Ultrastructure of Egg Envelopes in Self-Sterile and Self-Fertile Species of Tunicates[1]

In some tunicates, the eggs of a given individual cannot be fertilized by sperm of the same individual. *Ciona intestinalis* (see ORTOLANI[2], for references), *Styela partita*, and *Ascidiella aspersa* are representatives of such self-sterile forms. In other species, such as *Ascidia nigra*, *Ascidia curvata*, *Ascidia mentula*, and *Microcosmus exasperatus*, self-fertilization occurs readily. The block to self-fertilization in some of the self-sterile species, particularly *C. intestinalis*, can be eliminated by removing the membranes encapsulating the egg.

We have searched for an ultrastructural basis in the membranes for this difference in fertilization behavior. For comparison, we chose self-sterile individuals of *C. intestinalis* (Naples and Woods Hole) and self-fertile *A. nigra* (Bermuda). Eggs were obtained by puncturing the oviducts, and after thorough washing in sea water either observed under the light microscope or processed for electron microscopy as described elsewhere[3].

Under the light microscope, the 2 eggs differ conspicuously in the nature of their chorion cells (Figures 1a and 2a). The chorion cells of *Ciona* project as long, slender processes as opposed to the chorion cells of *Ascidia*, which are shorter and more rounded. Both species also possess a chorion and a layer of test cells directly on the surface of the egg. The overall organization of the egg envelopes appears to be similar in the 2 species.

Electron micrographs confirm this general impression, but also reveal differences in the organization of the egg envelopes (Figures 1b and 2b). The area of contact between adjacent chorion cells is much larger in *Ciona* than in *Ascidia*. It is between these areas of contact that spermatozoa pass in order to penetrate the layer of chorion cells in both species (SCHABTACH and URSPRUNG, unpublished). The ultrastructure of the chorion differs somewhat in the 2 species. In *Ascidia*, a dense core is covered on either side with fibrillar material. In *Ciona*, the dense core is also present, but there is much less fibrillar

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[2] G. ORTOLANI, Ricerca scient. *27*, 2150 (1957).

[3] E. SCHABTACH and H. URSPRUNG, J. exp. Zool. *159*, 357 (1965).

