SOME ASPECTS OF THE EMBRYOLOGY OF *TETRASTICHUS PYRILLAE* CRAW—AN IMPORTANT CHALCID EGG PARASITE OF *PYRILLA PERPUSILLA* WALKER—A SERIOUS PEST OF SUGAR-CANE *

by KUNDAN LAL, *Division of Entomology, Indian Agricultural Research Institute, New Delhi*

(Communicated by G. P. Sharma, F.N.I.)

(*Received 2 February 1967 ; after revision 4 March 1968*)

Some aspects of the embryology of the parasite *Tetrastichus pyrillae*—an egg parasite of *Pyrilla perpusilla* Walker—have been studied. *Tetrastichus pyrillae* egg 0·25 mm in length is contained in *Pyrilla* egg which is 0·90 mm long. The inner layer in case of the developing egg of *Tetrastichus pyrillae* represents a common mesoendoderm rudiment. The mesoderm is derived from the inner layer. Anterior and posterior endodermal rudiments are formed from inner layer 26, 28 hours respectively after oviposition, at the anterior and posterior blind ends of the embryo. The mid-section, i.e. mesenteron, is formed as a sac from the inner layer, i.e. from the anterior and posterior endodermal rudiments, and the mid-ventral endodermal strand, i.e. the alimentary canal is formed of Stomodaeum and Proctodaeum, of ectodermal origin, and mesenteron of endodermal origin.

INTRODUCTION

In the published literature we have large number of fine contributions on insect embryology. Yet there are many problems on which there has been no unanimity amongst the workers. For instance, there is a gap in our knowledge of the origin of the midgut epithelium and the number of segments in the head region, etc. Some aspects of the embryology of *Tetrastichus pyrillae* have been studied with a view to finding out some of the complexities of its embryonic development.

MATERIAL AND METHOD

The descriptive embryology has been studied with two techniques, i.e. the study of the entire egg as a whole mount and serial sections of egg of parasite which is (0·25 mm long) contained in the host egg which is 0·9 mm long. The eggs were fixed *in situ* in Cornoy's solution within the host egg. Then the eggs were dehydrated in alcohol series, kept in cedar-wood oil and then passed through benzene and embedded in paraffin. The eggs were then cut, along with the host egg, into serial sections of 6 to 8 μ. The ribbons were

* Part of the thesis approved for the award of Ph.D. degree of the Panjab University.

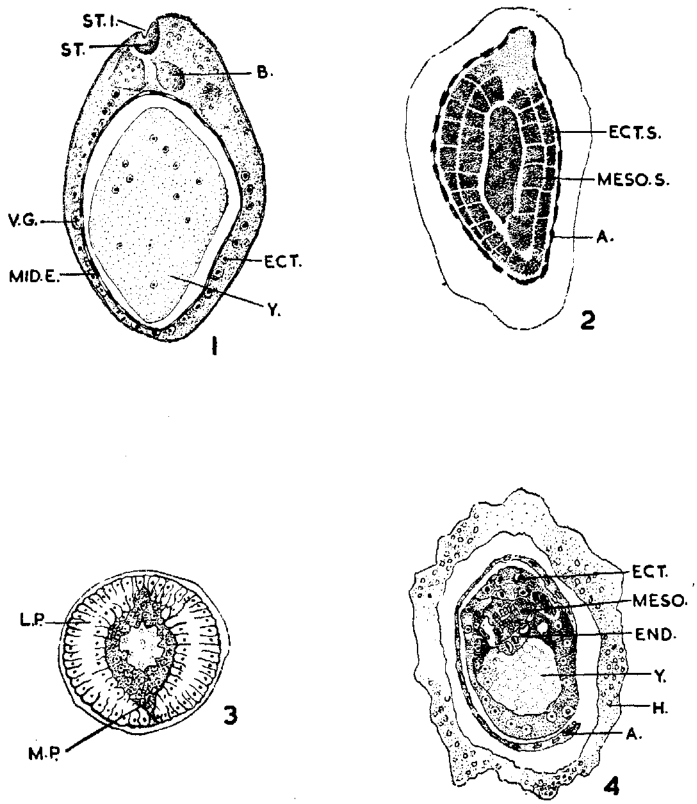
fastened to the slide with Mayer's albumen adhesive and were stained and mounted. Critical stages, both early and late, were stained with Delafield's haematoxylin stain as the variety of stains such as eosine, acid fuchsin, etc., did not give good results. For earlier stages, the age of the egg was known within hours. For later stages, their ages were not quite accurately known in all cases. Thus the later stages of eggs of the parasite, contained in the host egg, were incubated under constant temperature, i.e. 18 °C. temperature and 80 per cent relative humidity, for varying length of time from two hours to two days. The duration of embryonic period of control under the conditions was approximately two days. There was slight variation in time taken to hatch, but it was never longer than two days for the eggs to hatch successfully.

