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THE BREEDING AND DEVELOPMENT OF *STYELA MAMMICULATA* CARLISLE

By H. WALLACE
Zoology Department, The Queen's University, Belfast

(Text-fig. 1)

Styela mammiculata was discovered in Plymouth Sound in 1953 (Carlisle, 1954). It has become established there, although only locally abundant, and has recently been recorded from the Hampshire coast (Houghton & Millar, 1960). The adult form is solitary and stalked, clearly assignable to the genus *Styela*. Millar (1960)[1] believes that it is identical with *S. clava* Herdman. The development and larval structure of ascidians are of sufficient phylogenetic importance, concerning the relationships of genera and families, to merit a brief description. Other observations reported here may be of some value in assessing the geographic range of this species.

The breeding season is not known with certainty. Specimens failed to spawn in aquaria during October to December 1957, and July 1960. Fertile eggs were obtained by dissecting specimens of all sizes during late July 1960. This is taken to be immediately prior to a breeding season, possibly extending through August and September. By analogy to the local summer-breeding ascidians (Berrill, 1950; Marine Biological Association, 1957), *S. mammiculata* is inferred to be a boreal species, whose potential range of distribution lies mainly to the south of the English Channel, but which may prove capable of spreading farther north round the British coasts. The branchial baskets and oesophagi of forty-seven specimens were searched for commensal copepods, in the vain hope of establishing the region from which the Plymouth population originated. Only one copepod was found, a non-ovigerous female which R. V. Gotto (of this department) considers to be *Paranthessius cynthiae* (Brian).

Although natural spawning has not been observed, the anatomy of *S. mammiculata* shows it to be oviparous. Fertile eggs are 155 μ in diameter. Yellow test cells (12 μ diameter) form an incomplete layer within the thin but tough chorion, which is covered by a complete layer of clear follicle cells (20 μ diameter). The egg is rendered opaque by grey yolk and a cortical yellow

[1] Millar's article, providing the evidence that *S. mammiculata* and *S. clava* are identical appeared while this report was in proof. My inference that *S. mammiculata* is a boreal species is consonant with it being native to Japanese waters. It would be particularly interesting to learn if *Paranthessius cynthiae* occurs in Japanese specimens of *S. clava*, although the host-specificity of this copepod is unknown.

pigment. The majority of eggs obtained by dissection were immature. The most successful fertilizations were obtained by mixing the gametes of several specimens (50–90% of the fertile eggs were self-sterile) and by adding alkali to increase sperm activity. Fertilization is followed by the maturation divisions and the formation of a yellow crescent. The pattern of cleavage and segregation of coloured plasms are virtually identical with those of *S. partita* (see Conklin, 1905), nicely illustrating the mesodermal fate maps obtained by the more accurate method of marking blastomeres with chalk grains (Ortolani, 1955). The yellow colour forms a complete crescent (presumptive muscle), the presumptive mesenchyme being restricted to two colourless patches (Fig. 1 A).

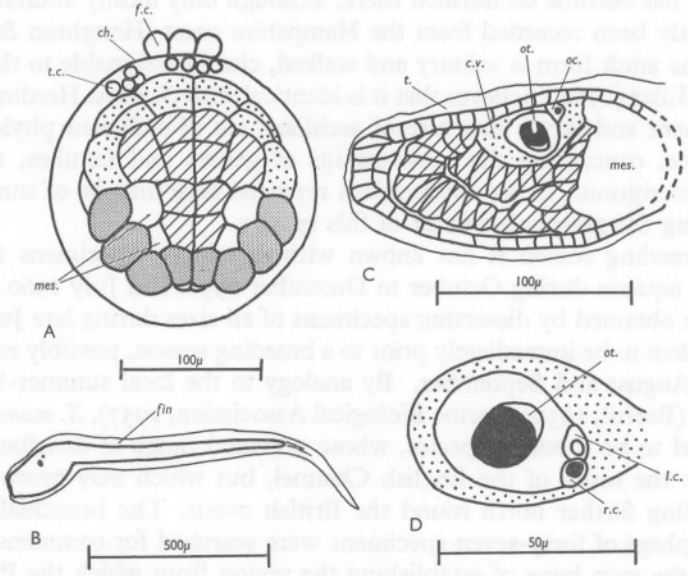

Fig. 1. A, Vegetal view of an embryo at the onset of gastrulation, most of the follicle cells and test cells omitted; B, larva; C, near-sagittal section of larval trunk; D, dorsal view of cerebral vesicle (whole mount). *Cross-hatched*: dark grey endoderm cells; *sparse stipple*: light grey crescent notochord and nerve cells; *close stipple*: yellow crescent muscle cells. *c.v.*, cerebral vesicle; *ch.*, chorion; *f.c.*, follicle cell; *l.c.*, lens cell; *mes.*, mesenchyme; *oc.*, ocellus; *ot.*, otolith; *r.c.*, retinal cell; *t.*, test; *t.c.*, test cell.

