

# Growth, survival and color segregation of F2 hybrids between selected “Haida no.1” and Orange-shell lines of the Pacific oyster

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## ABSTRACT

In aquatic breeding work, much attention has been paid to the performance of the F1 hybrids. However, fewer studies have been conducted on the characteristics of F2 and F<sub>n</sub> hybrids, especially in the intraspecific hybridization of the Pacific oyster *Crassostrea gigas*. In this study, the two purebreds, F1 hybrid families and F2 hybrid families was developed utilizing “Haida No.1” line (HH), Orange-shell line (OO) and their reciprocal hybrids (HO and OH) of *C. gigas* as the parents. The growth (shell height and wet weight), survival and shell color traits of the six groups were systematically investigated. Our results demonstrated the two F1 hybrid families exhibited significantly heterosis in shell height and wet weight compared to the purebred groups, with mid-parental heterosis at 9.85–36.49% and –16.50–50.07%, respectively. Moreover, the traits of two F2 hybrid families were gradually inferior to F1 hybrid families from 6th month. Two F2 hybrid families exhibited inbreeding depression in wet weight compared to F1 hybrid families through the whole grow-out stage, with the values from –13.13% to –7.34%. However, the growth and survival advantages were existed in F2 hybrid families compared to their two parental counterparts. Meanwhile, the survival rate of the F2 hybrid families were greater than those of the parental groups during the grow-out stage, with superiority rate of 1.55–19.59%. Furthermore, some orange-shell and purple-shell individuals were detected in the F2 hybrid families. The rates of orange-shell individuals to non-orange shell individuals and purple-shell individuals to non-purple shell individuals were both 1:3. Surprisingly, the growth traits of orange-shell and purple-shell individuals in the F2 hybrid families were significantly superior than those of their corresponding controls (Orange-shell line and wild population). Through this comprehensively comparison analysis, we confirmed that F2 hybrids of *C. gigas* had obvious advantages in terms of shell height, wet weight and survival rate compared to the purebred lines, and the orange and purple individuals obtained from the F2 hybrid families have great potential to be used for the development for new strains.

## 1. Introduction

Crossbreeding is a classical breeding method for genetic improvement in both of plants and animals, mainly utilizing the non-additive genetic variation (heterosis) to improve the growth and survival traits of progenies, and is commonly used to cultivate new varieties with succulent meat, improved stress resistance and high yield (Bartley et al., 2001). Hybridization comprises intraspecific hybridization and interspecific hybridization. Among them, interspecific crosses can generate more genetic variation in the offspring and supply more basic materials for cultivating new varieties (Qin et al., 2021). Moreover, intraspecific crosses do not result in offspring with poor fertility and have a wider

range of applications. In aquaculture, crossbreeding has been mainly employed to improve flesh quality, raise yield and enhance stress resistance (Bartley et al., 2001; Tan et al., 2020; Hedgecock et al., 1995; Hedgecock and Davis, 2007; Rawson and Feindel, 2012; Wang and Côté, 2012). For example, crossbreeding between the Chinese and American populations of Kumamoto oysters can yield hybrids with faster growth and higher survival rates (Ma et al., 2022). The growth and survival of offspring bred by crossing the oyster *Crassostrea hongkongensis* with *C. sikamea* are significantly better than those of *C. sikamea* (Zhang et al., 2017a). Furthermore, the environmental tolerance and resistance to pathogens of abalone can be remarkably improved by crossbreeding (Alter et al., 2017; Dang et al., 2011). The growth and survival of *C. gigas*

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can be improved by crossbreeding between different geographical populations or strains which have been selected over multiple generations (Kong et al., 2017; Han et al., 2020). In addition, the genetic diversity of mollusks also can be increased through crossbreeding (Lu et al., 2012; Liang et al., 2023).

