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Chromosome inheritance in triploid Pacific oyster *Crassostrea gigas* Thunberg

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Reproduction and chromosome inheritance in triploid Pacific oyster (*Crassostrea gigas* Thunberg) were studied in diploid female × triploid male (DT) and reciprocal (TD) crosses. Relative fecundity of triploid females was 13.4% of normal diploids. Cumulative survival from fertilized eggs to spat stage was 0.007% for DT crosses and 0.314% for TD crosses. Chromosome number analysis was conducted on surviving progeny from DT and TD crosses at 1 and 4 years of age. At Year 1, oysters from DT crosses consisted of 15% diploids ($2n = 20$) and 85% aneuploids. In contrast, oysters from TD crosses consisted of 57.2% diploids, 30.9% triploids ($3n = 30$) and only 11.9% aneuploids, suggesting that triploid females produced more euploid gametes and viable progeny than triploid males. Viable aneuploid chromosome numbers included $2n+1$, $2n+2$, $2n+3$, $3n-2$ and $3n-1$. There was little change over time in the overall frequency of diploids, triploids and aneuploids. Among aneuploids, oysters with $2n+3$ and $3n-2$ chromosomes were observed at Year 1, but absent at Year 4. Triploid progeny were significantly larger than diploids by 79% in whole body weight and 98% in meat weight at 4 years of age. Aneuploids were significantly smaller than normal diploids. This study suggests that triploid Pacific oyster is not completely sterile and cannot offer complete containment of cultured populations.
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Introduction

Triploidy has played an important role in the evolution of plants, with allotriploidy often being the first step during polyploidization and speciation (DeWit, 1980). Polyploidization is a prevailing process in plant evolution, and the majority of plants are recent polyploids. Polyploidy is less frequent in the animal kingdom. Polyploidization has clearly occurred in some invertebrate and lower vertebrate taxa (White, 1978; Schultz, 1980; Komaru *et al*, 2000). It has been suggested recently that two rounds of genome duplication (or tetraploidization) have occurred during the evolution of vertebrates (Furlong and Holland, 2002; Spring, 2002). There is renewed interest in the biology and evolutionary significance of polyploid animals, which is poorly understood at this time.

As a chromosome number mutation, triploidy occurs spontaneously at low frequencies in almost all animal species. While it is lethal or often associated with severe abnormalities in mammals and birds, triploidy is viable and often morphologically indistinguishable from normal diploids in most invertebrates, amphibians and fish (Fankhauser, 1945; Thorgaard, 1983; Komaru *et al*, 2000). Triploid animals are sterile in some organisms, but produce functional gametes and viable progeny in others. If triploids are not completely sterile, what are the genetic and evolutionary consequences of triploidy in animals? Studies on the reproduction and inheritance of triploidy in a variety of animal species may provide some insight.

The Pacific oyster, *Crassostrea gigas* Thunberg, is a marine bivalve native to the west Pacific, and now cultured in many coastal regions of the world. It is a diploid species without any known history of polyploidization. It is protandric dioecious with rare hermaphroditism (Amemiya, 1929; Guo *et al*, 1998). Triploidy occurs spontaneously in the Pacific oyster at low but consistent frequencies (Guo *et al*, 1992; Guo and Allen, 1994a) and as in most other organisms, it can also be artificially induced by blocking meiosis in newly fertilized eggs (Allen *et al*, 1989). Triploid Pacific oysters are fully viable and morphologically inseparable from diploids, although they have larger body size or faster growth (Allen and Downing, 1986; Guo *et al*, 1996). Triploids in most molluscs studied so far are bigger than diploids, a common phenomenon that has been referred to as polyploid gigantism (Guo and Allen, 1994b). Despite retarded gonadal development, triploids in most molluscs, including the Pacific oyster, produce mature and functional gametes in at least one sex (Allen, 1987; Komaru and Wada, 1989; Guo, 1991). Gametes produced by triploid Pacific oysters are primarily aneuploid and when fertilized, some of them develop into viable progeny (Guo and Allen, 1994a).

