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External versus Internal and Self- versus Cross- Fertilization in Bryozoa: transformation of the view and evolutionary considerations

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1. History of research

Fertilization (actually, insemination – sperm-oocyte fusion) in Bryozoa has been one of the most obscure topics for about two and a half centuries. As soon as the gametes were recognized, naturalists tried to understand where they met. Different points of view were considered, and we intend to follow this story.

Red and orange embryos are often seen through the semitransparent walls of the cheilostome brood chambers (ovicells), which is why Pallas in 1766¹ suggested that the ovicells were ovaria. He stated that, together with avicularia, they somehow could serve for fertilization, and even called them “Nectariums”. Following this idea, one could think that fertilization took place inside the ovicells.

The view about ovicells, containing embryos, as female gonads has been readily accepted by many famous and prominent naturalists like Lamaroux,² Milne-Edwards,³ Lamarck,⁴ Reid,⁵ Johnston⁶ and Hincks,⁷ and this view survived for more than a century. It was Huxley⁸ who understood their true function, stating that the ovicell was merely the ‘marsupial pouch’.

However, not all bryozoans possess ovicells. The simultaneous presence of both mature ovaries and testes inside the same zooids was considered as evidence of intrazooidal self-fertilization in them. It looked especially obvious when spermatozooids were concentrating around the ovarium.⁹⁻¹⁰ I was not able to find who was the first, suggesting self-fertilization in Bryozoa, but, for instance, van Beneden stated this in 1844.¹¹

Additionally, some of the scientists recorded an appearance of vitelline membrane around oocytes just after their ovulation in the body cavity, also considering it as a sign of fertilization.¹²⁻¹³ Calvet in his famous monograph published in 1900¹⁴ even stated that he observed intrazoooidal self-fertilization inside the zoooidal cavity in *Bugula simplex*.

Interestingly Farre saw sperm release in the ctenostome, *Alcyonidium duplex*, but he confused the male cells with ‘parasites’ and ‘cercariae’, and was not able to ascertain the exact locus of their expulsion. Farre wrote that they were ‘issued from the centre of the tentacula’.¹⁵

Hincks¹⁶ recorded sperm release through the ciliary intertentacular organ in the cheilostome, *Electra pilosa*. This contradicts with the modern knowledge that the sperm is released through the terminal pores on the tips of the tentacles (see below). However, the description of Hincks is so detailed and convincing that one can be in no doubt of whether the sperm expulsion may really be sometimes possible through the intertentacular organ in this species. Later Prouho¹⁷ speculated that this could happen if the rest of the sperm was moved out at the end of the reproductive period. Hincks also suggested that the intertentacular organ could be used for the release of eggs after their ovulation and fertilization in the body cavity.

One way or another, both these observations and ideas were abandoned, and the opinion about intrazoooidal self-fertilization in the body cavity has been accepted by most of the researchers since the middle of the 19th century.¹⁸⁻³⁸ The last mention I could find was in a paper by Smith, Werle and Klekowski on the ctenostome, *Pottsiella erecta*, published in 2003.³⁹

The first researcher, who disputed the general opinion and stated that cross-fertilisation occurs in some species, ctenostomes as well as cheilostomes, was Joliet.⁴⁰ Protandrous zoooidal hermaphroditism or zoooidal gonochorism, massive production of spermatozooids, their release and possibility to actively swim in the surrounding water lead him to believe that cross-fertilization is the rule. He suggested that fertilization by the alien sperm has a place in different species (1) inside the maternal zooid (within the tentacle sheath in the brooding ctenostomes studied or within the zoooidal cavity), (2) during oviposition, or even (3) in the ovicell (depending of where he recorded the vitelline membrane). Joliet thought that the sperm is released through the thin wall of the tentacle sheath during a sharp withdrawal of the polypide [he probably meant a rupture of non-calcified body wall].

