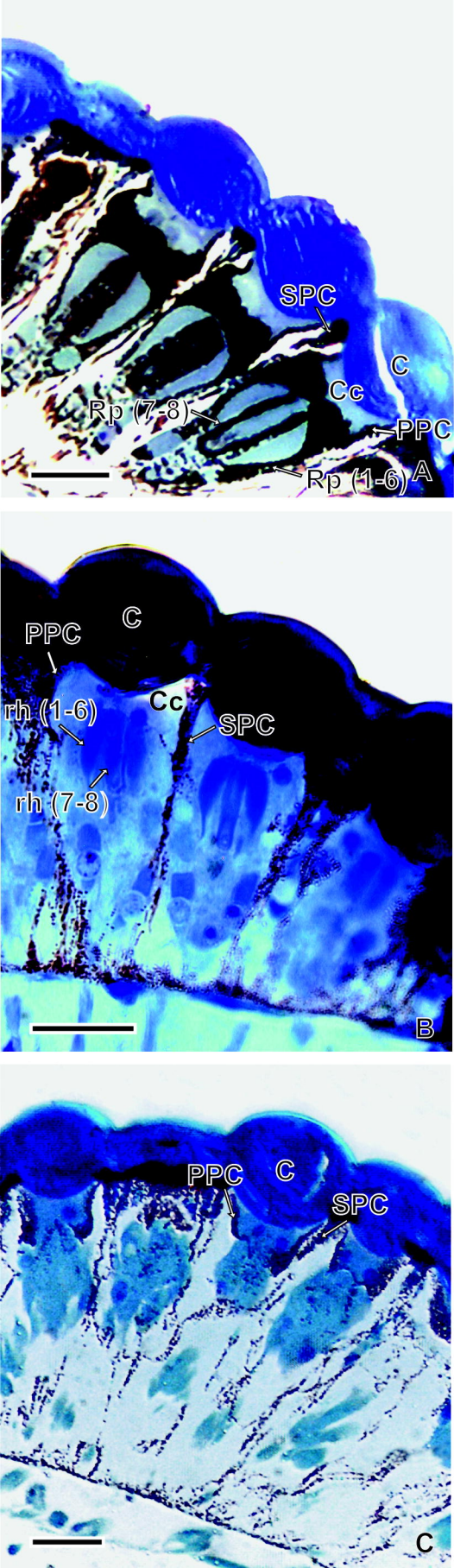
Cc
SPC
rh (1-6)
rh (7-8)
B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Rp (7-8)
PPC
Rp (1-6)
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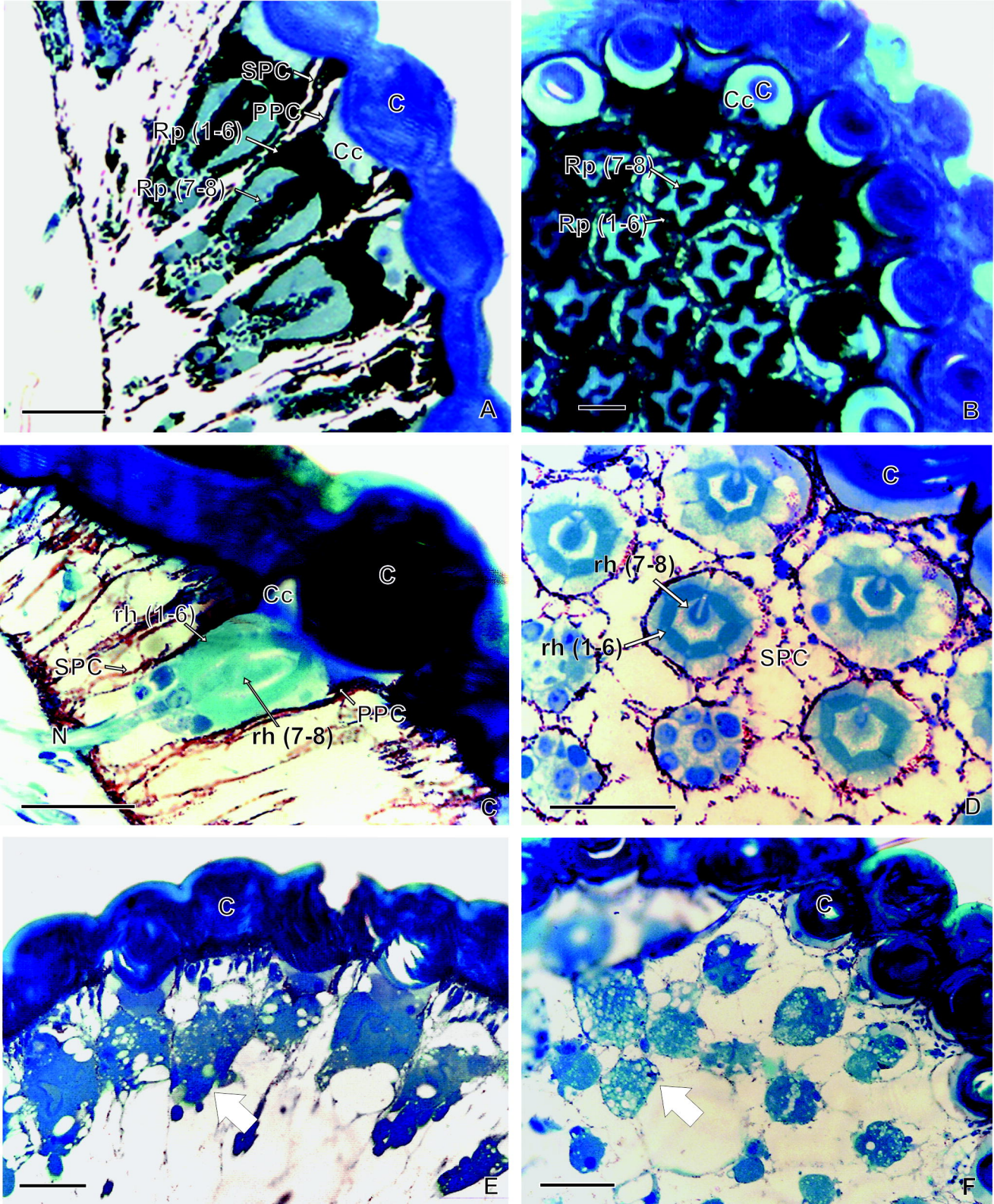
rh (7-8)