Triploid molluscs are useful for aquaculture production because of their 'sterility', improved meat quality and superior growth. They are widely used for commercial production (Allen *et al*, 1989; Chew, 1994; Guo *et al*,

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2001). Triploids are also under development for aquaculture use in several other molluscs. When triploids are completely sterile, the use of triploids for aquaculture may protect biodiversity by preventing escape of cultured stock to wild populations. For triploids that are not sterile, their reproductive potential and genetics are relevant to our understanding of potential impacts of triploids on wild populations. A previous study in the Pacific oyster has provided one estimate of the reproductive potential and gamete chromosome numbers of triploids produced by blocking meiosis (Guo and Allen, 1994a). Here, we provide an estimate of reproductive potential of mated triploid Pacific oysters produced from tetraploids and chromosomal identities of their progeny.

Materials and methods

Confirmation of triploids by flow cytometry

Reproduction and chromosome inheritance of triploid Pacific oysters were studied in diploid × triploid crosses. Triploid Pacific oysters used from this study were produced by diploid female × tetraploid male mating (Guo *et al*, 1996). Diploid oysters used were from the same base population that was originally from Washington State and had been maintained at Rutgers University for several generations. Triploid and diploid oysters were 3 years old at the time of spawning. Before making crosses, all triploids were individually certified by flow cytometric analysis (Guo and Allen, 1994a).

Spawn, larval and oyster culture

Two types of crosses, diploid female × triploid male (DT) and triploid female × diploid male (TD), were produced in triplicate. No diploid control was used in this study because of space limitations and the fact we had accumulated considerable data on diploid crosses over the years.

Gametes of the diploid and certified triploid oysters were obtained by dissecting gonads. Eggs were passed through an 80-μm screen, collected and rinsed on a 20-μm screen to remove tissue debris. Sperm suspension was passed through a 20-μm screen to remove large tissue debris. Since fecundity of triploids was limited, gametes from several triploids were combined for each cross. Filtered (1 μm) seawater was used throughout this study. All gamete preparation, fertilization and embryo incubation were conducted at 24–25°C.

Embryos were cultured in 210-l tanks according to standard protocols routinely used in our hatchery (Breese and Malouf, 1975). Starting on day 2, larvae were fed with freshly harvested algae, *Isochrysis galbana* and *Chaetoceros calcitrans*. Water was changed every other day. When oyster larvae were ready to set, they were treated with epinephrine for the production of cultchless oysters (Coon *et al*, 1986). Eyed larvae were collected on a 200-μm nytex screen and immersed in a solution of 10^{-4} M epinephrine in seawater for 16 h. Metamorphosed larvae were rinsed and held in downwellers with 200-μm mesh screens until they reached a shell length of about 1 mm, when they were transferred to upweller nursery systems. Survival of fertilized eggs to day 2 (D-stage), day 12 and day 60 (spat) were recorded.

Relative survival of DT and TD crosses was calculated by dividing the survival of DT and TD crosses by the survival of normal diploids. We did not have diploid controls in this study, but used existing survival data for diploid crosses. Under our hatchery conditions, survival of diploid crosses usually varied between 10 and 30% from fertilized eggs to spat. We used 20% as the survival for diploid crosses, which is about the same as what was observed in a previous study (Guo and Allen, 1994a).

Chromosome number in surviving oysters

Chromosome number of surviving oysters was determined at 1 and 4 years of age. A total of 20 oysters were sampled from each replicate of each group. Each oyster was measured (for size), labeled and processed for chromosome number analysis using a protocol similar to Guo and Allen (1994c). Oysters were first kept at 12–15°C for 24 h to synchronize cell divisions. They were then cultured at 23–25°C with intensive feeding for 48 h and subsequently treated with 0.005% colchicine for 6–10 h. The treated oysters were dissected, and a piece of stomach and gill tissues, about 0.5 cm^3, were taken. Tissue samples were treated with 0.075 M KCl for 10–20 min before fixed in Carnoy fixative (1 : 3 acetic acid and methanol). The fixative was changed three times, and samples were stored at 4°C. To make cell suspension, a piece of tissue was chopped fine in 50% acetic acid in water. Three to five drops of cell suspension were dropped onto clean slides and left air-dry. When more spreading was desired, cell suspension was dropped onto slide at 50–53°C. Then slides were stained with Leishman's stain for about 5–10 min. The working solution was a 1:4 dilution of the stock solution (100 mg Leishman stain/100 ml methanol) with PBS (0.025 M KH_2PO_4, pH 6.8).

