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The effect of interstrain hybridization on the production performance in the Pacific oyster *Crassostrea gigas*

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ABSTRACT

Intra-specific hybridization was carried out using three strains (C, J and K) of *Crassostrea gigas*, which were successively mass selected for two generation from three culture stocks collected from China, Japan and South Korea. Detailed comparison on larval growth development and the growth and survival of the spats and adult were carried out among six hybrids and three parental groups. By way of the serial growth traits measurements (shell height, shell length and the whole body weight), the obtained results reveal that six hybrid crosses were inferior to those of parental crosses to different extend at larval stage, while at spat and adult stages some differences were observe. It was noted that the cross CJ (C♀ × J♂) outperformed all the other groups in all growth traits ($P < 0.05$) and at harvest, the shell height, shell length and the whole body weight reached to 75.64(±11.68) mm, 46.35(±9.06) mm, 42.11(±10.53) g, respectively. For spats and adults survival rate, the hybrid crosses CJ and CK (C♀ × K♂) were larger than three parental crosses ($P < 0.05$) and that of the reciprocal hybrids crosses CK and KC (K♀ × C♂) were significantly ($P < 0.05$) larger than the parental crosses CC (C♀ × C♂) and KK (K♀ × K♂), which were 65.14 ± 5.50% and 77.33 ± 4.16% (spats), 57.59 ± 4.17% and 71.33 ± 4.16% (adults), respectively. Considering the growth and survival conditions, the results concluded that the crosses CJ, CK and KC in this work were deemed to be the better hybridization combinations for genetic improvement, which could have significant implications for the development of oyster aquaculture.

Statement of relevance: Three different culture stains of *Crassostrea gigas*, which were successively selected for two generations, were evaluated for growth and fitness traits, as well as for their reciprocal crosses, to determine if heterosis exists in growth and survival traits at different stages by rearing them under the same conditions. Our results could have significant implications for the development of oyster aquaculture.

1. Introduction

Hybridization is one of the most effective approaches for genetic improvement. Most studies on hybridization in plant and animal have been carried out to combine desirable characteristics (Falconer and Mackay, 1996). In aquaculture, hybridization was also used to increase growth rate, manipulate sex ratios, produce sterile animals, improve flesh quality, increase disease resistance, improve environmental tolerance and obtain desirable traits (Bartley et al., 2001). In fact, few successes in inter-specific hybrid were obtained (Stiles, 1973; Bray et al., 1990), so more attentions focused on intra-specific hybridization, which generally has higher spawn rate, hatch rate and viability compared with the inter-specific hybrids (Hulata, 2001). Due to the potentially genetic variation among difference populations, intra-specific hybrids can produce heterosis to improve productivity and combine desirable characteristics found in one population with those of another (Cruz and Ibarra, 1997; Zheng et al., 2006; Wang and Li, 2010).

Heterosis is the biological phenomenon whereby F_1 hybrids of two genetically dissimilar parents show an increased vigor, at least over the mean of both parental species (Rahman et al., 2005). It was first suggested in commercial shellfish species by the information derived from a population genetic study of American oyster *Crassostrea virginica*, where a positive correlation between growth and heterozygosity had been observed (Singh and Zouros, 1978). Recently, heterosis of hybridization among different populations has been reported in aquatic animals, such as scallops (Liu et al., 2004; Zheng et al., 2006; Zhang et al., 2007; Wang and Li, 2010), abalone (Deng et al., 2007; You et al., 2009, 2015) and shrimps (Tian et al., 2006, 2008), although the genetic mechanisms of heterosis are still poorly understood (Griffing, 1990). In theory, heterosis can be rapidly accumulated in the cross due to the accumulation of some different non-additive genetic variation in the parent lines, when the base populations were separately directed selected for several generations (Sheridan, 1997). Some researchers also recommended a combination of within line and reciprocal recurrent

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Table 1
Larval shell height (μm) and standard deviation (SD) for the nine experimental groups at larval stage.