While hybridization can produce exploitable heterosis to improve yield and quality traits of the offspring, the heterosis is mostly expressed in the F1 hybrids. The segregation of phenotypes and genetic traits in the F2 and Fn generations is usually caused by independent assortment and recombination of genes when F1 hybrids self-fertilize to produce the F2 generation and subsequent generations (Mendel, 1941). In one of the few examinations of the phenotypic traits of the F2 hybrids. The growth of the F2 hybrids of channel catfish (*Ictalurus punctatus*) and blue catfish (*I. furcatus*) was lower than that of the F1 and the self-cross groups (Argue et al., 2014). Compared to European carp (*C. carpio*) and silver carp (*C. gibelio*), their F2 hybrids exhibited significantly lower resistance to viruses (Šimková et al., 2022). In shellfish, the Fn ( $n \geq 2$ ) similarly presented high levels of genetic variation. For example, The F2 hybrids between *C. ariakensis* and *C. hongkongensis* had distinct advantages in terms of salinity tolerance and growth traits over their grandparent species (Qin et al., 2021). In contrast, the survival and growth of the F2 generations between *C. gigas* and *C. hongkongensis* were significantly lower than those of the parental self-cross group and the backcross group (Zhang et al., 2016). Moreover, studies on phenotypic and genetic traits segregation in F2 and subsequent generations in aquatic animals have mostly focused on interspecific or intergeneric crosses, such as *Argopecten purpuratus*  $\times$  *A. irradians irradians* (Feng et al., 2012), and *Oreochromis niloticus*  $\times$  *Sarotherodon melanotheron* (Fan et al., 2012; Guo et al., 2014). However, the phenotypic performance of F2 and higher hybrid generations of intraspecific crosses in shellfish have rarely been documented.

As one of the most important economically aquatic species, the Pacific oyster *C. gigas* have been widely cultivated in northern China. A fast-growing line “Haida No. 1” and an inbreeding line with orange shell color were successfully established through selective breeding (Li et al., 2011; Han et al., 2020). In a previous study, we evidenced that the F1 hybrids of the “Haida No. 1” and Orange-shell line presented a remarkable heterosis with respect to growth and survival rate compared to both two parental lines (Liang et al., 2022a, 2022b). As the two lines have undergone a dozen generations of mass selection for different characteristics during the selective breeding process, significant genetic differences existed between the two lines (Liang et al., 2023). Studies have shown that the degree of decline of heterosis in F2 and subsequent generations depends on the genetic distance between the grandparents, the environment and the degree of linkage between recombinant genes (Emlen, 1991; Lynch, 1991). Therefore, these two lines can be served as useful material to study the segregation of traits in the F2 hybrids of *C. gigas*.

In this study, the “Haida No.1” line, Orange-shell line and their reciprocal hybrids (“Haida No.1”  $\text{♀} \times$  Orange-shell line  $\text{♂}$ , Orange-shell line  $\text{♀} \times$  “Haida No.1”  $\text{♂}$ ) were employed as parents. The goal of this study was to investigate the growth metrics (shell height and wet weight), survival traits of the F1 hybrids, F2 hybrid families and their grandparent lines (“Haida No.1” and Orange-shell line). Moreover, segregation of growth, survival and shell color traits of F2 hybrid families were also systematically examined.

## 2. Materials and methods

### 2.1. Parental origins and experimental designs

The two-year-old Pacific oyster harvested from the Rushan breeding stock in Shandong Province, China (36.4°N, 121.42°E), was utilized in 2007 to develop the first generation of “Haida No.1” targeting rapid growth (Li et al., 2011). Up to the end of 2020, the 13th generation of “Haida No.1” (H) has been successfully bred through mass selection, in

which every generation contains >50 males as well as 50 females oysters to be parents. Four mutants (two males and two females) with solid orange color on both left and right shells were found accidentally from the offspring of black shell line and purple shell line crosses of *C. gigas* in 2011. Two full-sib families were established from these four oysters as the first generation of the Orange-shell line (O). Subsequently, two consecutive generations of family selection and seven generations of mass selection were established for fixing shell color and improving growth rate from 2012 to 2020, thereby the Orange-shell line was developed (Han et al., 2019). The hybrids of “Haida No.1” and Orange-shell line (HO, H  $\text{♀} \times$  O  $\text{♂}$  and OH, O  $\text{♀} \times$  H  $\text{♂}$ ) were established in both 2019 and 2020. Encouragingly, both two reciprocal hybrids exhibited significant heterosis in survival and growth (Liang et al., 2022a, 2022b).