The Pacific oyster has a diploid number of 20 chromosomes (Ahmed and Sparks, 1967). Oysters with 20 and 30 chromosomes were considered as euploid–diploid and triploid, respectively. Oysters with any other chromosome number were classified as aneuploids. For each oyster, 10–20 metaphases that show no signs of chromosome loss were counted. A chromosome number was accepted when five to 10 high-quality metaphases from the same oyster showing the same chromosome number. Variation in chromosome number, almost all showing losses, was mostly associated with 'bad' metaphases and considered as artefact from overspreading. Slides were screened with a Nikon ECLIPSE E600 microscope, and photographs were taken using Kodak professional B&W film with speed set at 100 ASA.

Since oysters were small at 1 year of age, body size was measured only by shell height and whole body weight. At 4 years of age, the following size measurements were collected on each oyster: shell height, shell length, shell width, whole body weight and meat weight. A condition index was calculated as a percent meat weight of whole body weight. Oysters were observed for abnormal morphology and coloration. Body size data for each chromosome number were pooled across groups and analyzed with ANOVA and multiple comparisons of means. Significance level was set at $P<0.05$ unless otherwise noted.

Results

Crosses and survival

A total of 66 confirmed triploids and 16 diploids were used for this study. All triploids sampled except for one were ripe and contained gametes. The triploid oysters consisted of 24 (36.4%) females, 24 (36.4%) males, 17 (25.8%) hermaphrodites and one (1.5%) individual without gametes. The diploid oysters consisted of six (37.5%) females and 10 (62.5%) males.

The number of parents used, number of eggs fertilized, fertilization level and percent survival to day 2 (D-stage), day 12 (eyed stage) and day 60 (spat) are presented in Table 1. Six diploid females were used for three DT groups, producing 49.1 million eggs or 8.2 million per female. A total of 21 triploid females were used in three TD groups, and they produced 23.4 million eggs, averaging 1.1 million per female. The relative fecundity of triploid females was 13.4% of that of normal diploids.

There was no difference in fertilization level between DT and TD crosses. Fertilization levels in DT and TD groups were 79 and 85%, respectively. Survival of fertilized eggs to day 2 (D-stage) was 21.2% in TD and 6.8% in DT groups, both lower than the 60–80% expected for normal diploids under our hatchery conditions. Most of larval mortality occurred during the first week. One DT group, DT2, suffered complete mortality before reaching spat stage. Cumulative survival to day 60 (spat) stage was 0.007% for DT groups and 0.314% for TD groups. Two-sample *t*-test using transformed percentage data showed that survival to spat of TD was significantly higher than that of DT groups ($P = 0.006$). Relative survival of DT and TD groups was 0.035 and 1.57%, respectively, of the normal diploid rate (assumed to be 20%).

Spat survived well after metamorphosis. Cumulative survival of spat to 3 years of age was 63.1% for two DT groups and 78.7% for three TD groups. TD2 and TD3 were accidentally lost in Year 3 (not due to mortality), and only TD1 was available for chromosome analysis at Year 4.

Chromosome number

At Year 1, chromosome number was successfully determined for 99 of the 100 oysters sampled (20×5 groups). One individual in TD2 did not produce enough metaphases. In the DT groups, the majority of oysters (85%) had aneuploid chromosome numbers (Table 2). In

Table 1 Number of parents used (for gamete pooling), number of eggs fertilized, percent fertilization and cumulative survival of fertilized eggs to day 2 (D-stage), day 12 and day 60 (spat stage) in diploid female × triploid male (DT) and reciprocal (TD) crosses in the Pacific oyster

Group	*Number of parents*		*Egg no.*	*Fertilization*	*Survival (%)*		
	Maternal	*Paternal*	*($\times 10^6$)*	*(%)*	*Day 2*	*Day 12*	*Day 60*
DT1	1 (2*n*)	6 (3*n*)	21.4	88	5.9	0.2	0.004
DT2	3 (2*n*)	4 (3*n*)	17.4	69	2.8	0.1	0
DT3	2 (2*n*)	8 (3*n*)	10.3	81	11.6	2.6	0.017
Mean	**2**	**6**	**16.4**	**79**	**6.8**	**1.0**	**0.007**
TD1	6 (3*n*)	1 (2*n*)	6.6	94	6.5	5.2	0.242
TD2	6 (3*n*)	1 (2*n*)	6.2	75	43.9	8.2	0.522
TD3	9 (3*n*)	1 (2*n*)	10.6	86	13.1	2.2	0.179
Mean	**7**	**1**	**7.8**	**85**	**21.2**	**5.2**	**0.314**

