



Crossbreeding of three different shell color lines in the Pacific oyster reveals high heterosis for survival but low heterosis for growth

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ABSTRACT

Heterosis is important for improving productive traits in aquaculture, but the heterosis between selected lines remains unclear in bivalve molluscs. Here, a diallel crosses among black shell (B, 7th generation), white shell (W, 7th generation) and orange shell (O, 6th generation) lines of the Pacific oyster were performed to examine the potential for exploiting heterosis of productive traits by crossbreeding of selected lines. Heterosis and combining abilities for growth and survival of 27 full-sib families were analyzed at both larval and juvenile stages, of which the juvenile stage was examined at two environments (Rongcheng and Rushan) for 10 months. The variance of specific combining ability accounts for the majority of the total variance for larval and juvenile survival and juvenile growth, which revealed the importance of non-additive genetic effects (heterosis) among these lines. Moreover, all reciprocal hybrids showed low or no heterosis for growth at both larval and juvenile stages, but a high heterosis for larval survival. This finding suggested that inter-line crossbreeding can be applied to improve the larval survival in hatcheries. Furthermore, four of six hybrid crosses showed positive *HPH* in Rushan but negative *HPH* in Rongcheng. The higher *HPH* at Rushan suggested that inter-line crossbreeding in *C. gigas* has more application value in Rushan. In addition, the survival of both black shell and white shell lines have been improved by crossing with the orange shell line. Especially, the hybrid cross WO ($W♀ \times O♂$) can improve the survival rate of purebred WW by nearly 130% at larval stage and nearly 40% at juvenile stage. Overall, this study reveals the high heterosis for survival among selected lines in the Pacific oyster, and will be helpful for the utilization of non-additive genetic effects among existing selected lines in bivalves.

1. Introduction

Selection and crossbreeding are the two major conventional breeding methods commonly used for intraspecific genetic improvement of aquaculture (Hulata, 2001). In cultured bivalve molluscs, selection is the most used approach for stock improvement (Langdon et al., 2003; Nguyen et al., 2014), while crossbreeding is often used to establish a composite base population at the beginning of a breeding program (In et al., 2017; Wang and Côté, 2012; Zhang et al., 2017). Currently, with selective breeding programs for bivalve species being developed worldwide, more and more lines have been successfully improved by family and/or mass selection (Gjedrem and Rye, 2018). Additive genetic variation for productive traits has been accumulated during repeated selection. Nevertheless, a large fraction of genetic variation in productive traits are non-additive component, such as dominance and epistatic effects (Hedgecock et al., 1995). Non-additive genetic component can be utilized to gain favorable heterosis in the

offspring cohort through mate selection in the short term, and to protect the genetic assets of breeders through F2-breakdown in the long-term (Joshi et al., 2018). Crossbreeding among selected lines offers the possibility for exploiting both the additive genetic variation accumulated within a line and the non-additive genetic variation between lines. Inter-line crossbreeding is a strategy that has been widely used in plants and other animal breeding programs, e.g., pig and poultry (Carena, 2005; Wientjes and Calus, 2017), and this strategy may bring further improvements to bivalves.

Substantial non-additive (heterosis) for growth and survival have been demonstrated experimentally by crossbreeding of inbred lines in oyster, and the heterosis for yield is a pervasive phenomenon (Hedgecock and Davis, 2007; Hedgecock et al., 1995). Favorable heterosis has also been obtained by the crossbreeding among three non-inbred lines of the Pacific oyster, which were successively mass selected for two generation from three geographically distant populations (Kong et al., 2017). In the Eastern oysters, however, the heterosis for growth

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and survival between three genetically improved (non-inbred) lines is neither pervasive nor consistent across sites and years (Rawson and Feindel, 2012). Therefore, the expression of non-additive genetic variation is related to parental inbreeding, and susceptible to environmental factors, which has been proved in fish crossbreeding (Fjalestad, 2005; Thoa et al., 2016). Except the effect of rearing environments of progeny and parental genetic distance, the bivalve lines are easily contaminated with wild individuals during grow out. An average of 10% of prospective parents were contaminants in the crosses reported by Hedgecock and Davis (2007). Contamination is thus another major factor that can make the results disturbed without validating parent pedigrees (Hedgecock and Davis, 2007). Therefore, the estimation of non-additive variance is related to the parental genetic distance, parental inbreeding, growing environments of progeny, and contamination of experimental populations. Considering that there were so many factors influencing non-additive genetic variance, it is necessary to examine the hybrid performance between more selected lines to exploit inter-line heterosis in bivalve.

The Pacific oyster (*Crassostrea gigas*) is one of the most widely farmed bivalves worldwide. In our previous work, three selected lines of *C. gigas* with solid black (Xu et al., 2019b), white (Xing et al., 2017) and orange shell color (Han et al., 2019) were obtained by six or seven generations of artificial selection with the targets of fast growth and solid shell color. Shell color, as a natural marker, can be used to distinguish and discard the contamination from wild germplasm or other lines during the growth of a purebred line in bivalves. This character simplifies the management of various lines and helps to improve the accuracy of the estimates of heterosis. Therefore, these different shell color lines are appropriate to study the non-additive variance between selected lines in *C. gigas*. In addition, the orange color is a recessive trait compared to black and white in *C. gigas*, and the shell color of the hybrids between orange and black or orange and white will still be black or white, respectively (Han and Li, 2020). However, the productive performance of the hybrid crosses between black shell and orange shell lines and between white shell and orange shell lines remain unknown.

