

STUDIES ON THE PUPATION OF *MUSCA
DOMESTICA NEBULO* FABR.
(DIPTERA—CYCLORRHAPHA—MUSCIDAE)

BY K. R. KARANDIKAR, M.Sc., Ph.D. (EDIN.)

(M.A.C.S., Poona-4)

AND

D. R. RANADE, M.Sc., Ph.D., F.E.S.I.

(Department of Zoology, University of Poona, Poona-7)

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INTRODUCTION

THE pupation is one of the most outstanding features of Holometabola. The pupa is defined as the resting or inactive stage of the holometabolous insects. It is the intermediate stage between the larva and the adult. The animal is helpless and delicate at this time, though externally the pupa appears inactive and lethargic, it is the period during which the most conspicuous changes take place, viz., histolysis of the larval organs and histogenesis of the imaginal organs. Actually, this period is that of transformation, the rapidity and nature of which vary considerably in different insects. In Holometabolous insects, the change from the larva to the adult is so great that a distinct resting period or pupa is essential.

The pupal stage is normally preceded by a prepupal stage, which retains the larval form and slight mobility, but ceases to feed. The pupae of insects are normally classified on the basis of the degree of their development, of freedom of appendages and of the nature of their covering.

In the Cyclorrhaphous Dipteran flies, the pupa is typically an adecticous coarctate pupa. The body wall of the third instar larva is retained and transformed into the *Puparium*, which serves as a protective covering to the enclosed pupa. Regarding the process of pupation of these flies, there are few important contributions, such as, by Lowne (1890-95) for *Calliphora erythrocephala* Meig.; Hewitt (1914) for *Musca domestica* L.; Snodgrass (1924) for *Rhagoletis pomonella* Walsh. and for *Drosophila melanogaster* Meig. by Robertson (1936). Hewitt (1914) in his description of the pupation

of *Musca domestica* L. has given a meagre account of the external changes that take place in the body of the third instar larva, when it begins to pupate. His account appears to be rather short and incomplete. It was therefore felt necessary to re-examine this process, and observations were made in an allied species, the common Indian housefly, *Musca domestica nebulo* Fabr. This insect was reared in the laboratory, under controlled conditions and a detailed study of the pupation was made.

During its life-cycle, this fly passes through three larval instars the duration of which varies according to the temperature and humidity conditions. Under controlled conditions, at a temperature of 28° C. the duration of each instar is as follows:—

- First instar larva—20 hours,
- Second instar larva—24 hours, and
- Third instar larva—96 hours.

While feeding the larvae never leave the nidus kept in the petri dishes, and all remain hidden well within it. These never expose themselves to the light and thus show a negative phototropic behaviour. After the feeding is complete and the time for pupation approaches, the larvae show certain changes in their behaviour. When ready for pupation, the larvae leave the nidus and crawl to the drier situations. Under cage conditions, it was noticed that the larvae leave the nidus, enter the dry soil and prefer to lie just underneath the petri dishes containing the nidus. In the absence of any such concealing places, they wriggle in the soil and try to burrow in it as much as possible. When such a burrowing is not possible, the larvae simply crawl over the soil rather impatiently. After some time, the larvae become a little lethargic and finally come to rest. Some changes both external and internal take place in the larval body. The larva begins to contract its body segments and assumes a barrel-shaped form. It also changes its colour from creamy white to dark brown or black. The body wall hardens considerably. This process of contraction, darkening and hardening of the larval body wall is rather quick. The hardened larval cuticle is now called as the *Puparium*. This event of puparium formation is often called as the *pupation* (Hewitt, 1914), which is however, incorrect. At most it represents as the preparatory phase. When the puparium is formed the larva actually moults within it into the next instar called the *Prepupa*. It is thus the prepupal moult which takes place and not the pupal moult. The actual pupation takes place at a much later stage, within the puparium. The series of changes which take place during the prepupal moult are as follows:—

