Genet. Res., Camb. (1996), **68**, *pp.* 109–116 *With 3 text-figures*

The negative correlation between somatic aneuploidy and growth in the oyster *Crassostrea gigas* and implications for the effects of induced polyploidization

E. ZOUROS[1,3]*, C. THIRIOT-QUIEVREUX[2] AND G. KOTOULAS[1]

[1] *Department of Biology, The University of Crete, and Institute of Marine Biology of Crete, Iraklion, Crete, Greece*
[2] *Observatoire Oceanologique, Université P. et M. Curie – CNRS, B.P. 28, F-06230, Villefranche-sur-Mer, France*
[3] *Department of Biology, Dalhousie University, Halifax, N.S., Canada B3H 4J1*

(*Received 28 November 1995 and in revised form 28 February 1996*)

Summary

This study extends previous observations that chromosome loss in somatic cells of juveniles of the pacific oyster *Crassostrea gigas* is associated with reduced growth rate. All four studies designed to examine this association (two using random population samples and two using full sibs) produced the same result. This consistent effect appears to be unrelated with the commonly, but not consistently, observed correlation between degree of allozyme heterozygosity and growth. We propose that the inverse relationship between aneuploidy and growth is due to the unmasking of deleterious recessive genes caused by 'progressive haploidization' of somatic cells. Because unmasking of deleterious recessive genes by random chromosome loss is unlikely in polyploid cells, this hypothesis may also provide an explanation for the observation that artificially produced polyploid shellfish usually grow at faster rates than normal diploid ones.

1. Introduction

Several studies have shown that cytogenetic abnormalities arising at either meiosis or mitosis may be common in bivalves (Longwell & Stiles, 1968; Stiles & Longwell, 1973, Thiriot-Quievreux, 1986). The effects of these abnormalities on viability, growth or fertility are expected to be serious. Direct evidence for this exists for the Japanese oyster *Crassostrea gigas*. In this species Thiriot-Quievreux (1986) has shown that a substantial proportion of somatic cells (varying from 10% to 30%) contain fewer chromosomes than the full complement of $2n = 20$. She also examined two groups of juveniles from the same population – a 'fast-growing' group that consisted of individuals that had achieved a given body size at an early age and a 'slow-growing' group that achieved the same size a month later – and found that the number of aneuploid cells (i.e. cells with one or more chromosomes missing) was higher in the slow-growing group. A similar result was obtained from two full-sib families (Thiriot-Quievreux *et al.* 1988).

Growth rate in bivalves has been extensively studied in relation to two other genetic parameters: allozyme heterozygosity and induced polyploidy. Natural populations of these species very often have high levels of homozygote excess (i.e. contain more homozygous individuals than the Hardy–Weinberg expectation) and also show a positive correlation between size and degree of heterozygosity at allozyme loci. The general significance of these correlations has been intensively studied and debated (see, for example, Zouros & Foltz, 1987; Houle, 1989; Pogson & Zouros, 1994; Ruiz & Barbadilla, 1995; David *et al.* 1995). Chromosome set manipulation in bivalves has also received much attention, mainly because of its potential for applications in aquaculture (see review by Beaumont & Fairbrother, 1991, and recent study by Guo & Allen, 1994). With few exceptions, triploids were found to grow faster than diploids. Increased levels of heterozygosity, energy reallocation from gonad to somatic tissues and larger cell volume (with no compensatory reduction of cell number) in triploids are some of the various explanations for the polyploidy effect on growth, but here, again, there is no general consensus (Guo & Allen, 1994).

In this study we have asked, first, whether the original observation of a correlation between somatic aneuploidy and reduced growth rate in a natural population of oysters (Thiriot-Quievreux, 1986) could

* Corresponding author.

be repeated. Following the first observation, the phenomenon was demonstrated in two separate studies (Thiriot-Quievreux *et al.* 1988, 1992). But because both these studies involved sibs, the results could not be extended to natural populations. Secondly, we have asked whether the degree of aneuploidy varied according to the degree of allozyme heterozygosity and whether there was a relationship between the effects of aneuploidy and heterozygosity on growth. A negative answer to these questions was obtained in a family study (Thiriot-Quievreux *et al.* 1992), which again could not be extended to natural populations. Finally, we propose an explanation for the somatic aneuploidy effect on growth and suggest that the same explanation may apply to the effect of polyploidy on growth rate.

2. Materials and methods

(i) *Animals*

Oyster spat was collected from a wild population in Bonne-Anse, near Royan in the French Atlantic coast, in August 1993. After removal from the collectors, the spat was reared at the IFREMER laboratory in La Tremblade for 6 months. A random sample of 400 juveniles were then sent to Villefranche-sur-Mer, from which, by visual inspection, 40 individuals of 'small' size, 40 of 'medium' size and 40 of 'large' size were removed. These animals were used for a joint size, chromosome and allozyme analysis. Size was scored as the combined weight of the two empty shells, which at the juvenile stage comprise most of the animal's total weight.