Groups	1 d	6 d	11 d	16 d	21 d	26 d
CC	66.18 ± 1.65^{b}	113.10 ± 8.77^{bcd}	154.60 ± 13.03^{bc}	213.60 ± 29.34^{ab}	264.80 ± 25.99^{ab}	286.50 ± 18.20^{ab}
JJ	62.37 ± 4.03^{a}	104.80 ± 10.02^{a}	145.20 ± 9.90^{ab}	210.90 ± 25.83^{a}	264.00 ± 30.41^{ab}	289.27 ± 37.76^{ab}
KK	65.82 ± 2.97^{b}	105.00 ± 8.45^{a}	149.30 ± 15.47^{ab}	210.40 ± 25.53^{a}	251.00 ± 30.75^{a}	276.90 ± 27.15^{a}
CJ	65.82 ± 2.80^{b}	122.60 ± 4.15^{e}	158.70 ± 18.95^{c}	234.40 ± 29.38^{c}	292.10 ± 43.66^{c}	317.30 ± 34.25^{c}
JC	62.37 ± 4.51^{a}	109.30 ± 6.18^{abc}	142.40 ± 14.22^{a}	216.90 ± 24.28^{ab}	282.60 ± 60.35^{bc}	311.50 ± 28.59^{bc}
CK	63.56 ± 4.49^{ab}	115.40 ± 5.66^{bcd}	156.50 ± 13.42^{bc}	217.80 ± 20.79^{ab}	272.80 ± 29.86^{ab}	300.50 ± 22.10^{bc}
KC	65.73 ± 2.95^{b}	113.60 ± 7.55^{bcd}	155.70 ± 11.91^{bc}	215.10 ± 27.92^{ab}	271.60 ± 24.65^{ab}	299.40 ± 32.65^{bc}
JK	65.73 ± 2.48^{b}	107.70 ± 8.20^{ab}	150.10 ± 13.17^{abc}	215.10 ± 31.44^{ab}	272.00 ± 23.20^{ab}	312.60 ± 27.44^{bc}
KJ	65.64 ± 3.67^{b}	116.80 ± 7.83^{de}	150.20 ± 11.16^{abc}	216.90 ± 31.28^{ab}	290.40 ± 31.81^{c}	317.20 ± 31.09^{c}

Means that share the same superscript letters within a column are not significantly difference ($P < 0.05$). C, J and K represent Chinese, Japanese and Korean strain respectively. Three parental groups: CC = C♀ × C♂; JJ = J♀ × J♂; KK = K♀ × K♂. Six reciprocal hybrid groups: CJ = C♀ × J♂; JC = J♀ × C♂; CK = C♀ × K♂; KC = K♀ × C♂; JK = J♀ × K♂; KJ = K♀ × J♂.

selection for improving crossbred performance (Bell, 1982; Wei and van der Steen, 1991).

The Pacific oyster, *Crassostrea gigas*, which originated from Asia and was introduced to many countries, is one of the most important aquatic species in the world. Selection and cross-breeding programs for genetic improvement in *C. gigas* have initiated in several countries (Langdon et al., 2003; Evans and Langdon, 2006; Boudry et al., 2004; Hedgecock et al., 1995, 1996, Hedgecock and Davis, 2007) and heterosis for growth and survival of *C. gigas* was demonstrated experimentally in crosses among inbred lines (Hedgecock et al., 1995, 1996). In China, the Pacific oyster is economically important and has also been widely adopted for farming with the development of efficient hatchery techniques. It originally propagated from wild-caught broodstock and now had cultured for many generations. In order to make the Pacific oyster industry to be competitive and sustainable in China or even the worldwide, breeding methods expect to be applied to improve production performance traits, especially crossbreeding, where heterosis might be achieved by crossing inbred lines or genetically diverged populations.

In the present study, three different culture stains of *C. gigas*, which were successively selected for two generations, were evaluated for growth and fitness traits, as well as for their reciprocal crosses, to determine if heterosis exists in growth and survival traits at different stages by rearing them under the same conditions. From this study, it was planned to choose one or more hybrid crosses that can efficaciously increase production traits such as growth and survival in the pacific oyster.

2. Materials and methods

2.1. Selection of broodstock

Three different culture strains were initiated in 2007. Two-year-old Pacific oysters were collected from three cultured stocks in Rushan in Shandong province, China (36.4°N, 121.3°E), Onagawa Bay in Miyagi Prefecture, Japan (38.3°N, 141.3°E), and Pusan, South Korea (35.1°N, 129.1°E) in January–February 2007, and transferred to Wendeng Aquatic Comprehensive breeding Base, Weihai, Shandong. Microsatellite analysis had indicated that the three cultured stocks were genetically different from each other (Yu and Li, 2007; Wang et al., 2012). The first generation of selection was carried out by mass selection for shell height on three cultured stocks from China, Japan, and Korea in July 2007. When harvested on day 360, the three selected lines grew by 7.9%–12.2% larger than the control lines, and the genetic gain ranged from 7.2% to 13.2% (Li et al., 2011). A second-generation mass selection was conducted in the three breeding lines in July 2008. After nursing for 2 months, the oysters were inserted into nylon ropes and cultured on suspended strings in Weihai Bay, Shandong, China (37.3°N, 122.1°E) (Wang et al., 2012). Pedigree of selected lines indicating founder stock and selection intensity for a successive two-generation selection was showed in Wang et al. (2012); Fig. 1 therein).