B
PPC
C
SPC
C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C
A
Cc C
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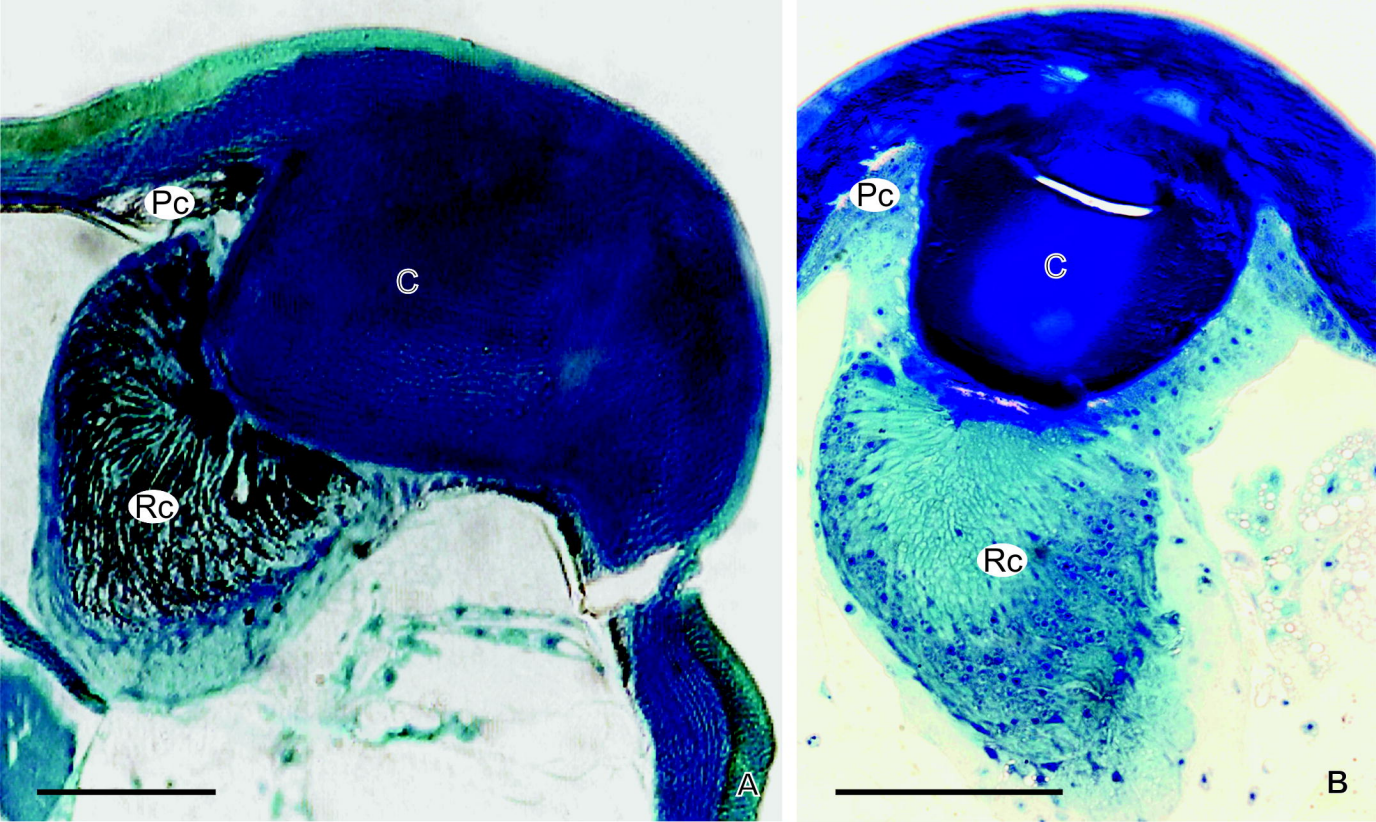
Rp (1-6)
B
C
Cc
rh (1-6)
SPC
N
PPC
rh (7-8)
C
C
rh (7-8)
rh (1-6)
SPC
