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The Little Dragon Sculpin *Blepsias cirrhosus*, Another Case of Internal Gametic Association and External Fertilization

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Abstract In the copulating cottid species, *Blepsias cirrhosus*, the point at which fertilization occurred was determined experimentally. Ovulated eggs were obtained directly from the ovary of impregnated females. Eggs maintained in ovarian fluid did not show any signs of development, whereas most of the eggs which had been placed in seawater had developed to the 4-cell stage after 24 hrs. The eggs kept in ovarian fluid initiated segmentation when later transferred into seawater. Histological examination showed that a number of spermatozoa had entered the micropyle, but that penetration did not occur in eggs that were not immersed in seawater. In these eggs, the metaphase spindle of the second meiotic division was observed in the ooplasm, at the animal pole. These results indicate that, in impregnated females, the spermatozoa associate with the eggs in the ovarian cavity, but that fertilization occurs externally when the eggs are spawned.

Although there is little information on the reproductive habits of marine cottids, it is known that internal fertilization occurs in some species (Breder and Rosen, 1966). In an earlier study of the copulating cottid, *Alcichthys alcicornis*, we found that fertilization occurred externally after spawning of the eggs which had previously been associated with spermatozoa in the ovary (Munehara et al., 1989). These results suggested that other cottids, which are regarded as internally fertilizing species, may also exhibit internal insemination followed by external fertilization.

In the present study, the point at which fertilization took place was determined in the little dragon sculpin, *Blepsias cirrhosus* (Pallas), in order to confirm the possibility that internal gametic association is common in these copulating cottids.

Materials and methods

Adult females of *Blepsias cirrhosus* were collected, using trammel nets, from the coastal waters of Usujiri, southern Hokkaido in June, 1986 and 1987. They were kept in an indoor tank (1 m^3 capacity) supplied with fresh seawater until the commencement of the experiments.

After careful incision of the ovarian capsule, ripe eggs were removed directly from the ovary of females which were known to have ovulated. Ovarian fluid obtained together with the eggs was removed by gentle suction with a pipette for use in the following tests. To determine if egg development started before or after contact with seawater, the eggs were placed in a petri dish containing either ovarian fluid

Table 1. Embryonic development of *Blepsias cirrhosus* eggs 24 hrs. after immersion in seawater or ovarian fluid.

Rearing medium	Percent (no.) of 4-cell stage eggs	Percent (no.) of undeveloped eggs
Seawater	84 (31)	16 (6)
Ovarian fluid	0 (0)	100 (20)
Another 24 hrs. after transference from ovarian fluid to seawater	65 (13)	35 (7)

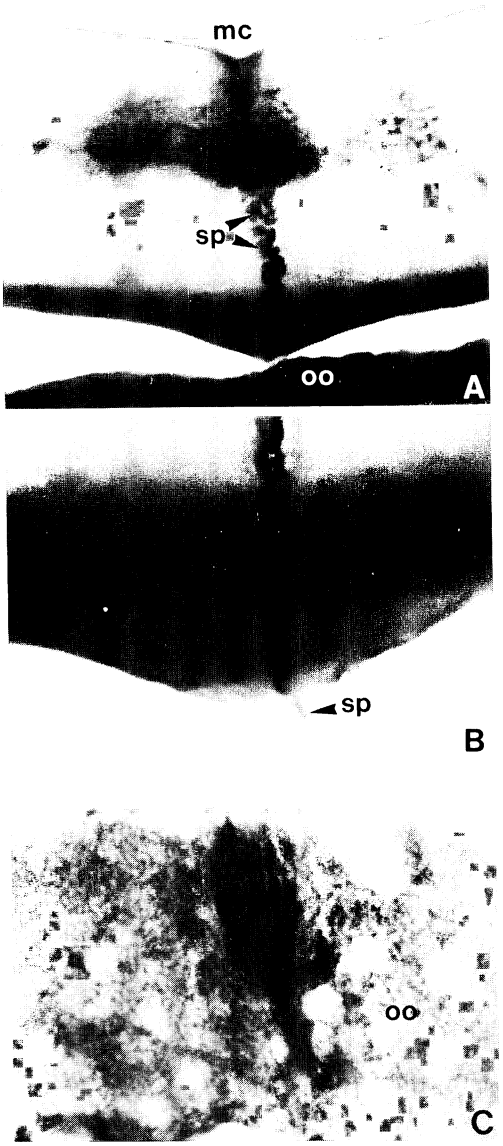

Fig. 1. Photomicrograph of internal gametic association in *Blepsias cirrhosus*. A: Spermatozoa (sp) entering micropylar canal (mc). oo, ooplasm. ×1,000. B: Fertilizing spermatozoon located at the apex of the micropylar cone. ×2,800. C: Chromosomes and metaphase spindle of second meiotic division. ×1,700.

or seawater at a water temperature of 10°C. After 24 hrs., the number of developing eggs were counted.

