Development of *Tethya serica* Lebwohl, a Tetraxonian Sponge

I. Observations on External Changes

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Introduction

The development of *Tethya serica* Lebwohl, a tetraxonian sponge, was studied for the first time by Nagai in 1910, and his results were re-examined by Kume in 1952. According to them, the sponge shows some peculiarities in its development. These are ; 1) the species belongs to the dioecious type and the fertilization takes place outside the body ; 2) the egg, when shedded, has numerous fine cytoplasmic processes radiating out from its surface, and when the egg is fertilized and the fertilization membrane is elevated, these processes, after bending down towards the egg surface, are perfectly involved in the perivitelline space ; and 3) as the fertilized egg discharges some sticky substances, the egg continues to develop while adhering the substratum.

According to Nagai, he tried to cultivate the eggs which adhered to the inner wall of a cup of glass by submerging them in the sea, and he found that they grew up into young little sponge in the following spring season. But, he could not make clear the way of their development.

The present article deals with the external changes of this sponge during its development from fertilization to the young sponge with osculum, of which cultivation and observation was made by the writer from 1954 to 1956 when she worked at Misaki Marine Biological Station.

Before going further, the writer wishes to express her hearty thanks to Professor Matazo Kume, Ochanomizu University, for his continuous direction throughout the work, to Professor Katsuma Dan, Tokyo Metropolitan University, for his invaluable advice on the culture method of the sponge, and to the staff of Misaki Marine Biological Station, for their kind help to her study.

Materials and Method

The materials used in the observation were collected at Aburatsubo Bay and Koajiro Bay near the Misaki Station. The breeding season of the species lasts from July to September ; but the observations were mostly done in August, when it breeds most actively. During the season, eggs are discharged almost every day through the osculum of the sponge, and these eggs are easily fertilized by the sperms also discharged from the male sponges in the same manner. As the fertilized egg has the character to stick to the substratum, the egg was made to adhere to the surface of the slide-glasses and thus the observation was made under the microscope. During the first five or six days, the eggs on the slide-glasses were cultured under the running sea water in the laboratory. But after a week, they were put into the wooden box and were submerged in the sea for further culture.

Cleavage

Since the eggs (about 0.18 mm in diameter) are so densely laden with yolk and are quite opaque, the process of cleavages was examined mostly by the reflected light. Generally, the eggs segment not partially but totally and equally ; however, there are many variations in cleavage patterns. The first cleavage takes place, in most cases, vertically to the surface of the substratum to which the egg adheres. The second one also segments vertically and crosses the first one with right angle (see Fig. 1, a and b). Even after the third cleavage, they are in most cases either vertical or somewhat oblique, and horizontal cleavages rarely

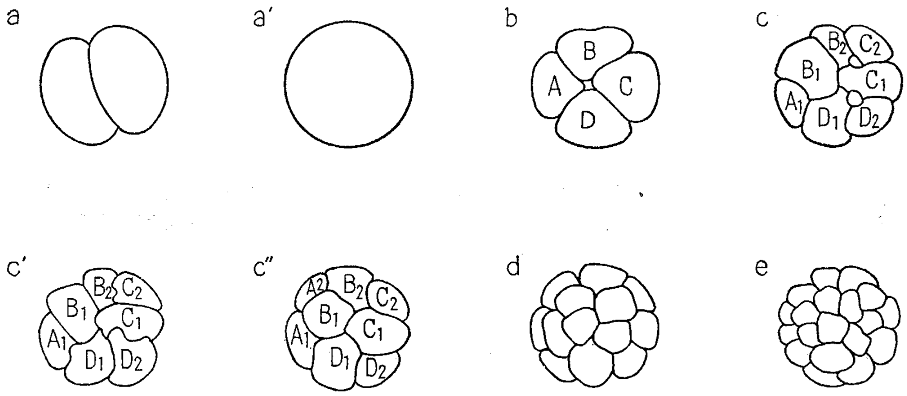


Fig. 1. An example of cleavage pattern (×85)

a. 2-cell stage; two hours after fertilization.
a′. Twenty minutes after a; cleavage furrow becomes obscure.
b. 4-cell stage (3°30′)
c–c″. 3rd cleavage.
c. Small cytoplasmic globules were extruded from C_2 and D_2 (4°10′).
c′. The globules were taken into C_2 and D_2 (4°20′).
c″. Blastomeres changed their position, 8 blastomeres being placed parallel to the adhered plane (4°30′).
d. 4th cleavage (6°20′).
e. 5th cleavage (6°50′).