In this study, a diallel cross among black shell line, white shell line and orange shell line of *C. gigas* was carried out, the growth and survival performances were compared among parental lines and their reciprocal hybrids under two rearing environments. The aims of this study were (1) estimating the heterosis and combing abilities among these three lines; (2) evaluating whether the productive traits such as growth and survival of black shell and white shell lines can be improved by crossing with the orange-shell line.

2. Materials and methods

2.1. Broodstock lines

Three selected lines used in this study were black shell line, white shell line and orange shell line of the Pacific oyster. Both black shell line and white shell line were initiated in 2010, based on relatively black shell and white shell oysters collected from wild populations of *C. gigas* in Rushan, Shandong Province, China, respectively (Xing et al., 2017; Xu et al., 2019b). Selective breeding, targeting at solid shell color (black or white) and rapid growth rate, were established by four consecutive generations of family selection from 2010 to 2013. In 2010, one hundred oysters (50 sires and 50 dams) were selected to found 50 full-sib families as the first generation (G1) of black shell and white shell lines, respectively. From 2011 (G2) to 2013 (G4), about 30 families were produced in each generation for black shell and white shell lines, respectively. After four generations of family selection, solid black shell and solid white shell in the whole shell have been achieved, whereas there was no superior productivity observed in black shell and white shell lines. In order to improve the growth traits of black shell line, five preferable full-sib families were selected from G4 in 2014, and 60

oysters (30 sires and 30 dams) with solid black shell and greatest shell height were selected from these five families as parents to produce the first generation of mass selection (G5) (Xu et al., 2019b). In the same year, five preferable full-sib families were selected from the fourth generation of white shell line, of which oysters with solid white shell as well as greatest shell height were used as parents to produce the first generation of mass selection with the purpose of improving the growth trait (Xing et al., 2017). In the first generation of the mass selection, the black and white selected lines grew by 9.04% and 9.01% larger than the control lines when harvest on day 490, respectively (Wang et al., 2016). In 2015 and 2016, the next two successive generations of mass selection were produced for black shell and white shell lines, respectively. In each generation, the number of parents was ranged from 75 to 100, and the selection intensity was about 1.40, as well as the genetic gain for shell height was about 10% (Wang et al., 2016; Xu et al., 2019a). The growth performance of black shell and white shell lines have been significantly improved compared to the wild population after generations of selection (Xing et al., 2017; Xu et al., 2020). Moreover, the genetic diversity of black shell and white shell lines had been assessed by microsatellites as well as mitochondrial COI region in previous studies. For the G5 to G7 of the black shell line, only two haplotypes were observed in each generation, and the mean allelic richness was ranged from 5.60 to 5.80, as well as the expected heterozygosity was ranged from 0.65 to 0.68 (Xu et al., 2019b). For the G5 to G7 of the white shell line, the mean allelic richness was ranged from 6.80 to 7.10, and the expected heterozygosity was ranged from 0.67 to 0.70 (Xing et al., 2017).

Orange shell line was established based on only four *C. gigas* (two males and two females) with orange left and right shells. These four orange shell oysters were used as parents to produce two full-sib families as the first generation in 2011. Next, two consecutive generations of family selection were established from 2012 to 2013 to fix the shell color. In order to enhance the growth performance, three generations of mass selection were performed from 2014 to 2016. In each generation of mass selection, the number of parents was about 100, and the selection intensity was about 1.90 (Han et al., 2019). Despite the orange shell line has been selected for six generations, it still has no advantages in growth and survival compared to the wild population (Han and Li, 2018). For the genetic diversity of the orange shell line, only one haplotype was observed in each generation of G4 to G6, and the mean allelic richness of G4 to G6 was ranged from 3.51 to 4.08, as well as the expected heterozygosity of G4 to G6 was ranged from 0.48 to 0.50 (Han et al., 2019). In July 2017, one-year-old sexually matured oysters were randomly chosen as broodstock from the black shell line (7th generation), white shell line (7th generation) and orange shell line (6th generation), respectively.

2.2. Crossing design and rearing

A complete 3 × 3 diallel cross among oysters from black (B), white (W) and orange (O) shell lines produced nine experimental groups, including three purebred groups BB (B♀ × B♂), WW (W♀ × W♂), OO (O♀ × O♂) and six reciprocal hybrids BW (B♀ × W♂), WB (W♀ × B♂), BO (B♀ × O♂), OB (O♀ × B♂), WO (W♀ × O♂) and OW (O♀ × W♂). Each experimental group was replicated by three independent, pairwise matings, and a total of 27 full-sib families were used in this study.

For all mating experiments, artificial fertilization and larval rearing management were conducted following the standard procedure described by Li et al. (2011). Briefly, oysters of each line were separated by sex. Three dams and three sires were randomly chosen and ranged in shell height for each of the lines as follows: B, 100.31 ± 6.00 mm, W, 98.49 ± 7.32, O, 66.05 ± 3.47. For each parent, the gametes were collected into separate buckets by stripping the gonads. Eggs from each dam were divided into three equal portions and fertilized by matching sperm according to the crossing design at a sperm: egg ratio of 50,

