

EYE PIGMENTS OF THE BLOOD-SUCKING INSECT, *Triatoma infestans* KLUG (HEMIPTERA, REDUVIIDAE)

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(With 1 figure)

ABSTRACT

The pigmentation of black (wild) and red (mutant) eyes of *Triatoma infestans* was studied spectrophotometrically and compared with red-eyed (wild) and white-eyed (mutant) forms of *Drosophila melanogaster*. The spectral absorption profiles of the black and red eye pigments of *T. infestans* were similar to each other and to that of the wild-type eyes of *D. melanogaster*. The similarity to the wild form of *D. melanogaster* indicated that both eye forms of *T. infestans* contained ommochromes of the xanthommatin type, a finding confirmed by ascending paper chromatography. Pteridines, melanins, and ommins were not detected as eye pigments in *T. infestans*. The eye color difference in *T. infestans* was assumed to be a function of the xanthommatin concentration, with a smaller content of ommochrome in red eyes, although this probably did not affect the insect's visual acuity. These data support other findings regarding the similarities between black- and red-eyed specimens of *T. infestans* for other characteristics.

Key words: *Triatoma infestans*, eye color, black eyes, red-eyed mutant, ommochrome, spectral absorption profiles.

RESUMO

Pigmentos de olho em *Triatoma infestans* (Hemiptera, Reduviidae)

A pigmentação de olhos pretos (forma selvagem) e vermelhos (forma mutante) de *Triatoma infestans* foi estudada por espectrofotometria e comparada à de olhos vermelhos (selvagem) e brancos (mutante) de *Drosophila melanogaster*. Os perfis do espectro de absorção dos pigmentos de olho preto e vermelho de *T. infestans* foram semelhantes entre si e aos dos olhos de tipo selvagem de *D. melanogaster*. A similaridade com a forma selvagem de *D. melanogaster* indicou que ambos os tipos de olho de *T. infestans* continham omocromos do tipo xantomatina, o que foi confirmado por cromatografia ascendente em papel. Não foram detectadas pteridinas, melaninas e omimas como pigmentos de olho em *T. infestans*. A diferença na cor de olho em *T. infestans* foi considerada uma função da concentração de xantomatina, sendo menor o conteúdo de omocromo nos olhos vermelhos, embora isso provavelmente não afete a acuidade visual do inseto. Esses resultados estão de acordo com dados de outros autores quanto a semelhanças envolvendo outras características entre espécimes de olho preto e vermelho de *T. infestans*.

Palavras-chave: *Triatoma infestans*, cor de olho, olhos pretos, mutante de olho vermelho, omocromo, espectro de absorção.

INTRODUCTION

Eye color in *Triatoma infestans* is genetically controlled at a single autosomal locus in such a way that black eyes are produced by the dominant gene (wild) and red eyes by the homozygous recessive gene (mutant) (Wygodzinsky & Briones, 1954; Dujardin & Bermúdez, 1986). No significant differences in oviposition or egg hatching rates have been associated with the parental eye-color phenotypes in this species (Pires *et al.*, 2002). Although no impairment of mating attractiveness or mating success has been related to the red-eyed color, it has been suggested that sperm cells from red-eyed males have a lower viability compared to those of black-eyed specimens, thus explaining why red-eyed mutants of *T. infestans* are uncommon in natural populations (Pires *et al.*, 2002).

At the cellular level, nuclear phenotypes in the Malpighian tubules of red-eyed *T. infestans* do not differ from those of black-eyed individuals, at least under full nourishment conditions (Gonçalves *et al.*, 1997).

Although investigations have dealt with red-eyed mutants of *T. infestans*, no information has up to now been available on the nature of the pigments in the red and black eyes of this species. The only reduviid hemipteran in which the eye pigments have so far been studied is *Rhodnius prolixus* for which, based on paper chromatography, the presence of ommins and xanthommatin has been suggested (Butenandt *et al.*, 1960; Linzen, 1974). On the other hand, there are numerous studies of eye pigmentation in dipterans that have demonstrated the occurrence of ommins, ommatins, and pteridines, and their relevance in mutations, insect physiology, and evolution, particularly in *Drosophila* species (Ephrussi & Herold, 1944; Cordeiro, 1959; Rasmuson *et al.*, 1960; Fuge, 1967; Connolly *et al.*, 1969; Stark & Wasserman, 1974; Nash & Henderson, 1982; Pfeiderer, 1992; Lewin, 2000). The typical ommatin in this case is xanthommatin (Linzen, 1974).

In this work, we used spectrophotometry to study the pigmentation of black and red eyes of *T. infestans*. Since fluorescence characteristics of the eye pigmentation was not the primary focus of the present study, they were not assessed.

MATERIAL AND METHODS

Black-eyed (wild) and red-eyed (mutant) adult males of *Triatoma infestans* Klug (1834) (Hemiptera, Reduviidae) were used. The insects were reared at 30°C and 80% RH in a laboratory at Superintendência de Controle de Endemias (Sucec), Mogi-Guaçu (São Paulo State). Adults were used because the pigment content of their eyes was greater.

