THE DEVELOPMENTAL HISTORY OF *MUTELA BOURGUIGNATI* (ANCEY) BOURGUIGNAT (MOLLUSCA: BIVALVIA)

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(*Communicated by C. M. Yonge, F.R.S.—Received* 13 *January* 1961—*Revised* 11 *April* 1961)

CONTENTS

An account is given for the first time of the development of *Mutela bourguignati* (Ancey) Bourguignat, an African freshwater bivalve of the family Mutelidae.

Eggs are shed in large numbers into the inner demibranchs. There they develop into minute larvae similar in some respects to the lasidium larvae of certain South American freshwater mussels, but differing in several ways. Each larva consists of a rounded body covered dorsally and laterally by a thin pellicle and provided anteriorly with two lobes clothed with cilia. Posteriorly, and on the ventral surface, are two sets of minute hooks. Anteriorly is a remarkable elongate, flaccid and colourless tentacle more than seventy times as long as the larva itself. There is no gut, nor can endodermal or mesodermal tissues be recognized as such at this stage.

After liberation via the exhalant siphon these larvae settle on the cyprinid fish *Barbus altianalis radcliffi* and there metamorphose and begin a parasitic phase of development. The larval pellicle folds, and its opposed edges fuse so as completely to enclose the larva. Two tubular outgrowths grow from its anterior end into the superficial tissues of the fish and serve both as organs of attachment and nutrition. In their vicinity skeletal tissues of the host are broken down. The main body of the larva, now designated the haustorial larva, elongates and becomes differentiated into a stalk and a bud. The stalk protrudes from the fish and bears the bud clear of the host. Within the bud the adult bivalve eventually develops. The stalk is traversed by long prolongations of the mantle which run into the haustorial tubes and function as absorptive tissues. The cuticle is not calcified nor is it composed of conchiolin. As differentiation proceeds the definitive mantle, which is the lineal descendant of the larval mantle, begins to secrete first periostracum then calcareous matter to form the rudiments of the valves, and a rudiment of the ligament is also formed. As adult features gradually

appear the bud is burst and a young bivalve, little more than 1 mm in length, attached to a long stalk emerges. Independent, particulate feeding commences at this stage.

Rupture of both cuticular and mantle elements eventually takes place at the point of juncture of stalk and young bivalve and the latter falls away to begin an independent existence. At the time of release it is capable of active locomotion and is able to produce a slender byssus thread.

A detailed, illustrated account of these changes, both external and internal, is given, and the development of certain individual organs is traced. The nature of the cuticle of the haustorial larva, the effect of this larva on its host, and the affinities and probable evolution of the larval stages are discussed, and the importance of taking larval development into account in classification is emphasized.

Introduction

While several features of the life history of the freshwater bivalves of the family Unionidae were early established, such as Leeuwenhoek's observations on their embryos in 1695, Rathke's finding of the glochidium larva in 1797 and Leydig's eventual unravelling of the life cycle in 1866, no comparable information is available concerning the African members of the related family Mutelidae. The only relevant reference is that of Franc (1949) who mentions that Monod had seen glochidia in the ctenidia of two species of *Mutela* from West Africa. In view of the existence of a different kind of larva in the South American mutelids, to which the African forms are believed to be closely related (von Ihering 1891/92) this report caused surprise to Haas (1954) and is not in accord with the facts related here.

Some information has been collected about the development of non-African species at present regarded as representatives of this family, but this too is fragmentary and conflicting. Thus some Australian freshwater bivalves, currently assigned to the Mutelidae, produce glochidia similar to those of the European Unionidae and with similar parasitic habits (Percival 1931; Hiscock 1951; McMichael & Hiscock 1958), while others, from South America, also assigned to the Mutelidae, produce a different type of larva known as a lasidium (von Ihering 1891/92, 1893; Bonetto 1951) whose subsequent development is, however, unknown. Development of the African *Mutela bourguignati* (Ancey) Bourguignat involves stages completely different from any hitherto described for other alleged mutelids. This paper describes the course of metamorphosis and the anatomy of these developmental stages, some of which are unique not only within the Bivalvia but within the Mollusca as a whole, and gives a detailed account of the life cycle, of which a brief preliminary outline has been given elsewhere (Fryer 1959).

Material and methods

Adult mussels were collected from the situations described below. From the inner demibranchs of their ctenidia, eggs and larvae at various stages of development were collected. Parasitic stages were obtained from the cyprinid fish *Barbus altianalis radcliffi* Boulenger, which serves as host. Most of these fishes were collected in the Victoria Nile but a few were obtained in Lake Victoria. After elucidation of the life history from material collected in nature, the entire cycle was followed in aquaria, and additional material thus obtained. As many stages as possible were studied alive, and almost all the figures of the entire organism have been prepared from live material. For material to be sectioned several fixatives, but particularly 4% neutral formaldehyde and Bouin's fluid, were employed.

In Africa, better results were obtained when low viscosity nitro-cellulose was used as the embedding medium than when ester wax was employed, but good sections were obtained from material embedded in the latter medium and kindly cut for me in the Department of Zoology, University of Glasgow. Of the stains employed Ehrlich's haematoxylin proved successful in Africa, while some of the sections cut at Glasgow were well stained with alcian blue, haemalum and eosin and others with Mallory's stain.

A NOTE ON THE ADULT OF *MUTELA BOURGUIGNATI* AND ITS ECOLOGY

M. bourguignati, which in general appearance resembles mussels of the genus *Unio*, has been recorded with certainty only from Lake Victoria and the Victoria Nile. Conchological details are given by Mandahl-Barth (1954), and anatomical notes by Bloomer (1932). Almost all the adult specimens utilized in the present study were collected in the Victoria Nile quite close to its origin. From the few specimens examined *in situ* it appears that the adult mussel lives in rocky situations largely buried in shingle and coarse sand from which only those portions of the valves which surround the siphons protrude. Most specimens came from water up to 8 ft. deep, and perhaps more, where the flow was moderately rapid, and were collected from the spoil brought up by grabs working in connexion with the Owen Falls Dam hydro-electric scheme. These specimens were living on a rocky bottom and were associated with a rocky-bottom fauna of insects, molluscs and fishes.

In life the mussels, although capable of locomotion, are essentially sessile, though they are not fixed by a byssus. The siphons, when extended, protrude for a distance equal to some 10% of the length of the valves. They are undoubtedly light-sensitive for, although sections have revealed no obvious light receptors, they respond to movements of external objects. Thus, if a mussel be kept in a suitable vessel and a hand is carefully passed over it above the water surface in such a manner as to cause no vibration, the siphons are rapidly withdrawn.

