The Supposed Pre-pupa in Cyclorrhaphous Diptera

By JOAN M. WHITTEN

(From Queen Mary College, University of London)

SUMMARY

The pre-pupal cuticle generally described for Diptera Cyclorrhapha is here considered to be an inner layer of larval endocuticle rather than the cuticle of a distinct pre-pupal instar. Chemical and microscopical evidence is given. The mechanism of rupture of the operculum is also discussed in connexion with endocuticle secretion, sclerotization, and subsequent water loss.

INTRODUCTION

The secretion of a pre-pupal cuticle before pupation would appear, from the literature, to be general for the Diptera Cyclorrhapha. It is referred to in all the current textbooks of insect morphology and physiology, including Wigglesworth (1950) and Roeder (1953). The instar has also been used as a subject for physiological investigations, including that on Drosophila by Ellenby (1953). It is mentioned specifically for Rhagoletis by Snodgrass (1924), for Calliphora by Tate (1953) and Wolfe (1954a, b, 1955), for Sarcophaga by Dennell (1946, 1947), for Drosophila by Robertson (1936) and Bodenstein (1950), for Psila by Ashby and Wright (1946), and by Fraenkel (1938) for Calliphora, Lucilia, Phormia, and Sarcophaga.

There is amongst the large literature on the pre-pupal cuticle no consistent account of its macroscopic structure. Snodgrass (1924), who appears to have been the originator of the term 'pre-pupal cuticle', describes a distinct pre-pupal stage in Rhagoletis, which he considers to represent a reduced fourth larval instar. According to him the pre-pupa, enveloped in its cuticle, can be removed from the puparium at a stage between the beginning of puparium formation and the formation of the true pupa. On the other hand, Fraenkel (1938) describes the pre-pupal cuticle in Calliphora in the abdominal region only. Robertson (1936) in Drosophila describes the pre-pupal cuticle as completely enveloping and extending into the larval stomodaeum and proctodaeum. Ashby and Wright (1946) are obviously puzzled over the spatial relationships of the cuticle in Psila and are more critical than other authors of its relationships. Hinton (1946) in discussing the pre-pupal cuticle in Diptera Cyclorrhapha accepts Snodgrass's (1924) conception of a reduced fourth larval instar. Hinton's diagrammatic representation (see fig. 4, A, p. 248) is, moreover, reproduced in Brauns (1954).

Dennell (1946, 1947), in detailed work on the cuticle of Sarcophaga, simply refers to the fact that Fraenkel and Rudal (1940) noted a pre-pupal moult in Calliphora, and mentions a pre-pupal moult in Sarcophaga in vague, unconvinced terms. Similarly, Wolfe's (1954, 1955) detailed account of the origin

and fate of the third larval (1954a) and of the pupal and adult cuticles (1954b) in Calliphora, makes only passing mention of the pre-pupal cuticle (1955). In spite of the large literature on the deposition, sclerotization, and moulting of the cuticles of the various instars in different species of Cyclorrhapha, no account exists of the chemical composition or cuticular nature of this very fine membrane, except the brief mention by Wolfe (1955).

**RESULTS**

**Drosophila melanogaster Meigen**

The cuticle was first encountered by the author during an investigation into the development of the tracheal system in *Drosophila melanogaster* (Whitten, 1957). In this small species a pre-pupal cuticle was isolated with difficulty and it appeared to lie free in the abdominal region, in a similar manner to that described by Fraenkel (1938) for Calliphora, but anteriorly it appeared, in Drosophila, to be continuous with the inner surface of the operculum. As the tracheal lining is continuous with the general cuticle of the body surface, detailed study of the former necessarily involved consideration of the cuticles in the different instars. I became increasingly aware of the anomalies presented by this cuticle, particularly with respect to the tracheal system: although distinct functional tracheal systems are present in each of the larval, pupal, and adult instars, there is no evidence for a pre-pupal tracheal system. Since the tracheal system of Calliphora erythrocephala Meigen was also under consideration, the latter species, because of its larger size, has been used for further investigation into the extent and possible nature of the pre-pupal cuticle. The following represents the results of this work, and the conclusions which have been drawn from it and from a simultaneous study of Phormia terrae-novae Robineau-Desvoidy.

