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Fine Structure of the Sterile Testis of Hybrid Threespine Stickleback between Marine and Landlocked Forms

Yoshiharu Honma, Akira Chiba and Eimitsu Tamura

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Abstract The process and cause of male sterility of a hybrid between female marine (M) and male landlocked (L) forms (M ♀×L ♂) of the threespine stickleback were studied by examining the testes of this hybrid and backcross offspring between M ♀ and F_1 (L ♀×M ♂) (MOLM ♂). In the M form numerous spermatids were elaborated and contained in the apical processes of the Sertoli cells, whereas in the ML and MOLM forms there were a number of macrophages in the lobular cavity with numerous projected pseudopodia engulfing voraciously the spermatids. As a result of this heavy phagocytosis the seminiferous lobules were quite vacant. Curiously, the Sertoli cells also actively project its processes and pseudopodia to catch the spermatids. In addition to morphometrical, behavioral and biochemical-genetic differences, these findings of sterility seem to suggest that the M form and L form represent independent species.

Sterility in both sexes of the offspring is known in the hybrids of threespine stickleback from marine (M) and landlocked (L) forms (Honma and Tamura, 1984). The male shows macrophage hyperactivity and the female, atresia of maturing ovum. Since Honma and Tamura's (1984) study was made with light microscope, the process of phagocytosis by the macrophages or other components, such as Sertoli cells was not made known. Although phagocytosis of Sertoli cells on the residual spermatids, relict sperms and degenerated germ cells are well-known for fish (Griel *et al.*, 1978; Griel *et al.*, 1980; Umeda and Hasegawa, 1984), it has never been demonstrated that such cells phagocytize the spermatogenetic and spermiogenetic cells.

The present work was therefore undertaken to clarify the process of phagocytosis and the cause of male sterility.

Materials and methods

The breeding procedure for getting the sterile males is the same as described by Honma and Tamura (1984). In addition, mating between F_1 hybrids (ML ♀×LM ♂; LM ♀×LM ♂) for obtaining F_2 offspring and 4 series of backcross experiments were also performed. The backcross pairing were as follows: LM ♀×M ♂, LM ♀×L ♂, M ♀×LM ♂ and L ♀×LM ♂. Table 1 summarizes the results of these crossing and backcrossing experiments.

For light microscopical studies, pieces of testis were fixed in Bouin's fixative, dehydrated through alcohol series, and embedded in paraplast. Serial sections were made at 6 to 8 μm thickness, and stained chiefly with hematoxylin-eosin, and azan trichrome. For electron microscopical examination, small pieces of testis were first immersed in

Table 1. Results of experiments on hybrids of the threespine stickleback between the marine (M) and landlocked (L) forms and their backcrossing.

Parental forms	Offspring forms	Results
1. M ♀+L ♂	F_1ML	male sterile
2. L ♀×M ♂	F_1LM	female sterile
3. ML ♀×LM ♂	F_2MLLM	eyed period, but did not hatching
4. LM ♀×LM ♂	F_2LMLM	non-development
5. LM ♀×M ♂	B_1LMMO	non-development
6. LM ♀×L ♂	B_1LMLO	non-development
7. M ♀×LM ♂	B_1MOLM	hatching, but male sterile
8. L ♀×LM ♂	B_1LOLM	died 3 months after hatching

2% glutaraldehyde solution in 0.1 M cacodylate buffer (pH 7.4) for 2 days, and postfixed in 1% OsO_4 in the same buffer for 2 hours. The specimen was then dehydrated by graded alcohol, and embedded in epon 812. Semithin sections (1 μm thick) were stained with toluidine blue and examined with a light microscope (Leitz Orthoplan), but ultrathin sections were stained with uranyl acetate and lead nitrate, and examined with a Hitachi H-500 transmission electron microscope and a Hitachi S-500 scanning electron microscope.

Results

As described by Honma and Tamura (1984) for the case of ML combinations, the adult fish produced through our backcross combination (MOLM ♂) were also sterile, with a drastic infiltration of macrophages in the seminiferous tubules. Therefore, the detail of phagocytosis found both in F_1 hybrid (ML) and backcross offspring (MOLM) were studied with the aid of electron microscope.

