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A 'G' chromosome banding study of three cupped oyster species: *Crassostrea gigas*, *Crassostrea angulata* and *Crassostrea virginica* (Mollusca: Bivalvia)

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Abstract – The G-banding technique was performed on chromosomes from gill tissue of three cupped oyster species: *Crassostrea gigas, Crassostrea angulata* and *Crassostrea virginica*. Identification of the ten individual chromosome pairs was obtained. Comparative analysis of G-banded karyotypes of the three species showed that their banding patterns generally resembled each other, with chromosome pair 3 being similar in all three species. However, differences from one species to another were also observed. The G-banding pattern highlighted greater similarities between *C. gigas* and *C. angulata* than between these two species and *C. virginica*, thus providing an additional argument for genetic divergence between these two evolutionary lineages. *C. gigas* and *C. angulata* showed a different G-banding patterns on the two arms of chromosome pair 7, which agrees with their taxonomic separation. The application of this banding technique offers a new approach to specific problems in oyster taxonomy and genetics. © Inra/Elsevier, Paris

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Résumé – Étude du marquage chromosomique en bandes G chez trois espèces d'huîtres creuses : *Crassostrea gigas*, *Crassostrea angulata* et *Crassostrea virginica*. Le marquage chromosomique en bandes G a été réalisé sur des chromosomes obtenus à partir de tissu branchial de trois espèces d'huîtres creuses, *Crassostrea gigas*, *Crassostrea angulata* et *Crassostrea virginica*, et a permis l'identification des dix paires de chromosomes de ces espèces. L'analyse comparée des caryotypes marqués en bandes G a montré que les principales bandes G présentaient un modèle proche chez les trois espèces, la paire de chromosome 3 étant identique. Cependant quelques différences ont pu être observées. Les caryotypes de *C. gigas* et *C. angulata* ont révélé plus de similitudes entre eux qu'avec celui de *C. virginica*. Ceci apporte un argument supplémentaire à la divergence génétique entre ces deux lignées évolutives. *C. gigas* et *C. angulata* montrent un marquage en bandes G différent sur les deux bras de la paire du chromosome 7, ce qui corrobore leur séparation taxinomique. L'application de cette technique apporte une nouvelle approche pour la taxinomie et la génétique des huîtres.

chromosome / bandes G / *Crassostrea gigas* / *Crassostrea angulata* / *Crassostrea virginica*

1. INTRODUCTION

Cytogenetic investigations in oysters were first mainly concerned with data on chromosome number and gross morphology (e.g. [1, 22]). Later, morphometric analyses of karyotypes provided the characterisation of chromosome morphology based on centromeric position (e.g. [13, 17, 31, 37]). These studies showed that oyster karyotypes were symmetrical and interspecific differences consisted in the occurrence and differing proportions of metacentric and submetacentric chromosomes [18, 21].

The application of differential staining techniques, such as Ag-NORs for nucleolar organiser regions and C-banding for constitutive heterochromatin allowed the identification of some specific chromosome pairs in the karyotypes of oyster species [14–16, 18, 19, 39]. More recent techniques, such as fluorescent in situ hybridization, have been tested in *Crassostrea gigas* [5, 9], and others, such as fluochrome staining and restriction endonuclease treatment, have been carried out in other bivalve species [25, 26]. But although the data obtained using these differential staining techniques provide a better knowledge of the karyotypes of bivalve species, they do not allow the identification of all individual chromosomes.

The G-banding technique, defined as a system of alternating dark and light bands throughout the length of the euchromatic parts of chromosomes [35], allows the identification of each individual chromosome which enables one to prepare precise and detailed karyotypes. This technique has been routinely used in vertebrate cytogenetics, especially in mammals (e.g. [11, 12, 34, 41]). Only a few studies have focused on lower vertebrates, such as fishes (e.g. [2, 6, 7, 24]) and on invertebrates such as insects (e.g. [4, 23, 33]). Among bivalves, G-banding patterns have been attempted in *Mytilus* [25, 27] and in the oyster *Crassostrea virginica* [32].

In this study, G-banding patterns are described in three cupped oysters: *Crassostrea gigas* (Thunberg), the Pacific oyster, *Crassostrea angulata* (Lamark), the Portuguese oyster and *Crassostrea virginica* (Gmelin), the Eastern American oyster.