In March 2021, the wild population (WP), “Haida No.1” line (HH), Orange-shell line (OO) and their reciprocal hybrids (HO, H  $\text{♀} \times$  O  $\text{♂}$  and OH, O  $\text{♀} \times$  H  $\text{♂}$ ) were collected from Rushan, Shandong Province, China (36.4°N, 121.42°E). All groups used in this study were one-year-old gonadally mature *C. gigas*. The HH, OO, HO and OH have been established in 2020 (Liang et al., 2022b), and the WP were collected from Rushan Bay. The five groups were sampled and transported to Yantai Litaos Seedling Limited, Shandong Province, China (37.3°N, 119.9°E). The broodstocks were artificially cultured with conditioning water (temperature: 24.0–25.0 °C; salinity: 30 psu) for two weeks prior to the experiments.

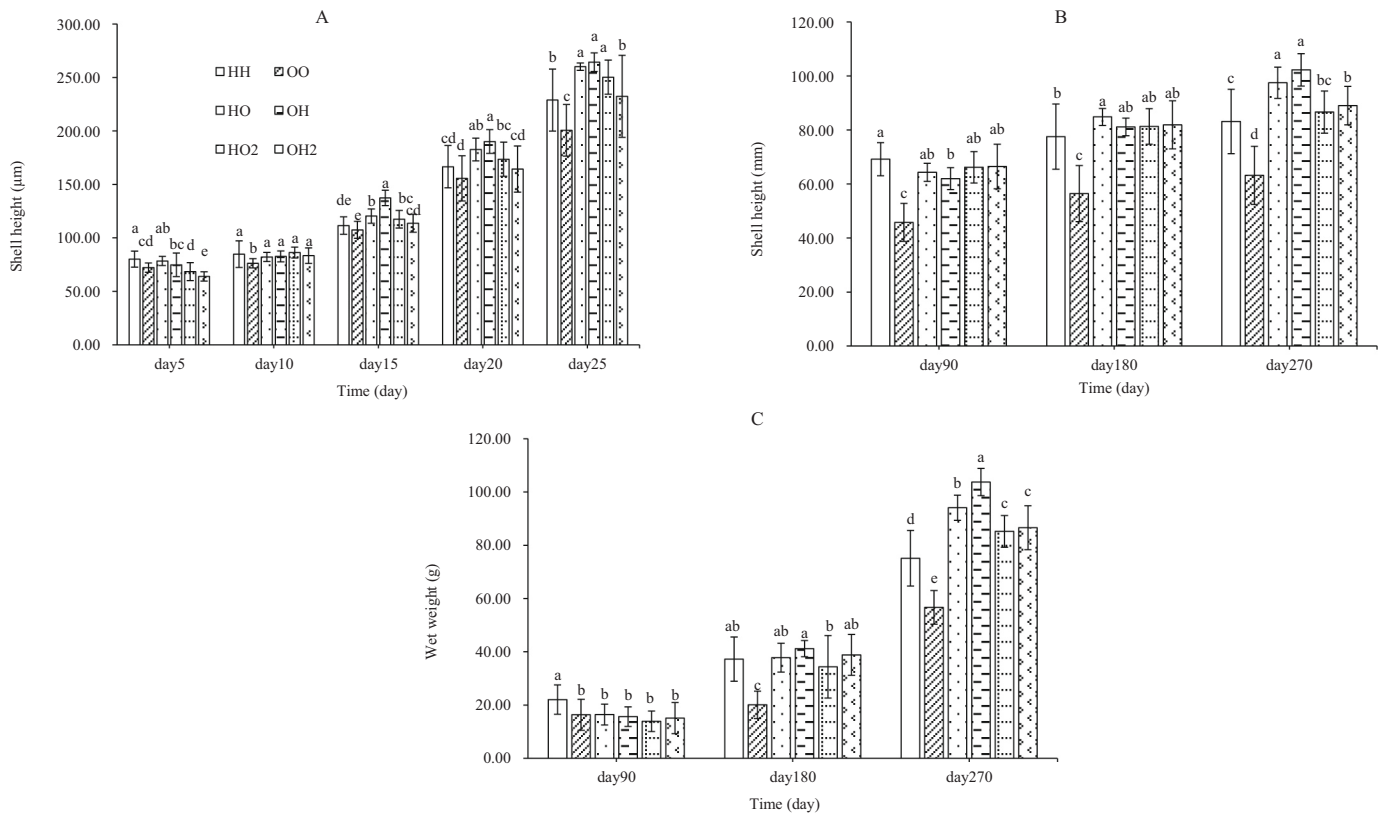
Sexually mature oysters were selected from the five groups (WP, HH, OO, HO and OH) based on shell height (Table 1). For each group, eggs from one maturely oyster were obtained by dissection and examined under microscope to verify that no uncontrolled fertilization had taken place. After the eggs were activated in a seawater container, the sperm from one male of each group were collected. Then, fertilization is accomplished using the following methods (Fig. 1). Firstly, eggs from “Haida No.1” and Orange-shell line were fertilized with the sperm from the two parental line to accomplished inter-line and intra-line hybridization. Thus, four combinations were generated: two purebred families (HH, H  $\text{♀} \times$  H  $\text{♂}$  and OO, O  $\text{♀} \times$  O  $\text{♂}$ ), and two F1 hybrid families (HO, H  $\text{♀} \times$  O  $\text{♂}$  and OH, O  $\text{♀} \times$  H  $\text{♂}$ ). Secondly, eggs from HO group or OH group were only fertilized with the sperm from the same group. Thus, two F2 hybrid families were generated: (HO<sub>2</sub>, HO  $\text{♀} \times$  HO  $\text{♂}$  and OH<sub>2</sub>, OH  $\text{♀} \times$  OH  $\text{♂}$ ). Thirdly, females of WP group were only fertilized with the sperm from males of the same group to establish the control group (WP, WP  $\text{♀} \times$  WP  $\text{♂}$ ). All combinations were obtained by mating one female and one male at a sperm: egg ratio of 30–50. The experiment was conducted in triplicates using three sets of parents.

