The Sexual Nature of a Rhizocephalan, *Peltogasterella socialis*[1]

By

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(*With 4 Text-figures*)

At present, all the known cirripedes of the order Rhizocephala, except members belonging to the family Sylonidae, are considered as hermaphroditic animals. On the other hand the existence of the complemental males such as known to exist among certain Pedunculata, has repeatedly been pointed out by several authors. F. Müller (1863) was the first to notice the fact that the cypris larvae attached at or in the mantle opening of the young hermaphrodite might be complemental males come to fertilize them. And this view was accepted by Delage (1884) who found that the complemental males, which to all appearance are identical with the ordinary cypris larvae of *Sacculina*, were fixed round the mantle opening of very young hermaphrodite. But neither Müller nor Delage ever succeeded in seeing these cmplemental males alive or in discovering any trace of spermatozoa, so that the manner of fertilization, if it occurs, is really obscure. While on the contrary, Smith (1906) has adopted the conclusion that these cypris larvae no longer play any part in the reproduction of their species. The recent study of Reinhard (1942) concerning the complemental males of *Peltogaster paguri*, suggests that the spermatozoa derived from the cypris larvae mingle with the self-produced sperm of the hermaphrodite to effect fertilization of the first batch of eggs. If this interpretation is correct, the mode of reproduction of the hermaphroditic rhizocephalids will be a very peculiar type.

Fortunately we were able to make researches with ample material of the parasites, the species name of which is *Peltogasterella socialis* Krüger.[2] It was thus possible to ascertain several important matters in connection with the sexual nature of the parasite. It is our conviction, then, after examining the structure of the so-called testes of the parasite, and after searching the spermatogenetic nature of the cypris cells which enter the "testes", that the organs represent in a certain sense seminal receptacles and not true testes; we need therefore describe the observations we have made on this subject in considerable details.

Observations

The materials on which this report is based, were collected from two localities, namely the shore of Atsuta and of Oshoro Bay in Hokkaido, on the side of the

1) Contribution No. 390 from the Zoological Institute, Faculty of Science, Hokkaido University, Sapporo, Japan.

2) Kindly identified by Dr. Sueo M. Shiino of the Prefectural University of Mie, to whom the authors' heartiest thanks are due.

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Japan sea. *Peltogasterella socialis* is parasitic on the hermit crab *Pagurus lanuginosus* de Haan[1] and also found on the other common hermit crabs of these localities. The shore of Atsuta, however, was favoured for collecting purposes, since the percentage of infection was higher there than at Oshoro Bay. For example, the highest rate of 74.8 per cent for 362 hermit crabs (*P. lanuginosus*) which were collected during the months of September and October, 1956, was left on record.

For a short time after the endoparasitic *Peltogasterella* breaks through to the exterior from the crab's abdomen, the external sac (the externa) is approximately 2.5–3.5 mm in length, but carries all the essential adult organs. Anatomically it consists of a visceral mass and an external mantle. Between the mantle and the visceral mass there is a mantle cavity or brood pouch. At the anterior end of the cylindrical body pointing to the head of the host is the mantle opening by means of which the mantle cavity communicates with the exterior. The "testes" are small tubes lying in the mesentery by means of which the visceral mass is broadly connected with the mantle, while the ovaries full of young oocytes occupy the main bulk of the visceral mass. The opening of the oviducts (col-

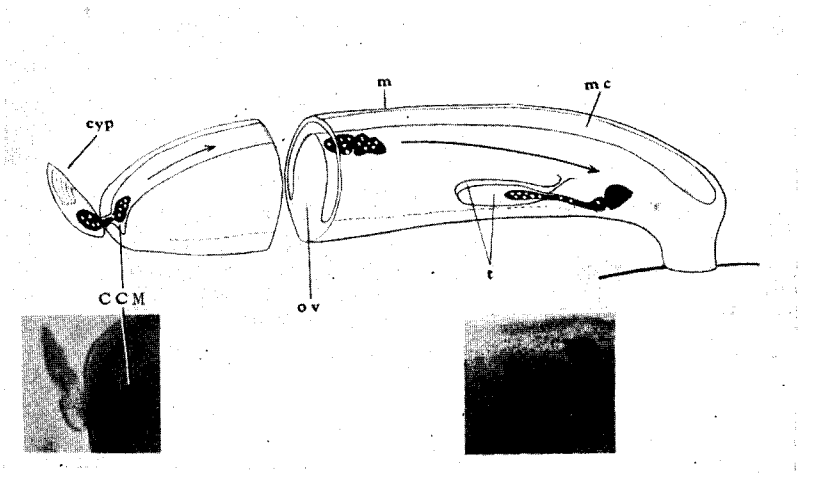

Fig. 1. Diagrammatic representation of immature externa of *Peltogasterella socialis* with cypris larva. The arrows show the probable migration of the cypridian cellular mass to the "testis". The insets show photographs illustrating the cypridian cellular mass in the host body. CCM, cypridian cellular mass; cyp, cypris larva; m, mantle; mc, mantle cavity; ov, ovary; t, "testis".