(ii) *Chromosome scoring*

The animals were incubated for 10 h in sea water containing 0·005% colchicine. Then, the gills were dissected in sea water and the remaining soft part was frozen at −80 °C for allozyme electrophoresis. Shells were weighed to the nearest milligram. The dissected gills were treated for 30 min in 0·9% sodium citrate and fixed in a freshly prepared mixture of absolute alcohol–acetate (3 : 1) with three 20 min changes. Slides were made from one individual gill following the air-drying technique of Thiriot-Quievreaux & Ayraud (1982). The preparation was stained for 10 min with Giemsa (4%, pH 6·8) and scored with a Zeiss III photomicroscope.

Chromosome counts were made directly on 30 well-spread metaphases randomly chosen from an individual animal. This information was used to obtain two measurements for each individual animal: the number of aneuploid cells, i.e. the number of cells with one or more chromosomes missing, and the number of missing chromosomes, i.e. the total number of missing chromosomes in the 30 cells examined.

(iii) *Allozyme scoring*

The frozen soft parts of all 120 animals were shipped to Iraklion, Crete, Greece, where they were stored at −80 °C until electrophoresis. Electrophoretic separation of allozymes was done on horizontal starch gels. The gel buffer TRIS–citric buffer pH 7·0 (Thiriot *et al.* 1992) was used for aspartate aminotransferase (Aat, EC 2.6.1.1), adenlyl kinase (Adk, EC 2.7.4.3), isocitric dehydrogenase (Idh, EC 1.1.1.42), leucine aminopeptidase (Lap, EC 3.4.11.1) and phosphoglucose isomerase (Pgi, EC 5.3.1.9). For phosphoglucose mutase (Pgm, EC 2.7.5.1) we used the buffer TRIS (0·1 M), maleic acid (0·1 M), EDTA (0·01 M), $MgCl_2$ (0·01 M) for electrodes and in dilution 1/11 for the gel. Staining was performed according to Pasteur *et al.* (1988). Because of the small amount of soft tissue from each animal, particularly those of small size, these were the only polymorphic allozyme loci that could be scored reliably. Thirteen animals could not be scored for one or more enzyme loci and were removed from the study of correlations between heterozygosity and growth or between heterozygosity and aneuploidy.

(iv) *Statistical analysis*

All statistical tests were performed using the SYSTAT 5·02 for WINDOWS software package from SYSTAT Inc. (Evanston, Ill., USA).

3 Results

The distribution of the number of aneuploid cells (NAC) and of the number of missing chromosomes (NMC) in the three size classes and in the total sample of 120 animals is given in Table 1. As expected, the two measures of aneuploidy were strongly correlated ($r = 0{\cdot}922$ on $n = 120$, $P < 0{\cdot}001$). The parametric correlation between the log-transformed weight (LnW) of an individual and its NAC was highly significant ($n = 120$, $r = -0{\cdot}282$, $P = 0{\cdot}002$), but the correlation between LnW and NMC was not ($n = 120$, $r = -0{\cdot}142$, $P = 0{\cdot}123$). Kendall's non-parametric rank correlation coefficient between NAC and the mean of LnW of individuals of the same NAC was $-0{\cdot}709$ ($P < 0{\cdot}005$). For NMC this correlation coefficient was $-0{\cdot}371$ ($0{\cdot}025 < P < 0{\cdot}05$; these are one-tailed probabilities testing the null hypothesis of a negative correlation rather than of a negative or positive correlation). The non-parametric tests produced the same result as the parametric ones which were based on a weight distribution that consisted of three size classes. Thus, there is clear evidence of a deleterious effect of somatic aneuploidy on body size (Fig. 1*a*). NAC, in particular, accounts

https://doi.org/10.1017/S0016672300033991 Published online by Cambridge University Press

Table 1. *Aneuploidy scores in a sample of juvenile oysters*

	Number of animals observed					Number of animals observed			
NAC	Large class	Medium class	Small class	Pooled	NMC	Large class	Medium class	Small class	Pooled
0	0	0	0	0	0	0	0	0	0
1	3	0	1	4	1	1	0	1	2
2	4	4	2	10	2	4	2	1	7
3	5	4	4	13	3	4	4	2	10
4	7	10	3	20	4	2	7	5	14
5	5	7	5	17	5	5	8	2	15
6	7	7	5	19	6	3	9	6	18
7	6	3	6	15	7	2	3	2	7
8	3	5	6	14	8	8	2	5	15
9	0	0	4	4	9	2	1	5	8
10	0	0	3	3	10	4	4	4	12
11	0	0	0	0	11	3	0	1	4
12	0	0	0	0	12	2	0	2	4
13	0	0	0	0	13	0	0	2	2
14	0	0	0	0	14	0	0	1	1
15	0	0	1	1	15	0	0	0	0
					16	0	0	0	0
					17	0	0	1	1
Total	40	40	40	120		40	40	40	120

NAC, number of aneuploid cells; NMC, number of missing chromosomes.

for about 8% of the variance in body weight in our sample, which is 2 times larger than that seen in most studies that have demonstrated a positive effect of multiple locus heterozygosity on growth (e.g. Koehn *et al.* 1988).

Table 2 provides locus-specific statistics for each of the six loci based on 107 animals scored for all these loci. There is no correlation between body weight and allozyme heterozygosity. This is true from the comparison of the means of homozygotes and heterozygotes (Table 2), as well as from the regression of the log-transformed weight of an individual and its number of heterozygous loci (NHL; Fig. 2*a*).