Differentiation of Germ Band into Different Layers and Formation of Alimentary Canal

According to germ layer theory, midgut epithelium is endodermal in origin and many investigators have established this homology and deriving the condition found in the insect egg, from the typical gastrula. This has been found so difficult that Nelson (1915) quoting Wiesman says, 'It becomes more and more evident that nowhere in the entire animal kingdom is the ontogeny so distorted and coenogenetically degenerate, as in insects, so that scarcely anywhere are germ layers so difficult to recognize as here.' The difficulties of interpreting the homologies of germ layers, especially in insects, are so great that writers from time to time have questioned the validity of the germ layer theory. Besides, it is a difficult task to find out these facts in the minute egg of this parasite (*Tetrastichus pyrillae* egg, 0·25 mm long) contained in the egg of the host (*Pyrilla* egg, 0·90 mm long).

Examination of sections of a large number of *Tetrastichus pyrillae* eggs, in which blastoderm was formed, showed the presence of yolk nuclei. An anterior ventral groove is seen in the germ band on its ventral side (Fig. 3) 22 hours after oviposition. Round the bend of the groove is seen the inner layer or middle plate and on the side of this anterior ventral groove is seen the outer layer or lateral plates. The inner layer begins by invagination of the median plate and as invagination progresses, cells become completely separated off from the lateral plates. The lateral plates soon close in completely (Fig. 4) under the invaginated mass. This anterior ventral groove may represent the gastrocœle of other animals. Thus the embryo or germ band becomes two-layered which may be considered as a modified method of gastrulation as true gastrulation is wanting in this case. This is seen 22 hours after oviposition (Fig. 3).

Formation of Mesoderm.—The embryo becomes three-layered by the formation of mesoderm from the inner layer (Fig. 4). The inner layer represents a common mesoderm-endoderm rudiment since part of the endoderm is usually formed from mesoderm, which is in close association with it. Mesoderm lies in between ectoderm and endoderm (Fig. 4). The mesoderm somites are seen between midgut and ectodermal segments (Fig. 2). Thus embryo is a three-layered structure as shown by experimental evidence. The mesoderm becomes arranged in segmental masses (Fig. 2).



FIGS. 1–4. 1, L.S. of egg—24 hours old (B., brain; ECT., ectoderm; MID.E., midgut epithelium; ST., stomodaeum; ST.I., stomodaeum invagination; V.G., ventral ganglia; Y., yolk); 2, L.S. of egg—35 hours old (A., amnion; ECT.S., ectodermal somite; MESO.S., mesodermal somite); 3, T.S. of egg—22 hours old (L.P., lateral plate; M.P., middle plate); 4, T.S. of egg—24 hours old (A., amnion; ECT., ectoderm; END., endoderm; H., host; MESO., mesoderm; Y., yolk).

The Anterior Endoderm Rudiment.—The anterior ventral groove is formed posterior to the cephalic lobes. The stomodaeum has not as yet appeared. This is the place where the anterior endoderm rudiment is formed. It manifests itself as a rapid proliferation of cells which result in the formation of nearly rounded mass or heap near the middle of cephalic lobe. Soon after the formation of endoderm rudiment near the cephalic lobe, a further invagination of the ventral groove occurs, which pushes the rudiment still further inward into the yolk. This is the stomodaeum (Fig. 1). This is 26 hours after oviposition.

The Posterior Endoderm Rudiment.—This is seen 28 hours after oviposition. The inner margin of the posterior end of germ band gives rise to the posterior endodermal rudiment. This rudiment can be distinguished from proliferation, along the middle line by the fact that its constituent cells do not migrate towards the yolk but are all aggregated together to form a heap of cells like the anterior rudiment.

