Intra-puparial development of flesh fly *Sarcophaga dux* (Thomson) (Diptera, Sarcophagidae)

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Intra-puparial development of forensically important and myiasis-producing flesh fly *Sarcophaga dux* (Thomson) (Diptera, Sarcophagidae) was studied. In the laboratory, second-generation pupae ($n = 240$) were dissected and photographed using digital camera and SEM for more elaborative description. Intra-puparial development of this species was studied with the description of larva–pupa apolysis phase, cryptocephalic pupa, phanerocephalic pupa and pharate adult stages. Total time for pupal development was about 252 h under laboratory conditions.

Keywords: Cryptocephalic and phanerocephalic pupa, intra-puparial development, larva-pupa apolysis, *Sarcophaga dux* (Thomson).

FLESH fly *Sarcophaga dux* (Thomson) is abundant in all zoogeographical regions throughout the world. These flies are attracted to human excrement and dead bodies of different animals. Adult flies are generally attracted to offered bait of rotten fish and meat. The larvae develop in carrion and are able to produce myiasis in different animals. The 'flesh fly' larvae are potentially the most useful insects for investigation of suspicious human death[1]. A forensically important fly is reported to complete its entire life cycle on a dead carcass along with the autolysis, putrefaction, fermentation and diagnosis process in the dead[2]. Few human death investigations have recovered *S. dux*[3]. This fly was recognized as forensically important in Switzerland and other parts of the world, where adult *S. dux* was found associated with human corpse[4]. Life history of this fly species and larval stages have been studied by many workers[3,5–9]. Samerjai *et al.*[10] described puparia of five flesh fly species by morphometric analysis on the length and width of puparia. They examined the number and arrangement of papillae in the anterior spiracle, the shape of the intersegmental spines and the pattern of spiracular tufts at the posterior spiracle. Their observations along with SEM photomicrographs were involved only with the external morphology of the puparium. This article describes the gradual morphological changes that take place during intra-puparial development of *S. dux*. Morphology of intra-puparial developmental stages of this forensically important and myiasis-producing species has great relevance. It may be possible to identify a species by studying intra-puparial developmental stages, even if only a few pupae are available. The terminology and chronology of the events described here are after Pujol-Luz and Barros-Cordeiro[11] who studied intra-puparial development of *Chrysomya albiceps* (Wiedemann) (Diptera, Calliphoridae).

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Materials and methods

A mating pair was collected from the Sonamukhi College campus, West Bengal, India and reared in the laboratory. The second-generation gravid female flies from pure culture deposited larvae on the breeding material (raw chicken meat) under laboratory conditions (temperature $22 \pm 2°C$, RH $56\% \pm 2\%$). Some of the fully mature, sluggish, third-instar larvae which ceased feeding were collected. After pupation of the second-generation larvae ($n = 240$), 4–5 pupae were collected and fixed in Carnoy's solution for 48 h and preserved in 70% alcohol for the next 6 h. Then the pupae were immersed in 5% formic acid for 48 h and finally kept preserved in 70% alcohol. Collection was done every 6 h starting from the larva–pupa apolysis period up to the emergence of adult. The preserved pupae were then dissected under stereoscopic binocular microscope with the help of fine needles and photographed with a camera (Nikon SLR Coolpix L820) with proper light adjustment. For each dissected pupa, photographs were taken of the dorsal, ventral as well as lateral view for more appropriate presentation. The pupae were preserved in 70% alcohol. Preparation for SEM involved dehydration through 90% and 99.5% alcohol. Then the pupae were treated with a mixture of absolute alcohol and amyl acetate (3 : 1); then absolute alcohol and amyl acetate (2 : 2) and lastly absolute alcohol and amyl acetate (1 : 3), each for 30 min. Finally the pupae were kept in amyl acetate for another 30 min following critical point drying to complete the dehydration process. The pupae were eventually sputter-coated with gold using IB2ion coaters and SEM photomicrographs

were taken (HITACHI S-530 Scanning Microscope; Tokyo, Japan).

