The Embryonic Development of Trichogramma evanescens, Westw., Monembryonic Egg Parasite of Donacia simplex, Fab.

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With Plates 10, 11, and 12.

Introduction.

Since the description of polyembryony in some parasitic Hymenoptera by Marchal (1) the attention of a few zoologists[1] has been turned to the interesting problems these forms offer.

[1] Sir Ray Lankester has kindly drawn my attention to the writings of the Polish Embryologist, Ganin, whose work on Platygaster, carried out forty years ago, is of great interest. Platygaster is a parasite on the larvæ of some Diptera (Cecidomyia), and its larval form is curiously modified in early stages. According to Ganin the larva has neither nervous, vascular, nor respiratory systems (Compare p. 20 of this paper), and its last abdominal segment terminates in a curious caudal organ of a tree-like nature, almost certainly concerned in nutrition. The larva undergoes a number of moults, looses its caudal organ, and gradually becomes vermiform. I have lately noticed that the larva of an Apanteles parasitic on Porthesia has a remarkably modified ultimate abdominal segment, which is very large, vesicular, and formed of hypertrophied cells. The gut of this larva is in all the early stages completely blind, and the animal depends ou the swollen abdominal segment for its nutrition. Like Ganin's larvæ this form looses the vesicular segment just before pupation. It is a very remarkable fact that the ultimate abdominal segment should be modified for this purpose (Zeit. f. Zool., Bd. xv.).

Silvestri (**2**) has contributed some important papers on the subject, but these have appeared in an Italian agricultural journal only taken by a small number of the scientific libraries of this country. Considering the vast number of parasitic Hymenoptera which exist, and their diversity and remarkable instincts, a rich field, only now being explored, is opened to zoologists. But it is a field full of difficulties, for the Trichogrammids, to mention one group alone, are, as Perkins (**4**) has said, among the smallest of known insects. In several other groups of parasitic Hymenoptera there are to be found numbers of forms whose life history and habits are of absorbing interest. The pure observer finds problems and instincts of wonderful diversity, and the embryologist is impressed with the remarkable adaptations for the modus vivendi which these forms follow.

The remarkable oogenesis of some of these parasites has been the subject of some interesting papers by R. Hegner (**3**).

The parasite, a part of whose embryology I have described in this paper, is a member of that important family the Chalcididæ, a numerous and highly interesting assemblage of minute Hymenoptera. These insects are of great importance to the economic entomologist, because among them one finds forms which aid the agriculturist, and which often injure. Trichogramma might be said to aid.

It is a pleasant duty to express my thanks to Mr. Goodrich for his kind interest in this work, and for advice and criticism, which has been of great value.

The Host[1] (Donacia simplex, Fab.).

Donacia simplex is quite common around Oxford in the early summer. Commander Walker informs me that he has occasionally taken this species at Oxford in winter. In the early summer one can always find the beetles on the water-

[1] Kindly identified by Mr. H. Britten, Assistant of the Hope Department, Oxford Museum.

reeds which grow from shallow ponds and the sides of streams; they may be observed copulating and laying their eggs. The latter are laid in masses in a regular manner, the whole group forming a rectangular mass containing a varying number of eggs. In one mass eggs of several shades of brown may occur in patches, as if a number of beetles had oviposited in the same place. Whether this is so I do not know. The egg groups do not adhere very closely to the surface of the reed, and they are easily removed by bending the surface upon which they are laid. From the number of parasitized eggs which one can find there is no doubt that this Trichogrammid must cause a great deal of destruction among the broods of beetles, and were the Donacia a pest on valuable plants it would be quite easy and worth while to rear batches of parasites. This has been done in the case of parasites of injurious insects, particularly in America, and such methods of attacking pests have so far met with a good deal of success. In the case of Donacia almost the entire number of eggs laid in a locality where the parasites are common will be found parasitised. In Pl. 10, fig. 3, is drawn an enlarged figure of Donacia simplex; in *A* the egg mass (*OV.*) viewed in profile upon the reed (*R.*) is shown, and resting on the lower eggs a Trichogramma (*P.P.*) is seen, drawn to about the same scale as the beetle.

The Parasite (Trichogramma evanescens, Westw.)

I have to thank Commander Walker for drawing my attention to some literature on Trichogramma. The Rev. J. Waterston, B.D., of the Imperial Bureau of Entomology, in kindly identifying this insect, writes that Trichogramma evanescens is generally found as a parasite upon the eggs of insects whose habits and place of oviposition are similar to that of Donacia.

As is common with many of the parasitic Hymenoptera, Trichogramma evanescens has very gaudy colouring. The wings, which are a shiny blue, at once attract attention

to the insect as it walks over the Donacia egg mass. In collecting my material I found it most convenient to examine the rushes for Donacia egg-masses from a boat, and those upon which parasites were seen were removed from the water-plant and placed in a box. A most unfortunate circumstance, unknown to me then, was the fact that the time[1] taken to walk to the laboratory with the material was just long enough to allow the newly-laid eggs to form polar bodies, segment, and enter upon the blastoderm stage. Except in the case of a small number of eggs laid in the laboratory, all my sections begin from the blastoderm stage onwards, and some important stages are missing. If the insect is taken into the laboratory and placed with an egg mass of Donacia, it is possible to watch oviposition taking place. The little parasite may be observed to walk somewhat rapidly over the eggs, continually tapping them with its geniculate antennæ. When is satisfied with the egg it has chosen it stops, unsheaths its ovipositor, and moves its abdomen backwards and forwards with a sawing motion about eight times, until the chorion of the Donacia egg is pierced. When this happens the parasite may be seen to depress its abdomen, thrusting home the ovipositor. It pauses about five seconds while the egg passes down the evipositor into the Donacia egg, withdraws its ovipositor, and generally begins on the next egg in the row. Though the parasite does not seem to work systematically along the rows, in many cases all the eggs in a mass are parasitised, though more often a few are left untouched.

In cases where all the eggs have been parasitised several parasites may have laid in one mass. It is quite common to observe two or three Trichogrammids on one Donacia egg-mass. In very rare cases there are two eggs laid in the same Donacia egg; one so seldom finds this that it is probable that a parasite is able to tell whether one of its fellows has previously given attention to an egg. What

[1] Added to the fact that the fixative I used does not penetrate the chorion of the beetles' eggs as quickly as desirable.

happens in development when two eggs are laid in the same Donacia egg I do not know, but one generally finds the two eggs in different stages of development. Probably the older embryo succeeds in the end in killing the other, for I have not yet found more than one insect imerging from one egg.

I am unable to say whether there is more than one brood of parasites during the summer, but it is possible to collect at the same time eggs containing parasites ready to emerge, and some containing newly laid eggs. This points to there being more than one brood. There are two or three species of Donacia fairly common at Oxford, and they appear one after the other, so that this strengthens the view that several broods occur in one season. During the winter months I have not found the empty egg-cases of Donacia on the stems of the reeds, and I have not been able to satisfy myself as to whether the parasite hibernate in the egg-cases or whether they emerge in summer and creep into crevices with a view to wintering there. Nearly every year the reeds upon which the egg-masses are laid are submerged in the floods, and become withered and torn, and thoroughly soaked. For this reason it is unlikely that the parasites would remain in the eggs which they have destroyed.

In remarking on the parasite and its host, I do not overlook the possibility of T. evanescens being found on the eggs of other insects.[1]

Technique.

The egg of Donacia is covered by a thick chorion which, added to the yolk, makes sectioning a very difficult business. The parasitised egg-masses were generally preserved in Petrunchekewitsch, with a little more nitric acid than usual. This often gave splendid results. A mixture of Petrunchekewitsch[2] and Bouin[2] was also tried with about equal results.

[1] Prof. Poulton informs me that this Chalcid parasitises the eggs of Dragon flies. I have since been able to observe this interesting fact myself.

[2] For these fixatives see Bolls Lee's Microtomists' Vade-Mecum.

In some cases the eggs were pricked and the whole thrown into picro-nitric.

After some trials Petrunchekewitsch was almost exclusively used, and in most cases it gave a fine fixation, but not always. In using this fixative it is not necessary to prick the eggs. Ordinary preservatives like Bouin, corrosive acetic, or Flemming will not penetrate the chorion. This at once causes difficulties, for alcoholic fixatives are not always reliable. The eggs were left over night in the Petrunchekewitsch and washed out in 70 per cent. alcohol.