This view was strongly supported by the observations of Vigelius,⁴¹ who also carefully studied the dynamics of sexual changes in bryozoan colonies, although it seems he considered intracolony self-fertilization. Vigelius thought that sperm release is possible through the zoooidal aperture only after polypide degeneration and destruction of the body wall. Fertilization itself was supposed to occur externally, inside the ovicell in brooding cheilostomes. However, the simultaneous presence of male and female gametes in the same zooids forced him to admit intrazoooidal self-fertilization in *Bugula calathus*.⁴²

Colonial sex structure and its dynamics were the first evidence against the old scheme. Repiahoff⁴³ and Calvet⁴⁴ found that sperm is organized in clusters, but it is not clear if they really found so-called spermatozeugmata. Bonnevie⁴⁵⁻⁴⁶ described the structure and

behaviour of these clusters in *Membranipora* and *Electra*. She noted that spermatozeugmata move as if they were a single thing, and speculated that the clustering of spermatozooids could enhance their locomotory power, admitting that this contradicted her own belief in either intrazoooidal or intracolony self-fertilization. Bonnevie also considered spermatozeugmata as an adaptation for 'Polyspermie': fertilization by several spermatozooids that was suggested to happen just after ovulation.

Thus, concerning the site of fertilization it was suggested that it happens either in the body cavity, right after ovulation, or outside it, in the tentacle sheath, ovicell or during oviposition. Strangely enough, reported findings of the sperm heads in the ovarian oocytes were almost not discussed in the literature. For instance, Harmer⁴⁷ encountered the sperm in the ovary of the cyclostome bryozoan, *Tubulipora phalangea*, and suggested that the so-called "paranuclear body" found in the ovarian oocytes is a male pronucleus. Borg⁴⁸ also found the sperm head inside the ovarian oocyte of cyclostome *Crisiella producta*.

Sperm has been also found in the ovaria of phylactolaemates *Plumatella*, *Fredericella* and *Lophopus*.⁴⁹⁻⁵¹ In one female gonad of *L. crystallinus* Marcus counted up to 150 spermatozooids, and 18 oocytes were inseminated in there.

In his 1938 paper Marcus⁵² described precocious intraovarian fertilization—fusion of the male and female cells before the onset of vitellogenesis—in a number of cheilostomes. The ovary contains several inseminated oocytes, and the sperm heads were also found between the ovarian cells. Marcus also recorded the sperm heads in the ovarian oocytes 'which are still growing' in two ctenostome species.⁵³ However, it is not very clear in which stage of growth these oocytes were. This led him to the admittance of two possibilities: (1) a fully grown ovary in zooids without testes may already contain the [alien] sperm (that means cross-fertilization in a case of protogyny); and (2) distinct protandry 'by no means indicates that there must be reciprocal fertilization' (self-fertilization is possible in a case of protandry).⁵⁴ However, the most important conclusion here is that simultaneous maturation of the gametes cannot be an evidence of self-fertilization if the fusion of the male and female cells is precocious. It means that young oocytes could be inseminated before sperm maturation in the same zooid or colony. Corrêa⁵⁵ found the sperm head in the early intraovarian oocytes in *Bugula flabellata* and Mawatari⁵⁶ detected it in the growing oocyte in *Watersipora subtorquata*.

Cori⁵⁷ found and depicted spermatozooids in the coelomic lumen of the tentacles of the ctenostome species, *Zoobotryon verticillatum*. Later Brien⁵⁸ mentioned this fact, suggesting that sperm is released via the terminal tentacular pores. Also, Silén (Figure 1) discussed in his papers some facts that favoured cross-fertilization.⁵⁹⁻⁶⁰ In contrast with Brien, considering the coelomopore and the intertentacular organ as probable routes for the alien sperm, he suggested that terminal tentacular pores could be also used as an entrance for the sperm in some species.⁶¹ Since no ripe spermatozooids have been found in testes of the ctenostome *Labiostrum gisleni*, it was suggested that the sperm recorded in the ovulated oocytes came from outside, fusing with eggs in the distal part of the zooid.⁶²

Finally, Silén⁶³ described the liberation of sperm via the terminal pores of two distomedial tentacles in three species of *Electra* and *Membranipora membranacea*.

