Physiological and molecular basis of gametogenesis in triploid Pacific oysters, *Crassostrea gigas*.

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Introduction:
Genetic improvement of *C. gigas* through triploidization:

- reduced (but variable) gonad development
- **better growth** (Gouletquer et al., 1996; Nell, 2002) and **survival** (Degrémont, 2003)
- limited propagation in the wild
Triploidy induction in *C. gigas*

**« Normal » diploid fecondation**

Oocyte 2 x 2n → Reduced 1n Oocyte + 1n Spermatozoa

PB1 2n → PB2 1n

**« Chemical » induction of triploidy (inhibition of the 2nd PB release)**

(Beaumont and Fairbrother, 1978):

Oocyte 2 x 2n → Unreduced 2n Oocyte + 1n Spermatozoa

PB1 2n

**« Natural » induction of triploidy (2n x 4n cross)** (Guo and Allen, 1994):

Oocyte 2 x 2n → Reduced 1n Oocyte + 2n Spermatozoa

PB1 2n → PB2 1n
Part one: Comparative histological study of reproduction in 2n, 3n c & 3n n spat
Material and Methods

BREEDERS

STUDIED OYSTERS

Ploidy (flow cytometry)

Reproductive Effort (histology)

Chemical induction

5 months in nursery

M 4n

F 2n

M 2n

3n n

2n

3n c

OYSTERS
Quantitative analysis of reproductive effort

Histological slides:
- Davidson Fixator
- Hematoxilin-eosin staining
- 3 cross-sections of the visceral mass /ind.

30 ind./group

Gonad occupation = gonad surface / total surface
High individual variation
Reduced gonad development in 3n oysters (≈ -50%)
No difference between 3n n and 3n c

<table>
<thead>
<tr>
<th>Group</th>
<th>Gonad occupation</th>
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<tbody>
<tr>
<td>2n</td>
<td>29.18% ± 10.61</td>
</tr>
<tr>
<td>3n n</td>
<td>14.57% ± 7.27</td>
</tr>
<tr>
<td>3n c</td>
<td>15.24% ± 8.11</td>
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</tbody>
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Results

Retardation in gonad development of triploids

- Some 3n individuals showed active gametogenesis (i.e. stage 2)

Stage 0: proliferation of germ cells
Stage 1: proliferation of gonias
Stage 2: maturation of germ cells
Stage 3: maturity
Stage 4: spawning and resorption
Conclusions (part one)

- Altered gonadogenesis in 3n compared to 2n oysters:
  - reduction of gonad development
  - retardation of gonad development
  - … but normal pre-meiotic proliferation of the germ cells

- Triploid oysters are not fully sterile, even at the spat stage (study on 1 year-old oysters currently in progress)
- No difference between 3n n and 3n c oysters

Altered gonadogenesis in 3n compared to 2n oysters: \(\text{correlated} \quad ?\)
Part two: Study of the expression of the gene Oyster Vasa-like (OyvIg) in 2n & 3n spat
**Oyvlg: specific marker of the germline**  
(Fabioux et al, 2004)

- ATP-dependent RNA helicase DEAD-Box protein
- Vasa-expression restricted to Germline lineage:
  - in the embryo (ontogenesis)
  - in the adult gonad (proliferation and differentiation of germ cells)

**Embryos**  
*in toto hybridization qPCR*

**Adults**  
*in situ hybridization qPCR*
**Material and Methods**

**BREEDERS**
- M 4n
- F 2n
- M 2n

**STUDIED OYSTERS**
- 3n n
- 2n

3 sampling dates:
(December / February / April 2006)

Control by flow-cytometry
- q-PCR
- ISH (localisation)

2-6 months in the field

Ploidy
Expression of Oyvlg
• Mean level of expression is higher in 3n than in 2n oysters (x3.6): the expression of Oyvgl is additive in triploids but dosage effect varies over time

• Variation is higher between triploids than between diploids
Variation of Oyvlg expression between 3n
Results / Perspectives of part two:

• Paradoxal « dosage effect »: the mean reproductive effort is lower in 3n vs. 2n, but the expression of Oyvlg in additive

• Can Oyvlg be used as a predictor of R.E. within triploids? (temporal study in progress)

• At what stage is gonadogenesis affected by triploidy? (after stage 1)

• Which other gene might better follow and predict gonadogenesis in triploids? (TGFβ, vitellogenine…)
Expression of TGFβ increases with maturity stage, it is maximal for ripe oysters (E. Fleury, unpublished datas)
Conclusion

Triploid oysters are not fully sterile:

- Mitotic proliferation of germ stem cells (and gonias?) seems to occur normally

- Their reproduction process is however altered at a later stage but some individuals showed active gametogenesis

- Environmental factors (food, temperature..) are likely to strongly influence reproductive effort in triploids (Shpigel et al., 1998)

- Previously published results about reproductive allocation in diploid oysters (Ernande et al., 2004; Samain et al., in press) supports the possible existence of a genetic basis of the variation of gonadogenesis between triploids (currently under study)