

Short communication

The growth, survival and ploidy of diploid, triploid and tetraploid of the Pacific oyster (*Crassostrea gigas*) in larval and juvenile stages

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ABSTRACT

Slow growth and genomic instability are the main problems facing tetraploid oyster breeding. In response to the above problem, we developed tetraploid by using ‘Haida No.3’, a new variety with rapid growth and black shell color, and analyzed the growth, survival and ploidy of diploid × diploid (DD), diploid × tetraploid (DT: triploid) and tetraploid × tetraploid (TT) in different periods. The results showed that there was no significant difference between the fertilization rate of DD, DT and TT, but the hatching rate of TT was significantly lower than that of DD ($P < 0.05$). The shell height of TT was significantly lower than DD, and the shell height of DT was significantly higher than that of DD at 13 days ($P < 0.05$). The survival rate of TT was lower than that of DD and DT in larval stage ($P < 0.05$), while there was no significant difference in the mean survival rate of DD, DT and TT in juvenile stage. The tetraploid rate in TT was 100% during the larval and juvenile stages. The shell color of all progeny in TT and DT was black and consistent with ‘Haida No.3’. These results indicated that black shell color of ‘Haida No.3’ could be stably passed on to triploid and tetraploid offspring, suggesting that establishing genomically stable tetraploid populations was feasible by screening tetraploid parents.

1. Introduction

Polyploid oysters were first reported in 1981, and the triploid oyster industry has been developing rapidly in the last two decades (Matt and Allen, 2014; Nell, 2002; Stanley et al., 1981). Triploid oysters play an important role in improving oyster yields and economic benefits because of their sterility and better growth performance (Buestel et al., 2009; Hand et al., 2004; Jeung et al., 2016; Nell, 2002; Normand et al., 2008; Piferrier et al., 2009). Triploid was initially produced by physical or chemical induction, but the high mortality and no guarantee of 100% triploid rates made it difficult to apply in commercial production (Gérard et al., 1999; Guo et al., 2002; Quillet and Panelay, 1986; Scarpa et al., 1994; Yang and Guo, 2006; Yamamoto and Sugawara, 1988). The crossing of tetraploid and diploid can produce 100% triploid, which is an ideal way for triploid to be applied in large-scale production (Guo and Allen, 1994a; Guo et al., 1996). However, when oyster producers are immersed in the production of triploid oysters, thinking that they can get the tetraploids once and for all, they do not realize that there might be a problem with the tetraploid itself.

The growth rate of tetraploid oyster is

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transformation from triploid to mosaic type has also been described by Zhang et al. (2010). This suggested that genomic instability in oyster polyploids exists. Genomic or chromosomal instability could lead to aneuploidy and mosaic individuals, posing a serious challenge to establishing stable tetraploid lines. The presence of aneuploidy in the offspring of tetraploid x tetraploid might indicate that part of tetraploid oysters suffers from reversion (Benabdelmouna and Ledu, 2015). Therefore, it is worth considering whether the stable tetraploid lines can be established by selecting tetraploids whose chromosomes are not reverted.

As a new variety of multi-generation selection, ‘Haida No.3’ variety of *C. gigas* with black shell color and rapid growth is an ideal material for producing tetraploids (Xu et al., 2017; Xu et al., 2020), having the potential to improve the tetraploid growth performance. Through our previous work, we have successively produced the tetraploids of ‘Haida No.3’ with black shell color. However, the inheritance of production traits in polyploid offspring is not clear. In order to assess the production performance among diploid, triploid and tetraploid ploidy of *C. gigas*, we compared and analyzed their insemination, growth, survival and ploidy. In addition, we improved the detection method of tetraploid parents to establish a stable tetraploid population.

1. Introduction

‘Haida No.3’ variety of *C. gigas* was originally derived from the black-shell individuals discovered in the cultured population in 2010. After seven generations of growth improvement and shell color fixation, ‘Haida No.3’ variety of *C. gigas* with rapid growth and black shell color was successfully developed (Xu et al., 2017; Xu et al., 2020). The tetraploid broodstock were produced using eggs from triploids of ‘Haida No. 3’ variety of *C. gigas* that were fertilized with haploid sperm taken from diploids of ‘Haida No.3’ variety of *C. gigas*.