respectively. Twenty-four hours after fertilization, D-larvae of each family were collected and reared at 23–25 °C in a 100 L plastic bucket. The larvae density of each bucket was initially set to 2 larvae mL⁻¹ and will not be adjusted later. This density ensures that each larva has enough space to reduce the effect of density on growth. When 50% larvae appear eyespots, strings of scallop shells were placed into the bucket to provide a substrate for eyed larvae to set. After all eyed larvae metamorphosed to spats, they were transferred to an outdoor nursery tank for a one-week temporary rearing. Then spats from each family were split and transferred to two major commercial grow-out sites for oyster farming in Shandong province: Rongcheng (37.11° N, 122.35° E) and Rushan (36.45° N, 121.42° E). Rongcheng and Rushan are two main farming areas of *C. gigas* in China, and there are significant differences between the two sea areas. In Rongcheng, the average annual water temperature is 12.95 °C, the average annual salinity is 32.20 psu, the average annual PH is 8.15, the average annual wave height is 0.3 m, and the average annual tidal range is 0.7 m; while in Rushan, the average annual water temperature is 14.20 °C, the average annual salinity is 30.00 psu, the average annual PH is 8.00, the average annual wave height is 0.5 m, and the average annual tidal range is 2.4 m (Gao et al., 2012; Sun et al., 2014). All spats were cultured by the long-line method by 4 months, and then placed in 8-layer lantern nets with 30 individuals per layer. This density can ensure that there is no squeezing between individuals, thereby reducing the impact of density on growth.

2.3. Recorded traits

At larval stage, each family was sampled on days 4, 8, 12, 16 and 20 after fertilization. A 100 mL sample was collected randomly from each bucket after the water was completely mixed, and fixed by the addition of 1% Lugol's solution to record the survival rate and the shell height. Larval survival rate was calculated on the basis of the total number of filled larval shells (distinguishing from empty larval shells) in each sample. Shell heights of 30 larvae, which were randomly selected from each sample, were measured in a microscope (100×) fitted with an ocular micrometer.

During grow-out stage, 30 oysters were randomly selected from each family on 4-month-old (December 10, 2017), 7-month-old (March 15, 2018) and 10-month-old (June 5, 2018) ages, respectively. The shell height of each oyster was measured by a digital caliper (0.01 mm). At each sampling, the dead oysters will be removed. Juvenile survival rates were calculated based on the total number of living oysters from one lantern net for each family at 10-month-old.

2.4. Statistical analyses

Differences in shell height and survival rate among purebred lines

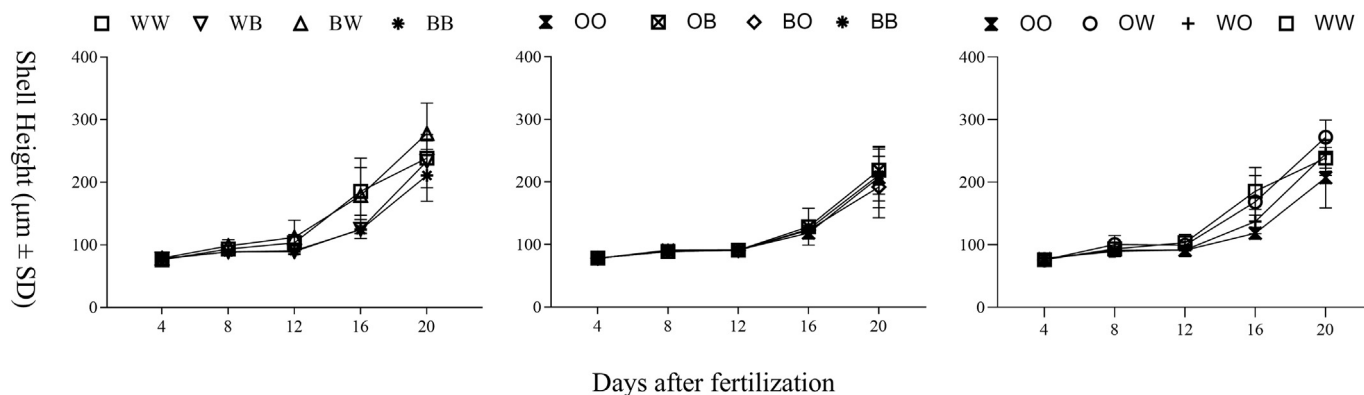


Fig. 1. Shell height of the three hybrid combinations at larval stage. W, O and B represent white shell color, orange shell color and black shell color lines of *C. gigas*, respectively. Three parental groups: WW = W♀ × W♂; OO = O♀ × O♂; BB = B♀ × B♂. Six reciprocal hybrid groups: BW = B♀ × W♂; WB = W♀ × B♂; BO = B♀ × O♂; OB = O♀ × B♂; WO = W♀ × O♂; OW = O♀ × W♂.

and their hybrid crosses within each kind of hybrid combination were analyzed with one-way analysis of variance followed by multiple comparison Tukey test. Analyses were performed using SPSS (Statistical Package for Social Science) 20.0 software. Differences were considered statistically significant if $P < .05$.

Combining abilities and heterosis analysis were based on the larval shell height and larval survival at 20-day-old, and juvenile shell height and juvenile survival at 10-month-old. General combining ability (GCA) and special combining ability (SCA) for shell height and survival rate were estimated using best linear unbiased prediction in ASReml-R software (Gilmour et al., 2002). The following statistical model was used for analysis of shell height and survival.

$$y_{hijk} = \mu + E_h + S_i + D_j + SD_{ij} + e_{hijk}$$

Where y_{hijk} = individual data; μ = common mean; E_h = environment_h; S_i = sire_i; D_j = dam_j; SD_{ij} = crossbred of sire S_i × dam D_j ; and e_{hijk} = residual effect. For the survival rate at both larval and juvenile stages, S_i and D_j were excluded from the above model, because the variance compositions of these two factors were too small or negative. For the same reason, S_i was excluded from the above model for shell height at juvenile stage in Rongcheng. The E_h was excluded from the above model, when analysis for traits at one specific environment. In addition, the relative importance of the special combining ability over the total combining abilities was calculated as the ratio of the variance composition: $V_{sca} / (V_{gca} + V_{sca})$, where, V_{gca} = general combining ability variance, V_{sca} = special combining ability variance.

