

A 'Fertilization Membrane' in the Ascidian *Herdmania momus* and its Relation to Self- and Cross-Fertilization

It is a characteristic of certain ascidian species that the eggs are refractory to the sperm from the same individual. Other species exhibit typical intergradation between this complete self-sterility to complete self-fertility^{1,2}.

One of the conclusions resulting from MORGAN'S extensive work (see review³) on genetic and physiological aspects of self-sterility in *Ciona intestinalis* was that the block to self-fertilization is associated with the chorion. He was able to increase the self-fertilizability of the eggs by several different treatments, among which were exposing them to acid sea water or to proteolytic enzymes. TYLER⁴ suggested that a lytic agent from the sperm might be linked with an immunological type of egg-sperm interaction like that known to act at the egg surface in other species. The existence of such a mechanism appears probable in light of the experiments of MINGANTI⁵, who demonstrated ascidian sperm agglutination by a fertilizin-like substance obtained from trypsin-treated eggs. ORTOLANI⁶ has provided further evidence for a surface-located blocking mechanism by abolishing self-sterility in *C. intestinalis* with the chelating agent versene.

Since no fertilization membrane elevation has been described in ascidians, the usual criterion for self-sterility has been failure of the egg to cleave. But in fertilized eggs of *Herdmania momus* (from the Hawaiian Islands) a 'fertilization membrane' does exist; in un-inseminated or in sterile eggs it is absent. Failure of the membrane to elevate in cases of sterility provides a more logical criterion for the membrane-located block than does cleavage failure. It adds credence to the chorion-block theory of self-sterility, without experimentally altering the egg surface, as was done in the other work.

The typical ascidian chorion bears the inner follicle cells and encloses the perivitelline fluid, which suspends the test cells. It rises from the egg surface during the 2 h following release of the egg from the oviduct. In most species only this membrane elevates, but in some members of the families Ascidiidae and Pyuridae a second, more delicate, membrane (inner chorion or inner membrane) separates from the inner side of the chorion shortly after the latter has elevated^{7,8}.

In the Hawaiian *Herdmania momus* (family Pyuridae) the virgin chorion elevates after the egg is released from the oviduct (Figure 1), as in other ascidians, but the inner chorion acts like a fertilization membrane and becomes visibly separate within 4 min after fertilization (Figure 2). This inner chorion is unlike a classical fertilization membrane, for it separates from the inner surface of the existing chorion instead of from the egg surface. There is evidence⁷ that the material in the interchorionic space is a gel, and, thus, the fertilized egg of *H. momus* is reminiscent of that of *Neanthes (Nereis)*, which also forms a gelatinous coat after fertilization.

There are conflicting reports on the structure of the *Herdmania* egg from the Indian Ocean. DAS⁸ anatomical study of *H. pallida*—which apparently is synonymous with *H. momus*⁹—described the mature egg with both inner and outer chorion; no mention was made of the egg being fertilized. SEBASTIAN¹⁰, on the other hand, de-

¹ T. H. MORGAN, Biol. Bull., Wood's Hole 82, 161 (1942).

² M. M. ESPOSITO SEU, R. C. Accad. Lincei [8] 6, 502 (1949).

³ T. H. MORGAN, J. exp. Zool. 90, 199 (1942).

⁴ A. TYLER, Physiol. Rev. 28, 180 (1948).

⁵ A. MINGANTI, Pubbl. Staz. zool. Napoli 23, 58 (1952).

⁶ G. ORTOLANI, Ric. sci. 27, 2450 (1957).

⁷ N. J. BERRILL, Phil. Trans. [B] 218, 37 (1930).

⁸ S. M. DAS, Indian zool. Mem. 5, 95 (1936).

⁹ W. G. VANNAME, Bull. Amer. Mus. nat. Hist. 84, 341 (1945).

¹⁰ V. O. SEBASTIAN, Proc. Indian Acad. Sci. [B] 37, 174 (1953).