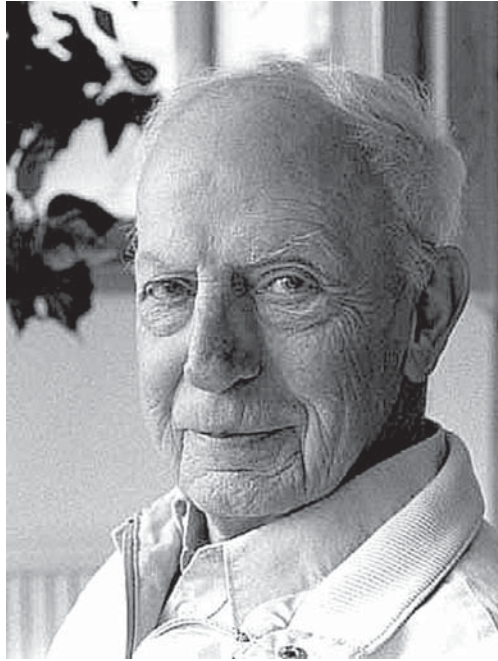


Figure 1. Lars Silén (photograph courtesy of the Department of Zoology, University of Stockholm).

Additionally, he mentioned that sperm and the eggs are released non-simultaneously in the same colony, which he argued was additional evidence in a favour of cross-fertilization.

Silén recorded that the fertilization envelope appears approximately one hour after the egg's release in *Electra posidoniae*. Based on this, he suggested that fertilization in this species takes place externally. In *E. crustulenta* sperm was observed inside the intertentacular organ, where fertilization has been suggested to occur. Nevertheless, Silén admitted that theoretically sperm can enter the zooidal cavity through the intertentacular organ or supraneural pore too, and cross-fertilization could occur in the body cavity. Returning to the old idea of Joliet,⁶⁴ Silén speculated that fertilization in larviparous species could be achieved during oviposition. Strangely, the data of Marcus who discovered precocious intraovarian fertilization in Gymnolaemata (see above) have been overlooked or ignored, despite his paper⁶⁵ being cited. Also Silén's⁶⁶ own finding of the male pronuclei inside the ovulated oocytes in *Labriostomella gisleni* was not mentioned or discussed.

Later Silén⁶⁷ and Bullivant⁶⁸ recorded the liberation of sperm via the tentacular terminal pores in nine more cheilostomes, one ctenostome and two cyclostome species. It should be noted that only malacostegans release their sperm via two distomedial tentacles, whereas in others the sperm leaves the parental zooid via terminal pores in all tentacles.

Additionally, the ultrastructural studies of Franzén⁶⁹⁻⁷⁷ showed that the morphology of

bryozoan sperm is indicative of internal fertilization.

Ultrastructural investigations of *Dyrynda* with Ryland,⁷⁸ and with King,⁷⁹ and also of Hughes⁸⁰ showed intraovarian precocious fertilization and sperm in ovaries in three cheilostome brooders, thus supporting the data of Marcus.⁸¹ Together with the observations of Silén⁸²⁻⁸³ and Bullivant⁸⁴ this proved that the type of fertilization occurring in Bryozoa could be termed 'external-internal'. One type of gametes—spermatozooids—leaves the parental zooid in searching for the female cells, whereas oocytes wait to be fertilized internally.

The above authors also showed that oocytes develop in doublets in cheilostome brooders.

The excellent observations made by Temkin⁸⁵⁻⁸⁶ resulted in a revision of our view on fertilization in Bryozoa. It was shown that fertilization is internal in all Gymnolaemata: either intracoelomic or intraovarian. In three egg broadcasters studied: *Membranipora membranacea*, *Alcyonidium* sp. and *Electra pilosa*, sperm fuses with late stage ovarian oocytes at or near ovulation. In the ctenostome brooder, *Bowerbankia gracilis*, sperm were only found inside the late stage ovarian oocytes (one per ovary). Temkin suggested that since only late stage oocytes are inseminated, the sperm possibly fuses with partially ovulated oocyte. However, Marcus⁸⁷ wrote that sperm was found in the oocytes, which are still growing in ctenostome brooders *Alcyonidium* sp. and *Nolella stipata* (see above). So, it is possible that insemination could occur earlier in these species, but this needs clarification.