Table 2 Chromosome number composition (%) of Pacific oysters produced from diploid female × triploid male (DT) and reciprocal (TD) crosses observed at Year 1 and Year 4 of age

Group	N	*Chromosome number*							*Group summary*				
		20	*21*	*22*	*23*	*28*	*29*	*30*	*20–23*	*28–30*	*An*	*An*/2n	*An*/3n
Year 1													
DT1	20	20.0	35.0	30.0	15.0	0	0	0	100.0	0	80.0	80.0	0
DT3	20	10.0	25.0[a]	45.0	5.0	10.0	5.0	0	85.0	15.0	90.0	75.0	15.0
Mean		**15.0**	**30.0**	**37.5**	**10.0**	**5.0**	**2.5**	**0**	**92.5**	**7.5**	**85.0**	**77.5**	**7.5**
TD1	20	60.0	10.0	0	0	5.0	5.0	20.0	70.0	30.0	20.0	10.0	10.0
TD2	19	31.6	5.3	0	0	0	10.5	52.6	36.8	63.1	15.8	5.3	10.5
TD3	20	80.0	0	0	0	0	0	20.0	80.0	20.0	0	0	0
Mean		**57.2**	**5.1**	**0.0**	**0.0**	**1.7**	**5.2**	**30.9**	**62.3**	**37.7**	**11.9**	**5.1**	**6.8**
Year 4													
DT1	13	7.7	61.5	30.8	0	0	0	0	100.0	0	92.3	92.3	0
DT3	24	25.0	58.3	12.5	0	0	4.2	0	95.8	4.2	75.0	70.8	4.2
Mean		**16.4**	**59.9**	**21.7**	**0**	**0**	**2.1**	**0**	**97.9**	**2.1**	**83.7**	**81.6**	**2.1**
TD1	17	47.1	0	0	0	0	11.8	41.2[b]	47.1	53.0	11.8	0	11.8

[a]Including one 21/26 mosaic.
[b]Including one 20/30 mosaic.
The diploid number in this species is 20, and aneuploids (An) at diploid and triploid levels were labeled as An/2*n* and An/3*n*, respectively.

DT1, four of the 20 oysters (20%) sampled had the diploid number of 20 chromosomes, and the other 16 (80%) were aneuploids: seven with 21 ($2n+1$, trisomic), six with 22 ($2n+2$) and three with 23 ($2n+3$) chromosomes. No triploids or hypotriploids were observed. In DT3, two of the 20 oysters (10%) were diploid, and all others (90%) were aneuploids. A total of 15 of the aneuploids were hyperdiploids with 21, 22 and 23 chromosomes. One oyster in DT3 was a mosaic for 21/26 chromosomes. Three of the aneuploids were hypotriploids: two with 28 ($3n-2$) and one with 29 ($3n-1$) chromosomes. Representative metaphases are present in Figure 1.

The majority (88.1%) of oysters from TD groups were euploid, and aneuploids only accounted for 11.9% of the TD oysters analyzed (Table 2). Among the euploids, about two-thirds (57.2% of the total) had 20 chromosomes, and about other third (30.9% of the total) were triploids with 30 chromosomes. There were some variations among the three TD groups in the frequency of chromosome number observed. TD3 had primarily diploids and no aneuploids, while TD2 had more triploids than diploids. TD1 had frequencies that were similar to group means. Among the seven aneuploids observed in TD groups, three were trisomics ($2n+1$) and four were hypotriploids ($3n-1$, $3n-2$). Overall, it was clear that TD groups produced more euploids (88.1%) and fewer aneuploids (11.9%) than DT groups (15 and 85%, respectively), and the difference is highly significant (χ^2, $P<0.0001$).

At Year 4, chromosome number was successfully determined for 54 oysters: 13 for DT1, 24 for DT3 and 17 for TD1 (Table 2). Several oysters in DT1 suffered unexpected mortality before chromosome analysis. Overall, the number of diploid, triploid and aneuploid oysters observed at Year 4 were similar to that observed at Year 1 (Figure 2). For DT groups, frequencies of diploid, triploid and aneuploid oysters at Year 4 were 16.4, 0 and 83.7%, respectively, compared with 15.0, 0 and 85% at Year 1. Within the aneuploid group, however, there was a noticeable change in frequencies of different aneuploid chromosome numbers. Aneuploids with 23 and 29 chromosomes were observed at Year 1, but disappeared in Year 4. The frequency of oysters with 22 chromosomes decreased, while the frequency of trisomics increased. In TD1, the frequency of triploids increased. Some of the changes may be incidental due to sampling error.

