Extracellular electrical activity from the photoreceptors of midge

A A Babrekar, G R Kulkarni*, B B Nath[†] and P B Vidyasagar*

*Department of Zoology, *Department of Physics, University of Pune, Pune 411 007, India*

[†]*Corresponding author (Fax, 91-20-25690087; Email, bbnath@unipune.ernet.in)*

The ontogeny of photosensitivity has been studied in a holometabolous insect, the midge *Chironomus ramosus*. The life cycle of midges shifts from an aquatic environment to a non-aquatic environment. Extracellular electrical activity of photoreceptor organs was recorded at larval and adult stages. We found an increase in photosensitivity as the larva metamorphosed to the adult stage. This is the first report of changes in photosensitivity during the development of any insect described in an ecological context.

1. Introduction

Light is detected by insects through different types of photoreceptors. Some insects exhibit a combination of different photoreceptors at different stages of development. In hemimetabolous insects, larval forms have single lens eyes known as the ocelli, in addition to compound eyes. On the other hand, in the larval form of holometabolous insects, morphologically different type of single lens eyes are found known as the stemmata (Horridge 1975; Land 1989; Gilbert 1994). Light is a major environmental cue influencing the spatial distribution, dispersion, taxes and kineses in insects (Frankel and Gunn 1960). Photobehavioural pattern of dipteran insects – flies, midges and mosquitoes – have been studied due to their relevance either as agricultural pests or as vectors of diseases. Attempts have also been made to develop 'light-traps' to aid in developing strategies for pest and vector control (Hutchins 1940; Belton and Pucat 1967; Husbands 1976; Ali *et al* 1986; Service 1993). In the last two decades, advancement in the genetics and molecular biology research in *Drosophila* led to a better understanding of the photoreceptor organs in dipteran insects (Godoy-Herrera *et al* 1994; Chou *et al* 1999).

Among the dipteran insects, aquatic larval forms of mosquitoes and midges (Suborder: Nematocera) soon after hatching exhibit well-developed photoreceptor organs called the stemmata or lateral ocelli visible on both sides of the head (Gilbert 1994). In general, photoreceptor organs of holometabolous insects are excellent model systems to study (i) ontogenesis of photoreceptor organs and (ii) developmental transitions of photosensitivity. Among nematoceran insects, only larval ocelli of mosquito (Family: Culicidae) have been investigated for spectral sensitivity (Seldin *et al* 1972; Gilbert 1994), whereas similar studies on midges (Family: Chironomidae) have never been attempted.

Chironomus, commonly called midges are primitive insects (order: Diptera). Adult midges have been known for their attraction to light. Miall and Hammond (1892) for the first time, studied the head structures of adult and larval Chironomids and described the compound and lateral eyes as organs of photoreception. There had been no published work till Oehring studied photobehaviour in *Chironomus* larvae in 1934. He reported the responsiveness of larval eyes to light, based on blinding experiments in which larval swim path had altered when eyes were blinded (Oehring 1934). From an ecological point of view, Baker and Ball (1995) studied the influence of light in *Chironomus tentans* larvae on microhabitat selection along with many other variable biotic factors and abiotic factors. They found out the positive influences of light in the selection of habitat by 3rd and 4th instar larvae. All these studies

Keywords. *Chironomus*; electroretinogram; insect development; midge; photoreceptor

———————————

have indirectly indicated larval response to light. However, no electrophysiological studies on larval stemmata have so far been carried out in *Chironomus* to establish the photosensitivity of larval photoreceptor organ.

Larval stages of *Chironomus* are aquatic and consist of four larval instars. Adult midges emerge out from pupae and swarm near aquatic vegetation (Oliver 1971; Pinder 1986). Unlike larval and pupal stages, adult photobehavioural responses in *Chironomus* have been a subject of investigation for a long time (Ali 1996). However, there have been no electrophysiological accounts of the compound eye of any adult *Chironomus* sp.

In the present study, response of the photoreceptor organs of larval and adult stages of *Chironomus* has been studied using electrophysiological approach.

