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Cover Photo by Parixit Kafley of *Balinta octonotata*

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Abstract

Respiratory horns differ between fly species, implying that the trait is imperative for effective respiration. It establishes communication between atmospheric air and the pupal tracheal system. Also, it provides a potential function for the development of eye colour at the time of pupation. We observed the formation of respiratory horns in the intra-puparial development of the Latrine fly *Chrysomya megacephala* (Fabricius), which can lead to pigment screening in the eyes of the pupal stage.

Keywords: Colour development, respiratory horns, Diptera.

Introduction

Respiratory horns (RH) are a pair of prong-like structures found on the dorsal side of the cephalic half in various Diptera pupae. De Meijere (1902) was the first to attempt a systematic explanation of the structure of the RH in different Diptera families. The length of the RH varies between the flies: short ones are found in the large flies like blowflies, *Hypopygiopsis tumrasvini*, *Chrysomya megacephala*, *C. putoria* and *Lucilia cuprina* (Siriwattananurongsee *et al.*, 2005; Sukontason *et al.*, 2006b; Mendonca *et al.*, 2014); long ones in small dipterans like scuttle flies, *Megaselia scalaris* (Loew), *M. spiracularis* Schmitz, *M. rufipes* (Meigen) and *Diplonevra peregrina* (Wiedemann) (Sukontason *et al.*, 2006a; Disney & Aguiar, 2008; Feng & Liu, 2012a, 2012b). Phorid flies (*Pseudacteon* spp.) parasitize and pupates inside the empty head capsule of fire ants (*Solenopsis* sp.), from which two respiratory horns extend diagonally

(Porter 1998)—implying that the longhorn trait is essential for effective respiration and that a larger number of thoracic horns and tracheal tubes allow for better oxygen supply (Rossaro *et al.*, 2007). In pupa of *Chrysomya megacephala* (Fabricius), Siriwattananurongsee *et al.* (2005) discovered a group of 38 globules at the end of the first abdominal segment. It was described using SEM photographs that pupal RH had slightly protruded from the centre of those globules in some older pupae. Several papillae were found on the RH, each with a longitudinal opening and a slightly convex base. The fifth segment of the puparium is pierced by paired pupal RH, which establishes communication between atmospheric air and the pupal tracheal system (Karandikar & Ranade, 1965). The function of RH in the puparial development of Latrine Fly, *Chrysomya megacephala* (Fabricius) was demonstrated in this study.

Material and Methods

The results obtained from this study were derived primarily from our previous study on intra-puparial development of Latrine fly *Chrysomya megacephala* (Sinha & Mahato, 2018), where the terminology and concepts to describe the processes of pupariation and pupation, as well as the puparium morphology, were adapted from Sinha & Mahato (2016). The larvae of *C. megacephala* were reared and observed in the captive chamber (temperature $22 \pm 2^\circ\text{C}$, relative humidity $58 \pm 2\%$). The pupae were separated into developmental stages and placed in Carnoy's solution for 48 hours before being preserved in 70% alcohol for 6 hours. They were then treated with 5% formic acid for 48 hours before being preserved in 70% alcohol. The experiment involved dissecting 102 preserved pupae using dissecting instruments under a stereoscopic binocular microscope. The sequence of intra-puparial development of *C. megacephala* was observed, which includes cryptocephalic pupa, phenerocephalic pupa, pharate adult, imago, and head detail, was photographed with a camera (Nikon SLR Coolpix L820).

Observations

The paired respiratory horn (RH) was located at the end of the first abdominal segment of the Pharate adult stage (Fig. 1A). The cephalic capsule was relatively prominent in the Phenerocephalic pupal stage. Then there was the Pharate adult, which was the longest of the three stages that remained 120-122 hours when the successive development of the pupal eye colouration happened. RH was visible near the base of the cephalic structure on the dorso-lateral side (Fig. 1A). Yellow eyes began to turn red after 60 hours of pupal development. From the base of the respiratory horn, red pigmentation on both lateral parts of the cephalic region started to spread (Fig. 1B). Around 68 hours, the pigmentation had covered nearly half of the eye area, and the red-eye had become more prominent, turning carmine-red before finally turning burgundy.