Chromosome number and body size

Shell height and whole body weight of 1-year old oysters are grouped by chromosome numbers and presented in Table 3. ANOVA revealed significant effects of chromosome number on body size (ANOVA, $P<0.001$). Triploids (30 chromosomes) were significantly bigger than diploids (20 chromosomes) in both shell height (by 18%)

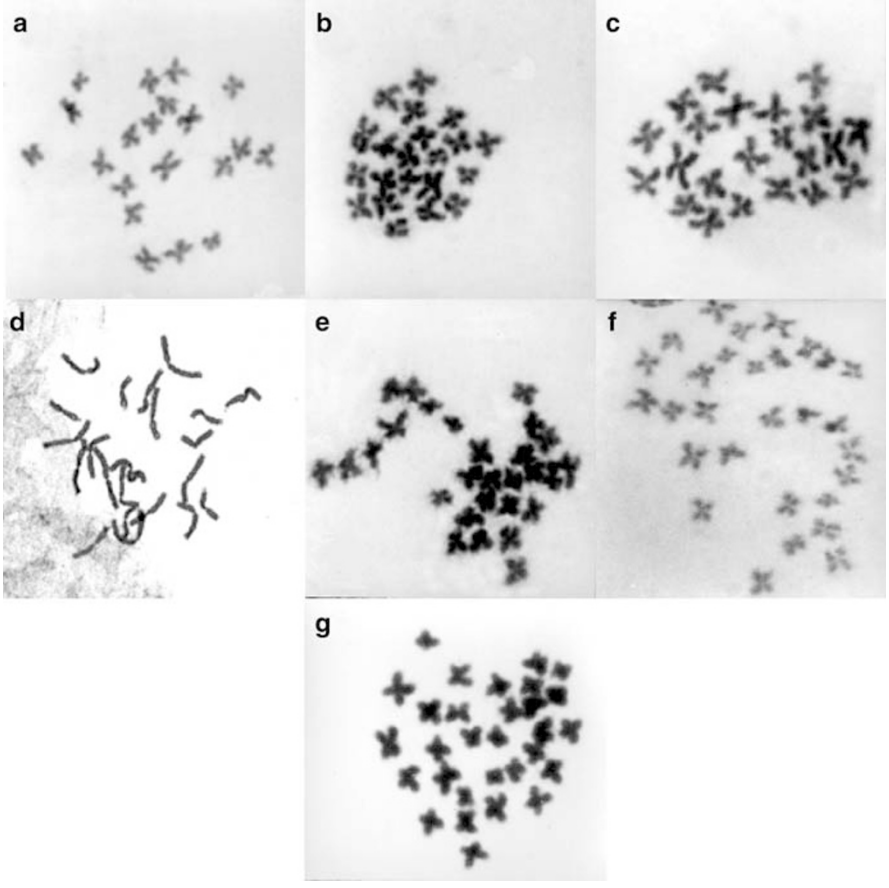

Figure 1 Representative metaphases of diploid, triploid and aneuploid Pacific oysters produced from diploid × triploid crosses: (**a**) $2n=20$; (**b**) $2n+1=21$; (**c**), $2n+2=22$; (**d**), $2n+3=23$; (**e**), $3n-2=28$; (**f**) $3n-1=29$; (**g**) $3n=30$ chromosomes.

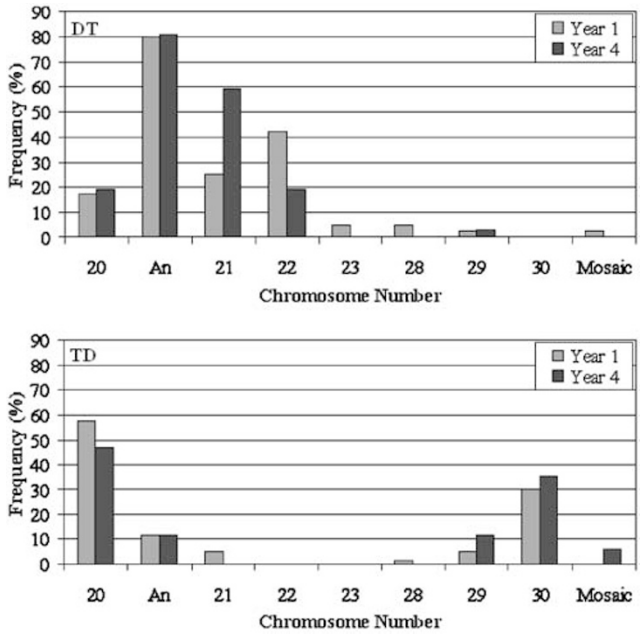

Figure 2 Frequency of oysters with different chromosome numbers produced from diploid female × triploid male (DT) and reciprocal (TD) crosses in the Pacific oyster observed at Year 1 and Year 4. An refers to all aneuploids combined.