At a low power magnification of SEM, the seminiferous tubules of ML and MOLM were empty without any spermatogenic cells (Fig. 1). In an earlier stage of spermatogenesis, there were numerous, spherical macrophages with many protrusions and blebs scattered over the surface of the tubular wall (Fig. 2). However, later in the spermiogenesis, spermatids and sperms were found gathered around a macrophage to form a ciliary ball-like mass (Fig. 3).

TEM study of this mass of macrophagocytosis disclosed that phagocytosis had occurred (Figs. 4, 5, 6). There were several, large vacuoles (the lacunae) in a macrophage, in which various kinds of phagocytized cells being dissolved by phagolysosomes were seen. Some of these phagolysosomes maintained the state of dense nucleus with several mitochondria in the neck region, while other showed somewhat less dense condition (Figs. 4, 5). In the state of advanced dissolution, the nuclear body changed into a residual body (3rd lysosomes) with lamellar configuration. These bodies close to the final stage of digestion exhibited myelinated structure (Figs. 5, 6).

On the other hand, the section of macrophage also showed several pseudopodia and surface and marginal folds projecting into the tubular lumen. The cytoplasm of the macrophage was filled with countless vesicles and tubules which seemed to have originated from the cross sections of agranular endoplasmic reticulum (ER) and Golgi apparatus. Rarely, tubular ER formed graceful curvilinear patterns and concentric whorls and circles that resembled the annulate lamellae (Fig. 6). Occasionally, in the center of this type of circle there is a phagolysosome located in a lacuna.

The Sertoli cell of fish from the controlled marine type (M) was tall, extending from the basal lamina to the lumen of the seminiferous tubule, and contained an irregular, indented nucleus (Fig. 7). The marginal fold were remarkably projected into the lumen. There were elongated mitochondria, a large Golgi apparatus consisting only of a simple, but parallel stacks of cisterna, and rather numerous vesicular and/or tubular components of agranular ER and a small amount of granular ER.

The processes of marginal folds surround the spermiogenetic cells (Fig. 7 shows the spermatids in a later period). As nuclear condensation proceeds, the karyoplasm showed a granular condition and the mitochondria gather near the future neck region that surrounded the base of the flagellum (Fig. 7). In other words, the spermiogenetic cells anchored deeply into the depth of Sertoli cells.

On the other hand, in the germinal epithelium of ML and MOLM, there was active phagocytosis of spermatids prior to the elaboration and release of sperms by the Sertoli cells. The spermatid was grasped and enveloped by the pseudopodia and slender process projected from the margin of the Sertoli cell. After taken into the cell, dessociation of karyoplasm and then mitochondria in the spermatid occurred (Fig. 8).

Discussion

Although many papers have been published dealing with the fine structure of fish Leydig (interstitial) cell, reports dealing with the detail of Sertoli (sustentacular) cell of fish are notably scarce. Several investigators have described the ultrastructural design and behavior of Sertoli cells from several species of teleosts, among them are Gresik *et al.* (1973) on medaka, van den Hurk *et al.* (1974) on black molly, Grier *et al.* (1978) on goodeid, Grier *et al.* (1980) on several species of bony fishes and Grier (1984) on atherine fish. The companion cells found within the lumina of the tubules of the brook stickleback described by Ruby and McMillan (1970) seem to be homologous to the Sertoli cells.