After one year culture, the second-generation offspring from the three strains (named "C, "J" and "K") were selected as broodstocks to produce all 9 possible reciprocal strain crosses and pure strains in a complete diallel cross design. The broodstocks were transferd from Weihai bay to hatchery of Wendeng Aquatic Comprehensive breeding Base in May–June 2009. The culture conditioning temperature at the beginning was 12 °C and kept for 5–7 days, raised 0.5–1.0 °C daily, and then gradually went up to 16 °C. Water was changed twice a day. The diet consisted of fresh *Nitzschia closterium* (Ehrenb) and the powder of *Spirulina*. After approximately one month, most breeders were almost reached ripeness stage.

2.2. Mating design

In June 2009, three brood stocks were dissected and 10 females and 10 males sexually matured were chosen to use in the experiment from each strain. The complete diallel cross produced nine distinct groups in this study, including six reciprocal hybrid groups CJ (C♀ × J♂), JC (J♀ × C♂), CK (C♀ × K♂), KC (K♀ × C♂), JK (J♀ × K♂), KJ (K♀ × J♂) and three parental groups CC (C♀ × C♂), JJ (J♀ × J♂) and KK (K♀ × K♂).

Twenty-Four hours after fertilization, D-larvae of each crosses were collected by sieving and reared in two 100 L tanks. The larvae densities were initially set to 10 larvae ml^{-1} and decreased with larval growth. Water temperature was kept at 23–24 °C, and fresh air was continuously pumped into each culture. During the initial period, 30% of the culture water was changed once a day, and changed-water volume was

Table 2
Analyses of variance (ANOVA) in shell height (SH), shell length (SL), the whole body weight (W) and survival rate (S).

Stage and age	Trait	*F*	*P*
Larvae			
1 d	SH	6.351	0.000
6 d	SH	17.191	0.000
11 d	SH	4.226	0.000
16 d	SH	1.895	0.061
21 d	SH	3.737	0.000
26 d	SH	7.180	0.000
Spat			
2 months	SH	16.126	0.000
	SL	14.714	0.000
3 months	SH	20.477	0.000
	SL	14.911	0.000
5 months	SH	22.088	0.000
	SL	17.550	0.000
9 months	SH	14.911	0.000
	SL	11.575	0.000
	S	93.787	0.000
Adult			
11 months	SH	13.140	0.000
	SL	12.221	0.000
13 months	SH	9.316	0.000
	SL	11.495	0.000
	W	20.254	0.000
15 months	SH	12.681	0.000
	SL	15.076	0.000
	W	21.852	0.000
	S	92.822	0.000

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Table 3
Shell height (SH) and shell length (SL) of reciprocal and parental crosses at spat stage.