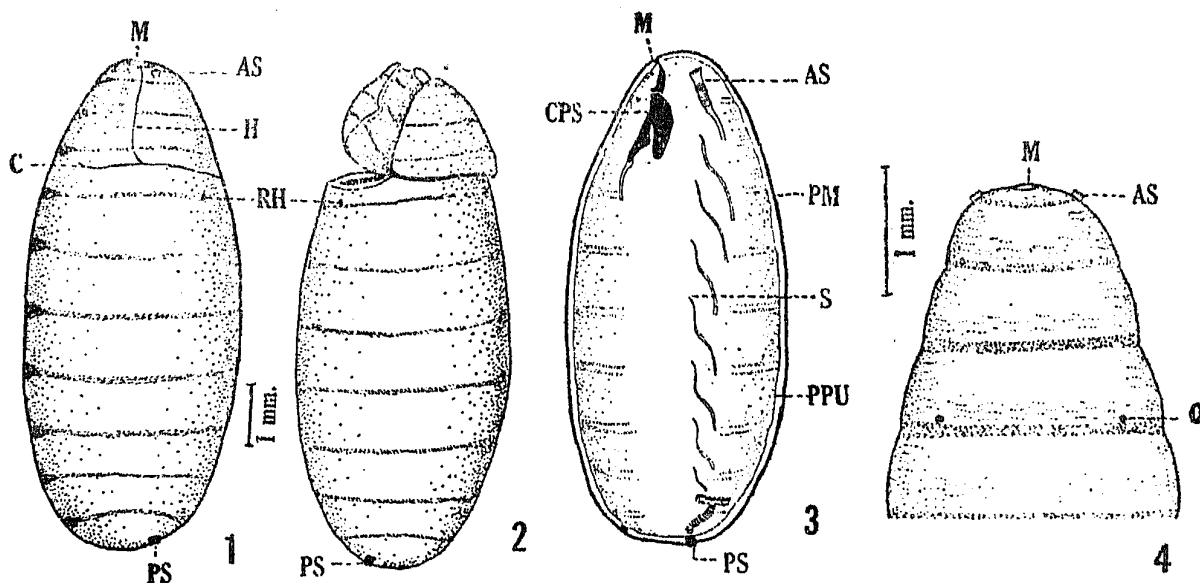
The external surface of the puparium (Fig. 1) retains many of the larval characters, though some of them are slightly modified. The segmentation of the body is still evident and the twelve segments though in a contracted condition can easily be counted on the puparium. The average length of the puparium is 6.5 mm. The spinous locomotary pads of the larva can easily be seen ventrally on the intersegmental areas. Due to the contraction of the oral and the post-oral segments, the larval mouth is also involuted and is closed by the hardening of the indrawn parts which form a chitinous plug (M). Similarly, the anterior larval spiracles (AS) come to project from the antero-lateral margins of the puparium. The posterior spiracles (PS) do not show any change except that the papillae around them are reduced in size. The anal lobes borne ventrally, by the last segment of the larva, have become densely chitinized and closely appressed, but not fused.

Certain novel characters which also appear simultaneously on the surface of the puparium are as follows: In the middle of the fifth segment, there appears a transverse, seam-like sutural line (C) which almost encircles the segment except on the ventral side. Because the oral segment is invaginated this seam-like suture is found apparently on the fourth segment, which however, is really the fifth segment. From this transverse suture, there arise two other lateral sutures (H), which extend anteriorly and meet each other round the anteriormost end of the puparium, in the region of the oral plug. These sutures represent the cleavage lines along which the puparial case is later on broken open by the imago, at the time of emergence (Fig. 2).

On the posterior border of the fifth segment, one can mark out two circular yellowish spots (Fig. 4, O), as large as the pin heads. The spots are situated dorso-laterally on either side and just behind the transverse seam-like sutures. The spots are bordered by brown ring and their cuticular portion is thin and fragile. They appear on the puparial surface at about three hours after the puparium formation, and are later on pierced through by the knob-like respiratory horns of the pupa (Roddy, 1955; Temporary pupal spiracles, Hewitt, 1914), as soon as the pupation takes place within the puparium (Fig. 1, RH).