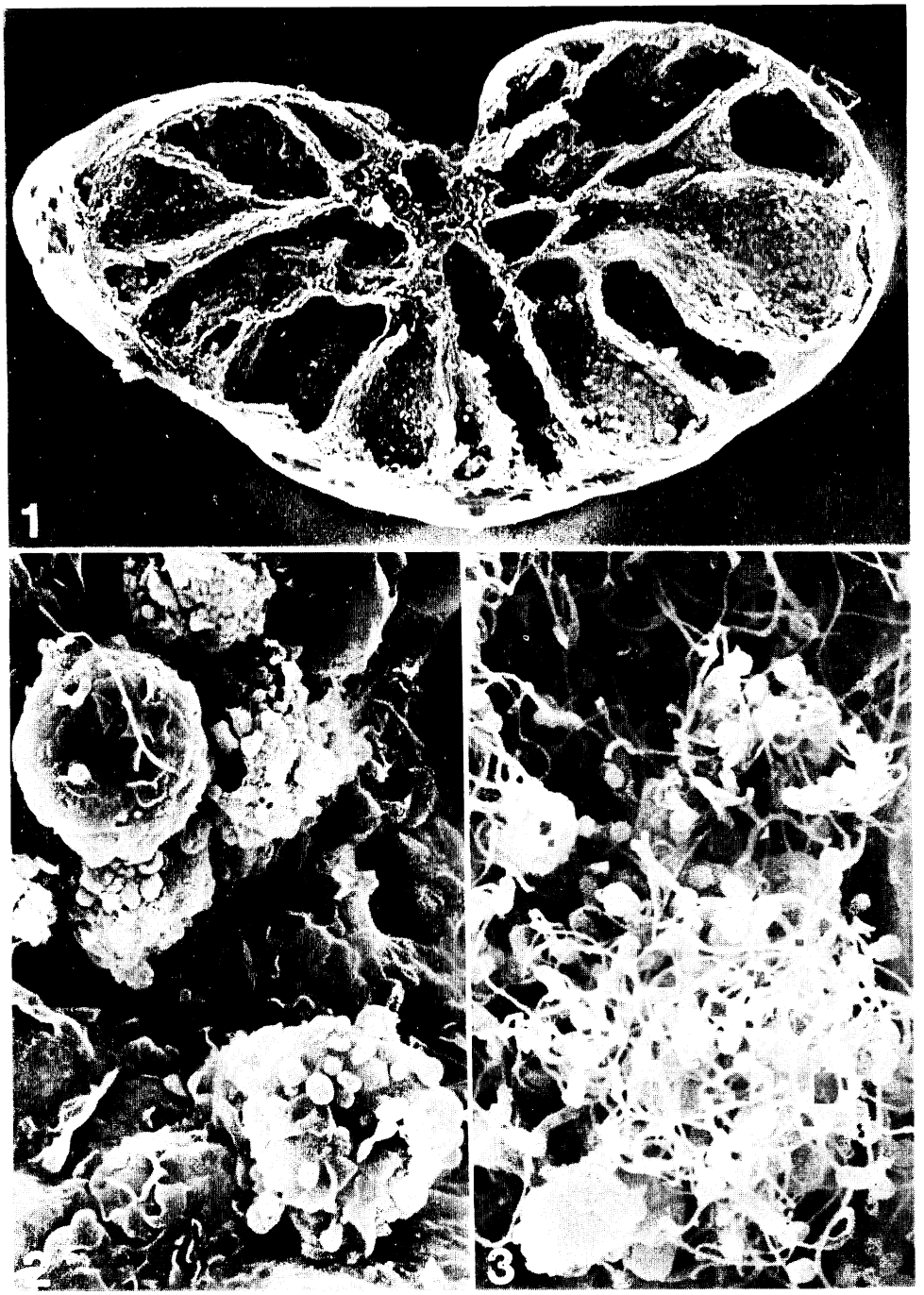


Fig. 1. Scanning electronmicrograph (SEM) of a sectioned testis of the threespine stickleback backcrossed between female marine (M) and male LM (female landlocked form (L)×male M form) (MOLM). Note every seminiferous tubule is void of spermiogenetic cells. ×100.

Fig. 2. SEM image on the tubular wall of the earlier period of spermatogenesis showing spherical macrophages with many protrusions. (MOLM) ×2700.

Fig. 3. SEM image of a late period of spermiogenesis, showing macrophages catching numerous spermatids and sperms (MOLM). ×2700.

