

REMOVAL OF THE BLOCK TO SELF-FERTILIZATION IN THE
ASCIDIAN *CIONA*

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The eggs of *Ciona intestinalis* found on the Atlantic and Pacific coast of this country cannot be fertilized, as a rule, by sperm of the same individual. A similar phenomenon has long been known in flowering plants. In the latter case it has been shown that the block is due to factors in the pistil that retard the growth of the pollen tube; or else failure to self-fertilize is due to the absence of factors that cause an acceleration of growth in cross-fertilization. In *Ciona* the nature of the block was unknown despite many attempts to determine it. During the past summer at Woods Hole I have succeeded in bringing about self-fertilization in *Ciona* by the simple process of freeing the egg from its membranes. The eggs were taken from the oviduct, put into sea water in a flat bottomed watch glass, and by means of two very fine needles the membrane around the egg was torn open. Sometimes the entire egg squeezed out of the slit, sometimes only a part of it pushed out. In either case the extruded protoplasm rounded up into a sphere.

Sperm was taken from the vas deferens of the same individual and diluted. A small amount of the sperm-suspension was added to the water containing the naked eggs and egg-fragments. Most of the eggs and fragments segmented in the course of an hour. The first cleavage was equal; the second equal or nearly equal, also the third. These divisions had every appearance of normal cleavage. Later stages were observed, but not carefully followed.

The same sperm-suspension was added in equal amounts to the eggs of the same individuals that had not been opened and were therefore still surrounded by their membranes. Not one of the eggs divided.

It is obvious from these results that self-fertilization in *Ciona* is blocked either by the membrane, or by the follicle cells (these stand like a palisade over the outer surface of the egg membrane) or by the test-cells (that lie scattered between the surface of the egg and the membrane), or by some secretion produced by one or by both of these surrounding layers of cells. That the block is not caused by the membrane is evident, since sections of eggs, inseminated by their own sperm, show that the spermatozoa pass to the inside of the membrane. Removal of the follicle cells by shaking them off had shown that such eggs cannot be fertilized. It follows that the block must be caused by the test-cells or by some substance produced by them.

It had been known for some time that the test-cells are derived from maternal tissue. They are not budded off from the egg as some of the earlier observers had claimed. The test-cells stand, therefore, in much the same relation to the spermatozoa as does the maternal tissue of the pistil of plants to the pollen tube. Their secretion must be supposed either to bring the sperm to rest, or to interfere in some other way with the union of the sperm with the egg.

In self-sterile plants it has not been possible to demonstrate whether the pollen could fertilize the egg-cell if it reached it. In *Ciona* the results here recorded demonstrate that there is no difficulty in self-fertilization if the surrounding cells and membranes are removed. It is true that some self-sterile plants will occasionally set seed at the end of the season, (as shown by East) owing in all probability to the weakening of the block; but such results do not exclude the possibility that the egg itself may have changed. It is interesting, therefore, to find that eggs of *Ciona*, that can not be self-fertilized while in the membrane, can be self-fertilized if the surrounding cell-envelopes are removed. It seems probable that here the block slows down the spermatozoa or changes their mode of swimming. In plants, on the other hand, East suggests that there is an actual acceleration of the pollen tube in cross-fertilization. In *Ciona* there is no need for such an hypothesis, since eggs removed from their membranes are as promptly self-fertilized as are the same eggs cross-fertilized with membranes intact.

I have suggested elsewhere that in the self-sterility of *Ciona* we are dealing with a genetic problem. The present results indicate (as I had suggested) that this relation involves a reaction between maternally derived substances (that have the same genetic inheritance as the cells from which the spermatozoa are derived) and their "own" spermatozoa. In other words, self-sterility in *Ciona* is not a case of selective fertilization, if by this term we means a selective reaction between the egg and sperms themselves.

The method of obtaining fragments by tearing the membrane will make it possible to study the development of egg-fragments of the unfertilized ascidian egg. A comparison of the development of such fragments with the highly specialized type of development shown by the isolated blastomeres of these eggs should give an opportunity to find out how early the mosaic character of the cleavage is initiated. It is my purpose to examine this question, if possible, this summer.