Development proceeds rapidly: the maturation divisions and first cleavage occupy the first hour after fertilization; early cleavages follow at half-hourly intervals; the embryo is gastrulating after 5 h and hatches within 14 h, at 21° C. The tail is flexed laterally to encircle the trunk of the embryo. A hatching enzyme is secreted which will digest the chorions of adjacent unfertilized eggs.

A recently hatched larva is shown in Fig. 1 B. The caudal fin runs above and

below the tail and projects behind it. On the trunk, a continuation of the ventral fin reaches the ventral papilla; the dorsal fin bifurcates, sending an arm to each of the two dorsal papillae. The muscle cells of the tail retain the yellow pigment. The cerebral vesicle (Fig. 1 C, D) contains a large otolith and a relatively small ocellus. The otolith is a single cell distended by a mass of black pigment 15 μ in diameter. It is difficult to discern the structure of the ocellus from longitudinal sections: Fig. 1 C shows the single retinal cell with its pigmented sphere (4 μ diameter). A single lens cell, containing a refractive body, may also be seen in fortunately orientated sections or dorsal views of whole mounts (Fig. 1 D). The lens cell lies to the right of the retinal cell, projecting very little above or in front of it.

The development of *S. mammiculata* appears to be as typical of its genus as is the adult form. Such features as the coloration of the egg and test cells, and general larval structure, are common also to *S. partita* (see Conklin, 1905) and to *S. plicata* (personal observation at Naples). Self-sterility and the hatching enzyme are found in these three species and the majority of other solitary ascidians. The occurrence of a visibly coloured mesodermal crescent may suggest an affinity between the Styelinae and Pyuridae (Millar, 1951, 1954). In addition to the instances given by Millar, a total of four species of *Styela* are known to form yellow crescents—the three cited above and *S. barnharti* Ritter & Forsyth (Berg & Humphreys, 1960). The only enterogonid ascidian known to do so is *Ascidiella scabra* (see Fautrez, 1940).

The phylogenetic value of the larval ocellus has been admirably demonstrated by Berrill (1948, 1950), but its structure in *Styela* required confirmation. Grave (1944), after a histological technique that resolved the cellular nature of only the muscles and notochord, recognized a pigmented cell as the ocellus of *S. partita* but homologized it to one of the three lens cells of enterogonid and pyurid larvae. Since the larva is light-sensitive, Grave was compelled to conclude that other unpigmented cells form a retina. Berrill (1948) described the ocellus of *S. yakatutensis* Ritter as possessing a single lens cell and a small pigmented retina (i.e. a reduced ocellus of the two typical cellular components, as described above). His later statement that 'the ocellus is reduced to a single pigmented lens cell in *Styela* species' (Berrill, 1950, p. 45) is at variance to his own results, the present description and optic theory. A lens transmits and refracts light: it would not be expected to carry an opaque pigment mass. A retina is commonly pigmented, to absorb light in the ascidian ocellus, or partly surrounded by a shield of pigment as in the vertebrate eye and possibly the botryllid photolith. Whether or not a lens cell is present in *S. partita*, Grave (1944) misconstrued the pigment cell which can only be the retina. The contention (Berrill, 1948) that all ascidian ocelli are homologous is supported here, for the small ocellus of *S. mammiculata* could easily be derived from that of the Pyuridae and Enterogona by a reduction of the number of both lens and retinal cells.

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SUMMARY

Styela mammiculata breeds during the summer, probably in August and September. Some eggs are self-sterile. Embryos show a yellow crescent of presumptive muscle cells. The larvae hatch by means of an enzyme. The larval sensory apparatus includes a large unicellular otolith and a small ocellus, which is composed of a single pigmented retinal cell and a single lens cell.

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