2. MATERIALS AND METHODS

2.1. Biological material

Specimens of each taxon were reared at the Ifremer hatchery in La Tremblade (Charente-Maritime, France). Specimens of *Crassostrea gigas* were collected from the Seudre estuary, where this species was introduced from Japan [8] and is currently farmed on a large scale. Specimens of *Crassostrea angulata* were collected in Setubal bay (Portugal), then acclimated at the Ifremer hatchery. Their taxonomic status was assessed using mitochondrial DNA markers as described in Boudry et al. [3]. Specimens of *Crassostrea virginica* were imported from a wild population located in Shippagan, New Brunswick (Canada) and acclimated at the Ifremer hatchery. These oysters were maintained in common quarantine facilities until reproduction, and their progenies were sampled for chromosome analysis.

2.2. Chromosome preparation

Whole juvenile animals were incubated for 7–9 h in a 0.005 % solution of colchicine in seawater. Because cell cultures are not yet available for molluscs, we used growing somatic tissues such as gills as a source of mitoses. After dissection, gills were treated for 30 min in 0.9 % sodium citrate. The material was fixed in a freshly prepared mixture of absolute alcohol and acetic acid (3:1) with three changes of 20 min each. Slide preparations were made from pieces of gill tissue from each individual, using an air drying technique [38].

2.3. G-banding

G-banding was performed by the ASG method (acetic/saline/Giemsa) after Sumner et al. [36]. Chromosome preparations were treated for 1 h at 60 °C in 2 × SSC (0.3 M sodium chloride: 0.03 M trisodium citrate). After rinsing in distilled water, the slides were stained with 2 % Giemsa-stain in phosphate buffer, pH 6.8 for 90 min. Best results were obtained by banding within 5–10 days of chromosome preparation.

Photographs of G-banded metaphases were taken with a Zeiss III photomicroscope. Karyotypes were made on the basis of length, centromeric position and banding pattern. Because we were working with somatic tissues, we had to use many animals to obtain a sufficient number of mitoses. Moreover, the latter showed different levels of condensation making the number of cells we could work on even smaller. Thus, in total, 18 G-banded karyotypes were examined in *C. gigas*, 20 in *C. angulata* and 21 in *C. virginica*.

3. RESULTS

Establishing a repetitive G-banding pattern requires a similar degree of condensation of chromosomes to be compared. Although a large number of metaphases was observed, we selected only those with similar degrees of condensation for interpretation.

Figure 1 presents an example of a G-banded metaphase of one of the three species studied, *C. gigas*. In *figure 2*, haploid G-banded karyotypes are shown to facilitate comparison of homologous chromosome pairs between the three species studied. The karyotype of *C. gigas* consists of ten metacentric chromosomes, that of *C. angulata* has nine metacentric and one submetacentric (no. 8) and the karyotype of *C. virginica* includes eight metacentric and two submetacentric (nos 4 and 8) chromosomes [18].

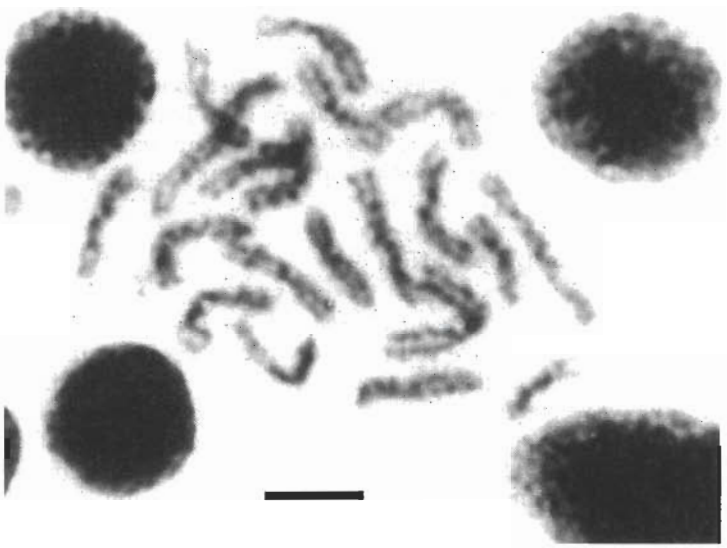

Figure 1. G-banded metaphase of *Crassotrea gigas*. Bar = 5 μm.

