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Experimental evidence for a genetic basis to differences in aneuploidy in the Pacific oyster (*Crassostrea gigas*)

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Abstract − Aneuploidy has been previously reported in wild and cultivated Pacific oysters, *Crassostrea gigas*, and has been shown to be negatively correlated with growth. This is especially important since high variability of growth rate is one of the major problems in the aquacultural production of this species. The existence of a genetic basis for the observed differences in aneuploidy was first investigated through the comparative study of six full-sib families, with mean individual weights ranging from 0.59 to 1.49 g. The slowest growing family was found to have the highest level of aneuploidy. Significant differences in aneuploidy were also found among families when individuals with the same growth rate were sampled. This supports the hypothesis of the existence of a genetic basis for the control of aneuploidy level. Additionally, the possible inheritance of the level of aneuploidy was studied in four full-sib progenies originating from crosses within and between two different populations with contrasting levels of aneuploidy. The limited number of parental oysters ($N = 6$) in which the scoring of aneuploidy was possible did not allow demonstration of the inheritance of the level of aneuploidy. However, a genetic difference in aneuploidy could be attributed to the origin of the parental populations. As in the first experiment, significant differences in aneuploidy were observed between progenies when sampling individuals of the same weight. Thus, the results of our study of full-sib progenies of *C. gigas* lend support to the hypothesis of a genetic basis for the level of aneuploidy. © 2001 Ifremer/CNRS/Inra/IRD/Cemagref/Éditions scientifiques et médicales Elsevier SAS

aneuploidy / *Crassostrea gigas* / full-sib families / genetics / growth / oyster

Résumé − Preuve expérimentale d'une base génétique pour les différences de taux d'aneuploïdie chez l'huître creuse (*Crassostrea gigas*). Des travaux antérieurs ont montré, chez des huîtres creuses *Crassostrea gigas* sauvages et cultivées, la présence d'aneuploïdie et sa relation négative avec la croissance. En effet, la grande variabilité de croissance est un problème important pour la production aquacole de cette espèce. L'existence d'une base génétique pour les différences d'aneuploïdie est tout d'abord estimée par l'étude comparative de six familles dites de « plein-frères », dont le poids moyen des individus varie de 0,59 à 1,49 g. Le taux d'aneuploïdie de la famille montrant la croissance la plus lente s'avère le plus élevé. Des différences significatives d'aneuploïdie sont également notées parmi les familles où les individus échantillonnés ont le même taux de croissance. Ceci est en faveur de l'hypothèse de l'existence d'une base génétique pour le contrôle du taux d'aneuploïdie. De plus, l'éventuelle transmission du taux d'aneuploïdie est étudiée chez quatre familles de plein-frères, issues de croisements réalisés au sein et entre deux populations ayant montré des taux d'aneuploïdie contrastés. Le nombre limité ($N = 6$) de parents, pour lesquels il a été possible d'évaluer le taux d'aneuploïdie, ne permet pas de démontrer l'hérédité de ce phénomène. Cependant, le caractère génétique associé à l'origine des parents peut être suggéré. Comme dans la première expérience, des différences significatives de taux d'aneuploïdie sont observées parmi les descendances où les individus échantillonnés avaient un même poids. Nos résultats sur l'étude des familles de plein-frères de la deuxième génération permettent donc de proposer une base génétique du phénomène d'aneuploïdie. © 2001 Ifremer/CNRS/Inra/IRD/Cemagref/Éditions scientifiques et médicales Elsevier SAS

aneuploïdie / *Crassostrea gigas* / famille de plein-frères / génétique / croissance / huître

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1. INTRODUCTION

The high variability of growth rate is one of the major problems for the aquacultural production of the Pacific oyster, *Crassostrea gigas*. Environmental conditions vary tremendously from one area to another and, more locally, competition for food or space can be responsible for part of the observed variation. However, part of this variation in growth rate is also assumed to have a genetic basis, as selective breeding for a higher growth has been shown to be effective (Sheridan, 1997; Nell et al., 2000; Ward et al., 2000).