Observation

Under laboratory conditions, first-instar larvae transformed to second-instar stage after an average time-span of 11 h. Second-instar larvae then transformed to third-instar stage after 35 h on an average. Duration of third-instar stage was long – about 114.15 h – which the larvae remained active for 40 h and immobile for 74.15 h. When third-instar larvae were fully mature and sluggish, they stopped feeding and tried to hide themselves in darker places and generally burrowed in the underlying substratum (mixture of sawdust or sand as used in the laboratory). They burrowed the substratum with the help of their mouth hooks of cephalopharyngeal sclerite. During this pupation process, retraction of segments took place and body length also reduced anterio-posteriorly. Posterior spiracles collapsed and became fused to anal tubercle. Cuticular pigmentation did not show any remarkable change. The newly formed barrel-shaped pupa was about 11 ± 0.5 mm in length (Figure 1). At the end of the pupation process, larva–pupa apolysis took place. At this stage, larval skin transformed to pupal sheath (puparium). Pigmentation of puparium changed from creamy white to yellowish–orange. The larva–pupa apolysis stage lasted for about 6 h.

After the apolysis was complete, pupal morphology gradually changed with time. At every hour a pupa transformed to maturity. The cephalic regions developed with gradual disappearance of cephalopharyngeal sclerite. The eye capsules developed with formation of legs and wing buds. Thoracic and abdominal segmentation became more prominent with time. Eye colouration changed from creamy-white to reddish colour; successively bristles and hairs appeared on thorax and abdomen. External colouration of the puparium rapidly turned from yellowish-orange to light brown, then deep brown; after 36 h, the puparium became blackish-brown and remained so until emergence of adult (Figure 2).

The entire period of pupal development inside the puparium may be divided into cryptocephalic, phenerocephalic and pharate adult.

Cryptocephalic pupa (Figure 1 and 2A–C)

Duration of this phase was about 24 h. In this phase the head remained hidden and thoracic appendages were also not found externally. After 12 h of apolysis, pupae showed little demarcation of the cephalic capsule (Figure 2 *A–C*). Mouth hook of cephalopharyngeal sclerite was visible both from dorsal and ventral view (Figure 24).

Phanerocephalic pupa (Figure 3 and 4A–C)

This phase lasted for only 24 h and rapidly changed into the next. Cephalic capsule was clearly visible. Two pairs of thoracic appendages were also seen from ventral view. Cephalopharyngeal sclerite was visible in ventral position after 36 h (Figure 25) of apolysis, and was also visible both from dorsal and ventral positions after 48 h. No change was found in the posterior portion of body up to 36 h. Wing buds and lateral projection of spiracles developed within 42–48 h.

Pharate adult (Figure 5–20 A–C)

This phase was lengthy. Head, thorax and abdomen were clearly distinguishable during this phase. Wing buds and legs became more developed. Abdominal segmentation became clearer. This phase can be sub-divided according to the colouration of pupal eye as follows:

(a) Phase with transparent eyes (Figure 5–12A–C): This phase lasted for four days with very slow morphological changes. Demarcation between two eyes was not prominent. Wings were more enlarged. Leg segments were visible. A small part of mouth hook of cephalopharyngeal sclerite also found up to day 3 (Figure 26). Eye facets were visible.