Finally, it is found that two anterior and posterior rudiments are situated at the anterior and posterior blind ends of the embryo. These rudiments are connected by the ventral mid-strand of endoderm (Fig. 7). 28 hours after oviposition the posterior rudiment begins to differentiate. The proctodaeum starts as in pushing of the ectoderm against this mass. The anterior and posterior endoderm rudiments, along with ventral middle strand of endoderm which may represent yolk cell membrane of other workers, give rise to the definitive lining of the midgut by rapid division and spreading of cells from the anterior and posterior endodermal rudiments. There is reason to believe that enclosure of yolk by endoderm cells derived from mesoderm and endoderm rudiment, i.e. inner layer, does not strictly represent the process of true gastrulation in *Tetrastichus pyrillae.*

Stomodaeum.—This is the first to appear as an oval depression in the middle of head segment 26 hours after oviposition (Fig. 1). Later the depression becomes crescentic. This develops immediately in front of the anterior rudiment as also seen by Nusbaum and Fulinski (1906) in *Phyllodromia.*

Proctodaeum.—It arises rather later after the formation of posterior endodermal rudiment 28 hours after oviposition. It arises as dorsoventral invagination a little in front of the posterior endodermal rudiment from the hind end of germ band (Fig. 6).

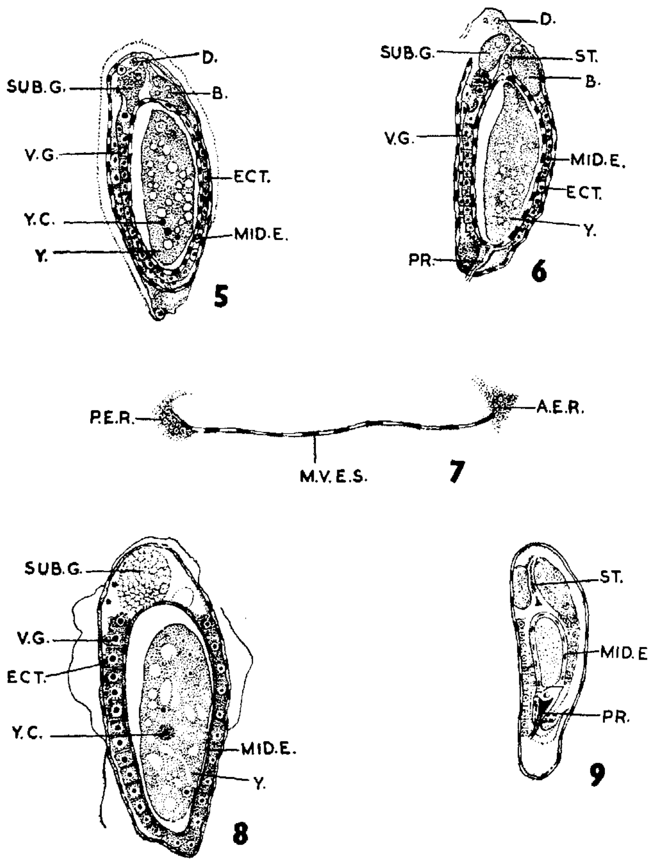
Alimentary Canal.—The alimentary canal is formed of three sections. The anterior section or stomodaeum is formed by ectodermal invagination at the anterior extremity of the germ band, while the posterior section is formed somewhat later by a similar invagination at the hind end.

The mid-section or mesenteron is formed as a sac from the inner layer, i.e. from the anterior and posterior endodermal rudiments and the mid-ventral endodermal strand. It has been observed that proctodaeum has not joined the midgut (Fig. 9) which shows that the posterior portion of midgut does not take its origin from the extremity of proctodaeum. The latter is ectodermal in origin. In it is definitely seen (Fig. 9) that the stomodaeum and proctodaeum are quite separate from midgut, i.e. the midgut does not originate from these and as such is not ectodermal in origin. The stomodaeum has joined with the anterior portion of the midgut and its walls are separate. A continuous passage through stomodaeum is established, by the absorption of the adjoining wall of the stomodaeum and the midgut, where they are opposed to one another. It has been observed that the endodermal rudiments are cut off from the germ band before the formation of stomodaeum and proctodaeum of ectodermal origin. It may be seen that stomodaeum is formed by invagination, after the germ layers—ectoderm, endoderm and mesoderm—have already been formed (Fig. 1). Thus the anterior and posterior endodermal rudiments and mid-ventral endodermal strand derived from the inner layer contribute to midgut formation. The yolk cells seem to be non-germic.