Groups	2 months		3 months		5 months		9 months	
	SH (mm)	SL (mm)	SH (mm)	SL (mm)	SH (mm)	SL (mm)	SH (mm)	SL (mm)
CC	3.14 ± 0.95^{bc}	2.25 ± 0.91^{bc}	8.56 ± 1.80^{cd}	6.91 ± 1.88^{de}	18.13 ± 6.76^{c}	14.88 ± 5.84^{c}	23.54 ± 6.81^{bc}	17.06 ± 5.52^{cd}
JJ	3.20 ± 0.70^{bc}	2.61 ± 0.59^{de}	7.15 ± 1.93^{bc}	5.80 ± 2.48^{bcd}	15.96 ± 4.94^{bc}	11.88 ± 3.19^{abc}	19.88 ± 5.24^{ab}	13.92 ± 3.73^{abc}
KK	2.18 ± 0.61^{a}	1.62 ± 0.52^{a}	5.13 ± 1.74^{a}	3.95 ± 1.66^{a}	11.60 ± 3.51^{a}	9.41 ± 2.61^{a}	17.93 ± 4.97^{a}	12.88 ± 3.95^{a}
CJ	4.12 ± 1.30^{d}	3.15 ± 1.22^{e}	9.58 ± 3.17^{d}	7.28 ± 2.30^{e}	26.70 ± 9.48^{d}	20.48 ± 8.02^{e}	30.52 ± 7.32^{e}	21.85 ± 5.41^{e}
JC	2.73 ± 0.67^{b}	2.08 ± 0.58^{b}	5.98 ± 1.27^{ab}	4.53 ± 1.26^{ab}	13.23 ± 4.35^{ab}	10.92 ± 3.57^{ab}	18.44 ± 3.84^{a}	13.12 ± 4.08^{ab}
CK	3.12 ± 0.60^{bc}	2.49 ± 0.45^{bcd}	8.82 ± 2.08^{cd}	6.33 ± 1.53^{de}	19.22 ± 6.81^{c}	14.87 ± 6.20^{c}	27.37 ± 6.56^{de}	19.42 ± 4.78^{de}
KC	2.85 ± 0.62^{bc}	2.17 ± 0.52^{bc}	8.32 ± 2.65^{cd}	6.33 ± 2.20^{cde}	19.04 ± 6.17^{c}	15.19 ± 3.86^{cd}	24.37 ± 8.93^{bc}	17.35 ± 7.02^{bcd}
JK	3.45 ± 0.63^{cd}	2.66 ± 0.51^{de}	9.51 ± 3.42^{d}	7.15 ± 2.66^{de}	24.21 ± 6.17^{d}	19.14 ± 5.37^{de}	27.46 ± 8.14^{c}	19.32 ± 6.90^{de}
KJ	2.74 ± 0.65^{b}	2.17 ± 0.54^{bc}	6.15 ± 1.77^{ab}	4.97 ± 1.70^{abc}	15.59 ± 3.18^{bc}	13.97 ± 6.38^{bc}	19.26 ± 6.90^{ab}	14.41 ± 5.70^{abc}

Means that share the same superscript letters within a column are not significantly difference ($P < 0.05$). C, J and K represent Chinese, Japanese and Korean strain respectively. Three parental groups: CC = C♀ × C♂; JJ = J♀ × J♂; KK = K♀ × K♂. Six reciprocal hybrid groups: CJ = C♀ × J♂; JC = J♀ × C♂; CK = C♀ × K♂; KC = K♀ × C♂; JK = J♀ × K♂; KJ = K♀ × J♂.

increasing as larvae grow up. All seawater used for larval rearing was filtered through sand filters and polypropylene. During the whole larvae period, larvae were lived on *Isochrysis galbana*, and later stage feed were added on *Platymonas* sp. and *Chaetoceros muslleri*. To minimize environmental effects, all progenies in this study reared under common conditions, and the equipment used were thoroughly washed with fresh water to prevent contamination among crosses.

When 30% larvae reached eyed-stage and appeared ready to settle at around day 22, the settling substrates (strings of scallop shells) were set into the larval tanks and spats on the settling substrates were continuously cultured in the tanks for 10 days, and then the spats were placed in 9-layer lantern nets (40 cm in diameter and 10 cm per layer) with 100 spats on each layer, suspended in an outdoor nursery tank (200 m^3) for one month to avoid settling of oyster larvae in the nature seawaters. The spats were transferred to the field (Shuangdao Bay, China, 37.28°N, 122.57°E) in September 2009. The measurements of growth data were recorded every 2 month, and the lanterns were cleaned to remove the attachment at the same time. The spats were transfer to new lanterns in March 2010, when the density was adjusted to 50 individuals for each layer for all lanterns.

2.3. Measurement

For larval stage, shell height of 30 larvae per group was measured using a microscope (100×) equipped with an ocular micrometer every 5 day. During spat and adult stages, shell height and shell length of 30 individuals randomly sampled from each group were measured using a vernier caliper on different days, until 15-month age, and the whole weight was measured on 13 and 15 month age. Spat and adult survival rates were calculated based on the total number of live individuals from the 2, 5 and 8 layers of lantern nets for each group at 9-month-old and 15-month-old ages, respectively.

2.4. Statistical analyses

The raw growth data (Shell height, shell length and the whole weight) were transformed to nature logarithms to get normality and homoscedasticity (Neter et al., 1985). Percent survival values were transformed to angular values to stabilize the variances of errors (Rohlf and Sokal, 1981). Differences in growth data and survival rates among the genetics groups were analyzed with one-way analysis of variance and Post-hoc mean comparisons were then done with a Tukey test. Analyses were conducted using SPSS (Statistical Package for the Social Science) 16.0 software. Significance level for all analyses was set to $P < 0.05$.

The equation to determine heterosis was taken from Falconer and Mackay (1996):

$$Heterosis(\%) = [(F_1 - P)\ 100]/P$$

where, P = average phenotypic value of the two parental populations; F_1 = the mean value of one hybrid cross.

