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Effect of inbreeding on performance and genetic parameters of growth and survival traits in the Pacific oyster *Crassostrea gigas* at larval stage

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ABSTRACT

The occurrence of inbreeding over generations cannot be avoided in breeding practice because of limited effective population sizes, and inbreeding is expected to decrease the heritability within populations. The Pacific oyster (*Crassostrea gigas*) is the most widely cultured shellfish species worldwide, however, the effects of inbreeding on the heritability of its economic traits remain unclear. In this study, the effect of inbreeding on the performance and heritability of growth and survival traits were investigated by an inbred line of the Pacific oyster. Two inbreeding groups, a low inbreeding group containing 25 F1 families and a high inbreeding group containing 25 F2 families, were constructed by this inbred line. The performance and heritability of shell height, shell length and survival traits of two inbreeding groups were compared with that of the wild group including 25 families at larval stage. The performance of growth and survival traits was affected negatively by inbreeding. Using the animal model, heritability was estimated for shell height, shell length and survival. Lower heritability in growth traits was observed in two inbreeding groups. However, for survival rate, the estimations of heritability were not influenced by inbreeding obviously. The medium heritability of larval survival rate (0.29–0.34) observed in the inbreeding groups indicates that their survival trait still has a good potential for selection. These results will helpful for the management of inbreeding in shellfish breeding programs.

1. Introduction

Aquaculture is the fastest growing food production sector and is rapidly becoming the primary source of seafood for human diets (Gratacap et al., 2019). However, in comparison to terrestrial livestock, relatively little (less than 10 %) aquaculture production is underpinned by modern selective breeding programs (Gjedrem, 2012). Most farmed aquatic species are either still sourced from the wild or in the early stages of domestication, suggesting a substantial potential for genetic improvement in aquatic productions (Gjedrem and Rye, 2018). Molluscan shellfish have traditionally been a major product of world aquaculture, and many selective breeding programs for oyster (Langdon et al., 2003), clam (Zhao et al., 2012), scallop (Zheng et al., 2012), and mussel (Nguyen et al., 2014) species have been initiated worldwide.

For selective breeding to be effective, there must be additive genetic variation present in the current population. Heritability (h^2), the proportion of additive genetic variation to total phenotypic variance, is the central parameter to predict the potential of improving desirable traits through selective breeding (Falconer and Mackay, 1996). Heritability is various with traits, populations, culture conditions as well as different life stages, thus reliable estimate of the heritability is essential for planning a reasonable selective breeding program. In molluscan shellfish, heritability has been reported for many economic traits such as growth (Nguyen et al., 2011; Kong et al., 2015), survival (Dégremont et al., 2007, 2010), shell sharp (Nguyen et al., 2014), shell color (Evans et al., 2009), as well as meat quality (Wan et al., 2020), and the growth and survival as the most important production traits have been found to be generally medium to high.

Selective breeding is a long-term work that requires repeated selection within a closed population over multiple generations, which makes it difficult to avoid inbreeding (Leroy, 2014). Moreover, most aquatic animals are high fecundity and high variance in reproduction success, resulting in higher possibility of inbreeding, especially when there are high selection intensities and without pedigree records (Boudry et al., 2002; Hedgecock and Davis, 2007). Inbreeding increases expression of deleterious recessive alleles and reduces the additive genetic variance within lines, under the assumption of neutrality and purely additive gene action (Falconer and Mackay, 1996). The expression of deleterious

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recessive alleles leads to the reduction of performances of fitness traits, which have been well documented in aquatic animals (Anderson and Hedgecock, 2010; Gao et al., 2015), including many species of shellfish (Evans et al., 2004; Zheng et al., 2012). In theory, declining genetic variance will limit the potential for response to selection in a selective breeding program (Keller and Waller, 2002; Kristensen et al., 2005; Leroy, 2014). Heritability is the central parameter to predict the potential of improving desirable traits through selective breeding as mentioned above; however, the effects of inbreeding on the heritability, especially on different economic traits, are rarely studied in marine shellfish.