When in xylol the eggs were pricked with a fine needle and placed in the paraffin bath. It was not always possible to successfully prick the eggs. but unless this was done it was necessary to leave the masses longer in the bath. This hardens the eggs and makes sectioning a dreadfully difficult task. The eggs were cut in their groups, 5 μ in thickness, on a Yung microtome, each section being painted with celloidin and ether. One could not be sure that the eggs were not parasitised until after staining, and three or four batches would often be cut without finding any stages. It was only by staining overnight in Iron Hæmatoxylin that a a suitable differentiation could be got. Ehrlich and the carmines were useless. In some cases alternate slides were counterstained in orange G. or dilute acid fuchsin.

General Facts Concerning the Appearance of the Material in Sections and in Whole Mounts.

In Pl. 11, fig. 8, there is drawn a part of the section of a parasitised egg-mass. The larval parasite (*D.P.*) lies in the yolk of the Donacia egg, and a little to the right and lower edge of the larva is the remains of the embryonic gut of the host (*G.*). At *N.S.* are the remains of the Donacia larva's nervous system, and below at *L* is a still recognisable degenerate leg. The parasite has reached the stage just before it begins to swallow the yolk in which it lies. Abutting against the chorion of the egg in the middle of the field are

the chorions of the neighbouring eggs, all of which were parasitised.

It will be seen that when these eggs were attacked the contained embryos had become far advanced and were almost ready to hatch. Though one can observe a Donacia ovipositing, and a parasite on the same mass piercing and depositing its eggs in the newly laid beetle's eggs, it is possible to find eggs parasitised at any stage. If one removes Donacia embryos from their chorion by means of fine needles and stains them in paracarmine, one can often find the developing parasites as in Pl. 10. fig. 2, at *D.P.* Now this embryo lies at the posterior pole of the embryo beetle, and is too far down to have been oviposited there. It may be that this egg was an outside one of the mass and that the parasite bored it from the side; but such cases occur too frequently in sections of eggs in the middle of the mass, and I am inclined to think that in those Donacia eggs laid in a horizontal position the developing Trichogramma embryo may sink downwards. I cannot otherwise explain how parasites' eggs are found in this position, because the beetle's eggs seem too closely applied to one another to allow the parasite to get its ovipositor between them, and reference to Pl. 10, fig. 1, will show how short the little insect's ovipositor is. (Both fig. 1 and 2 are drawn to the same scale: × 75.)

The Effect of the Deposition of the Egg in the Developing Embryo's Body.

Primarily the effect is to arrest further development of the host, but all life is not killed immediately, for living nuclei are to be found much later on as the parasite develops. As is well known, the nuclei in the yolk of an insect's egg are very large, and such vitellophags become larger than the other cells almost from the time they are established. I believe that it is the vitellophags which manage to live longest after the parasite has oviposited in the beetle's egg, and in

some cases degenerate, but evidently still living yolk cells can be found in the gut of the young larval parasite.

In degeneration the nuclei become hyperchromatic, large stainable masses collecting in both nucleus and cytoplasm, the cell finally becoming a black shapeless mass. I am inclined to believe that the large cells forming the serosa also live longer than the ordinary embryonic cells, after the Trichogramma embryo has been developing some time.

The Ovarian Egg when Ready to be Laid.

In Pl. 11, fig. 9, a longitudinal section of the nearly mature ovarian egg is drawn. The egg is of an elongated oval shape, the anterior pole (*A.*) being somewhat broader than the posterior, and the cytoplasm appears homogeneous except for the occurrence at the posterior pole of a large dark mass (*G.C.D.*), the so-called germ-cell, or germ line, determinant. The probable nature, mode of appearance, and the fate of this protoplasmic inclusion will be dealt with under a separate heading. The follicle cells are much drawn out in Pl. 11. fig. 9, and it is very difficult to distinguish between the wall of the ovary and the follicular layer. The nucleus lies slightly towards the anterior yolk of the egg in the mid line. It consists of a large condensed mass of chromatin surrounded by a clear nucleoplasmic zone. In the latter minute stainable granules may be found. The manner in which this condensed form of nucleus is produced is, as far as I am able to judge from my material of adult insects, the same as that described by Hegner for Copidosoma (**3**).

The Newly Laid Egg.

In the eggs at this period I have found a small body near the surface, which, I think, is the spermatozoon. In Pl. 11, fig. 10, this darkly staining body is seen to be surrounded by a number of small granules. I have been unable to find any signs of activity around this body as one would expect if it

were a spermatozoon, though in some insects no clearing of the cytoplasm around the male pronucleus, or other event, takes place at this period. In the stage later, during the formation of the polar bodies, the granules which were present in Pl. 11, fig. 10, around the male pronucleus (*M.P.N.*) cannot be seen, but the latter has penetrated further into the egg. In all the sections of newly laid eggs that I have found, the cytoplasm towards the central region of the egg has become partially vacuolated and thinner, while the germ cell determinant has become much more faintly staining. My collection of newly laid eggs is not complete enough to show whether this thinning out of the central region of the egg is the rule, and it should be observed that in Pl. 11, fig. 11, which shows the formation of the polar bodies, this vacuolisation was quite absent. In Pl. 11. fig. 7, I have drawn a transverse section of an egg which shows the nucleus lying in a central clear region, and quite close a denser part of the cytoplasm containing a cloud of granules (*G.G.*).

The egg, when laid, lies almost always towards the top of the Donacia ovum, and it never has a definite orientation, for in a section of a group of the host's eggs one cuts across eggs in all directions. In a brood of parasites which I caught emerging, some had their heads downwards in the Donacia egg, some their abdomens. At the stage when the larva begins to feed, it is forced to lie lengthwise in the host's egg, because it has by then become too long to lie in any other way. It is obvious that the orientation of the pupating Trichogramma larva in relation to the Donacia egg is not governed by any special circumstance. Nevertheless it is possible, though to my mind unlikely, that the larva may be able to turn around at will within the Donacia egg.

The newly laid egg is provided with a vitelline membrane and a thin chorion (*C.H.*).

Formation of the Polar Bodies.

In the one egg I found at this stage there were two polar bodies (Pl. 11, fig. 11). One polar body (*P.B*^{1}.) has been

extruded and lies on the surface of the egg. The spindle of the second polar body is in the telophase and the chromosomes seem fused. No aster or centrosomes could be seen. Around the neighbourhood of the forming polar body is a clear zone, and a little above the dumb-bell shaped figure are two large granules. I do not know exactly how these granules arise, but I think that they are possibly extrusions of the polar figures, for expulsion of granules from the nuclei can be observed in later stages. The fate of the polar bodies is not known. In Oophthora and Encyrtus they eventually degenerate (Hegner, **3***a*).

The Stages between Formation of Polar Bodies and the Blastoderm.

These are not described in the present paper; through lack of material last spring I have been unable to get the stages. This spring I was able to procure a great deal more material, with which I hope to describe the early segregation of the germ-cells and the accompanying phenomena.

The Blastoderm Stage.

The material from the blastoderm stage onwards to the formation of the young larva is very complete. The germ-cell determinant at the posterior pole of the egg has, by the time of formation of the polar bodies, become more faintly staining, and considerably broken up (Pl. 11, fig. 20, broken pieces *P.P.*). Such broken pieces come apart, and the whole determinant loses almost all affinity for stains of any kind. The exact time at which the determinant usually disappears is at present unknown, but very rarely one can find rather darkly staining patches in the germ-cells of the blastoderm stage, which may be the remains of the germ-cell determinant (Pl. 12, fig. 36 *G.*)

In Pl. 11, fig. 12, the earliest blastoderm stage is seen in longitudinal section. The number of germ-cells is very difficult to determine, for at about this stage the latter lose almost

all affinity for stains. I feel quite sure that there are at least six germ-cells in the blastoderm stage, but often one counts more during later stages, in some cases as many as nine. Mitotic division of the germ-cells between the blastoderm stage and the adult larva I have not yet found, and I feel the more certain of this at the period of germ layer formation, because one never finds the germ-cells assuming a greater affinity for chromatin dyes as they would do if they were in the prophases of mitosis. From the time of their segregation onwards to the formation of the larva the germ-cells are resting. At the time when the larva has swallowed almost all the yolk (see Pl. 12, fig. 38 and p. 25) the germ-cells seem to become active again, but though I believe they begin to divide by amitosis, I have not enough material of this stage to feel quite certain of this. The germ-cells have the appearance of fig. 36 of Pl. 12, and the granules (*G.*), which one can in rare cases discover, may be the remains of the germ-cell determinant. In Pl. 11, fig. 21, the structure of the germ-cells and the blastoderm nuclei is shown. The arrangement of the latter is very peculiar and characteristic. The nucleus consists of an oval nucleoplasmic zone, Pl. 11, fig. 21 *A.* (*N.P.*) in which is placed excentrically, and always towards the periphery of the blastoderm, a large chromatin nucleolus (*M.G.*). This large granule is rounded on the side touching the edge of the nucleus and generally more irregular on its inner surface. Placed on the periphery of the nucleus, and always pointing towards the central region of the egg, is a granule, or two, much smaller, and quite spherical (*G.R.C.*) (P. 11, fig. 21). This remarkable arrangement and the peculiar orientation of the nucleus and its granules is quite clear. Observe also the transverse section in Pl. 11, fig. 12, and in fig. 17.