Table 3 Body size (±SE) by chromosome number of oysters produced from diploid × triploid crosses measured at 1 year of age

Chromosome	N	*Length (mm)*	*Whole weight (g)*
20	39	33 ± 2^{b}	3.4 ± 0.5^{b}
21	15	25 ± 3^{c}	2.3 ± 0.5^{bc}
22	15	19 ± 2^{c}	1.0 ± 0.2^{c}
23	4	19 ± 2^{c}	0.9 ± 0.2^{b}
28	4	31 ± 4^{abc}	3.0 ± 0.9^{abc}
29	4	23 ± 3^{bc}	1.1 ± 0.3^{bc}
30	18	39 ± 3^{a}	5.6 ± 1.3^{a}

Groups that do not share any letter are significantly different from each other.

and whole body weight (by 65%). Hyperdiploids were significantly smaller than normal diploids in shell height. In body weight, oysters with 22 chromosomes were smaller than diploids, while the other hyperdiploids were not significantly different from diploids. The lack of significant difference is probably due to the small number of aneuploids observed.

Body size measurements by chromosome number of 4-year old oysters are presented in Table 4. Effects of chromosome number on body size at Year 4 were highly significant in all measurements (ANOVA, $P<0.001$ or $P<0.0001$). Diploid oysters measured 70 mm in shell height, 42.4 g in whole body weight and 9.3 g in wet tissue weight. Triploids were significantly bigger than diploids in all measurements except in condition index. Triploid gigantism (percent increase over diploids) was 23% in shell height, 24% in shell length, 23% in shell width, 79% in whole body weight and 98% in meat weight. Hyperdiploids were significantly smaller than normal diploids in all direct measurements. In condition index, oysters with 22 chromosomes were smaller than diploids, while trisomics were not significantly different from diploids. Oysters with 29 chromosomes were significantly smaller than triploids, despite the small sample size. Aneuploids, when combined into a single group ($n=42$), were significantly smaller than diploids in all measurements ($P<0.01$).

Although aneuploids and triploids differed significantly from diploids in group means, there was considerable variation in body size within each chromosome number group (Table 1 and 4). Most chromosome number groups overlapped each other in size. Further, we could not detect any visible phenotypic difference among diploid, triploid and aneuploid oysters. Sampling at Year 1 was conducted during the spawning season, and all oysters sampled showed close to normal levels of gonadal development for 1-year old oysters. It was not possible to distinguish triploids and aneuploids from diploids by the appearance of gonads alone.

Discussion

Reproductive potential of triploids

This study confirms early observations that triploid Pacific oysters produce functional gametes (Allen, 1987; Guo, 1991) and viable offspring (Guo and Allen, 1994a). The relative fecundity of triploid females observed in this study, 13.4%, is higher than the 2% observed in the previous study (Guo and Allen, 1994a). Triploids used in the previous study were produced from diploids by blocking polar body II. Triploids used in this study were mated triploids from diploid × tetraploid mating, and the tetraploids were produced from selected triploid females with high fecundity (Guo and Allen, 1994c). The mated triploids may have inherited genes responsible for high fecundity in triploids through their highly fecund triploid grandmothers. Despite the artificial selection on fecundity through the triploid grandmother, reproductive potential of mated triploids is relevant because they are being used for commercial aquaculture production (Guo *et al*, 2001).