The Pacific oyster (*Crassostrea gigas*) is the most widely cultured shellfish species worldwide, and many selective breeding programs have been initiated to improve its growth rate, disease resistant, shell and meat quality traits (Langdon et al., 2003; Dégremont et al., 2007; Wan et al., 2020). A medium to high heritability for growth and survival traits, as well as the effects of inbreeding on these traits during larval and adult stages have been revealed by an abundance of studies (Kong et al., 2015; Evans et al., 2009; Dégremont et al., 2007). However, the effects of inbreeding on the heritability of these economic traits in *C. gigas* are remain unclear. Both the great fecundity and very high larval mortality of *C. gigas* bring a huge risk of inbreeding to its breeding programs, especially for these programs based on mass selection (Hedgecock and Davis, 2007). Therefore, understanding the effects of inbreeding on the heritability of economic traits is essential to the design of efficient long-term breeding strategies of *C. gigas*. Furthermore, the characters of high fecundity and high larval mortality are common in most marine shellfish, thus taking *C. gigas* as the studied species can also provide a reference for the breeding of most marine shellfish.

In our breeding practice of *C. gigas*, four oysters with solid orange shell color were found by chance (Han and Li, 2018). Based on these four orange-shell oysters, an orange-shell line of *C. gigas* was established with three generations of family selection for fixing orange shell color, and followed by five generations of successive mass selection to promote the growth (Han et al., 2019). The orange-shell line is a typical inbred line, and only one mtCOI haplotype was found in its three generations of mass selection populations (Han et al., 2019). Moreover, the special shell color of this line protects it from being contaminated by wild germplasm during culture. Therefore, the orange shell line is an ideal material to study the effects of inbreeding on performance and heritability of economic traits in *C. gigas*.

In this study, a low inbreeding group (25 F1 families) was constructed by the 8th generation of the orange-shell line, and a high inbreeding group (25 F2 families) was constructed using the progeny of 25 full-sib families as parents. Besides, one control group including 25 families was constructed by wild *C. gigas*. The performance and heritability of growth and survival traits were compared among the two inbreeding and one control groups at larval stage, in order to reveal the different effects of inbreeding on the performance and heritability of growth and survival traits in *C. gigas*.

2. Materials and methods

2.1. Broodstock collection

The orange-shell line was constructed on only four orange-shell individuals (two females and two males) in 2011. To fix the orange-shell trait and expand propagation, three generations of family selection were established from 2011 to 2013. Next, five generations of mass selection were established from 2014 to 2018 to enhance the growth performance of the orange-shell line. In each generation of mass selection, the selection intensity was maintained at around 1.8, and about 50 females and 50 males were used as parents. In a previous study, the genetic variability of three successive generations of mass selections were evaluated. Compared to wild populations, significant reduction in expected heterozygosity (He: 34.21–39.24 %), average number of alleles (Na: 69.55–76.92 %) and allelic richness (Ar: 68.17–74.91 %) as well as increased mean pairwise genetic relatedness (R: 6.87–25.79 times) were observed due to the extra small genetically-effective population size (Han et al., 2019).

To compare the larval performance of different experimental groups simultaneously, all the 75 families were produced in 18 June 2019. The parents of the low inbreeding (F1,) group were selected from the 8th generation orange-shell line randomly, and 25 families were produced by crossing one female to one male. The parents of the high inbreeding (F2) group were selected from the progeny of 25 full-sib families, which were established based on the 7th generation orange-shell line by crossing one female to one male in June 2018. One male and one female were selected randomly from each family constructing in 2018 to establish 25 full-sib F2 families in 18 June 2019. The 25 families of non-inbred control (CF) group were established by crossing one female to one male, using the wild individuals collected from Laizhou Bay as parents. The average shell height of parents of three groups were 51.87 $\pm$ 7.89 mm for CF, 38.53 $\pm$ 6.64 mm for F1 and 30.19 $\pm$ 5.94 mm for F2.

2.2. Spawning and rearing

Eggs and sperm of each parental pairs were individually collected and fertilized separately. Fertilized eggs were hatched and fostered in separate plastic buckets. In the whole process of rearing, the larval density of each family was kept roughly the same. Initial larval density was adjusted to 15 larvae/mL, then reduced to 5 larvae/mL and 1 larvae/mL on day 7 and 14 after fertilization, respectively. For each family, the density was adjusted by reducing or increasing the quantity of seawater, if the density was lower or higher than the target density. And ratios of adjustment were recorded to calibrate the survival rate after adjustment. Half volume of water was changed twice per day with fresh filtered seawater for each family in the morning and night, and the seawater temperature was held at 25 °C. Veliger larvae had been provided with of Isochrysis galbana before length of larvae reached 120 μm, and at later stage provided with Isochrysis galbana and Platymonas helgolandica. Food consumption is almost the same for each family.