One other adult feature merits mention, namely, the extensive fusion of the mantle edges along their ventral borders in the posterior region and over about one-third of their length. Thus the pedal aperture is confined to the anterior end.

THE FREE-LIVING LARVA

Only the inner demibranchs of the ctenidia serve as marsupia within which eggs and larvae are brooded. The eggs are very yolky, minute, and spherical, the largest seen in the ovaries having a diameter of only 43 μ. After shedding they swell to a diameter of 180 to 190 μ. They are shed into the ctenidia in large numbers—several thousand per demibranch. While development of almost all the eggs of a batch takes place at the same rate, larvae with retarded (or arrested?) development occur in most broods. It has not been possible to study early embryological development in detail.

By the time the larva is sufficiently differentiated to reveal definite structure two outgrowths clothed with cilia have arisen at the anterior end of the still approximately spherical body and a thin pellicle covers its dorsal surface (figures 1 and 2). The two outgrowths develop into lobes which become distinct from the more voluminous posterior region, the pellicle takes on a definite form, and a long thin tubular structure destined to

Settlement and metamorphosis of the free-living larva

Following the free-living stages is a period of intense protelean parasitism. The host is the cyprinid fish *Barbus altianalis radcliffi* Boulenger, and parasitic larval stages have so far been found only on this species. The fins are the most favoured sites of attachment, particularly the caudal fin, but specimens also settle elsewhere and have been found attached to scales in various parts of the body and to bony head structures. Specimens settling on the caudal fin do so particularly near its upper and lower regions. This distribution suggests that the free-living larvae lie on the bottom, possibly anchored loosely by the tentacle, and are stirred up by passing fishes. If, as often happens, a fish lies near the bottom maintaining its position by occasional gentle movements of the tail, then larvae lying near the tail will be readily disturbed and the caudal fin will become more heavily infected than other parts of the body.

Initial, and very temporary, attachment to the host is almost certainly effected by means of the tiny hooks with which the larva is provided posteriorly. Mucus produced by the host quickly surrounds the still minute larva and it is to enclosure in this rather than to its hooks, in any case soon rendered functionless by subsequent development, that the larva owes its firm anchorage. Several changes take place at this stage. The tentacle is lost, the ciliated lobes are withdrawn by contraction of the retractor muscles, and the adductor muscle contracts so as to fold the larval shell along the mid-dorsal line into a distinctly bivalved structure (figure 17). The shell is still composed of a sheet of organic material and there is no suggestion of differentiation into valves and ligament. Preservation of mature larvae sometimes causes the shell to fold in this manner and sections of these specimens have enabled some of the internal changes to be seen clearly (figure 18). As a result of folding the ventral margins of the shell are brought into contact with each other in the mid-ventral line. Here fusion takes place. This process, while recalling the peculiar conditions which prevail in the adults of *Teredo* and more particularly *Brechites*, has a closer parallel in the development of the Scaphopoda (Lucaze-Duthiers, cited by Dawydoff 1928), but is carried further in that fusion is *complete*, and the result is a completely enclosed structure. Attachment is now maintained solely by virtue of the mucus produced by the host.

The parasitic stages

(*a*) *The earliest stages*

After settlement the ciliated lobes, their retractor muscles, and the adductor muscles are broken down. Growth takes place in both anterior and posterior directions (figure 19). Anteriorly two outgrowths (*PH*) arise and posteriorly there is a gradual elongation of the entire body. The internal rudiments from which the growing tissues of the larva (termed simply post-larva in a preliminary communication—Fryer 1959) arise can be seen in figure 17. Growth of these tissues is accompanied by secretory activity and the enclosing pellicle also begins to extend. The two anterior prolongations (figure 19, *PH*) bear a distinct relationship to the former anterior margin of the larval shell (figure 19, *AE*) whose sculpture can still be detected in very early parasitic larvae. The anterior outgrowths (figures 20, 21 and 22, *H*) quickly penetrate the host's tissues. Nutritional and allied problems are discussed separately (p. 288); here it is sufficient to say that

(*c*) *The culmination and termination of parasitism*

The commencement of particulate feeding foreshadows the complete independence of the young mussel. Severance occurs between stalk and bud where a distinct line of weakness has been forming for some time. Here, too, rupture of the mantle prolongations takes place where these are narrowest. The line of weakness lies at the anterior extremity of the periostracum which, as noted, is here very thick, and is along a more or less straight line at the anterior extremity of each valve. Growth here must gradually stretch that part of the haustorial larval cuticle fused to the valves while not greatly affecting that of the adjoining parts of the stalk. In one specimen about to separate from the stalk, alternate contraction and relaxation of the anterior adductor muscle was observed. Such activity will probably assist in breaking the larval cuticle, for the stalk is rigid while the part attached to the valves will be moved at each contraction. The haustorial larval cuticle splits cleanly, as can be seen both from the released mussel and, particularly clearly, from the stalk which remains attached to the host.

Rupture of the mantle prolongations is apparently due to autolysis and not, at least initially, to muscular contractions. Partition leaves the projecting ends of the prolongations bluntly rounded. Observations made on one specimen may be significant. In this individual, which had been removed from the host by severance of the stalk near its extremity, the cuticle had ruptured as had one mantle prolongation and the young bivalve remained attached to the stalk by one prolongation. As a consequence it lay more or less at right-angles to the stalk, in which condition it remained for several hours. From time to time the tip of the foot was inserted into the open end of the stalk and used as a lever to force stalk and animal apart.

Experimental infection of fishes in aquaria showed that the parasitic phase of the life cycle is passed through in about 25 days at a temperature of 24 to 25 °C. These temperatures are similar to, or possibly slightly lower than, those likely to be experienced in nature in the vicinity of Lake Victoria. Breeding takes place throughout the year.

The free-living juvenile

By virtue of its mobile foot the juvenile mussel (figure 65) is immediately capable of active locomotion. In nature, however, the situation in which it is shed would often lead to its being carried downstream were it not for the ability to secrete a byssus thread for temporary attachment. This thread consists of a single coiled hyaline filament 7 or 8 μ in diameter and usually longer than the animal. In some cases the attached extremity is somewhat flattened. As the thread is flexible the coils confer elasticity and, once anchored, the young mussel is able to move over a circumscribed area, the byssus thread acting as a life-line. When irritated the animal can sever the byssus thread at its origin.

When shed naturally young mussels are probably about 1·3 to 1·4 mm long but those prematurely released when somewhat smaller are capable of feeding and crawling, and survived equally well. Specimens kept in dishes and fed on a suspension of minute algae ('green water') lived for up to a month, by which time the valves had grown noticeably at their margins and showed distinct lines of growth. The length of the largest specimen,

1 month old at the time of death, was about 1·65 mm. Even after a month the remnant of the haustorial larval cuticle persisted around the dorsal parts of the shell. The spines present near the shell edges when the young bivalve is still attached to the host quickly disappear, suggesting that they are only important towards the end of the parasitic stage. The ligament (*RL*) is still represented by the structure already described.