3. Results

3.1. Larval growth

Shell height data at the larval stage are shown in Table 1. On the first day of D-larvae, no significant differences ($P > 0.05$) in the mean shell height were observed among the experimental groups except the groups JJ and JC (Tables 1 and 2). From day 6, the mean shell lengths of cross CJ and KJ were larger than that of other experimental groups, especially starting from 21 days, were significantly larger than that of other groups ($P < 0.05$, Tables 1 and 2). The group CJ and KJ was considered the better combination in growth at the larval stage.

Table 4
Shell height (SH), shell length (SL) and the whole body weight (W) of reciprocal and parental crosses at adult stage.

Groups	11 months		13 months			15 months		
	SH (mm)	SL (mm)	SH (mm)	SL (mm)	W (g)	SH (mm)	SL (mm)	W (g)
CC	29.38 ± 9.07^{bc}	24.22 ± 8.39^{bc}	54.59 ± 10.65^{bcd}	37.59 ± 7.31^{bcd}	18.58 ± 8.86^{b}	62.34 ± 9.03^{ab}	41.57 ± 9.34^{bcd}	32.90 ± 11.61^{cd}
JJ	26.38 ± 10.07^{abc}	19.79 ± 7.87^{ab}	53.67 ± 10.81^{abc}	36.26 ± 7.63^{abc}	16.62 ± 8.91^{ab}	61.31 ± 8.64^{ab}	40.23 ± 6.90^{bcd}	28.23 ± 13.08^{bc}
KK	20.87 ± 5.75^{a}	15.74 ± 4.40^{a}	48.93 ± 6.92^{a}	30.73 ± 7.78^{a}	11.22 ± 6.69^{a}	56.72 ± 8.06^{a}	29.97 ± 5.35^{a}	15.97 ± 6.13^{a}
CJ	38.86 ± 12.66^{d}	28.82 ± 8.98^{c}	64.57 ± 8.74^{e}	44.85 ± 7.93^{e}	31.49 ± 10.16^{c}	75.64 ± 11.68^{d}	46.35 ± 9.06^{d}	42.11 ± 10.53^{d}
JC	21.21 ± 7.35^{a}	15.84 ± 5.22^{a}	49.26 ± 11.41^{a}	33.08 ± 8.16^{ab}	12.28 ± 8.76^{a}	58.07 ± 8.96^{ab}	36.62 ± 5.28^{b}	22.08 ± 8.61^{b}
CK	33.30 ± 13.33^{cd}	26.76 ± 12.28^{bc}	62.88 ± 13.09^{cde}	43.39 ± 8.33^{de}	29.68 ± 15.59^{c}	64.43 ± 8.69^{bc}	42.59 ± 6.85^{cd}	35.60 ± 16.11^{cd}
KC	32.54 ± 10.28^{cd}	25.40 ± 8.23^{bc}	58.33 ± 10.28^{bcde}	40.43 ± 8.28^{cde}	21.70 ± 10.23^{bc}	63.90 ± 8.01^{bc}	41.91 ± 6.69^{cd}	33.09 ± 9.75^{cd}
JK	37.27 ± 12.54^{d}	27.09 ± 10.26^{c}	62.94 ± 11.17^{de}	43.72 ± 9.71^{de}	29.95 ± 9.87^{c}	70.70 ± 9.59^{cd}	45.54 ± 8.12^{d}	41.82 ± 18.13^{d}
KJ	24.05 ± 10.18^{ab}	18.22 ± 7.70^{a}	51.63 ± 10.71^{ab}	36.64 ± 7.45^{bc}	16.24 ± 7.72^{ab}	59.17 ± 7.57^{ab}	40.02 ± 11.30^{b}	24.55 ± 11.96^{b}

Means that share the same superscript letters within a column are not significantly difference ($P < 0.05$). C, J and K represent Chinese, Japanese and Korean strain respectively. Three parental groups: CC = C♀ × C♂; JJ = J♀ × J♂; KK = K♀ × K♂. Six reciprocal hybrid groups: CJ = C♀ × J♂; JC = J♀ × C♂; CK = C♀ × K♂; KC = K♀ × C♂; JK = J♀ × K♂; KJ = K♀ × J♂.

3.2. Spat and adult growth

The shell height, shell length and the whole body weight at spat and adult stages were summarized in Table 3 and Table 4. Compared three parental groups, the group CC grown significantly faster than the group KK, and no significant differences was observed between the group CC and JJ at the spat and adult stages. For six hybrid crosses, the cross CJ outperformed other five groups in all growth traits, and the size parameters were significant larger than the three parental crosses (Tables 2, 3 and 4). At harvest, the shell height, shell length and the whole body weight of the group CJ reached to 75.64(±11.68) mm, 46.35(±9.06) mm and 42.11(±10.53) g, respectively.