Discussion

There is a gap in our knowledge of the origin of the midgut epithelium. The interpretation of middle layer has been much discussed. Heymons (1895) derives the midgut in Pterygota from stomodaeum and proctodaeum and identifies the endoderm with yolk cells. The observation on the development of *Tetrastichus pyrillae* eggs has revealed that the inner layer is considered as mesoendoderm on the ground that midgut arises from it, from the cells of the median part of the inner layer, as well as from cells localized in the anterior and posterior endodermal rudiments. Here the yolk cells are described as parablast. They have been observed (Fig. 8) in yolk and most probably their function is to liquefy the yolk for the assimilation of embryo and they take no part in the formation of the germ layer of embryo. In *Tetrastichus pyrillae* egg it has been observed that inner layer plays an important part in the development of the midgut. The midgut has not been seen arising from stomodaeum or proctodaeum which are without any doubt ectodermal in origin. Thus it has been observed that the midgut is derived from inner layer, along its whole length on the ventral side which is in the form of middle ventral strand of endoderm as well as anterior and posterior rudiments of endoderm. It means in addition to the bipolar origin of the midgut from anterior and posterior endoderm rudiments, the middle ventral strand of endoderm also goes to form the midgut. The histological feature is that the inner layer has acquired a lighter stain than the outer layer, while staining with Delafield's haematoxylin. The part of the inner layer, i.e. the endoderm which surrounds the yolk and forms the midgut, has nuclei slightly flattened and enlarged (Figs. 5, 6 and 8) and nuclei of the outer layer are almost round in shape. Thus there is adequate reason for identifying any part of the midgut of *Tetrastichus pyrillae*. Inkmann also (1933) derives the midgut from the innermost cells of inner layer and regards them as endoderm. He distinguishes the inner layer by histological features from the outer layer.

Thomas (1936) has observed in stick-insect, *Carausius morosus*, that the primitive endoderm cells are proliferated from the middle of germ band and form a membrane between germ band and yolk. He has further observed that masses of cells at the anterior and posterior end of germ band are to be endodermal rudiments from which midgut epithelium has been formed. Thus the formation of midgut from endoderm in case of *Tetrastichus pyrillae* shows



FIGS. 5–9. 5, L.S. of egg—28–30 hours old (B., brain; D., dorsal organ; ECT., ectoderm; MID.E., midgut epithelium; SUB.G., subaesophageal ganglia; V.G., ventral ganglia; Y., yolk; Y.C., yolk cell); 6, L.S. of egg—28 hours old (B., brain; D., dorsal ganglia; ECT., ectoderm; MID.E., midgut epithelium; PR., proctodaeum; ST., stomodaeum; SUB.G., subaesophageal ganglia; V.G., ventral ganglia; Y., yolk); 7, A.E.R., anterior endoderm rudiment; M.V.E.S., mid-ventral endodermal strand; P.E.R., posterior endoderm rudiment; 8, L.S. of egg—28 hours old (ECT., ectoderm; MID.E., midgut epithelium; SUB.G., subaesophageal ganglia; V.G., ventral ganglia; Y., yolk; Y.C., yolk cell); 9, L.S. of egg—28 hours old (MID.E., midgut epithelium; PR., proctodaeum; ST., stomodaeum).

some resemblance to the condition described by Thomas (1936) in case of stick-insect, *Carausius morosus*. Tiegs (1938) while dealing with embryonic development of *Calandra oryzae* has stated that stomodaeum and proctodaeum arise as simple ingrowth of the outer layer. He further remarked that the midgut is formed from the blind end of stomodaeum and proctodaeum, quite independently of the inner layer, i.e. it is ectodermal in origin. He has further said that the development of midgut remains one of the most controversial problems of descriptive embryology. As regards the suggested ectodermal nature of midgut epithelium as seen by Tiegs (1938) it should be noted that endodermal rudiments may be so small as to be readily mistaken for proliferation from ectoderm. The endoderm cells are distinguished from the ectoderm by difference of appearance which largely depended upon the fixative and the stain used.