Some anatomical features of the juvenile are shown in figure 65 which also gives an indication of the extensibility of the foot (*F*). The heart (*HT*) beats at a rate of 26 to 28 beats/min. The ctenidia (*C*), while still consisting of only a single row of filaments on each side, are now long and conspicuous and have begun to lay down skeletal supporting

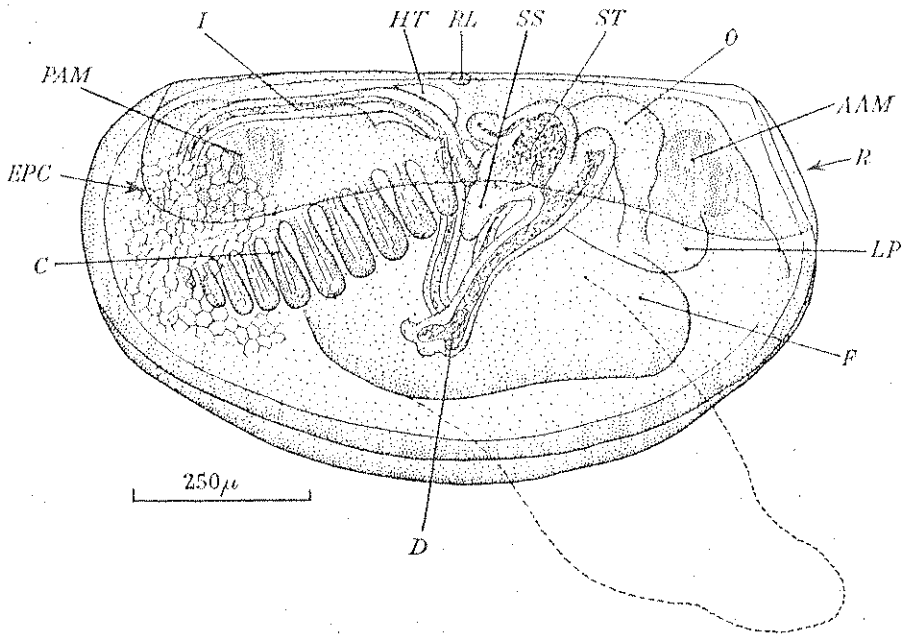
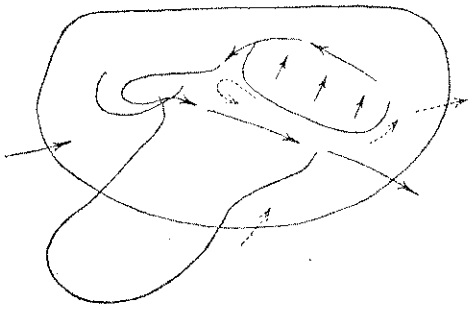


FIGURE 65. Juvenile *Mutela bourguignati* shortly after release from the stalk. The ornamentation of the shell valves is indicated near the posterior end only, and the internal organs are shown more clearly than they can be seen in life at any given level of focusing. The dotted line indicates the extensibility of the foot. *AAM*, anterior adductor muscle. *C*, ctenidium. *D*, digestive diverticulum of alimentary canal. *EPC*, edge of persistent cuticle of haustorial larva. *F*, foot. *HT*, heart. *I*, intestine. *LP*, labial palp. *O*, oesophagus. *PAM*, posterior adductor muscle. *R*, region of former attachment to stalk. *RL*, rudiment of ligament. *SS*, style sac. *ST*, stomach.

rods. The alimentary canal approaches more closely the adult condition, oesophagus (*O*), stomach (*ST*), style sac (*SS*), digestive diverticulum (*D*) and intestine (*I*) being well defined. In the presence of algal food much of the gut often shows up clearly by virtue of its green contents.

There are no signs of fusion of the mantle edges either to form the siphons or ventrally. However, feeding currents are set up. These, and particularly the inhalant current, are not absolutely constant. The ill-defined inhalant stream passes into the mantle cavity from various directions, but essentially the flow is through the mantle cavity from anterior to posterior. Currents set up by the ctenidia and labial palps are, however, well defined. The essential features of these feeding currents, as indicated by a milk suspension (Crisp & Southward 1956), are shown in figure 66.

Food can be observed rotating steadily and almost continuously in the stomach region in a clockwise direction when viewed from above as the result of the activity of the crystalline style. From time to time particles pass rapidly into or out of the digestive diverticulum (*D*) the location of which is shown in figure 65. Here they move to and fro as indicated by arrows in figure 67. Muscular contractions have been observed in the dorsal region of the digestive diverticulum, but even if these had not been seen both the speed at which particles move within the diverticulum and the velocity with which they enter it indicate that muscular activity rather than ciliary action is responsible. Sections reveal no trace of cilia in the diverticulum, which as yet possesses none of the complexity which characterizes the digestive diverticula of adult bivalves.



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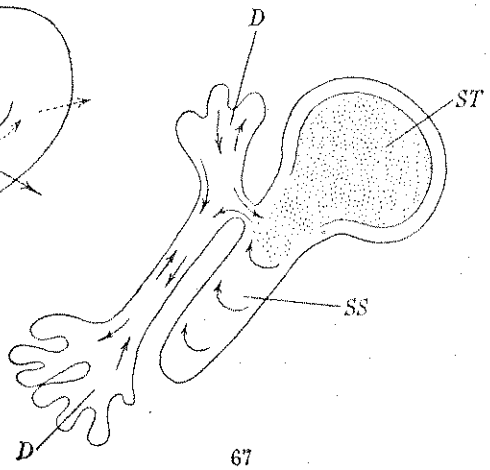


67

FIGURE 66. The feeding currents of the juvenile.

FIGURE 67. Diagrammatic representation of stomach and associated structures from a stage a little older than the specimen shown in figure 65, to show the increase in complexity of the digestive diverticula, the movement of food within them, and the rotation of the crystalline style. *D*, digestive diverticulum of alimentary canal. *SS*, style sac. *ST*, stomach.

That muscular activity should be observed is interesting in view of recent discussions on the relative importance of muscular and ciliary activity in the movement of food particles within the digestive diverticula of bivalves (see Owen (1955) for discussion). In general it seems that in adults most movement is the result of ciliary action, but in the larva of *Ostrea edulis* L. muscular activity has been shown to be all important (Millar 1955).

