Fluorescence in situ hybridization (FISH) of rDNA genes (5S and 18-5.8-28S) as a tool for chromosomal tagging and to assess the occurrence of somatic and sperm aneuploidy in Pacific oyster *Crassostrea gigas*.

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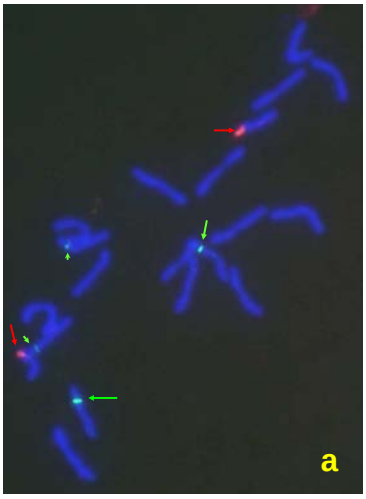
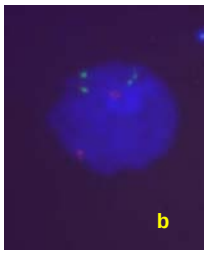
Introduction

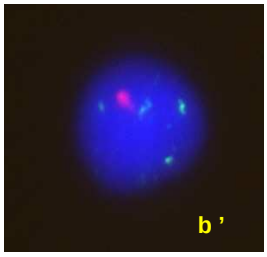
Aneuploidy (or alteration of chromosome number) has been frequently described in diploid, triploid and tetraploid *C. gigas*. In diploid Pacific oysters, aneuploidy affects 1 to 3 chromosomes in up to 35% of the somatic cells and is positively correlated with inferior growth of the animal and thus could be associated with significant economic loss in this major aquaculture species. In polyploid Pacific oysters, aneuploidy was shown to be more heavily frequent and is linked to reversion to lower ploidy levels (mozaics) and is at the origin of aberrant gametes formation. In this context, identification of individual chromosomes involved in aneuploidy events both in somatic and germinal cells would be an important step towards the study of the exact occurrence of this phenomenon and would provide means of screening genitors and gametes in order to establish aneuploid-free tetraploid stocks dedicated to breeding programs and/or serving as male genitors in hatchery-based triploid production.

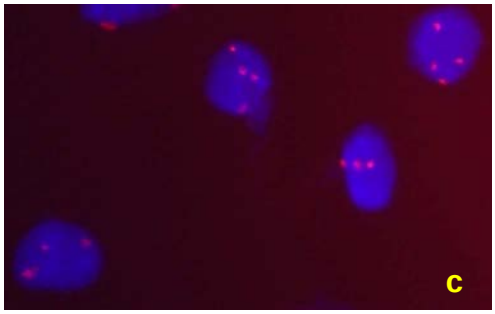
Methods

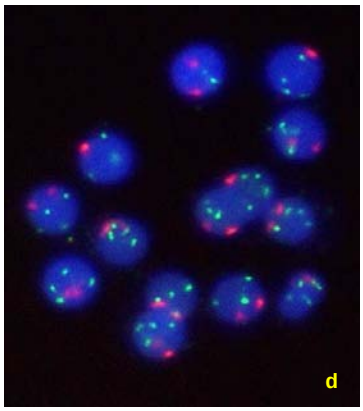
Metaphase plates and nuclei preparations were made from fast growing larvae or from adult Pacific oysters according to standard protocols. Sperm collection and decondensation were done as described in Vidal et al. (1993) with modifications concerning the duration of DDT treatment. *C. gigas* 5S rDNA was obtained by PCR amplification of total genomic DNA in the presence of 5S-specific primers described by Cros et al. (2005). The obtained PCR fragments were cloned and labelled by digoxigenin-11-dUTP by means of nick translation. For the 18S-5.8S-25S rDNA probe we used two recombinant plasmids (HM123, HM456), containing fragments of *Xenopus laevis* rDNA (Meunier-Rotival et al. 1979) together in a probe mix. This probe mix was labelled by means of nick translation by direct incorporation of tetramethylrhodamine-6-dUTP. FISH procedure was then performed as described in Benabdelmouna et al. (2001).











Results and Conclusion

• Fluorescent in situ hybridization (FISH) of rDNA genes (5S and 18-5.8-28S) onto somatic metaphasic plates was successfully used to tag three out of ten chromosomes (Fig a). Red labelled 18-5.8-28S rDNA probe showed clear signals onto the terminal part of the chromosome pair 10 (red arrows). Green labelled 5S rDNA probe revealed two signals in interstitial position onto two different chromosome pairs : a major site located onto chromosome 4 (green arrows) and a minor site located onto chromosome 5 (green arrowheads).

• The same probes when applied onto nuclei preparations from gill tissues showed i) typical pattern of diploïd nucleus (2n = 20) with two red and four green foci (b) and ii) patern typical of aneuploïd (2n = 19) nucleus with four green signals and only one red signal corresponding to the loss of one of the two chromosomes 10 (b').

• Mozaic status can also be evidenced by FISH using the same rDNA probes. Figure c shows nuclei from tetraploïd-triploïd mozaic labelled with 18-5.8-28S rDNA probe.

• Sperm decondensation was effective after treatment with 5 mM DDT during 1 min. Sperm-FISH with the two rDNA probes appears to be also effective when used onto decondensed tetraploïd sperm nuclei. In addition to typical patterns of tetraploïd sperm nuclei with four green and two red signals, some sperm nuclei appear to be aneuploïd by chromosome loss (d).

These preliminary results open the way for a reliable and fast study of the mosaicizm and aneuploidy in both somatic and sperm cells and further analyses are currently in progress in order to obtain additional chromosomal tags (BAC clones, SSR, EST…) and to accelerate the procedure of aneuploid detection by the mean of PRINS (primed in situ labelling) -based approaches.

References

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