Heterosis was calculated with the following two formulas (Hallauer et al., 2010):

(1) Mid-parent heterosis (MPH): $MPH (\%) = [(F_1 - MP) \times 100] / MP$.

Where F_1 = the mean value of one hybrid cross; MP = average performance of its parents.

(2) High-parent heterosis (HPH): $HPH (\%) = [(F_1 - HP) \times 100] / HP$.

Where F_1 = the mean value of one hybrid cross; HP = the performance of its better parent.

3. Results

3.1. Larval and juvenile growth

No significant differences ($P > .05$) in the mean shell height were observed among the purebred lines and their hybrid crosses in each of the three kind of hybrid combinations throughout the larval stage (Fig. 1). At juvenile stage, the growth of all experimental groups cultured in Rushan were faster than that in Rongcheng, and the magnitude of differences among the parental lines and their hybrid crosses were

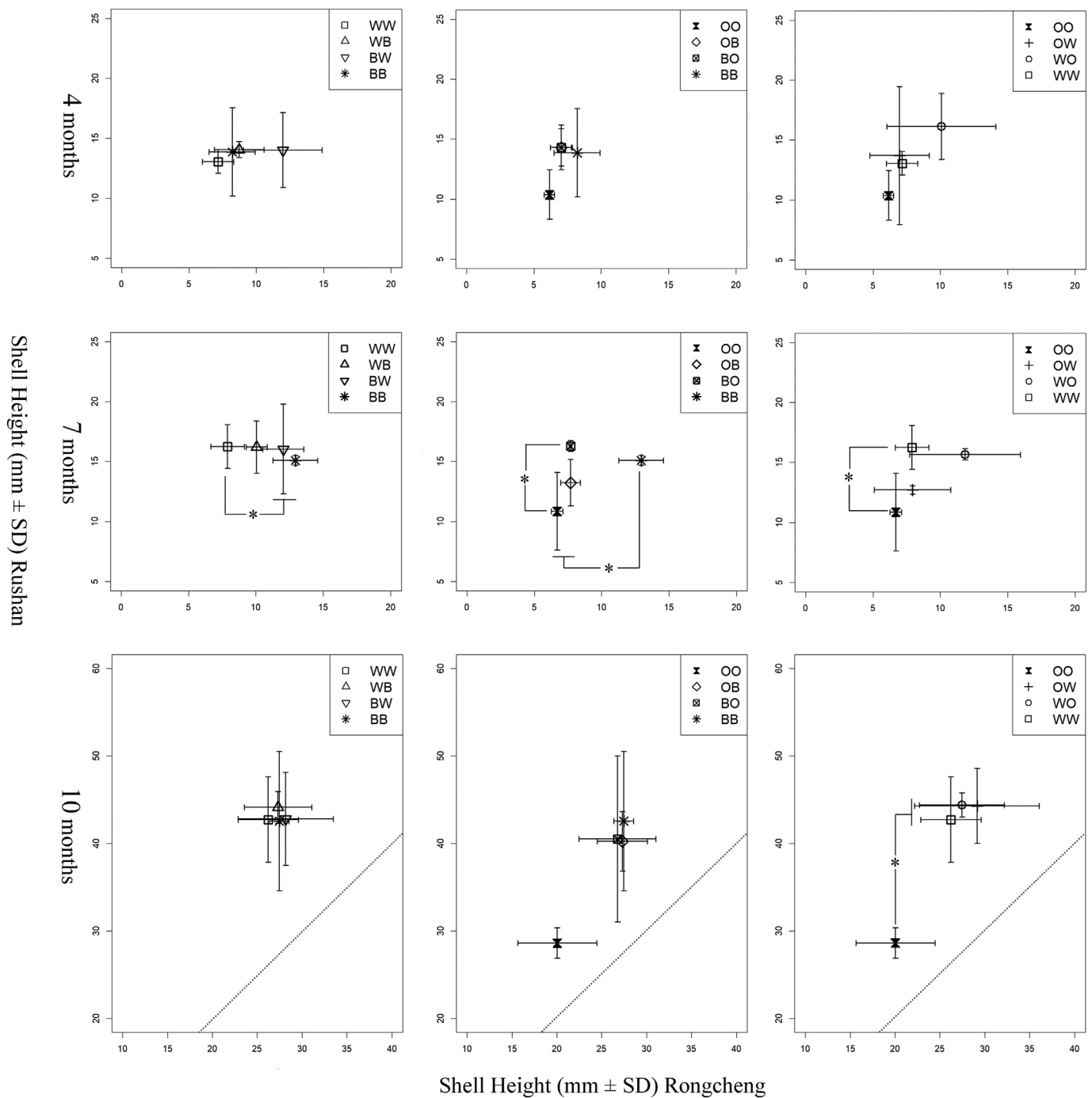


Fig. 2. Shell height of the three hybrid combinations on 4 months, 7 months and 10 months at Rongcheng (horizontal axis) and Rushan (vertical axis), * $P < .05$. W, O and B represent white shell color, orange shell color and black shell color lines of *C. gigas*, respectively. Above the dotted line indicates that the value at Rushan is larger than that at Rongcheng. See Fig. 1.

affected by the environments (Fig. 2). At 4-month-old, no significant differences ($P > .05$) for juvenile shell height were observed within each of the three kinds of hybrid combinations in both Rushan and Rongcheng. At 7-month-old, the shell heights of WB and BB were significantly ($P < .05$) larger than that of WW in Rongcheng. Meanwhile, the shell height of BB was significantly ($P < .05$) larger than that of OO in Rongcheng, and the shell height of OB was significantly ($P < .05$) larger than that of OO in Rushan. In addition, the shell height of WW was significantly ($P < .05$) larger than that of OO in Ruhsan. At 10-month-old, no significant difference within hybrid combinations between W and B and between O and B were observed.