While the larva is contracting its body segments, its cuticle begins to separate from the hypodermis. This process goes on for some time and a stage is reached when the cuticle is separated from its hypodermis except at certain points, *e.g.*, anteriorly, at the oral plug, anterolaterally, at the paired larval anterior spiracles and posteriorly, at the paired posterior larval spiracles. This shedding of the larval cuticle completes the process of moulting. The moulted larval cuticle becomes dark brownish in colour and

hardens to give rise to the characteristic *puparium*. Due to this moult, the third instar larva now enters the fourth instar, called the *Prepupa*, which remains within the hardened puparium. The prepupa shows the following characters:



FIGS. 1-4. Fig. 1. Puparium (Lat. view). Fig. 2. Puparium from which the adult has emerged. The anterior end of the cap is lifted (Ventro-lateral view). Fig. 3. Median lengthwise section of the puparium and the prepupal cuticle (Pupa is removed). Fig. 4. Anterior portion of the puparium showing the paired openings for the respiratory horns (Dorsal view).

The Prepupa.—The prepupa which is enclosed by the puparium is not entirely separated from the latter but remains attached to it at various points as mentioned above. It therefore gets a firm anchorage to the puparium. It is very difficult to remove the prepupa intact from the puparium. If with a fine surgical knife, small pieces of the puparium are cut away and then the puparium is treated with 70% alcohol, one can easily see a whitish film of the coagulated moulting fluid between the old loosened puparium (moulted skin of the third instar larva). The newly secreted prepupal cuticle is thin and papillated.

After removal from the puparium, if the prepupa is examined under the binocular microscope, it shows the following characters. It is milky white in colour and 12-segmented. It is intermediate in appearance between the larva and the pupa. It therefore combines in itself some larval and some prospective pupal characters. The *larval characters* shown by the prepupa are as follows: (1) It is 12-segmented, (2) it retains the cephalo-pharyngeal skeleton of the third instar larva, within its body, (3) it has no distinct head, (4) it has no thoracic appendages, like the legs and the wings, and (5) it

obtains air through the paired anterior and the posterior spiracles of the larva, which are attached to the outer wall of the puparium. These spiracles are functional and are joined with the prepupal tracheal system.

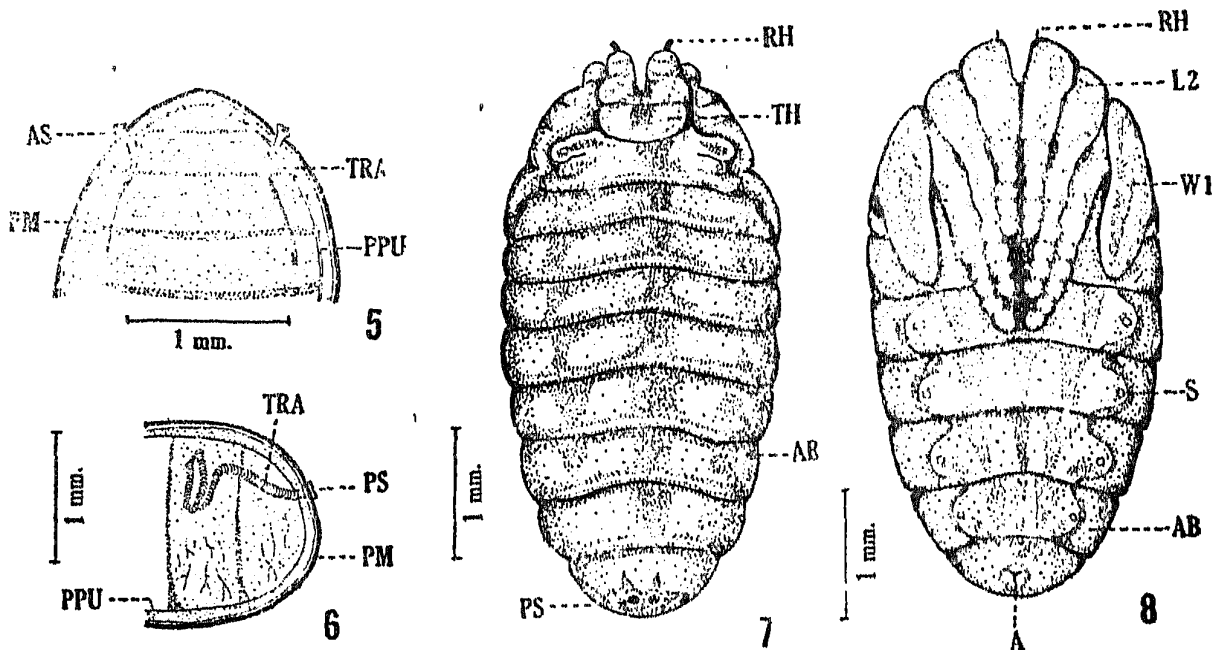
The prepupa also shows an important prospective *pupal character*. In addition to the paired anterior and the posterior spiracles, the prepupa also possesses eight pairs of rudimentary lateral spiracles, which are borne by the IV–XI body segments. The lateral spiracles (Fig. 3, S) are minute, non-functional and represent the rudiments of the prospective pupal and the adult spiracles.

