The Fixation of the Cypris Larva of Sacculina carcini (Thompson) upon its Host, Carcinus mænas.

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With 6 Text-figures.

The fixation of the Cypris larva of Sacculina has hitherto been successfully observed only by Delage (1), and since a considerable degree of doubt has been thrown on his account it may be of interest to record that I have been able this autumn, at Plymouth, to rear the larvæ and observe all the stages of fixation upon their host. My inability to obtain the Cypris larvæ of any of the Rhizocephala at Naples had left a gap in my observations, which I am now in a position to fill, and thus I am able to present a complete account of the life-history of Sacculina from my own observations (2).

It is extremely easy to obtain large batches of the Nauplii of Sacculina or Peltogaster by selecting a crab whose parasite is nearly ready to emit its Nauplii from the brood pouch, and keeping it for a few days in a vessel of sea water. The readiness of the parasite to emit the Nauplii can be judged in Sacculina by the purple colour of the mantle, and in Peltogaster by the mantle becoming a paler pink than usual.

The Nauplii of Peltogaster are not actively heliotropic, but they are negatively geotropic, and in a short time all of them reach the surface film of the water and perish, and I have been unable to obviate this difficulty in rearing the larvæ. The Nauplii of Sacculina are actively heliotropic, but there is no marked negative geotropism; nevertheless, with the

Neapolitan races of Sacculina which infest Inachus mauritanicus[1] (Lucas) and Pachygrapsus marmoratus (Fabr.), I did not succeed in keeping them alive for more than four days, or in observing the transformation into the Cypris stage.

The Nauplii of the Sacculina on P. marmoratus appeared to be hardier than those of the parasites on I. mauritanicus, and I suggested (2, p. 44) that this might be due to the former crab being a distinctively littoral species, and thus subjected to more varying conditions than the Spider-crabs; this suggestion is probably correct since the Nauplii of the Sacculina of Carcinus mænas are evidently the hardiest of all, and this crab is more decidedly littoral in habit than P. marmoratus.

The successful rearing of the Nauplii of Sacculina carcini was effected by selecting "purple" Sacculinæ and obtaining a batch of healthy Nauplii; they were then transferred to sea water which had been purified by mixing with powdered charcoal and filtering in the manner used by the Director of the Plymouth Laboratory, Dr. E. J. Allen, in his preparations of pure cultures.

It is of extreme importance that water of great purity should be used, since the larvæ are to live in this water for nearly a fortnight, and the multiplication of Infusoria and Algæ in the water is highly injurious to them. The larvæ, of course, require no food, since they possess no gut and subsist entirely on the yolk reserves which they contain.

The Nauplii, actively swimming the whole time, undergo four successive moults in four days; they are then transformed by a single moult into the Cypris larvæ. Before these larvæ fix upon their host, Delage observed that they had to spend at least two more days in a free-swimming state; fixation also only takes place in the dark. These two points I am able to confirm from my own observations.

The fixation of the Cypris larvæ, as followed by myself,

[1] By an error in nomenclature I. mauritanicus (Lucas) was called I. scorpio (Fabr.) throughout in my monograph (2).

takes place exactly in the manner described and figured by Delage (1). In every case the larva was fixed by one of its antennulæ to the base of a hair, most frequently on the legs of the crab, and preference was shown for the sparsely scattered, plumose hairs upon the flat surfaces of the proximal joints of the legs. The larvæ appeared to avoid fixing on the dense hairs which fringe the edges of the appendages. This fact is fortunate, since the Cypris when fixed upon an isolated hair on the smooth surface of an appendage is a most conspicuous and unmistakable object.

The crabs which I placed with the Cypris to be infected were about 7—15 mm. in breadth, and I selected specimens which had recently undergone a moult, since the skin of these individuals is clean and easy to manipulate.

Soon after fixation the Cypris larva casts away bodily all its thoracic appendages with their attached muscles; I was fortunate to obtain this stage, the particular specimen exactly resembling Delage's Plate 23, fig. 21 (2). During the next two days a process goes on inside the Cypris shell which leads to the formation of the Kentrogon larva. According to Delage, the ectoderm of the larva draws away from the Cypris shell and comes to surround a mass of mesodermal cells lying in the anterior region of the body; the ectoderm secretes a layer of chitin externally to the whole, the Cypris shell falls off, and with it the degenerated remains of the larval muscles, pigment, sense-organs, and broken-down food material, and the so-called Kentrogon larva, a little oblong sac encased in chitin, is left attached to the hair of the crab by means of the antennule of the Cypris.