However, the shell heights of WO, OW and WW were significantly larger than that of OO in Rushan ($P < .05$).

The combining abilities (*GCA* and *SCA*) and heterosis (*MPH* and *HPH*) for shell heights of three kinds of hybrid combinations at both larval and juvenile stages were showed in Table 1. The order of the general combining ability (*GCA*) of the three lines was $W > B > O$ regardless of sire and dam, except that the *GCA* of sire was $W > O > B$ at larval stage. Moreover, the variance of special combining ability (*SCA*) accounts for 30.73% of the sum of *GCA* and *SCA* at larval stage, and increased to 95.08% at juvenile stage regardless of the different environments. For the heterosis at larval stage, all the mid-parent

Table 1

General combining ability (GCA), specific combining ability (SCA), mid-parent heterosis (MPH %) and high-parent heterosis (HPH %) for shell height of the three kind of hybridization combinations at both larval and juvenile stages.

Group ^a	Larvae			Juvenile ^b						
				RC		RS		RC & RS		
	GCA			GCA ^c		GCA		GCA		
Sire_B	-5.09			-		39.40		4.90		
Sire_O	-0.05			-		-115.79		-11.46		
Sire_W	5.15			-		76.39		6.56		
Dam_B	-9.85			8.84		17.87		10.28		
Dam_O	-15.32			-25.43		-38.87		-22.29		
Dam_W	25.17			16.58		21.00		12.01		
	SCA	MPH	HPH	SCA	MPH	HPH	SCA	MPH	HPH	SCA
WB	10.95	3.92	-2.09	105.72	4.88	2.51	75.55	0.45	0.23	116.60
BW	6.86	23.61	16.46	44.11	1.75	-0.55	178.71	3.49	3.28	166.23
OB	-19.05	4.67	3.69	13.15	12.56	-2.62	-43.24	13.84	-4.79	-48.19
BO	1.26	-8.26	-9.12	31.50	14.87	-0.62	-13.67	13.08	-5.43	-14.64
WO	8.15	10.01	2.74	177.63	25.85	11.50	339.99	24.18	3.67	233.37
OW	21.07	22.05	13.98	102.12	18.55	4.61	260.21	24.40	3.86	213.27
$V_{SCA} / (V_{GCA} + V_{SCA})$	30.73%			92.94%		82.23%		95.08%		

^a B, O and W represent black shell, orange shell and white shell lines of *C. gigas*, respectively. BW = B♀ × W♂; WB = W♀ × B♂; BO = B♀ × O♂; OB = O♀ × B♂; WO = W♀ × O♂; OW = O♀ × W♂.

^b The juvenile stage was reared at two environments, RC = Rongcheng; RS = Rushan.

^c The variance component of sires in Rongcheng were too small to be retained in the model.

heterosis (MPH) were positive except that of BO was negative. In hybrid combinations between W and B and between B and O, only the hybrid crosses BW and OB showed positive high-parent heterosis (HPH), but both reciprocal hybrids WO and OW showed positive HPH (2.74% and 13.94%, respectively). Heterosis of juvenile shell heights at Rushan (MPH: 0.45%–24.40%; HPH: -5.43%–3.86%) were similar to that at Rongcheng (MPH: 1.75%–25.85%; HPH: -2.62%–11.50%), despite the growth of all experimental groups cultured in Rushan were faster than that in Rongcheng (Fig. 2). Overall, all the MPH of six hybrid crosses were positive, but the HPH of three kinds of reciprocal hybrids were low or negative. Both the reciprocal hybrids OW and WO had positive HPH (3.67%–11.50%) at both Rongcheng and Rushan. However, both OB and BO had negative HPH (-5.43% to -0.62%) at both environments. The HPH of reciprocal hybrids between B and W (-0.55%–3.28%) were between that of hybridization between B and O and hybridization between W and O. The result of HPH was consistent with the ranking of SCA, in which the SCA were highest for the hybrids of W and O, intermediate for hybrid of W and B, and lowest for the hybrids of B and O.

3.2. Larval and juvenile survival rate

At larval stage, the mean survival rates of six reciprocal hybrid crosses (82.00%–85.00%) were higher than that of three parental lines (37.00%–55.00%) (Fig. 3A). The mean survival rates of hybrid crosses BW and WB were significantly ($P < .05$) higher than that of parental line WW at day 20. Similar result was observed in the hybridization between B and O that the mean survival rates of hybrid crosses BO and OB were significantly ($P < .05$) higher than that of OO. While, no significant ($P > .05$) difference was observed within the hybridization between W and O. Juvenile survival rates of nine experimental groups were affected by the environments (Fig. 3B). The mean survival rates of these three purebred lines at Rushan were lower than that at Rongcheng. In the hybridization between W and B, the mean survival rates of hybrid crosses (BW: 65.83%, WB: 69.17%) were between that of WW (65.00%) and BB (85.83%) in Rongcheng; however, the mean survival rates of hybrid crosses (BW: 79.17%, WB: 74.17%) were higher than that of their parental lines (WW: 55.00%, BB: 60.83%) in Rushan (Fig. 3B). Similar results are also observed in the hybridization between B and O that the mean survival rates of hybrid crosses (OB: 75.83%, BO: 80.00%) were between OO (55.83%) and BB (85.83%) in Rongcheng,