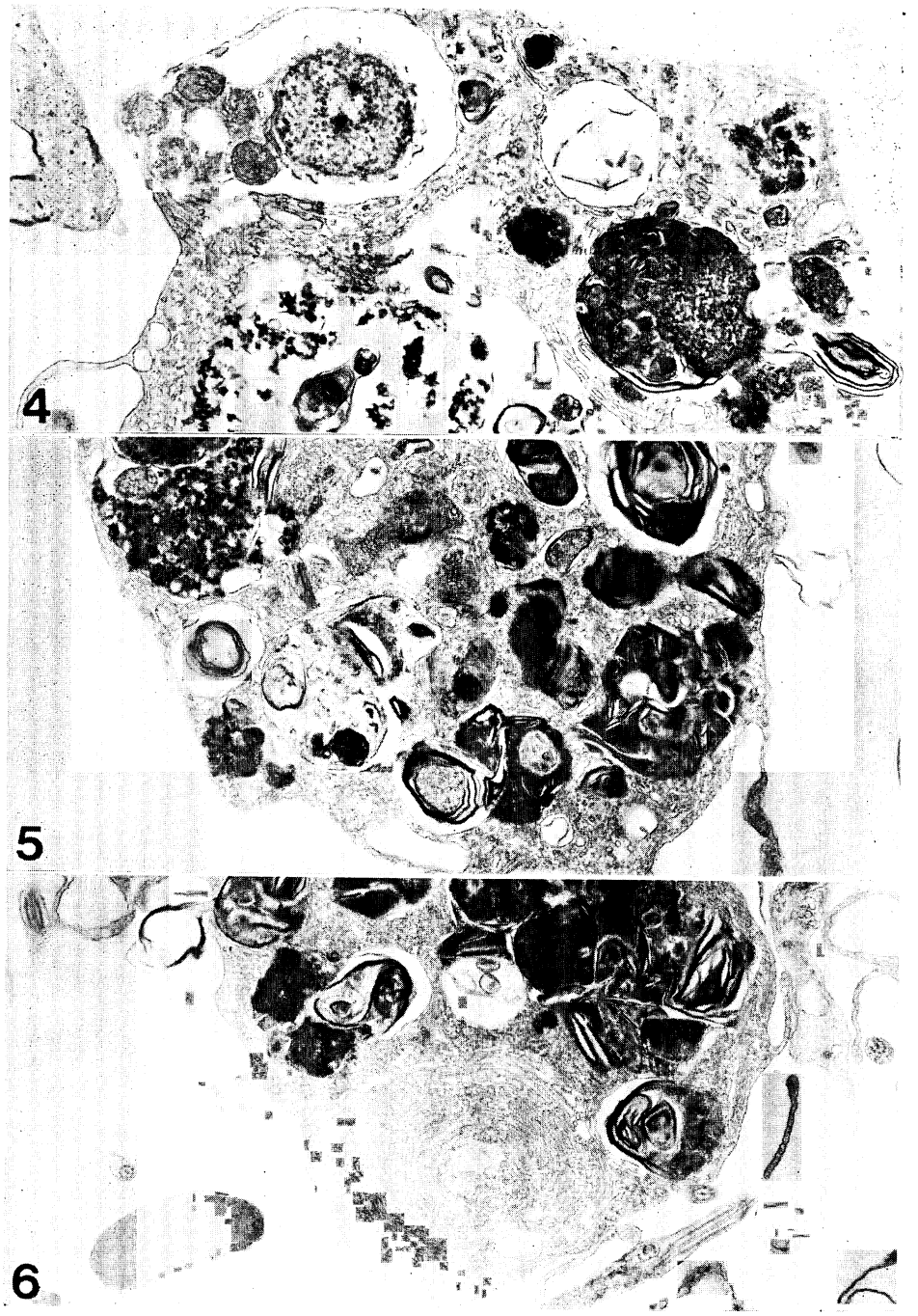
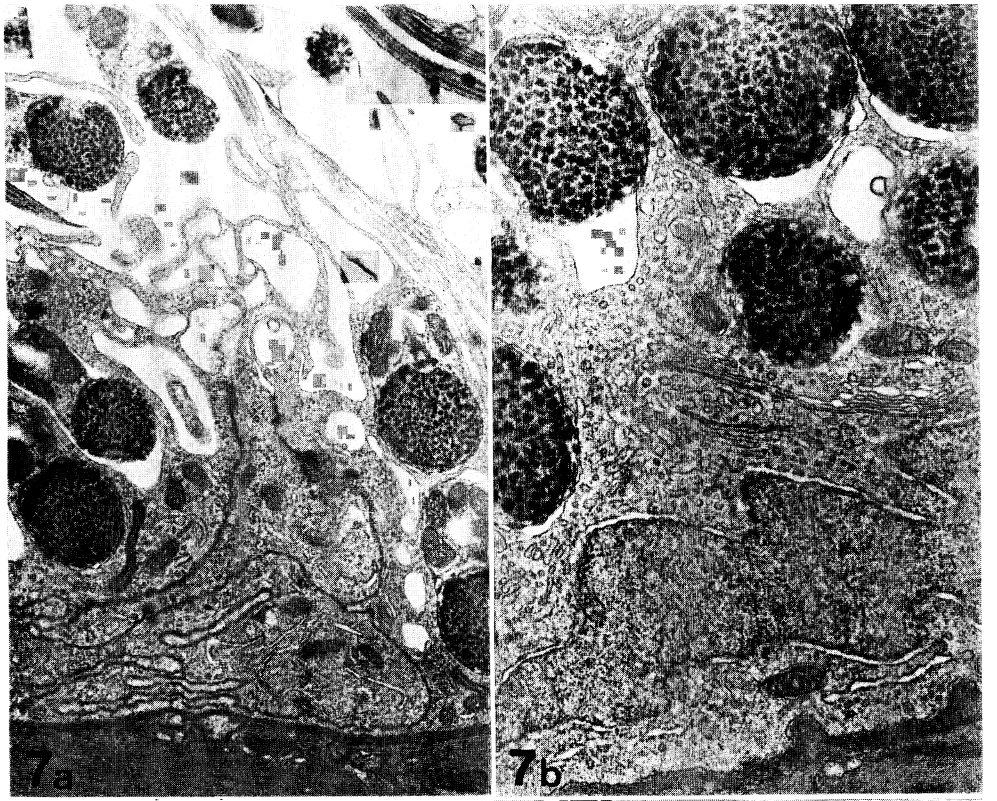


