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The Adherent Filaments of Eggs of Isaza (*Chaenogobius isaza* Tanaka, Pisces)

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ABSTRACT The adherent filaments of eggs of Isaza (*Chaenogobius isaza* TANAKA) are formed during the tertiary yolk globule stage between the egg membrane and the granulosa cell layer. The long granulosa cells are arranged along the meridional line from the micropyle to the antipodes. Since the filaments are formed between the granulosa cell rows, the arrangement of the filaments is dependent on the arrangement of the granulosa cells. Thus, the filaments show a reticular structure around the micropyle, and run almost parallel to the meridional lines on the rest of the egg surface. Once the egg is released into water and sticks to the wall of the vessel, the adherent filaments detach from the egg membrane and turn inside out, except around the micropyle and in this way form the stalk of the egg. In other species of gobiid fish, the structure and origin of the adherent filaments is essentially the same. The eggs of osmerids and salangids, though only distantly related to gobiids, also have adherent membranes (filaments) of similar structure. (*Zool. Mag. 87 216-220, 1978*)

The eggs of Isaza *Chaenogobius isaza* TANAKA) are laid as a sheet of one close layer on the under side of stones on the stony shore of Lake Biwa. Each deposited egg has a tuft of adherent filaments on one end of the oval face, and sticks itself on the stone surface by this filament tuft (Fig. 1). This report describes the shape and the origin of the adherent filaments of Isaza in comparison with the adherent structures of other kinds of fishes which have been reported.

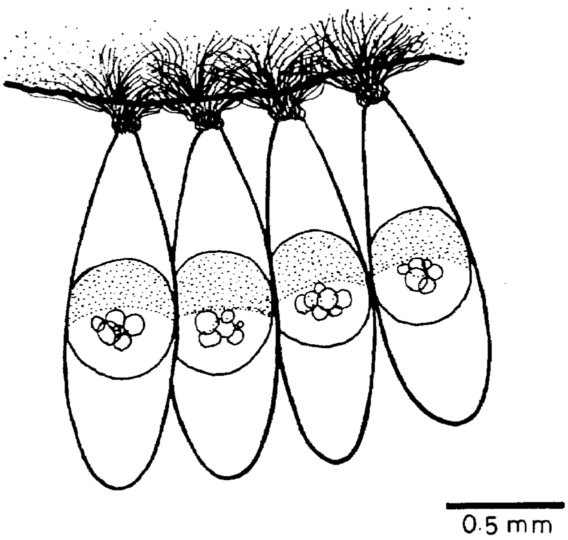

Fig. 1. Deposited eggs of Isaza.

Materials and Methods

For the observation of ovarian eggs in the process of maturation, the fish were caught with a trawl net or trap called "Eri" in Shiozu Bay situated in the north end of Lake Biwa from September 1974 to August 1975. Their ovaries, fixed in Hollande's solution, were sectioned by the usual paraffin method and stained with Mayer's haematoxylin-eosin or Mallory-Heidenhain's azan triple stain. To observe the whole structure of completed adherent filaments and the adhering manner of the egg, the live ovarian eggs just before and after ovulation were also supplied from the fish caught with the trap "Eri" in April and

May 1977.

Results

Observations of the ovarian eggs of the tertiary yolk globule stage showed that a fibrous structure was formed between the granulosa and egg membrane as a parallel ridge, and the fibers alternated with granulosa cell rows (Fig. 2).

Though the arrangement of the fibrous structure was closely related to that of the granulosa cells, until the end of the peri-nucleolus stage (October-November) it was difficult to confirm any definite arrangement of the cells. From the yolk-vesicle stage (December), however, the long granulosa cells could be seen arranged in a definite direction (Fig. 3). From the secondary yolk stage on, micropyle could be seen very clearly. At this stage, the oval granulosa cells were arranged radially around the micropyle (Fig. 4). Fibers were surrounding each of the granulosa cells around the micropyle, so that the fibers showed a reticular structure (Fig. 5). A similar reticular structure was observed in the basal part of the adherent tuft of the deposited eggs. The long granulosa cells were arranged along the meridional line, and the fibrous structure developed along these granulosa cells (Fig. 6). In the earlier phases, the fibers ran in a curve along the outline of the spindle-shaped granulosa cells (Fig. 7), and afterward they became parallel to each other except in the region close to the micropyle.

With maturation of the eggs, the fibrous structure became conspicuous while the granulosa cells became indistinct except in the region around the micropyle.