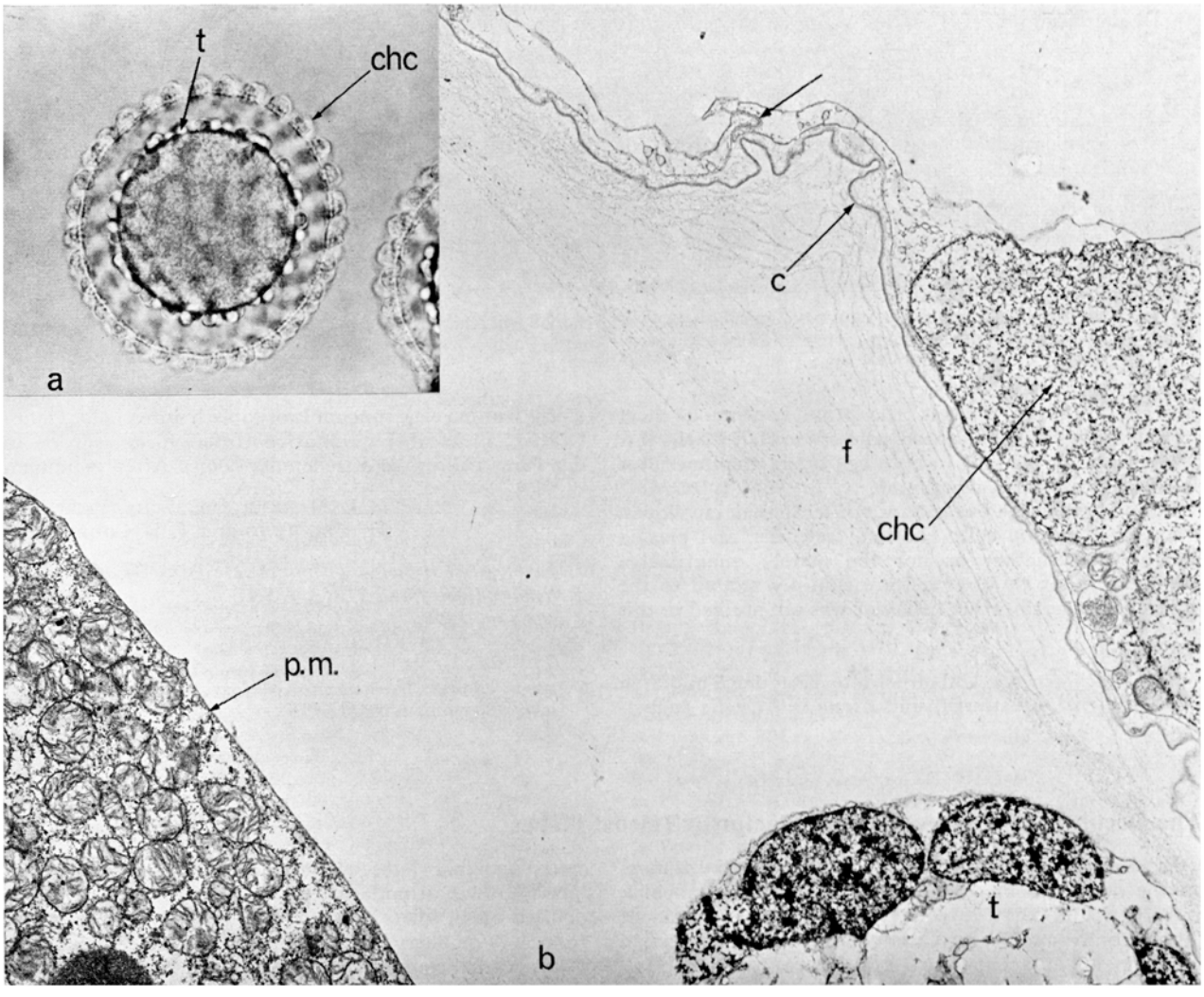

Fig. 1. Egg of *Ascidia nigra*. Magnification (a) × 220; (b) × 9500.