As the juvenile mussel grows, the extremities of the digestive diverticulum increase in complexity because of branching. The general trend of this process can be seen by comparing the condition of the diverticulum shown in figure 65, which is from a newly shed juvenile, with the diagrammatic representation of the same structure shown in figure 67 which is based on an older mussel. Changes also take place in the ctenidia. The number of filaments increases—in a large specimen thirteen, plus two small posterior papillae, were counted—and the tips of adjacent filaments fuse laterally.

Notes on anatomy and organogenesis

To supplement the general picture of development, certain details require brief comment.

(*a*) *Mantle and shell*

Complete continuity of the mantle is maintained from the free-living larva, throughout the parasitic stages, to the adult. With the exception of the supposed teloblast derivatives, the mantle and its secretions are the only differentiated structures to persist from the free-living to the haustorial larva. The original larval shell does not, however, become incorporated in the adult shell, and an interesting point arises concerning its homology. In bivalves generally the larval shell represents the periostracum (Raven 1958) and normally the calcified layers of the shell are added to it. This sequence does not apply to *Mutela* where a thick cuticle is added to the larval shell and the definitive periostracum is not produced until later. Probably the larval pellicle is to be regarded as homologous with the periostracum of other larval bivalves, but cannot, as in many of these cases, function as a base to which adult shell material can be added directly, because a new and additional stage has been incorporated into ontogeny. As a result a second primary layer has to be secreted towards the end of the parasitic phase, and it is on this base that the adult shell is laid down in a manner typical of bivalves.

(*b*) *Ctenidia*

In spite of the drastic modifications which have taken place during the evolution of the present ontogenetic sequence, the origin and early development of the ctenidia of *Mutela* are typical of that group of bivalves in which these structures originate as papillae. This method of ctenidial development appears to be of deep-seated genetical origin in certain bivalves for it has also been retained by those species whose development takes place via a glochidial stage (Schierholtz 1889; Harms 1909, etc.).

(*c*) *Heart, pericardium and kidney*

The development of these structures is similar to that which takes place in the Unionidae (Harms 1909) and in *Dreissensia* (Meisenheimer 1901). During development the inner arms of the looped kidney tubules, whose cells are markedly vacuolate at this stage, unite beneath the intestine (figure 68, *K*). The reno-pericardial aperture (*RPA*) of each kidney can be clearly seen during the later stages of parasitic life and here is located a tuft of very long cilia (figures 69 to 71).

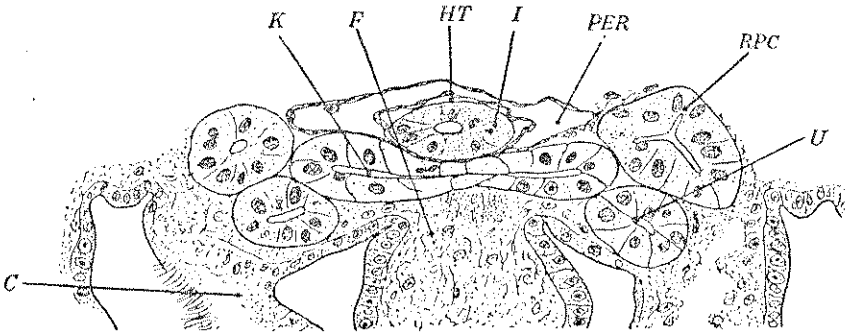
Host/parasite relationships and the question of nutrition

Mucus secretion by the host as a response to the presence of the very early parasitic stages has been described. By the time the host tissues are penetrated this mucus forms a white speck visible to the naked eye. Without such specks it would be almost impossible to locate the minute early parasitic stages.

Around the stalk of larger, firmly anchored, individuals at the point where they emerge from the host, is usually an area of suppurating host tissue often tinged with blood, indicating the rupture of small blood vessels. In aquarium experiments, where a heavy infestation was obtained on the tail fin of several host fishes, considerable areas of fin

membrane were completely eroded so that the posterior edge of the fin gave the impression that pieces had been bitten out of it. Just how much of this erosion was due to the parasite and how much to bacterial infection of the wound is not known.

Broken-down tissues are lost from the wound surrounding the stalk. This source of food has been exploited by stalked colonial ciliate protozoans (Peritricha: *Opercularia?*) which



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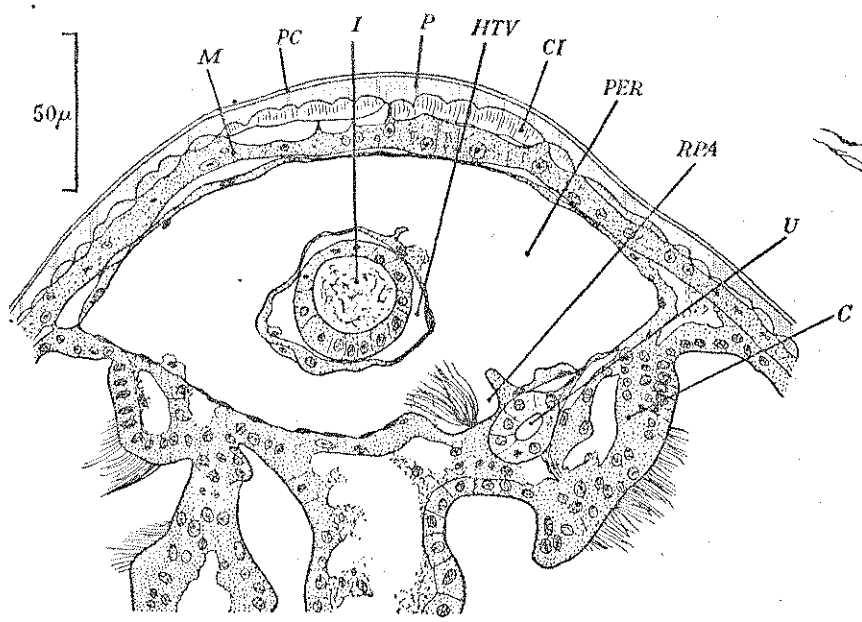

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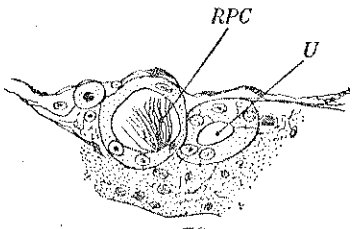

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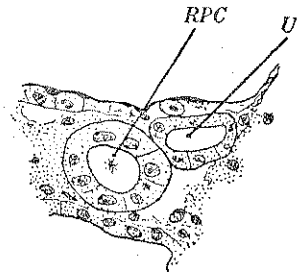