The fertilized eggs for each group were separately hatching in 100-L polyethylene plastic buckets. The larvae and juveniles were nurtured in accordance with the methodology introduced by Li et al. (2011). Basically, after a 24-h incubation period, each group of D-larvae was screened out through a 300-mesh mesh and cultured separately in conditioned seawater (temperature: 24.0  $\pm$  1 °C; salinity: 30.0  $\pm$  1 psu). Larvae were fed with *Isochrysis galbana* during the D-larvae stage, and appropriate *Platymonas* sp. were supplied as the larvae grew. The larvae density was initially to be set at 2–4 larvae ml<sup>-1</sup> per bucket, decreasing to 0.5 larvae ml<sup>-1</sup> with larval growth. Once 30% of the larvae attained the eyed-stage, bunches of scallop shells were suspended in buckets to be used as substrates for larval settlement and metamorphosis. After all the larvae finished attaching, the substrates were moved to an outdoor nursery pond for a two-week temporary rearing. Afterwards, the

**Table 1**

The shell heights and wet weights of five broodstocks used in this study.

Items	HH	OO	HO	OH	WP
Shell height (mm)	106.04 $\pm$ 9.44	90.32 $\pm$ 11.5	107.92 $\pm$ 8.07	109.72 $\pm$ 11.1	33.82 $\pm$ 7.01
Wet weight (g)	87.64 $\pm$ 13.52	51.88 $\pm$ 10.15	86.95 $\pm$ 14.76	99.15 $\pm$ 12.14	15.51 $\pm$ 2.55



**Fig. 1.** Diagrammatic representation of Pacific oyster “Haida No. 1”, Orange-shell line, and the mating strategy in this study. The dotted box contains the breeding process for two lines. The red box contains the mating strategy. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

juveniles were transferred to Rongcheng, Shandong Province, China (37.11°N, 122.35°E) for breeding. After one month of culturing, the spats were deposited into 10-layer lantern nets with 30 spats per layer at July 2021. The initial shell heights for HH, HO, OH, OO, HO<sub>2</sub>, OH<sub>2</sub> and WP were 16.37 ± 5.79 mm, 15.65 ± 3.70 mm, 17.07 ± 2.44 mm, 16.44 ± 3.90 mm, 14.49 ± 2.30 mm, 17.32 ± 4.70 mm and 15.94 ± 3.49 mm, respectively.