Survival to spat of TD crosses (0.314%) was about 44 times higher than that of DT crosses (0.007%). The same pattern was observed in the previous study, where TD groups survived 65 times better than DT crosses (Guo

Table 4 Body size (±SE) by chromosome number of oysters produced from diploid × triploid crosses measured at 4 years of age

Chromosome	N	*Height (mm)*	*Length (mm)*	*Width (mm)*	*Whole wt(g)*	*Meat wt(g)*	*CI (%)*
20	15	70 ± 2^{b}	38 ± 2^{b}	22 ± 1^{b}	42.4 ± 4.2^{b}	9.3 ± 1.0^{b}	21.9 ± 0.6^{a}
21	22	54 ± 3^{c}	33 ± 1^{c}	18 ± 1^{c}	27.9 ± 3.0^{c}	5.6 ± 0.6^{c}	20.0 ± 0.6^{b}
22	7	50 ± 3^{c}	30 ± 3^{c}	18 ± 1^{c}	19.8 ± 3.8^{c}	3.3 ± 0.6^{c}	16.9 ± 1.2^{c}
29	3	59 ± 7^{bc}	33 ± 3^{bc}	19 ± 3^{bc}	26.5 ± 7.6^{bc}	5.9 ± 1.9^{bc}	21.4 ± 1.3^{ab}
30	6	86 ± 4^{a}	47 ± 2^{a}	27 ± 2^{a}	75.7 ± 9.6^{a}	18.4 ± 2.5^{a}	24.1 ± 0.7^{a}

Groups that do not share any letter are significantly different from each other.

and Allen, 1994a). This observation is not accidental and may be a reflection of real differences in chromosome inheritance between triploid females and males (see below). Assuming that triploid males have about the same relative fecundity as triploid females (13.4%), reproductive potential of triploids in DT and TD mating is about 0.0046 and 0.2104%. On average, triploids have a relative reproductive potential of 0.1075% (about 1/1000), when mated with normal diploids. The reproductive potential estimated here is higher than the previous estimate of 0.0046% or 1 in 22 000 (Guo and Allen, 1994a). The difference is partly due to the increased fecundity (by about seven-fold) of mated triploids and partly due to increased survival. Larval survival is variable, and estimates provided here are provisional and for hatchery conditions only. Nevertheless, this study provides the first estimate of the reproductive potential of mated triploids produced from tetraploids. The use of existing diploid data may be an additional source of variation, but it should not affect the magnitude of the estimates. It is clear that triploid Pacific oysters are fertile, and more estimates are needed for an accurate understanding of their reproductive potential.

Chromosomal inheritance

The finding of aneuploids among progeny from DT and TD crosses is not surprising. Triploid Pacific oysters produce primarily aneuploid gametes with an approximately normal distribution of chromosome numbers between 10 ($1n$) and 20 ($2n$) and around a mean of 15 ($1.5n$) (Guo and Allen, 1994a). It has been shown that the Pacific oyster tolerates a variety of chromosome numbers including: $2n-1$, $2n+1$, $3n-2$, $3n-1$, $3n$, $3n+1$, $3n+2$, $3n+3$, $4n-2$, $4n$-1, $4n$ and $4n+1$ (Guo and Allen, 1994c; Wang *et al*, 1999). This study adds two more viable chromosome numbers, $2n+2$ and $2n+3$, to the list of viable chromosome numbers in this species. Since DT and TD crosses cover all possible chromosome number variation between $2n$ and $3n$, the absence of chromosome numbers between 24 and 27 suggests that they are lethal in the Pacific oyster. It seems that the Pacific oyster can tolerate aneuploidy with two-chromosome additions and one-chromosome loss to its diploid genome. Similarly, the pearl oyster *Pinctada martensii* can tolerate two-chromosome gains to its diploid genome (He *et al*, 2000). *Drosophila* can tolerate about 10% chromosome gain and 3% chromosome loss, and human for 6% gain and 3% loss (Hecht and Hecht, 1987).

A new and rather surprising finding from this study is that oysters from DT crosses were primarily aneuploid (85%), while oysters from TD crosses were primarily diploid and triploid (88%). Triploids with 30 chromosomes accounted for 30.9% of oysters from TD crosses, but were absent in DT crosses. These differences suggest that chromosome inheritance differs significantly between triploid females and males. Triploid females somehow produced more diploids and triploids than triploid males, which produced more aneuploids. There is no comparable data in the literature. In a previous study, triploid females produced more triploid progeny than triploid males when mated with diploids (Guo and Allen, 1994a), but the ploidy was determined by flow cytometry, which was not sensitive enough to detect aneuploids. Guo and Allen (1994a) have suggested that meiotic segregation in triploid males is accurate and produces strictly aneuploid gametes by random segregation of the three sets of chromosomes; they propose that segregation in eggs, on the other hand, is selective, favoring the production of euploid gametes. Our data seem to support Guo and Allen's hypothesis.