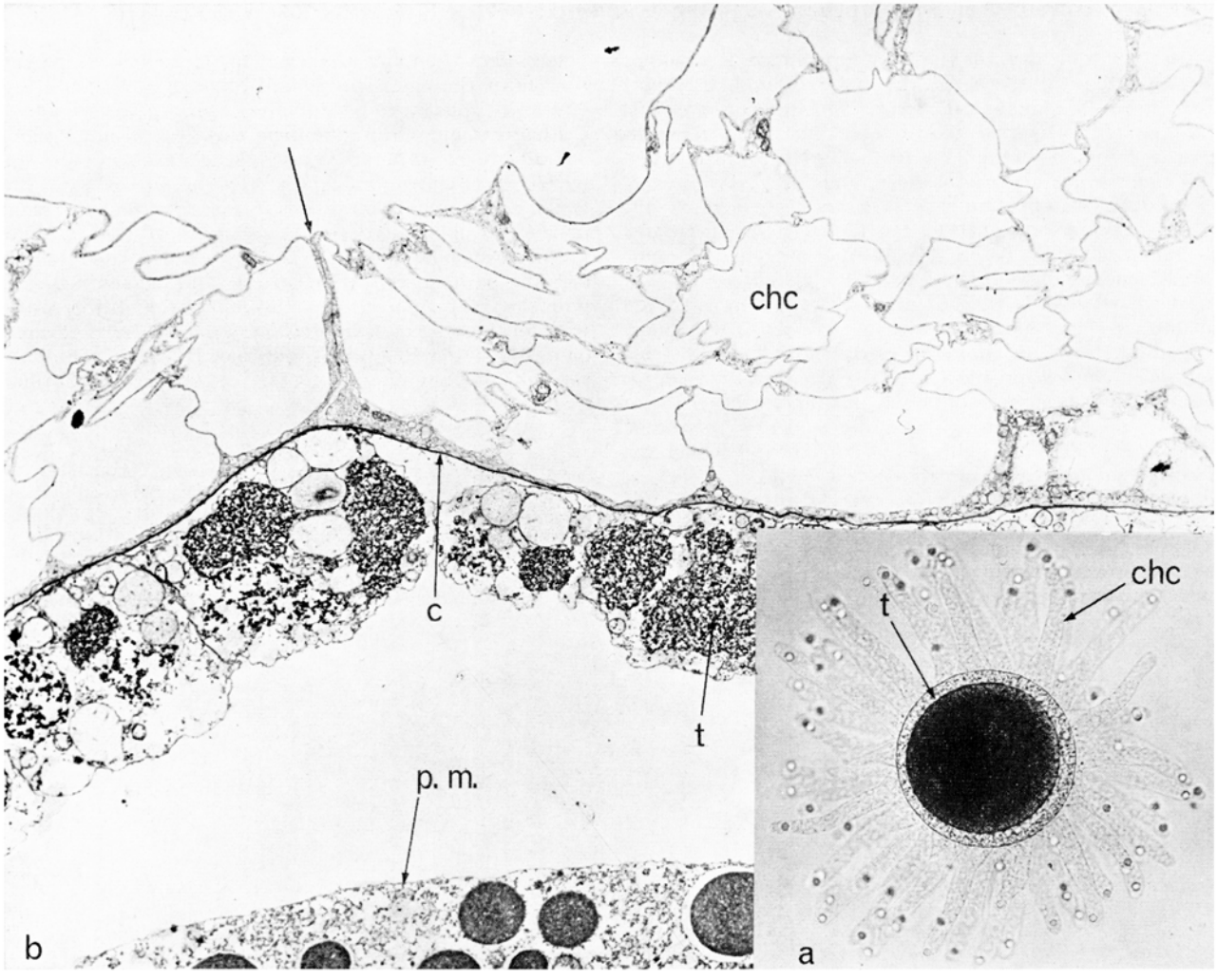

Fig. 2. Egg of *Ciona intestinalis*. (a) × 145; (b) × 4900. c, dense core of chorion; chc, chorion cell; p.m., plasma membrane; t, test cell. Arrow: area of contact between 2 chorion cells.

material. In *Ciona*, the test cells form a continuous sheet around the perivitelline space, whereas in *Ascidia* the test cells are widely spaced. In both egg types, the innermost envelope is a plasma membrane.

Basically then the 2 eggs do not differ in their envelopes. Both have chorion cells, chorion, test cells, and plasma membrane. Whether or not the merely quantitative differences that we observed are causally related to the difference in fertilization behavior was not studied in this analysis.

Zusammenfassung. Unbefruchtete Eier der Tunikaten *Ascidia nigra* (selbstfertil) und *Ciona intestinalis* (selbststeril) wurden elektronenmikroskopisch untersucht. Quantitative, nicht aber qualitative Unterschiede wurden in der Feinstruktur der Eizellen der beiden Arten gefunden.

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The Occurrence of Nerve Growth Factor in Teleost Fishes

BUEKER[1] investigating the effects of implanted tumors on the development of the nervous system of the chick embryo found that sarcoma 180 caused enlargement (hypertrophy and hyperplasia) of the spinal ganglia in the segments adjacent to it. LEVI-MONTALCINI and HAMBURGER[2] found that the sarcoma stimulated growth of the sympathetic ganglia to a greater extent than the spinal ganglia. Subsequently these workers isolated a 'nerve growth stimulating factor' from the sarcoma and devised an in vitro bioassay in which a spinal ganglion from a 7- or 8-day chick embryo was placed in hanging drop culture containing tissue culture medium, rooster plasma and the fraction to be assayed[3]. At optimum concentrations of the nerve growth factor (NGF) a dense