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SHORT COMMUNICATIONS

FINE-STRUCTURAL CHANGES IN THE EGG CHORION OF *BRADINOPYGA GEMINATA* (RAMBUR) INDUCED BY PAPER MILL EFFLUENT (ANISOPTERA: LIBELLULIDAE)

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The egg chorion of the dragonfly *B. geminata* undergoes major structural changes when incubated in paper mill effluent. The exochorion becomes blemished, marred and perforated. It bunches into a granular condition and looses its jelly-like original identity. The endochorion develops cracks and is pitted with holes. The hexagonal demarcations of the endochorion plates are obliterated and replaced by a network of angular surface reticulations. The micropylar stalk and the circular basal ridge dissolve and distort the micropylar apparatus.

INTRODUCTION

The insect egg chorion serves various functions such as protection from pathogens, predators and mechanical stress and from environmental hazards such as desiccation and hydration. It allows sperm entry and provides elasticity for easy oviposition. Later, it protects the growing embryo, ensures sufficient oxygen and carbon dioxide exchange and helps in liberation of the larva. The egg chorion, in insects, is therefore an extensively modified dynamic structure with major functional significance (HINTON, 1981; MARGARITIS, 1985).

The environmental pollution due to small and medium scale pulp and paper industries is multidimensional causing serious problems, not only to the landmass fertility but also to the natural flora, fauna as well as aquatic bodies (KSIBI et al., 2003). This results in serious threats to aquatic organisms (GHOSE & KONAR, 1980; RAO & NAADHAKUMAR, 1982; CHANDRASEKARAN et al., 1989)

including dragonflies (SUBRAMANIAN & VARADARAJ, 1993; VARADARAJ et al., 1993; SUBRAMANIAN & MURALIDHARAN, 1999).

A review of literature reveals that very few attempts have been made to study the effect of paper mill effluents (PME) on dragonflies and these reports are mostly related to histopathological, histochemical and biochemical parameters (SUBRAMANIAN & VARADARAJ, 1993; VARADARAJ et al., 1993). Furthermore, information on the eggs of dragonflies has mostly been undertaken with respect to the effect of different pH, temperature and oxygen on hatching. (PUNZO, 1988; SOEFFING, 1990; MILLER, 1992). The fine structure of the odonate chorion has been investigated mostly to understand the "structure-function" relationship with respect to the process of fertilization and oviposition [see ANDREW (2002) for detail references]. No major attempts have yet been made to study the effect of PME on the egg chorion of aquatic fauna. The present investigation has therefore been undertaken to study the effect of PME on the fine structure of the egg chorion of the dragonfly, *Bradinopyga geminata*.

MATERIAL AND METHODS

The egg-laying females were collected near small local ponds at Nagpur, during the post-monsoon periods (September-November) of 1999-2000. Egg dumping was initiated by passing a thin needle through the thorax or by holding the wings flat and dipping the abdominal tip in water tubes and petri-dishes (ANDREW & TEMBHARE, 1996; ANDREW, 2002).

The eggs passed out of the genital opening and accumulated on the sub-genital plate. The eggs were divided into two groups. The first group of eggs was placed in petri-dishes containing pond water while the second group was placed in paper mill effluent (PME) procured form the local industrial area (MIDC, Hingna, Nagpur District). After five days, the eggs of both the groups were prepared for scanning electron microscopy (SEM). Eggs were dehydrated in ethanol, transferred to acetone, air-dried, mounted on stubs, coated in a gold-coating unit (E-5200) and examined under a stereoscan 250MK II Cambridge Scanning Electron Microscope.

OBSERVATIONS

The eggs incubated in pond water hatched in 8-12 days and had only 8% mortality whereas 100% percent mortality was found in the eggs incubated in PME.

Various structural disintegration of the egg chorion with respect to the exochorion, endochorion and micropylar apparatus was observed in the paper mill effluent treated eggs (Figs 1-4). The exochorion became blemished, marred and perforated. It bunched into granular condition and lost its jelly-like original identity. The endochorion developed cracks and it was pitted with holes. The hexagonal demarcations of the endochorion plates were obliterated and replaced by a network of angular surface reticulations. The micropylar stalk and the circular basal ridge dissolved and distorted the micropylar apparatus.

A comparative account of this degeneration is given in Table I.

