

Study on Intra – puparial development of Latrine fly *Chrysomya megacephala* (Fabricius) (Diptera, Calliphoridae)

Shuvra Kanti Sinha^{1*} and Santanu Mahato²

¹Department of Zoology, Sonamukhi College, Sonamukhi, 722 207, West Bengal, India

²Institute of Environment Education and Research, Bharti Vidyapeeth Deemed University, Pune, 411043, Maharashtra, India.

ABSTRACT:

Intra-puparial development of Latrine fly *Chrysomya megacephala* (Fabricius) (Diptera, Calliphoridae) was studied for the first time. Gravid female fly was collected from field and reared in laboratory following suitable method. After pupation of the second generation larvae (n=140), pupae was collected in every six hours. The collected pupae fixed in Carnoy's solution and preserved in 70% alcohol. Preserved larvae were dissected under stereoscopic binocular microscope with help of fine needle and photographed with camera (Nikon SLR Coolpix L820) making proper light adjustment. Intra-puparial development of this species was observed with description of larva-pupa apolysis phase, Cryptocephalic pupa, Phenerocephalic pupa and pharate adult stage including fascinating changes of colouration with maturity towards adult. Total time for pupal development was about 153 hours in laboratory conditions (Temp $22 \pm 2^\circ\text{C}$, RH $58 \pm 2\%$).

Keywords: Intra-puparial development, Cryptocephalic pupa, Phenerocephalic pupa, Pharate adult, *Chrysomya megacephala* (Fabricius).

INTRODUCTION:

Most of the studies on *Chrysomya megacephala* (Fabricius) was confined to its life cycle and description of egg, larval instars and puparium. Ismail et al.² studied development of forensically important *Chrysomya megacephala* in laboratory, concluding that successful development of this fly was closely dependent on temperature and humidity of the surroundings. Singh et al.⁶ focused on a totally different concept of pupal development of *C. megacephala*. They described that specific gravity of a puparium gradually changes with its age. Thus age of pupa can easily be determined by study of floating pattern of a puparium in solution of water and alcohol. Pujol-Luz and Barros-Cordeiro⁴ did experiment on intra-puparial development of *Chrysomya albiceps* and compared its developmental stages as described for *Musca domestica*, *Calliphora erythrocephala*, *Sarcophag abullata*, *Cuterebra tenebrosa*, *Oestrus ovis* and *Dermatobia hominis*. Proenca et al.³ analyzed intra-puparial development of *Chrysomya putoria* (Wiedemann, 1830) in laboratory conditions. Barros-Cordeiro et al.¹ studied intrapuparial development of blowflies *Cochliomyia macellaria* and *Lucilia cuprina* in Laboratory. Development of *Lucilia eximia* inside puparium was also studied by Ramos-Pastrana et al.⁵.

The present work describes gradual morphological changes that took place during development of pupa inside the puparium of *Chrysomya megacephala* (Fabricius) in laboratory conditions. Study of intra-puparial development of this forensically important fly species has tremendous relevance. Terminology and chronology of the events followed and described in this article are according to Pujol-Luz and Barros-Cordeiro⁴, Sinha and Mahato⁷.

MATERIALS AND METHODS:

Gravid female fly collected from field was reared in laboratory using method followed by Sinha & Mahato⁷. After successful mating between first generation mature male and female flies in captivity (temperature $22 \pm 2^\circ\text{C}$, RH $58 \pm 2\%$), second generation flies were produced. Gravid female flies of second generation were transferred to a clean breeding jar containing raw chicken as breeding material. Female flies deposited eggs on that. The larvae grow on breeding material and started pupation therein. A few of fully matured and sluggish larvae which ceased feeding on the fourth day of larval development were collected. Starting from that collection, 5-6 larvae were consecutively collected in every six hours up to the emergence of adult flies. Collected pupae (n=102) were fixed in Carnoy's solution for next 48 hours and preserved in 70% alcohol for next 6 hours. Then the pupae were treated with 5% formic acid for 48 hours and finally kept preserved in 70% alcohol. The preserved pupae were dissected under stereoscopic binocular microscope with help of dissecting instruments and photographed with camera (Nikon SLR Coolpix L820).

OBSERVATION:

In laboratory conditions, egg hatched in to first instar larva after 11 hour of deposition. First instar larva transform in to second instar after 21 hour. Average life span of 2nd instar larva was 58 hours. Then it was transformed in to third instar larva (Fig. 1). Duration of third instar was 32 hours. Throughout the period of second instar and first 20 hours of third instar stage, larva remained very active in feeding but gradually it became immobile. In the late hours of third instar stage, larva was fully matured and very much sluggish. It tried to keep itself hide in underlying substance. So it started to make burrow and move to a deep darker place. Where larva started retraction of body segments. Gradually posterior spiracles collapsed and fused to anal tubercle. Finally it took a shape of barrel-shaped pupa. The larval skin transformed in to cream-white color and progressively to orange-yellow. The entire process of larva-pupa apolysis stage took an average time of 1.5 hours. The outer colouration of puparium transformed to deep brown and finally to blackish-brown that remained same until the emergence of adult (Fig. 2).

After completion of apolysis, pupal morphology was increasingly changed with time. Cephalopharyngeal sclerite disappeared, cephalic region developed very fast. Head, preliminary wing buds and legs appeared. Proboscis developed. Colouration of eyes changed successively. Thoracic and abdominal segmentation appeared. Body coloration gradually changed. Bristles and hairs developed with progression of pupation hour. Total time of development starting from end of apolysis to emergence of adult was an average of 153 hours.