**Calliphora erythrocephala and Phormia terrae-novae**

**Puparium formation and the spacial relationships of the cuticular layers**

As in all Diptera Cyclorrhapha, puparium formation begins with cessation of feeding, eversion of anterior spiracles, and darkening of the cuticle.

Darkening is a gradual process and takes place progressively inwards. In the present instance it has been found possible to peel away the outer sclerotized inner layers. Whilst darkening is still in progress it is not possible to remove the operculum; this is quite clearly due to factors involved in the sclerotization and subsequent loss of water in the ‘line of fracture’, and will be discussed later. As darkening continues the body contents at the posterior end are withdrawn from the anal papillae (fig. 1, A); withdrawal at the anterior end has not yet occurred and the larval tracheae are still intact.

Later, the mouth armature and living contents anteriorly are drawn inwards, and the pupal spiracles make their appearance median to the larval spiracular tracheae. At this stage the space between the larval cuticle and the developing pupal spiracles is filled with fluid. Slightly later the intima of the
spiracles can be seen secreted simultaneously with that surrounding the larval trunks anteriorly (fig. 1, B).

**Fig. 1. Phormia terrae-novae.** A, posterior end of early puparium showing contraction of living contents away from cuticle (compare fig. 3, B). Semi-diagrammatic. B, anterior end of slightly later stage showing spatial relationships including retracted larval mouth armature, and retracted larval spiracular tracheae in relation to newly formed pupa (compare fig. 3, D). Semi-diagrammatic. C, posterior end of fully hardened air-filled puparium showing spatial relationships of the pupal cuticle, the 'membrane' or so-called 'pre-pupal' cuticle, and the puparium wall; larval tracheae and proctodaeum withdrawn (compare fig. 3, E). Semi-diagrammatic.
Whitten—Supposed Pre-pupa in Cyclorrhapha

Dissection of the contents of the puparium at this stage reveals an object intermediate in appearance between larva and pupa. Anteriorly, as already mentioned, the pupal spiracles are visible; the wings and legs are only partially, at most, evaginated, and the head is invaginated. The larval mouth armature and stomodaeum are still retained within the body, and can either be withdrawn from the invagination lying between the pupal spiracles, or left within this by severing their connexion with the puparium anteriorly. The rest of the body consists of the long abdomen, only slightly shorter than the corresponding larval abdomen. The larval tracheae, in both abdomen and thorax, are still retained and the spiracular tracheae connect with the spiracles on the puparium. Successively older puparia, when dissected, reveal specimens intermediate between this and the normal pupa. Quite evidently evagination of the head and thoracic appendages and relative increase in length of the thoracic region is accompanied by progressive contraction of the enveloping cuticle in the abdominal region. Complete contraction of the abdomen to characteristic pupal form is accompanied by withdrawal of the larval tracheae, which also seems to be brought about in part by the release of the air-bubble, hitherto contained within the abdominal region. Tate (1953) describes in detail the part played by the air-bubble in removal of the tracheae, although he offers no explanation of its origin. Bodenstein (1950) illustrates a pre-pupa of Drosophila which is clearly comparable with this early pupal stage in which appendages and head are still inverted, larval tracheae retained, and abdomen long and larva-like. It is clearly not a pre-pupa as the inner surface of the puparium at this stage always has the membrane corresponding to the 'pre-pupal cuticle' of other authors lying on its inner surface. Also, the spiracular tracheae which pass through the membrane have either to be severed or artificially withdrawn from the young pupa before it can be removed from the puparium. At all stages the membrane is loose posteriorly and closely applied to the wall of the puparium anteriorly. In older, air-filled puparia, air frequently separates the membrane from the puparial wall. The cast larval tracheae always lie on its inner surface.

Endocuticle secretion and sclerotization in specialized areas

In certain areas of the puparium the endocuticle evidently remains unsclerotized. This is the case with the pupal 'windows' (fig. 2, A) which remain transparent and unsclerotized. Wolfe (1954) states that in Calliphora none of the endocuticle remains unsclerotized, but he is specifically concerned with the abdominal region, and in this region the so-called pre-pupal cuticle lies loosely and is unconnected with the puparium wall. The significance of this will be discussed later.