2. Methods

Fourth instar larvae and adults of a tropical midge, *Chironomus ramosus*, were used for this study. The eggs laid by *C. ramosus* females were collected from a laboratory-reared inbred population. The larvae and adult midges were maintained in the laboratory using a mass rearing technique (Nath and Godbole 1998). Histological studies were carried out for morphometric analysis of photoreceptor organs of fourth instar larval stage and one-day-old adult stage. Fourth instar larvae were studied because the stemmatal unit of earlier instars are not morphologically distinct. Moreover, in *C. ramosus*, fourth instar constitutes the longest larval stage in the life cycle (Nath and Godbole 1998). For histological studies, a modified method of Yajima (1970) was followed.

To record the extracellular electrical activity from the photoreceptors of aquatic larvae, moist tissue paper was put on a wax tray. Fourth instar larvae were immobilized using an adhesive tape without causing any damage to the organism. On the other hand, adults were immobilized using the same procedure but without any moist tissue paper support.

The experimental set-up comprised of microelectrodes, signal amplifier, cathode ray oscilloscope (CRO) and light emitting diode (LED) along with a stimulator (Deshpande 1985). The microelectrodes were prepared from tungsten wire with tip diameter 8–9 μm and inserted in the stemmata of *C. ramosus* larvae with the aid of micromanipulators (Leitz, LABORLUX2, Germany). The electrophysiological signals were picked up by the microelectrodes and amplified with high-input impedence, low-noise amplifier with a gain factor of 10 and frequency response from 10 Hz to 2 kHz. Signals were observed on storage CRO on a 50 ms/cm time scale. The stemmata were stimulated with a LED of 3 mm diameter, kept at a distance of 3 cm. The light stimulus is of ON-OFF type with ON time of 1 s duration. The responses were recorded during ON period. Refer fig-

ure 2 for the response pattern from the photoreceptor organs for 0·4 s duration. Intensity of 4 lux was given correspondingly in all the preparations, after the insect was dark-adapted for 5 min. The peak amplitude and duration of the transient responses were measured according to the scale of CRO for calibration. A total of 50 larval and 50 adult samples were analysed. Paired comparison '*t*' tests were carried out to determine the difference between the means of paired values as described by Clarke (1980).

3. Results and discussion

Histological studies were carried out for morphometric analysis of larval stemmata and adult compound eyes. Figure 1a shows a pair of stemmata (lateral ocelli) on the lateral side of the larval head. All stemmata were nearly of equal size, 32 μ (± 3 μ) length and 15 μ (± 2 μ) diameter. Figure 1b shows each of the stemmata comprised of 9 photoreceptor cells, 2·5 μ diameter. From the longitudinal sections, average depth of each photoreceptor was 5 μ (data not shown). In adults, total number of facets (ommatidia) in the compound eye were 350 (± 15). All om-
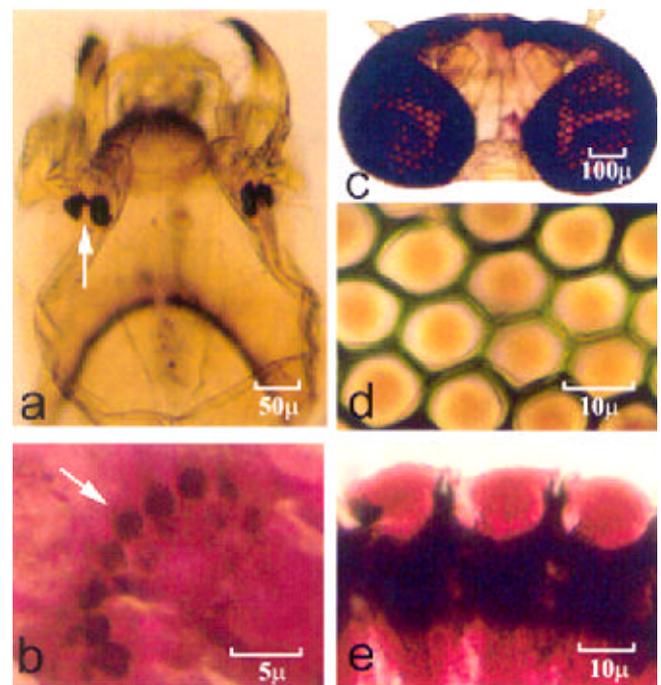
Figure 1. Photoreceptor organs of larval and adult stages of *C. ramosus*. (**a**) Whole mount preparations (arrow indicating stemmata). (**b**) Transverse sections of larval stemmata (arrow showing phtoreceptor cells). (**c**) Whole mount preparations of adult compound eyes showing ommatidial organizations; while the hexagonal arrangements of ommatidial units are evident at higher magnifications in (**d**). (**e**) Longitudinal section through the adult compound eye.

matidia were arranged hexagonally (figure 1c, d). The length of compound eye was 450 μ (± 8 μ) with a width of 300 μ (± 5 μ). The diameter of single ommatidium was 12 μ (± 2 μ). The longitudinal section through the compound eye showed 45 μ depth of the optic nerve (data not shown). The lens diameter was 15 μ (figure 1e).