The occurrence of aneuploidy (i.e. cytogenetic abnormality characterised by the alteration of the normal diploid chromosome number, 2n = 20 in *Crassostrea gigas*) is now well documented in this species (Thiriot-Quiévreux, 1986; Thiriot-Quiévreux et al., 1988, 1992; Guo and Allen, 1994; Zouros et al., 1996; Wang et al., 1999). It is of special importance, as a negative relationship between growth rate and aneuploidy has been consistently observed in this species in thirteen populations studied over 10 years (Leitão et al., 2001). The causes of aneuploidy are still unclear. In human and some plant species, there is evidence that genetic factors are involved in the origin of the aneuploidy, where genes have been found to be responsible for the non-disjunction phenomenon (Bond and Chandley, 1983; Verma, 1990).

In the present paper, the genetic basis of the aneuploidy phenomenon is investigated in the Pacific oyster, *Crassostrea gigas*, through the comparison of aneuploidy in hatchery-produced full-sib families chosen for their differential growth performance, and in hatchery-produced full-sib progenies of parents of known aneuploidy level.

2. MATERIAL AND METHODS

2.1. Origin of the studied oysters

2.1.1. Full-sib families chosen for their growth performances

A nested half-sib mating design was used to produce full-sib families of oysters during March 1998 (IFREMER, La Tremblade, France). Six males were crossed with 24 females producing 6 half-sib families (to test for sire effects), each of them containing 4 full-sib families (to test for dam within sire effect). Twenty-one full-sib progenies were obtained out of these 24 crosses. After 5 months of rearing under standard uniform conditions (IFREMER, Bouin, France), 100 animals per family were individually weighed in order to estimate the growth performance of each family. Six families were then selected to represent the full range of variability observed for growth rate. Two different kinds of sampling were performed in each family: 1) for the 'within-family' sample, 10 individuals with an individual weight equal to the mean of each selected family were sampled, and 2) for the 'between-family' sample, 10 individuals with an individual weight equal to the mean over all 6 families were sampled. This sampling strategy was designed to separate any family effect from the well-established aneuploidy–growth relationship.

2.1.2. Full-sib progenies from parents of known aneuploidy level

The oysters used as parents for the study of the possible inheritance of aneuploidy originated from two different locations (Scotland and France). Significantly different levels of aneuploidy had previously been reported in these populations, 16% and 25% respectively in 'slow-growing' oysters (Leitão et al., 2001). In the present study, 8 oysters were used as G1 parents (2 males and 2 females from each population) and were scored for aneuploidy. However, only one female from each population gave enough results to estimate aneuploidy (because 30 mitoses per animal are required). Four crosses were performed: one within the Scottish population (cross No. 1), one within the French population (cross No. 2), and two between the two populations (crosses No. 3 and 4). The study of aneuploidy was made on the 8-month-old progenies of these 4 crosses. For each progeny, the same sampling strategy was performed as above, 10 animals with the within-family mean weight, and 10 animals with the between-family mean weight were prepared for aneuploidy study.

Larvae and spat from these crosses were reared at the IFREMER hatchery (La Tremblade, France) for approximately 3 weeks, under standard conditions (30 L tanks, temperature: 23–24 °C), and fed with a mixture of three different phytoplankton species (*Chaetoceros pumilum*, *Isochrysis galbana* and *Tetraselmis suecica*: 60 cells·μL^{-1}·day^{-1}) (Walne, 1974; Robert and Gérard, 1999). Larvae were settled in clutch, and spat were reared in the IFREMER nursery (Bouin, France), where they were fed *Skeletonema costatum*.

