CROSS- AND SELF-FERTILIZATION IN THE ASCIDIAN MOLGULA MANHATTENSIS

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The rule for the "single" ascidian *Ciona intestinalis* is no self-fertilization and 100 per cent cross-fertilization, but there are rare cases of the reverse and it is these that may give a clue to the genetic problem involved. Another single ascidian, *Cynthia,* has been shown to self-fertilize frequently but no cases of cross-sterility have been reported so far. A third single ascidian, *Molgula manhattensis,* has been shown to give a high percentage of self-fertilization, mainly from my own earlier examination (*Jour. Exper. Zool.,* 1904), but no thorough comparison of the extent of selfing with the extent of crossing has been made. It seemed worth while to undertake such an examination in the hope that some degree, at least, of self-sterility might be found and, if so, fall in line with the situation in the other two species. In my former paper there were cases of 100 per cent self-fertilization and also a few in which no selfing occurred.

During September, 1941, *Molgula* being abundant on the float in the eel pond at Woods Hole, a number of them were tested. There is an ovotestis on one side, in a loop of the intestine. It is smaller than the one on the other side, and its central slate-colored ovarian portion is, as a rule, more nearly surrounded by the white testis than is the ovotestis on the other side, and this makes it more difficult to remove the ripe eggs without contamination from the testis. The larger ovotestis on the opposite side is generally only partly surrounded by the testis, and the eggs can frequently be removed by a small puncture in the ovarian portion without contamination.

Each individual was first washed in tap water, the test was split open, and the interior mass removed and also washed. A small puncture in the ovarian part allows the eggs to exude. They were then collected in a dish of sea water (15 cc.).

In a preliminary test of six individuals (August 28) three lots of eggs gave no cleavage, showing that no sperm had come out with the eggs, or at least not sufficient sperm for selfing. Three sets gave a high percentage of cleavage, which must have been due to undetected sperm that

had come out with the eggs. The three sets of eggs that had not cleaved were cross-fertilized and all cleaved.

In later experiments the eggs were first removed from an individual (*A*) to a Syracuse dish of sea water; one-half of these was transferred to another dish and later cross-fertilized with sperm from the "reciprocal" individual (*B*). More eggs and some sperm were then removed together from the same individual to another dish. The latter were heavily inseminated with their own sperm. At the same time similar dishes were prepared from individual (*B*), some of whose eggs were crossed with sperm from (*A*). If none of the unfertilized eggs in *A* and *B* cleaved, it would mean that no spermatozoa (or too small an amount to be effective) have been introduced into this dish. Hence, if any or all eggs in the cross-fertilized dish cleaved this was due entirely to cross-fertilization. If any or all eggs cleaved in the self-fertilized dish, this must have been due to its own sperm. As an illustration, the results for three such tests were as follows:

(A) The unfertilized eggs of *A* gave no cleavage; those selfed gave 25 per cent cleavage; those crossed by *B* gave 100 per cent.

(B) The unfertilized eggs of *B* gave no cleavage; those selfed gave 100 per cent; those crossed by *A* gave 100 per cent.

(C) The unfertilized eggs of *C* gave no cleavage; those selfed gave no observed cleavage (but later one coiled embryo was present and a few abnormal embryos); those crossed by *D* gave 100 per cent.

(D) The unfertilized eggs of *D* gave no cleavage; those selfed gave 1 per cent; those crossed (to *C*) gave 75 per cent.

(E) The unfertilized eggs of *E* gave 10 per cent cleavage; hence to this extent some of the "cross-inseminated" eggs may have been selfed before foreign sperm was added; the selfed eggs gave 100 per cent; and the crossed eggs (by *E*) gave 100 per cent.

(F) The unfertilized eggs of *F* gave no cleavage; the selfed eggs gave no cleavage; those crossed to *E* gave 100 per cent.

It is evident from these tests that the amount of selfing that occurred varied from 0 to 100 per cent, while the cross-fertilized eggs gave 100 per cent cleavage except in one case (75 per cent).

Two of the preceding combinations (A–D) are recorded in the first two pairs of Table I. This table gives the results from 38 pairs (or 76 individuals). The respective pairs used in reciprocal crossing are bracketed. With three exceptions the cross-fertilized eggs gave 100 per cent cleavage. The three exceptions (75, 50, 80) were in different pairs. There was nothing peculiar in the selfing of these three cases unless it be

TABLE I

Control	Self	Cross	Control	Self	Cross	Control	Self	Cross
0	25	100	5	100	100	50	100	100
0	100	100	0	100	100	0	100	100
0	0	100	0	100	100	0	2	100
0	1	75	2	75	100	0	40	100
0	100	50	90	100	100	0	90	100
0	0	100	0	80	100	100	100	100
0	35	100	100	100	?	0	12	100
0	100	100	0	0	2	30	100	100
0	100	100	0	100	100	50	100	100
0	10	100	2	75	100	0	0	100
0	90	100	90	100	100	0	100	100
0	15	100	0	80	100	80	100	100
50	100	100	0	50	100	4	8	100
50	50	100	100	100	100	0	8	100
20	20	?	15	15	100	0	0	100
4	14	100	70	80	100	0	20	100
0	100	95	0	0	100	1	0	100
0	20	90	0	10	100	20	75	100
0	80	80	0	10	100	20	100	100
0	80	100	0	20	100	100	100	100
15	25	100	5	100	100	100	100	100
70	80	100	0	100	100	0	100	100
0	0	100	2	5	100	100	100	100
0	10	100	0	100	100	4	100	100
0	10	100	20	100	100			
0	0	100	100	100	100			

that the 50 per cent crossed eggs gave 100 per cent selfed. In addition there were two cases in which, by oversight, the crossed eggs were not recorded, but there can be no doubt that they gave 100 per cent cleavage or attention would have been drawn to them.

