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THE DEVELOPMENT AND EARLY STAGES OF THE ASCIDIAN *PYURA SQUAMULOSA* (ALDER)

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(Text-fig. 1)

In the family Pyuridae the larval and post-larval stages are known only in *Halocynthia pyriformis* (Rathke) and *Boltenia echinata* (L.). Berrill (1948) has pointed out that this is the least specialized family of the Pleurogona and that its larvae are therefore of particular interest.

On 1 August 1950 specimens of *Pyura squamulosa* (Alder), up to 36 by 30 mm., were collected from boulders at low-water level in the Sound of Kerrera, Argyll. They were taken on the following day to the Marine Station, Millport, and there kept in running sea water until 4 August, when artificial fertilizations were carried out.

Egg (Fig. 1 A). The ripe ovum is spherical, orange-pink, and 160 μ in diameter. It is surrounded by loosely arranged inner follicle cells, a chorion, and a complete layer of rounded outer follicle cells. When the egg is freshly drawn from the ovary there is a narrow perivitelline space, but after some minutes in sea water the space is enlarged by the osmotic uptake of water and the diameter of the chorion increases from 175 to 190 μ. The final external diameter of the layer of outer follicle cells is about 220 μ.

Sperm (Fig. 1 D). The form of the sperm is typical of that of many ascidians. The rod-like head is 9 μ long and has a protoplasmic bulge near the point of attachment of the tail. The tail is a flagellum about 50 μ long.

Fertilization and development. The eggs and sperm from two individuals were mixed in sea water, and in spite of the alleged difficulty in obtaining fertilized eggs of pyurid ascidians (Berrill, 1950), development proceeded, and apparently normal larvae were obtained in large numbers. Cleavage follows the pattern which is now well established in ascidians. A deep orange crescent (Fig. 1 B) appears in the fertilized egg like the yellow crescent of *Styela partita* (Conklin, 1905) and the bright orange crescent of *Boltenia echinata* (Berrill, 1948); pigmentation of this kind is unknown in other ascidians. The pigment is later localized in the tail of the larva (Fig. 1 C). Most of the eggs fertilized about 11.30 a.m. on 4 August, and kept in Petri dishes at 18–19° C. had hatched as active larvae by 9.30 a.m. on the following day.

Larva (Fig. 1 E). The trunk of the larva is about 200 μ long and the tail about 730 μ long, inclusive of the posterior projection of the fin. There are two dorsal and one ventral anterior papillae of conical shape. The fin runs round the