Electrophysiological experiments have been carried out to understand the developmental change of photosensitivity of photoreceptor organs in *C. ramosus*. Herein the aquatic larvae metamorphose to non-aquatic adult forms. The
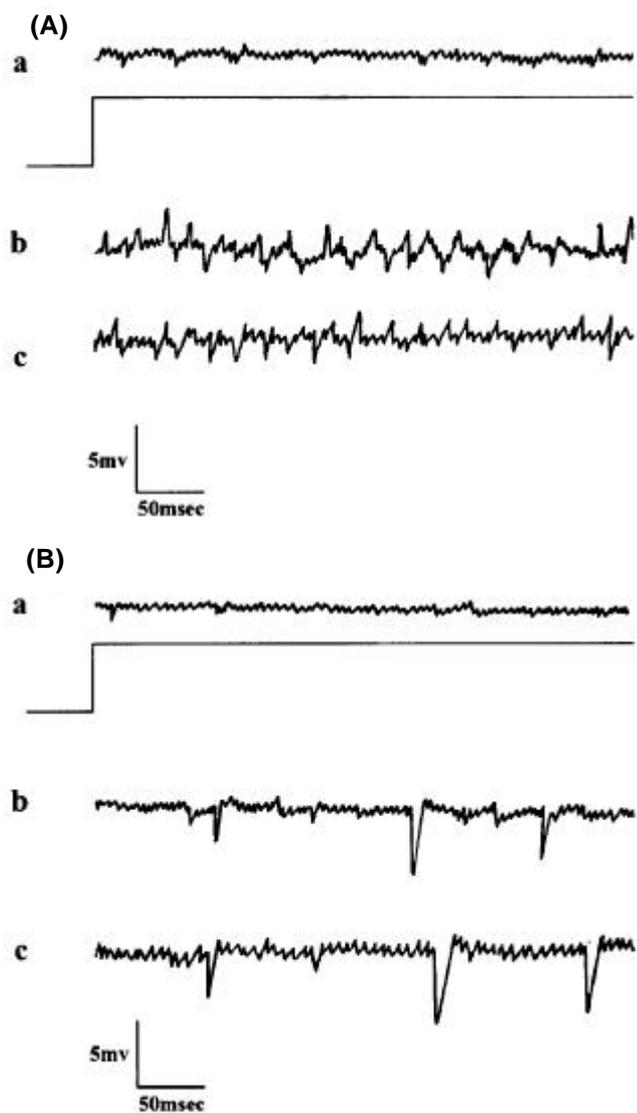
Figure 2. Extracellular electrical activities from the photoreceptor organs of larval (**A**) and adult stages (**B**) of *C. ramosus*. (a) Responses recorded under dark adapted condition. (b, c) Responses obtained with light-ON stimulus after dark-adapted conditions in both the developmental stages (the sustained light flash was 'ON' for 1 s and the waveforms shown in (b) and (c) are representative records of 40 ms duration).

response of photoreceptor to light-ON condition was investigated in *C. ramosus* larvae and adults. Extracellular electrophysiological responses were obtained from both larval and adult stages under dark-adapted conditions. Figure 2 shows only a representative sample response of typical waveform pattern (for 400 ms light-ON condition) from both larval and adult eye. The average amplitudes of electrical transients generated in the photoreceptor organs of larvae and adults showed considerable quantitative differences. The results of these analyses are shown in table 1. The magnitude of electrical activity of adult eye in response to light-ON flash stimuli was found to be higher than corresponding larval photoreceptor organ. The differences between larval and adult photosensitivities were found to be significant (paired comparison 't' test; $P < 0.05$).