An examination of this table shows that no cleavage took place in 44 lots of eggs removed from the ovary and not inseminated. There were 32 other lots with percentages ranging from 1 to 100 per cent selfing. This means that the amount of sperm that had been unintentionally re-

moved with the eggs was too small in amount in most cases to self-fertilize any of the eggs. An examination of the table shows that, in cases where no cleavage occurred in the dishes of control eggs, it also did not take place in 9 of the selfed dishes, which must mean that 10 per cent of the individuals were totally incapable of self-fertilization. Of those lots in which none of the control eggs cleaved (44 cases), and some eggs at least cleaved when selfed, the percentage of eggs cleaved ranged from 2 to 100 per cent. This may be interpreted to mean that although plenty of spermatozoa were present, only a percentage of them were actually effective as measured by the cleavage. I interpret this to mean that in addition to the spermatozoa having the same genetic composition as the eggs, there are varying percentages of sperm with a different genetic composition, and these are able to fertilize eggs from the same individual. This interpretation is also consistent with the evidence from those cases where a small percentage of the eggs in the control uninseminated dishes cleaved. As the table shows, there were 20 cases in which the control "unfertilized" eggs that cleaved gave a lower percentage of cleavage than did the "self-fertilized" eggs, and only 10 cases in which they were the same. These results are consistent with the assumption above, viz., that there are present a smaller and variable number of spermatozoa whose genetic make-up differs from the majority of sperm, the latter incapable of fertilizing their own eggs, but the former having the ability to do so. This conclusion is consistent with the more completely worked-out case of *Ciona*.

It should be noted that I did not come across a single case of cross-sterility, but the numbers may have been too small to expect to meet such cases. There is one case recorded where no eggs were selfed and only two eggs cleaved when cross-fertilized, but I am inclined to think that the sperm was not in condition to self or to cross. It is noticeable here that there is a question mark in the table when the sperm of this individual was used for crossing the reciprocal.

Normal and Abnormal Development

In some of the preceding experiments the dishes were examined when the tadpoles were due to hatch (or after that time) in order to find out whether the development was normal or abnormal. The observations are not here reported since it became apparent that unless the embryos are followed closely before and at the time of expected hatching and later, the records of what constitutes abnormals cannot be accurately defined. For instance, the tadpoles are due to emerge ten to twelve hours after fertilization depending on temperature. Besides being a

rather inconvenient hour for taking records, many of the embryos without passing through a free swimming stage undergo metamorphosis in place, and the time may extend over a considerable interval. This makes it difficult to record normal versus abnormal embryos. In general, it may be said that in a large number of cases there were many abnormal embryos as compared with the number of those that cleaved. This applies mainly to embryos that did not pass into the complete tadpole stage. Those that underwent a metamorphosis without passing through the free-swimming tadpole stage were so variable that without fuller records it is not possible to make a clear statement about them. Further observations are needed. There was no evidence that the selfed lots behaved differently from the cross-fertilized lots.

Two Unisexual Molgulas

Two individuals were found that had no testis on either side but well-developed ovaries. These came from a small bunch of some 35 to 50 individuals, and may therefore have been related. They were full-sized individuals and normal in all other respects. Eggs were taken out separately from each side of each individual. One-half of the eggs of each were kept in case undetected sperm was present, but none of them cleaved. The cross-fertilized eggs taken from one side of one individual cleaved irregularly. Later they gave rise to a few tadpoles and some metamorphosing embryos. The eggs from the other side when crossed gave abnormal cleavage (polyspermic?) and later a few metamorphosed individuals. Eggs taken from the other individual and not fertilized did not cleave. Those from one side that were cross-fertilized gave 100 per cent cleavage and later many metamorphosed individuals. Those from the other side that were fertilized gave 90 per cent cleavage and later a few normal tadpoles and many metamorphosed embryos. The eggs of these two individuals when cross-fertilized did not behave differently from those of bisexual Molgulas. If a new generation could be reared from such unisexual forms, it might be possible to find out if the condition is inherited.

Summary

Tests of self- and cross-fertilizations of the single ascidian *Molgula manhattensis,* were made during August and September, 1941, at Woods Hole. It was found that 100 per cent self-fertilization occurred in less than half the cases (see Table I), but in others the percentages ranged from 1 to 90 per cent. One hundred per cent of cross-fertilization oc-

curred in practically all cases. The occurrence of normal and abnormal development was observed, and many cases of abnormal embryos as well as normal tadpoles were found, but, owing to the fact that many embryos metamorphosed directly, or at least without passing through a free-swimming tadpole stage, the numerical results are not reported here. A closer set of observations are needed to give reliable data as to whether self-fertilized eggs give rise to such development more often than do cross-fertilized eggs. Two mature individuals were found that had only ovaries. Their eggs gave 100 per cent cross-fertilization.