Micropyle in the Developing Eggs of the Anchovy, *Engraulis japonica*

Akio Hirai and Tadashi S. Yamamoto

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The teleost demersal egg is usually equipped with a specialized envelope apparatus that appears to be useful for embryonic development in natural circumstances. Examples of such an apparatus are an adhesive layer enclosing the herring egg (Kanoh, 1949) and filamentous threads extending from the vegetal surface of the medaka egg (Yamamoto, 1961). Since the apparatus in some cases shows a species-specific structure, it gives a clue for identifying the species of parental fish. Electron microscopic observations of the envelope have recently shown that the structure of micropyle is also species-specific in salmonid eggs (Szöllösi and Billard, 1974; Riehl and Schulte, 1977, 1978; Riehl, 1980). With the exception of a few fish groups, however, specialized envelope apparatus are usually not observed by light microscopy of pelagic eggs (Ahlstrom and Moser, 1980). In order to investigate the possibility that the micropylar structure in the pelagic fish egg can be used for the identification of the parental species, electron microscopy was performed on the envelope of anchovy eggs. We found an unusual conformation of the micropyle in the developing eggs of this fish.

Materials and methods

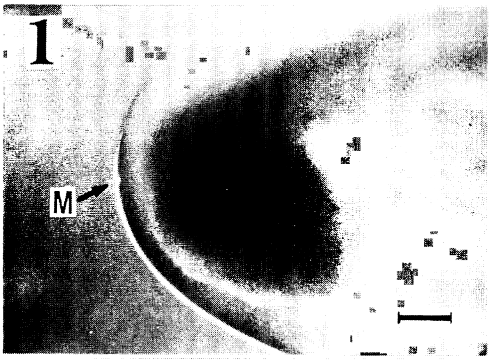
In May 1984, the developing eggs of the anchovy, *Engraulis japonica*, were collected off the northern coast of Kyushu near Genkai-cho, Saga Prefecture, by means of a plankton net. These were fixed and preserved in 10% formalin in sea water. The eggs examined were at the early gastrula stage or the eyed period of embryonic development. In preparation for electron microscopy, the eggs were cut into halves with a Wecker's scissors to separate the envelope from the embryonic body. Pieces of the egg envelope containing the micropyle were rinsed in 0.1 M cacodylate buffer (pH 7.4) and postfixed in 1% OsO_4 in the same buffer. For the purpose of scanning electron microscopy (SEM), these were dehydrated in ethanol, critical point dried in CO_2 and sputter-coated with gold. The samples were then examined in a JEOL JSM-T20 scanning electron microscope. For transmission electron microscopy (TEM), the egg envelope postfixed in OsO_4 was dehydrated in acetone and embedded in Epon 812. Thin sections were cut and mounted on collodion-coated copper grids. Following stain in uranyl acetate and lead citrate, these were observed in a JEOL JEM-100S transmission electron microscope.

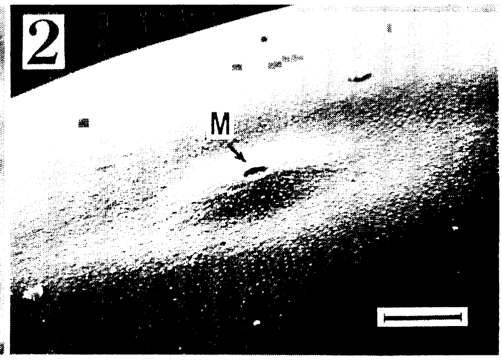
Observations

The female anchovy spawns pelagic eggs. The developing egg is elliptical in shape and measures about 1.4 and 0.7 mm on its long and short axes, respectively. The egg envelope is almost transparent and relatively thin (about 2 μm in thickness). At one end of the elliptical egg, however, the envelope is apparently thicker than at other parts of the egg (about 4 μm in thickness). Since the micropyle is observed in the thick part, the long axis of the ellipsoid represents the egg axis of this fish (Fig. 1). Under the light microscope, no specialized apparatus useful for fixing the egg onto the substratum is detected on the surface of the envelope.