The demonstration of a negative effect of aneuploidy on growth raises the question of whether this effect interferes with the detection of NHL on growth. To examine how the effects of NAC and NHL are related we fitted the model

$$\mathrm{LnW} = \mathrm{C} + \mathrm{NAC} + \mathrm{NHL} + \mathrm{NAC}*\mathrm{NHL},$$

where C is a constant. The interaction term, NAC*NHL, was not significant ($P = 0{\cdot}863$), and when removed the analysis produced a non-significant effect for NHL ($r = 0{\cdot}016$, $P = 0{\cdot}825$) and a significant effect for NAC ($r = -0{\cdot}114$, $P = 0{\cdot}01$), consistent with the results obtained when NAC and NHL were considered separately. To test for the effects of individual loci, the model

$$\mathrm{LnW} = \mathrm{C} + \mathrm{NAC} + \mathrm{H} + \mathrm{NAC}*\mathrm{H}$$

was used for each locus separately, with H taking the value 1, when the individual was heterozygous and 0 when it was homozygous for the locus under consideration. The NAC term was significant for all loci and the H term was significant for the *Pgm* locus ($r = 0{\cdot}498$, $P = 0{\cdot}001$). The interaction term was not significant for any locus, but the term between NAC and *Pgm* was the largest ($P = 0{\cdot}193$). The lack of significance means that the slopes of the regression of weight against NAC among *Pgm* heterozygotes and *Pgm* homozygotes are not statistically different from each other, but it does not imply that one or the other or both of these slopes are different from zero. Indeed, the correlation coefficient between NAC and weight is $-0{\cdot}049$ among *Pgm* heterozygotes ($P = 0{\cdot}315$) and $-0{\cdot}133$ among *Pgm* homozygotes ($P = 0{\cdot}002$), suggesting that the aneuploidy effect is more pronounced among the latter.

While there is no clear evidence for a positive correlation between enzyme heterozygosity and growth rate in our sample, the data share several trends with studies in which such correlations were found. First, there is a tendency for the mean size to increase with increasing NHL, even though this is not statistically significant (Fig. 2*a*). Secondly, the locus whose heterozygosity has a significant effect on growth, *Pgm*, is also the locus with a large, statistically significant excess of homozygotes in the sample (Table 2). Third, there is a clear correlation between the ratio of mean weight of heterozygotes to mean weight of homozygotes at a locus and the locus-specific estimate of fixation index (Fig. 2*b*). This correlation is a common characteristic of studies that have demon-

https://doi.org/10.1017/S0016672300033991 Published online by Cambridge University Press