The growth tendencies in shell height of three kind hybridization combinations are shown in Fig. 1. It is elicited from the hybridization between C and J that shell height of the hybrid cross CJ was significantly larger than parental crosses CC and JJ (Fig. 1.a; Tables 2, 3 and 4). For the hybridization between C and K, the growth of hybrids from two direction (CK and KC) was significantly faster than the parental cross KK, but the two hybrid crosses did not significantly from each other at the two stage (Fig.1.b; Tables 2, 3 and 4). The shell height and shell length of cross JK was significant larger than that of the reciprocal hybrid cross KJ and parental cross KK (Fig.1.c; Tables 2, 3 and 4). At 15 months of age, the shell height, shell length and the whole body weight for cross JK reached to 70.70(±9.59) mm, 45.54(±8.12) mm, 41.82(±18.13) g, respectively.

3.3. Spat and adult survival

The survival rate of hybrid crosses CJ and CK were higher than the three parental crosses and other hybrid crosses, while that of hybrids crosses JK and KJ were lower than that of three parental and other four hybrids crosses (Fig. 2 and Table 2). The cross CJ exhibited the highest survival rate at 69.45 ± 2.97% at spat stage and 81.33 ± 4.16% at adult stage. Survival rate of the reciprocal hybrids crosses CK and KC were significantly larger than the parental crosses CC and KK, which were 65.14 ± 5.50% and 77.33 ± 4.16% (spats), 57.59 ± 4.17% and 71.33 ± 4.16% (adults), respectively (Fig. 2 and Table 2).

3.4. Heterosis

Heterosis values assessed from the performance traits and survival rate of three kinds of the reciprocal hybrids at the different periods are presented in Table 5. Among the different hybrid crosses, growth traits and growth stages there were distinct differences in the magnitude of heterosis expression. At larval stage, average heterosis of shell height of any hybrid cross was positive, and the degree of heterosis (1.61–8.66%) was different among the hybrid crosses. During the spat and adult stage, the differences of heterosis for growth and survive existed among the six hybrid crosses and in most hybrid crosses the growth heterosis value tended to increase with age. The heterosis of cross CJ for all performance indicators are positive (14.56–78.92%), whereas those of the reciprocal cross JC are negative, ranging from −6.07% to −30.23%. Both hybrid crosses CK and KC had positive heterosis value in growth traits, with the larger value observed in the cross CK. For the reciprocal crosses JK and KJ, the cross JK had larger heterosis value of shell height, shell length and the whole body weight than those of the cross KJ at spat and adult stage, of which were contrary to the larvae stage.

For survival rate, the heterosis value in cross CJ, CK and KC were positive at the spat and adult stages, which were 27.02% and 21.99%, 57.23% and 51.63%, 39.87% and 39.86% respectively, while the other three crosses expressed negative heterosis value.

4. Discussion

In the present work, the genetic variation of different culture stains in Pacific oyster *C. gigas* was as the foundation for establishing crossbreeding plans. The amount of heterosis in a cross between two particular lines or populations depends on the square of the difference in gene frequency between the populations, and the larger difference of crossbreds and the higher homozygosis frequency, the greater heterosis would cause (Falconer, 1981). Although it is difficult to except stable levels of heterosis, we combined the mass selection in hybridization experimental in order to provide “purer” hybridization parents to obtain the best hybrid progeny. After being introduced to China, the three culture stocks were maintained separately whilst practicing within mass selection for two generation, and non-additive genetic variation