From SEM, it was observed that the outer surface of the envelope is not smooth but possesses a number of small knobs. With the exception of the micropylar region, these show a uniform distribution throughout the egg surface (Fig. 2). In the micropylar region, the envelope shows a circular elevation of the surface measuring about 15 μm in diameter. Thin sections reveal that the envelope consists of concentric layers organized in different ways (Fig. 3) (cf. Kobayashi, 1982). The outer layer—zona pellucida externa—is extremely thin and composed of high electron-dense material. The inner layer—zona pellucida interna—forms the bulk of the envelope and consists of alternating dark and light lamellae of different thicknesses (Fig. 3); the outermost lamella shows medium electron density. Each lamella is composed primarily of fibrillar material. Since the innermost lamella looks like an accumulation of granular material showing medium electron density, the arrangement of fibrillar material seems to be different from that in the other lamellae of the interna.

High magnification by SEM of the micropylar





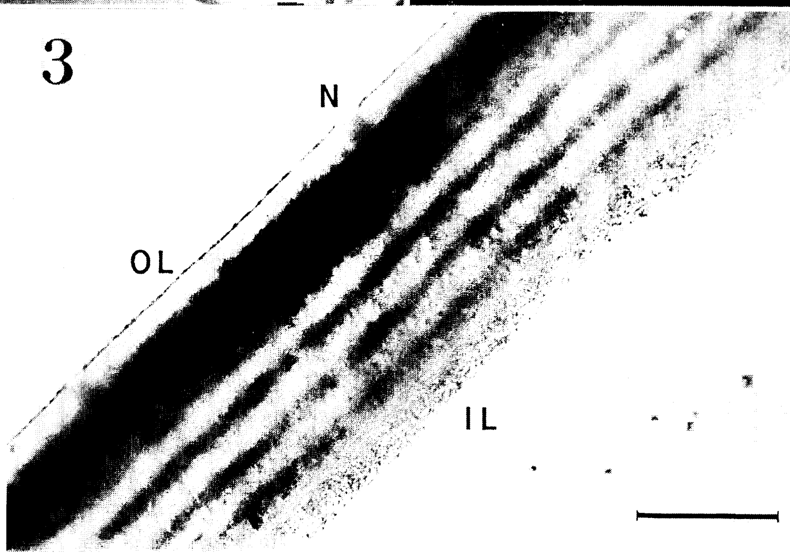


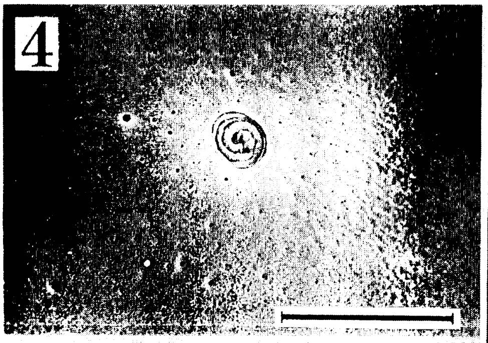
Fig. 1. Microphotograph of a part of the formalin-preserved egg. M, micropyle. Bar = 100 μm.

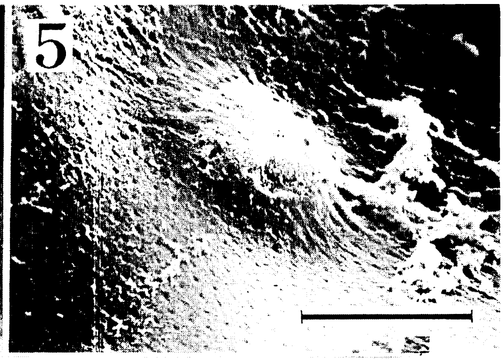
Fig. 2. SEM of the animal pole region of the egg showing the circular elevation of the envelope surface at the micropylar region. M, micropyle. Bar = 10 μm.