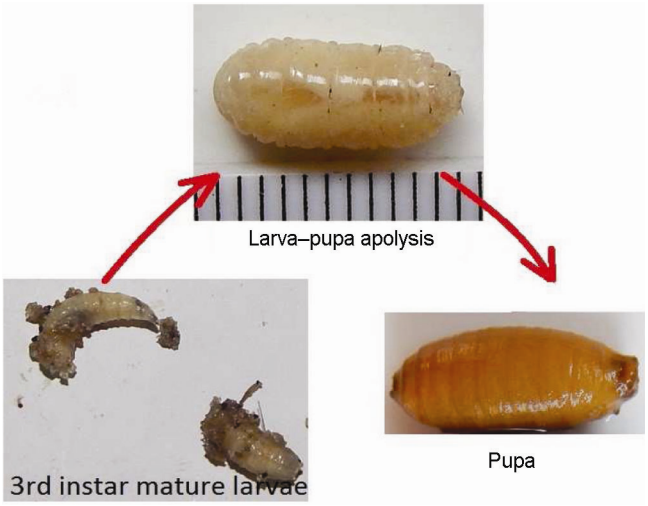


Figure 1. Transformation of matured larva to pupa through larva–pupa apolysis.

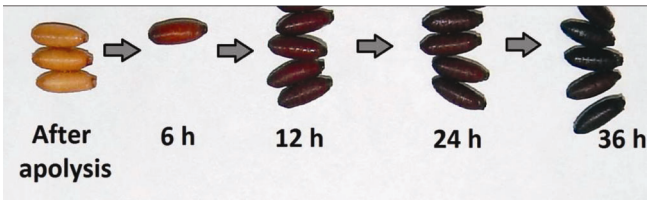
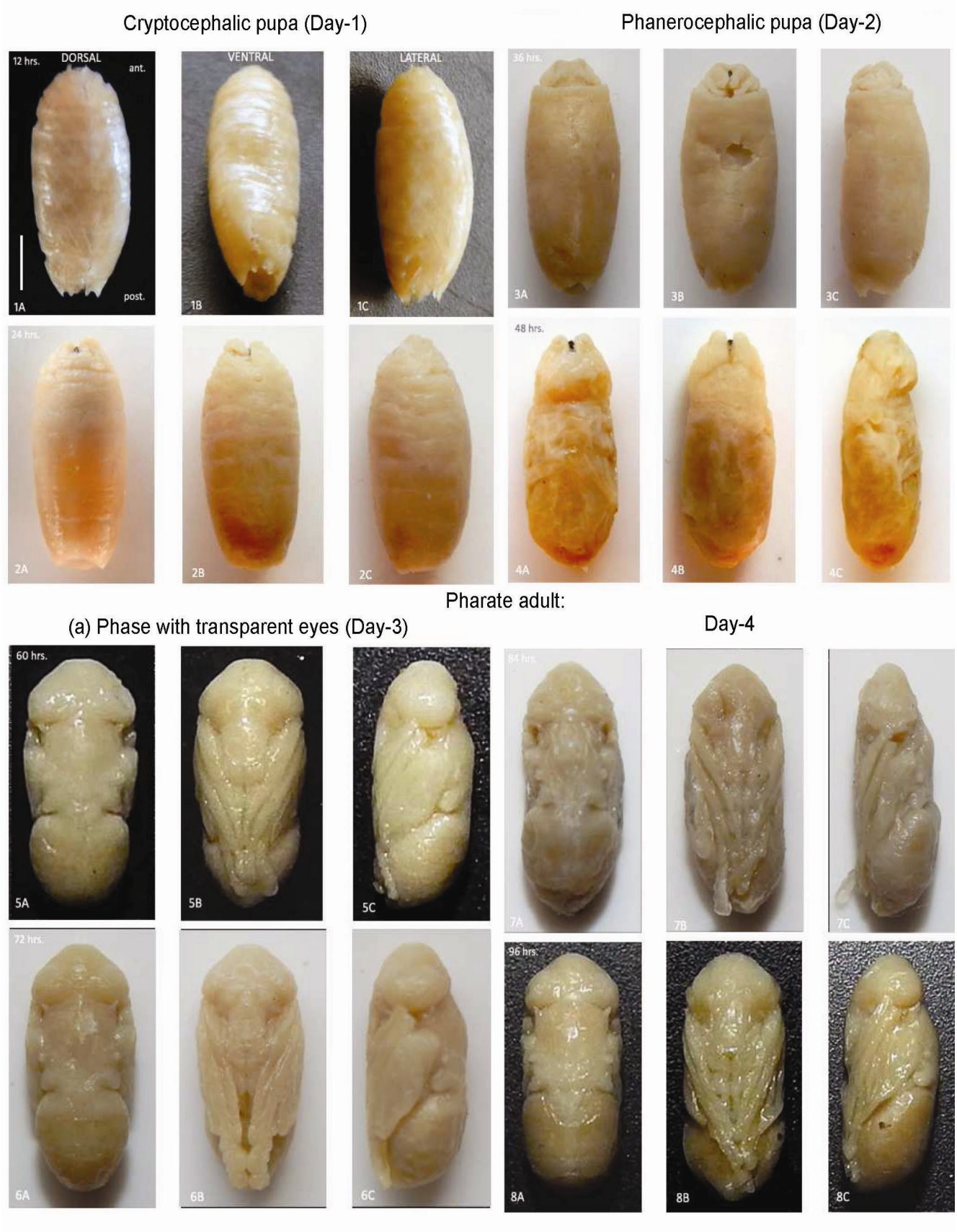