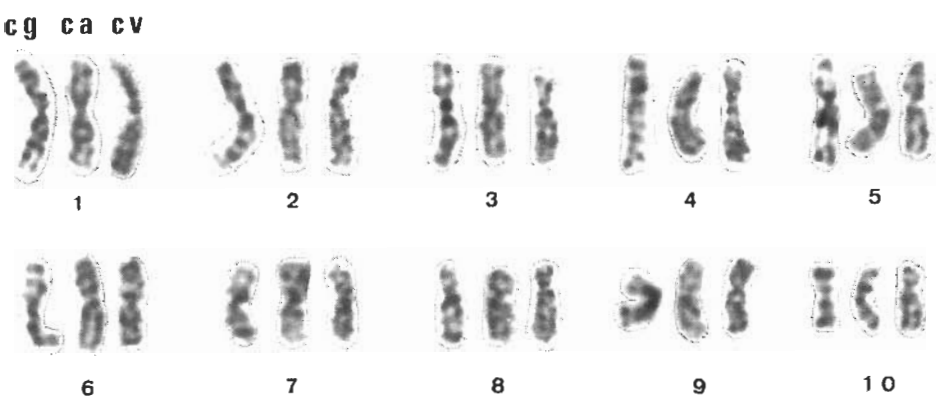

Figure 2. Haploid G-banded karyotypes. cg: *Crassostrea gigas*, ca: *Crassostrea angulata*, cv: *Crassostrea virginica*. Bar = 5 μm.

Figure 3 gives a schematic representation of the G-banding patterns obtained for the three species. Owing to differences in condensation of chromosomes only

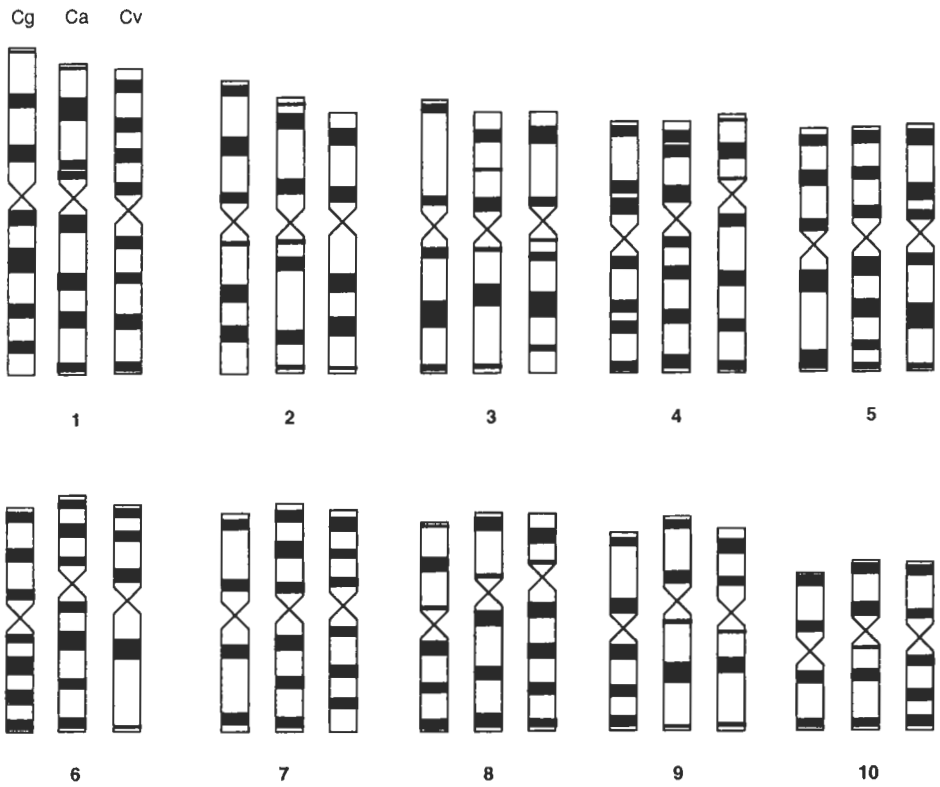

Figure 3. Comparative ideogram of G-banding patterns in *Crassostrea gigas* (cg), *Crassostrea angulata* (ca) and *Crassostrea virginica* (cv)

major G-bands were used to compare the three species, with an emphasis on their number rather than their position.

Chromosome 1: on the short arm, both *C. gigas* and *C. angulata* show two major bands, while in *C. virginica* four major bands are present. All three species present four bands on the long arm.

Chromosome 2: on the short arm, *C. gigas* shows three bands while in *C. angulata* and *C. virginica* two major bands are present. On the long arm, the three species are characterised by two major bands.

