

Study of the reproductive potential of triploid Pacific oysters (*Crassostrea gigas*, Thunberg)

J. Normand, F. Cornette, C. Ledu & P. Boudry

Ifremer - Laboratoire de Génétique et Pathologie, Mus du Loup, 17390 La Tremblade - France

Introduction

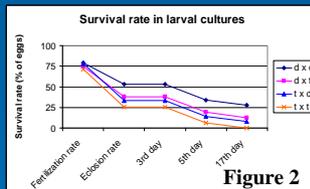
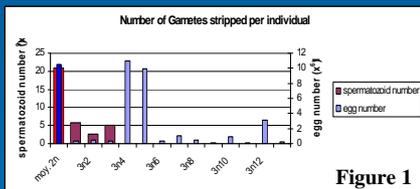
Triploidy (i.e. the addition of a haploid genome to a diploid genome) leads to faster growth, better survival and partial sterility in oysters.

Despite their relative sterility, previous studies reported some gonadic maturation in triploid oysters (Gong *et al.*, 2004). This study was conducted to examine (1) the reproductive potential of triploids (compared to diploids) (2) early development and ploidy in their progeny

Methods:

- Ploidy analysis of the parental oysters, embryos and larvae by flow cytometry
- Early embryonic development by epi-fluorescence observations
- Fecundity by image analysis
- Eclosion rate, fertilisation rate and larval mortalities
- Course of egg meiosis and embryonic development

Reproductive potential of triploid oysters



- The number of fully matured gametes is generally much lower in triploids but it varies between individuals (Figure 1),
- Fertilisation capacity of gametes of triploids is equivalent to those of diploids,
- Larvae from triploid parents are significantly less viable than those from diploid oysters (Figure 2).

Meiosis of female gametes and early embryonic development

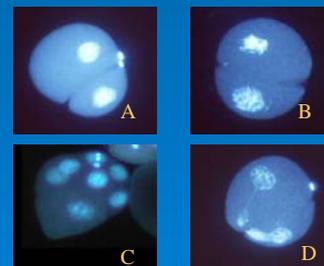
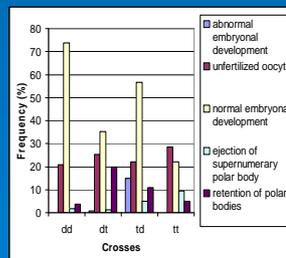
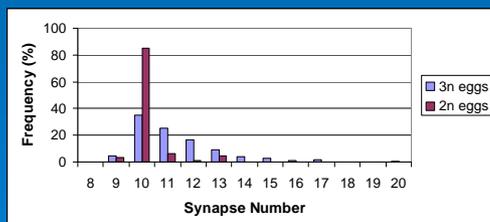


Figure 3: Chromosomal association at Meiosis:

- A. Diploid egg at P1 with regular chromosomal association (showing 10 bivalents)
- B. Triploid egg at P1 showing irregular chromosomal association with 12 synapsis (probably 8 trivalents, 2 bivalents, 2 univalents)

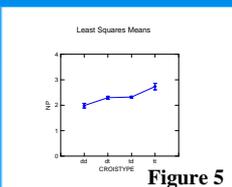
Figure 4 : Classification of embryonic development :

- A. Normal embryonic development: 2 nucleus, 2 polar bodies (PB)
- B. Embryo showing a retention of its polar bodies: 2 Nucleus, 0 PB
- C. Embryo showing supernumerary polar body ejection: 8 Nucleus, 3PB
- D. Abnormal embryonic development

• Association of chromosomes at meiosis is slower and more irregular in triploid than in diploid oocytes (Figure 3). This might lead to high aneuploids rates in female gametes of triploids, which carry to high variability for the larval ploidy level.

• Structural (epigenetic) characteristics of the gametes from triploid oysters cause considerable abnormalities during the firsts stages of embryonic development (Figure 4). This discrepancies can be lethal (bad condensation lead to aberrant cellular division) or drive to strong distortions of the ploidy of the embryos (e.g. retention of polar body induce polyploidy).

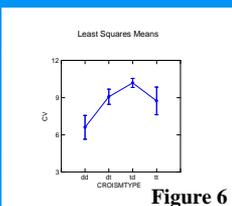
Ploidy in the progeny of triploids



| Crosses | Ploidy Level | Standard deviation |
|---------|--------------|--------------------|
| d x d | 1,98 | 0,07 |
| d x t | 2,29 | 0,14 |
| t x d | 2,32 | 0,33 |
| t x t | 2,73 | 0,12 |

Relation between ploidy level and crosses:

Anova: F = 8,886 et P < 0,0001



| Crosses | Coefficient of Variation | Standard deviation |
|---------|--------------------------|--------------------|
| d x d | 6,61 | 2,45 |
| d x t | 9,08 | 1,45 |
| t x d | 10,18 | 3,05 |
| t x t | 8,74 | 2,92 |

Relation between the variability of the ploidy level (C.V.) and crosses:

Anova: F = 4,513 et P = 0,005

• Ploidy level of larvae from diploid-triploid crosses is comprised between 2n and 3n (Figure 5) and is very variable (Figure 6).

• This suggests that chromosome segregation at meiosis must be aleatory (gametes from triploid parents should display a ploidy level about 1.5 n). This assumption is corroborated by previous studies (Guo and Allen, 1994; Gong *et al.*, 2004).

• The resulting larvae are diploid, polyploid or aneuploid, progeny from triploid females showing the highest rates of polyploid progeny.

Conclusion

Triploids oysters are not completely sterile. They exhibit a reproductive potential lower than those of diploids but triploidy cannot ensure a total genetical containment for these individuals (whereas no polyploids have ever been found in natural populations).

Progeny from triploid oysters are aneuploids, diploids, or polyploids. Whereas aneuploids exhibits low viability, polyploids -if fertile- could favor the production of triploids in subsequent generations.

Literature cited:

Gong N, Yang H, Zhang G, Landau B J, Guo X. (2004) Chromosome inheritance in triploid Pacific oyster *Crassostrea gigas* Thunberg, *Heredity*, 93: 408-415.
Guo X et Allen Jr SK. (1994). Reproductive potential and genetics of triploid oyster, *Crassostrea gigas* (Thunberg), *Biol Bull*, 187: 309-318