D
C
E
C
F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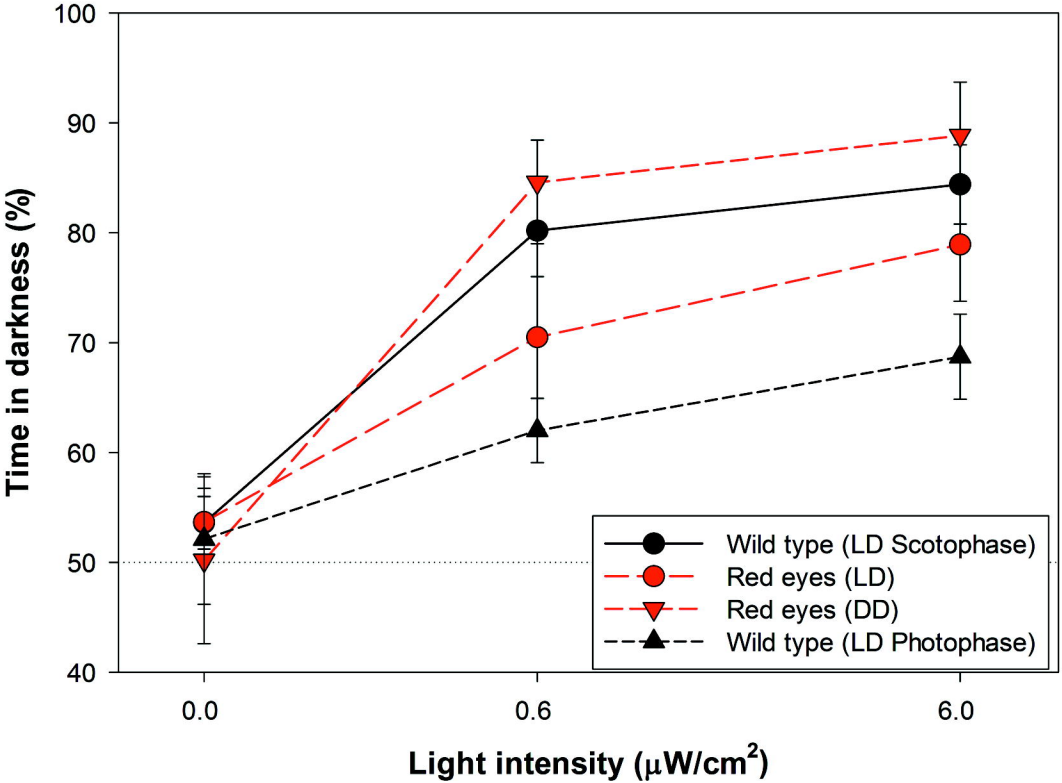
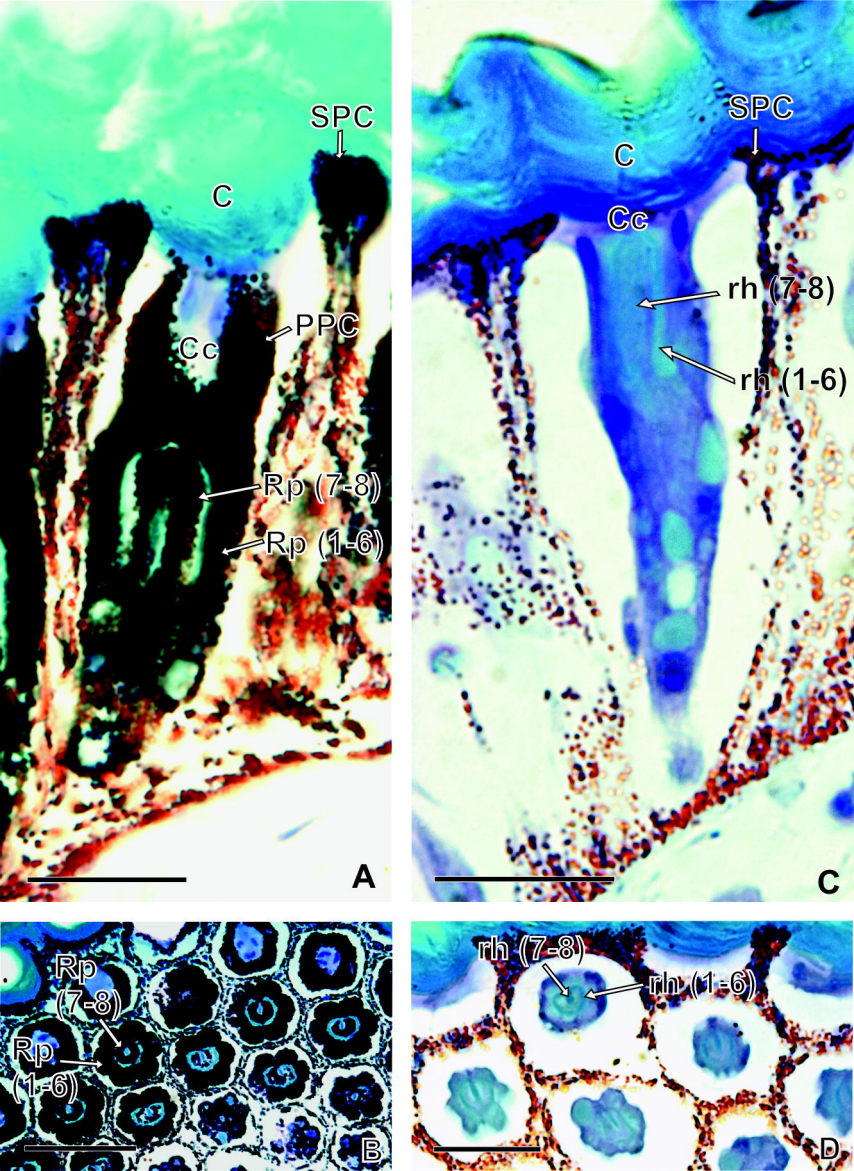

100
90
80
70
60
50
40
Time in darkness (%)
0.0
0.6
6.0
Light intensity (μW/cm²)
Wild type (LD Scotophase)