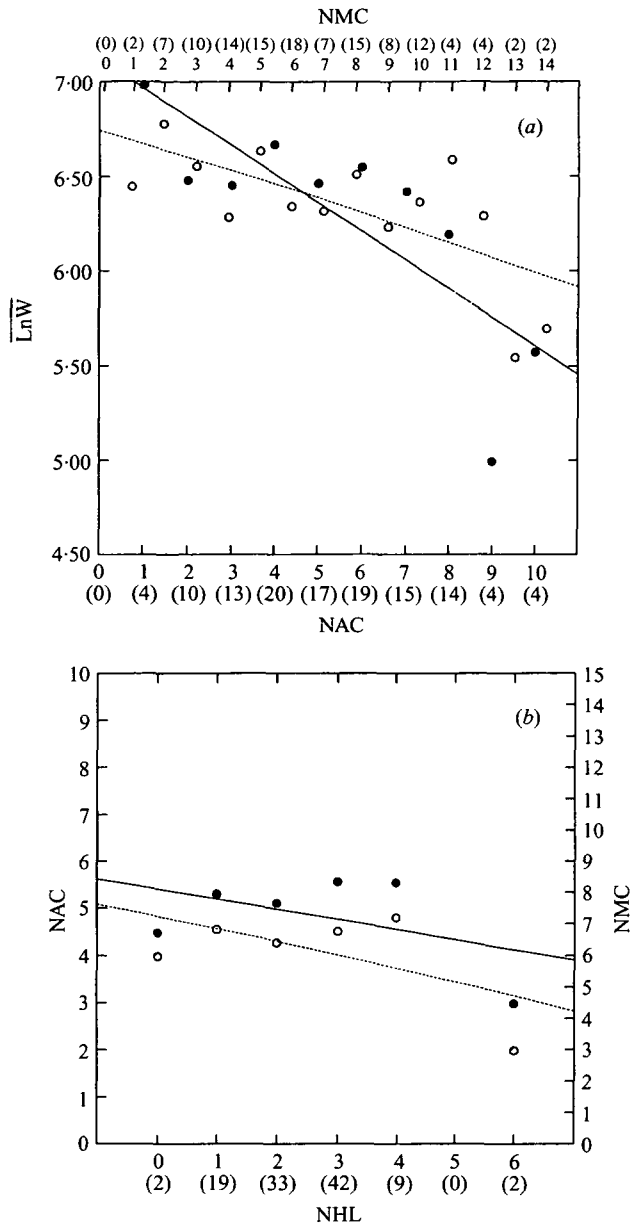

Fig. 1. (*a*). A plot of the mean of log-transformed weights, LnW (summed weight of both valves), of animals with the same number of aneuploid cells (NAC, filled circles) or missing chromosomes (NMC, open circles). Numbers in parentheses give the number of animals with the same NAC or NMC. Class NAC = 10 also contains one animal with NAC = 15, and class NMC = 14 one animal with NMC = 17. The regressions of individual LnW ($n = 120$) against NAC and NMC (lines not shown) are, respectively, $r = -0{\cdot}282$, $P = 0{\cdot}002$ and $r = -0{\cdot}142$, $P = 0{\cdot}123$. The regressions of mean LnW against NAC and NMC are, respectively, $n = 10$, $r = -0{\cdot}796$, $P = 0{\cdot}006$ (continuous line); and $n = 14$, $r = -0{\cdot}681$, $P = 0{\cdot}007$ (dotted line). (*b*) Plot of number of aneuploid cells (NAC) or number of missing chromosomes (NMC) against number of heterozygous loci (NHL). Numbers in parentheses are the number of individuals with the same NHL. The regressions of individual NAC and NMC, respectively, against individual NHL (lines not shown) are $n = 107$, $r = -0{\cdot}001$, $P = 0{\cdot}991$, and $n = 107$, $r = -0{\cdot}039$, $P = 0{\cdot}693$. The regressions of the means of NAC and NMC, respectively, against NHL are $n = 6$, $r = -0{\cdot}471$, $P = 0{\cdot}346$ (filled circles, continuous line) and $n = 6$, $r = -0{\cdot}586$, $P = 0{\cdot}222$ (open circles, dotted line).

strated a correlation between degree of heterozygosity and growth (Zouros, 1987).

4. Discussion

(i) *The lack of heterozygosity/growth correlation*

The lack of a correlation between degree of allozyme heterozygosity and growth in our sample is not an unusual observation. The correlation is known to vary substantially among loci and to emerge only as a statistical trend over many loci in large population samples (Zouros & Foltz, 1987). It is also known to be more common in populations with pronounced heterozygote deficiencies (Zouros, 1987; Gaffney *et al.* 1990). In the sample examined here only the *Pgm* locus showed a heterozygote deficiency and this was also the only locus in which heterozygosity was positively correlated with growth. A positive correlation over all six loci was found between excess of homozygosity and the difference of the mean weights of homozygotes and heterozygotes. Thus, whereas the hypothesis of a genuine absence of a correlation between individual degree of allozyme heterozygosity and growth rate cannot be rejected, its presence could hardly be detected given the small size of the sample, the fact that it was made of three visually selected size classes and, most importantly, the small number of loci scored. Another possibility is that the sample was drawn from a population with food abundance. Several studies have shown that the heterozygote/growth correlation is more likely to appear under stressful conditions (Rodhouse & Gaffney, 1984; Gentilli *et al.* 1988; Scott & Koehn, 1990; Borsa *et al.* 1992; Stiven, 1995; Audo & Diehl, 1995). The observation that the correlation between degree of aneuploidy and growth is stronger among *Pgm* homozygotes than among *Pgm* heterozygotes might also be explained in terms of stress. If somatic aneuploidy is assumed to impose an internal developmental stress on the organism, then we would expect that homozygotes will suffer more from chromosome loss than heterozygotes. Why this effect should be confined to one locus remains unexplained. We know from the work of Pogson (1991) that the *Pgm* locus has indeed an overdominant expression in the Pacific oyster, but it is less clear if this translates to overdominance for fitness.

(ii) *The negative correlation between somatic aneuploidy and growth and a hypothesis for its explanation*

The results of this study confirm the existence of a negative correlation between somatic aneuploidy and growth rate in juvenile oysters. This correlation is clearly not family-specific, as it has now been observed in two different population samples and in three different families. Both heterozygote deficiencies and

https://doi.org/10.1017/S0016672300033991 Published online by Cambridge University Press

Table 2. *Locus-specific statistics in a sample of juvenile oysters*

		n	LnW	NAC	NMC	*F*
Aat	Ho	51	6·529 (0·813)	5·510 (2·541)	6·745 (3·236)	0·049
	He	56	6·505 (0·859)	5·143 (2·203)	6·536 (3·098)	(1·000)
Adk	Ho	73	6·536 (0·833)	5·123 (2·223)	6·548 (3·060)	0·006
	He	34	6·474 (0·846)	5·735 (2·632)	6·824 (3·380)	(0·934)
Idh	Ho	43	6·700 (0·776)	5·140 (2·494)	6·744 (3·193)	−0·060
	He	64	6·393 (0·854)	5·438 (2·288)	6·563 (3·147)	(0·367)
Lap	Ho	75	6·478 (0·872)	5·427 (2·201)	6·880 (3·036)	0·097
	He	32	6·607 (0·743)	5·063 (2·735)	6·063 (3·388)	(0·165)
Pgi	Ho	92	6·580 (0·805)	5·185 (2·107)	6·500 (2·933)	0·014
	He	15	6·127 (0·927)	6·133 (3·563)	7·467 (4·291)	(0·826)
Pgm	Ho	49	6·213 (0·848)	5·653 (2·720)	6·714 (3·391)	0·164
	He	58	6·773 (0·735)	5·034 (2·000)	6·569 (2·962)	(0·001)

n, observed number of homozygous (Ho) or heterozygous (He) animals at a given enzyme locus (see text for enzyme names); LnW, log-transformed weight; NAC and NMC as defined in Table 1; standard errors in parentheses. *F*, the fixation index (defined as $F = 1 - H_{ob}/H_{ex}$, where H_{ob} is the number of observed heterozygotes and H_{ex} is the number of heterozygotes expected under Hardy–Weinberg equilibrium). The numbers in parentheses under *F* give the probability of the sample assuming Hardy–Weinberg equilibrium.