but the mean survival rates of hybrid crosses (OB: 75.00%, BO: 65.83%) were higher than that of their parental lines (OO: 34.17%, BB: 60.83%) in Rushan. Moreover, the mean survival rate of OO was significantly ($P < .05$) lower than that of OB, BO and BB in Rushan. In the hybridization between O and W, the mean survival rates of both hybrid crosses (OW and WO) were higher than that of their parental lines (WW and OO) in both Rongcheng and Rushan.

For the survival rate at both larval and juvenile stages, no GCA was detected because the variance compositions of sire and dam were too small or negative (Table 2). The SCA and heterosis values assessed from the survival rates of the three kinds of reciprocal hybrids were variable depending on stages and environments (Table 2). At larval stage, all the SCA for each hybrid crosses were positive (6.40–8.70), and all the MPH (86.81%–154.55%) and HPH (49.09%–133.33%) were positive and at a high level. At juvenile stage, the SCA of both hybrid crosses WB and BW were negative in Rongcheng, but turned positive in Rushan. The SCA of hybrid cross WO ranked first, regardless the difference in environments. This result consists with the order of SCA for juvenile shell heights that WO also ranked first. For heterosis of juvenile survival, the MPH of all hybrids in Rushan (28.06%–73.83%) were larger than that in Rongcheng (-12.71% to 53.10%). Meanwhile, the HPH of four (WB, BW, OB and BO) of six hybrid crosses were negative in Rongcheng (-23.30% to -6.80%) but turned positive in Rushan (8.22%–30.14%). However, both the hybrid crosses OW and WO showed consistently positive HPH (21.21%–42.91%) in both environments. Furthermore, WO had the greatest heterosis for survival throughout the larval (MPH: 154.55%; HPH: 133.33%) and juvenile (MPH: 53.10%–73.83%; HPH: 40.91%–42.91%) stages.

4. Discussion

Heterosis for growth and survival in the Pacific oyster have been documented by crossing inbred lines made by selfing hermaphrodites or by brother-sister mating within pedigreed families (Hedgecock and Davis, 2007; Hedgecock et al., 1995). Crossbreeding of such inbred lines, however, is difficult to apply in practice, mainly due to inbreeding depression of these inbred lines (Hedgecock and Davis, 2007). The lines, which have been selected over generations, have favorable performance and a relatively homogenous genetic background. However, the magnitude of heterosis in crossbreeding of selected lines