This arrangement is quite constant and typical, but in one blastoderm alone did I find a difference, and this lay in the presence of other granules (*O. G.*) near the large main nucleolar granule (*M. G.*), Pl. 11, fig. 16. It may be possible that the blastoderm was younger than its fellow drawn in

Pl. 11, fig. 12, and that the exceptional nucleus is an intermediate form.

The nuclei at the anterior end of the egg are orientated in relation to the centre, as are those of the posterior. In the central region of the egg are found a number of black masses (Iron Hæmatoxylin staining) of approximately the same size and shape as the excentric nucleolar mass of the blastoderm nuclei. That these masses are extrusions from the latter is proved by the fact that all stages in their expulsion can be found. In Pl. 11, fig. 12, there were twenty-three in the egg. When the nucleolar mass is shot out towards the centre of the egg the nucleoplasm and the other granules break apart. The former disappears, the latter may be found in the egg (Pl. 11, fig. 12, *G. R. C.*). In Pl. 11, fig. 17, at *X.*, there is a space left in the row of nuclei; exactly on the same level, and quite near, are two nucleoli labelled *Y.* I believe that the empty space was occupied by the chromatic masses, both of which have lost their nucleoplasmic zone and their small granule. Additional proof that my conclusion concerning the character of these masses is correct will be mentioned below. In Pl. 11, figs. 12, 13, 16, and 17, the central part of the egg is seen to contain extruded nucleoli. I have been able to count the number extruded in various eggs.

In Pl. 11, fig. 12, there were twenty-three; in Pl. 11, fig. 13, there were fifty-three; in Pl. 11, fig. 16, there were twenty-four; in Pl. 11, fig. 17, there were thirty; and so on, the number usually varying from twenty to fifty. In Pl. 11, figs. 12, 16, and 17, are younger than Pl. 11, fig. 13, so fewer nucleoli have been expelled. It is generally true that the younger the blastoderm, the fewer the extruded nucleoli. Examination of the figures of blastoderm stages will fail to reveal any dividing nuclei, and none are ever found in the sections. It is quite obvious that if it is true that nuclei are extruded and no division takes place, one should find a decrease in the number of nuclei in the growing blastoderm. Up to a certain point this is so. In Pl. 11, figs. 12 and 14 are both longitudinal

sections through the egg, the former at a time when most nuclei are present, the latter when fewest are present and just before multiplication begins again. Pl. 11, fig. 12, has thirty-eight nuclei in the section ; Pl. 11, fig. 14, has thirty. Counts of a large number of sections yield similar results, though the total number of nuclei in a number of blastoderm stages varies a good deal. From Pl. 11, fig. 12 to fig. 14, it will be noticed that the egg has broadened and contracted in length a good deal. Measured roughly from the camera lucida drawing, Pl. 11, fig. 12, is a centimetre longer than the much older stage Pl. 11, fig. 14. We then realise that two curious processes take place at this time ; one, the expulsion of as many as fifty nuclei, the other, an obvious shortening and broadening of the egg. Explanations for both occurrences are difficult to formulate. In cases where no shortening can be shown to have occurred it is equally true that no lengthening has taken place, so that it remains correct that the developing egg departs from the proportions which it had when laid. A relative shortening always occurs, i. e., in comparison of lengths and breadths of the eggs at different stages, for Pl. 11, fig. 8, is one and three-quarter times as broad again as Pl. 11, fig. 12, and a little shorter. Pl. 11, fig. 13, is the later stage of the blastoderm. The egg has become relatively broader and shorter, and important changes have been taking place in the nuclei. It has already been remarked that in this egg fifty-three of these have been extruded. The germ-cells now stain quite faintly, but their arrangement is still unaltered. Most of the blastoderm nuclei in Pl. 11, fig. 13, are the same as those in Pl. 11, figs. 12 or 21, but others show differences. Many of them have lost their small spherical granule, which was directed centrally, and in these the large nucleolar mass has shifted from its position in the periphery of the nucleoplasmic wall (Pl. 11, fig. 21A) to the middle of the nucleoplasmic zone (Pl. 11, fig. 13A, 2 and 3). The latter figure is much enlarged and shows three stages in the alteration of the nuclear arrangement. At a later stage these changes become

widespread, and by the stage in Pl. 11, fig. 14, no granules are left and all nucleolar masses are found in the mid-region of the nucleoplasmic mass. I have not discovered the early blastoderm form of nucleus in any other stages.

Pl. 11, fig. 18, is a transverse section of an interesting stage. It shows that the blastoderm nuclei have grown and that changes have taken place in their disposition, while the mass of extruded nuclei which, in Pl. 11, figs. 12, 13, 16, and 17, was situated in the centre of the egg, appears to be shifting outwards. Now, an examination of all later stages after the blastoderm will reveal the fact that the extruded nuclei leave their central position in the egg, and pass to the periphery (see Pl. 11, figs. 14, 15, 18, 19, 24, 25, and 27, *E. X. N.*).

It is just after the stage drawn in Pl. 11, fig. 12, that this occurrence takes place, and Pl. 11, fig. 18, shows what happens. The central mass containing the nuclei, as is seen in Pl. 11, fig. 13, is somewhat vacuolated. Almost the whole of this central region streams out to the periphery, carrying the extruded nuclei with it, and breaking through and disarranging the layer of blastoderm nuclei on one side; in the process several healthy nuclei are carried out as well (Pl. 11, figs. 18 and 19, *L. E. N.*). The space left by the out-streaming mass is soon closed up, and the disarranged nuclei resume their places; the new membrane appears between the re-formed blastoderm and the extruded mass (*M. B.*, in Pl. 11, fig. 19).

Regarding the position in which this final expulsion of extruded nuclei takes place, though no absolute regularity exists, it is a fact that the outbreak appears generally towards the middle at any place, but more often than not on the future dorsal side of the embryo. In Pl. 11, figs. 14, 18, and 19, it was ventral; in Pl. 11, figs. 15 and 25, it was dorsal. In Pl. 11, fig. 14, it was near the posterior pole; in the others about median.

As will be seen in Pl. 11, figs. 15, 18, 19, and 27, at *E. X. N.* this extruded mass is quite large and consists of the

wider reticulate central part of the egg. After its expulsion the widely reticulate central part of the egg (Pl. 11, fig. 13) disappears (observe Pl. 11, figs. 14, 18, and 19). A partial vacuolisation may reappear secondarily, as in Pl. 11, fig. 15, but this is rare. Further description of the fate of this extruded mass will be postponed, but it remains for a good while lying between the chorion (Pl. 11, fig. 19, *V.M.*) and the re-formed blastoderm, often becoming much flattened.

The Appearance of the Germ Layers.

The expulsion of the inner waste mass is a preliminary to the incipient formation of the germ layers. On what is later the dorsal surface of the embryo a longitudinal groove appears, and beneath this groove the regularity of the arrangement of the blastoderm nuclei becomes disturbed. On a space oocupied by about five or six nuclei broad and seven or eight nuclei long a gradual sinking-in begins. In Pl. 11, fig. 18 and 19, the groove is marked *I. N. V.* and the sinking nuclei *N. S. I.* In Pl. 11, fig. 14, the egg at this period is seen in longitudinal section.

This process is undoubtedly gastrulation, though in view of the fact that the representative of the blastula is solid the event is somewhat disguised. Pl. 11, fig. 19, has a striking resemblance to a gastrulating blastula, though there is no segmentation cavity or blastocœle. That the groove represents the early blastopore (*I.N.V.*) I have no doubt, and were the depression to become deeper it would form the mesenteron. As it happens, this never takes place, the cavity of the gut being formed in a different way.

It has already been shown that about the stage in Pl. 11, figs. 13, 14, the nuclei loose their granule, and the large nucleolus becomes placed in the centre of the nucleoplasmic zone. By the stage in Pl. 11, fig. 14, this has taken place in every nucleus. In this figure the blastopore (*I.N.V.*) appears on the dorsal surface of the anterior end of the egg, but its

position varies little. The row of nuclei which will form most of the gut, and which are now sinking in (*N.S.I.*) are, on the average, a little bigger than the other nuclei. At the anterior pole of the egg, near the letters *E.X.N.*, is seen an extruded nucleus. It is a fact that though the main expulsion of nuclei occurs between the stages in Pl. 11, fig. 12 and fig. 13, even after the throwing out of the central part of the egg which contains these large granules, sporadic extrusion may take place. That these later extrusions do really occur is shown by comparing the size of expelled granules. In Pl. 11, fig. 14, the granule in the anterior end of the egg is twice as large as those extruded earlier at the posterior region. (Compare also Pl. 12, fig. 32.)