When ovulated eggs were artificially taken out from the ovary, the fibrous structure was very adhesive. Once the egg stuck to the wall of the vessel or to something solid, the whole fibrous structure, except for a part around the micropyle, became detached from the egg membrane by the mechanical action of the movement of water and turned inside out. This detachment and turning out of the fibrous structure often also occurred in the course of taking eggs out of the ovary. In any case, the fibers stuck and got tangled with each other but they never stuck to the egg membrane. The fiber was so elastic that it rarely broke. The egg membrane then swelled up gradually taking a roughly ellipsoidal shape with the egg floating in the middle of it, as observed with eggs spawned in the field. This swelling of the egg membrane occurred regardless of fertilization.

Discussion

These observations clearly demonstrate the identity of the fibrous structure on the surface of ovarian eggs and the adherent filaments of the deposited eggs. Although the author could not observe the spawning in the field, the released eggs must stick to the stone in the same manner as observed in the laboratory. That the adherent filaments do not stick to the egg membrane is an important factor for the eggs to be arranged on the stone in a single sheet. As the egg is heavier thah water, Isaza must spawn keeping its genital orifice in contact with the stone ceiling.

The demersal teleost eggs adhering to the substrate can be classified into two groups. One consists of those which have no special structure on the egg membrane and stick to the substrate with mucus. The other consists of those which have some special structure on the egg membrane as in Isaza. The author proposes to divide the latter group according to the phylogeny into 1) Gobiidae (including Isaza), 2) Cyprinodontida and Belonida, 3) Osmeridae and Salangidae.

The egg of *Oryzias latipes* (Cyprinodontida), has long sticky filaments near the vegetal pole and short jointed papillae all over the egg surface (Kamito, 1928). Adherent filaments of the egg of belonid fish are found sparsely all over the egg. The eggs of the other fishes of Belonida have a common character of having a fine filaments group and one thick filament (Matsubara and Ochiai, 1965). The structure of the adherent filaments of Isaza, which are jointless and form a tuft on the animal pole, differs from those of Cyprinodontida or Belonida. Therefore their origin and the mode of formation also must be different.

The egg of *Plecoglossus altivelis* (Osmeridae) has an adherent membrane over the animal hemisphere, and the membrane is perforated with many pores (Iwai, 1962). The eggs of the other osmerid fishes like *Hypomesus* and *Osmerus* have similar adherent membranes. Though those are not filamentous, the membranes are attached to the egg membranes at the micropyles and adhere to the substrate in turning inside out, just as seen in the filaments of Isaza egg.

Wakiya and Takahashi (1937) reported that the egg of Salangidae had an external egg membrane. The external egg membrane of those salangid fishes also has a function of adhering to the substrate in turning inside out. The morphology of the membrane varies with species. In most cases it consists of filaments radiating from the micropyle and resembling the adherent filaments of Isaza. Only the membrane of *Salangichthys ishikawae*, unlike those found in eggs of the other salangids, overlies the animal hemisphere and has some large holes arranged radially. The other part of this membrane is perforated with many pores. This configuration resembles that of the adherent membrane of *Plecoglossus altivelis* eggs.

In the Gobiidae other than Isaza, *Leucopsarion petersi* lays eggs with a reticular external egg membrane which, fixed at the animal pole, adheres by turning inside out (Kitahara, 1904).

Te Winkel (1935) reported a similar filamentous structure in *Mistichthys luzonensis* extending out from the animal pole. She did not describe the manner of formation and the arrangements of the filaments over the egg membrane in she ovary, but the mentioned that "in sections there is a single layer o. granulosa cells between every two villi."

On *Gobius minutus* the similar observation was reported (Guitel, 1892).

Matyjewicz-Juszczyk (1973) reported in his study concerning capillary networks of a ovarian follicles in *Mesogobius batrachocephalus* and *Neogobius melanostomus*, that granulosa cell rows and adhesive fibers were arranged alternately and ran meridionally.

In the other gobiid fishes, deposited eggs adhere to the substrate with filaments starting from one extremity of the egg. All these filaments can be regarded as having a similar origin and structure as well as function to the filaments found in Isaza eggs.

As mentioned above, eggs of most gobiid fishes have adherent filaments similar to those of Isaza. The adherent membrane of eggs of osmerids and salangids also share the same characters with the eggs of Isaza. It is therefore likely that all these structure are homologous. The adherent filaments found in Cyprinodontida and Belonida, on the contrary, are of different origin.

The process of formation of these adherent filaments or membranes has not been reported. That, in the case of Isaza, the form of adherent filaments is determined by the arrangement of granulosa cells suggests that the filament is formed by the secretion of these cells. The adherent filaments in Gobiidae, Osmeridae and Salangidae may be formed likewise, with differences of form depending on the different arrangement of the granulosa cells and a different mode of secretion. Closer comparative studies are now in progress.