In May 2021, the one-year-old diploid and tetraploid parents used in this study were transferred to oyster hatchery in Laizhou (Shandong Province, China) three weeks before the experiment started. During the temporary rearing period, all broodstock were conditioned at the temperature of 24 ± 1 °C and salinity of 30 ± 1 psu and fed with

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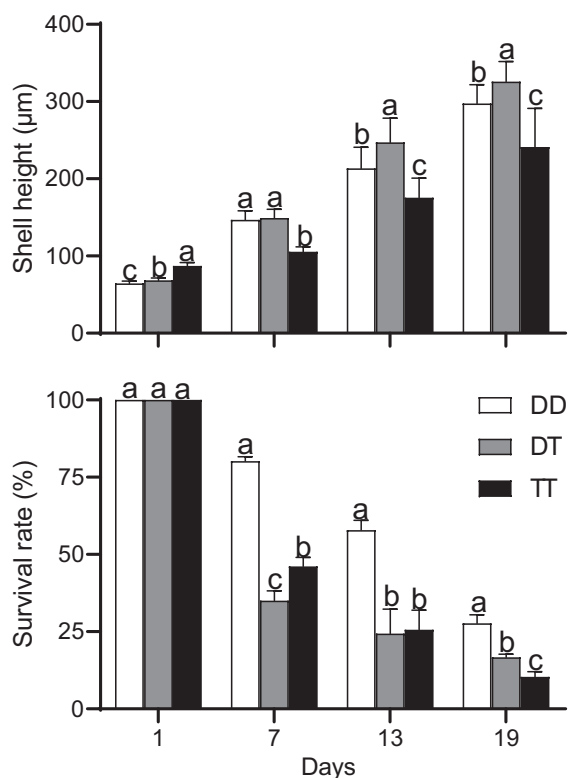


Fig. 1. Survival rate and shell height of diploid x diploid (DD), diploid x tetraploid (DT) and tetraploid x tetraploid (TT) crosses of during the larval stage. Different lowercase letters indicate significant differences ($P < 0.05$).

between DD and DT groups ($P > 0.05$). From day 13, the mean shell height of DT ($247.29 \pm 31.18 \mu\text{m}$) was significantly higher than that of DD ($213.53 \pm 27.22 \mu\text{m}$) and TT ($175.36 \pm 25.42 \mu\text{m}$), and that of DD was significantly higher than that of TT ($P < 0.05$). From 50 days to 120 days, the mean shell height of crosses DD, DT and TT increased by 28.26 mm, 31.73 mm and 17.16 mm, respectively (Table 2). The mean shell height of DT cross was significantly higher than that of DD and TT crosses ($P < 0.05$), and the mean shell height of DD cross was significantly higher than that of TT cross ($P < 0.05$).

During the larval stage, the survival rates of DD, DT and TT crosses at day 19 dropped to 27.73%, 16.74% and 10.41%, respectively (Fig. 1). The survival rate of DD cross was higher than that of DT and TT crosses throughout the larval period. At day 7, the survival rate of DT and TT occurred a sharp decrease and the survival rate of TT cross was higher than that of DT ($P < 0.05$), while the survival rate of DT was higher than that of TT at day 19 ($P < 0.05$). In contrast to the low survival rate of larval stage, DD (88.65%), DT (87.74%) and TT (87.84%) had a high survival rate between 50 and 120 days (Table 2).

Table 2. Shell height and survival rate of diploid x diploid (DD), diploid x tetraploid (DT) and tetraploid x tetraploid (TT) crosses of on days 50 and 120. Different superscript letters in each column indicate significant difference ($P < 0.05$).

Cross	Shell height (mm)		Survival rate (%)
	50 d	120 d	120 d
DD	4.47 ± 0.89 ^b	32.73 ± 3.17 ^b	88.65 ± 3.72 ^a
DT	5.83 ± 1.39 ^a	37.56 ± 2.08 ^a	87.74 ± 2.46 ^a
TT	3.62 ± 0.47 ^c	20.78 ± 5.14 ^c	87.84 ± 3.50 ^a

The triploid and tetraploid rates of DT and TT crosses were 100% in larval and juvenile stages, respectively (Fig. 2). Triploid and tetraploid progenies showed black shell color, being the same as diploid 'Haida No. 3' (Fig. 3).

A large number of malformed larvae in DT and TT crosses was the main reason for the low hatching rate, while there were almost no malformed larvae in DD cross, suggesting that the abnormal larvae in DT cross were caused by the increase of intracellular chromosome. The malformed larvae in the TT cross might be the offspring of the first-generation of tetraploid and tetraploid hybrids, which have not yet adapted to the doubling of the chromosome (Comai, 2005). Low hatching rates of tetraploids have also been reported in previous studies (Guo et al., 1996; Zhang et al., 2022). The development of tetraploids based on selective breeding strains might be beneficial to the survival of their offspring, but the adverse effects of chromosome doubling still played an important role in the hatchability of offspring, such as more complex pairing and segregation interactions leading to mitotic abnormalities (Comai, 2005).