The germ-cells in Pl. 11, fig. 14, have changed their position somewhat, becoming arranged towards the ventral edge of the posterior pole. In this figure the germ-cells are drawn a little darker than they should be. Pl. 11, fig. 19, is drawn from such a transverse section as that through *K.* in fig. 14. The insinking nuclei (*N.S.I.*) are shown.

Such an arrangement does not last long, for as the nuclei sink inwards they lose their order. This is caused by the fact that some lag behind while others penetrate more quickly towards the centre of the egg. This is shown in Pl. 11, fig. 15, at *N.S.I.* By this time these nuclei have become very large. The relationship of the various nuclear elements in the egg now becomes more complicated, because at intervals around the periphery other nuclei grow larger and sink inwards (Pl. 11, fig. 15, at *X.Y.*). All these nuclei are quite distinct from those which were the first to begin sinking inwards, and I feel sure that some of them at least contribute to the formation of the gut. Others form loose cells lying in the cavity between the gut and the ectoderm. Often just before and at this stage amitotic division of nuclei is found taking place. Moreover, the chromatic arrangement of some of the nuclei changes curiously. In these the large centrally placed nucleolus becomes ragged at the edges and pieces break off and become arranged around the periphery

of the nucleoplasmic zone. This process may go on till the nucleus becomes normal, that is, until a rough reticulum is produced, and sometimes the chromatin becomes very sparse. These changes are shown in Pl. 12, figs. 40–43. In Pl. 11, fig. 15 such nuclei are marked *N.*, and in Pl. 11, fig. 25, there is a large group of them towards the centre of the embryo. I do not know the reason for this reversion to the usual chromatic arrangement, but at a much later stage during pupation the early abnormal form of nucleus gives place to the normal one. The nervous system of the larva, for instance, is formed of nuclei having quite a different chromatic arrangement from that of the adult. I feel convinced that the curious form of nucleus found in the larva is connected with the unusual metabolic conditions to which the developing egg is exposed. Inspection of Pl. 11, fig. 15, will show that a large number of nuclei are sinking inwards, but among them the nuclei marked *N.S.I.*, which are the original endoderm, are remarkable for their size. In this figure the extruded nuclei and the inner mass of the egg have been thrown out on the mid-dorsal side, and lie in the space formed by the gastrulating periphery of the ovum (*INV.*). The germ cells lie towards the ventral edge of the posterior pole of the egg and have sunk inwards; at *Z.* the edge of the blastoderm tends to embrace the pocket in which the germ cells lie. The latter stain very faintly, and form a light area on the posterior pole of the egg. Fig. 23 of Pl. 11 is a transverse section through this part of the egg, near the letters *A*-*A* in Pl. 11, fig. 15. The latter figure is a little earlier than the former. In Pl. 11, fig. 22, drawn at twice the magnification of either Pl. 11, figs. 9 or 23, is an oblique longitudinal section of the posterior pole of the egg to show both the manner in which the germ cells sink into the egg in the form of a pocket (*GCP.*), the neighbouring blastoderm nuclei (*X.X.*) surrounding and protecting the pocket, and the relative staining power of the egg cytoplasm and the germ cell cytoplasm. Up to the stage drawn in Fig. 15 of Pl. 11, the somatic nuclei of the egg are scattered in a syncytium; in

Fig. 24 of Pl. 11 a transverse section of the embryo is drawn at a stage when the cell outlines begin to appear. At the places where the body cavity is formed, the syncytium becomes thin and vacuolated, and between the future cell elements, cell walls are deposited. In Pl. 11, fig. 24, the large endoderm cells have become arranged in a definite manner (*END.N.*), and the beginning of the lumen of the future gut is seen at *GL.* Beneath the ring of endoderm nuclei (*END.N.*) a large cavity (*CAV.*) has already appeared, but otherwise the separation into regions is still slight. On what is the ventral side of the embryo, at the letters *NCN*, will be noticed three rows of nuclei. The upper row (*MCN.*) just beneath the embryonic body cavity (*CAV.*) becomes detached by further vacuolisations in the region marked *X*, *X*, and in the larva becomes loose in the body cavity (Pl. 12, fig. 37, *MCN.*).

Of the two lower rows, the bottom one, and at least some of the upper row nuclei, form the nerve chain of the adult. Fig. 39 of Pl. 12 should be compared with this figure.

In Pl. 12, fig. 39, the body cavity is better formed (*CAV.*). It will be noticed in Pl. 11, fig. 18, that there are four large nuclei marked *Z* which do not seem to be included in the forming gut. The upper two may form such large glandular cells as those marked *Z* in Pl. 12, fig. 39, for in this figure it will be noticed that in places the wall of the gut (*GL.*) is formed of two rows of cells. One can often find very large unattached cells in the newly-formed body-cavity, and these may break up later on (Pl. 12, fig. 39, *XX.*). Immediately after the final sorting up of the cell elements, and after each nucleus has taken its place, there is an expulsion of superfluous cells, which degenerate either in the hæmocœl, or are cast from the surface of the ectoderm (Pl. 12, fig. 39, *X*, *X.*).

The Formation of Stomodæum, Mesenteron, and Proctodæum.

As far as one can tell in a case where such wide variation occurs, the large dorsal mass of nuclei which sinks inwards

takes part in the formation of no organ except the mid-gut, but, as I have already pointed out, some of the cells forming the mesenteron may conceivably be of another origin, namely from nuclei which sporadically wander in from the periphery on other parts of the surface of the embryo (Pl. 11, fig. 15, *XY.*). From the first the nuclei destined to form the mid-gut are conspicuous by their large size and rapid growth. The lumen of the mesenteron appears just after the stage drawn in Pl. 11, figs. 27 and 28. It seems to be formed by an internal delamination of the solid endodermal cell mass in some cases, but in others it looks as if, during growth, the ring of cells, gradually enlarging, left a lumen in their centre, just as the lumen is known to appear in an ordinary duct. In any case there is always a residuum left in the developing lumen (Pl. 12, figs. 27 and 28). After the endodermal cells have grouped themselves as shown in Pl. 11, fig. 24, the proctodæum and stomodæum begin to be quite recognisable; and there is no doubt that the latter is formed by a regular invagination (Pl. 12, fig. 31, *ST.*). The manner in which the proctodæum is formed is a little more doubtful. In the case of the stomodæum the invagination is normal (Pl. 11, fig. 27). The inpushing cells meet the roughly disposed endoderm cells, and when the final dissolving out and disintegration of that part of the embryo which forms the body-cavity takes place the connection between the stomodæal and mesenteron cells remains unbroken. The same thing applies to a region where the proctodæum is formed, but it is difficult to be sure of a true invagination such as occurs with the stomodæum. The latter is formed of much smaller cells than the proctodæum, and is longer, while the demarcation between mesenteron and proctodæum is quite indistinct. In Pl. 12, fig. 30, which is a horizontal section of the front region of a larva of the same age as that drawn in Pl. 12, fig. 38; the stomodæum, mouth, and mesenteron are shown. In Pl. 12, fig. 34, there is a longitudinal section of the proctodæum of a somewhat younger larva, but it serves to show how short the hind gut is. This

seems to be the rule in many Hymenopterous larvæ. In the oldest larvæ I have found there is no œsophageal valve formed, nor is there any differentiation in the proctodæal end of the gut.

As the larva grows it swallows all the host's yolk in the egg, and no defecation takes place until every yolk disclet has been swallowed; by this time the animal is enormously stretched, and the body-wall and gut-wall are so thin as to be overlooked unless care is taken. Pl. 12, fig. 38, is drawn when the swallowing is well advanced, Pl. 12, fig. 33, when the first food has reached the mesenteron. When the larva has finished swallowing the yolk, it occupies almost the whole extent of the egg.

The Head Region of the Larva of Trichogramma.

In Pl. 12, fig. 30, I have drawn the horizontal section of the head region. The mouth (*MTH.*) is a simple opening; but pointing forwards and outwards are two extraordinary horn-like processes (*PRC.*). These are seen to protrude from a pair of lateral thickenings—one on each side of the head. These thickenings arise quite early, and are closely associated with the inner side of the epidermis. In Pl. 12, fig. 29, (*TH.*) I have drawn a transverse section of a younger head to show the thickenings before the horn is secreted from them. Beyond this curious organ I have been unable to discover any other mouth parts whatsoever.

The Late Larva.

In the stage when the larva has swallowed all the yolk several facts may be noticed.