1) Kindly identified by Dr. S. Miyake of the Kyushu University, to whom our heartiest thanks are due.

leteric gland) is not still established in these young specimens.

The young externae described above are invariably visited by the cypris larvae of the same species. These larvae do not simply attach to the body of the externae, but fix themselves firmly by their antennae at the mantle opening. Now if we study the larva a little time after fixation at the mantle opening, we may obtain the appearance shown in Fig. 1. The undifferentiated cells of cypris origin ("cypridian celluar mass" or "embryonic cells") have drawn together into a compact pigmented mass (Fig. 2) and are beginning to pass into the mantle cavity of the externa through the antenna by which the cypris is fixed. This process is already finished when we find the empty cypris consisting of mere chitinous cuticle at the mantle opening. On the other hand, the ejected cypridian cellular mass migrates through the mantle cavity and then enters one of the paired receptacles which are used to be designated as the testes, distal parts of which are known as the vasa deferentia.

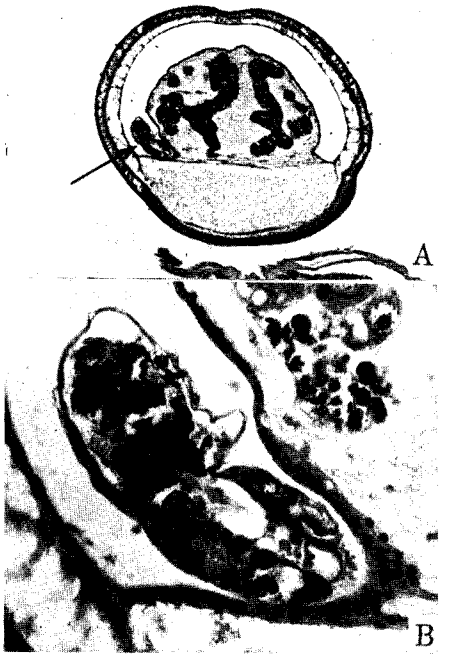

Fig. 2. A. Photomircograph of a cross-section of immature externa. Note the cypridian cellular mass in the mantle cavity (arrow). ×60, about. B, A high-power view of the cypridian cellular mass. ×400.

In this connection, it will be necessary to describe the histology of the immature "testes". As shown in Figs. 3 and 4, the wall of the tubular testis consists of an outer and an inner cell layer which are sharply separated from one another by a chitinous membrane of considerable thickness. The outer thick epithelium seems to correspond to "cellules nutritives" which Duboscq (1901) has described in *Sacculina*. On the other hand, the inner single layered epithelium is morphologically unique. The cells of the epithelium are characterized by signs of hypertrophied degeneration, that is to say, the nuclei show pycnosis, cell boundaries become obscure and moreover the basophilic granules positive to the Feulgen reaction are scattered here and there indicating the destroying fragments of the nuclear substances. At any rate, the inner layer seems to have suffered from profound degeneration.

It is to be noticed that if the immature externae measured 2.5–3.5 mm in length had not taken cypris larva, the further development of the parasite would have discontinued and eventually would have disintegrated. After the hermit crabs with immature externae were brought to the laboratory, they were kept separately in rather small glass vessels. Thus, several groups of parasites were kept under observation for a considerable length of time, *viz.* about one month. When the externae with the cypris larvae fixed at the mantle opening were reared up in the laboratory, they had reached a length of approximately 4 mm after