2.2. Chromosome scoring

The animals of full-sib families and full-sib progenies were incubated for 8–10 h in seawater containing 0.005% colchicine. Adult oysters used as G1 parents were treated the same way immediately following the stripping of their ripe gonads. The gills were then dissected in seawater, treated for 30 min in 0.9% sodium citrate and fixed in a freshly prepared mixture of absolute alcohol–glacial acetic acid (3:1) with three 20 min changes. Slides were made from one individual gill following the air-drying technique of Thiriot-Quiévreux and Ayraud (1982). The preparation was stained for 10 min with Giemsa (4%, pH 6.8). Chromosome counts were made directly by microscope observation (Zeiss III photomicroscope) on apparently intact and well-spread metaphases.

2.3. Data analyses

The level of aneuploidy was estimated by counting 30 randomly chosen, similarly well-spread metaphases per individual. This is the minimal statistical number usually accepted in cytogenetic studies (Stallard et al., 1981; Wenger et al., 1984).

Because the number of metaphases per individual studied was the same in all the studied material (30 per individual), it was possible to analyse aneuploidy as a quantitative trait. The statistical analyses were carried out through the application of ANOVAs and Tukey multiple comparisons using SYSTAT 9.0 to test for the significance of family effects.

3. RESULTS

3.1. Full-sib families chosen for their growth performances

Mean aneuploidy and mean weights in the within-family and the between-family samples of the 6 selected full-sib families are given in *table I*. The aneuploidy level (26%) of the within-family sample of family No. 10, with the lowest mean weight (0.59 g), was much larger than the aneuploidy level (13%) of family No. 24, which had the highest mean weight (1.49 g). Pairwise comparisons between families (Tukey multiple comparisons) revealed 2 groups among the 6 families: family No. 10 in one group, and the 5 other families in the other. The statistical analyses showed that the aneuploidy level of the animals from the between-family sample (animals with the same weight) was also highly significantly different ($P < 0.001$), and showed the same 2 groups.

Table I. Evaluation of aneuploidy in *Crassostrea gigas* full-sib families known to have different growth rates.

Family No.	Within-family sample		Between-family sample	
	Mean aneuploidy (%)	Mean weight (g)	Mean aneuploidy (%)	Mean weight (g)
10	26	0.59	25	0.98
18	15	0.75	16	0.98
19	17	0.91	15	0.98
3	14	1.01	*	
22	13	1.22	11	0.98
24	13	1.49	14	0.98

Percentages given are means from 10 animals based on 30 metaphases scored per animal. *: the mean weight of the within- and between-family samples being very close, aneuploidy has been scored only in one series.

3.2. Full-sib progenies from parents with known aneuploidy level

Scoring of aneuploidy was carried out in the 8 parents immediately following the stripping of their ripe gonads for the crosses. *Table II* shows the percentage of aneuploidy scored in each parent. Because these animals were sexually mature, very few mitoses were available in gill tissue. Consequently it was only possible to score the full 30 mitoses in 6 out of these 8 animals.

Results from the progenies are given in *table III*. Looking at the within-family samples, the level of aneuploidy in the progeny of the Scottish parents (cross No. 1) was the lowest, and that in the progeny of the French parents (cross No. 2), the largest. The aneuploidy in the progenies of the crosses between French and Scottish parents (crosses No. 3 and 4) showed intermediate values. Similarly to the experiment on selected full-sib families, animals from the between-families samples showed significant differences of aneuploidy ($P = 0.017$).

4. DISCUSSION

The investigation of the genetic basis for growth and for the variation in aneuploidy level in the Pacific

Table II. Evaluation of aneuploidy in G1 parents of *Crassostrea gigas* originating from two populations showing contrasting levels of aneuploidy.