The first is absence of tracheæ; the second, absence of any external sign of segmentation; and the third, the absence of completely differentiated muscles or heart.

The larva is merely an ovoid sac, provided in front with two horn-like processes, and with an opening at either end, for taking in food and casting out waste matter; internally there is a gut divided as usual into three regions; and finally

there is the single median ventral germ-cell pocket, beneath the proctodæum.

In Pl. 12, fig. 30 (*CU.*), a distinct cuticle could be seen. It dipped into the pockets from which the horn-like jaw-processes protruded, and the latter are probably cuticular in nature. The thickening (*TH.*) is ectodermal. Cuticle (chitin) was found in the stomodæum, but I am not quite sure of its presence in the proctodæum. It is possible that the processes are used for scooping up the yolk of the host as the larva feeds, and they are probably much modified mandibles.

When the larva has swallowed all the yolk, very often not the smallest particle can be found outside its gut, and exactly how the yolk at the posterior end of the host's egg is worked to its mouth is impossible to say; but it is probably by means of movements of the body that the unswallowed parts are brought forward.

The Fate of the Extruded Matter.

In Pl. 11, fig. 15, the extruded mass still lies within the vitelline membrane of the egg. As the larva grows the membrane becomes stretched and the waste mass flattened; but, though it remains intact for a good time, it eventually bursts. The extruded mass then floats free in the yolk of the Donacia egg. In Pl. 11, fig. 27, *EM.*, it is shown to the right of the ventral side of the posterior pole of the embryo. In Pl. 12, fig. 35, it is seen quite close to the embryo at *EM.*

Curiously enough these fragments seem to live a good while, and nuclear changes, such as those undergone in the blastoderm, take place in some cases.[1] The mass may becom spherical, as in Pl. 12, fig. 32, and may resemble the egg itself. Eventually the mass either degenerates outside the

[1] One is tempted to entertain the view that this peculiarity may be in some way or other connected with a faculty that culminates in the establishment of polyembryony. Were the extruded mass to contain enough live nuclei it might partially follow the development of the embryo.

embryo or is swallowed by the latter. The live nuclei, to which the temporary persistence of the extruded mass is due, may develop the microsome granule (*GRC.*) drawn in Pl. 11, fig. 21 A. This is the case with the nuclei marked *LEN.* in Pl. 12, fig. 32. (See addendum, p. 30.)

The Nervous System.

The nervous system can be recognised very early; it arises from the multiplication of ectodermal cells in the usual manner found in insect larvæ, but it never becomes properly separated off from the ectoderm. Even in late larval life the nervous system seems "coarsely" made; that is to say, it is formed of comparatively few cell elements which are not differentiated in the characteristic manner, and there are no such things as nerves in the sense of offshoots or twigs to organs, such as exist in other larvæ, such as Vespa. The nerve-cells do not differ in any way from other cells in the body, always excepting germ-cells. In Pl. 11, fig. 18, the nerve-chord is seen in a rudimentary condition, and consists of the bottom row of nuclei marked *N. C. N.*, and an unknown number of the row above. In Pl. 11, fig. 21, the brain (*BR.*) and nerve-chord (*N. C.*) are cut longitudinally. In Pl. 11, fig. 22, Pl. 12, figs. 33 and 38, a better view of the chord in transverse section is seen, and in Pl. , fig. 29, the brain (*BR.*) is cut transversely, to illustrate its close connection with the epidermis (*EP.*) and œsophagus (*STD.*). No such things as ganglia exist, and the chain ends a little before the germ-pocket; it does not reach the proctodæum. In late stages (Pl. 12, fig. 38, *N. C.*) it becomes an increasingly difficult matter to recognise the chain, so stretched does it become, and by the time the larva has swallowed all the yolk in the Donacia egg, the nervous chain is for most of the hinder part of its length quite unrecognisable. The œsophageal connectives seem to consist of single cells applied to one another (Pl. 12, fig. 30, *ŒS. CON.*), and are extremely rough.

The Amitotic Division in the Developing Embryo.

It has been shown that the number of nuclei in the blastoderm stage becomes subsequently reduced, but that soon afterwards, at about the stage in Pl. 11, fig. 15, amitosis can be found. Mitosis never occurs in the stages I have examined, and I suspect that it never occurs at any stage of development; but between polar bodies and blastoderm, and larva and pupa, I have no stages. I cannot find mitosis in the ovary of the imago, but my series is not satisfactory, and subsequent work may cause me to alter my views. In the dividing nucleus the large median chromatic body may be seen to elongate (Pl. 10, figs. 6A and 6B), while the nucleoplasmic zone (*NP. Z.*) is unaltered in shape. The nucleoplasmic zone soon constricts and becomes elongate. The chromatin mass becomes roughly dumb-bell-shaped, and the nucleus divides into two by a constriction (Pl. 10, figs. 15A and B).

From the scanty evidence afforded by Pl. 11, fig. 11, it seems probable there is no proper mitotic figure in the polar bodies. The figure draw in Pl. 11, fig. 11A, closely resembles the stages of amitosis in the embryouic nuclei, except for the absence of the nucleoplasmic zone. It is probable that mitotic figures will be found during and after the formation of the pupa. The probable reason for the absence of mitosis during early development is evidently connected with the explanation of the form of the nucleus. (See the discussion, p. 26.)

Mesoderm.

In the section of the young larva one always finds loose cells in the body cavity. These I believe to be mesoderm; such cells are shown in Pl. 11, fig. 27, *MC.*; Pl. 12, fig. 33, *MC.*; fig. 34, *X.* The formation of mesoderm is quite unaccompanied by the appearance of mesoblastic somites; these cells which form the mesoderm are derived from nuclei which sink inwards from the periphery in the stage of fig. 15, Pl. 11, but as the disposition of such nuclei varies I find it impossible to state exactly where they arise. It will be clear, after

an examination of Pl. 12, fig. 39, that the cells marked *MCN.*, which form the mesoderm, appear in a scattered manner, being set free by vacuolisations which rise around them as the body-cavity is formed. It has already been noticed that at this stage many such cells degenerate completely (*X*, *X.*, Pl. 12, fig. 39), and the number which persists in the young larva is never constant.

It is the body cavity cells which most usually exhibit that curious resumption of the reticulum of the nucleus shewn in Pl. 11, figs. 27 and 28, *X*, and in Pl. 12, fig. 34, *X*. The fate of these cells, and the part they play, if any, in histolysis, I do not know at present, but at the stage when the larva has swallowed up all the yolk in the Donacia egg, they seem few in number and much compressed, while their nuclei never show the reticulate structure. Some of the loose cells in the body-cavity also form muscles, becoming slightly flattened under the ectoderm.

The Germ Cells.

Trichogramma evanescens is one of those remarkable animals where a definite difference can be seen very early to exist between germ cells and soma cells; the difference between the two lies in the presence of a germ cell determinant in the former. At the time of segregation we know, from the cases of such insects as Chironomus or Calligrapha, the germ cell determinant becomes included in the pole cells which later form the gonads, and in some special examples pieces of the broken-up determinant can be found in fairly late stages of development. The germ cells at the blastoderm stage (Pl. 11, fig. 12, fig. 14, and fig. 15) have been described. They have already lost a great deal of affinity for any stains, and in bad preparations the nuclei can hardly be found. Not long after the extruded centrally-placed nuclei are finally thrown out to the periphery of the egg, the germ cells begin to sink inwards. Exactly what causes them to move in this manner I am quite at a loss to say, but it is easy to watch the event taking place. In the fully formed

larva the germ cells lie in a pocket beneath the proctodæum, that is, on the ventral edge of the body-cavity. In the earliest stages the germ cells may be seen moving in this direction (fig. 14 of Pl. 11, in the direction of the arrow). One germ cell (*M.*) has begun its migration. By the stage in Pl. 11, fig. 15, the germ cells have sunk right into the ventral edge of the posterior pole, pushing aside the blastoderm nuclei. In Pl. 11, fig. 22, which is a somewhat oblique longitudinal section, this inpushing is finished, and the germ pocket is formed by the nuclei (*X.*, *X.X.*). The latter are quite early set aside for this work, and continue in that position in late larval life. During the time the other organs are being differentiated the germ cells remain closely embraced by these cells; and just when the lumen of the gut is appearing (Pl. 11, figs. 27 and 28) the germ pocket has the appearance drawn in Pl. 12, fig. 37, in transverse section, and in Pl. 12, fig. 34, in longitudinal. The germ cell socket is enclosed by about four cells, and contains the germ nuclei in what appears to be a syncytium, though faint cell outlines and slight vacuolisations can sometimes be noticed. The germinal cytoplasm stains very faintly in plasma dyes. In Pl. 12, fig. 34A, I have drawn an enlarged view of the pocket in order to show the staining reactions. In the case of nearly every nucleus the nucleolus alone can be made to stain. Regarding the number of nuclei in the pocket I could count seven in one, and in another six, but there were always doubtful nuclei at or on the edge of the syncytum, which may or may not have been germ nuclei; it is probable that the number of germ cells is subject to variation, though I have never found less than six.