Another region of the puparium in which both secretion and sclerotization of endocuticle is specialized and differs from the general surface is along the lines of rupture of the operculum. In partially sclerotized puparia a lighter band can be seen running down from the pro-thoracic spiracles on each side. When darkening is complete, together with loss of water by the larval cuticle
and replacement of the fluid internally by air, the region appears as an air-filled line (fig. 2, A); and the split now occurs with application of only light pressure. Ease of removal would appear to be dependent on drying out of the puparial cuticle. Before this, more effort is required to peel away the operculum. The explanation can be found in a transverse section of this region. Frozen sections before hardening and loss of water appear as in fig. 2, B. The sclerotization of the exocuticle is uneven, or rather the actual secretion of endocuticle which will become sclerotized (future exocuticle) would appear to be uneven. In consequence, the underlying region also appears to be secreted unevenly. A later stage, when drying out has occurred and the operculum is easily removed, reveals the separated edges of the operculum and antero-

---

**Fig. 2. Phormia terrae-novae.** A, anterior end of air-filled puparium in lateral view showing future line of fracture of operculum. B, transverse section of larval cuticle in region of line of fracture: early stage of sclerotization and puparium formation. C, anterior end of late air-filled puparium with removed operculum. D, enlarged view of section of torn edge along line of fracture of operculum. A-D all semi-diagrammatic.
Whitten—Supposed Pre-pupa in Cyclorrhapha

FIG. 3. Diptera Cyclorrhapha: diagrammatic reconstruction of the spatial relationships of the cuticular layers and the hypodermis (in dotted line) at the various stages between the beginning of puparium formation and secretion of the adult cuticle. A, third instar larva in immobile condition at white puparium stage. B, third instar larva with anterior spiracles as yet unretracted but with living contents withdrawn from anal papillae; darkening of puparium beginning. C, third instar larva, darkening proceeding, showing relative positions of outer endocuticle (forming sclerotized exocuticle) and newly secreted inner endocuticle (later forming the membrane formerly considered to be the pre-pupal cuticle). D, early pupa with inverted head and thoracic appendages; larval mouth armature, stomodaeum, proctodaeum, and tracheal system still retained within newly secreted pupal systems; darkening of puparium almost complete. E, late pupa with head and thoracic appendages everted; puparium air-filled and fully sclerotized with larval stomodaeum, proctodaeum, and tracheal system withdrawn; cuticular layers dehydrated and reduced in thickness; exocuticle hard, dark, and brittle; endocuticle transparent and membrane-like. F, adult stage within pupal cuticle, with newly secreted adult tracheal linings surrounding pupal tracheal linings.
ventral surface of the puparium as in fig. 2, c, d. The hard, darkened exocuticle at the separated edge is extremely thin, and is separated by a space from a thin membrane also apparently torn along its edge. The elongated air-space seen in surface view is presumably the area between the two layers. Except along this line the inner layer lies close to the hard sclerotized layer. Loss of water and resulting decrease in thickness of the two layers in this region would appear to cause the separation, the resulting space becoming the elongated air-space after entry of air into the puparium. The membrane is undoubtedly the inner endocuticle reduced in thickness by loss of water, separated at the line of fracture but elsewhere in contact with the sclerotized exocuticle.

Chemical nature of the cuticle

Chemical tests applied to this membrane (so-called pre-pupal cuticle) removed from the abdominal region where it is loose, have given the following results: it is stained pale blue with Mallory, whereas the outer exocuticle stains orange; it stains very faintly with methylene blue; gives a positive test with aniline blue, but a negative one with acid fuchsin. The same results were given by the withdrawn larval proctodaeum and peeled-off inner layers of the puparium before sclerotization of the larval cuticle. These results differed from those given by the pupal cuticle after emergence of the adult, in which the endocuticle has been dissolved leaving epicuticle only (Wolfe, 1954).

Finally, it is soluble in concentrated sulphuric acid, and gives a very positive reaction for chitin with the chitosan test.

DISCUSSION

The evidence so far obtained substantiates the hypothesis that this membrane in *Phormia* and *Calliphora* is in fact modified larval endocuticle and not the cuticle of a distinct pre-pupal instar as is generally supposed.