**2.2. Performance evaluation**

At the larval stage, 30 individuals were randomly picked from each of the six groups (two purebred families: HH and OO; F1 hybrid families: HO and OH; F2 hybrid families: HO<sub>2</sub> and OH<sub>2</sub>) and photographed with Olympus BX53 on the days 5, 10, 15, 20, 25 after fertilization. The shell height of the 30 oysters were then measured through Image-ProPlus 6.0. Moreover, the number of surviving larvae was counted by randomly selecting 100 ml from each group at the sampling day and dividing by the number of living oysters in 100 ml at the time of D-larvae to estimate the survival rate during the larval stage.

A sample of 30 oysters per replicate per group were randomly sampled to evaluated the growth metrics (shell height and wet weight) at 3rd month, 6th month and 9th month. The shell height of the samples was gauged with vernier calipers (accuracy 0.01 mm) and the wet weight was monitored with an electronic scale (accuracy 0.01 g). The survival rate was calculated as the percent difference of living oysters at time T relative to living oysters at time 0:

$$SR_T (\%) = N_{(T)} \times 100 / N_0$$

Here, SR<sub>T</sub> indicates the survival rate of a given cross at time T; N<sub>(T)</sub> represents the living oysters per lantern net at time T; N<sub>0</sub> is the total number of oysters in each lantern net of a given group at July 2021.

To determine the heterosis of F1 hybrid families in growth or sur-

vival, the mid-parent heterosis (*M*) was calculated according to the formula below (Wang et al., 2011; Zheng et al., 2012):

$$M_{F1} (\%) = [(F1 - MP) \times 100] / MP$$

Where F1 and MP denote the growth metrics (shell height or wet weight) or survival rate of F1 hybrid families and parental groups (HH and OO), respectively. *M*<sub>F1</sub> stands for the mid-parent heterosis of hybrids.

To explore the gain in phenotypic values of the F1 hybrid families compared to their “Haida No.1” counterparts, the high-parental heterosis (*H*) was derived by adapting the underlined model from Cruz and Ibarra (1997):

$$H_{(F1/HH)} (\%) = (P_{F1} - P_{HH}) \times 100 / P_{HH}$$

Where *P*<sub>HH</sub> is the mean performance value (shell height, wet weight or survival rate) of the “Haida No. 1” of *C. gigas*. *P*<sub>F1</sub> represents the mean phenotypic value of the two F1 hybrid families.

Furthermore, inbreeding depression and superiority were introduced to judge the performance of the F2 hybrids in this study, inbreeding depression rate (*I*) of F2 hybrid families was calculated through the modified equation (Zheng et al., 2012):

$$I (\%) = (P_{F2} - P_{F1}) \times 100 / P_{F1}$$

Here, *I* indicate the inbreeding depression rate of F2 hybrid families compared to their F1 hybrid family counterparts. *P*<sub>F2</sub> and *P*<sub>F1</sub> represent the mean phenotypic value of F2 hybrid families and F1 hybrid families, respectively.

In addition, the superiority rate (*S*) of the F2 hybrid families was defined as the percentage increasing of the phenotypic values between the F2 hybrid families and the two pure parental lines (HH and OO) and was considered in accordance with the equation below (Wang et al.,

2011):

$$S (\%) = (P_{F2} - P_P) \times 100 / P_P$$

Where *S* is the superiority rate. *P<sub>F2</sub>* and *P<sub>P</sub>* represent the mean phenotypic value of the two F2 hybrid families and the pure parental progeny (HH and OO), respectively.

### 2.3. Statistical analyses

Data are presented as the means ± standard deviation (SD). The mean growth metrics (shell height and wet weight) were log transformed by base 10 and the survival rate of each group were transformed by arcsine to improve the normality and homoscedasticity. Then, the data were analyzed using SPSS 26.0. Differences in shell height, survival rate or wet weight between different groups were analyzed by one-way ANOVA and multiple comparison Tukey test. Significance was determined at *P* < 0.05 unless otherwise specified.