heterozygosity/growth correlations could, in principle, result from aneuploidy. If the level of enzyme activity in an individual is an inverse function of the number of aneuploid cells for the chromosome carrying the scored locus, then high levels of aneuploidy could lead to the miscoring of a heterozygous individual as a homozygote. By analogy with null alleles (Zouros *et al.* 1980; Foltz, 1986), this would lead to an underestimation of heterozygosity in the population and to a correlation of apparent degree of heterozygosity with growth rate. An indirect way through which the same correlations could arise is if the degree of allozyme homozygosity were an index of overall homozygosity of the individual and if the degree of chromosome loss were correlated with overall homozygosity (e.g. through increased probability for homozygosity for recessive mutations at genes controlling mitosis). Our results suggest that somatic aneuploidy is an unlikely explanation of either the heterozygote deficiency or the heterozygosity/growth correlation, which are often jointly observed in natural populations of bivalves.

What explanations could we offer for the high occurrence of aneuploid somatic cells in this species and for the negative correlation between individual degree of aneuploidy and growth rate? A hypothesis that can explain both these observations is presented in Fig. 3. An important part of the hypothesis is that newly formed zygotes contain large numbers of mutations. We suggest that to a large degree these are novel mutations that have occurred in the germ line of the zygote's parents. Marine bivalves that broadcast their gametes in the water column are known to produce a vast number of gametes (the number of eggs produced by a female oyster is of the order of 10^7 per spawning: (Strathmann, 1987). This implies a large number of germ cell divisions, which (assuming a constant rate of error per DNA replication) would lead to the accumulation of a high number of mutations per gamete. This accumulation of mutations has been proposed as an explanation for the high amounts of mitochondrial DNA variation in the Pacific oyster (Beckenbach, 1994). Zouros *et al.* (1980) have also suggested high mutation rate per gamete as one of several hypotheses for the heterozygote deficiency and the heterozygosity/growth correlation in the American oyster. Amounts of allozyme variation in natural populations of marine bivalves are, indeed, on the high side compared with that in other animals (e.g. Koehn *et al.* 1988), but this could be due to many other reasons, such as large effective population size (Hedgecock & Sly, 1990) or balancing selection (Karl & Avise, 1992). Even though direct evidence for a high frequency of mutations in organisms with very high gametic output is missing, this hypothesis is supported by the findings of Shimmin *et al.* (1993) and Chang *et al.* (1994), who have observed that mammalian DNA sequences of the *Y* chromosome evolve at a higher rate than homologous sequences of the X chromosome, presumably because of the much larger number of cell divisions in the male germ line. In most bivalves the female germ line is also a fertile source of new mutations because it too undergoes a high number of cell divisions.

If we assume that gametes carry a large number of mutations, then among these mutations there will be mutations at loci involved in the control of the process of mitosis. The fact that we have observed no juvenile with all 30 sampled cells aneuploid suggests that aneuploid zygotes are not formed or, if they are, die very early in development. Most of the mitosis-affecting mutations should, therefore, have a post-