Figure 2. Gradual changes of puparium colour with time.


Cryptocephalic pupa (Day-1)
Phanerocephalic pupa (Day-2)
12 hrs.
DORSAL
ant.
post.
1A
VENTRAL
1B
LATERAL
1C
36 hrs.
3A
3B
3C
24 hrs.
2A
2B
2C
48 hrs.
4A
4B
4C
Pharate adult:
(a) Phase with transparent eyes (Day-3)
Day-4
60 hrs.
5A
5B
5C
84 hrs.
7A
7B
7C
72 hrs.
6A
6B
6C
96 hrs.
8A
8B
8C

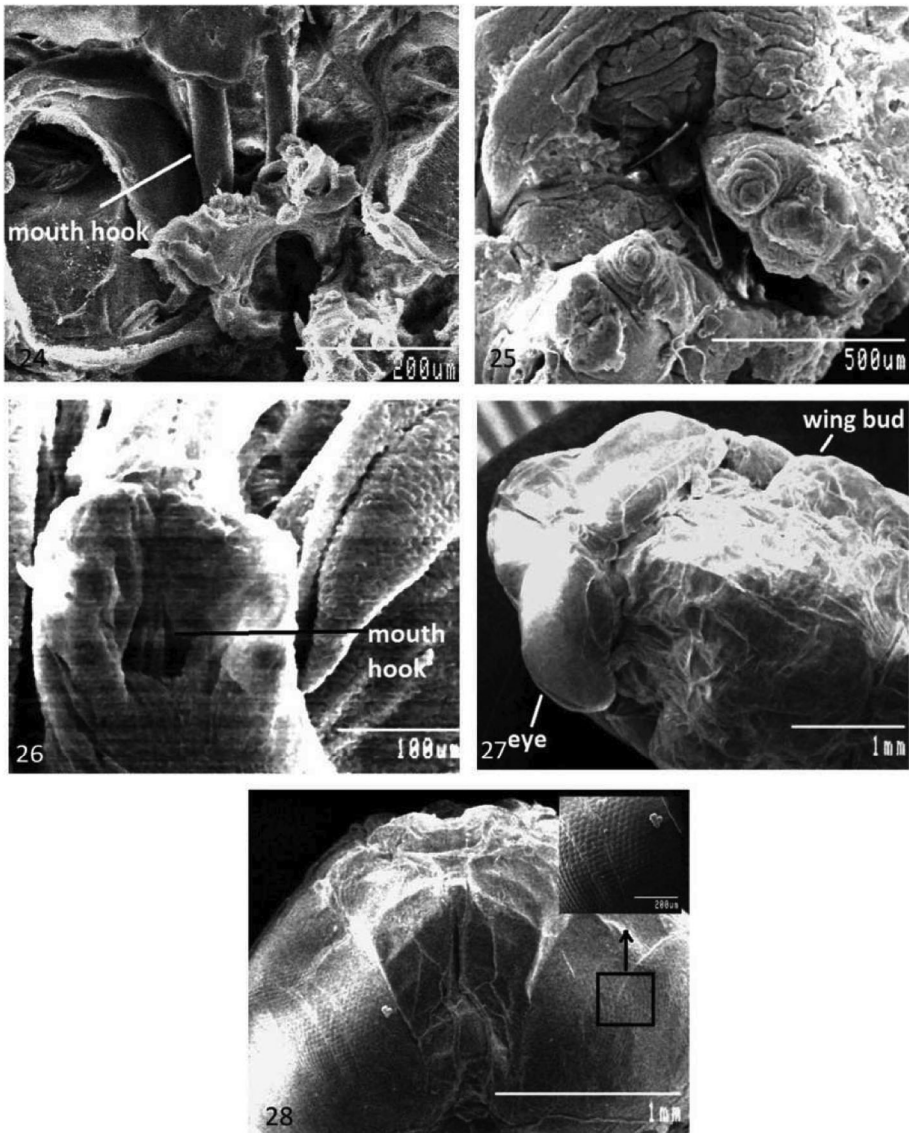
(*Contd*)