The black-eyed specimens, which were from colonies reared at Sucec beginning in 1975, originated from domestic insects collected in the states of São Paulo (north) and Minas Gerais (south), Brazil. The colonies have periodically been crossed with specimens collected in southern Minas Gerais. The red-eyed colony originated from specimens collected in Argentina and was homozygous recessive for eye color mutation.

Red-eyed wild forms and white-eyed mutant adults of *Drosophila melanogaster*, strain Canton S, kindly provided by Dr. Louis Bernard Klaczko (Unicamp, Campinas) were used as standards. In the white mutant of *D. melanogaster*, the synthesis of ommochrome and pteridines is affected (Linzen, 1974).

Forty eyes of *T. infestans* (20 wild and 20 mutant specimens) and 200 eyes of *D. melanogaster* (100 wild and 100 mutant specimens) were used for each assay. The eyes were initially treated, overnight at 25°C, with 5 ml of 30% ethanol acidified to pH 2.0 with concentrated HCl (AEA). This procedure extracts pteridines. The eyes were then removed and placed in a second solution consisting of 5 ml of absolute methyl alcohol acidified with concentrated HCl to 1% (v/v) (AMA). This procedure, based on Ochando's (1981) modification of the method described by Ephrussi & Herold (1944), extracts ommochrome pigments after 24 h at 25°C. All extractions were done in a dark room to avoid changes in the spectral properties of the extracted pigments.

Spectral absorption curves were obtained for the eye pigment solutions using an HP8452A diode array spectrophotometer (Roseville, CA, USA) over the wavelength range of 200 through 700 nm. The spectral profiles were compared with each other and with those reported by Ephrussi & Herold (1944) and Ochando (1981) for *D. melanogaster*.

Since treatment with acidified methanol extracts ommochromes and melanins, and since the latter are not soluble in concentrated sulfuric acid and HCl (Ajami & Riddiford, 1971), a pigment extract was obtained with these acids in order to assess whether melanins were present in *T. infestans* eyes.

To confirm the conclusions resulting from the analysis of the spectral absorption profiles, eye pigment solutions were also subjected to ascending chromatography on Whatman n. 1 paper, using n-propanol-ammonia (5%) (2:1, v/v) as the solvent. The chromatograms were run at room temperature in the dark (Brown & Nickla, 1977) until the solvent front was approximately 15 cm from the point of sample application. The spots were detected using a short wavelength (254 nm) UV lamp (LF215S, UVItec Limited, Cambridge, UK) and their R_f were calculated.

RESULTS

Pigments were not extracted from *T. infestans* eyes when ethanol was used as the solvent, thus excluding the presence of drosoperins (Ephrussi & Herold, 1944). However, when pigments were obtained with acidified methanol, which extracts ommochromes and melanins (Ephrussi & Herold, 1944), pigments were revealed. The treatment of *T. infestans* eyes with concentrated sulfuric acid resulted in total extraction of eye pigments. Since such a procedure does not extract melanins (Ajami & Riddiford, 1971), these results indicate that ommochromes are the pigments present in both black and red eyes of *T. infestans*.

The absorption spectra of the eye pigments extracted from *T. infestans* and *D. melanogaster* with acidified methanol are shown in Fig. 1. Comparison of the absorption profiles of the ommochromes present in the eyes of both species revealed similarities. As expected, based on well-characterized ommochrome absorption profiles (Schäfer & Geyer, 1972; Linzen, 1974 – review), four peaks were evident: A ($\lambda = 210\text{--}225$ nm), B ($\lambda = 265\text{--}275$ nm), C ($\lambda = 350\text{--}360$ nm), and D ($\lambda = 440\text{--}450$ nm). These peaks were not well defined in the curves for black-eyed *T. infestans*, but they were for red-eyed *D. melanogaster*, whose ommochromes belong to the xanthommatin family

(Linzen, 1974), and for red-eyed *T. infestans*. Indeed, the black eye pigmentation of *T. infestans* is characterized by decreased absorption at region D and enhanced absorption in region C, a typical response of concentrated ommochromes to the H_2O_2 present in the AMA extractive solution (Ochando, 1981) (Fig. 1).

Chromatography of the pigments extracted from black and red eyes of *T. infestans* and from red eyes of *D. melanogaster* showed a main spot ($R_f = 0.15$) close to the point of sample application. Weakly absorbing spots with higher R_f values were observed in the material from the three sources and were assumed to be pigment precursors or metabolic by-products.

DISCUSSION

The extraction properties of the *T. infestans* black and red eye pigments and the similarity of their spectral absorption profiles with those of eyes from wild *D. melanogaster* under identical experimental conditions indicated the presence of ommochromes and absence of melanins in *T. infestans*. Since xanthommatin is the ommochrome occurring in wild *D. melanogaster* eyes, we concluded that xanthommatin also occurs in both black and red eyes of *T. infestans*. The chromatographic data supported this hypothesis (Brown & Nickla, 1977).