Red eyes (LD)
Red eyes (DD)
Wild type (LD Photophase)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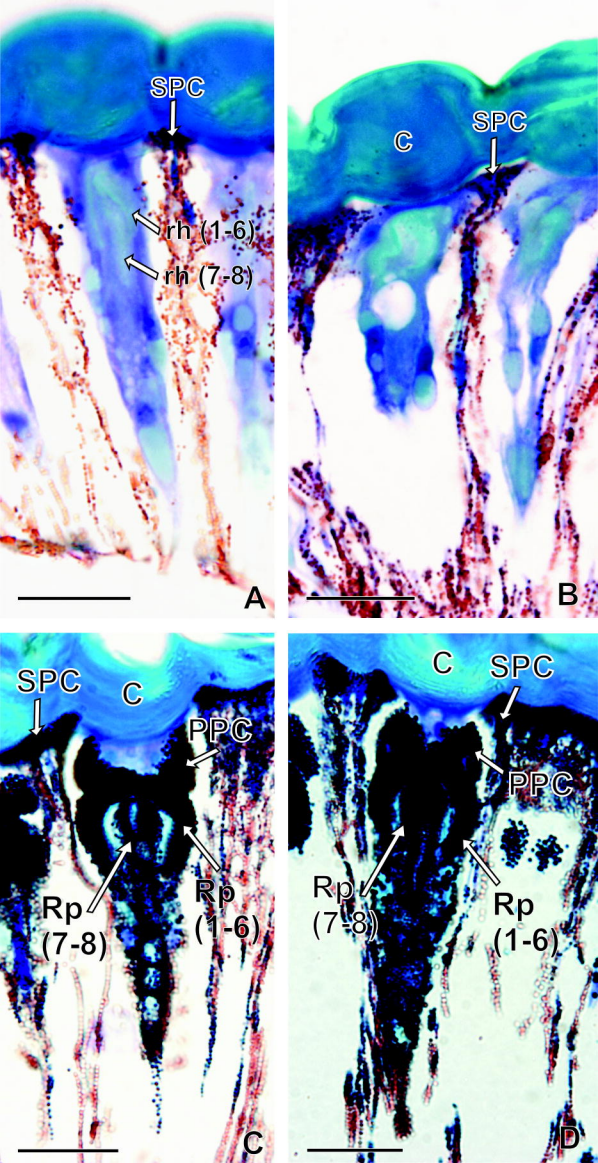
C
Cc
PPC
Rp (7-8)
Rp (1-6)
A
SPC
C
Cc
rh (7-8)
rh (1-6)
C
Rp (7-8)
Rp (1-6)
B
rh (7-8)