Day-5
Day-6
108 hrs.
9A
9B
9C
132 hrs.
11A
11B
11C
120 hrs.
10A
10B
10C
144 hrs.
12A
12B
12C
(b) Phase with yellow eyes (Day-7)
(d) Phase with red eyes (Day-8)
156 hrs.
13A
13B
13C
180 hrs.
15A
15B
15C
(c) Phase with pink eyes (Day-7)
168 hrs.
14A
14B
14C
192 hrs.
16A
16B
16C

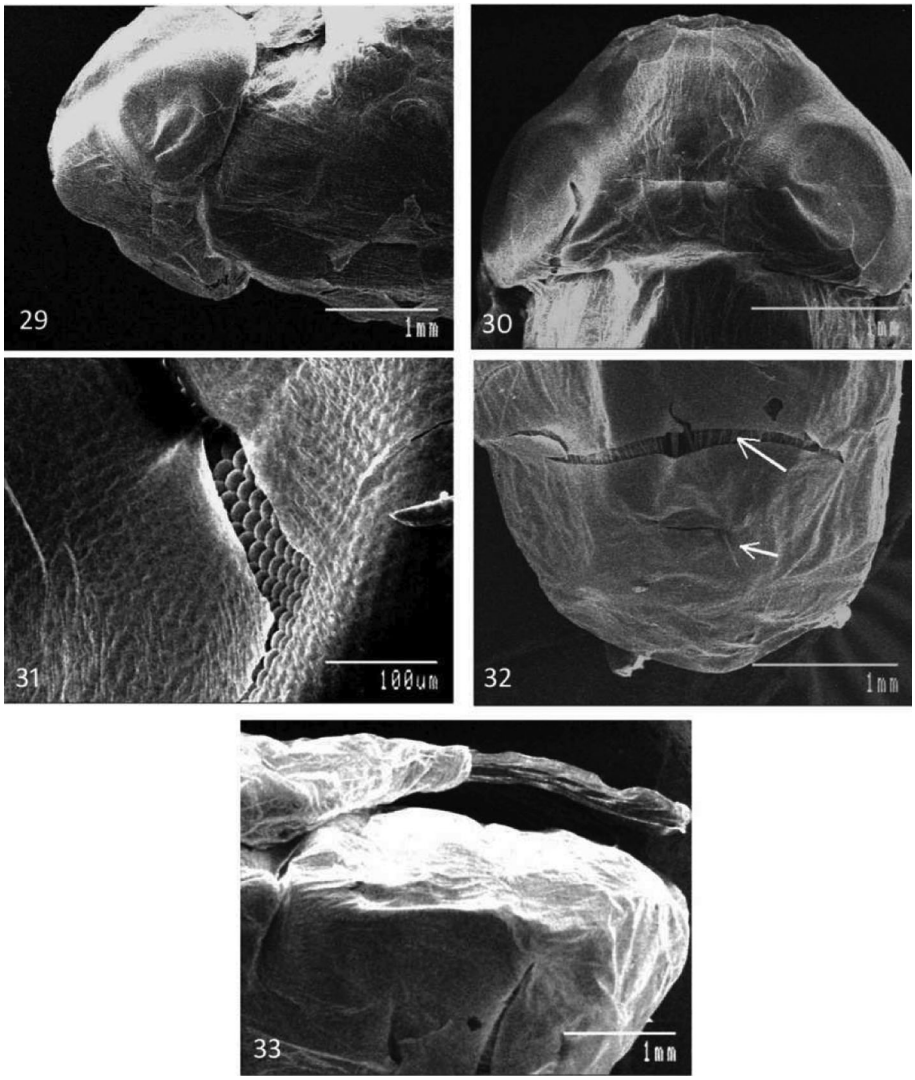
(*Contd*)