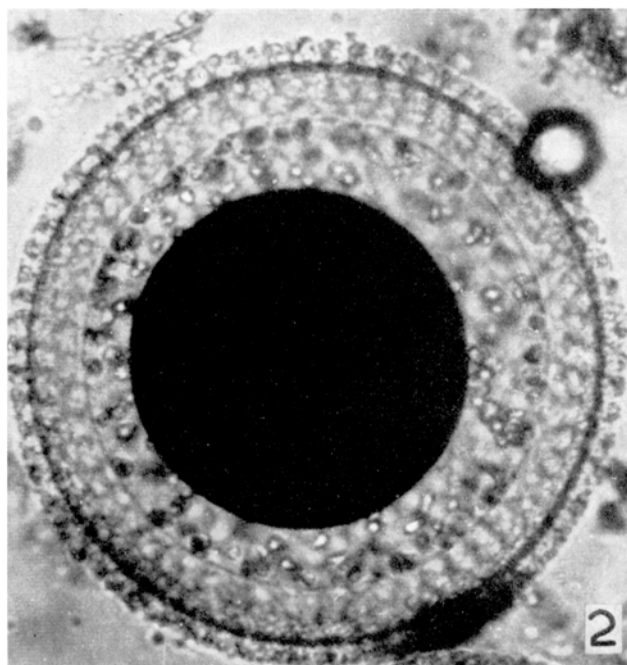
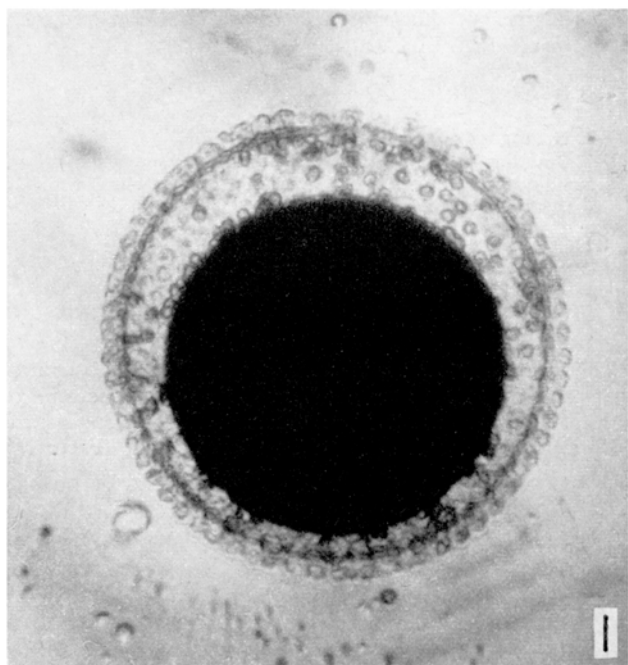


Fig. 1. Unfertilized egg of *Herdmania momus* showing the uninseminated chorionic membrane.

Fig. 2. Fertilized egg of *Herdmania momus* showing the separation of the inner chorion ('fertilization membrane').

scribed the development of *H. pallida* and illustrated the fertilized egg with only one membrane; he made no reference to a change in the membrane upon fertilization.

Experimental. After dissecting the gonads from an individual, the eggs were pressed from the oviducts into a Syracuse dish where they were washed with one or more changes of sea water. Aliquots of eggs were pipetted to five other Syracuse dishes, each containing 10 ml of sea water. A number of eggs were left in the original dish as a control against accidental cross-insemination. The seminal fluid was drawn from the testes by puncturing them with glass capillaries. The sperm was diluted just prior to use with 1 ml of sea water.

To examine the membrane in relation to self- and cross-fertilization, eight groups (five ascidians per group) were inseminated according to MORGAN's 5 × 5 test procedure¹¹. The eggs from each animal were tested with their own sperm and with the sperm of each of the other four animals in the group. The data from these eight groups were then pooled (Figure 3) to show the correlation between membrane elevation and cross- and self-fertilization.

One dish of cross-sterile eggs was found, but it was omitted from Figure 3, as it contained only 28 eggs.