Considering that almost all larvae in DD cross are D-shaped larvae when DT cross is still largely composed of trochophore larvae, the most likely reason for the difference in the size of newly hatched D-shaped larvae between the DT and DD crosses was that the triploid oyster cells were larger than the diploid ones, or more precisely, it's caused by an increase in cell size without a decrease in cell number (Guo and Allen, 1994b). In oysters, triploid growth usually exceeds diploid growth after a year, when diploids begin to allocate large amounts of energy resources to reproduction (Stanley et al., 1984; Allen and Downing, 1986). In this study, the mean shell height of triploid oysters was always higher than that of diploid oysters from day 13. This might be related to the increased heterozygosity of the triploid gene (Wang et al., 2002). More importantly, triploids might inherit superior growth performance from their parents (Leeds and Weber, 2019). Tetraploid oysters could inherit the excellent production performance of their parents. For example, tetraploid oysters could inherit disease resistance (Guo et al., 2002). In this study, tetraploid offspring still remained black shell color, indicating that black shell color could be inherited. Rapid growth and black shell color are traits specific to 'Haida No.3' of , which mean that rapid growth may also be inherited, just like black shell color.

Generally, survival or vigor of neopolyploids was low in synthetic populations (Gaeta et al., 2007; Matsushita et al., 2012). It has also been reported that the survival rate of tetraploid aquatic animals was lower than that of diploid animals (Cassani et al., 1990; Hörstgen-Schwark, 1993; Zhou et al., 2010). In this study, the survival rate of tetraploid larvae was found to be higher than that of previous study (Guo et al., 1996). This might mean that selective breeding strain for the development of tetraploids is beneficial to improve the survival performance of tetraploids. In the present study, the differences of survival rate between DD and TT groups in larval and juvenile stages suggested that tetraploids adapted to chromosome changes could survive like diploids.

If chromosome loss of tetraploid oysters was real, and not due to the testing method, then tetraploid oyster genome instability could be divided into the following two situations. Firstly, tetraploid oyster genome instability is general, but the timing of chromosomal loss is

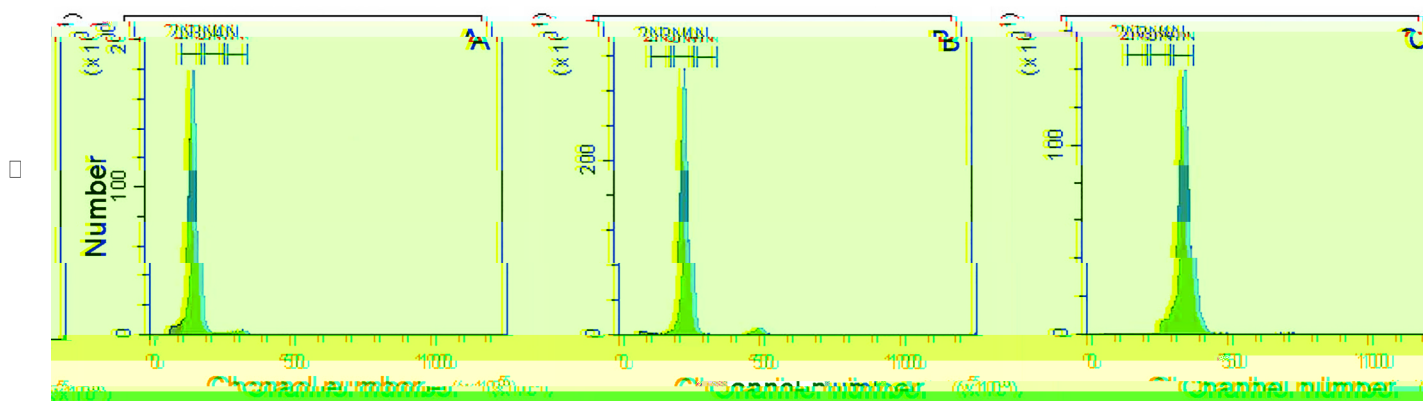


Fig. 1. Flow cytometry graphs of offspring in diploid × diploid (DD), diploid × tetraploid (DT) and tetraploid × tetraploid (TT) crosses of *Pinctada fucata* at day 120. A, DD offspring; B, DT offspring; C, TT offspring.