Regarding the occurrence of the prepupal stage in the Cyclorrhaphous Dipteran flies, there is some difference of opinion among the different authors. Snodgrass (1924) has described a distinct prepupal stage, in *Rhagoletis*, which according to him, represents a reduced fourth larval instar. The prepupal instar is mentioned specifically, for *Calliphora*, by Tate (1953) and Wolfe (1954, 1955); for *Sarcophaga*, by Dennell (1946, 1947); for *Drosophila*, by Robertson (1936), and Bodenstern (1950); for *Psila*, by Ashby and Wright (1946), and by Fraenkel (1938) for *Calliphora*, *Lucilia*, *Phormia* and *Sarcophaga*. Hinton (1946) in discussing the prepupal cuticle in Diptera Cyclorrhapha, has accepted Snodgrass's (1924) conception of a reduced fourth instar larva. Brauns (1954) has also supported Hinton's (1946) views. But Whitten (1957) has recently shown by the chemical and microscopical evidences, that the prepupal cuticle generally described for Diptera Cyclorrhapha (*Phormia*, *Calliphora*) is actually an inner layer of larval endocuticle, rather than the cuticle of a distinct prepupal instar. According to her there is no distinct prepupal instar in these flies. The present studies have however indicated the presence of a prepupal instar in the housefly.

The prepupal instar has a short duration of about 6 hours after which it moults within the puparium and becomes the *Pupa* proper. The hypodermis of the prepupa secretes a new and thicker cuticle which is called the pupal cuticle. Due to the rhythmic contractions of the body of the prepupa, the old prepupal cuticle gets loosened from the new pupal cuticle and finally is completely separated from the latter. The prepupal cuticle which is extremely thin and papillated then gets itself firmly attached to the inner wall of the puparium and thus reinforces it. The events involved in the process of pupal moult are described below.

The Pupal Moult.—By muscular contractions, the prepupa contracts its body at its anterior and the posterior ends and begins to shed its cuticle. The cephalo-pharyngeal skeleton of the larva, which still lies within the body

of the prepupa, is also attached anteriorly to the chitinized, hardened oral plug of the puparium. When the prepupa begins to contract anteriorly, this cephalo-pharyngeal skeleton is incompletely pulled out of the body of the prepupa. As mentioned earlier, the anterior and the posterior spiracles of the prepupa are firmly attached to the outer wall of the puparium and cannot therefore be withdrawn by the contracting prepupa. During the contractions of the prepupal body at the anterior and the posterior ends, the atrial tubes of these spiracles are partly pulled out of the body of the prepupa. When more contractions of the prepupal body take place, the anterior and the posterior portions of the dorsal longitudinal tracheal trunks situated just behind the atrial tubes are broken and remain attached to the inner wall of the moulted prepupal cuticle (Fig. 5, TRA). The remaining greater portion



FIGS. 5-8. Fig. 5. Inner surface of the puparial cuticle. Fig. 6. Cast-linings of the dorsal tracheal trunks connected with the posterior spiracles. Fig. 7. The cryptocephalic pupa (Dorsal view). Fig. 8. The cryptocephalic pupa (Ventral view).