Since the first description of electroretinogram (ERG) of insects by Crescitelli and Jahn (1939), ERG has provided an important physiological index to detect photosensitivity of insect eye. A comparative ERG study in diverse groups of insects revealed similar waveforms of electrophysiological responses (Yinon 1970). Studies in butterflies and caddies flies have indicated developmental fate of larval photoreceptor organs and have demonstrated its functionality (Pappas and Eatan 1977; Hagberg 1986; Ichikawa 1991). Among the dipteran insects, ERG studies were carried out only to analyse the visual sensitivity of adult eyes (Yinon 1970; Seldin *et al* 1972; Agee and Park 1975; Markow and Merriam 1977). However, only in *Aedes* mosquitoes, two independent ERG studies revealed photosensitivity of adult (Brammer and White 1961) and larval eyes (Seldin *et al* 1972) without any comparison between the two developmental stages. In this paper, we have presented the developmental transitions in photosensitivity using an insect model system.

Vision and photoreception are important from a developmental standpoint, and developmental changes of photobehaviour of lower organisms require investigations. Visual abilities at different developmental stages of an insect depend on how the species are adapted. Holome-

Table 1. Amplitude measurements of transients obtained before and after sustained light stimulus under dark-adapted conditions in two major developmental stages of *C. ramosus*.

Developmental stages	Mean (± SD) amplitude (in mv)	
	Before stimulus	After stimulus
Larva (*n* = 50)	0·83 ± 0·13	1·92 ± 0·11
Adult (*n* = 50)	0·87 ± 0·12	3·16 ± 0·38

Sample size (*n*) for each stage = 50. *P*-values were < 0·05, suggesting that observed difference between the mean amplitude of transients obtained from larval and adult samples after light stimulus are significant.

tabolous insects offer an opportunity to study photoreception capability in distinct developmental stages. Chironomid midges have been used for this study since developmental stages show ecological transitions. Aquatic bottom-dwelling tubicolous larvae metamorphose to a transient pupal stage and, subsequently, eclose to terrestrial low-flying adult midges (Oliver 1971). We found the photosensitivity of larvae was lower than the adults. This could be due to the tube-dwelling photophobic life style of larvae. Adults are known for their positive phototactic behaviour (Ali *et al* 1984, 1986). However, our study, for the first time, demonstrated adult eye's photosensitivity in electrophysiological terms. Therefore, our electrophysiological data link sensitivity of developmental stage-specific photoreceptor organs (of both larvae and adults) to its ecological adaptations.

Variability of extracellular electrical activities can probably be explained by changes in the anatomical structure of the photoreceptor organs during metamorphosis. Perhaps future electron microscopic studies are required for clarifying whether electrophysiological differences in larvae and adults are indeed related to ultrastructural differences in photoreceptor organs. In general, animal vision depends on light absorbing rhodopsins – the protein portions of which are called opsins. Opsin genes have been reported to influence photosensitivity in *Drosophila* (Carulli *et al* 1994; Chou *et al* 1999). It would be interesting to find out whether differential expression of opsin gene/s play a role in controlling variable photosensitivity in the developmental stages of *Chironomus*.

Chironomus larvae are known as pests and adults are known for creating a nuisance in human habitats (Ali 1996). Adult midges also cause allergic reactions to human (Libers and Baur 1994). Therefore, we believe that our findings will help in designing 'light-trap' as an effective pest control strategy based on developmental stage-specific photosensitivity.

Acknowledgements

References

Agee H R and Park M L 1975 Use of the electroretinogram to measure the quality of vision of the fruit fly; *Environ. Lett.* **10** 171–176

Ali A, Staford S R, Flower R C and Stanley B H 1984 Attraction of adult Chironomidae (Diptera) to incandescent light under laboratory conditions; *Environ. Entomol.* **113** 1044–1009

Ali A, Stanley B H and Chaudhuri P K 1986 Attraction of some adult midges (Diptera: Chironomidae) of Florida to artificial light in the field; *Florida Entomol.* **69** 644–650

Ali A 1996 A concise review of chironomid midge (Diptra: Chironomidae) as pests and their management; *J. Vector Ecol.* **21** 105–121

Baker R L and Ball S L 1995 Microhabitat selection by larval *Chironomus tentans* (Diptera: Chironomidae); effects of predators, food, cover and light; *Freshwater Biol.* **34** 101–106

Belton P and Pucat A 1967 A comparison of different lights in traps for Culicoides (Diptera: Ceratopogonidae); *Can. Entomol.* **99** 267–272