2.3. Measurement

Replicates were sampled every 5 days from day 1 to day 21. After the water was mixed, a 50 mL sample was collected randomly and fixed by the addition of 1% Lugol's solution. The total number of filled larval shells (distinguishing from empty larval shells) and shell length and shell height of 30 larvae randomly selected in each replicate sample were quantified using a light microscope (100×) fitted with an ocular micrometer (Han and Li, 2018).

2.4. Statistical analysis

The shell length, shell height and survival rate of larvae were separately analyzed at different age of days by one-way ANOVA of SPSS (Statistical Package for Social Science) 20.0 software with family as fixed factor. Differences were considered statistically significant if $P < 0.05$.

The software package ASReml 3.0 was used to estimate the heritability (h^2) and phenotypic and genetic correlations ($r_{P/G}$). . For each trait, the model was applied using Restricted Maximum Likelihood (REML) algorithm as follows:

$$y_i = \mu + a_i + d_j + e_i$$

In this model, observation y from the individual i, was predicted from variables on the right-hand side of the equation. The μ is the mean value of the trait, whereas a_i is the additive effects for the ith animal, d_j is random effect common to full-sibs (a combination of maternal and environmental effects from dam j) and e_i stands for the residual error.

The subsequently logarithmic likelihood ratio test confirmed that the d_j was not significant for all traits. As a result, the d_j was removed from the model. The reduced model was applied as follows:

$$y_i = \mu + a_i + e_i$$

Under this model, heritability estimations of growth traits were calculated as $h^2 = \sigma_a^2 / (\sigma_a^2 + \sigma_e^2)$, and heritability estimations of survival rate were calculated as $h^2 = \sigma_a^2 / (\sigma_a^2 + \sigma_e^2 * 3.28978)$, where σ_a^2 is the additive genetic variance, and σ_e^2 is the residual variance. Genetic and phenotypic correlations between shell length and shell height were calculated as $r_{P/G} = \sigma_{12} / \sqrt{\sigma_1^2 \cdot \sigma_2^2}$, where σ_{12} is estimated additive genetic or phenotypic covariance between the two traits, and σ_1^2 and σ_2^2 are the additive genetic or phenotypic variances of trait 1 and 2, respectively (Xing et al., 2018).

3. Results and discussion

3.1. Performance of larvae growth and survival

The performance of growth and survival rate of larvae was present for each group in Fig. 1. From day 6, shell length and shell height in CF group were higher than that in two inbreeding groups, and lowest in F2 groups ($P < 0.05$, except shell height on day 6, Fig. 1A, B). The survival rates were significantly different in three groups ($P < 0.05$, Fig. 1C), in which CF group was the highest, whereas F2 group was the lowest ($P < 0.05$). In addition, the survival rate of the inbreeding groups decreased more than its growth trait compared with the CF group (Fig. 2). F1 group showed relatively small reduction in shell length (from −2.62 % to −12.78 %) and shell height (from −4.19 % to −8.01 %) compared with the reduction in survival rate (from −31.11 % to −56.89 %) (Fig. 1).

The planktotrophic larval stage of most cultured bivalve species is more vulnerable than their juvenile and adult stages (Keller and Waller, 2002). Inbreeding depression at larval stages has already been studied in bivalves (Hedgecock et al., 1995; Launey and Hedgecock, 2001; Taris et al., 2007; Zheng et al., 2012). These results observed in this study support previous finding that inbreeding cause the reduction of growth and survival traits, and inbreeding depression is expected to be stronger in traits more closely associated with fitness, such as survival rate (Evans et al., 2004; Han and Li, 2018; Huisman et al., 2016; Zheng et al., 2012).

3.2. Genetic parameters

The CF group showed a similar heritability for growth (shell height: 0.27 ± 0.14; shell length: 0.25 ± 0.14) and survival (0.29 ± 0.06) traits at the end of larval experiment (Table 1). Compared with the CF group, both the two inbreeding groups showed lower heritability for shell height and shell length, while the heritability of these growth traits of the F2 group was lower than that of F1 group (Table 1). Different from these growth traits, the heritability of the survival rate was not decreased by inbreeding, and the F1 group (0.34 ± 0.03) showed a higher heritability than that of the CF group (0.29 ± 0.06), while the F2 group (0.29 ± 0.11) showed a same heritability with that of the CF group.