71

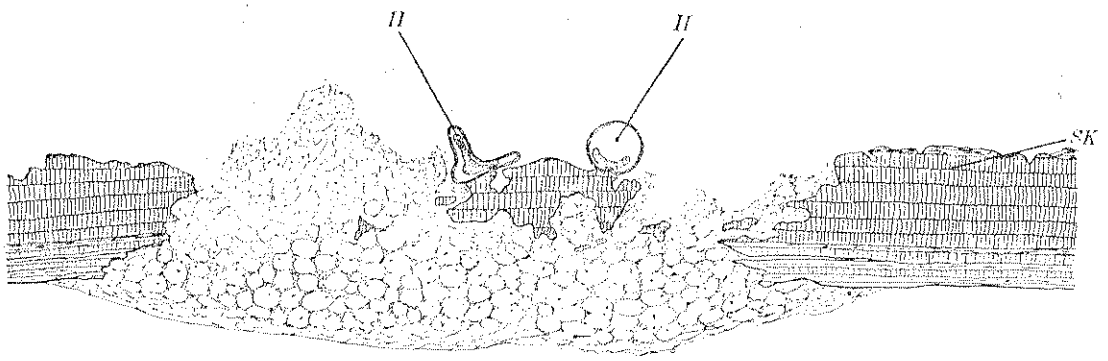
FIGURE 68. Transverse section through heart region of a young bivalve still attached to the stalk, to show the developing kidney and the heart and pericardium. *C*, ctenidium. *F*, foot. *HT*, heart. *I*, intestine. *K*, inner arms of kidney tubules uniting beneath intestine. *PER*, pericardium. *RPC*, reno-pericardial canal. *U*, ureter.

FIGURE 69. Transverse section of a slightly older specimen showing details of early shell structure and the reno-pericardial aperture. *CI*, calcareous island of definitive shell. *HTV*, ventricle of heart. *M*, mantle. *P*, periostracum. *PC*, persistent cuticle of haustorial larva. *RPA*, reno-pericardial aperture. Other lettering as in figure 68.

FIGURES 70 AND 71. Sections adjacent to that shown in figure 69, showing the beginnings of the reno-pericardial canal. Lettering as in figure 68.

are almost always present as a 'necklace', sometimes voluminous, around the stalk. This niche is virtually identical with that provided by freshwater parasitic copepods of the genus *Lernaea* around the thorax of which, near the point where they emerge from the host, similar colonies, possibly of the same species, regularly occur and utilize particles escaping from the wound caused by the parasite.

The most striking effects of the haustorial larva, however, are on skeletal structures. Figures 72 and 73 show sections through a scale to which a haustorial larva has attached



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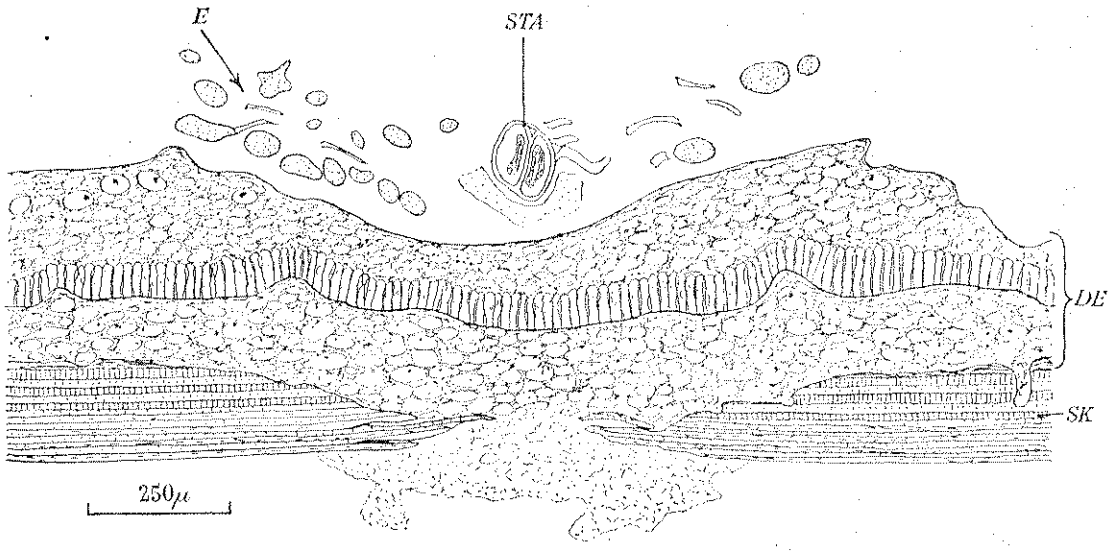

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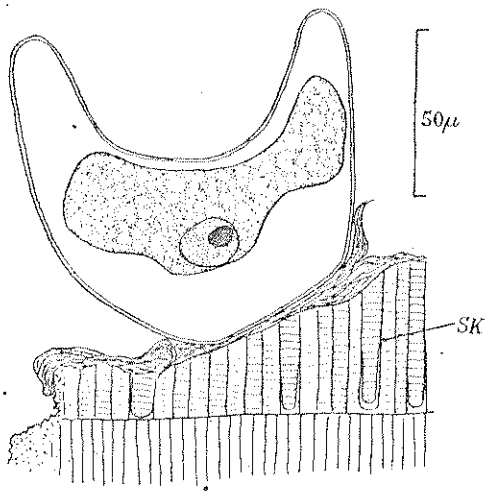
FIGURE 72. Transverse section of a haustorial larva attached to a fish scale, and cut through the haustoria. This section corresponds to the region shown in section in figure 75. The overlying dermis and epidermis of the fish scale have fallen away during preparation of the section. *H*, haustorium. *SK*, skeletal substance of fish scale.

FIGURE 73. The same, cut through the stalk region (in the region indicated by *AA* in figure 75). This section shows how digestive juices exuded through the haustorial tubes affect regions remote from them, for the erosion of the scale is the result of this process. (See figure 75 and text for further explanation.) *DE*, dermis and epidermis of fish scale. *E*, fragments of epizooic protozoans attached to stalk of haustorial larva. *SK*, skeletal substance of fish scale. *STA*, stalk of haustorial larva.

itself. Figure 72 is cut at the level shown in section in figure 75, and 73 in the region indicated by the line *AA* in figure 75. These show how the haustoria become firmly attached to a scale (see also figure 74) and how in their vicinity the skeletal substance of the scale (*SK*) becomes eroded and broken down. It is curious that those parts of the scale actually in contact with the haustorial tubes are not affected so much as are adjacent regions. The digestive juices which must be exuded by the haustoria appear to take effect at some distance from the tubes as indicated in figures 73 and 75. Here the scale substance has broken down, presumably as a result of seepage of material in the direction indicated by