Fig. 3. Section through the envelope of the animal pole region showing a niche (N) of the outer surface. Note the granular organization of the innermost lamella of interna. IL, z.p. interna; OL, z.p. externa. Bar = 1 μm.

region reveals that the elevated region of the outer surface of the envelope possesses distinct pores. The outer opening of the micropyle measures about 3.2 μm in diameter and is observed in the center of the region (Fig. 4). The inner surface of the envelope also shows a circular elevation in the region surrounding the inner opening of the micropyle (Fig. 5), although the size of the region is about one half of that observed on the outer surface of the envelope. Probably owing to precipitates produced during the preparation of samples, however, we are unable to observe any details of the surface structure in this region of the envelope. In thin sections through the elevated region of the envelope, the externa is disrupted at intervals; the envelope thus shows niches on the surface. Judging from the width of these niches, the pores observed by SEM of the elevated surface of the envelope represent the sites where the externa is disrupted. It is of interest that the outermost lamella of the interna invariably contains a small amount of electron-dense material in the region beneath the niche of the surface.

An important fact observed by SEM of the envelope is that the outer opening of micropyle is almost completely filled with convoluted structures (Fig. 4). No canal traversing the envelope





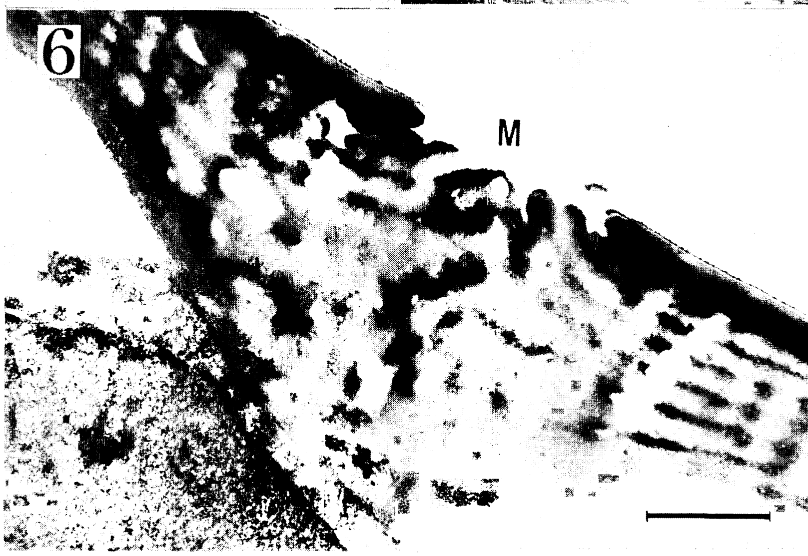


Fig. 4. A high magnification of the micropylar region. Note convoluted structures in the outer opening of micropyle. Bar = 10 μm.

Fig. 5. SEM of the inner surface of the envelope showing the circular elevation that contains the inner end of the micropyle. Bar = 10 μm.

Fig. 6. Section through the micropyle. Note annular ridges of the interna which close the outer opening of the micropyle (M). Bar = 1 μm.

is detected in serial sections of the micropylar region. It is assumed that the micropylar canal in the developing anchovy egg collapses as a result of swelling of the interna of the envelope. In fact, annular ridges of the interna are observed in the outer opening of the micropyle (Fig. 6). There is no doubt that the convoluted structures filling the micropyle observed by SEM of the envelope are formed by the material of interna. The presence of many vacuoles in the envelope also suggests that the inner layer tends to swell at the micropylar region.

Discussion

The envelope of the anchovy egg consisted of concentric layers of different electron density, zona pellucida externa and interna. A similar structure of the envelope was reported in both demersal and pelagic eggs (Busson-Mabillot, 1973; Dumont and Brummet, 1980; Davenport *et al.*, 1981; Kobayashi, 1982; Ohta *et al.*, 1983; Groot and Alderdice, 1985). No specialized apparatus which could be useful for the fixation of the egg onto the substratum in natural circumstances was recognized by SEM and TEM of the anchovy envelope. The interna showed the lamellar organization and did not contain radial canals.

Although there are no reports on the differentiation of envelope in oogenesis of this fish, microvilli of the oocyte may traverse the thickness of the interna during the formation of the envelope (Kobayashi, 1985). Since microvilli form a passive barrier to deposition of material for the egg coat (Kobayashi and Yamamoto, 1985), radial canals with these villi in their lumen may be observed in the envelope enclosing the young oocyte. Concurrently with the withdrawal of the microvilli from the canal lumen at the final stage of oocyte maturation, however, the radial canals may disappear from the interna. Since deposition of material for the externa secreted from the follicular cells (Flügel, 1967) is also disturbed by the presence of the villi, the disruption of externa (surface niches) may represent the outer orifice of the previous radial canals.