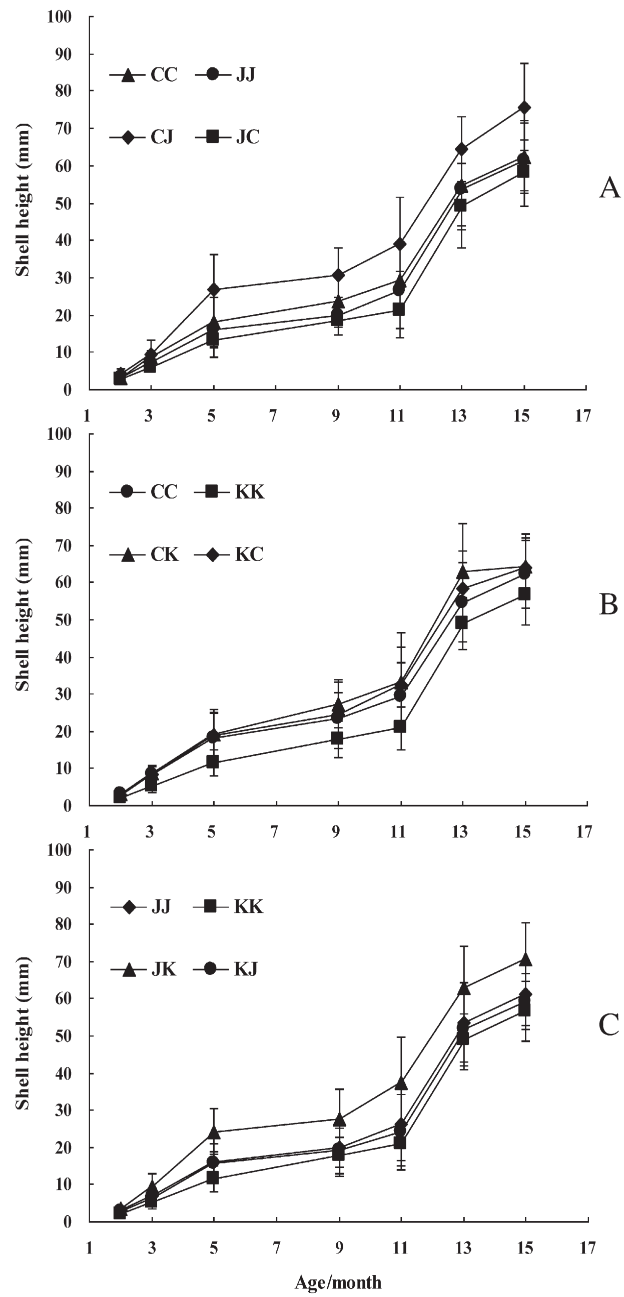

Fig. 1. Comparison of the growth of hybridization between different strains in *C. gigas* at spat and adult stages. (A) The shell height of Chinese and Japanese strains with four experimental crosses. (B) The shell height of Chinese and Korean strains with four experimental crosses. (C) The shell height of Japanese and Korean strains with four experimental crosses. C, J and K represent Chinese, Japanese and Korean strain respectively. (C, J and K represent Chinese, Japanese and Korean strain respectively. Three parental groups: CC = C♀ × C♂; JJ = J♀ × J♂; KK = K♀ × K♂. Six reciprocal hybrid groups: CJ = C♀ × J♂; JC = J♀ × C♂; CK = C♀ × K♂; KC = K♀ × C♂; JK = J♀ × K♂; KJ = K♀ × J♂.).

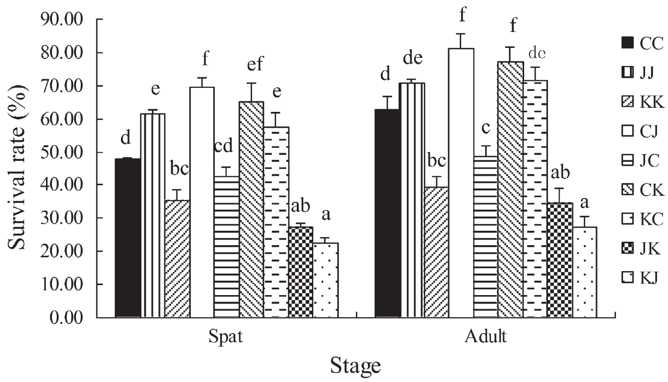

Fig. 2. Comparison of survival rate of nine experimental crosses of *C. gigas* at spat and adult stages. (C, J and K represent Chinese, Japanese and Korean strain respectively. Three parental groups: CC = C♀ × C♂; JJ = J♀ × J♂; KK = K♀ × K♂. Six reciprocal hybrid groups: CJ = C♀ × J♂; JC = J♀ × C♂; CK = C♀ × K♂; KC = K♀ × C♂; JK = J♀ × K♂; KJ = K♀ × J♂.). Means that share the same superscript letters within a stage are not significantly difference ($P < 0.05$).

may be larger among the parent strains. Therefore, heterosis may rapidly accumulate in the cross between the selected strains.

In our study, the six hybrids cross all showed positive mean heterosis for shell heights at larval stage although the degree of heterosis value was different among them and the hybrid crosses CJ and KJ outperformed all the other parental and hybrid crosses in shell height at larval stage, of which mean value of heterosis were 8.66% and 7.26%, respectively. This result may be due to the combination of selection and hybridization, the further study needs to be adopted.