After the stage drawn in Pl. 12, fig. 34, my material is not very good, but at a stage a little after the time the larva has distended itself with the yolk of the host, the germ cells seem to become almost similar to the somatic cells, and amitotic division begins. The exact details and further confirmation of the facts cannot be given at present.

It will be noticed in Pl. 11, figs. 21, 22, and Pl. 12, figs. 34

and 35, that the germ nuclei gradually lose all staining power, except that of the chromatic nucleolus, the reticulum disappearing. In later stages, when the germ cells begin to stain more heavily, only the nucleolus can be made to take up chromatin dyes.

With regard to the migration of the germ-cells from outside the embryo inwards (Pl. 11, figs. 21 and 22, no pole canal could be recognised. The germ cells seem to sink in passively, and never become amœboid as in Calligrapha (3).

The Germ Cell Determinant.

The origin of the germ cell determinant, even in those insects where the eggs are larger and technique easier, is still in doubt. I have examined several Hymenopterous insects parasitic upon Aphids, and find that the determinants appear as a cloud of granules towards the posterior pole of the egg.

In Trichogramma the determinant is densest and most darkly staining during the period in which it still lies in the ovarian tubule, but is just about ready to lay (Pl. 11, fig. 9). By the time the egg has been laid and the polar bodies are in process of formation the determinant loses a great deal of its affinity for stains, and begins to break into pieces (Pl. 11, fig. 26, *P*, *P*.) At the blastoderm stage the determinant has completely disappeared, and with the exception of the rarest cases nothing of it remains (Pl. 12, fig. 36, *G*.). Indeed at at this stage the cytoplasm of the germ-cells, instead of staining more heavily than that of the somatic syncytium, as one would expect, has lost a great deal of staining power, both in nucleus and cytoplasm. This soon becomes very accentuated (Pl. 11, fig. 22). In the newly laid eggs in Pl. 11, figs. 10 and 20, the germ cell determinant has become rather shrunken and faintly staining, though in the case of Pl. 11, fig. 11, the determinant has a good deal more affinity for stains.

Discussion.

The Significance of the Nuclear Changes during Blastoderm Stage.—Many nuclei (from twenty-five to fifty-five) are cast out altogether. Others, as far as I can tell all of them, extrude the microsome or small chromatin granule, marked *GRC.* in Pl. 11, figs. 16 and 21. In some cases there are two granules of the same size, both of which are expelled into the cytoplasm. No granule can be found to be extruded from the germ cells, and it might follow therefore that the latter, at this period at least, contain more chromatin than the ordinary blastoderm nucleus. In Miastor, Kahle (**5**) and Hegner (**3**) have described a definite chromatin diminution process whereby the somatic nuclei are deprived of a part of their chromatin during certain divisions. Though I do not overlook the possibility of a homologous occurrence taking place in Trichogramma evanescens, I am more inclined to believe that another explanation should be attached to the remarkable chromatin diminution in the parasite. In the first place the chromatin diminution in Miastor takes place quite early, before the blastoderm is formed completely, and, moreover, the process is brought about in a different manner, not by extrusion of a granule, but by the discarding of the larger part of the chromosomes during the mitotic division, only the extreme ends of the chromosomes going to the opposite spindles at the telophase. The residual mass in the middle of the spindle undergoes degeneration.

No satisfactory explanation of the occurrence in Miastor has been advanced, but in Trichogramma evanescens I would suggest that the process is connected with the curious metabolic influences which must affect the nuclei. It must be remembered that all nourishment which is necessary for the development of the egg, and which is ordinarily provided by the central mass of yolk of the insect-egg, is, in the case of this parasite, derived from the yolk of another insect's egg and without the aid of vitellophags. Such nourishment

must be received over the surface of the ovum, and it follows that the surface nuclei must be partly engaged in the taking up of the food matter. A glance at Pl. 11, fig. 7, and fig. 28, will show how enormously the egg has grown during development. Both figures are drawn to the same scale, and the embryo in Pl. 11, fig. 28, had not yet begun to swallow food. All the food necessary for this growth has been derived through the surface of the embryo and of the developing egg, and without the help of yolk cells, which are so characteristic in hexapod embryology. The form of nucleus in the blastoderm must be the one suited to the requirements of the developing embryo, and the occasional expulsion of whole nuclei, and the constant extrusion of the granule, is probably due to the fact that the nuclei become hyperchromatic. That this nuclear arrangement is artificial and temporary is shown, in the first place, because it is not found in the adult insect (follicle cells of ovary excepted); and secondly, because there is always a tendency for the nuclei to regain the normal reticulate arrangement. It is as if the forces which suppressed the usual chromatic arrangement were overcome now and again, but soon recovered their power. To illustrate this suggestion it may be mentioned that the changes shown in Pl. 12, figs. 40–43 take place sporadically. Nuclei like that figured in Pl. 12, fig. 43, occurred in the embryos in Pl. 11, figs. 27 and 28 (*X.*), were absent in Pl. 12, fig. 33, but were common in Pl. 11, fig. 15 (*N. N.*), and were found to occur in a scattered manner right up to the formation of the larva, when they became suppressed. It was particularly in the loose cells in the body cavity that such nuclei were found, and it seems fair to conclude that these are the cells which would be least affected by the metabolic influences surrounding the embryo.

The occurrence of the modified nucleus in the follicle cells of the adult insect's ovary is due to the fact that such cells are exposed to somewhat the same conditions as the nuclei in the embryo, and are engaged in passing on food to the ovum (Pl. 11, fig. 9, *FN.*).

Hyperchromatic nuclei are known to occur in nurse cells of insects, in various cells of vertebrate fœtal membranes, and in many tissues concerned in nourishment, and where these nuclei do not become noticeably hyperchromatic, they generally hypertrophy.

The extruded granules are, therefore, to be regarded as superfluous chromatin, which has arisen through the peculiar conditions to which the blastoderm nuclei are exposed.

Formation of the Germ Layers.—In view of the fact that the egg of Trichogramma is not provided with yolk the formation of the germ layers is of great interest, for the yolk profoundly alters the organogeny in the usual hexapod development. That one would receive a faithful representation of the ancestral mode of development of the insect from the case of Trichgramma is too much to expect, because the method of development, though primitive in some respects, is overshadowed by the effects of the parasitic mode of life. The blastoderm stage is without doubt quite normal, and except for minor nuclear phenomena differs not at all from that of the host or of Miastor (**3**), but the events leading to the formation of the endoderm are interesting. That the progress figured in Pl. 11, figs. 14 and 19, is one of gastrulation one hardly doubts. In the case of Polygnotus minutus Marchal (**7**) describes how the embryo is formed by a complete invagination of one side of the hollow blastula, to form a two-layered gastrula. The method of gastrulation in the parasite treated in this paper is somewhat less distinct than in the case of Polygnotus, and before the process is far advanced a secondary insinking of other peripheral nuclei almost completely obscures it (compare Pl. 11, figs. 14 and 15.)

The manner in which the endoderm is formed in Trichogramma is of very considerable interest in view of the discussions which have been caused by the different opinions expressed by several authors (Dohrn, Kowalevsky, and Ganin (**7**)), but it is not intended here to review their widely different suggestions in the light shed by Trichogramma.

Germ Cell and Determinant.

In the ordinary Hymenopterous larva (e.g. Vespa) the germ cells lie about two-thirds way in the length of the body and above and resting upon the mid-gut.

In the Trichogramma larva the germ cells are situated at the posterior pole and ventral to the proctodæum. In the adult insect the ovaries occupy the same position as they do in the Vespa imago. Migration of germ cells is very small in the developing embryo. In most insect embryos the germ cells are carried into the tail fold, and may be said to either migrate or be passively carried a good distance, but except for the early insinking of the germ cells and the formation of the germ pocket in Trichogramma the position of these cells is hardly altered.

I have looked carefully at my sections of the adult ovary, and find that the germ cell determinant appears as a cloud of granules, which become more and more heavily staining, and denser and denser, until the determinant resembles a dark spherical ball at the posterior pole of the egg. The whole history of the germ cell determinant, in so far as the ovary is concerned, has been exhaustively treated by Hegner (**3**) in more suitable insects. I have examined a number of sections of the Hymenopterous parasites common on Aphids, and I am able to substantiate most of his remarks; but in the nurse cells, as well as in the developing oocyte, I have found curious large spherical granules which have not hitherto been mentioned. These seem to appear after synezesis in the oocyte, and whether they have anything to do with the germ cell determinant I cannot at present say. If suitable material is procured I hope to examine this point.