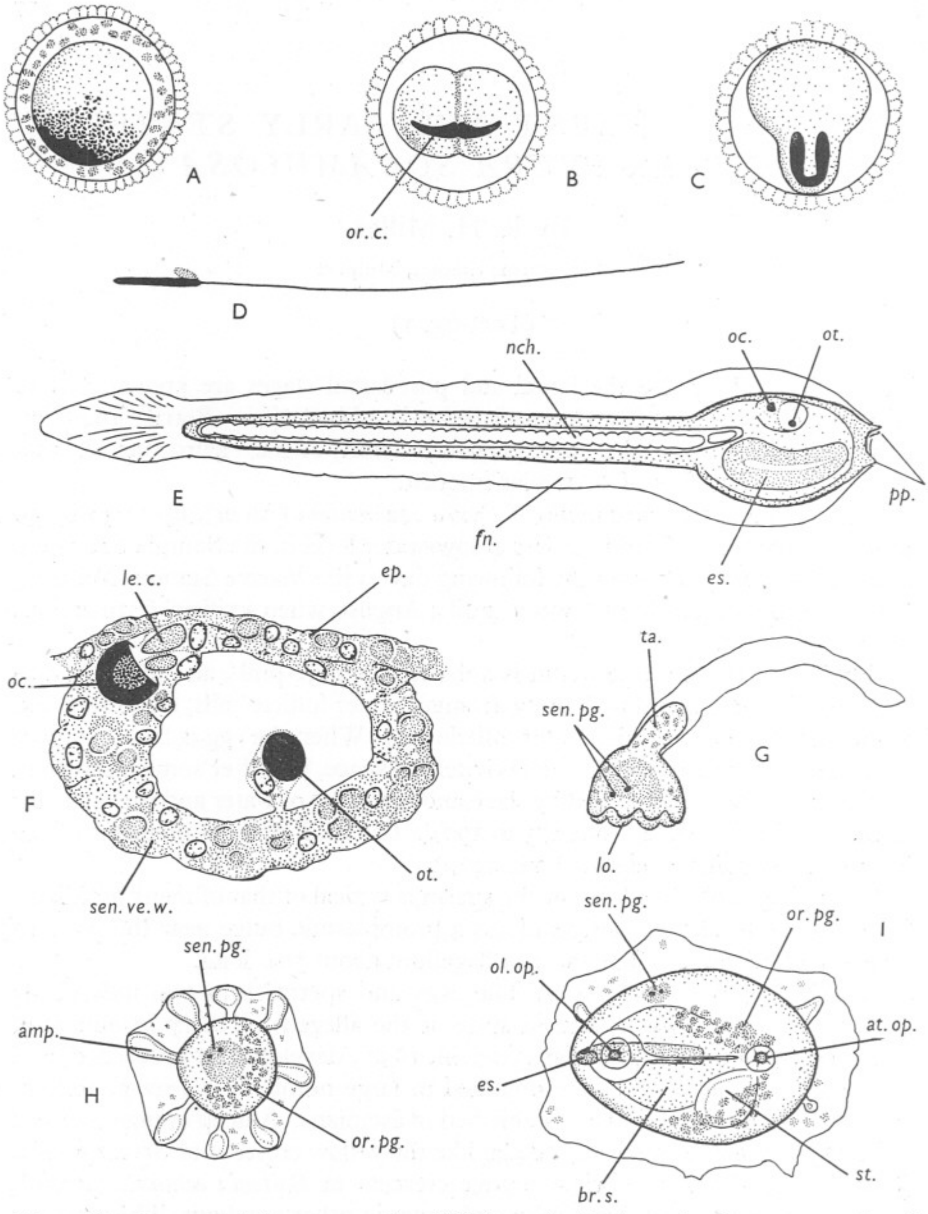

Fig. 1. Development of *Pyura squamulosa* (Alder). A, fertilized egg showing concentration of pigment forming orange crescent. B, first cleavage, showing orange crescent. C, embryo with trunk and tail differentiated and orange pigment localized in tail. D, sperm. E, larva. F, sagittal section through larval sensory vesicle. G, metamorphosing larva. H, post-larval stage with ampullae. I, young ascidian. *amp.*, ampulla; *at. op.*, atrial opening; *br.s.*, branchial sac; *ep.*, epidermis; *es.*, endostyle; *fn.*, fin; *le.c.*, lens cell; *lo.*, anterior lobes; *nch.*, notochord; *oc.*, ocellus; *ol.op.*, oral opening; *or.c.*, orange crescent; *or.pg.*, orange pigment; *ot.*, otolith; *pp.*, papillae; *sen.pg.*, remains of larval sensory pigment (otolith and ocellus); *sen.ve.w.*, wall of sensory vesicle; *st.*, stomach; *ta.*, tail being resorbed.

trunk in the sagittal plane and bifurcates on the dorsal surface of the trunk to send a short ridge forward to each dorsal papilla. On the ventral surface of the trunk the fin remains simple and runs forward to the single ventral papilla. The rudiment of the endostyle occupies the ventral part of the trunk, and the sensory vesicle lies near the dorsal surface. Both otolith and ocellus are present and well developed (Fig. 1F). The otolith is contained in a single cell projecting up from the floor of the sensory vesicle, and consists of a spherical or ovoid mass of black pigment. The mean value of the greatest diameter of this pigment mass is $13{\cdot}2\,\mu$. On the postero-dorsal wall of the sensory vesicle lies the ocellus. This has a cup-shaped mass of black pigment of which the mean diameter across the opening is $14{\cdot}3\,\mu$. In addition to a number of retinal cells there are, opposite to the opening of the pigment cup, three cells each containing a large clear body acting as a lens. The distal ends of the lens cells are closely applied to the lower surface of the dorsal epidermis. No trace was found of the rudiments of siphons, or peribranchial sacs. The tail has the usual single row of notochordal cells surrounded by muscle cells, and also contains the pigment which formed the orange crescent of the fertilized egg. Before the larva hatches the tail is coiled in a horizontal plane.