Chromosome 3: the pattern is very similar across the three species, being characterised by two major bands at the extremities of the short arm and one large band at the centre of the long arm.

Chromosome 4: on the short arm, in *C. gigas* and in *C. angulata*, the bands are observed in subtelomeric and near centromeric positions while *C. virginica* is characterised by one centrally located major band. Four bands are located on the long arm of the three species differing slightly in their position, which was probably caused by the different degrees of condensation of the chromosomes.

Chromosome 5: the three species present three bands on the short arm. On the long arm, the G-banding pattern is different between the three species.

Chromosome 6: on the short arm, three bands are observed in the three species. On the long arm, *C. gigas* and *C. angulata* show four successive bands, while in *C. virginica* the banding pattern is characterised by the presence of only one major median band.

Chromosome 7: *C. gigas* shows two bands on the short arm and two on the long arm. In *C. virginica* and *C. angulata*, three bands are seen on both the short arm and the long arm. These differ slightly in intensity and position.

Chromosome 8: the short arm in all three species is characterised by the presence of one major band. There are three major bands on the long arm of *C. gigas* and *C. angulata* and four bands in *C. virginica*.

Chromosome 9: on the short arm, two bands are seen in the three species. On the long arm, *C. gigas* presents three near equivalent bands differing from *C. angulata* and *C. virginica* which are characterised by the presence of one major band.

Chromosome 10: the three species are characterised by two bands at the extremities of the short arm. Two major bands are seen on the long arm of *C. gigas* and *C. angulata* while three are present in *C. virginica*.

4. DISCUSSION

The application of G-banding to three species of oysters: *C. gigas*, *C. angulata* and *C. virginica*, allowed individual identification of the chromosomes which makes it possible to prepare accurate karyotypes and describe the respective idiograms.

Comparison with previous G-banding analysis of *C. virginica* [32] showed that the number of G-bands identified as black bands (i.e. the most distinct) was similar in chromosome pairs 5 and 9 but quite different in the remaining chromosome pairs. This can be explained by i) different karyotypes of the oyster populations studied (6 m-4 sm in Tabasco, Mexico; 8 m-2 sm in our population), ii) different techniques used, iii) different condensation of chromosomes.

The major G-bands of the three *Crassostrea* species studied here show a similar pattern on the whole chromosome 3, on the short arms of chromosomes 5, 6, 8, 9 and 10 and on the long arms of chromosomes 1, 2 and 4. *C. gigas* and *C. angulata* present additional similarities on the short arms of chromosomes 1 and 4, and on the long arms of chromosomes 6, 8 and 10. These two taxa, often considered as the same species [28], have been differentiated by mitochondrial DNA analysis [3, 30] and karyotype analysis [18]. G-banding highlights similarities between these two taxa, except for chromosome 7 where both arms are shown to be different. This difference corroborates their taxonomic separation. *C. angulata* and *C. virginica* also display additional similarities in the number of major G-bands on the whole chromosome 7, the short arm of chromosome 2 and the long arm of chromosome 9, but they differ on the short arms of chromosomes 1 and 4, and on the long arms of chromosomes 5, 6, 8 and 10. Karyological differences between these two species have been previously observed [18]. *C. virginica* contrasts with *C. gigas* on the short arms of chromosomes 1, 2, 4 and 7, and on the long arms of chromosomes 5, 6, 7, 8, 9 and 10. Genetic divergence between *C. gigas* and *C. virginica* has been demonstrated by molecular phylogenies [20, 29] and by karyotype analysis [18]. The

differences in G-banding pattern between *C. gigas* and *C virginica* substantiate their genetic difference

Therefore, from the analysis of the banding karyotypes of the three species, we can conclude that they generally resemble each other with chromosome pair 3 being similar in all the three species. However, differences were observed from one species to another, showing that there is a higher resemblance between the banded karyotypes of *C. gigas* and *C. angulata* than between these two species and *C. virginica.*

Because of the economic and ecological importance of oysters, genetic investigations are of special interest. In this respect, the identification of structural chromosomal features could be very useful in gene mapping, hybrid breeding or stock conservation programmes. The individual identification of the chromosomes by G-banding will provide a better knowledge of the aneuploidy phenomenon reported in oysters (e.g. [40]) by identifying missing chromosomes. Similarly, G-banding could also provide a very valuable technique for chromosome segregation studies on triploid and tetraploid oysters [10]. The applications of G chromosome banding are therefore numerous and represent a useful new tool in oyster genetics.

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