According to Temkin⁸⁸ sperm fuses with early ovarian oocytes in all cheilostome brooders studied. Only one sibling in each oocytic doublet is inseminated, and this cell will become an egg. There is only vitellogenic doublet in each ovary, but all previtellogenic doublets possess sperm heads too. Spermatozooids are obviously entering the maternal coelom through the supraneural pore, accumulate on the ovarian surface, further moving between ovarian cells.

All these findings show that internal fertilization is a characteristic for all Gymnolaemata, providing high levels of fertilization success in this group. Activation is delayed and this is considered as a possible adaptation for liberation/oviposition of the egg through the small opening of the intertentacular organ or supraneural pore.⁸⁹⁻⁹⁰

My data support the results of Temkin. For instance, 14 spermatozooids were found in one ovary of *Tegella armifera* (not counting those in oocytes). In ovaries of *Bugulopsis monotrypa* and *Pacificincola insculpta* there were up to 15 spermatozooids in the female gonad. They obviously enter it in the areas with loose cell packing, but they are also found (rarely) between tightly packed follicular cells. Part of the male cells fuse with early primary oocytes, the rest stay between the ovarian cells, probably fusing with new forming oocytes in the course of their appearance.

In some species the diameter of the youngest fertilized previtellogenic oocytes were just a little bit longer than the length of the sperm head. The large male pronucleus is forced to keep the shape of a comma, often being placed around the female nucleus like a belt. It obviously means that insemination occurs just after the oogonial division. This division

leads to an early oocytic doublet in which siblings are connected by a cytoplasmic bridge. The fertilized female cell will become an egg, non-fertilized—a nurse-cell.⁹¹

In most species the fertilization envelope is observed in brood chambers, whereas sometimes it was seen inside the zooid. Similar to Pergens⁹² and Prouho,⁹³ I recorded the fertilization envelope around the late stage oocytes, either completely or partially ovulated, in some brooding species. Obviously, it is very elastic, and does not prevent the oviposition.

The method to check a possibility for intracolony self-fertilization is an experiment. It has been described first in the cheilostome *Epistomia bursaria*, whose colonies produced embryos growing in isolation.⁹⁴⁻⁹⁵

In the experiments of Temkin⁹⁶ reproduction was successful in isolated colonies of *Membranipora* sp. This was also in accordance with the results of Maturo,⁹⁷ in whose experiments five gymnolaemate species produced larvae, when grown from single ancestrulae in isolation. However, this contradicted the data of Cancino, Castañeda and Orellana,⁹⁸ in whose experiments oogenesis failed to complete in isolated colonies of *Membranipora isabelleana* or never started in *Celleporella hyalina*. Also in the latter species self-fertilization led to a frequent embryonal abortion and reduced fitness of the offspring in the experiments of Hunter and Hughes.⁹⁹ In general, cross-fertilisation is considered as a rule among Bryozoa, and selfing, if it really exists, might be used in an ‘emergency’ situation. Inbreeding with normal progeny has been recorded in one population of *C. hyalina* only.¹⁰⁰⁻¹⁰¹

Precocious insemination and the recently found ability to store sperm make it important to carefully isolate colonies grown from ancestrulae at an early stage of their development in experiments. For instance, the mechanisms of allosperm storage and translocation are already present at the three-zooid stage of astogeny. Colonies consisting merely of the ancestrula and two autozooids obtained and stored alien sperm, further using it to fertilize eggs for a maximum period of 4-6 weeks in *C. hyalina*.¹⁰² Returning to the old idea of Marcus,¹⁰³ it was suggested that the sperm can travel through the colony from autozooids (that “catch” it), to female zooids via communication pores, using the funicular system. However, this is doubtful since pores are closed by the pore cell-complexes.

The same authors revealed that egg growth is absent in reproductively isolated colonies, and allosperm is a trigger of vitellogenesis.¹⁰⁴ We supported these data in a course of anatomical research of *Callopora lineata*.

2. Brief evolutionary analysis

Sperm heads have been found inside the ovarian oocytes in Cyclostomata, Phylactolaemata and all brooding Gymnolaemata studied. All these groups possess non-feeding endotrophic larvae.