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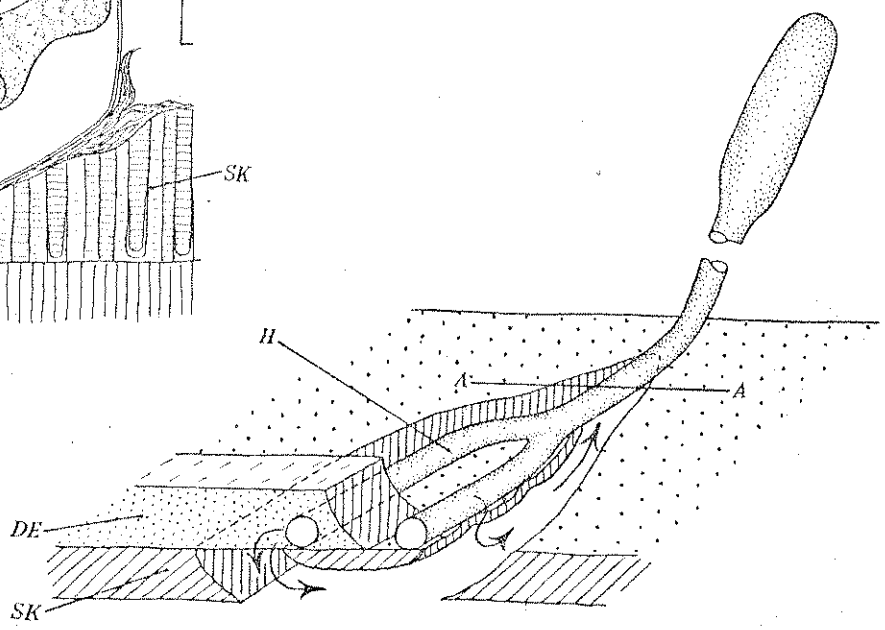

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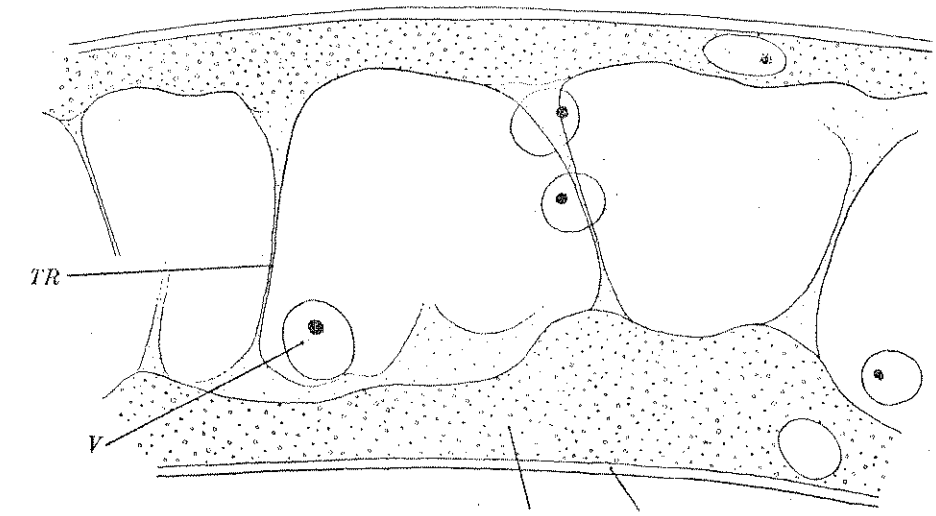
FIGURE 74. Transverse section near the tip of a haustorial tube *in situ* on a fish scale. *SK*, skeletal substance of fish scale.

FIGURE 75. Diagrammatic representation of a haustorial larva *in situ* on a fish scale, showing the effects of the digestive juices secreted by the haustoria whose course is shown by arrows. For further explanation see text. *DE*, dermis and epidermis of fish scale (shown only in one region). *H*, haustorium. *SK*, skeletal substance of fish scale.

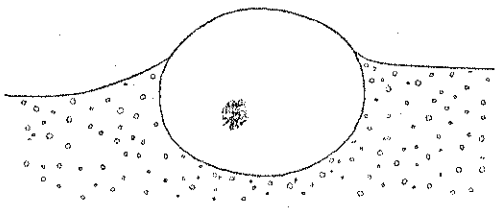
the arrows in figure 75. The soft tissues of the host (*DE*) are, however, not entirely unaffected and often respond by undergoing local proliferation in the vicinity of the haustoria thereby filling the gap eroded in the skeletal substance of the scale (figures 72 and 73).

The parasitic larva of *Mutela* stands alone among described molluscs in absorbing nutriment through the haustorial tubes. While it is remarkable that tissues derived from the mantle, and therefore ectodermal in origin, should fulfil a nutritional function, this state of affairs has a parallel in the development of the Unionidae via the glochidium in

which both larval and definitive mantles are employed for this purpose. There, however, the mantle is applied directly to the host tissues and, while some extracellular digestion almost certainly takes place initially, it has been established for several species that intracellular digestion involving pseudopodial activity plays an important part in the process (Faussek 1895, 1901, 1903; Blystad 1923). Conditions imposed by the complete enclosure of the mantle prolongations in *Mutela* preclude any possibility of intracellular digestion and all the nutriment absorbed must diffuse through the walls of the haustorial



76



77

FIGURE 76. Optical section, to which certain details revealed by deeper focusing have been added, of part of haustorial tube not far from its tip, of a haustorial larva in which foot and ctenidia are beginning to be differentiated. From a living specimen. *HLC*, haustorial larval cuticle. *MP*, mantle prolongation. *TR*, trabecula. *V*, vesicle of haustorium.

FIGURE 77. A vesicle in process of extrusion into the central cavity of the haustorium.

tubes. The nearest, though nevertheless quite different, and less specialized, approach to this condition is to be found in the glochidium of *Lampsilus luteola* (Lamark) where a placenta-like arrangement between larval mantle and host tissues is established (Blystad 1923).