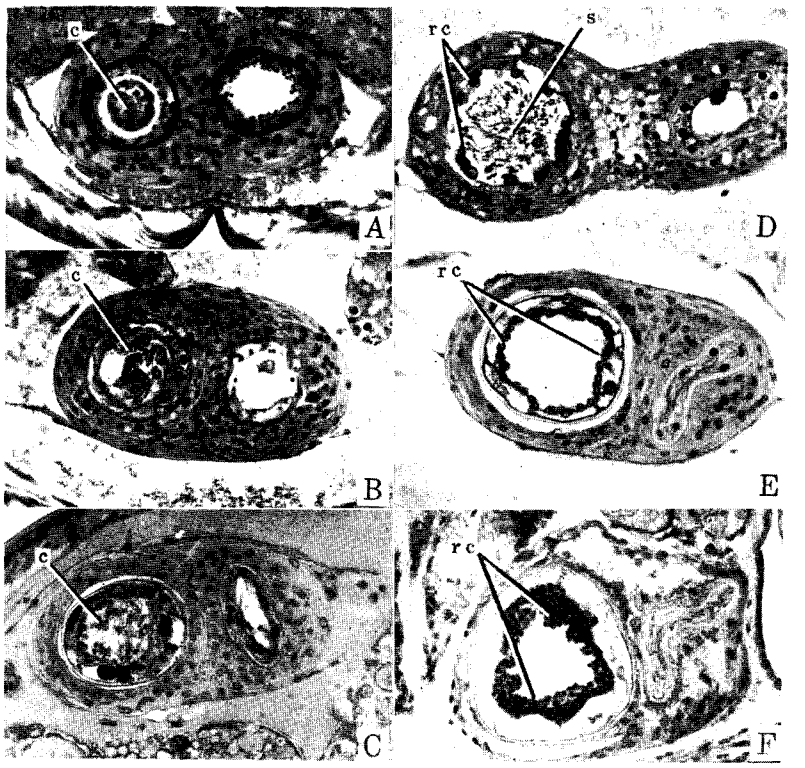

Fig. 3. A. Photomicrograph through the "testes" of the immature externa, immediately after the entrance of the cypridian cellular mass (c) into one of the paired "testes". B. Note that the cypridian cellular mass (c) is increasing. C. Cypris cells (c) undergo mitotic divisions and give rise to numerous spermatocytes. The empty "testis" of the opposite side is degenerating. D. Cypris cells give rise to a number of spermatozoa (s). Note the presence of some residual germ cell of cypris origin (rc). E and F. After spermatozoa have been released, the residual germ cells remaining in the testis (rc) begin to divide and give rise to numerous spermatocytes. ×150, about.

15 days. However, in all 360 cases of the externae which have no cypris, we could not notice any increase of the body length in about the same lentgh of time.

And in fact, after about two more weeks, the majority of the externae arrested in development, disintegrated and eventually dropped off from the host body. Furthermore, the parasitized hermit crabs in which the endoparasitic *Peltogasterella* was still invisible from without, were kept under observation. After the parasite broke through the exterior, the externa did not have an opportunity to meet with cypris larva and was arrested in development as in the former case. Thus, the above observations strongly support the view that only immature externae which have received cypris larvae would be secured further development.

We now turn to the fate and the function of the cypridian cellular mass which enters the "testis" and at the end we will attempt to give an explanation of the sexual nature of the externa of *Peltogasterella*. We examine, either in total preparations or in serial sections, the "testes" either before they have received the cypridian cellular mass or afterwards. In consequence of the increase of the cypridian cellular mass, the epithelium inside of the chitinous membrane, showing unique degeneration, becomes thin and is faintly stained (Fig. 3, A and B). On the other hand, the cypris cells which have drawn together into a mass are beginning to show spermatogenetic nature and finally the cavity of the tubular "testis" is filled with the ripe spermatozoa (Fig. 3, C and D), whereas, so far as our observations go, nothing like this has ever been observed in the cell which constitute the "testis". In all of the mature *Peltogasterella* measuring 4 mm up to 8 mm in length, the cypris cells have been found either in one side or in both sides of the paired "testes" (Fig. 4). Thus, in the former case it appears that only one "testis" usually functions at a time (Fig. 3, D, E, and F). These ripe spermatozoa derived from the cypris cells are released into the mantle cavity and effect fertilization of the first batch of eggs. At that time, the residual germ cells of cypris origin have been left in the functional "testis" and the fertilization of the succeeding batches of eggs may be effected by the spermatozoa derived from them (Fig. 3, rc). On the other hand, the empty "testis" of the opposite side, having the appearance shown in Fig. 3,

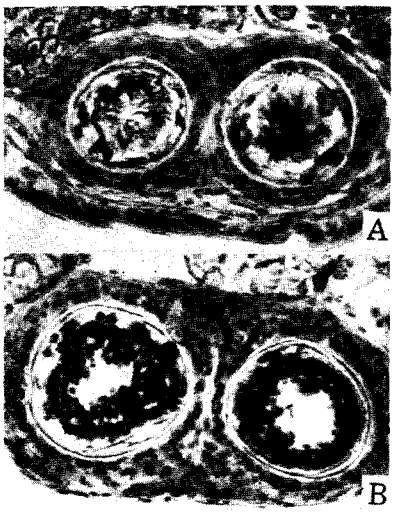

Fig. 4. A and B. Photomicrographs of cross sections of the paired "testes". In these cases the germ cells of cypris origin are to be seen in both of the paired "testes". ×ca. 150.

F has degenerated and has become entirely useless.

Conclusion

The Rhizocephala which have been monographed by Krüger (1940) consist of five families: Peltogastridae, Lernaeodiscidae, Sacculinidae, Clistosaccidae and Sylonidae. With the exception of the members of the family Sylonidae all the known Rhizocephala are, at present, considered to be hermaphroditic animals. *Peltogasterella socialis* with which this inquiry is concerned, belongs to Peltogastridae which may be considered to retain most typical organization of the Rhizocephala. One of the great controversies related to the so-called hermaphroditic Rhizocephala is the question of cross-fertilization. In spite of the presence of the complemental males (cypris larvae) in some genera (*Sacculina* and *Peltogaster* etc.), the existence of cross-fertilization has never been established. Reinhard (1942) provided the first decisive evidence of the reproductive capacity of the complemental male in *Peltogaster paguri*, but we are unable to look upon the rôle of the complemental male the way he described, namely: "the spermatozoa derived from the cypris presumbly mingle with the self-produced sperm of the hermaphrodite to effect fertilization of the first batch of eggs."

At present, we would aviod a too positive statement, but hitherto described observations strongly suggest that adult *Peltogasterella socialis* is structurally female and propagates by a continuous round of cross-fertilization by means of the dwarf male (cypris larva). We therefore suggest the term seminal receptacle to designate the so-called testis of *Peltogasterella*.

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