rh (1-6)
D


SPC
rh (1-6)
rh (7-8)
A
C
SPC
B
SPC
C
PPC
Rp
(7-8)
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C
C
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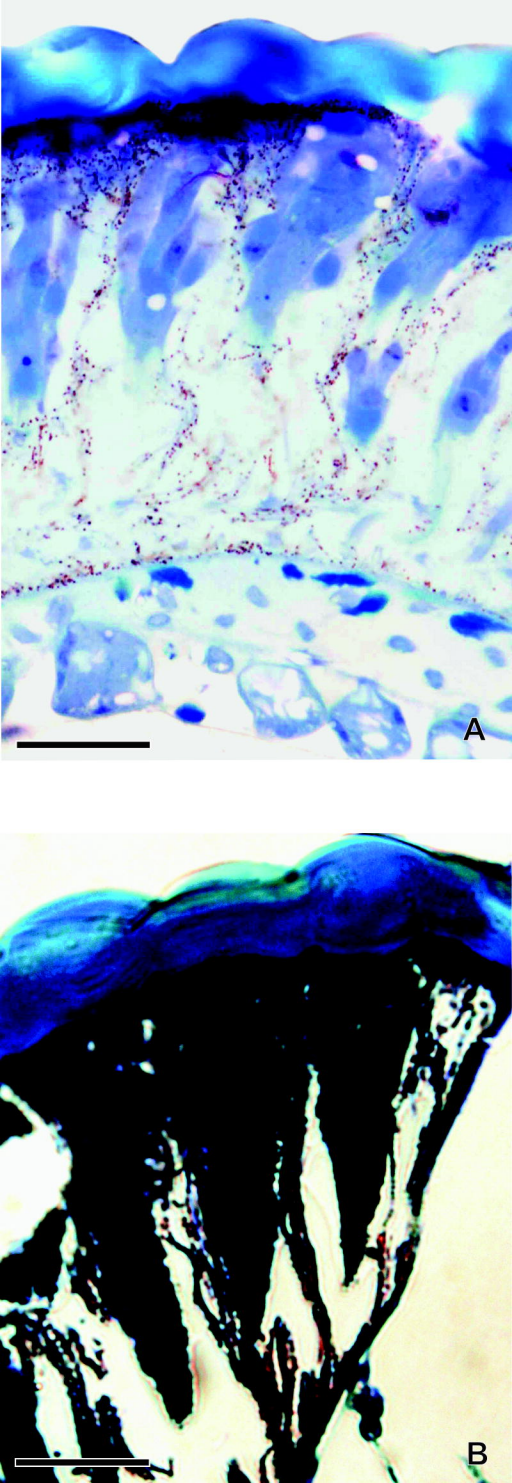
Rp
(7-8)
Rp
(1-6)
D


A
B