Delage's figures representing these changes were so completely reproduced in the larvæ observed that I have nothing to add to his description.

With regard to the contents of the Kentrogon larva a word is necessary. Delage considered that a layer of ectoderm is present surrounding a mass of cells which he calls the ovary. This conception I disputed (2, p. 43) after finding the earliest internal stages of the parasite in which no visible differentia-

tion of the cells into distinct layers or organs was present. I therefore suggested the term "embryonic cells" to designate the cellular contents of the Kentrogon. After observing in several instances the formation of the Kentrogon larva, it appears to me that the ectoderm of the Cypris is undoubtedly included in the Kentrogon together with the mesodermal cells, as Delage originally maintained. At the same time, his designation of the mesodermal mass of cells as the ovary is obviously a misnomer, since this mesodermal mass gives rise to all the mesodermal organs and tissues of the adult, and, at the early stages of the endoparasitic development, is quite undifferentiated (see **2**, pp. 47 and 55).

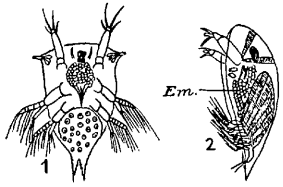
We may, therefore, retain the term "embryonic cells" for the cellular contents of the Kentrogon, with the admission that these embryonic cells are composed of embryonic ectoderm and embryonic mesoderm. It may be remarked that, in this manner, the Kentrogon of the Rhizocephala almost exactly corresponds to the post-nauplius stage of Monstrilla, as described by Malaquin (**3**), which, on penetrating the ectoderm of its host, loses all the larval organs, and consists of a little chitinous sac containing embryonic ectoderm and meso-endoderm, from which the adult organs are regenerated. In Monstrilla, of course, the endoderm is not entirely suppressed, as is the case in the Rhizocephala, though it is on the way to becoming so.

This interesting parallelism between the Rhizocephala and the Monstrillidæ has not been sufficiently insisted on.

In the extreme anterior region of the Kentrogon, where it is attached to the base of the crab's hair, I have been able to observe the formation of the dart which gives the name to this larval stage (κέντρον, dart; γόνος, larva). This hollow dart, as Delage described, elongates and pierces the base of the hair, thus opening a passage through which the embryonic cells of the Kentrogon can pass into the hæmocœl of the host. The dart is continuous with the inner cuticle of the Kentrogon larva, and is presumably a product of the ectoderm.

After the formation of the Kentrogon larva with its dart, a process obviously preparatory to the infection of the host, the next stage in the life-history which is known is that which I have described (2, p. 47) under the name Sacculina interna migrans.

The parasite at this stage consists of a mass of embryonic cells undergoing rapid division by mitosis; it has an irregular shape, a few small roots beginning to grow out from a central tumour, and the whole is enclosed in a thin chitinous cuticle. There is no visible differentiation of any of the adult organs. It is clear that the gap between this stage and the Kentrogon larva is very slight, since the morpho-



TEXT-FIGS. 1 and 2.—1. Nauplius of Sacculina.
2. Cypris of Sacculina.

logical structure is practically identical. These small migrant Sacculinæ were found in the hæmocœl of the crabs applied to the upper part of the intestine just below the stomach, that is to say, far away from the point where the body of the adult Sacculina externa is situated. This position of the earliest known internal stage of Sacculina is completely in accord with the indefinite position of the fixation of the Cypris larvæ upon the crab, and is irreconcilable with the theory that the Cypris fixes upon the under surface of the crab's abdomen, and is transformed into the adult Sacculina in situ.

We are still confronted with the problem how the cells of the Kentrogon that enter the hæmocœl of the crab at any

point are transported to the position of the Sacculina interna migrans. Delage, who did not observe the latter stage, believed that the cells of the Kentrogon, on entering the crab, at once began to send out roots, and to grow always in a determinate direction until the central tumour reached the point where the adult Sacculina was to be evaginated. But, since the youngest Sacculinæ of the migrant stages which I observed were not provided with elongated roots stretching to the skin at any point where the Cypris might have fixed, I incline to the opinion that the embryonic cells of the Kentrogon, after entering the hæmocœl, are carried passively about in the blood stream until they, sooner or later, reach the large blood spaces surrounding the intestine, and that, arrived there, they begin to throw out the root system, while the central tumour grows down toward the junction of thorax and abdomen where the body of the Sacculina externa is differentiated. The method of this differentiation, the formation of the adult organs, and the evagination of the Sacculina to the exterior have been fully described by Delage (1) and myself (2).