Metamorphosis and Post-larval Development

Within 12 hr. of hatching resorption of the tail is in progress both in larvae attached to the glass of the vessel and in those unattached (Fig. 1G). At the same time the trunk becomes shorter and wider. Eight lobes now appear round the anterior margin of the trunk, and the tail begins to shorten; the test round the tail, however, is not resorbed. By the time the tail has disappeared the eight anterior lobes have developed into eight ampullae growing out laterally over the substratum. At this stage two black pigment spots represent the remains of the larval otolith and ocellus, and the orange pigment from the larval tail forms a band across the body of the young ascidian (Fig. 1H). Later the ampullae become irregular in outline and constricted at the base, and soon some of them disappear. At about this time the endostyle becomes clearly visible. At the next stage the functional ascidian is recognizable, having oral and atrial openings, active body musculature, branchial sac with proto-stigmata, and stomach containing food masses (Fig. 1I). The ampullae are represented by one or two small irregular projections from the basal part of the body. The remains of the larval otolith and ocellus show as two black spots on the right side of the body, and not, as found by Berrill (1929) in *Halocynthia pyriformis*, near the adult ganglion.

Subsequent development was not followed, as all the major changes in organization from larva to adult were now complete.

The development time-table is given below.

4 August: 11.30 a.m., fertilization; 1.00 p.m., 1st cleavage; 2.30 p.m., 4th cleavage; 3.30 p.m., 5th cleavage; 5.00 p.m., gastrulation; 6.00 p.m., deep gastrula; 9.00 p.m., differentiation of trunk and tail. 5 August: 9.30 a.m., hatching of larvae almost complete; 7.30 p.m., tail resorption in progress. 7 August: tail completely resorbed; ampullae well developed. 8 August: diameter of post-larva over ampullae 0·33 mm. 10 August: diameter of post-larva over ampullae 0·40 mm. 11 August: diameter of post-larva over ampullae 0·48 mm. 14 August: oral and atrial openings, branchial sac and gut differentiated. 17 August: feeding first observed.

The temperature from the time of fertilization until the end of tail resorption was 18–19° C. and thereafter 23° C.

In Table I some of the important features in the development of *Halocynthia pyriformis*, *Boltenia echinata* and *Pyura squamulosa* are compared.

TABLE I

	Halocynthia	*Boltenia*	*Pyura*
Pigmented crescent in developing ovum	o	+	+
Diameter of ovum (mm.)	0·26	0·18	0·16
Larval sense organs	Otolith and ocellus	Otolith and ocellus	Otolith and ocellus
Number of lens cells in ocellus	3	3	3
Rudiments of peribranchial sacs in larva	+	+	o
Number of ampullae in post-larva	4	4	8

The Pleurogona (= Stolidobranchiata Lahille, 1886) comprises the families Styelidae, Pyuridae, and Molgulidae. In these families the sensory equipment of the larva is variously modified or reduced. Thus the ocellus has disappeared in the larvae of all the Molgulidae, the most highly developed of pleurogonid ascidians. In the Styelidae the ocellus may be combined with the otolith (subfamily Botryllinae), or absent (Dendrodoa, Polycarpa), or merely reduced (Styela). The larvae of the Pyuridae have hitherto been known only from *Halocynthia pyriformis* and *Boltenia echinata*. In both of these the ocellus is present and of normal structure (Berrill, 1950), though not so well developed as in the larvae of the enterogonid ascidians.

In *Pyura squamulosa* the larger size of the ocellus represents a more primitive condition. The larva is also less specialized in that it shows no trace of precociously developed peribranchial sacs or siphon rudiments such as those of *Boltenia*. It is indeed nearer to the enterogonid type of larva than that of any other known pleurogonid ascidian. The small size of the egg also indicates a condition at least as primitive as *Boltenia* and considerably more primitive than *Halocynthia*. The presence of a pigmented crescent in the developing egg of *Pyura squamulosa* and *Boltenia* suggests that these forms may be closer than *Halocynthia* to the styelid stock, at least one member of which shows a similar pigmented crescent.

Evidence gained from a study of the development of *Pyura* therefore indicates it to be more primitive than either *Boltenia* or *Halocynthia*, the other two pyurid ascidians whose development is known.

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