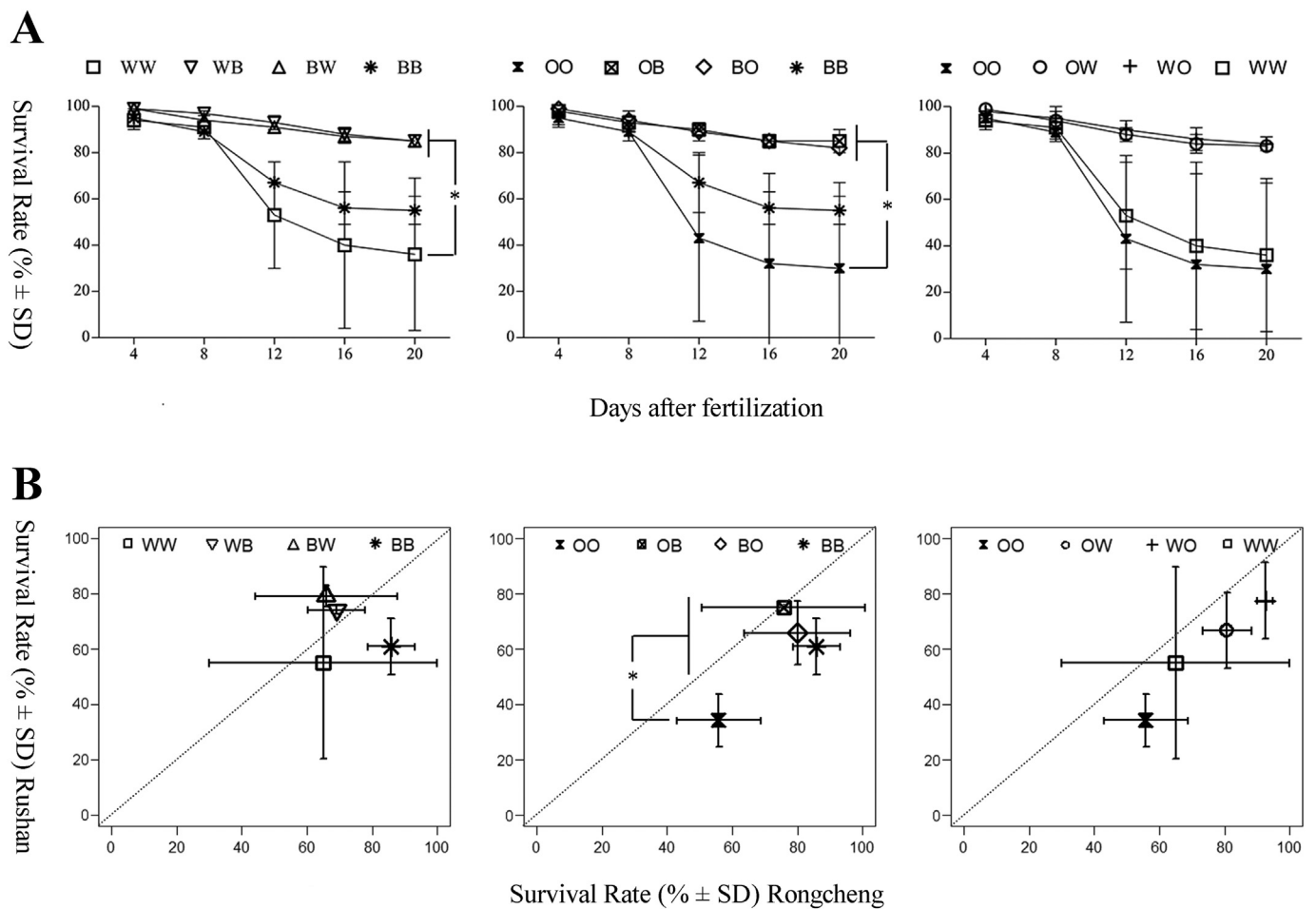


Fig. 3. Survival rate of the three hybrid combinations at larval (A) and juvenile stages (B) at Rongcheng (horizontal axis) and Rushan (vertical axis), * $P < .05$. W, O and B represent white shell color, orange shell color and black shell color lines of *C. gigas*, respectively. Above the dotted line indicates that the value at Rushan is larger than that at Rongcheng. See Fig. 1.

remains unclear. Here, we discussed the potential of applying crossbreeding between selected lines to improve the productive performance in *C. gigas*.

4.1. The heterosis and combing abilities among these three lines

There were three major observations on the performance of the crossbreeding among these three selected lines. Firstly, special combing ability (SCA) explained the majority of the total variance for larval survival, juvenile survival, and juvenile shell height. This result is consisted with the results of crossbreeding of inbred lines in *C. gigas*

(Hedgecock and Davis, 2007; Hedgecock et al., 1995), but different from previous studies about crosses among geographical divergent populations of aquatic animals, in which SCA explained only a small part of the total variance of the productive traits (Costa et al., 2019; Pongthana et al., 2010; In et al., 2017). GCA and SCA, which reflect the additive and non-additive effects respectively, are two basic indicators for crossing efficiency (Falconer and Mackay, 1996). The relatively high proportion of the variance explained by the SCA for all analyzed traits revealed the importance of non-additive genetic component (Hedgecock and Davis, 2007). The high levels of non-additive genetic component observed in present study suggested that more attention

Table 2

Specific combining ability (SCA), mid-parent heterosis (MPH %) and high-parent heterosis (HPH %) for survival rate of the six reciprocal hybrids at both larval and juvenile stages.

Group ^a	larvae			Juvenile ^b						
	SCA	MPH	HPH	RC			RS			RC & RS
				SCA	MPH	HPH	SCA	MPH	HPH	SCA
WB	8.70	86.81	54.55	-0.96	-8.29	-19.65	5.68	28.06	21.92	1.12
BW	7.64	86.81	54.55	-1.56	-12.71	-23.30	8.91	36.69	30.14	1.67
OB	7.05	100.00	54.55	0.23	7.06	-11.65	6.22	57.89	23.29	3.57
BO	6.64	92.94	49.09	0.98	12.94	-6.80	0.30	38.60	8.22	1.94
WO	6.99	154.55	133.33	3.21	53.10	42.91	7.84	73.83	40.91	9.84
OW	6.40	151.52	130.56	1.13	33.79	24.35	0.84	49.53	21.21	2.48

^a See Table 1.

should be paid to the utilization of the non-additive genetic effects (heterosis) among these lines in the future (Falconer and Mackay, 1996).

Secondly, the heterosis for growth of all hybrids were either no or low at both larval and juvenile stages, but for larval survival rates were at a high level in all hybrids. The high heterosis for larval survival between selected lines was similar with that reported in the crosses between inbred lines of *C. gigas* (Hedgecock et al., 1995), and higher than that reported in the crosses of wild populations that are likely not to be inbred in oysters (Zhang et al., 2017) and scallops (Wang and Côté, 2012; Wang et al., 2011). This finding suggested that the inter-line crossbreeding can be applied to improve the larval survival of purebred lines in hatcheries. The low (or no) heterosis for growth in all hybrids, however, is different from the results from crossbreeding of inbred lines, in which the heterosis for yield is a pervasive phenomenon and the *HPH* can be as high as approximately 100% (Hedgecock and Davis, 2007). Similarly, the low or negative *HPH* for growth also found in the crossbreeding of other selected lines in *C. gigas* (Kong et al., 2017) and *C. virginica* (Rawson and Feindel, 2012). These differences in magnitude of heterosis among crosses between inbred lines, crosses between selected lines and crosses between wild populations confirms that the level of inbreeding is a key component in productive heterosis for oysters (Rawson and Feindel, 2012).

Thirdly, for the juvenile survival, all the *MPH* at Rushan were larger than that at Rongcheng, and four of six hybrids (WB, BW, OB and BO) showed a positive *HPH* at Rushan but negative at Rongcheng. The different heterosis between two environments confirmed that it is important to evaluate the stability of heterosis by comparing the performance of different crosses in different environments (Fjalestad, 2005; Rawson and Feindel, 2012; Thoa et al., 2016). Furthermore, Rushan has a higher annual average water temperature and larger sea waves than that of Rongcheng, as mentioned in the Materials and methods. These factors may reduce the survival, because the mean survival rates of all three purebred lines at Rushan were lower than that at Rongcheng (Fig. 3B). The higher heterosis found at Rushan suggested that the heterosis for juvenile survival in *C. gigas* has more application value in Rushan, and inter-line crossbreeding has the potential to be used to increase the juvenile survival of purebred lines.