## 3. Results

### 3.1. Growth of purebreds, F1 hybrid families and F2 hybrid families

Significant difference in growth was detected among “Haida No.1” (HH), Orange-shell line (OO), their two reciprocal hybrids (HO and OH), and two F2 hybrid families (HO<sub>2</sub> and OH<sub>2</sub>) during the larval stage (*P* < 0.05) (Fig. 2A). The growth of HH was larger than the other five combinations in the first ten days after fertilization. However, the shell heights of F1 hybrid families and F2 hybrid families grew faster than that of HH and became significantly larger than that of OO from day 15 (*P* < 0.05). At day 25, the shell heights of the four groups are in the following order: OH > HO > HH > OO, with the mid-parental heterosis of 22.11%, high-parental heterosis of *H<sub>(HO/HH)</sub>* of 13.65% and *H<sub>(OH/HH)</sub>* of 15.50%, respectively. Two F2 hybrid families exhibited inbreeding depression in shell height compared to F1 hybrid families, with values of inbreeding depression rate from -13.63% to 2.93%. But, the shell heights of F2 hybrid families were larger than two parental lines during the larval stage, with the superiority rate of 12.37% at day 25 (Table 2).

The shell heights of the two F1 hybrid families were inferior to that of the HH group, but significantly exceeding that of the OO group at day 90 (*P* < 0.05) (Fig. 2B). However, the shell heights of F1 hybrid families were significantly higher than those of two purebreds from the 6th month (*P* < 0.05). Moreover, heterosis for shell height in the two F1

**Table 2**

Genetic indexes for F1 hybrids and F2 hybrids at larval stage.

Traits	Items	Day 5	Day 10	Day 15	Day 20	Day 25
Shell height	<i>M<sub>F1</sub></i> (%)	0.57	2.40	17.72	15.72	22.11
	<i>H<sub>(HO/HH)</sub></i> (%)	-2.13	-3.09	8.02	9.70	13.65
	<i>H<sub>(OH/HH)</sub></i> (%)	-6.67	-2.41	23.21	14.16	15.50
	<i>I</i> (%)	-13.63	2.93	-10.33	-9.36	-7.97
	<i>S</i> (%)	-13.14	5.40	5.56	4.89	12.37
Survival rate	<i>M<sub>F1</sub></i> (%)	/	0.92	10.96	16.42	41.32
	<i>H<sub>(HO/HH)</sub></i> (%)	/	-1.47	4.67	2.62	11.11
	<i>H<sub>(OH/HH)</sub></i> (%)	/	1.83	8.41	1.57	12.42
	<i>I</i> (%)	/	-0.37	-0.66	-0.51	-2.63
	<i>S</i> (%)	/	0.55	10.23	15.82	37.60

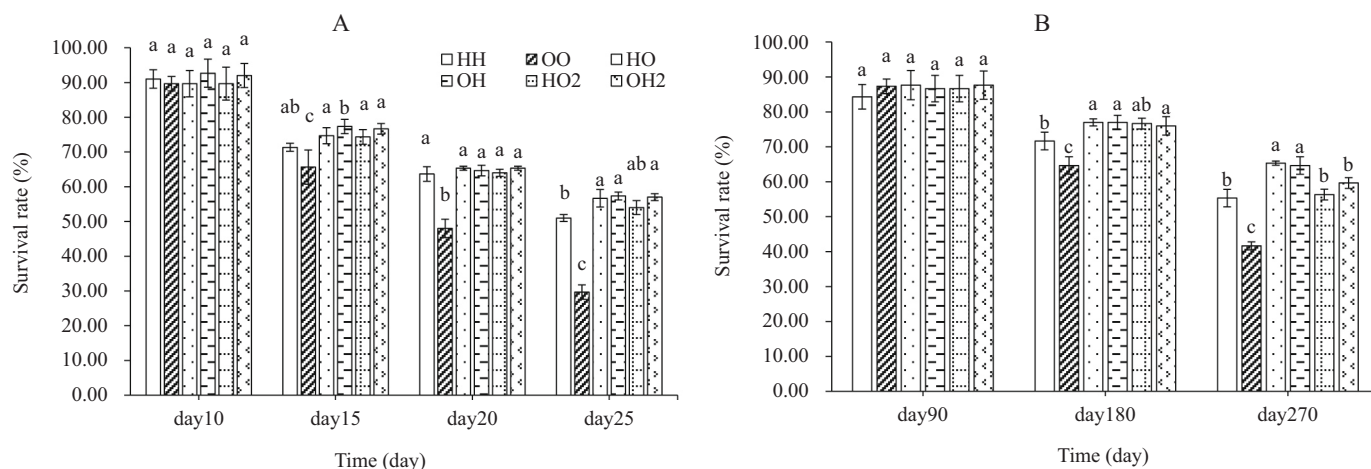
*M<sub>F1</sub>* represents the mid-parental heterosis of F1 hybrids; *H<sub>(HO/HH)</sub>* and *H<sub>(OH/HH)</sub>* represent the growth or survival advantage for HO and OH, respectively, compared to HH; *I* is the inbreeding rate; *S* indicates the superiority rate.