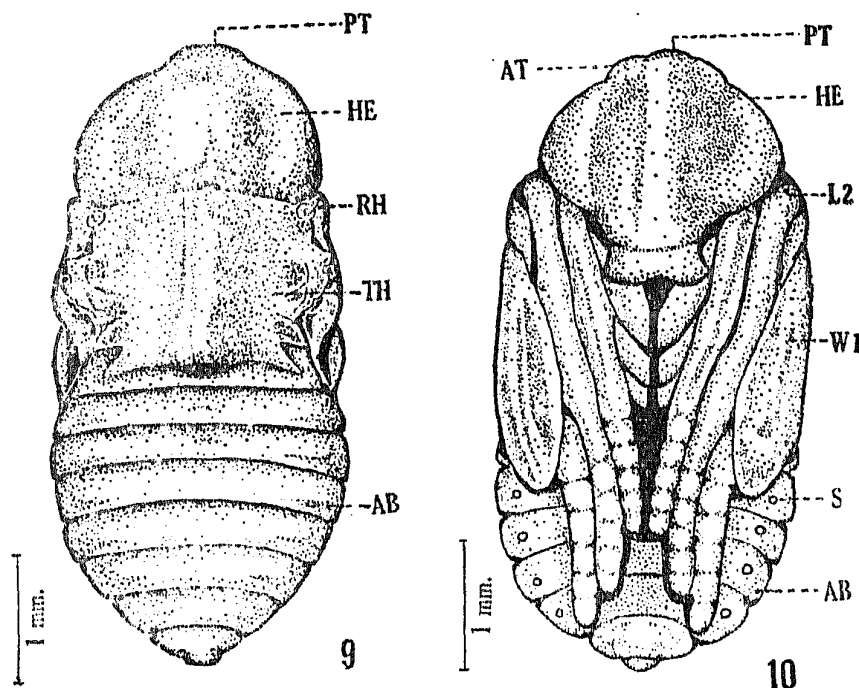
of the dorsal longitudinal tracheal trunks of the prepupa, remains within the body. When the anterior portion of the dorsal longitudinal tracheal trunks is broken, air rushes in, through the paired anterior spiracles and fills in the anterior and lateral empty spaces in between the prepupal cuticle and the newly formed pupal cuticle. This air is useful as an air cushion to the pupa, in order to absorb shocks. At the anterior broken ends of the dorsal longitudinal tracheal trunks, a pair of new pupal spiracles is developed in the prothoracic region. These pupal prothoracic spiracles remain enclosed with the puparium and are known as the internal spiracles. Each prothoracic

spiracle is composed of slightly protruding felt chamber, which is drawn out into a well-developed sclerotized *Respiratory Horn* (Figs. 7 and 9, RH). These paired respiratory horns are covered with spiracular papillae. Each horn is about 0.185 mm. long and 0.105 mm. broad. With further contractions of the prepupal body, these respiratory horns protrude through the small, circular apertures present on the wall of the puparium (Fig. 4, O), and establish communication between the atmospheric air and the pupal tracheal system. The posterior broken ends of the dorsal longitudinal tracheal trunks (Fig. 6, TRA) of the pupa, become closed and do not therefore give rise to any posterior spiracles in the pupal stage.

By the time the anterior and the posterior portions of the dorsal longitudinal tracheal trunks are broken, the leg and the wing buds of the moulting prepupa are also everted. These organs are rather short in length but gradually elongate during the later part of the pupal period. After the eversion of the leg and the wing buds, the prepupa sheds its cuticle on all sides and completes the pupal moult. The prepupa now becomes the *Pupa* proper.

The Pupa.—Immediately after the pupal moult, the pupa has, as yet no distinct head. A portion of the cephalo-pharyngeal skeleton of the larva still remains within its body. Due to this acephalic condition, the pupa is known as the *Cryptocephalic Pupa* (Figs. 7 and 8). It is a transitory phase and is of a very short duration. The pupa contracts its body from the anterior side more and the respiratory horns pierce through the cuticular apertures borne on the fifth segment of the puparium. This protrusion of the respiratory horns not only establishes a necessary connection between the atmospheric air and the pupal tracheal system, but also gives necessary anchorage to the pupa with the puparium. During the anterior contractions of the pupal body, the cephalo-pharyngeal skeleton of the larva is completely pulled out of the body of the pupa and simultaneously the invaginated head of the pupa is everted. The cephalo-pharyngeal skeleton remains pressed against the inner wall of the prepupal cuticle. The pupa now possesses a small, thin-walled head and is therefore known as the *Phanerocephalic Pupa* (Figs. 9 and 10). The antero-dorsal wall of the head capsule is known as the ptilinum (Figs. 9 and 10, PT). It is usually everted in the form of a cap-like lobe and below this lies a transverse bilobed ridge, within which are developed the antennae (Fig. 10, AT). The compound eyes are represented by laterally placed swollen areas on the head capsule. The thorax is much shrunken and consists of reduced pronotum and metanotum and well-developed mesonotum. The respiratory horns (Fig. 9, RH) are borne by the prothorax. There are two pairs of thoracic spiracles, a prothoracic

and a metathoracic pair. The legs and the wings have slightly increased in length (Fig. 10, L2, W1). The abdomen (Fig. 10, AB) is eight-segmented. It is cylindrical and bounded on all sides by membraneous walls. It bears seven pairs of lateral spiracles.



