Studies on the Fertilization of the Egg of the Flounder. II. The morphological structure of the micropyle and its behavior in response to sperm-entry

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Knowledge is very meagre about the entry of spermatozoa in fish eggs, a few past works only being referable as the literature on the subject. Probably, this is due to the difficulty of observation of the micropyle at the entrance of spermatozoa in living state. The writer has succeeded in devising a method to observe the entry of spermatozoa into eggs through the micropyle in fresh material. The present paper describes the morphological structure of the micropyle and its behavior in response to sperm-entry in eggs of the flounder, *Limanda schrenki*.

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The studies have been carried out with both fixed and living material. For sectioning, the eggs were fixed with sublimate acetic acid. The sections were cut 10 micra thick and stained with Heidenhein’s iron haematoxylin with counter-staining of eosin.

1. The structure of the micropyle. The envelopes which cover the egg surface consist of two layers, an outer adhesive layer and an inner membrane called the zona radiata. The outer adhesive layer is comparatively thin in sections and stained deeply by haematoxylin. In living eggs immersed in normal sea water the outer layer is distinctly found swollen and shows a broad layer. The inner membrane existing directly underneath the outer layer is moderately thick in appearance measuring about 20 micra; it is almost transparent when fresh, while

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in sections it is stained deeply by eosin. The membrane can be subdivided into two layers, an upper granulated layer and a lower striped-like one. The micropyle is formed penetrating the envelopes at the animal pole. The structure of the micropyle is shown in the half-diagrammatic figure (Fig. 1). The inner canal of the micropyle (mc in Fig. 1), is a simple and narrow tube. It assumes a truncate, funnel-shape; it has a rather wide opening, but the diameter becomes smaller towards the base. The inner space of the canal is seen to be very narrow; probably only a single spermatozoon can pass through it. Around the canal of the micropyle there are several apparatuses, viz., the outer disk (od), middle cavity (mc) and inner protuberance (ip). The outer disk is a zone of half-moon shape situated outside the inner egg membrane. It is placed on the middle cavity like a cap. The canal of the micropyle opens at the central part of the outer disk. In the fresh material the upper part of the canal of the micropyle is easily observable being protruded from the inner egg membrane, but the outer disk is difficult to observe. In the sectioned material, however, the outer disk is clearly detected, and it is difficult to observe the upper part of the canal of the micropyle. The middle cavity of goblet-shape is found embedded in the inner egg membrane. The canal of the micropyle runs through the middle part of the cavity. The latter takes a highly elongated, tube form, narrowing toward the proximal portion, with a wide opening close to the margin. In fresh eggs, both the canal of the micropyle and the middle cavity can be observed by close observation. The inner protuberance directly underlying the inner egg membrane is found protruded from the membrane as a small body. The existence of this protuberance is evident both in living and in sectioned material, in the latter case showing a specific affinity to stain. The canal of the micropyle passes through the protuberance at its middle region. The inner protuberance was also shown in the egg of herring by Kanoh (’49), but concerning the other structure with the exception of the canal of the micropyle no description has been made.

2. Behavior of the micropyle in response to sperm-entry. The spermatozoon of this fish is characterized by having a spheroidal head, a short and round middle piece, and a very long tail (Fig. 2).
Observed fresh in sea water, the spermatozoa are seen to move by rotation and to approach the egg. At the outer opening of the micropyle, the spermatozoa usually move along the mouth-edge of the micropyle for a while. One of the spermatozoa just entered into the outer mouth of the canal of the micropyle, advances at once passing towards the middle part of the tube, but it suddenly stops at the inner part of the canal. When many spermatozoa enter into the canal of the micropyle, therefore, there is formed a row of spermatozoa in the tube (Fig. 3-b). The one which has first entered into the canal appears as a bright body. It advances slowly downward in the canal and reaches the inner mouth. The entry of a spermatozoon can be recognized by its bright image at the opening mouth of the canal of the micropyle (Fig. 3-c). The spermatozoon takes about two minutes in passing the canal completely. In a moment the bright image disappears from sight, and then the boundary between the surface of the egg protoplasm and the inner margin of the protuberance becomes clear. Following this change, the inner part of the boundary becomes evident with the gradual formation of the perivitelline space. In a few minutes the canal of the micropyle becomes narrower and at the same time the spermatozoa lying in the canal are observed to be pushed out from the micropyle as if they are discharged from the egg (Fig. 3-d and Fig. 4-c). Along with the elevation of the egg membrane a ball-like body is frequently found formed at the mouth part of the micropyle. At first the ball is observed as a very minute one, but it grows with time into a moderate size, being greater than some 15 μ in diameter (see mb in Fig. 3-f and Fig. 4-d).

Fig. 3. Diagrammatic figures showing the behavior of the micropyle in response to sperm-entry.
From the above observations, it can be understood that there are discernible three significant occurrences in the change of the micropyle in response to sperm-entry. The first one is the prevention of the ad

![Fig. 4. All figures, except b, are photographed from living eggs.](image)

vance of many spermatozoa into the inner part of the canal of the micropyle. Probably this is attributable to the small size of the canal. Because of this smallness it becomes difficult for many spermatozoa to pass through the micropyle at the same time and this certainly serves to prevent pathological polyspermy. The second significant occurrence is the discharge of the spermatozoa from the canal. Obviously, in this manner the egg is also free from polyspermy. The canal of the micropyle becomes narrow with time due to the swelling of the inner egg membrane. Also, the contraction of the canal is observed to occur early at the inner part of the canal. This is evident by referring to the fact that when the eggs at this stage are reinseminated with fresh sperm, the spermatozoa are found lying at the middle part of the canal.

![Image of specimens](image)
without advancing further. The discharge of the spermatozoa from the canal, however, seems not to be due to the gradual contraction of the canal, but is concerned with some other causes. As the third significant occurrence there is the formation of the ball-like body at the mouth part of the micropyle. The ball-formation was observable in many eggs, though not all. Its formation was observed also in unfertilized eggs which have formed the perivitelline space. Nothing is known at present about the significance and mechanism of the ball-formation.

**LITERATURE**