The different effects of inbreeding on the heritability of growth and survival, confirmed that the genes controlling survival segregate independently from genes controlling growth. Contrary to expectations, our result suggested that some significant amount of additive genetic variance for survival, a character closely related to fitness, was maintained within this inbred population, revealing that the survival trait can potentially still improved by selection. It may be explained by the dominance hypothesis that inbreeding will expose deleterious alleles influencing survival to strong selection, which means homozygous recessive deleterious alleles will be purged, to maintain the heterozygosity, resulting in a lower than expected level of inbreeding (Leroy, 2014). Furthermore, the absence of deleterious alleles controlling

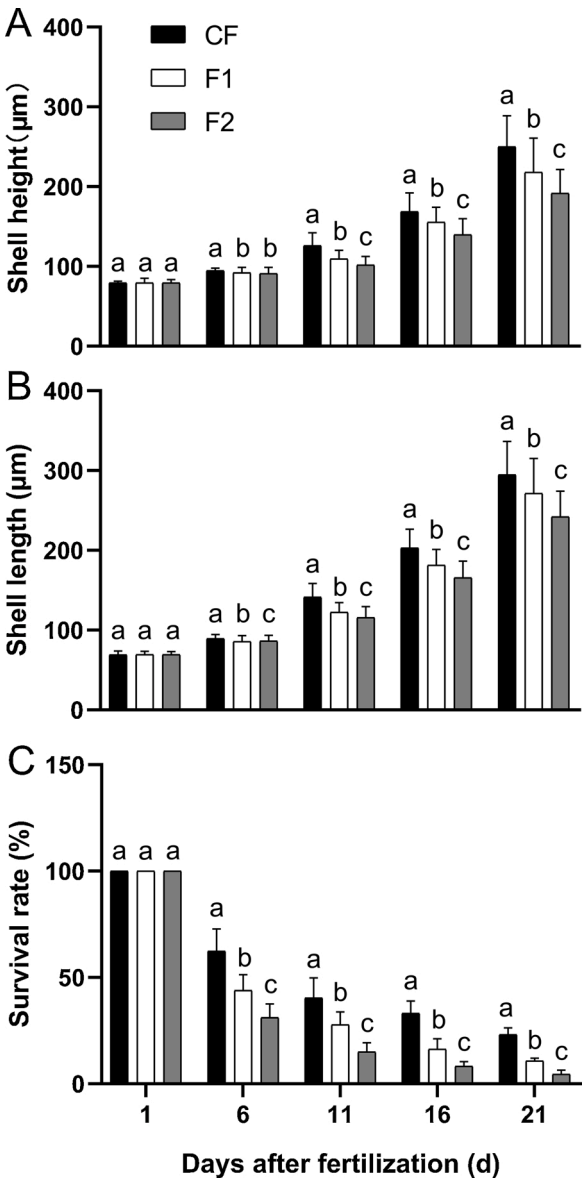

Fig. 1. Comparison of shell height (A), shell length (A) and survival rate (C) among three experimental groups. Different letters of the same day after fertilization indicated significant differences between the two groups ($P < 0.05$).

survival rate with large effects introduced through gene flow will promote elimination of deleterious alleles (Gulisija and Crow, 2007; Hedrick, 1994), which meant relatively higher heterozygosity was maintained at the genetic locus controlling individual survival due to purging genetic load, even if the level of mutational load is apparent (Plough, 2016). However, the heritability of growth traits seems to be affected by inbreeding negatively. In the previous study, no significant difference in Na (3.60–4.40), Ar (3.51–4.08), and He (0.48−0.50) occurred during three generations of mass selection, which means the homozygosity of recessive deleterious alleles of locus controlling growth will increase when inbreeding occurs (Han et al., 2019). Different from survival, these homozygous recessive deleterious alleles will not be purged at larval stage, which leads to reduced heterozygosity and lower heritability of growth traits.

In three experimental groups, both correlation parameters between shell length and shell height were positive with 0.84–1.00 for genetic correlations and 0.37–0.97 for phenotypic correlations (Table 2), and there was no obvious and regular distinction on genetic and phenotypic