hybrid families (9.85–36.49%) gradually increased with spat growth. The shell height of two F2 hybrid families were larger than those of the other four groups, with positive inbreeding depression and superiority at 5.05% and 15.40%, respectively, at day 90 (Table 3). However, two F2 hybrid families were gradually smaller than two F1 hybrid families but

**Table 3**

Genetic indexes for F1 hybrids and F2 hybrids during the grow-out stage.

Traits	Items	3th month	6th month	9th month
Shell height	<i>M<sub>F1</sub></i> (%)	9.85	23.87	36.49
	<i>H<sub>(HO/HH)</sub></i> (%)	-7.04	9.41	17.23
	<i>H<sub>(OH/HH)</sub></i> (%)	-10.42	4.64	22.97
	<i>I</i> (%)	5.05	-1.65	-12.06
	<i>S</i> (%)	15.40	21.83	20.03
Wet weight	<i>M<sub>F1</sub></i> (%)	-16.50	37.71	50.07
	<i>H<sub>(HO/HH)</sub></i> (%)	-25.50	1.42	25.24
	<i>H<sub>(OH/HH)</sub></i> (%)	-29.07	10.53	38.09
	<i>I</i> (%)	-9.62	-7.34	-13.13
	<i>S</i> (%)	-24.53	27.60	30.36
Survival rate	<i>M<sub>F1</sub></i> (%)	1.55	12.96	34.02
	<i>H<sub>(HO/HH)</sub></i> (%)	3.95	7.44	18.07
	<i>H<sub>(OH/HH)</sub></i> (%)	2.77	7.44	16.87
	<i>I</i> (%)	0.00	-0.87	-10.77
	<i>S</i> (%)	1.55	11.98	19.59



**Fig. 2.** Shell heights and survival rates for two purebreds (HH and OO), two F1 hybrids (HO and OH) and two F2 hybrids (HO<sub>2</sub> and OH<sub>2</sub>) during the whole life. A: the shell heights of six groups at the larval stage; B: the shell heights of six groups during the grow-out stage; C: the wet weights of six groups during the grow-out stage. H and O indicate “Haida No. 1” line and Orange-shell line of the Pacific oyster, respectively. Two purebred groups, HH (H♀ × H♂), OO (O♀ × O♂), their reciprocal F1 hybrids, HO (H♀ × O♂), OH (O♀ × H♂) and two F2 hybrids, HO<sub>2</sub> (HO♀ × HO♂), OH<sub>2</sub> (OH♀ × OH♂). Different superscript letters in the same day indicate significant difference (*P* < 0.05) among six groups.

still exhibited growth advantage compared to the two parental counterparts from 6th month.

At 3rd month, two hybrids exhibited negative heterosis in wet weight, with the values at  $-16.50\%$ . But, the wet weight of two reciprocal hybrids were larger than those of two parental lines from 6th month. Notably, two hybrid groups exhibited apparently heterosis in wet weight compared to two purebred groups, with the values of high-parental heterosis ( $H_{(HO/HH)}$  and  $H_{(HO/HH)}$ ) at  $25.24\%$  and  $38.09\%$ , respectively (Table 3). Two F2 hybrid families exhibited inbreeding depression in wet weight compared to F1 hybrid families through the whole grow-out stage, with the values from  $-13.13\%$  to  $-7.34\%$ . However, the wet weights of F2 hybrid families were larger than two purebreds from 6th month. Notably, F2 hybrid families was significantly heavier than two parental lines at 9th month (Fig. 2C).