Figure 1A–22C. Developmental stages with the puparium.

Figure 23. Emerged adult.



Figures 24–28. Scanning electron photomicrographs of developing pupa of *Sarcophaga dux*. (**24**) Mouth hook of cryptocephalic pupa on day 1(200 ×). (**25**) Frontal view of head of phenerocephalic pupa on day 2(100×). (**26**) Frontal view of head of pharate adult on day 3(30×). (**27**) Dorsal view of head and thorax of pharate adult on day 4 (30×). (**28**) Antero-dorsal view of pharate adult on day 6(50×) (Inset, Eye facets (300×).



Figures 29–33. Scanning electron photomicrographs of developing pupa of *S. dux*. (**29**) Lateral view of head of pharate adult on day 10 (30×). (**30**) Dorsal view of head of pharate adult on day 10 (40×). (**31**) Eye facets in pharate adult in day 10 (300×). (**32**) Dorsal view of posterior portion of abdomen of pharate adult on day 10 (80×). (**33**) Wing of pharate adult on day 6 (50×).

(b) Phase with yellow eyes (Figure 13A–C): This phase lasted for 9–11 h and rapidly changed to pink-coloured phase. Two compound eyes were easily distinguishable. Wings enlarged to the length of half of the abdomen.

(c) Phase with pink eyes (Figure 14A–C): This phase lasted for about 10–12 h. Projected terminalia was also visible. Eyes were more prominent and proboscis not fully developed.

(d) Phase with red eyes (Figure 15–20A–C): Rapid changes were found with time in this phase. Scutellar bristles developed first after 180 h, and thoracic, frontal and abdominal bristles developed successively. Antennae were also visible. Eyes turned to reddish-violet in about 192 h, and gradually became reddish. Eye facets were more developed (Figure 31). Body was not fully pigmented till 240 h. Wings were not fully developed.

(e) Imago and emergence of adult (Figure 21–22–C): After 240 h of pupal development rapid changes in pigmentation of the entire body were seen. Bristles developed all over the body, legs were fully developed, longitudinal stripes on thorax and abdominal markings appeared after 250 h. Imago (Figure 21A–C) was found on the ten and half days and adult fly with inflated ptilineal sac (Figure 22A–C) emerged on the same day.

Discussion

The successive changes during intra-puparial development in *S. dux* clearly show morphological changes from a mature larva to an adult. Total time for pupal development starting from larva–pupa apolysis to emergence of adult was recorded in about 252 h under laboratory conditions. Life cycle of this fly has been studied by many

workers[3,5–9] around the world with description of larval instars. Morphology of the puparium was also described by Samerjai *et al.*[10], but stages of intra-puparial development have not been studied before. The phase of development of *S. dux* within the puparium showed gradual morphological changes towards maturation with time. This knowledge may be helpful to forensic scientists. Detailed documentation of other forensically important species is also necessary.

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