Addendum.

When this work had been finished I had not had the opportunity of acquainting myself with Prof. Silvestri's writings, only knowing of them through short reviews in

other papers more accessible to me. Since then I have been enabled, through Mr. Goodrich's kindness, to read Silvestri's valuable articles. I have been impressed by the similarity between all stages in the development of Oophthora and of Trichogramma. To my eye, untrained in the appreciation of small systematic differences in Chalcids, the adult insects in these species are closely similar, and the peculiar larvæ of both species are structurally identical.

Apart from differences due to different interpretation there is no doubt that the course of organogeny in these parasites is parallel.

Silvestri ('Bolletino del Laboratorio de Zoologia Generale e Agraria,' vol. i and iii) identifies the darkly staining masses of the inner region of the blastoderm stage (Pl. 11, figs. 12 and 13 in my drawings) as a "piccolo numero di nuclei, che in seguito degenereranno," but has overlooked the small granule (*GRC.*) (if really present in Oophthora) which is so characteristic of stages such as that of Pl. 11, figs. 12, 13, and 21. In Oophthora the germ cells have sunk into the egg before any marked differentiation of the primary germ layers has taken place (vide Silvestri, vol. iii, p. 78, fig. xxx, vii, 5), for it will be remembered that in the stage drawn in my fig. 15, Pl. 11, the germ layers are distinctly forming and the germ cells still situated at the pole of the egg.

Regarding Silvestri's statement that the extruded masses are nuclei, it might be well to mention that these darkly staining masses are but a part (i.e. the nucleolus) of the original nuclei (see p. 11, and the figs. 13A and 21 of Pl. 11).

In Trichogramma I have not described the formation of an embryonic membrane, nor do I believe that such exists. In Encyrtus aphidivorus and in Oophthora Slivestri describes the formation of a "pseudoserosa" from a delamination of the surface cells of the embryo. He states: "L'involucro embrionale dell' Oophthora è in parte omologo a quello dell' Encyrtus, perchè in questo sembra che derivi completamente per delaminazione delle cellule embrionali, mentre nell'

Oophthora la parte di esso, che prima si forma, deriva dalla parte spugnosa del protoplasma che occupava, a blastoderma completo, il centro dell ovo. Intorno a tale differenza io però non voglio insistere troppo perchè potrebbe essermi sfuggito il primo vero periodo di formazione dell' involucro embrionale nell' Encyrtus, mentre ho potuto seguirlo con ogni precisione nell' Oophthora."

In Encyrtus Silvestri gives several figures (vol. iii, 1908, p. 67) of the "inizio della psedoserosa," which I find not unconvincing, but I cannot see any delamination taking place in fig. xxvi, 2, except at *P.*, which I think has little in common with the "pseudoserosa" drawn in fig. xxv, 3. I will leave my comment at this point because Encyrtus is in some ways different from Trichogramma, and will consider Oophthora (vol. iii, pp. 71, 79). Whether Prof. Silvestri's or my views concerning these forms are correct, I am convinced that we have to deal with two species whose development is closely similar. I find stages such as those drawn by Silvestri in figs. xxxvii and xxxviii, and in almost all others of his figures. Not only this, but the modified larvæ of both Trichogramma and Oophthora are similar.

He believes that one part of the pseudoserosa is formed by the extruded inner mass (protoplasma superficiale spugnoso), while the other is formed like that of Encyrtus, and is homologous with this membrane in the latter.

In my figs. 15, 18, 19, 24, and 25 of Pl. 11, I have drawn at *EXN.* what Silvestri calls the "pseudoserosa." Since reading the Professor's papers I have very carefully re-examined my sections, and find nothing to alter in my interpretations; but I have drawn Pl. 10, fig. 4, with a view to the clearer explanation of my view of the "pseudoserosa" of Silvestri.

The egg when laid is surrounded by a vitelline membrane and a thin chorion, which, however, is quite distinct (Pl. 10, fig. 6, *CH.*) As development goes on the waste nucleoli collect in the centre of the egg, and are soon extruded (Pl. 11, figs. 18 and 19). They come to the surface of the egg, and at first form a slight cavity in the ovum. But as

the egg grows rapidly the chorion becomes slightly stretched, and the lump of "protoplasma spugnoso" becomes pressed flat, and mechanically spreads around the egg (Pl. 10, fig. 4, *X, X, X.*). Now should the chorion by any chance burst, as it sometimes does, the extruded mass is released and lies near the egg and embryo (Pl. 11, fig. 27; Pl. 12, fig. 35, *EXN.*).

In Pl. 10, fig. 4, the extruded mass (*EXN.*) lies inside the chorion, and has been flattened out between the points *X, X, X.*, on the dorsal surface of the embryo, but on the ventral surface (*V.*) the chorion, though somewhat stretched and thinner, is still recognisable, and cannot be confused with any other structure. The "protoplasma spugnoso" of Silvestri is an extruded dead mass, and is in no way comparable or homologous with either the ammon or serosa of other insects, and since, as Silvestri shows, there is really a living embryonic membrane around the egg of Encyrtus, it is incorrect, in my humble opinion, to say that "L'involucro embrionale dell' Oophthora è in parte omologo a quello dell' Encyrtus." In his figure on p. 67 of vol. iii, he depicts a membrane (*P.*) which has nuclei evenly distributed, and the tout ensemble is far more convincing than his fig. xxxvii, 6, of Oophthora. In the latter figure there are no nuclei in the "pseudoserosa" except those on one side, which he had already declared were "in seguito degenereranno."

I feel convinced that in Trichogramma and Oophthora the "pseudoserosa" of Silvestri is merely an artefact produced by the mechanical flattening out of a waste mass of protoplasm and chromatin. If the chorion bursts early no "pseudoserosa" can be formed.

I agree with Silvestri's description of the larva except that his fig. *XL.*, p. 81, which, he says, is a sagittal section, he marks what I consider to be the longitudinal nerve-chord, as "cellule muscolari *M.*" It is true that no properly differentiated muscles seem to exist in the larva of either species, and the movements of the animal are brought about by flattened mesoderm cells lying here and there under the

ectoderm. These cells only differ from the other somatic cells in that they are more elongate, their nuclei and cytoplasmic structure being normal.

It is a curious fact that Silvestri, though not paying much attention to the formation of the germ layers, has not figured the invagination of the endoderm (Pl. 11, figs. 14 and 19). I cannot but believe that this happens in Oophthora, where all our other stages are almost identical.

In Oophthora that remarkable nuclear arrangement of early stages (Pl. 11, figs. 16 and 21) has not been described, and it possibly is absent; however, Prof. Silvestri does not appear to have paid great attention to the nuclei of early stages, and it may have been overlooked. I mention this because the early changes in the nuclei of Trichogramma are so striking.

As Silvestri has pointed out, Encyrtus aphidivorus is not a parasite on aphids, but a hyperparasite on one or two other true aphid parasites. With regard to the fate of the embryonic membrane which he figures enveloping the larva (on p. 69, fig. xxix) he says: "È in tale stato di sviluppo che la larva allungandosi rompe nella parte anteriore e nella posteriore la serosa e libera comincia a nutrirsi dei tessuti dell' ospitatore."

It will be seen that, with the exception of those parts of organogeny which Silvestri has not treated at length, his admirable work agrees fairly well with the few remarks I have been able to pass on the embryology of Trichogramma, and I have no doubt that when the Professor examines his stages in greater detail, his results will fall into line with my own.

Summary.

(1) Trichogramma evanescens lays its eggs on the egg mass of a beetle, Donacia simplex, a single parasite emerging from one host's egg.

(2) The ovum has a large germ cell determinant at its posterior pole, and in segmentation the determinant is

divided among the large cells at the posterior pole, which are the germ cells.

(3) In the single case found there were two polar bodies.

(4) The blastula is fairly normal except for the curious arrangement of the chromatin in the somatic nuclei.

(5) Many nucleoli are cast out into the centre of the egg, where they collect till from twenty-five to fifty are present; the mass is then extruded on the periphery of the egg.

(6) As the blastoderm grows it broadens without lengthening up to the stage where the germ layers begin to form.

(7) About thirty-five nuclei sink inwards from the dorsal surface of the embryo to form endoderm.

(8) From the blastoderm stage to that of the gastrula no nuclear division appears to take place.

(9) Shortly after the formation of the endoderm amitosis may be found, and from this onwards the number of nuclei increases.

(10) The mesoderm seems to be formed from peripheral nuclei, which sink in sporadically; no somites can be made out, nor does any segmental method of formation of the mesoderm occur.