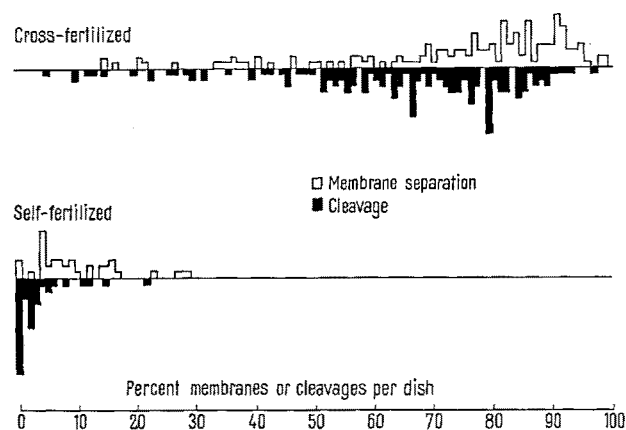


Fig. 3. Pooled data from eight 5 × 5 tests showing cross- and self-fertilization in relation to the percent membrane separation and to the percent eggs cleaved (2 to 4 blastomeres). Each unit of the histogram represents one dish, containing eggs with the indicated percent cleavage or membrane separation. The values are given to the nearest whole percent with values between zero and one shown as 1%. All samples shown had more than 30 eggs and averaged 77 eggs.

Fractionation of the Liver Extract on Sephadex G25

Dextran gels are used for desalting of macromolecular materials^{1,2} as well as separation of proteins, peptides and amino acids^{3,4} and nucleic acids and nucleotides⁵. Because of the mild conditions during gel filtration and the quantitative separation of low and high molecular compounds, this method is suitable for the isolation of low molecular materials from tissues. This study describes the separation of an extract of rat liver by gel filtration on Sephadex G25. 40 ml of rat liver extract (105.000 g; 60 min) was fractionated on a column of Sephadex G25 (3 × 95 cm) in a buffer (0.01 M NH₄HCO₃, pH 8.6) at a speed of 50 ml per hour and at a temperature of 3°C.

However, the appearance of the membranes was no different from that of the self-sterile eggs, as would be expected if the block to fertilization occurs at the chorion in both self- and cross-sterility.

In no case was an egg fertilized (as indicated by cleavage) without showing membrane separation, but membrane separation without cleavage did occur; it was found in an average of 10.3% of the cross-inseminated eggs and 6.8% of the self-inseminated eggs. Following MORGAN³, who suggested that self-fertilizing sperm may arise by mutations in the sperm tract, one might postulate the occurrence of *incomplete* fertility mutations. These could result in sperm deficient in some factor affecting specificity, such that chorion activation was permitted but nuclear activation was not. However, such a hypothesis does not explain the 3.5% greater ($P < 0.025$) membrane separation without cleavage in the cross- than in the self-inseminated eggs.

The close correlation between membrane separation and cleavage is evidence for a close connection between chorion activation and fertilization; the failure of the egg membrane to elevate in cases of self-sterility correlates with the existence of a block. Care must be taken, however, in interpreting this as evidence for a chorion-located block. While the present findings are in agreement with those of earlier investigators, no time sequence study has been made of membrane separation and cortical response in *H. momus* to exclude the possibility that the observed membrane separation may be a consequence of an antecedent reaction at the egg cortex or elsewhere¹².

Zusammenfassung. Im Ei der Ascidie *Herdmania momus* hebt sich nach der Besamung die Befruchtungs-membran von der äusseren Haut ab. Die Korrelationen, die bestehen zwischen Membranabhebung und Befruchtung, sowie ihrem Unterbleiben und der Sterilität, stützen wiederum die Chorionblocktheorie der Auto-sterilität.

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¹¹ T. H. MORGAN, J. exp. Zool. 78, 271 (1938).

¹² The author wishes to acknowledge the encouragement of the faculty of the University of Hawaii under whose auspices this investigation was made.

The fractions were analysed by measuring the absorption at 260 mμ and 280 mμ (Figure a), by conductometric determination of salts, by Bial's reaction for sugars and by a modified method for phosphorus assays⁶ (Figure b). The ninhydrin reaction was performed according to MOORE and STEIN⁷ (Figure c).

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