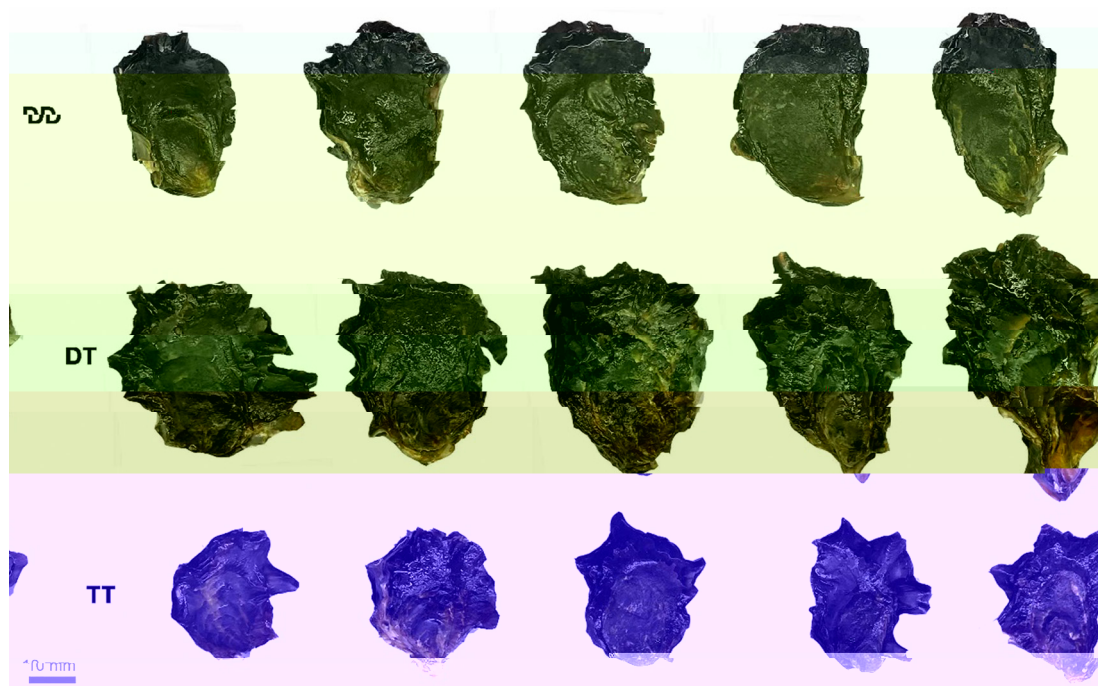


Fig. 2. The offspring of diploid × diploid (DD), diploid × tetraploid (DT) and tetraploid × tetraploid (TT) crosses of *Pinctada fucata* at day 160.

different in different tetraploids. In this case, the tetraploid oyster genome is relatively stable until the chromosomes started to decay. Secondly, the genome instability of tetraploid oyster is not general and part of the tetraploid oyster genome is always stable. Either way, genomically stable tetraploids exist. Therefore, selecting tetraploids with stable genomes to establish a tetraploid population would be important for improving tetraploid performance. In this study, the tetraploid rate in the TT cross was always 100%, proving that establishing genome-stable tetraploid population by screening tetraploid parents was feasible to some extent. The secretion and distribution of black pigmentation are controlled by two independent loci (Xu et al., 2019). And black pigmentation is identified as the foreground color, while gold and white are the background colors (Ge et al., 2015). The shell color of the offspring of ‘Haida no. 3’ of *Pinctada fucata* is normally black, but there is a possibility that ploidy changes may affect the expression of genes related to the shell color of the offspring. In this study, Triploids in DT cross and tetraploids in TT cross still retained the same black shell color as ‘Haida No.3’, suggesting that the ploidy change did not affect the trait of black shell color. This proves that the polyploid

development of selectively bred diploid strain with important economic traits had great potential in the oyster industry.

1. Introduction

The growth, survival and ploidy of diploid, triploid and tetraploid of ‘Haida No.3’ were studied. The significant differences in survival rate of different ploidy only appeared in larval stage. Triploids had a faster growth rate than diploids and tetraploids. Black-shell color trait of diploid ‘Haida No.3’ could be inherited stably in both triploid and tetraploid offspring. It is feasible to establish genomically stable tetraploids by screening tetraploid parents. This study provides meaningful implications for the development of tetraploids with important economic traits and the establishment of genomic stability of tetraploids.

2. Materials and methods

2.1. Investigation, methodology, completion of the

experiment, software, data analysis, formal analysis, writing-original draft, writing-review. : Conceptualization, experimental design, coordination and manuscript revision, visualization, supervision, review-editing, funding acquisition, project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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