The entire period of pupal maturity inside pupal shell may be divided in to three stages. These are Cryotocephalic Pupa, Phenerocephalic Pupa and Pharate Adult stage.

Cryotocephalic Pupa (Fig. 3)

This stage lasted for only 12 – 14 hours. Head remained hidden in this stage and thoracic appendages not found externally. Moreover the larval body segmentation remained unchanged in this stage. Cephalopharyngeal sclerite was visible in dorsal and ventral view.

Phenerocephalic Pupa (Fig. 4)

Duration of this stage was 18-20 hours. Cephalic capsule was more prominent in this stage. Cephalopharyngeal sclerite was visible up to some late hours. Thoracic appendages were clear only from ventral and lateral view. Wing buds were identified from ventral view. No remarkable change appeared in the posterior portion of body.

Pharate Adult (Fig. 5-8)

This stage was the longest of all three stages and took 120-122 hours to complete. Head, thorax and abdomen were clearly visible in this stage. Successive development of thoracic appendages took place. Bristles and hairs developed all over the body and legs. Depending on the colouration of pupal eye, this stage can be divided in to following phases:

- a) **Yellow-eye phase:** Cephalic portion dome-shaped. Respiratory horns were visible at dorso-lateral position near base of cephalic structure. Proboscis enlarged gradually with advancement of pupal hour. Curvature more prominent in between Cephalic part to thorax and thorax to abdomen. Segmentation on abdomen was lightly visible and pointed growth of terminalia appeared after 32 hours.
- b) **Transitional phase in between yellow to red eye:** About 60 hours of pupal development, the red pigmentation on both lateral parts of cephalic region started to spread from the base of respiratory horn. At about 68 hours, the pigmentation covered nearly half of the eye area and about 74 hours, most of an eye area was pigmented. Wings were more enlarged, squama was prominent. An elongated antero-posterior groove was visible on thorax dorsally. Legs were more developed but length and shape of proboscis remain unchanged.
- c) **Carmine red-eye phase:** Eyes were prominent with carmine-red in colour. Frontal part of head was orange-yellow. Antenna started to develop at about 92 hours. Colouration of thorax and abdomen turned to blackish-blue. Frontal part of prothorax, wings and posterior bands of abdominal segments were deep blue pigmented. Leg segments were more prominent, with basal parts darker in colour. Abdominal segments were dark-blue dorsally but light-yellow ventrally. Bristles on thorax and abdomen were appeared at about 92 hours.
- d) **Burgundy-eye phase:** Eye colour turned to burgundy. Both frons and gena were orange in colour. Vertical and frontal bristles on head more prominent. Proboscis was more developed. Body was more pigmented. Bristles and hairs on thorax and abdomen appeared clearly at about 98 hours. Eye facets were visible at about 104 hours. Legs remained completely black. Ventral side of abdomen was light-yellow up to about 110 hours and wings not completely developed. They reached only up to second abdominal tergite.

Rapid changes occurred in the puparium. Head, thorax, legs and abdomen developed completely. Bristles and hairs appeared all over the body. On the 12th day of egg deposition, new generation adult fly emerged out with inflated ptilineal sac by rupturing puparium. Expansion of wings and bluish –green iridescence on body happened within few seconds after emergence.

DISCUSSION:

The entire events of gradual development of forensically important *Chrysomya megacephala* (Fabricius) inside puparium were very interesting. Cryptocephalic and phenerocephalic stages were shorter in relation to pharate adult stage. The successive change of pigmentation on eyes in pharate adult was very fascinating (Fig. 9-13). Respiratory horn might play a key role for development of pigmentation which has to be investigated in future. With extensive works on life cycle and larval development on this species which have done before, present observation on intra-pupal development gives whole frame knowledge of development of *Chrysomya megacephala* (Fabricius) that must help forensic science.

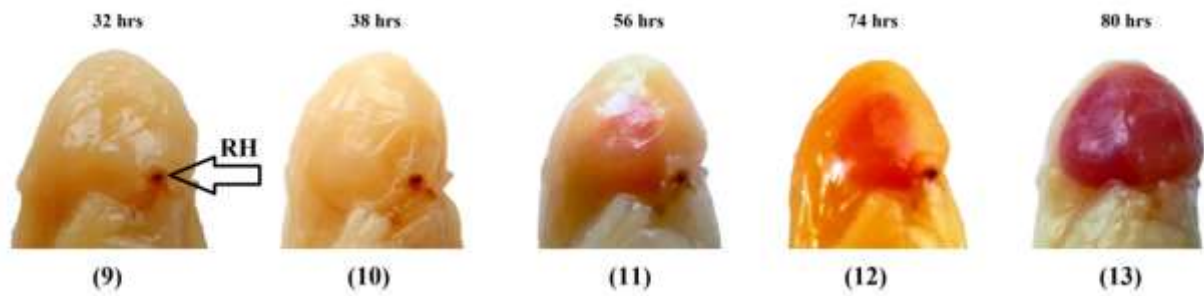
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Explanation of Figures:

Figure 1. First, second and third instar larva of *Chrysomya megacephala* (Fabr.)

Figure 2. Changes of external colour of puparium with advancement of pupal hour.

Figure 3. Cryptocephalic pupa.

Figure 4. Phenerocephalic pupa.

Figure 5-8. Pharate adults.

Figure 9-13. Spreading of pigmentation in eye from base of respiratory horn (RH) with progression of pupal development.