occur. But, after the segmentation is completed and the blastomeres reach the stage of resting, there appears in blastomeres a tendency to change their positions so as to be placed parallel to the adhered plane (Fig. 1, c″). The occurrence of the horizontal cleavage is probably inhibited by the adhesion of the eggs to the substratum.

Occasionally, the egg divides at once into four cells, without showing any sign of the first cleavage furrow. In such case, the cytoplasmic division of the first cleavage must have been omitted, since the time required for such division is about twice as much as that of normal segmentation. In later cleavage stages, there often exist 3, 5, 6 or 7 cell stages, as the rate of segmentation in each blastomere is not always synchronistic (Fig. 1, d and e).

After the segmentation is completed and the blastomeres are clearly formed, the cleavage furrows often become obscure before the onset of the following segmentation (Fig. 1, a′). Quite peculiar, but frequently observed occurrence is the extrusion of a small cytoplasmic globule out from the surface of the blastomeres during cleavages. Such extrusion, however, is not only limited to a definite region of the egg, but it is also of a temporal occurrence ; therefore, when the following segmentation begins, the globule is soon taken into the same blastomere from which it is extruded (Fig. 1, c–c″).

External Changes of Embryo

During the early cleavage stages, the protoplasmic processes which were involved in the perivitelline space, are still remaining as a form of glanules (Fig. 2, a and Fig. 3, a). But these granules become almost invisible when the embryo reaches the morula stage, and the cells lying along the periphery of the embryo move and elongate towards the membrane with a consequence of the perivitelline space becoming narrower (Fig. 2, b and Fig. 3, b). Then, from outer end of thus elongated cells, cytoplasmic process begins to grow out like a thread, which extends toward the membrane, penetrate it, and elongates freely outside of it (Fig. 3, c). At the time when these processes first develop, they grow out along various directions (Fig. 2, c) ; but some of them can reach and stick to the substratum to which the embryo has already adhered. Although this makes the embryo to be far more firmly fixed to the substratum than ever, their duration is quite short, and soon degeneration begins when root is newly formed.

The Formation of the Root

Soon after the processes are developed, the formation of a new kind of projections follows which appear from the surface of the embryo somewhere near the adhered plane. Each of these projections consists