Observation in *Tetrastichus pyrillae* on the formation of endoderm rudiment of the midgut thus agrees closely with observations of Eastham (1927) for *Pieris rapae,* Henson (1932) for *Pieris brassicae,* Wheeler (1889, 1893) in *Blatta,* Nusbaum and Fulinski (1906, 1909) in *Phyllodromia* and *Gryllotalpa,* Thomas (1936) in stick-insect, *Carausius morosus,* Mellanby (1936) in *Rhodnius* and Paterson (1936) in *Corynodes.* The results of Heymons (1895) for Orthoptera, Mansour (1927) and Tiegs (1938) for *Calandra* are contrary to the above observations.

Thus it can be noted that most of the recent workers on the development of midgut rudiments in insects are divided into two groups. One school of thought is of the opinion that midgut is ectodermal in origin from the blind ends of stomodaeum and proctodaeum while the other opines that the formation of midgut is derived from the endoderm. The author has clearly proved by experimental evidence that midgut has been formed from the anterior and posterior endodermal rudiments, and middle ventral endodermal strand, derived from the inner layer in *T. pyrillae.*

ACKNOWLEDGEMENTS

I take this opportunity of expressing my deep sense of gratitude to Dr. E. S. Narayanan, formerly Head of the Division of Entomology, Indian Agricultural Research Institute, New Delhi, for providing the necessary facilities to carry out this piece of research work and for guidance throughout the course of these investigations. Acknowledgement is also due to Shri A. K. Roy, Head Artist, for his accurate *camera lucida* drawings.

REFERENCES

Eastham, L. E. S. (1927). Contribution to the embryology of *Pieris rapae. Quart. J. micr. Sci.*, **71**, 353–394.

Henson, H. (1932). The development of alimentary canal in *Pieris brassicae* and the endodermal origin of Malpighian tubules in insects. *Quart. J. micr. Sci.*, **75**, 283–309.

Heymons, R. (1895). Die Embryonalentwicklung von Dermapteron Und orthopteron unter besondever Berucksichtigung der Keimbaltterbildung, pp. 1–136.

Inkmann, F. (1933). Beitrage zur Entwicklungsgeschichite des Kornkafers (Calandra granaria). Die Anfangsstadien der Embryogenese. *Zool. Jahrb.* (*Anat.*), **56**, 521–558.

Mansour, K. (1927). Development of larval and adult midgut of *Calandra oryzae. Quart. J. micr. Sci.*, **71**, 312–352.

Mellanby, H. (1936). The early embryonic development of *Rhodnius prolixus*. *Quart. micr. Sci.*, **78**, 71–90.

Nelson, J. A. (1915). 'Embryology of Honey Bee.' Princeton Press, Princeton, Humphrey Milford, Oxford University Press, London.

Nusbaum, J., and Fulinski, B. (1906). Ueber die Bildung der Mitteldarmanlage bei *Phyllodromia* (*Blatta*) *germanica*. *Zool Anz.*, **30**, 362–381.

——— (1909). Zur Entwicklungsgeschichte des, Darmdrusenblates bei *Gryllotalpa vulgaris*. *Z. Wiss, Zool*, **93**, 306–348.

Paterson, N. F. (1936). Observations on the embryology of *Corynodes pusis* Marsh. *Quart. J. micr. Sci.*, **78**, 91–131.

Thomas, A. J. (1936). Embryonic development of *Carausius morosis*. *Quart. J. micr. Sci.*, **78**, 487–511.

Tiegs, O. W. (1938). Embryonic development of *Calandra oryzae*. *Quart. J. micr. Sci.*, **80**, 159–284.

Wheeler, W. M. (1889). The embryology of *Blatta germanica* and *Doryphora decemlineata*. *J. Morphology*, **3**, 291–386.

——— (1893). Contribution to insect embryology. *J. Morphology*, **8**, 1–160.