Studied parents	Total mitosis	No. of cells with 2n = 20	No. of aneuploid cells			Total	Aneuploidy (%)
			2n = 19	2n = 18	2n = 17		
Scotland							
Parent No. 1 male	30	28	1	1	0	2	7
Parent No. 2 male	30	24	3	3	0	6	20
Parent No. 3 female	6	4	1	0	1	2	–
Parent No. 4 female	30	27	0	2	1	3	10
France							
Parent No. 5 male	30	25	4	1	0	5	20
Parent No. 6 male	30	28	2	0	0	2	7
Parent No. 7 female	30	23	2	3	2	7	23
Parent No. 8 female	7	5	2	0	0	2	–

Only the individuals where 30 metaphases could be scored were used as parents of the second generation.

Table III. Evaluation of aneuploidy in progenies of *Crassostrea gigas* originating from crosses of G1 parents.

	Progenies			
	Within-family sample		Between-family sample	
Crosses	Mean aneuploidy (%)	Mean weight (g)	Mean aneuploidy (%)	Mean weight (g)
No. 1: within Scottish parents female No. 4 × male No. 2	8	0.94	9	1.13
No. 2: within French parents female No. 7 × male No. 5	16	0.76	18	1.19
No. 3: between French and Scottish parents female No. 7 × male No. 1	13	1.32	13	1.29
No. 4: between Scottish and French parents female No. 4 × male No. 6	15	1.89	14	1.15

G1 parents: see *table II*. Percentages given are means from 10 animals based on 30 metaphases scored per animal.

oyster, traits that are negatively correlated (Leitão et al., 2001), is of particular importance since fast-growing individuals tend to be marketed at an earlier age than slow-growing ones. Because these slow-growing oysters remain longer (up to 1 or 2 more years) in the production areas, their relative participation to natural recruitment is increased. This is more likely to occur in overstocked areas where the biomass carrying capacity is a limiting factor (Héral, 1989). In the long term, this could lead to an undesired increasing frequency of slow-growing animals in the population, which might also enhance aneuploidy. This hypothesis is supported by the results of the present study, which provide the first experimental evidence of a genetic basis for differences in aneuploidy level.

Our results, obtained from the comparison of the aneuploidy level of selected full-sib families, showed that the negative relationship between aneuploidy and growth appeared both when scoring the within-family and between-family samples. This suggests that the level of determination of this phenomenon was intrinsic to the families and not just related to the growth performance of the studied individuals. The hypothesis of the existence of a genetic basis for the control of the aneuploidy is therefore supported.

The study of aneuploidy in two successive generations of *C. gigas* showed that the progenies of the Scottish parents (the population with the lowest level of aneuploidy) were less aneuploid than the progenies of the French parents (the population with the highest level of aneuploidy). Offsprings of the between-population crosses showed intermediate levels of aneuploidy. Thus, a relationship between aneuploidy and population origin is evident. Furthermore, looking at the between-family samples of the 4 full-sib progenies, with the same weight, significant differences of aneuploid values were also observed. This also supports a genetic basis for the phenomenon.

The direct relationship between aneuploidy of parents and their progenies is less clear. The inheritance of this phenomenon could not be demonstrated, due to the insufficient number of genitors in which the scoring of aneuploidy was possible. It is very difficult to obtain a good mitotic index in adult animals, especially during the reproductive period and a non-destructive method of studying aneuploidy, by which animals could be studied before reproduction, has yet to be successfully developed for oysters. Examining the parents themselves, male parents of the two within-population crosses showed the same level of aneuploidy (20%), while in the female parents used in these crosses, the aneuploidy level was different (10% for the Scottish cross and 23% for the French cross). The hypothesis of a predominant maternal effect could be suggested in the inheritance of aneuploidy and merits further examination in an experiment using a greater number of parents.

In conclusion, the study of aneuploidy in hatchery-produced *C. gigas* families leads us to propose a genetic basis for the aneuploidy phenomenon. It would be of great interest to establish a link between this genetic basis and the one reported for growth (Ward et al., 2000). This would imply establishing whether the same genes are involved in both phenomena. Such a genetic relationship between growth and aneuploidy would constitute important information for the aquaculture of certain bivalve species of commercial importance, especially those for which growth improvement by selective breeding programmes has been initiated.

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