As might be expected in an organ with such a function, the living tissues of the haustoria (*MP*) are located peripherally and are everywhere in contact with the walls of the tubes. This is shown in figure 76 which has been prepared from a living specimen. (In the figures of transverse sections of preserved material the cytoplasm has contracted away from the walls.) The ectoderm (*MP*) here consists of granular, watery cytoplasm forming a cylinder whose walls are of irregular thickness and which lies against the inner face of the cuticle

(*HLC*) comprising the haustorial tubes. Cell boundaries, at least in the later stages of development, are not apparent. The central cavity, which appears to be filled with fluid, is spanned by numerous cytoplasmic strands or trabeculae (*TR*). The cytoplasm contains numerous conspicuous inclusions of a characteristic type. These are ovoid to spherical vesicles (*V*) whose diameter may exceed 17 μ and which are bounded by a membrane. They contain clear, colourless cytoplasm, and as a rule one, or in some cases two, clumps of darker material. Sometimes this gives the impression of a cell nucleus though usually it can be seen to be granular in nature and sometimes appears as a rather loose aggregation of granules (figure 77). Occasional vesicles seem to lack these darker bodies. As indicated in figures 27 to 31 and figure 74, the granules stain darkly as a large nucleus-like mass. What are presumably the precursors of these vesicles are found in early parasitic stages. It appears that the vesicles are extruded from the inner wall of the cytoplasmic cylinder into the central cavity, and figure 77 shows one in process of extrusion. Within the central cavity they maintain their identity even in sectioned material, so their presence there seems to be no accident.

The true nature of the various parts of these vesicles is uncertain, but they probably serve as a means of transporting food obtained near the extremities of the haustoria to the basal regions where it is re-absorbed.

The cuticle of the haustorial larva

The cuticle which invests the haustorial larva is the linear descendant of the pellicle of the free-living larva. From an early stage it is thick as a result of deposition of material inside the original very thin pellicle. The two layers are distinct and maintain their identity throughout development, though the thin outer layer cannot always be made out clearly in sections and sometimes gives the impression of being illusory, the result of refringence at the outer margin of the cuticle. Of its reality, however, there is no doubt for in some sections it can be clearly seen and sometimes, as in figures 26 (particularly inset) and 35, it peels from the underlying cuticle. It can also be demonstrated by chemical means. As the superficial cuticle persists until a late stage of development it must either be continually produced, but by what process is difficult to appreciate as all secretory tissues are separated from it by the thick cuticle, or represent a change in the cuticle resulting from contact with the external environment—water or host tissues.

Although secreted by the mantle or its prolongations, the cuticle of the haustorial larva is not composed of conchiolin—a term of no precise chemical significance (Trueman 1949) —nor is it calcareous. The difference between it and periostracal conchiolin is strikingly apparent when the young bivalve begins to secrete the first adult periostracum. This clear amber-coloured material differs markedly from the almost colourless cuticle to which it becomes adpressed but with which it does not mingle.

Physically the cuticle is tough but pliable. Simple chemical tests reveal the following properties. It is very resistant both to concentrated mineral acids and caustic alkalis. It does not dissolve in cold concentrated hydrochloric, sulphuric, or nitric acids. In nitric acid it becomes only faintly brown and can hardly be said to give a positive result to the xanthoproteic test. It does not dissolve in hot concentrated hydrochloric acid but disperses completely in hot concentrated sulphuric acid (becoming an intense brown as it

does so) and in hot concentrated nitric acid. It is very resistant to a saturated aqueous solution of potassium hydroxide and resists boiling in such a solution for some time but eventually breaks down. When heated to 160 °C for 15 min in such a solution some portions dissolve but fragments remain. These readily dissolve in concentrated nitric acid and in acetic acid so the material is *not* chitin.

Even a 1% solution of sodium hypochlorite (the strongest at first available) causes disintegration of the cuticle: after about 20 h immersion the walls are much weakened and crumble when touched with a needle. Nevertheless, even after a week's immersion the walls retain their form. They are now, however, exceedingly thin and apparently composed only of the transparent superficial cuticle. To test this a stalk was cut into short pieces which were immersed in a 1% sodium hypochlorite solution. After 2 days these were still intact but the walls were very thin. That digestion had taken place from the *inside* and that the persisting remnant was the superficial cuticle was proved by the fact that, although most of the numerous attached peritrichs (see p. 289) growing on one section had fallen away, the stalk of one of these remained attached. If the superficial cuticle remaining after immersion for some time in sodium hypochlorite solution is broken into fine pieces these also gradually dissolve. These results and the fact that, although the xanthoproteic test is only very weakly positive, a positive result is obtained to the argentaffin test, suggests that the cuticle (including the superficial cuticle?) is composed of a quinnone-tanned protein. The production of such proteins is not unknown in the Mollusca, for Brown (1952) has shown that they comprise the material forming both the periostracum and the byssus of *Mytilus*. Possibly, therefore, in spite of their obvious differences, the cuticle and the periostracum may not be very different chemically.

Relationships of the larval stages

(*a*) *The free-living larva*

Superficially the free-living larva resembles a veliger, and it is tempting to suggest that it represents a larva of this type in which development of the foot, gut, and all tissues of mesodermal or endodermal origin has been suppressed and in which certain other structures, notably the tentacle and posterior hooks, have been developed. The differences, however, are deep seated and it is unwise to press homologies too far. Even the ciliated lobes, which immediately suggest the velum of a veliger, differ markedly from that structure. According to Pelseneer (1906) the velum of the bivalve veliger is never lobed: in the free-living larva of *Mutela* the ciliated lobes are separate entities. The ciliation of the velum also differs from that of the lobes and, while the two are analagous, it would be rash, in the absence of comparative embryological data, to homologize them.

Similarly it is difficult to relate the free-living larva to the glochidium larva of the Unionidae. Both are specialized, but in different directions. With the lasidium larva of the South American *Anodontites* (= *Glabaris*) the first larval stage of *Mutela* appears to have much greater affinity. Each consists basically of a simple body covered dorsally and, to some extent at least, laterally by a thin pellicle, with a cilia-bearing organ anteriorly, and with some form of hooks posteriorly. In the lasidium the cilia-bearing organ is a single median lobe. Nevertheless, this is more like the ciliated lobes of the free-living larva of *Mutela*, which could have been derived from such a structure by cleavage, than like the

velum of a veliger. Its ciliation is very similar to that of these lobes. The 'hooks' of the lasidium are different from, and perhaps less specialized than, those of the first larval stage of *Mutela*. Their presence, however, suggests that they fulfil a similar function and that the lasidium becomes parasitic.

The lasidium also produces a tentacle which, although broad and flat and considerably shorter than the tubular structure present in the larva of *Mutela* (though it may be up to ten times as long as the larva itself) (von Ihering 1891/92), is doubtless homologous with it. Virtually nothing is known about the internal anatomy of the lasidium and von Ihering's statement that the large cells to be seen internally are endoderm remains to be proved, as does his conjecture about the presence of an oesophagus.

The question of a name for the first larval stage of *Mutela* raises a problem. To refer to it as a lasidium might imply unwarranted affinities with the lasidium of South American mussels, while to give it a distinctive name could obscure its obvious relationships to the lasidium. This matter cannot be elucidated until the latter is better known. For the present it seems best to be non-committal and to refer to it simply as the free-living larva.