Histological observations were carried out on a number of eggs before exposure to seawater, in order to ascertain whether or not fertilization had occurred. After fixation in Bouin's fluid, 5 μm serial paraffin sections were prepared, and then stained with Delafield's hematoxylin and eosin.

Results

Initiation of egg development. Thirty one out of 37 eggs (84%) which had been placed in seawater were at the 4-cell stage after 24 hrs. (Table 1). On the other hand, the eggs maintained in the ovarian fluid did not show any signs of development. When these unchanged eggs were transferred into seawater, 13 out of 20 eggs developed to the 4-cell stage after 24 hrs. These results indicated that development was initiated only after the eggs had been in contact with seawater.

Occurrence of internal gametic association. The micropyle of the egg of *Blepsias cirrhosus* is composed of a micropylar vestibule, which is a funnel-like depression about 90 μm in diameter in the egg envelope, measuring about 39 μm in thickness (Fig. 1A). The external opening of the canal is centrally placed at the bottom of the vestibule and measures about 5 μm. The canal is slightly tapered, and its inner opening at the surface of the ooplasm is located at the center of the inner protrusion of the egg envelope (micropylar cone).

Serial sections of the micropyle area of the eggs fixed before immersion in seawater revealed the presence of a number of spermatozoa in the micropylar canal. Although spermatozoa were bunched linearly in the canal, some reaching the apex of the micropylar cone (Fig. 1B), no further progress such as penetration into the ooplasm was detectable by light microscopy. Furthermore, the chromosomes and metaphase spindle of the second meiotic division were observed in the ooplasm near the micropylar cone of the eggs (Fig. 1C).

Discussion

The present study revealed that eggs directly obtained from the ovarian cavity of female *Blepsias cirrhosus* showed no signs of development when maintained in ovarian fluid, but achieved fertilization and subsequent early segmentation when in contact with seawater. Histological observations of these eggs confirmed that the process of fertilization had not been initiated in the ovarian fluid, although a number of spermatozoa had entered the micropyle. It appeared that the mature females used in this

study had copulated and that the spermatozoa had already been introduced into the ovarian cavity.

In the copulating cottid species, *Alcichthys alcicornis*, reproductive behavior consists of a mating ritual, spawning by the female, and subsequent copulation with a male. Thus, the eggs laid by unimpregnated females are inseminated and fertilized externally (Munehara, 1988). On the other hand, the eggs of impregnated females are associated with spermatozoa in the ovarian cavity, but actual fertilization occurs externally after exposure to seawater (Munehara et al., 1989). Although the reproductive habits of *Blepsias cirrhosus* are as yet unknown, the species is clearly another example of "internal gametic association with external fertilization."

The males of cottids which are known to be copulating species are generally similar in so far as they have a huge penis compared with their body size: *Orthonopias triacis* (Bolin, 1941), *Clinocottus recalvus* (Morris, 1952), *Oligocottus snyderi* (Morris, 1956), *Synchirus gilli* (Krejsa, 1964), *Clinocottus analis* (Hubbs, 1966), *Taurulus bubalis* (Lamp, 1966), *Pseudoblennius cottoides* (Shiogaki and Dotsu, 1974; Shinomiya, 1985), *P. percoides* (Shinomiya, 1985), *Furcina ishikawae* (Shinomiya, 1985), *Artedius harringtoni* (Ragland and Fischer, 1987) and *A. alcicornis* (Munehara, 1988; Munehara et al., 1989). In *B. cirrhosus*, on the contrary, the penis is too diminutive to be noticed (Bolin, 1947). It should also be noted that the presence of huge external genitalia is often characteristic of copulating species, but such is not necessarily the case in cottids.

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イソバテングにおける体内配偶子会合および体外受精の実証

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交尾型カジカ，イソバテング *Blepsias cirrhosus* の配偶子がいつ，どこで会合し受精開始するかを明らかにした．排卵した雌の卵巣腔から卵と卵巣腔液を取り出し，一部の卵を海水または卵巣腔液に浸し，24時間後にそれぞれの胚発生状態を観察した．その結果，海水中の卵のほとんどは胚発生を開始していたが，卵巣腔液中の卵は全く発生しなかった．しかし，これらの未変化の卵を海水に移すと，その多くが胚発生を開始した．また，海水に

浸す前の卵を光顕観察した結果，多数の精子が卵門管内に侵入しているものの，卵細胞質内への貫入は認められず、卵も第二減数分裂の中期にとどまっていることが確認された．以上の結果から，本種では体内で両配偶子の会合は終えているが，受精は産卵後海水中で開始することが明らかとなった．

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