Figs. 9-10. Fig. 9. The phanerocephalic pupa (Dorsal view). Fig. 10. The phanerocephalic pupa (Ventral view).

(A, Anus; AB, Abdomen; AS, Anterior spiracle; C, Circular cleavage line; CPS, Cephalo-pharyngeal skeleton; H, Horizontal cleavage line; HE, Head; L2, Mesothoracic leg; M, Mouth (Oral plug); O, Openings for the respiratory horns; PM, Puparium; PPU, Prepupal cuticle; PS, Posterior spiracle; PT, Pilinum; RH, Respiratory horns; S, Lateral spiracles; TH, Thorax; TRA, Moulded tracheal linings of the preceding instar; W1, Fore-wing.)

The pupa is covered by three cuticular sheaths; an outer, heavily chitinized puparium (moulded cuticle of the third instar larva), a middle thin, prepupal cuticle and an inner newly secreted pupal cuticle. The pupa is therefore known as the *Coarctate pupa* or *Adecticous exarate pupa*. It can now be easily removed from the puparium. If an empty puparium from which the pupa is removed be examined under the binocular microscope, one can see the prepupal cuticle firmly adhered to the inner wall of the puparium (Fig. 3). The cephalo-pharyngeal skeleton (CPS) of the larva together with the chitinous linings of the stomodaeum is firmly fixed to the ventral wall of the prepupal cuticle. The moulded anterior portions of the dorsal longitudinal tracheal trunks extend backward as two conspicuous tubes lying below the level of the lateral seam-like cleavage lines of the puparium. The moulded posterior portions of the same trunks are disposed in an irregular

manner against the inner dorsal wall of the prepupal cuticle. The moulted tracheae of the eight pairs of rudimentary spiracles (S) form a tangled mass which remains on the lateral sides of the inner wall of the prepupal cuticle. When the imago escapes the pupal case, the pupal cuticle gets itself separated from the freshly deposited imaginal cuticle and remains attached to the inner wall of the prepupal cuticle.

SUMMARY

The third instar larva, after feeding, leaves the nidus and makes a few movements on the dry soil kept in the cages. It then begins to contract its body from the anterior and the posterior ends. Its cuticle begins to separate from the body. When it is completely separated except at two points (*i.e.*, at the anterior and the posterior spiracles), the process of moulting is completed. The organism which now lies in the moulted larval skin called the puparium, is now known as the *Prepupa*. Some novel characters now appear on the outer wall of the puparium. These are the transverse and lateral sutural lines and paired pin-head shaped circular spots situated on the fifth segment. The prepupa remains attached to the puparium through the anterior and the posterior spiracles. The prepupa though looks similar to the third instar larva, differs from it in the possession of eight pairs of non-functional lateral spiracles. The prepupal stage lasts for about 6 hours. The prepupa then undergoes a moult, and becomes the pupa proper. During this moult, the prepupa contracts its body from the anterior and the posterior ends. Due to these contractions, the anterior and the posterior spiracles, borne by the puparium, break their connection with the pupal tracheal system. New pupal spiracles are developed on the prothorax, where this breaking takes place. These prothoracic pupal spiracles also give rise to the respiratory horns, which pierce through the fifth segment of the puparium and establishes a communication between the atmospheric air and the pupal tracheal system. The eight pairs of lateral spiracles remain non-functional, in the pupal stage. During pupation, the head, the legs and the wings become everted. Immediately after pupation, the pupa does not possess a distinct head. This pupa is called the *Cryptocephalic pupa*. After some time the invaginated head is everted and the pupa then is known as the *Phanerocephalic pupa*. The pupal stage lasts for about 96 hours and then the adult emerges.

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