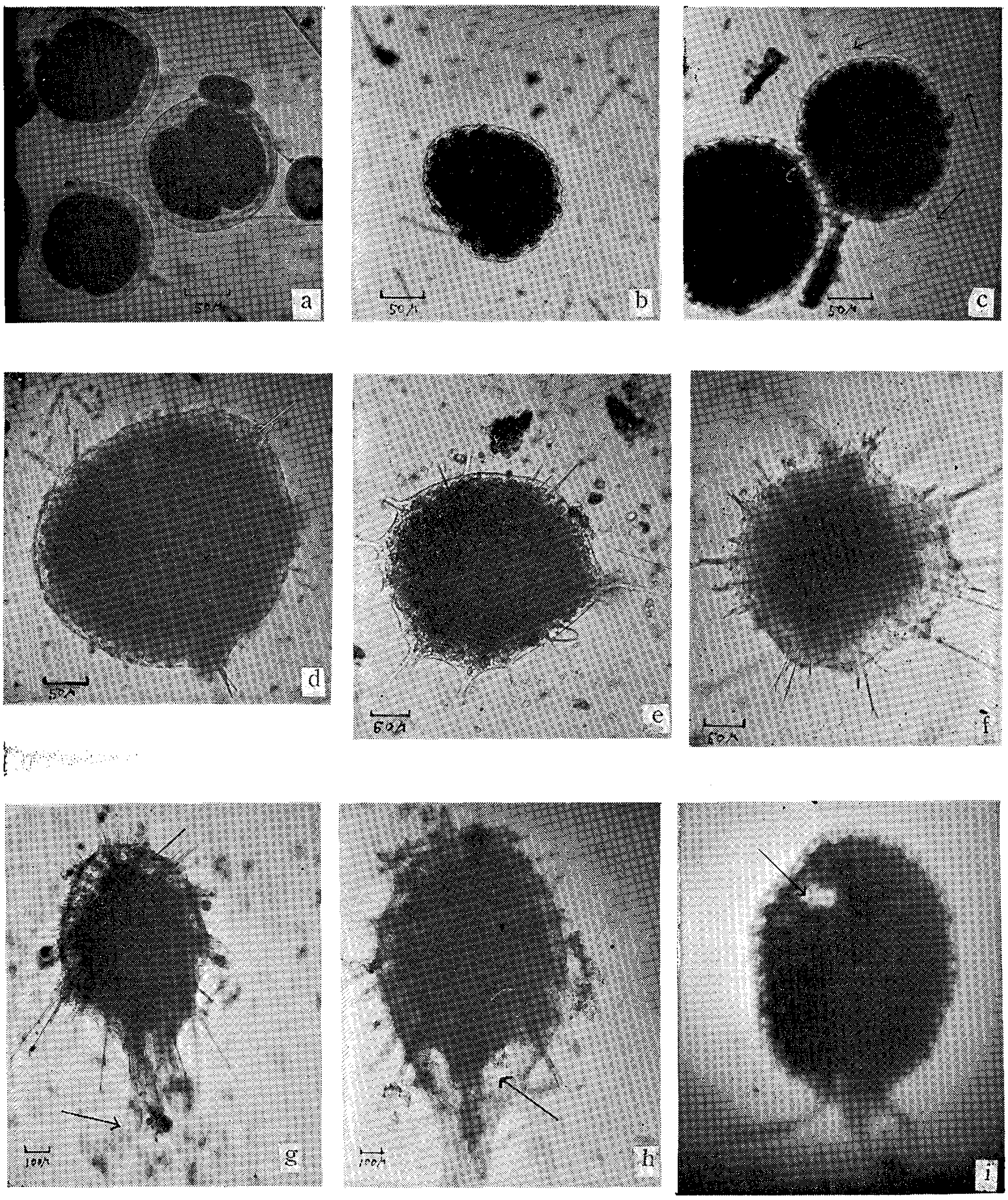


Fig. 2 (a–i). Successive stages in development of *Tethya serica*, from cleavage to young sponge with osculum.

a. Cleavage stage (2 cells).
b. Morula stage; 20 hours after fertilization.
c. 3-day embryo; cytoplasmic processes (indicated by arrows) begin to appear.
d. 4-day embryo; spicules extend outward and push up the membrane from interior.
e. $4\frac{1}{2}$-day embryo; projections of rudimental root region begin to appear.
f. 10-day embryo; trifurcated spicules arranged radially in bundles.
g. 15-day embryo; canal system (arrow upper) and root region (arrow lower) are developed.
h. 25-day embryo; opening of excurrent is formed near the base of root (arrow).
i. A young sponge 46 days old, with osculum (fixed specimen).

of a cell, and its tip, thin and roundish, moves forward by creeping like a pseudopodium of the protozoa upon the surface of the slide-glass

(Fig. 2, e). When these projections grow, some neibouring cells migrate into the projections, which as a consequence, becomes larger and multicellular, but still behave like an amoeba as a whole (Fig. 3, d).

The projections thus formed are the rudiment of the root region of the sponge. Usually, these projections are directed variously from near the adhered surface of the embryo, but in some cases they are directed in one and the same point. When these projections adhere firmely to the substratum, the membrane that envelops the embryo is stretched and the latter becomes flattened out evenly on the slide-glass (Fig. 2, e). But when embryos grow and elongate vertically, then basal parts of the projections are gathered and are united into one under the lower surface of the embryo. In this way, a rudiment of the root is formed. Distal ends of the projections that remain disunited are often bifurcated and constantly stretch and elongate on the slide-glass (Fig. 3, e). After the development of the root, the embryo adheres to the substratum only by the root, and the other part of the embryo becomes completely free from the substratum. It is ten days after fertilization that the root is thus formed.

