Oocyte activation in *Mytilus edulis* by *Crassostrea gigas* spermatozoa

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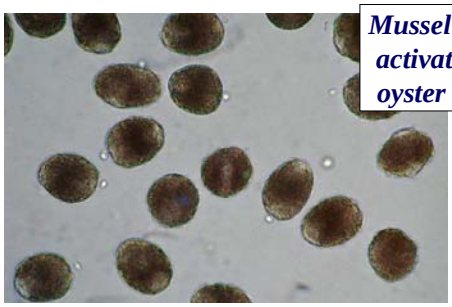
Background

Interspecific fertilisation experiments have rarely been made above the genus level in marine molluscs, probably because interspecific barrier mechanisms are known to act between gametes at the intrageneric level in some groups[1,2] including *Mytilus*[3].

Our study was initially motivated by the practical needs of hatchery mussel production but the activation of mussel oocytes by oyster sperm has further reaching implications both for genetic improvement techniques and ecology.

Hatchery production of the blue mussel usually requires controlled induction of spawning as gonad stripping has not given satisfactory results in this species. On a batch of spawned mussel oocytes we tested the effects of spawned and stripped mussel spermatozoa and stripped oyster spermatozoa.

Although we had initially considered oyster sperm as a potential spawning stimulant[4] for mussels, we first wanted to test for direct effects that it could have on their oocytes.



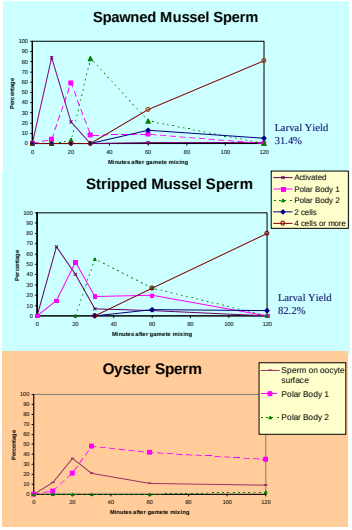
Mussel oocyte activation by oyster sperm

Surprisingly, contact with oyster sperm activates mussel oocytes. This photo was taken at 3h, though the stage illustrated is normally reached at 20-30 minutes with mussel sperm. Cleavage occured in a small number of embryos however, and the 4-cell stage was observed (below).

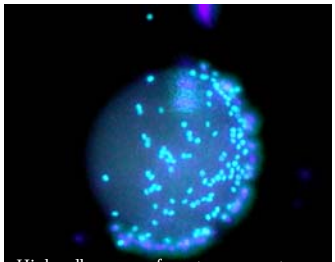
Comparative embryo development

Comparison of mussel sperm (spawned and stripped) and oyster sperm (stripped)

1000 spz/oocyte



Spawned and stripped mussel sperm gave similar results. **Oyster** sperm activated a smaller proportion of oocytes, there was slower development and no larvae in this trial.



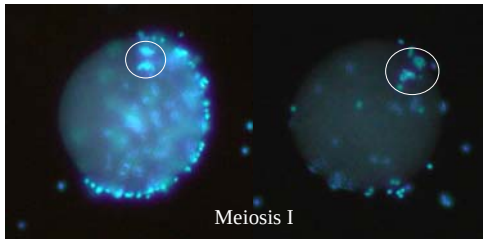
High adherence of oyster spermatozoa to mussel oocytes. No decondensation or penetration. (Hoescht staining[5])

Observations in follow-up experiments

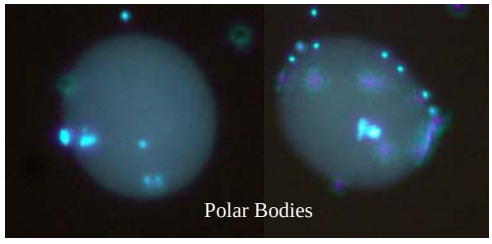
- Later observation at 4 and 15 hours revealed multicellular embryos in oyster sperm groups.
- With a greater oyster sperm concentration (~10x), the number of oocytes with sperm attached was improved to 100% by 10 mins but there was less activation (13% max) and this was further delayed.

Ploidy restoration

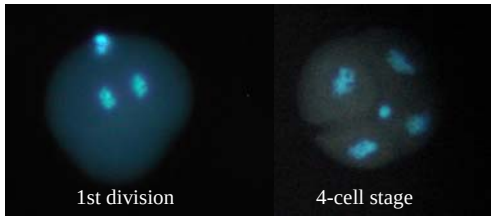
Following the embryo development timing, we made 300µM 6-DMAP treatments (15 or 20 min) to block polar body expulsion. However, only dead or deformed larvae were recovered after 2 days.



Meiosis I



Polar Bodies



1st division

4-cell stage

Potential mechanisms

Activation is mediated by a chemical reaction occurring when the sperm head fuses to the oocyte surface leading to ionic fluxes and charges within the cell[6,7]. The addition of certain ions or serotonin can bring about activation[8,9,10], and some of these substances are also spawning stimulants. It is possible that the presence of oyster sperm changes the chemical environment causing similar parthenogenic activation or that it contains substances similar to those in mussel sperm. Spontaneous activation has also been observed without sperm in Eastern oyster[11], indicating a certain sensitivity of such mechanisms. The possibility of hermaphroditism (and thus self-fertilisation), is unlikely in our trials as control oocytes with no sperm were not activated at all.

Both oyster[12] and mussel sperm[13,14] have been seen to activate echinoderm eggs, to penetrate and develop pronuclei. This is a still larger phyletic gap, and the development of echinoderm embryos is profoundly different.

Crosses between oyster oocytes and mussel sperm produced no similar effects in oyster, the effect of oyster sperm on other species remains to be tested.

Aquacultural and Ecological implications

- Activation without fertilisation should lead to haploids[15]
  - Combining oyster sperm and polar body blocking treatments could create gynogens, but in low numbers because of low activation %.
- Absence of previous intergeneric experimental observations:
  - Are activation and recognition mechanisms separate?
- Loss of mussel gametes by interspecific interference
  - Percentage affected is low but the 'competitive effect' is in favour of oysters.
  - The breeding seasons of the species do not normally overlap, though climatic change and invasiveness[16] of *C. gigas* could bring them into contact more frequently.

Literature cited: 1. Swanson & Vacquier 1995, PNAS 92:4957-4961; 2. Lu et al. 2006, JSR 25:509-514; 3. Riginos & MacDonald 2003, Mol Biol Evol 20:200-207; 4. Rice et al. 2002, JSR 21: 715-718; 5. Dubé et al. 1985, Cell 40:657-666; 6. Colas & Dubé 1998, Semin Cell Dev Biol 5:535-537; 7. Tosti &Boni 2004, Hum Rep Upd 10:53-65; 8. Hollingsworth 1941, Bio Bull 81:261-276; 9. Allen, 1953, Bio Bull 105 213-239; 10. Yi et al. 2002, J. Cell Sci 115:311-320; 11. Stiles & Chroromanski 2002, ICES CM U11; 12.Osanai & Kyozuka 1982, Gamete Research 5: 49-60; 13. Longo 1977, J Cell Biol 73:14-26; 14. Kenzi & Kyozuka 1984, Zoological Science 1:245-254; 15. Fairbrother 1994, Aquaculture 126: 25-34; 16. Nehring 2003, Aliens 17:02-21.

BLUE SEED Technology development for a reliable supply of high quality seed in blue mussel farming. 6th Framework Program EC Craft Project