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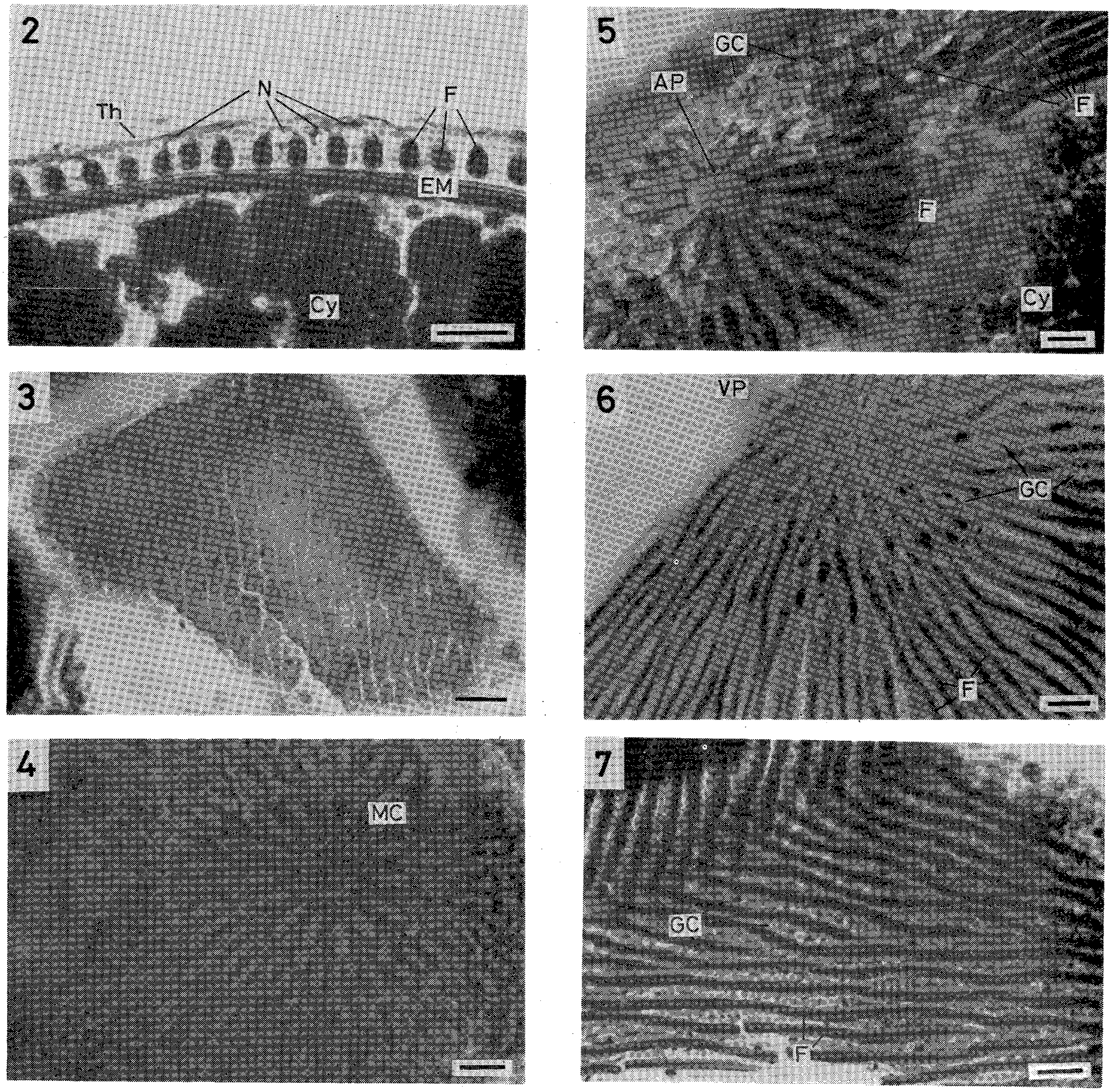

Figs. 2–7. Photomicrographs showing the arrangement of granulosa cells and fibers.

Fig. 2. Cross section of ovarian egg showing granulosa cells and fibers surrounding the egg. ('75 June 2, 1+)

Fig. 3. Granulosa cells arranged in a definite direction. ('75 Feb. 11, 0+)

Fig. 4. Granulosa cells at the pole with micropyle. ('75 Feb. 11, 0+)

Fig. 5. Fibers near the pole with micropyle. ('75 Mar. 20, 1+)

Fig. 6. Fibers near the pole opposite to micropyle. ('75 June 2, 1+)

Fig. 7. Fibers and granulosa cells arranged alternatively. ('75 June 2, 1+)

Th: theca, N: nucleus of granulosa cell, F: fiber, EM: egg membrane, Cy: cytoplasm, MC: mycropylar cell, GC: granulosa cell, AP: animal pole, VP: vegetal pole, The scales represents ten micrometers.