Fig. 4. Transmission electronmicrograph (TEM) showing a macrophage catching and phagocytizing many spermatids (MOLM). ×18,000.

Fig. 5. TEM image showing various processes of phagocytosis of spermatids in a macrophage. Note the residual bodies considered as third lysosomes (MOLM). ×10,800.

Fig. 6. TEM image of a macrophage showing residual bodies and tubular ER which is arranged in an annulate lamella (MOLM). ×9000.



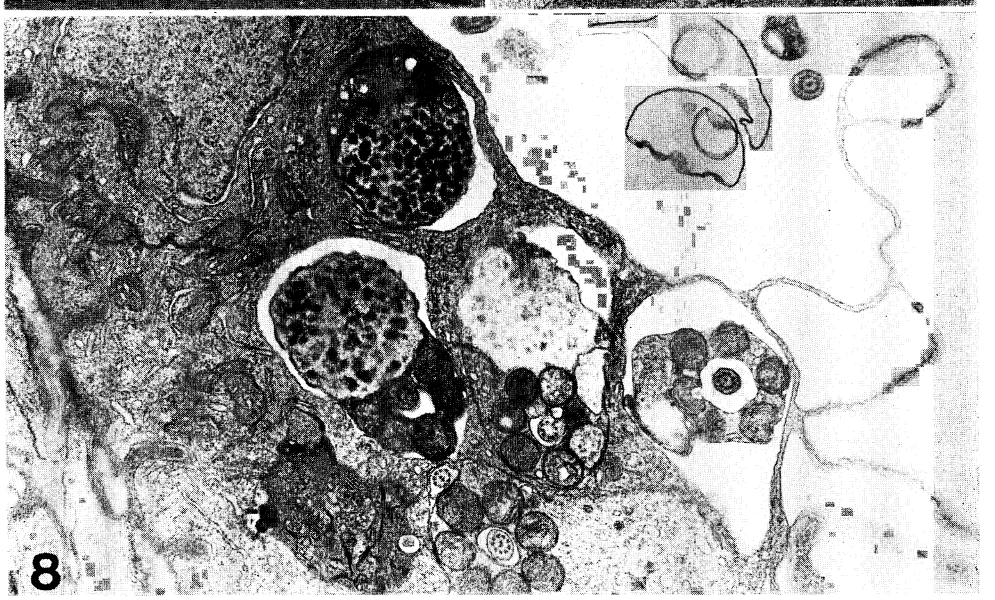


Fig. 7. TEM image of the Sertoli cell of the marine (M) form (control). Notice an irregular and indented nucleus, and remarkable projections of marginal folds of the cell. Several spermatids anchored deeply into the projections of Sertoli cell. a ×10,000, b ×18,000.