Brammer J D and White R H 1961 Vitamin A deficiency: effect on mosquito eye ultrastructure; *Science* **163** 821

Carulli J P, Chen D M, Stark W S and Hartl D L 1994 Phylogeny and physiology of *Drosophila* opsins; *J. Mol. Evol.* **38** 250–262

Chou W H, Huber A, Bertrop J, Schulz S, Schwab K, Chadwell L V, Paulsen R and Britt S G 1999 Patterning of the R7 and R8 photoreceptor cells of *Drosophila*: evidence for induced and default cell-fate specification; *Development* **126** 607–616

Clarke G M 1980 *Statistics and experimental design* (London: Edward Arnold)

Crescitelli F and Jahn T L 1939 The electrical response of the dark-adapted grasshopper eye to various intensities of illumination and to different qualities of light; *J. Cell. Comp. Physiol.* **13** 105–112

Deshpande G S 1985 *A study of electrical responses to ON-OFF and sine-wave modulated light stimuli in simple and compound eye system of cockroach Periplaneta americana*, Ph.D.Thesis, University of Pune, Pune

Frankel G S and Gunn D L 1960 *The orientation of animal* (New York: Dover)

Gilbert C 1994 Form and function of stemmata in larvae of holometabolous insects; *Annu. Rev. Entomol.* **39** 323–349

Godoy-Herrera R, Sauntander R and Figuroa J 1994 A developmental and biochemical analysis of larval photoresponse of *Drosophila*; *Anim. Behav.* **48** 251–262

Hagberg M 1986 Ultrastructural and central projections of extraocular photoreceptors in caddisflies (Insecta: Trichaptera); *Cell Tissue Res.* **245** 643–698

Horridge G A 1975 Optical mechanisms of clear zone eyes; in *The compound eye and vision of insects* (ed.) G A Horridge (Oxford: University Press) pp 255–298

Husbands R C 1976 Light traps and the significance of collection data; *Bull. Soc. Vector Ecol.* **3** 17–26

Hutchins R C 1940 Insect activity at a light trap during various periods of the night; *J. Econ. Entomol.* **33** 654–657

Ichikawa T 1991 Brain photoreceptors in the pupal and adult butterfly fat of the larval ocelli; *Zool. Sci.* **8** 471–476

Land M F 1989 Variations in the structure and design of compound eyes; in *Facets of vision* (eds) D G Stavenga and R C Hardie (Berlin: Springer verlag) pp 90–111

Libers V and Baur X 1994 Chironomidae hemoglobin chi-t-I characterization of an important inhalant allergen; *Clin. Exp. Allergy* **24** 100–108

Markow T A and Merriam J 1977 Phototactic and geotactic behaviour of counter current defective mutants of *Drosophila melanogaster*; *Behav. Genet.* **7** 447–455

Miall L C and Hammond A R 1892 The development of the head of the imago of *Chironomus*; *Trans. Linn. Soc. London* **5** 265–279

Nath B B and Godbole N N 1998 Technique for mass rearing of

Indian *Chironomus* species (Diptera, Nematocera, Chironomidae); *Studia Dipterol.* **5** 187–193

Oehring A 1934 Helligkeitsreaktionen der *Chironomus* Larve; *Zool. Jahrb. (Allg. Zool.)* **53** 343–366

Oliver D R 1971 Life history of Chironomidae; *Annu. Rev. Entomol.* **16** 211–230

Pappas L G and Eatan J L 1977 The internal ocellus of *Manduca sexta*: electroretinogram and spectral sensitivity; *J. Insect physiol.* **23** 1355–1358

Pinder L C V 1986 Biology of freshwater Chironomidae; *Annu. Rev. Entomol.* 1–23

Seldin E B, White R H and Brown P K 1972 Spectral sensitivity of larval mosquito ocelli; *J. Gen. Physiol.* **59** 415–420

Service M W 1993 *Mosquito ecology: Field sampling methods* (London: Elsevier)

Yajima H 1970 Study of the development of the internal organs of the double malformations of *Chironomus dorsalis* by fixed and sectioned materials; *J. Embryol. Exp. Morphol.* **2** 287–303

Yinon U 1970 Similarity of the electroretinogram in insects; *J. Insect Physiol.* **16** 221–225