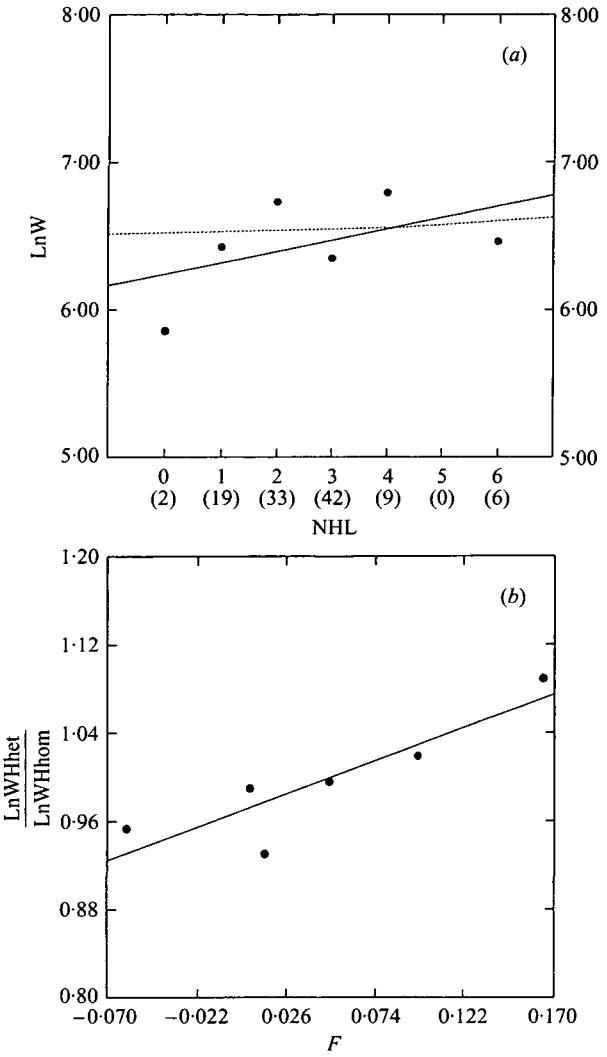

Fig. 2(*a*). Plot of the mean of log-transformed weights, LnW, of animals with the same number of heterozygous loci (NHL). Numbers in parentheses are the numbers of animals with the same NHL. The dotted line is the regression of individual weight against NHL ($n = 107$, $r = 0{\cdot}021$, $P = 0{\cdot}831$). The continuous line is the regression of mean weight against NHL ($n = 6$, $r = 0{\cdot}518$, $P = 0{\cdot}292$). (*b*) Plot of the ratio of the mean of log-transformed weight of homozygotes and heterozygotes (LnWHhet/LnWHhom) at a given locus against the fixation index (F) at that locus (regression: $n = 6$, $r = 0{\cdot}881$, $P = 0{\cdot}02$).

zygotic function, causing spontaneous chromosome loss in the somatic cells of the developing organism. This progressive haploidization will, in turn, result in the unmasking of growth-rate-affecting mutations and cause a retardation of growth. Mutations of the latter type are expected to be fairly common among gametes, because growth rate, as a quantitative character, is expected to have a polygenic determination. The only properties required from these mutations are that they be fully or partially recessive and that there be a monotonic relationship between their number and the deleterious effect on growth rate.

This appears to be the simplest explanation we can offer at present to account for both the high incidence of somatic aneuploidy and its effect on growth rate. It is consistent with the expected high mutation rate in animals of large gametic output and, also, with experimental evidence from *Drosophila*, where repeated rounds of replication of balanced chromosomes are known to result in the accumulation of substantial loads of recessive mutations for viability (Mukai, 1969). Recently, random somatic chromosome loss and gene recessiveness were also used to formulate a hypothesis for the evolution of genomic imprinting. According to the 'surveillance' hypothesis (Thomas, 1995) genomic imprinting evolved to cause the elimination of aneuploid cells which may, otherwise, become cancerous. Cells with an imprinted gene are effectively hemizygous and will become non-functional after spontaneous loss of the chromosome that carries the non-imprinted copy of the gene.

(iii) *Extension to polyploids*

Several hypotheses have been proposed for the increased body size in polyploid bivalves (for a recent review see Guo & Allen, 1994). They fall into three categories: histological, energy allocation and genetic. The first hypothesis, termed 'gigantism' by Guo & Allen (1994), suggests that the large size is caused by the increased volume of polyploid cells, not compensated by reduction in the number of cells. The second hypothesis postulates that in triploids, which have reduced fertility, energy that would otherwise be used to fuel the development of the gonad is diverted to the development of somatic tissues. The genetic hypothesis has at least two versions. One is the 'gene dose' hypothesis, which suggests that triploids grow faster simply because they have the same gene product in triple dose, whereas normal diploids have it in double dose. The other is the 'allelic variation' (or heterozygosity) hypothesis, which suggests that the advantage of triploids results from these individuals having a higher probability of carrying two different alleles at the same locus than diploids (in principle, triploids may even carry three different alleles, which is impossible for diploids).

The progressive haploidization hypothesis that we propose here to explain the effect of aneuploidy on growth rate suggests yet another genetic explanation. The important element in this hypothesis is not whether the cell will have the activity of a gene twice or three times or whether it will have this activity in more than one variation, but rather whether it will have any activity at all. Since there is no reason to suspect that triploid somatic cells are losing chromosomes at a different rate from diploid ones, the probability that a chromosome loss will expose a recessive deleterious mutation, and thus deprive the cell completely of the activity of a certain gene, will be

https://doi.org/10.1017/S0016672300033991 Published online by Cambridge University Press