In Gymnolaemata with planktotrophic development the mature oocytes are inseminated near the time or during ovulation, in cheilostomes with lecithotrophic larva, intraovarially, just after the oogonial division. The first case demonstrates the primitive condition, the

second is advanced. In the ctenostome, *Bowerbankia gracilis*, only one late stage ovarian oocyte per gonad contains a sperm nucleus, whereas all oocytic doublets are inseminated in cheilostome brooders.¹⁰⁵ In the latter, sperm triggers vitellogenesis, but there is no connection between insemination and vitellogenesis in *Bowerbankia*. So, *Bowerbankia* could be considered as an intermediate stage in the hypothetical series.

Extrapolating this suggestion to the evolution of bryozoans, I speculate that internal fertilization could have been acquired first by the ancestral form. For example, ovulated eggs are fertilized intracoelomically in the Recent phoronids.¹⁰⁶ Phylactolaemata 'accelerated' the process and acquired the intraovarian fertilization. Earliest ctenostome broadcasters probably possessed intracoelomic fertilization of the late stage ovarian oocytes at or near ovulation. Their brooding descendants could acquire the intraovarian fertilization of the late stage oocytes, but there are no data enough to support this suggestion. Since there are both brooders and broadcasting species within the ctenostome genus *Alcyonidium*, slightly different variants of the sperm-oocyte fusion might be expected.

Which state was initial for Cyclostomata is unclear. Their embryos develop intracoelomically, and this is absent in Ctenostomata. It might be that the first cyclostomes were broadcasters with intracoelomic or 'ovulatory' fertilization that further transformed to the intraovarian and led to the development of embryos inside the female gonad.

The cheilostome broadcasters could inherit fertilization of the ovulating oocytes from their ctenostome ancestors. Brooders went further acquiring the ultimate evolutionary stage, precocious intraovarian fertilization just after oogonial division. The intermediate stage (the intraovarian fertilization of the late stage oocytes) is unknown in them, although it can exist in such bryozoans as *Tendra*, a brooder with many primitive characters.

The above examples show that although internal fertilization in Bryozoa has probably evolved in their common ancestor, intraovarian fertilization could evolve several times. The reason for evolution of precocious fertilization is unclear, and could be connected with sperm competition. In Cheilostomata brooding evolved several times independently¹⁰⁷⁻¹⁰⁸ that is evidence of the independent evolution of lecithotrophy. All brooding cheilostomes studied up to now possess precocious intraovarian fertilization that suggests the possibility for it evolving several times within the order.

It is also possible that namely early fusion with a spermatozoid, triggering a cortical reaction and forming common fertilization envelope around both siblings,¹⁰⁹ evolutionary became a cause for origin of oocytic doublets. Further, fertilization began to acts as a trigger for the beginning of the vitellogenesis.

One consequence of the internal fertilization is that potentially it can trigger the embryonic development inside the maternal coelom. There are two records in the literature proving that it occurs incidentally in cheilostome brooders¹¹⁰ as well as broadcasters.¹¹¹ However, it is not the case with the vast majority of Gymnolaemata since the egg activation is normally delayed till the time when a zygote will be released.¹¹²⁻¹¹³ The zygote is very plastic in bryozoans being able to squeeze through the small supraneural coelomopore, that is impossible for an embryo. Nonetheless, intracoelomic

brooding (viviparity) evolved in Cyclostomata and the cheilostome family Epistomiidae. The larvae are released via the wide oeciostome of cyclostome gonozooids, and via the zooidal opening, possible, through a rupture of vestibular wall in epistomiids. It is clear that the intracoelomic (starting as intraovarian) brooding could only evolve in connection with endotrophy. In both, Cyclostomata and Epistomiidae there is endotrophy accompanied by extraembryonic nutrition in the ovary and further in the zooidal coelom.

Obviously, the most important consequence of the evolution of internal fertilization is that it allows fertilizing of nearly 100% of the oocytes in Bryozoa.¹¹⁴⁻¹¹⁵ The internal fertilization provides much higher probability for the meeting of the sperm and oocytes that is especially important for brooders with their small number of eggs. The Recent Bryozoa demonstrate the different stages of the transition from more primitive to more advanced state, towards very early sperm-oocyte fusion and complete dependence of the oogenesis from fertilization.

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