The Formation of the Spicule

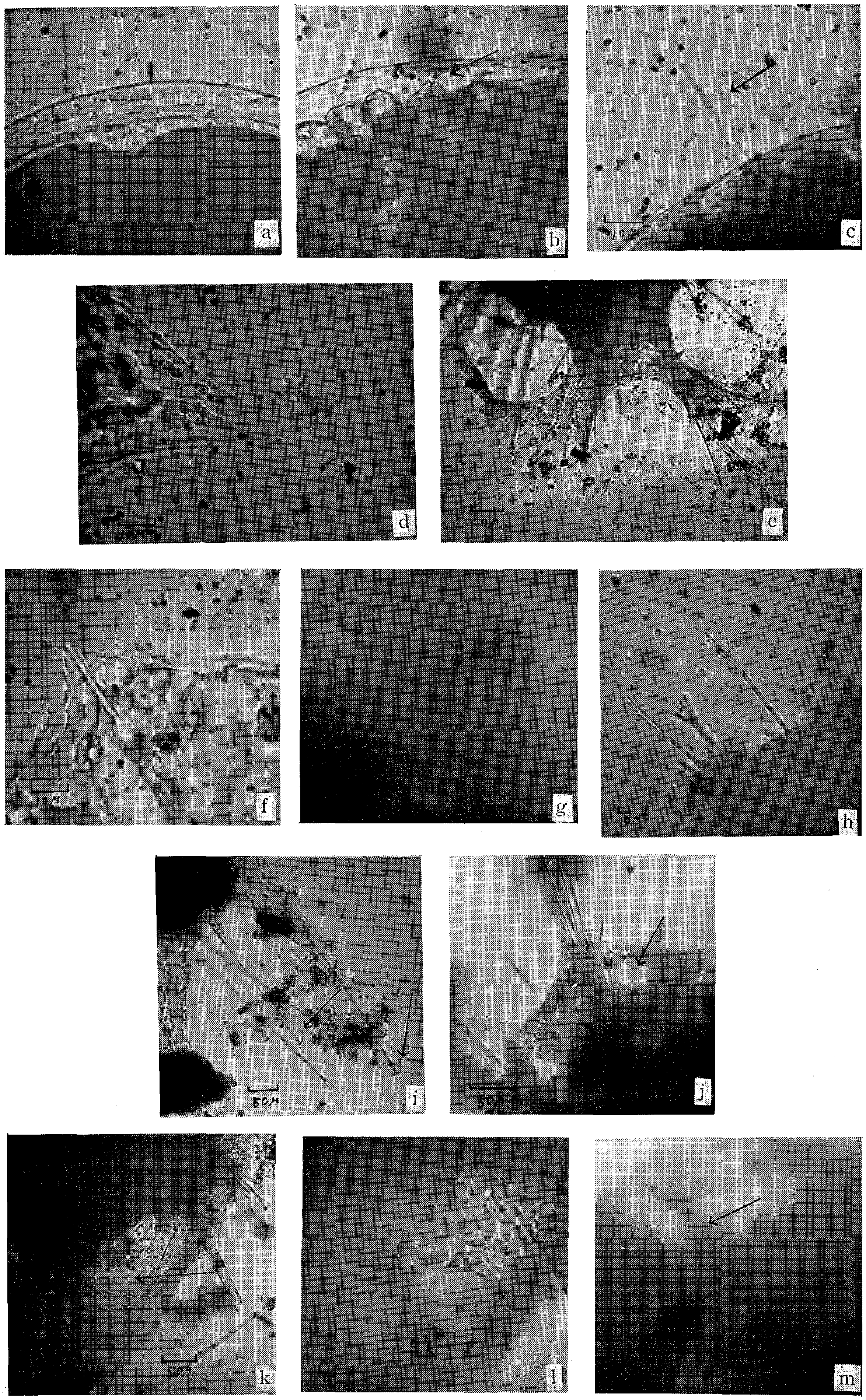
By crushing the two-day embryo under the cover slip, it is found that the thin and soft spicules are already formed at its center. In four-day embryo, these spicules extend outward from its center, and come to push up from interior the membrane which covers the surface of the embryo (Fig. 2, d and Fig. 3, f).