Table I
Fine-structural changes in the chorion components of the eggs of *Bradinopyga geminata* incubated in paper mill effluent (PME)

Egg chorion Components	Dry-egg*	Wet-egg*	PME-incubated egg
Exochorion (EX)	Thin, unsculptured, uniform layer	Thick unsculptured, jelly-like, spongy layer (Fig. 1)	Jelly-like, spongy nature lost. It bunches into small lobules or granules, becomes dissolved in places, detaches from the EN, and exhibits a grainy or sandy texture (Fig. 2)
Endochorion (EN)	White to pale cream; composed of hexagonal plates	Dark brown, sculptured with regular hexagonal plates (Fig. 1)	The hexagonal demarcation is lost; the surface develops cracks and is pitted with tiny circular holes (Fig. 2); some cracked pieces break off from the surface
Micropylar Apparatus (MA)	Triangular with a circular base; stalk possesses a pair of sub apical circular micropylar orifices. Differentiated from the EX by a thin circular ridge (Fig. 3)	As in dry condition	The MA sinks, and acquires a granular texture; the stalk disintegrates, while the circular ridge is obliterated (Fig. 4)

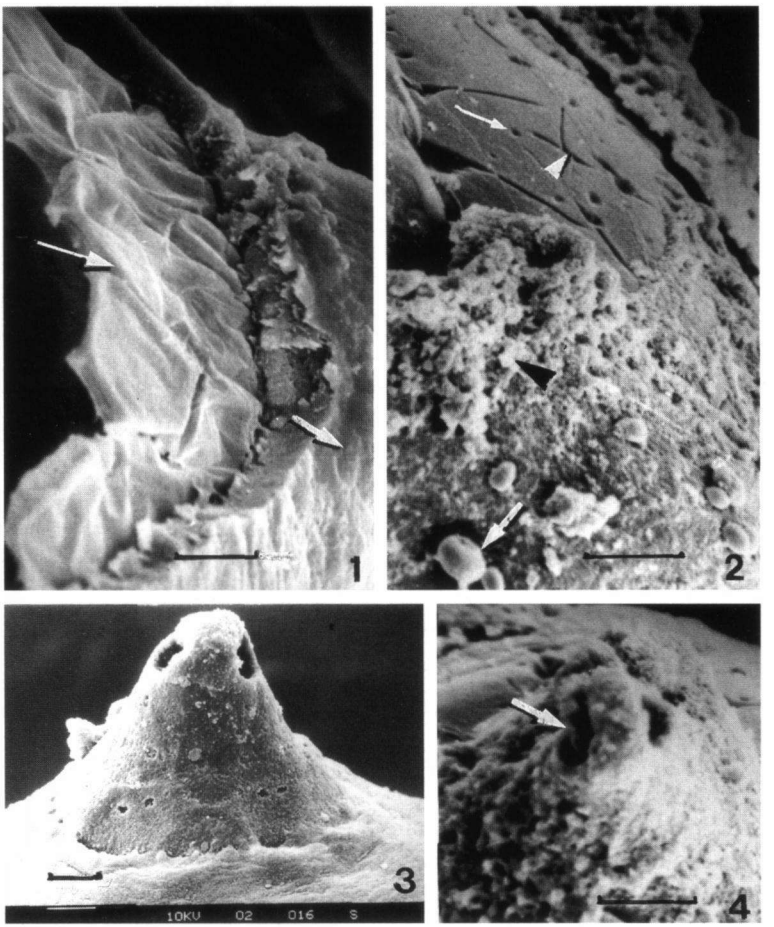
* ANDREW & TEMBHARE (1996)

DISCUSSION

Protection against environmental hazards during embryogenesis is the main function of the egg chorion after oviposition. This is fulfilled by the physical-mechanical properties of the egg. This property is the consequence of the chemical stability of the chorion, which depends upon the cross-links existing between the component molecules (MARGARITIS, 1985).

The insect egg chorion is predominantly composed of protein (> 95%) (PETRI et al., 1976) along with traces of organic (carbohydrates, lipids) and inorganic (Calcium, Iron, Ca^{+2}, Na^{+}, K^{+}) ions/atoms/molecules (REIGER et al., 1980, 1982). The cross-links between the chorion proteins are mainly disulphide bridges, dityrosine-trityrosine bonds and sclerotization through quinine tanning, which may occur separately or in combination (KAWASAKI et al., 1972; PETRI et al., 1976).

In the dragonfly chorion, disulphide bridges are the major cross-link between the protein molecules (KAWASAKI et al., 1974). The present investigation indicates that the paper mill effluent contains compounds that denature the chorion protein, probably by breaking these di-sulphide bridges. The variation in the intensity of denaturation between the exochorion and endochorion indicates that the exochorion proteins are mostly linked with di-sulphide bridges, since the intensity of protein denaturation by the PME is more severe in the outer exochorion



Figs 1-4. Normal and paper mill effluent (PME) treated eggs of *Bradinopyga geminata* (Scale bar = 5 μm): (1) SEM of fractured chorion to show thick exochorion (thick arrow) and endochorion composed of hexagonal plates (thin arrow); – (2) PME treated chorion shows granular (black arrow head) lobulated (white thick arrow) exochorion, while the endochorion is cracked (white arrow head) and pitted with circular holes (thin arrow); – (3) Normal micropylar apparatus; – (4) PME treated micropylar apparatus with disintegrating stalk and orifice (arrow).

then the inner endochorion. Variation in the action of denaturation between these two layers may also be because of the difference in the physical nature of the two chorions, the outer exochorion being spongy and the inner endochorion being composed of compact hexagonal plates (ANDREW & TEMBHARE, 1996).

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