(11) The nervous system, stomodæum, and probably proctodæum, are normally formed.

(12) The germ cells lie in a pocket formed by several somatic cells, which embrace them.

(13) Ordinary mouth parts, tracheæ, heart, and œsophageal valve are wanting; the head has two horn-like mandibular processes, which may assist in scooping forwards the food.

(14) The larva does not feed on the food little by little, defecating as it eats; instead, it begins by swallowing all the yolk at once, so that its body becomes enormously distended and stretched.

(15) Metameric external segmentation is absent, the body and head being continuous and sac-like.

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EXPLANATION OF PLATES 10, 11, AND 12,

Illustrating Mr. J. Bronté Gatenby's paper on "Trichogramma evanescens (W.): a Monembryonic Egg Parasite of Donacia Simplex."

Lettering.

ANT. Anterior pole. *B. C.* Body cavity. *BR.* Brain. *CAV.* Developing body cavity. *CH.* Chorion. *CU.* Cuticle. *D.* Dorsal surface. *D. P.* Developing parasite. *E.* Ectoderm. *ECD. N.* Ectodermal nuclei. *E. M.* Extended mass of cytoplasm and chromatin. *END. N.* Endodermal nuclei. *F.* Food. *F. N.* Follicle nuclei. *G.* Stainable granule. *G. C.* Germ cell. *G. C. D.* Germ cell determinant. *G. C. N.* Germ cell nucleus. *G. C. P.* Germ cell pocket. *G. L.* Gut lumen. *G. R. C.* Minor granule of nucleus. *GT.* Gut. *INV.* Invagination. *L.* Leg of host. *L. E. N.* Healthy nucleus extruded. *M.* Muscle cells. *M. C.* Cells of body cavity. *M. T. H.* Mouth. *N.* Nucleus. *N. C.* Nerve chord. N. P. Z. Nucleoplasmic zone of nucleus. *N. S. I.* Nuclei sinking inwards (endoderm). *ŒS.* Œsophagus. *ŒS. COM.* Œsophageal commissure. *OV.* Eggs of Donacia. *P.* Broken pieces of germ cell determinant. P. P. Parasite. *P. B.* 1st polar body.

PD. Proctodæum. *POST.* Posterior pole. *P. R. C.* Frontal process of larva. *R.* Reed. *S. L. N.* Mostly vitellophags of host. *ST.* Stomodæum. *TH.* Glandular thickening secreting frontal process. *V.* Ventral. *V. C.* Vacuoles in cells. *V. M.* Vitelline membrane. *Y.* Yolk.

[In reproduction all figures reduced by one-half.]

Figs. 7, 9, 10, 11, 12, 13, 14, 15, 17, 18, 19, 20, 23, 24, 25, 28, 29, 30, 32, 33, 34, 35, 37, drawn with a Zeiss $\frac{1}{12}$ oil immersion and compen. eye-piece 8. A camera lucida was used, with drawing-board slightly inclined towards the microscope, and at table level. Magnification about 1,760 diameters.

Figs. 27, 31, and 38 from Zeiss $\frac{1}{12}$ and comp. eye-piece 4.

Figs 10A, 11A, 13A, 16, 21, 22, 26 enlarged about twice from camera drawings with O. $\frac{1}{12}$ E. 8.

Figs. 36, 40, 41, 42, and 43 enlarged in the same way about four times.

Fig. 8 drawn with O. $\frac{1}{6}$ E. 2, drawing-board at table level. (Camera lucida).

Fig. 39 was drawn with Zeiss O. F. E. 4.

Fig. 4.—× 2400 (Koristka $\frac{1}{12}$th × Hug. oc. 5.

PLATE 10.

Fig. 1.—Trichogramma evanescens (Westwood), adult female (now × 75.)

Fig. 2.—Donacia embryo × 75 containing at its posterior pole a parasite (*D. P.*). Whole preparation. The parasite was at the stage drawn in Pl. 11, fig. 24.

Fig. 3.—Donacia simplex (*F.*) × 4.

Fig. 3A.—Egg mass of Donacia, viewed from side with parasite (*P.*). All to same scale as beetle.

Fig. 4.—Transverse section in mid region of an embryo when the gut lumen has formed, Shows the flattening out of the extruded mass (*EX. N.*) under the chorion (*CH.*).

Fig. 5.—Stages in amitosis of somatic cells.

Fig. 6.—Part of early blastoderm stage to show chorion (*CH.*) and extruded nucleolus (*EX. N.*).

PLATE 11.

Fig. 7.—Part of Donacia egg showing the newly-laid egg of the parasite in transverse section.

Fig. 8.—Part of egg mass of Donacia in transverse section showing a parasite at the stage drawn in fig. 28.

Fig. 9.—Nearly mature ovarian egg of parasite to show nucleus (*N.*) and germ cell determinant (*G. C. D.*).

Fig. 10.—Newly-laid egg, with spermatozoon (*M. P. N.*).

Fig. 11.—Formation of second polar body.

Fig. 12.—Typical blastoderm stage showing extruded nuclei (*EX. N.*) and germ cells (*G. C.*).

Fig. 13.—Later blastoderm to show stages in nuclei and shortening of egg.

Fig. 14.—Late blastoderm stage showing beginning of formation of endoderm (*N. S. I.*).

Fig. 15.—Stage after fig. 14 to show beginning of insinking of peripheral nuclei (× *Y.*), and penetration and change of position of germ cells.

Fig. 16.—Part of transverse section of blastoderm stage to show structure of nuclei.

Fig. 17.—Transverse section of a blastoderm stage to show expulsion of nuclei (*Y.*).

Fig. 18.—Transverse section showing final extrusion of nuclei (*EX. N.*) and beginning of gastrulation.

Fig. 19.—Gastrula stage in transverse section after expulsion of nuclei (*EX. N.*). Nuclei in this specimen a little larger than usual.

Fig. 20.—Transverse section of posterior pole of the same egg as that in fig. 7, to show germ cell determinant.

Fig. 21.—Posterior pole of blastoderm stage to show germ cells (*G. C.*) and structure of nuclei.

Fig. 22.—Posterior pole of egg just after sinking inwards of germ pocket (*G. C. P.*) and when the nuclei (*X. X.*) form a covering for the pocket.

Fig. 23.—Transverse section of same embryo as that in figs. 24, 25, and Pl. 12, fig. 31, to show germ cells. Such a section as this is through the points A . . . A in Pl. , fig. 15.

Fig. 24.—Transverse section of embryo during the formation of gut (*END. N.*) and nervous system (*N. C.*), etc.

Fig. 25.—Section through anterior region near stomodæum.

Fig. 26.—Enlarged view of posterior pole of the egg drawn in fig. 11, to show breaking up germ cell determinant (*P. P.*).

Fig. 27.—Obliquely sagittal section through embryo to show formation of gut (*G. T.*), stomodæum (*STD.*), proctodæum, brain, and nerve chord.

Fig. 28.—Section such as that through points × Y × Y in fig. 27. × is a cell whose nucleus has temporarily resumed the usual reticulate arrangement. Compare Pl. 12, figs. 40–43.

PLATE 12.

Fig. 29.—Transverse section through brain and thickening (*TH.*)-which secretes the horn-like process. Same age as embryo in Pl. 11, figs. 27 and 28.

Fig. 30.—Horizontal section through head region of a young larva to show horn-like processes (*P. R. C.*), mesenteron (*MES.*), and mouth (*M. T. H.*).

Fig. 31.—Transverse section through developing stomodæum. Same embryo as that in Pl. 11, figs. 23, 24, and 25.

Fig. 32.—Cast-out mass of cytoplasm with the extruded nuclei. Has become round, and the nuclei still live (*L. E. N.*).

Fig. 33.—Transverse section of larva near midgut when it begins to take in food (*F.*).

Fig. 34.—Longitudinal section through germ pocket (*G. C. P.*) of same embryo as that in Pl. 11, fig. 27.

Fig. 35.—Part of embryo and the extruded mass (*E. M.*) with some nuclei still living (*L. E. N.*).

Fig. 36.—A germ cell of blastoderm stage containing the faint remains (*G.*) of the germ cell determinant.

Fig. 37.—Transverse section through germ cell pocket (*G. C. P.*) in same embryo as that in Pl. 11, fig. 28.

Fig. 38.—Larva in transverse section after it has began to swallow yolk (*F.*), and when the body becomes stretched thereby.

Fig. 39.—Transverse section through embryo after the stage drawn in Pl. 11, fig. 24, to show formation of body cavity (*C. A. V.*).

Fig. 40–43.—Stages in the resumption by mesoderm nuclei of the typical reticulate arrangement. Compare fig. 34 at X.