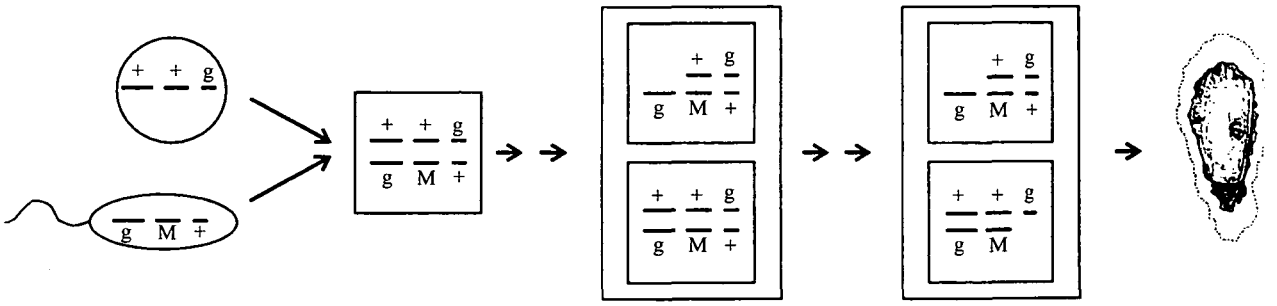

Fig. 3. A schematic presentation of the hypothesis for the deleterious effect of somatic aneuploidy on growth rate. Three pairs of chromosomes are shown, identified by different length, with one locus on each chromosome. Wild-type alleles are designated by + and mutant alleles by a letter. The second chromosome carries a locus that participates in the control of mitosis. The sperm shown carries a mutation (M) at this locus which is assumed to be partially dominant, and the resulting zygote (left-hand square) is predisposed to chromosome loss during mitosis. The first and third chromosomes each carry one locus that affects growth rate. The sperm carries a completely recessive mutation (g) at one of these loci and the egg such a mutation at the other. Two cell lineages are shown in the developing individual (rectangles). In the first lineage (left-hand upper square), the first chromosome of egg-origin is lost, thus unmasking the allelic deleterious g mutation of the sperm. Later, a second chromosome loss occurs in a different cell lineage (right-hand lower square) involving the third chromosome of sperm-origin, thus unmasking the deleterious g mutation of the egg. As the cumulative result of the expression of these deleterious genes, the animal achieves a smaller size than if it did not suffer from autosomal aneuploidies (dotted outline).

much smaller in a triploid than in a diploid cell. In other words, triploids are larger in size not because their growth is accelerated, but because the growth of diploids is depressed.

The main difficulty with the histological and the energy reallocation hypotheses is that they cannot account for the common observation that triploids resulting from blocking meiosis I grow faster that those resulting from blocking meiosis II. This observation is consistent with the genetic variation hypothesis, because di-allelic and tri-allelic individuals are expected to be more common among triploids of the first type, but only for loci whose recombination distance from the centromere is less than 2/3 (Guo *et al.* 1992). In a recent study, Hawkins *et al.* (1994) have indeed found that meiosis I triploids of the European oyster *Ostrea edulis* outperformed meiosis II triploids, but the latter were not statistically different from normal diploids. Beaumont *et al.* (1995) did not observe size differences at the juvenile stage between triploids and diploids in the blue mussel *Mytilus edulis* and, in particular, found no indication that tri-allelic triploids were different from di-allelic ones for several enzyme loci. The progressive haploidization hypothesis may account for some of these observations, but the available evidence is not enough for a critical evaluation of the various versions of genetic hypotheses. More specific tests would be needed for this. One such test would be to examine jointly chromosome loss and growth depression in cohorts of diploid, triploid I and triploid II in several sibships.

We thank Dr Andre Gerard for collecting and rearing the spat sample and Dr J. M. Legay for help with shell biometrics. We also thank Drs A. Magoulas, G. Markakis and C. Saavedra and Mr C. Batargias for comments. This research was partially supported by a grant from the National Science and Engineering Research Council (NSERC) of Canada to E.Z. and by a grant from CNRS (EP75) to C.T.-Q.

References

Audo, M. C. & Diehl, W. J. (1995). Effect of quantity and quality of environmental stress on multilocus heterozygosity–growth relationships in *Eisenia fetida* (Annelida: Oligochaeta). *Heredity* **75**, 98–105.

Beaumont, A. & Fairbrother, J. (1991). Ploidy manipulation in molluscan shellfish: a review. *Journal of Shellfish Research* **10**, 1–18.

Beaumont, A., Fairbrother, J. & Hoare, K. (1995). Multilocus heterozygosity and size: a test of hypotheses using triploid *Mytilus edulis*. *Heredity* **75**, 256–266.

Beckenbach, A. T. (1994). Mitochondrial haplotype frequencies in oysters: neutral alternatives to selection models. In *Non-neutral Evolution: Theories and Molecular Data* (ed. B. Golding), pp. 188–198. New York: Chapman and Hall.

Borsa, P., Jusselin, Y. & Delay, B. (1992). Relationship between allozyme heterozygosity, body size, and survival to natural anoxic stress in the palourde *Ruditapes decussatus* L. (Bivalvia: Veneridae). *Journal of Experimental Marine Biology and Ecology* **155**, 169–181.

Chang, B. H., Shimmin, L. C., Shyue, S. K., Hewett-Emmett, D. & Li, W.-H. (1994). Weak male-driven molecular evolution in rodents. *Proceedings of the National Academy of Sciences of the USA* **91**, 827–831.

David, P., Delay, B., Berthou, P. & Jarne, P. (1995). Alternative models for allozyme-associated heterosis in the marine bivalve *Spisula ovalis*. *Genetics* **139**, 1719–1726.

Foltz, D. W. (1986). Null alleles as a possible cause of heterozygote deficiencies in the oyster *Crassostrea virginica* and other bivalves. *Evolution* **40**, 869–870.

Gaffney, P. M., Scott, T. M., Koehn, K. & Diehl, W. J. (1990). Interrelationships of heterozygosity, growth rate, and heterozygote deficiencies in the coot clam, *Mulinia lateralis*. *Genetics* **124**, 687–699.

Gentilli, M. R., Beaumont, M. R. & Beaumont, A. R. (1988). Environmental stress, heterozygosity and growth rate in *Mytilus edulis*, L. *Journal of Experimental Marine Biology and Ecology* **129**, 145–153.

Guo, X., Hershberger, W. K., Cooper, K. & Chew, K. K. (1992). Genetic consequences of blocking polar body I with Cytochalasin B in fertilized eggs of the Pacific oyster, *Crassostrea gigas*. II. Segregation of chromosomes. *Biological Bulletin* **183**, 387–393.

Guo, X. & Allen, S. K., Jr (1994). Sex determination and polyploid gigantism in the dwarf surfclam *Mulinia lateralis* (Say). *Genetics* **138**, 1199–1206.