According to Riehl and Götting (1974), the micropyle of the fish egg is classified into three types. It consists of a deep depression (vestibule) and a short canal in Type I. The micropyle in Types II and III possesses a long canal. In these cases, however, the micropylar vestibule is shallow (Type II) or difficult to recognize (Type III). The micropyle in the anchovy, porgy (Hosokawa *et al.*, 1981) and eel eggs (Ohta *et al.*, 1983) is composed of a simple canal without clear depression of the vestibule. Although the unfertilized anchovy eggs were not observed in this study, the micropyle of this fish may belong to Type III of the classification. In spite of the similarity in shape, the micropyle of the anchovy and eel eggs is apparently smaller than that of the porgy egg. The outer opening of micropyle in the fixed material measures 3 μm in the former eggs but is 5 μm in the latter. The diameter of the circular elevation of the surface that contains the micropyle is 15 μm in the anchovy egg, whereas it is 20 μm in the porgy egg (unpublished measurement). A similar elevation is not observed in the micropylar region of the eel egg, although the outer surface of the envelope shows numerous knobs with central depressions (Ohta *et al.*, 1983). Furthermore, the envelope of the anchovy egg possesses distinct pores in the elevated region of the animal pole. These observations suggest that the fine structural organization of the micropylar region is species-specific also in the pelagic fish eggs.

The micropyle of the developing anchovy egg was almost completely closed by the material of the interna. The micropylar canal which allows the passage of a fertilizing spermatozoon traverses the thickness of the envelope in the teleost unfertilized eggs. In *Esox lucius*, however, Riehl and Götting (1975) described that the micropylar canal of the "ripe" egg collected from the ovary is usually closed with remnants of the micropylar cell. Judging from the observation on the micropyle of the oocytes taken from the preovulatory salmon ovary (Kobayashi and Yamamoto, 1985), we assume that the materials used by these workers are not fully matured eggs. The closure of the micropylar canal in the developing anchovy egg is not an artifact of the fixation of the eggs in formalin, because the micropylar structure in many fish eggs is well preserved in the same fixative (unpublished observation). A swelling of the canal wall may occur during embryonic development in natural circumstances. Since the structure was the same in the eggs at different stages of embryonic development (early gastrula and eyed embryo), the swelling may occur at the cleavage stage in the anchovy egg. The decrease in diameter of the micropylar canal is actually observed in the chum salmon egg shortly after initiation of embryonic development (Kobayashi and Yamamoto, 1981). It is therefore plausible that the physico-chemical property of the envelope in the micropylar region is different from that in other regions of the egg (cf. Kanoh, 1949; Yamamoto, 1958, 1963). We are now carrying out experiments which clarify factor(s) to induce the swelling of the interna in the micropylar region of the pelagic fish eggs in natural circumstances.

Acknowledgments

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(AH: Tokyo Kyuei Co. Ltd., Shiba Tsurugamaru, Kawaguchi 333, Japan; TSY: Zoological Institute, Faculty of Science, Hokkaido University, Sapporo 060, Japan)

カタクチイワシ発生卵の卵門

平井明夫・山本　正

初期のう胚および発眼期にあるカタクチイワシ卵の卵門を走査型，透過型電顕で観察した．卵膜は薄い外層と厚い内層より成り，卵付着装置はみられない．内層は明暗交互に重なる薄層構造を呈するが，放射管を持たない．楕円形の卵の一端は僅かに表面が隆起し，その中央に卵門がある．卵門周辺の隆起には少数の比較的明瞭な小孔がある．卵門は前庭を持たず，その内部は渦巻き状構造物で充たされている．この構造物は未受精卵の卵門管壁を作っていた卵膜内層の膨張によって生じたものである．膨張は発生初期に起るらしく，固定処理によるものではない．卵門およびその周辺部の形態から浮性卵の魚種の同定が可能であると考えられる．

(平井: 333 川口市芝鶴ケ丸 6906-10 (株)東京久栄技術センター; 山本: 060 札幌市北区北 10 西 8 北海道大学理学部動物学教室)