Fig. 8. TEM image of the phagocytosis of the spermatids by the Sertoli cell. Note dissociation of karyoplasm and mitochondria (MOLM). ×18,000.

In these papers, it was clear that the function of the Sertoil cell includes the mechanical support for the various stages of spermatogenetic cells, the protection of these spermatogenetic cell, elaboration and release of spermatids in the later period of metamorphosis and phagocytosis of residual sperms and spermatids. Yeung *et al.* (1985) discovered that in the Sertoli cells of the sex-reversing rice field eel there was less activity of steroidogenesis, and van den Hurk *et al.* (1974) found no indication of a steroid synthesizing function in the black molly.

As far as we are aware, only one published work is known dealing with the phagocytosis of residual sperms by the Sertoli cells. In it Umeda and Hasegawa (1984) dealt with cultured yellowtail in Japan. Their description and illustrations were rather brief and indistinct in disclosing such phagocytosis. Therefore, the matter of phagocytosis of spermatids before elaboration and release of sperms to the lobular lumen still remains unaccounted for. Moreover, the reason why the Sertoli cell recognized these spermatids as alien before accomplishment of spermiogenesis is unknown at present.

As to the process of phagocytic invasion and the activity of macrophages in the testis of fish, only one report is known, in which Ruby and McMillan (1970) documented this activity in the brook stickleback. In a previous paper, Honma and Tamura (1984) disclosed a drastic infiltration and an active phagocytosis of macrophages in the threespine stickleback. However in both works, the fine details of the process of phagocytosis of macrophages were undisclosed.

The cause of male sterility in the threespine stickleback occurring in F_1 offspring between M females and L males and backcross offspring between M females and LM males is assumed, based on our study, to be brought about by the phagocytosis of macrophages and Sertoli cells. This fact is considered to be related to reproductive, physiological, and also genetic isolation between M and L. With regard to the genetic difference of threespine sticklebacks between M and L. Taniguchi *et al.* (1985) reported that the value of genetic distance by electrophoresis was estimated as 0.680 (mean). This figure is equivalent to the values of interspecific and/or intersubspecific differences. They also calculated that the rate of heterozygote in the M is 0.040, whereas in the L it is 0.004. A remarkable low percentage of heterozygosis may suggest the fixation and homozygosis of genes in L. These information suggest that the present status of M and L forms may be regarded as an independent species.

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(YH: Faculty of Science, Niigata University, 8050 Igarashi-ninocho, Niigata 950–21, Japan; AC: Biological Laboratory, Nippon Dental University, 1–8 Hamaura-cho, Niigata 951, Japan; ET: Niigata Kita Senior High School, Oogata, Niigata 950, Japan)

イトヨの降海型と陸封型間の雑種不妊精巣に関する電子顕微鏡的観察

本間義治・千葉　晃・田村栄光

イトヨの降海型（M）と陸封型（L）の交雑を試みたところ，M♀とL♂との組合せでは雑種個体（F_1）の雄が不妊となった．光学顕微鏡で観察したところ，大食細胞の活動によるらしいことがわかった（Honma and Tamura, 1984）．F_2は得られず，4組の戻し交雑のうちM♀とLM♂の組合せのみ親魚にまで発育したが，これも雄は不妊となった．そこで，不妊の過程と原因を明らかにするため，走査並びに透過電子顕微鏡によって検索してみた．正常の降海型雄親魚では，多数の精子細胞がセルトリ（支持）細胞の突起によって包まれ，精子に変態していく過程がみられた．一方，不妊雄（MLとMOLM）では多数の大食細胞が出現して，精子細胞を貪食し活発に処理しているのが観察された．さらに，セルトリ細胞も盛んに精子細胞を捕食していた．これらの食作用により，雑種の精巣の精細管は精子が形成されないで空虚化してしまう．雑種不妊による隔離機構に加え，両型間の遺伝的距離も離れていることがわかったので（谷口・本間・川真田，1985），現在では両型はそれぞれ独立した種であると見なしてよい．

(本間: 950-21 新潟市五十嵐2の町 8050 新潟大学理学部; 千葉: 951 新潟市浜浦町 1-8 日本歯科大学新潟歯学部; 田村: 950 新潟市本所字居浦 847-1 新潟北高校)