The life-history of Peltogaster, the only other of the Rhizocephala that has been at all worked out (2), is very similar to that of Sacculina, save that it seems probable that the Cypris fixes upon the abdomen of the hermit-crab, and that the migrant phase of the internal development is not so marked.

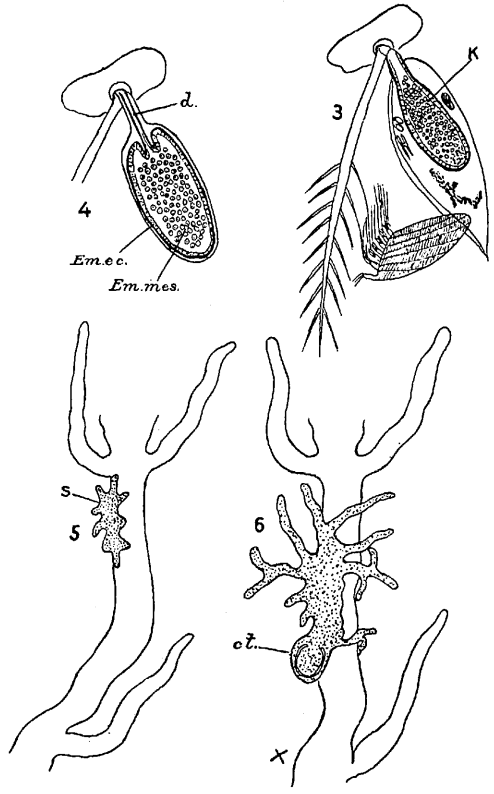
The life-history of Sacculina may be shortly summarised as follows:

The eggs undergo maturation in the brood pouch, and are self-fertilised.

Development up to the Nauplius stage proceeds in the brood pouch.

The Nauplii are expelled to the exterior, and lead a free-swimming existence for four days, undergoing four moults.

The Cypris stage is attained at the fifth day, and, after two or three days of free existence, the Cypris larvæ attach



TEXT-FIGS. 3—6.—Diagrams illustrating the life cycle of Sacculina. 3. Cypris fixed on hair; degeneration of larval organs, and formation of Kentrogon (*K*). 4. Kentrogon, formation of dart (*d.*); *Em.ec.* Embryonic ectoderm; *Em.mes.* Embryonic mesoderm. 5. Sacculina interna migrans (*S.*) on gut of host. 6. Sacculina interna, later stage, with central tumour (*ct.*) passing to position of evagination (*X.*).

themselves by their antennulæ to a hair upon any portion of a young individual of the host, preferably upon the appendages.

The Cypris casts off its thoracic appendages, the ectoderm draws away from the shell, and comes to surround a mass of mesodermal cells; it secretes a chitinous coat, and in this manner the Kentrogon larva is formed. The Cypris shell, together with all the larval organs, are thrown off.

The ectoderm of the Kentrogon secretes a hollow dart in the anterior region which projects up into the antennule by which the larva is fixed to the base of the hair on the crab, and gradually penetrates the base of the hair so as to open a means for the cells contained in the Kentrogon to enter the hæmocœl of the crab.

The embryonic cells of the Kentrogon, consisting of ectoderm and mesoderm, enter the hæmocœl of the crab, and are carried about in the blood-stream until they reach the large blood-spaces surrounding the intestine. They are enclosed in a thin chitinous cuticle. The Sacculina interna migrans now proceeds to grow rapidly, to throw out roots in all directions, while the central tumour grows down the intestine towards the junction of thorax and abdomen of the crab. As it grows in this manner, the adult organs are differentiated in the most posterior portion of the central tumour, which soon arrives at the position of evagination of the adult Sacculina. Here differentiation proceeds, and the pressure of the growing tumour upon the epithelium of the crab causes it to degenerate, and thus, when the crab next moults, a hole is left in the new chitin, through which the Sacculina protrudes, and so gains the exterior.

Literature.

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2. Smith, G.—'Fauna des Golfes von Neapel,' Monograph, No. 29, "The Rhizocephala," 1906.
3. Malaquin, A.—"Le Parasitisme Évolutif des Monstrillides," 'Arch. Zool. Expér.,' ser. 3, t. ix, 1901.