The difference in chromosome inheritance between triploid females and males explains the observation that TD crosses survived 44 times better than DT crosses. TD crosses survived better than DT crosses, probably because of the production of euploid progeny.

Chromosome number and body size

Results of this study demonstrate that chromosome number significantly affects body size in the Pacific oyster. Triploids are significantly bigger than diploids, while aneuploids are significantly smaller than diploids. Triploids have been produced and evaluated in over 20 species of molluscs and in most species studied so far, triploids are bigger than diploids (Guo *et al*, 2001). This phenomenon has been referred to as triploid gigantism, and several hypotheses have been advanced attributing triploid gigantism to sterility, increased heterozygosity or larger size of triploid cells (Guo and Allen, 1994b). The level of triploid gigantism observed here, 79% in whole body weight and 98% in meat weight, is among the highest reported for this and other species. Triploid gigantism is generally between 30 and 50% in most species studied so far (Guo, 1999). The data presented here are robust, considering that oysters were measured before ploidy determination and the comparisons were made within groups.

Aneuploidy or hyperdiploidy has a negative effect on body size in the Pacific oyster as has been shown in other organisms. In human, most trisomics are lethal, and Trisomy 21 (Down Syndrome) causes growth and mental retardation. In the pearl oyster *Pinctada martensii* (Dunker), aneuploids or hyperdiploids ($2n\pm1$ and $2n\pm2$) were also smaller than diploids (He *et al*, 2000). No comparable data exist in the Pacific oyster. Aneuploids at the triploid level were not significantly different from triploids, probably due to small sample size (Wang *et al*, 1999). Negative correlations between somatic aneuploidy (or hypodiploidy) and growth has been reported in the Pacific oyster (Thiriot-Quievreux *et al*, 1988; Zouros *et al*, 1996; Leitao *et al*, 2001). Zouros *et al* (1996) has proposed that the negative correlation between aneuploidy and growth is caused by unmasking of deleterious genes from progressive haploidization or chromosome loss. The observation in this study, that hyperdiploids are also smaller than normal diploids, argues against the unmasking hypothesis. The fact that the addition of a complete set of chromosomes in triploidy has no negative effects on body size, supports that prevailing hypothesis that aneuploidy negatively affects body size because of altered gene dosages or regulating systems (Guo and Birchler, 1994). Furthermore, we saw no evidence in this and other studies that somatic hypodiploidy was a real phenomenon. Loss of chromosomes is a common artifact of chromosome spreading by air-drying. In our experience, chromosome loss is apparently associated with 'bad' metaphases, and metaphases of high quality rarely show hypodiploid chromosome numbers (Wang *et al*, 1999).

Evolutionary and practical implications

It is clear that triploid Pacific oysters are not sterile. The reproductive potential of one in 1000 is small for a given individual, but may be significant at evolutionary scales. Triploids produce diploid, triploid and aneuploid progeny when mated with normal diploids. A previous study has shown that fertile tetraploids can arise from triploid × triploid crosses (Guo and Allen, 1994a). Triploids are not only viable and vigorous, but also have a size advantage over normal diploids. It is possible that triploidy, which occurs spontaneously in most organisms, represents a 'chromosome lottery' in evolution and possibly leads to the emergence of triploids, tetraploids and other new chromosome numbers. It is unknown to what extent triploidy has contributed to chromosome number changes in molluscs, but the results of this study suggest that such a possibility exists. The evolutionary significance of triploidy is probably limited in *Crassostrea* oysters, since all species studied so far had a haploid number of 10 chromosomes (Nakamura, 1985). However, triploidy is not an evolutionary dead end in the Pacific oyster and probably not in most other mollusks either. In other groups of molluscs, variation in chromosome number is common, and triploid species exist along with diploids (Patterson, 1969; Komaru *et al*, 2000).

Owing to their superior growth and 'perceived' sterility, triploids are being used for aquaculture production and field evaluation of non-native species. Most molluscs are cultured in open marine systems. Triploids, when completely sterile, can be used for the containment of non-native species or genetic-modified strains/organisms. In the Pacific oyster and probably most molluscs, triploids are not completely sterile and cannot provide complete containment. Since triploids have greatly reduced reproductive potential compared with diploid stocks, the use of triploids in aquaculture can still reduce 'genetic pollution' of wild populations by selected strains. On the other hands, the production of aneuploid progeny is unwanted. When large populations of triploids are deployed for aquaculture production, they could affect chromosome number of wild populations.

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