The spicules that develops at first, belongs to the monoaxon type. But presently, their outer end is furcated into three and takes a form of tetraxon type (protriaene), which is characteristic of Tetraxonidae (Fig. 3, g). After this occurrs, they begin to project out of the embryonic surface piercing through the membrane (Fig. 2, f and Fig. 3, h). It is noteworthy that the trifurcation of spicules always takes place when they are still under the membrane, and never occur after their projection through it.

In ten-day embryo, the spicules are already arranged radially in bundles (Fig. 2, f), and into the root, a different kind of spicules grow from the center of the embryo. These spicules are the anatriaene (Fig. 3, i), in which trifurcated points are directed inwardly instead of outwardly as is the case in protriaene.

The Formation of the Canal System

It is of course, difficult to study the development of the canal system only by external observation. However, in embryos older than


a
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eleven days, it is found by external observation that there develop the transparent portions between the radiating bundles of spicules. These portions are the incurrent cavities differentiated under the thin dermal layer (Fig. 2, g and Fig. 3, j), where there are seen a number of pores opening to the exterior. If powders of carmine are poured into the culture medium, they are observed streaming into incurrent cavities through the pores, and finally accumulated in the center of the embryo. The canal system at this stage, is well developed throughout the embryo, and no gastral cavity is as yet developed in the center. The central portion where powders of carmine are accumulated is only the central part of such canal system.

There is near the base of the root the opening of excurrent through which the carmine powders are poured out (Fig. 2, h and Fig. 3, k, l); but this is a structure quite different from the osculum which is in the adult, and it is in about forty-day embryo, when the osculum is first formed at the center of the upper surface of the embryo (Fig. 2, i and Fig. 3, m). The problem whether there exist some developmental relations between the excurrent opening near the base of the root and the osculum is not clear at present.

Summary

1. In this report, the external observations of developmental changes after cleavage in a tetraxonian sponge, *Tethya serica* Lebwohl, are described.

2. The egg segments totally and equally, but there are seen many variations in cleavage patterns, which probably owes to the adhesion of the egg to the substratum and to the presence of heavy yolk.

3. At the surface of the embryo where the embryo adheres to the substratum, there develops from marginal cells of the embryo a number of thread-like processes. These processes help to fix the embryo

Fig. 3 (a–m). More detailed changes of development of young sponge.

a–c. External changes of surface in early embryo.

a. Granules in the perivitelline space.

b. and c. Thread like processes (arrows) are gradually formed.

d. Multicellular projections grow out.

e. Rudiment of the root is completed.

f–i. Formation of spicules.

f. Monoaxon spicule extends outward, pushing up the membrane. g. Outer end of spicule is furcated into three. h. trifurcated spicule pierced through the membrane. i. Spicules in the root (anatriaene, indicated by arrows).

j. Subdermal cavity (arrow) is seen through the outer surface of the young sponge.

k–l. In k, the opening of the excurrent canal is found, and carmine particles are poured out through it in l (arrow).

m. Osculum is developed (46 days, indicated by an arrow).

more firmly to the substratum, but soon degenerate after the appearance of the multicellular projections, which are the rudiment of the root.

4. The multicellular projections come together under the growing embryo, and are united into one at their base, thus becoming the rudiment of the root. Distal ends of the projections which remains disunited, are often furcated, and constantly grow on the substratum.

5. The spicules are formed at the center of the embryo about two days after fertilization. At first, it is of the monoaxon type, but later becomes tetraxon type by trifurcation of its distal end. Most of the spicules are protriaene, but the spicules in the root belong to anatriaene.

6. Twelve days after fertilization, incurrent cavities are formed under the dermal layer of embryo, through which pores are opened outwardly.

7. The exit of excurrent canal is primarily formed near the base of the root and when the embryo is forty days old after fertilization, the osculum of the adult form is developed on the upper surface of the embryo.

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