According to Wolfe (1954), endocuticle is secreted throughout the third larval instar and is converted, at puparium formation, into sclerotized exocuticle, but he considers that no endocuticle remains unsclerotized. However, Wolfe is only concerned with the abdominal region and here the 'pre-pupal cuticle' (inner endocuticle) is loose and separate from the exocuticle (sclerotized outer endocuticle). Thus any relationship with the larval cuticle is not obvious. Frozen sections of early puparia show the two distinct zones of endocuticle; these are most clearly distinguishable in the region of rupture of the operculum (fig. 2, b).

In *Sarcophaga* Dennell (1946) has shown that endocuticle is secreted not only throughout the third larval instar, but also after the beginning of puparium formation. The evidence presented here suggests that this is also the case in *Phormia* and *Calliphora*; it is this endocuticle secreted during puparium formation which remains unsclerotized and subsequently, after loss of water, becomes the thin membrane hitherto known as the pre-pupal cuticle. Comparison of the inner unsclerotized endocuticle of *Sarcophaga*, as described by Dennell (1946), with this membrane in *Phormia* and *Calliphora* confirms
their homologous nature. The staining reactions are similar and, in both, pore canals are absent. In addition, both are not dissolved by the moulting fluid occupying the space between larval endocuticle and newly secreted pupal epicuticle. In contrast to this, both at the previous ecdyses and when the adult emerges, the moulting fluid dissolves the endocuticle, leaving only epicuticle as the cast skin (Wolfe, 1954).

The secretion of endocuticle after the beginning of puparium formation can in part explain the spatial relationships of the resulting membrane (inner endocuticle) to the exocuticle (outer endocuticle) of the puparium. Anteriorly, the two layers are closely applied although they can be peeled off from one another. This is not surprising since even in the outer endocuticle it is possible to peel off unsclerotized from sclerotized layers during darkening of the puparium, and in the present case the differing chemical and physical composition of the two layers, combined with loss of water by both, probably aids the separation process.

The peculiar separation of the membrane posteriorly could be explained as follows: At the beginning of puparium formation, while the anterior end with its mouth armature has not yet retracted, there is, posteriorly, a withdrawal of the larval tissues from the anal papillae and from the puparial wall (fig. 1, A). If secretion of endocuticle is continuing at the same time, as would appear, then this would result in a separation of the two layers. The separation is subsequently made more obvious after loss of water by both layers and entry of air into the puparium.

The structural relationships seen in Phormia and Calliphora have been found to be similar in Drosophila and Musca. As the description of a 'pre-pupal cuticle' has also been given, by various authors, for many other species, the results given here seem to be general for Diptera Cyclorrhapha. The specialized method of secretion and the subsequent fate of the cuticular layers at the lines of rupture of the operculum, described here, is also probably similar in other Cyclorrhapha; however, the actual extent and positions of the lines vary considerably within the group (Hennig, 1952; Brauns, 1954).
CONCLUSIONS

Since the pre-pupal cuticle is in effect the inner larval endocuticle and no pre-pupal instar exists, the pre-pupa of *Rhagoletis* (Snodgrass, 1924) is really still the third larval instar in an immobile condition. The same applies to each of the other examples given in the literature cited. On the other hand, the pre-pupa illustrated by Bodenstein (1950) is really an early pupa. Snodgrass’s pre-pupa would correspond to my fig. 3, c and Bodenstein’s to my fig. 3 d. Hinton’s diagram (1946, fig. 1) would be relabelled as in my fig. 4, b.

Only this interpretation of the membrane in question can resolve the anomalies presented first by the larval tracheal system and the larval stomodaeum and proctodaeum and, secondly, by the inconsistent and unconvincing reports of a distinct instar and its cuticle in the various members of the Cyclorrhapha.

I wish to thank Professor V. B. Wigglesworth for his continued encouragement and for kindly reading through the manuscript. The author is solely responsible for the views expressed.

REFERENCES


ROBERTSON, C. W., 1936. 'The metamorphosis of *Drosophila melanogaster*, including an accurately timed account of the principal morphological changes.’ *J. Morph.*, 59, 351.