The inability of acidified ethanol to extract eye pigments excluded pteridines as a component of the eye pigments in *T. infestans*, unlike what happened in the case of *D. melanogaster* (Cordeiro, 1959; Rasmuson *et al.*, 1960; Fuje, 1967; Connolly, 1969; Pfeiderer, 1992).

The lack of an absorption peak at $\lambda = 520$ nm or 547 nm in the spectral absorption profile of the eye pigments of *T. infestans* indicated that ommins may not contribute to the ommochromes involved (Linzen, 1974). This conclusion differs from the paper chromatography results for *Rhodnius prolixus*, which suggested that ommins were the main ommochrome in the eyes of this species (Butenandt *et al.*, 1960; Linzen, 1974).

The presence of xanthommatin as the main or only ommochrome in insect eyes has been reported for other species including *Musca domestica* (Strother, 1966), *Calliphora* sp. (Langer, 1967), and *D. melanogaster* (Linzen, 1974).

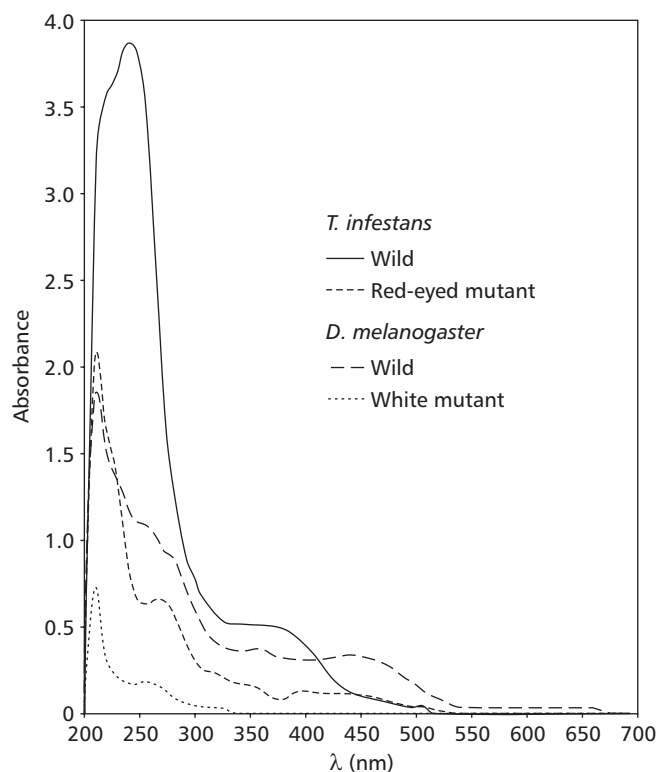


Fig. 1 — Spectral absorption profiles of pigments extracted from *T. infestans* and *D. melanogaster* eyes. White mutant and wild-type *D. melanogaster* were used as negative and positive controls, respectively, for xanthommatin.

Assuming that black and red eye pigments in *T. infestans* are the same, the red color may be caused by the smaller pigment amount. Indeed, the eye pigment of wild *T. infestans* changes from black-brown to red and then to yellow as the pigment extract is diluted. The lower absorption in the $\lambda = 440\text{-}450$ nm region (peak D), concomitant with higher absorption in the $\lambda = 350\text{-}360$ nm region (peak C) only in the spectral profile of pigments from black *T. infestans* eyes, is typical of redox properties of the ommochrome pigment (Schäfer & Geyer, 1972). Assuming that in *T. infestans* the ommochrome concentration in black eyes is higher than in red eyes, the H_2O_2 concentration required to completely stabilize the redox state should not be the same for both eye types.

Ommochromes are formed by the oxidative coupling of 3-hydroxykynurenine during tryptophan metabolism (Colombo *et al.*, 1973; Linzen, 1974).

The reaction generates molecules with peculiar solubilities, redox properties, and spectral characteristics (Linzen, 1974). Ommochrome pigments, including xanthommatin, arise from kynurenine precursors synthesized in organs such as Malpighian tubules and fat bodies during insect development. Kynurenine has been detected microspectrofluorimetrically in the Malpighian tubules of *T. infestans* (Mello & Vidal, 1985). With the development of the insect optical system, the precursors of eye pigments discharged from the insect's hemolymph are captured by the developing eyes and transformed and deposited in pigment granules (Shoup, 1966; Colombo *et al.*, 1973; Wigglesworth, 1984). Xanthommatin and other ommochromes are synthesized only in the eyes and in epidermal cells (Colombo *et al.*, 1973).

Mutations leading to less eye pigmentation reflect changes in the metabolism of tryptophan or

in the transit of ommochrome pigment precursors to the eye (Wigglesworth, 1984; <http://flybase.bio.indiana.edu>). However, in the case of red-eyed *T. infestans*, this may be not disadvantageous since ommochromes are still present although at a much lower level. The visual acuity of the compound eye has been reported to be reduced when ommochromes are lacking (Linzen, 1974).

In summary, these findings further support the similarities between black- and red-eyed specimens of *T. infestans* already demonstrated for other characteristics (Gonçalves *et al.*, 1997; Pires *et al.*, 2002).

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