Hawkins, A. J. S., Day, A. J., Gérard, A., Naciri, Y., Ledu, C., Bayne, B. L. & Héral, M. (1994). A genetic and metabolic basis for faster growth among triploids induced by blocking meiosis I but not meiosis II in the larviparous European flat oyster, *Ostrea edulis* L. *Journal of Experimental Marine Biology and Ecology* **184**, 21–40.

Hedgecock, D. & Sly, F. (1990). Genetic drift and effective population sizes of hatchery-propagated stocks of the Pacific oyster, *Crassostrea gigas*. *Aquaculture* **88**, 21–38.

Houle, D. (1989). Allozyme-associated heterosis in *Drosophila melanogaster*. *Genetics* **123**, 789–801.

Karl, S. A. & Avise, J. C. (1992). Balancing selection at allozyme loci in oysters: implications from nuclear RFLPs. *Science* **256**, 100–102.

Koehn, R. K., Diehl, W. J. & Scott, T. M. (1988). The differential contribution by individual enzymes of glycolysis and protein catabolism to the relationship between heterozygosity and growth rate in the coot clam, *Mulinia lateralis*. *Genetics* **118**, 121–130.

Longwell, A. C. & Stiles, S. S. (1968). Fertilization and completion of meiosis in spawned eggs of the American oyster *Crassostrea virginica* (Gmelin). *Caryologia* **21**, 65–73.

Mukai, T. (1969). The genetic structure of natural populations of *Drosophila melanogaster*. VII. Synergistic interactions of mutant polygenes controlling viability. *Genetics* **61**, 749–761.

Pasteur, N., Pasteur, G., Bonhomme, F., Catalan, J. & Britton-Davidian, J. (1988). *Practical Isozyme Genetics*. Chichester: Ellis Horwood.

Pogson, G. H. (1991). Expression of overdominance for specific activity at the phosphoglucomutase-2 locus in the Pacific oyster, *Crassostrea gigas*. *Genetics* **128**, 133–141.

Pogson, G. H. & Zouros, E. (1994). Allozyme and RFLP heterozygosities as correlates of growth in the scallop *Placopecten magellanicus*: a test of the associative overdominance hypothesis. *Genetics* **137**, 221–231.

Rodhouse, P. G. & Gaffney, P. M. (1984). Effect of heterozygosity on metabolism during starvation in the American oyster *Crassostrea virginica*. *Marine Biology* **80**, 179–187.

Ruiz, A. & Barbadilla, A. (1995). The contribution of quantitative trait loci and neutral marker loci to the genetic variances and covariances among quantitative traits in random mating populations. *Genetics* **139**, 445–455.

Scott, T. M. & Koehn, R. K. (1990). The effect of environmental stress on the relationship of heterozygosity to growth rate in coot clam *Mulinia lateralis* (Say). *Journal of Experimental Marine Biology and Ecology* **135**, 109–116.

Shimmin, L. C., Chang, H.-J. & Li, W.-H. (1993). Male-driven evolution of DNA sequences. *Nature* **362**, 745–747.

Stiles, S. S. & Longwell, A. C. (1973). Fertilization, meiosis and cleavage in eggs from large mass spawning of *Crassostrea virginica* (Gmelin), the commercial American oyster. *Caryologia* **26**, 253–262.

Stiven, A. E. (1995). Genetic heterozygosity and growth rate in the southern appalachian land snail *Mesodon normalis* (Pilsbry 1900): the effects of laboratory stress. *Malacologia* **36**, 171–184.

Strathmann, T. S. (1987). *Reproduction and Development of Marine Invertebrates of the Northern Pacific Coast*. University of Washington Press.

Thiriot-Quievreux, C. (1986). Etude de l'aneuploidie dans differents naissains d'Ostreidae (Bivalvia). *Genetica* (*The Hague*) **70**, 225–231.

Thiriot-Quievreux, C. & Ayraud, N. (1982). Les caryotypes de quelques espècers de Bivalves et Gastéropodes marins. *Marine Biology* **70**, 165–172.

Thiriot-Quievreux, C., Noel, T., Bougrier, S. & Dallot, S. (1988). Relationships between aneuploidy and growth rate in pair matings of the oyster *Crassostrea gigas*. *Aquaculture* **75**, 89–96.

Thiriot-Quievreux, C., Pogson, G. H. & Zouros, E. (1992). Genetics of growth rate variation in bivalves: aneuploidy and heterozygosity effects in a *Crassostrea gigas* family. *Genome* **35**, 39–45.

Thomas, J. H. (1995). Genomic imprinting proposed as a surveillance mechanism for chromosome loss. *Proceedings of the National Academy of Sciences of the USA* **92**, 480–482.

Zouros, E. (1987). On the relation between heterozygosity and heterosis: an evaluation of evidence from marine mollusks. *Isozymes: Current Topics in Biological and Medical Research* **15**, 255–279.

Zouros, E., Singh, S. M. & Miles, H. E. (1980). Growth rate in oysters: an overdominant phenotype and its possible explanations. *Evolution* **34**, 856–867.

Zouros, E. & Foltz, D. W. (1987). The use of allelic isozyme variation for the study of heterosis. *Isozymes: Current Topics in Biological and Medical Research* **13**, 1–59.