There remains the problem of the tentacle. This has a counterpart in some glochidia and also in the lasidium. Although the tentacle of some glochidia is extremely long (Lillie (1895) cites Forel as mentioning some 10 to 15 mm in length) it is doubtful if any are relatively, or perhaps even absolutely, as long as that of the free-living larva of *Mutela*. Although its probable homologue in the glochidium is still often termed the larval byssus it is certainly *not* a true byssus as Carrière showed as long ago as 1884, if only because the glochidium also possesses a true byssus gland. Carrière, who referred to it as a 'Klebefaden', regarded it as an organ *sui generis* as did Lillie (1895), and it is difficult to be more specific. In the glochidium it has generally been regarded as a means of assisting the larva to attach itself to its host; it is almost certainly not a flotation mechanism for the glochidium normally rests on the bottom. The manner in which the tentacle assists the larva to attach itself does not seem to have been observed, and Lefevre & Curtis (1912) hold that glochidia which have shed their tentacle are more successful in attaching themselves than are those which retain it. A new suggestion which would apply to such glochidia as possess a tentacle and occur in rivers, and perhaps to the lasidium, as well as to the larva of *Mutela*, namely, that it serves as an anchor, has been made on p. 267. Lillie (1895) suggests that its original function was excretory and that it is composed of insoluble excretory matter. His thesis is that the excretion of such insoluble matter represents an adaptation to development within a strong vitelline membrane where soluble excretory products would be undesirable and that the thread-like form is the mechanical result of the form of the excretory gland. With this suggestion it is difficult to agree, not only because other animals, such as certain fishes, are active while still within the egg membrane yet are able to rid themselves of soluble excretory products, but particularly because Schierholtz (1899) had already shown that some glochidia lack a tentacle. Such a lack was later shown to be typical of the glochidia of several North American mussels (Lefevre & Curtis 1912).

(*b*) *The haustorial larva*

The haustorial larva represents a hitherto undescribed stage which has been incorporated into the life cycle and increases the efficiency of a phase of protelean parasitism. Whereas

the glochidium serves as both a distributive and as a parasitic larva, specialization has here gone a stage further and the free-living larva, which originally must have served both functions, now fulfils merely a distributive role. The haustorial larva could have evolved as a development of the free-living larva which, after settlement, obtained its nutriment by the application of naked mantle tissue to the tissues of its host, much as does the glochidium today. From this stage specialization could proceed, with the mantle prolongations probably at first merely covered, and not completely enclosed, by an extension of the cuticle, and only later becoming enclosed—presumably after the evolution of digestive processes capable of functioning under such conditions. Increasing and more prolonged dependence on the host would result in a slowing down in development of adult organs and ultimately the evolution of an entirely new method of gut formation, for the alimentary canal would begin to function ever later in ontogeny and could not be formed by the usual methods. In spite of these great specializations, however, such organs as the definitive shell, foot, ctenidia and kidneys develop in a typical bivalve manner, and so apparently does the stomach, though in all cases the process is delayed.

The successful evolution of such a larval form stems largely from the versatility of the mantle which, apart from undergoing great morphological changes, has had to assume the role of an organ of nutrition and produce a cuticle of different composition from that of any of the layers of the shell. In fact the very possibility of exploiting parasitism as a mode of larval life was dependent from the outset on the ability of the mantle to fulfil an absorptive function, for it is difficult to conceive of the free-living larva as having passed through a stage in which it was capable of having absorbed host tissues by more conventional means.

Discussion

The differences in larval anatomy and ontogeny between *Mutela* and the Unionidae are remarkable when one considers the great similarity of the adults—a similarity which makes familial recognition on anatomical grounds difficult for any but the specialist. No more striking example is found in the animal kingdom. No case cited by de Beer (1958) as an example of caenogenesis is more remarkable. This, the result of divergent specialization of larval forms, should be given due weight in classification, and it may well be that the family Mutelidae will have to be revised. The so-called 'mutelids' of Australasia, for instance, produce glochidia, as do the Unionidae, a fact which, taken alone, indicates that their affinities may lie with the widespread Unionidae rather than with the African and South American mutelids whose relationships were recognized with great insight by von Ihering; relationships which seem to be borne out by the similarity of the larval forms. On the other hand these freshwater mussels must be regarded as persistent types, for alleged mutelids are known as fossils in the Northern Hemisphere from rocks as old as the late Palaeozoic (Modell, cited by McMichael & Hiscock 1958), and it is conceivable that larval divergence may have taken place within those members of the family which became isolated in Africa and South America (perhaps then united?) and which have been isolated for a long period from the Australasian forms. These latter are believed by McMichael & Hiscock (1958) to have colonized Australasia from the north in about Triassic times and, whatever their origin, must have been isolated from at least this period onwards. This divergence of larval forms, whatever the relative standing of the Unionidae and

Mutelidae, may be indicative of a new spurt of evolution in an ancient stock which had become morphologically stable in adult features but which began to exploit the opportunities presented by the adaptive radiation of teleost fishes which took place in post-Triassic times. Earlier than that, hosts of the type utilized today did not exist.

Too little is known of host preferences to permit speculation, but the association between *M. bourguignati* and a cyprinid fish is apparently of more recent origin than any time when South America and Africa may have been united, for cyprinid fishes are unknown in South America.

Heavier infestations of the host by the parasitic stages of *Mutela* are found in the Victoria Nile than in Lake Victoria. As the host is more abundant in the river than in the lake, free-living larvae are more likely to locate a host in the former situation. During its free-living larval stage, therefore, this bivalve is more favoured by riverine than by lacustrine conditions. Probably the present-day population of *M. bourguignati* in Lake Victoria was established by individuals carried there by fishes since the lake assumed its present form in comparatively recent times.

It is certain that other mutelids will be found to have a similar development to that of *M. bourguignati*, for, subsequent to the discovery of the parasitic stages of this species, search was made in Lake Tanganyika by Mr H. Matthes who has succeeded in finding similar but as yet unidentified parasitic stages on two species of cyprinid fishes. During the present work also two late haustorial larvae were found which were much broader and shorter than typical individuals of *M. bourguignati*. These may be deformed individuals of this species, though they were quite healthy, or may represent another species.

The interest shown in my work by Professor C. M. Yonge, C.B.E., F.R.S. throughout the time I was in the field has been a source of great encouragement to me. At this time too he gave help in a very practical way by having sections cut for me in his department. Some of these were dealt with personally by Dr Gareth Owen. I had the benefit of discussing my results with Professor Yonge and Dr Owen, and both very kindly read my manuscript and offered much useful advice and criticism. For these kind actions I should like to express my sincere thanks.

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