4.2. Effects of crossing with orange shell line on black shell and white shell lines

Another major focus of our study was to determine whether the productive performance of black shell and white shell lines can be further improved by crossing with orange shell line. The shell coloration of the progeny of the white and black shell oysters would not be changed by crossing with the orange shell oysters (Han and Li, 2020). For productive traits, positive *MPH* were observed in all four hybrid crosses (OB, BO, WO and OW), except the larval shell height of BO. However, *HPH* is more appropriate to detect whether the hybrid performance exceeds that of its better parent, compared with *MPH*. For shell height, only WO and OW showed consistently positive *HPH* at both larval and juvenile stages. Unfortunately, this low level of expressed heterosis in growth is of little significance for production. Noticeably, encouraging heterosis has been observed in the survival rate. For larval survival, all the four hybrid crosses showed a high level of *HPH*, especially WO and OW, with *HPH* values of 133.33% and 130.56%, respectively. The magnitude of *HPH* for survival at juvenile stage was reduced compared with that at the larval stage. Specifically, both OB and BO showed positive *HPH* only in Rushan and negative *HPH* in Rongcheng. However, both WO and OW maintained stable medium level of *HPH* across different environments. Moreover, The *HPH* for juvenile survival of WO (40.91%–42.91%) was approximately two times of that of OW (21.21%–24.35%) in both sites. Differences between reciprocal crosses are common, and are also observed in other inter-line crosses in *C. gigas* (Kong et al., 2017) and other aquatic

animals (Thanh et al., 2009). Overall, crossbreeding with the orange shell line can be used to improve the survival of black shell and white shell lines, although its contribution to growth is small. The survival rates of both WO and OW at both larval and juvenile stages were better than that of their parental line WW in Rushan and Rongcheng, and the hybrid cross WO can improve the survival rate of purebred WW by nearly 130% at larval stage and nearly 40% at juvenile stage. Encouraging results also been obtained in both OB and BO that the larval survival rate of purebred BB has been improved by nearly 50%.

4.3. Difference of heterosis between survival and growth

Orange shell line showed the poorest productive performance in terms of growth at the juvenile stage and survival at both larval and juvenile stages, compared with white and black shell lines (Figs. 2 and 3). This result is due to the fact that the orange shell line (based on only four individuals) is more inbred than the black and the white ones (Han et al., 2019; King et al., 2017; Xu et al., 2019b). Consequently, these diallel crosses in this study can be divided into two categories, one is the cross between non-inbred lines (i.e. cross between W and B), and the other is the crosses between non-inbred line and inbred line (i.e. cross between W and O and cross between B and O). Although the orange shell line is an inbred line, the inbreeding degree of this line is lower than that reported in Hedgecock and Davis (2007) and Hedgecock et al. (1995), and this line has been successfully bred for nine generations by 2020. In this study, no matter in the cross between non-inbred lines or crosses between non-inbred line and inbred line, the heterosis was mainly expressed in the increasing of survival, but little contribution to growth. Although density has an impact on growth, resulting in underestimation of the final heterosis of growth, this effect had been greatly reduced by reducing the initial density at larval and juvenile stages to ensure that each individual has enough space to grow. Thus, the impact of density in this study was minimal or non-existent, and could not have confounded our finding concerning heterosis. The difference in heterosis between survival and growth found in this study is not observed in the crosses between inbred lines (Hedgecock and Davis, 2007; Hedgecock et al., 1995) and crosses between two-generations of selected lines in *C. gigas* (Kong et al., 2017). The fact that heterosis is observed for survival but not for growth could be related to the level of inbreeding and expression of genetic load in the purebred progenies (Launey and Hedgecock, 2001; Plough and Hedgecock, 2011). As mentioned in the Materials and Methods, the breeding programs of these three lines only focus on improving growth and shell color, and did not incorporate survival into their breeding goals. Both black shell and white shell lines were selected from wild populations with abundant genetic variation, and a large amount of additive genetic variance for fast growth has been maintained during generations of selection. However, the survival rates of these two lines were not been directional selected during their selection, and thus the loci related to survival maybe more diverse and complementary than that of growth between these two lines. For the orange shell line, the initial genetic base is small, the loci related to survival within this line may be more complementary to that of black shell and white shell lines than the loci related to growth. Further research is needed to understand the genetic mechanism of the difference in heterosis between growth and survival.

In summary, this study reveals that the heterosis among selected lines is high in survival but low in growth in *C. gigas*, by systematic assesses the growth and survival of crosses between three multi-generation selected lines at larval and grow-out stages. The survival of all hybrid crosses exceeded 49% of their better parental line at larval stage, and exceeded their better parental line from 8.22% to 40.91% at juvenile stage at Rushan. Especially, the hybrid cross WO can improve the survival rate of purebred WW by nearly 130% at larval stage and nearly 40% at juvenile stage. These results, coupled with the advantages of shell color lines in multiple lines management, will facilitate the application of inter-line crossbreeding in commercial seed production of

C. gigas. Furthermore, these findings will be helpful for the utilization of non-additive genetic effects among existing selected lines in bivalves.

Declaration of Competing Interest

The authors declare no conflict of interest.

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