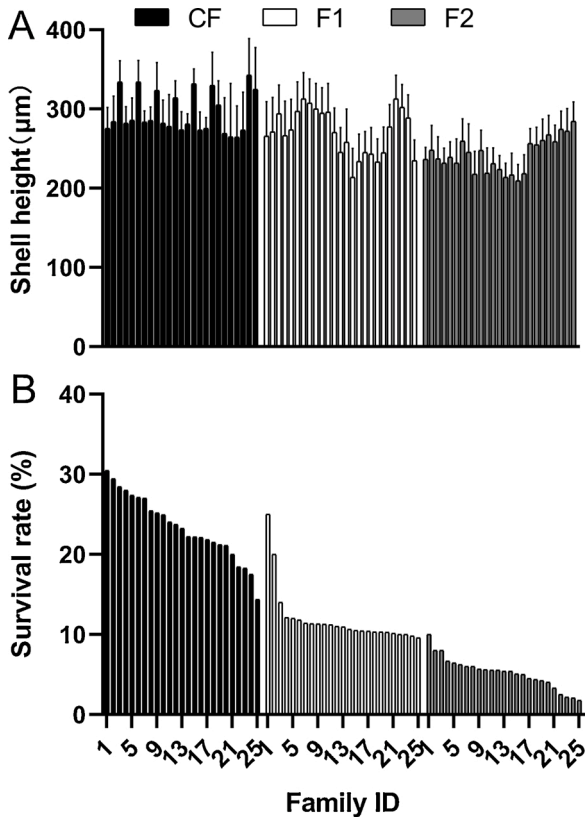

Fig. 2. The shell height (A) and survival rate (B) of each family in three experimental groups at day 21.

Table 1
Heritability estimate of growth and survival traits of different groups.

Traits	Experimental group	Days of age				
		1	6	11	16	21
Shell height	CF	0.45 ± 0.13	0.39 ± 0.13	0.36 ± 0.14	0.30 ± 0.14	0.27 ± 0.14
	F1	0.49 ± 0.12	0.35 ± 0.14	0.29 ± 0.14	0.25 ± 0.15	0.20 ± 0.15
	F2	0.52 ± 0.12	0.34 ± 0.14	0.26 ± 0.14	0.23 ± 0.15	0.18 ± 0.15
Shell length	CF	0.50 ± 0.12	0.44 ± 0.13	0.38 ± 0.13	0.29 ± 0.14	0.25 ± 0.14
	F1	0.46 ± 0.13	0.39 ± 0.13	0.33 ± 0.14	0.27 ± 0.15	0.19 ± 0.15
	F2	0.50 ± 0.12	0.32 ± 0.14	0.26 ± 0.14	0.21 ± 0.15	0.17 ± 0.15
Survival rate	CF	/	0.29 ± 0.05	0.34 ± 0.05	0.33 ± 0.05	0.29 ± 0.06
	F1	/	0.37 ± 0.05	0.36 ± 0.05	0.38 ± 0.06	0.34 ± 0.03
	F2	/	0.38 ± 0.05	0.37 ± 0.06	0.28 ± 0.08	0.29 ± 0.11

correlations in three groups. It thus implied that selection for shell height or shell length could result in favorable changes in another growth trait, and the inbreeding has no effect on the larval shape.

In conclusion, our results demonstrated that the heritability of survival rate was not influenced by inbreeding at larval stage, although inbreeding caused a significant reduction to the survival performance in *C. gigas*. The medium heritability of larval survival rate found in the inbreeding groups indicates that their survival trait still has quite a good potential for selection. These results will contribute to the management of inbreeding in shellfish breeding programs.

Table 2
Genetic correlation and phenotypic correlation for growth traits of different groups.

	Correlation parameter	Days of age				
		1	6	11	16	21
CF	GC	0.89 ± 0.06	0.84 ± 0.07	1.00 ± 0.01	0.97 ± 0.02	0.99 ± 0.01
	PC	0.37 ± 0.06	0.76 ± 0.03	0.95 ± 0.01	0.91 ± 0.01	0.97 ± 0.01
F1	GC	0.95 ± 0.03	0.91 ± 0.04	0.99 ± 0.01	0.94 ± 0.02	0.96 ± 0.02
	PC	0.49 ± 0.05	0.84 ± 0.02	0.87 ± 0.02	0.93 ± 0.01	0.93 ± 0.01
F2	GC	0.89 ± 0.06	0.92 ± 0.03	0.96 ± 0.02	0.99 ± 0.01	0.98 ± 0.01
	PC	0.44 ± 0.05	0.87 ± 0.02	0.85 ± 0.02	0.95 ± 0.01	0.91 ± 0.01

Note: GC means genetic correlation; PC means phenotypic correlation.

CRediT authorship contribution statement

Jiafeng Fang: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing - original draft, Writing - review & editing. **Ziqiang Han:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing - original draft, Writing - review & editing. **Qi Li:** Conceptualization, Formal analysis, Investigation, Methodology, Funding acquisition, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

No conflict of interest exits in the submission of this manuscript, and manuscript is approved by all authors for publication.

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