Discussion

During intra-puparial development of *C. megacephala*, RH appeared first at about 56 hours of development and remained visible up to the fully developed Pharate adult stage at about 110 hours (Fig. 1C). With successive development of the cephalic region, the eyes become developed progressively to obtain their normal shape and its colour changes from transparent to yellow, then to carmine-red step by step (Fig. 1B). It was found that, at about 60 hours of pupal development, the red pigmentation on both lateral parts of the cephalic region (i.e. preliminary eye) started to spread from the base of RH. During 68 hours of development, pigmentation covered nearly half of an eye area. At about 74 hours, most of the eye area was pigmented with carmine red colour (Sinha & Mahato, 2018).

It was established that ommochromes are responsible for eye colouration in insects. Some of the ommochromes produced at the time of pupation contribute to the screening pigment in the eyes of adult Diptera. Ommochromes are a group of pigments derived from amino acid tryptophan via Kynurenine and 3-hydroxykynurenine. It was found that 3-hydroxykynurenine is transported to the eyes during metamorphosis in *Drosophila* sp., where the ommochromes are found (Chapman, 2013). Ommochromes may be red dihydroxanthommatin and yellow xanthommatin. Xanthommatin is widely distributed in insects and is a screening pigment in the accessory cells of their eyes, usually in association with pterins which are abundantly found in pigment cells in compound eyes of insects and functions as a co-factor in ommochrome biosynthesis. Pterins are products of purine degradation, and their accumulation in the eyes of higher Diptera reflects the age (Shamim *et al.*, 2014). Ommochromes are produced by oxidative condensation of 3-hydroxykynurenine. It can be presumed that atmospheric oxygen enters

through the pupal RH helps in a condensation reaction to produce ommochromes.

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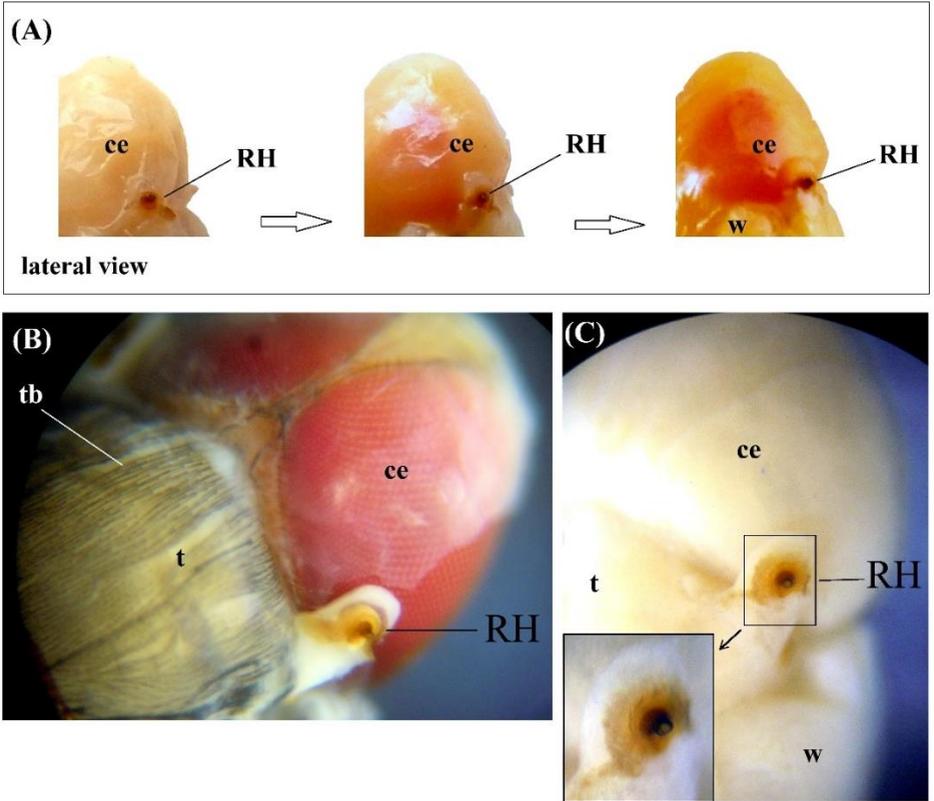


Fig. 1: (A), Spreading of pigmentation with gradual development towards adult; (B), Respiratory horn in fully developed Pharate adult; and (C), Showing knob-shaped respiratory horn (enlarged view inset) in early Pharate adult. Characteristics considered in this figure included the following: RH = respiratory horn, ce = compound eyes, t = thorax, tb = thoracic bristles and w = wing.