For the later growth and survival in the field in the study, the hybrids of different combinations and traits produced different heterosis effects. There were three major observations on heterosis at spat and adult stages. Firstly, differences of heterosis obviously consisted in the reciprocal crosses CJ and JC, the cross CJ had positive heterosis value (13.33–78.92%) at spat and adult stages for all traits, but those of JC were all negative (−6.07–−30.23%). Differences between reciprocal crosses are also commonly observed in fishes (Basavaraju et al., 1995; Bartley et al., 2001), sea urchins (Rahman et al., 2001, 2005) and scallops (Cruz and Ibarra, 1997). Difference in reciprocal hybrids performance may be due to the sex-linked genes, cytoplasmic inheritance, or parental effects. All these results suggest that the determination the male and female parent population is very important before hybridization applying in commercial seed production. Secondly, the heterosis of survival in CK (51.63% and 57.23%) and KC (39.87% and 39.86%) was larger than that of shell height and shell length in CK (8.23–33.93%) and KC (7.14–29.51%). Wang and Li (2010) obtained the similar result in Bay scallops *Argopecten irradians irradians*. In their study, the heterosis for survival rate was higher than that in growth at both the larval and adult stages. Falconer (1981) stated that heterosis depends on the presence of directional dominance for the loci involved in the specific trait, thereafter Derose and Roff (1999) also emphasized that morphological traits exhibit little or no directional dominance. The results of heterosis in this study may be interpreted as the coefficient of dominance variance is larger in survival rate than in morphological traits. Thirdly, the magnitude of heterosis was not constant among life history stages. Compared to other marine bivalves, heterosis for growth trait was reported to increase with ages (Zheng et al., 2006; You et al., 2009, 2015), however, in this study, the result was not completely consistent with other reports. The average heterosis value in later growth in most hybrid crosses was larger than that at larval stage, and the highest for the spat stage. The results may be due to the season factor such as seawater temperature. The growth rate at spat stage was lower than that at adult stage, so the gene expression for growth in hybrid cross may be more obvious relative to the parental groups at the spat stage, while at adult stage the differences among hybrid and parental crosses were little.

In summary, the major goal of our study in *C. gigas* is to obtain the seed with better growth and survival traits. Combining the results on growth and survival lead to the conclusion that the hybrid groups CJ, CK and KC were better combination for seed production.

Table 5
Heterosis for shell height (SH), shell length (SL), the whole body weight (W) and survival rate (S) in six hybrids crosses of *C. gigas*.

Stage and Age	Trait	Heterosis (%)					
		CJ	JC	CK	KC	JK	KJ
Larvae							
	SH	8.66	1.61	3.38	3.21	4.22	7.26
Spat							
2 months	SH	29.97	−13.88	17.29	7.14	28.25	1.86
	SL	29.63	−14.40	28.68	12.14	25.77	2.60
3 months	SH	21.96	−23.87	28.85	21.55	54.89	0.16
	SL	14.56	−28.72	16.57	16.57	46.67	1.95
5 months	SH	56.64	−22.38	29.30	28.09	75.69	13.13
	SL	53.06	−18.39	22.44	25.07	79.80	31.24
9 months	SH	40.58	−15.06	32.00	17.53	45.25	1.88
	SL	41.06	−15.30	29.73	15.90	44.18	7.54
	S	27.02	−22.14	57.23	39.87	−43.61	−53.36
Adult							
11 months	SH	39.38	−23.92	32.54	29.51	57.76	1.80
	SL	30.97	−28.02	33.93	27.13	52.49	2.56
13 months	SH	19.29	−9.00	21.48	12.69	22.69	0.64
	SL	21.56	−10.34	27.13	18.46	30.53	9.36
	W	78.92	−30.23	99.19	45.64	115.16	16.67
15 months	SH	22.35	−6.07	8.23	7.34	19.80	0.26
	SL	13.33	−10.46	19.07	17.17	29.74	14.02
	W	37.77	−27.76	45.69	35.42	89.23	11.09
	S	21.99	−27.00	51.63	39.86	−36.96	−50.31

C, J and K represent Chinese, Japanese and Korean strain respectively. Three parental groups: CC = C♀ × C♂; JJ = J♀ × J♂; KK = K♀ × K♂. Six reciprocal hybrid groups: CJ = C♀ × J♂; JC = J♀ × C♂; CK = C♀ × K♂; KC = K♀ × C♂; JK = J♀ × K♂; KJ = K♀ × J♂.

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