### 3.2. Survival of purebreds, F1 hybrid families and F2 hybrids

No significant difference was noticed in survival among the six groups at day 10 ( $P > 0.05$ ) (Fig. 3A). However, the survival rate of OO was significantly lower than the other five groups from day 15 during the larval stage. Two F1 hybrid families and two F2 hybrid families exhibited survival advantage compared to parental lines, with mid-parental heterosis and superiority at  $0.92\text{--}41.32\%$  and  $0.55\text{--}37.60\%$ . However, the survival rates of F2 hybrid families were lower than two F1 hybrid families. The inbreeding depression rate for survival of F2 hybrids were from  $-2.63\%$  to  $-0.37\%$  during the larval stage (Table 2).

The survival rate of HH was lower than the other five groups, however, with no significant difference at 3rd month ( $P > 0.05$ ) (Fig. 3B). From 6th month, four hybrid families (HO, OH, HO<sub>2</sub> and OH<sub>2</sub>) were gradually larger than two purebreds (OO and HH) in survival. Moreover, F1 hybrid families were significantly larger than F2 hybrid families, the same pattern was existed between two F2 hybrid families and two purebreds at 9th month. Two F1 hybrid families expressed heterosis in survival compared to parental lines, with mid-parental heterosis from  $1.55\%$  to  $34.02\%$  during the whole grow-out stage. Although the survivals of F2 hybrid families were inferior to F1 hybrid families ( $I: -10.77\text{--}0.00\%$ ), they were better than those of two purebreds, with superiority rates being at  $1.55\text{--}19.59\%$  (Table 3).

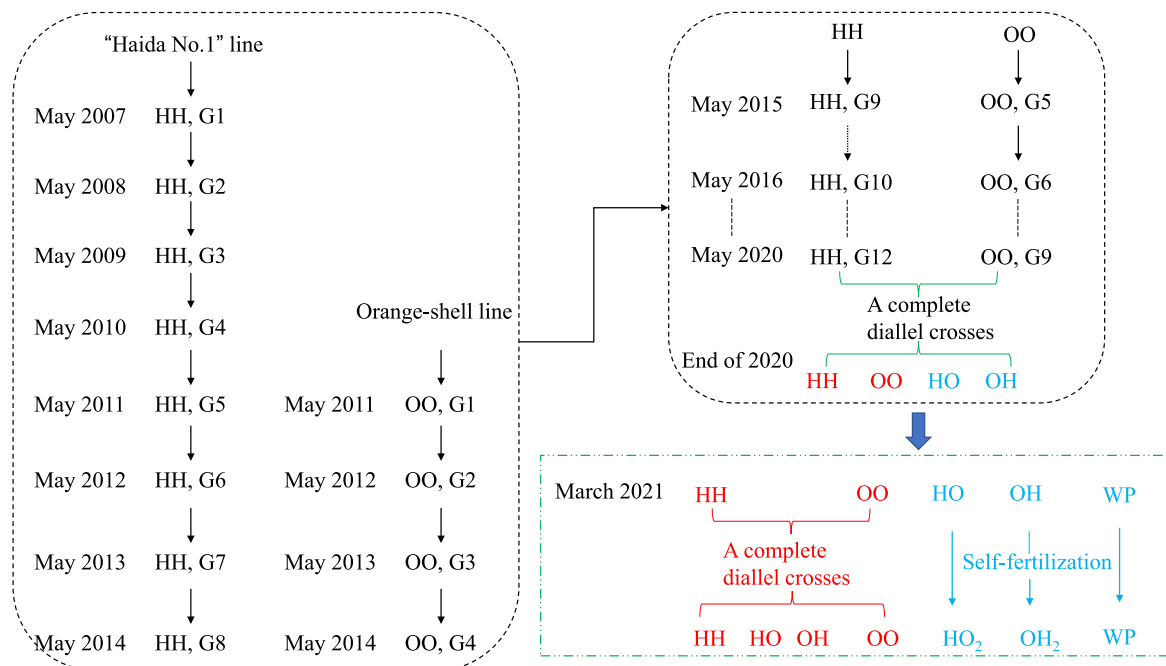
### 3.3. Separation of shell color in F2 hybrid families

Sixty oysters were randomly sampled from the F2 hybrid families to quantify shell color traits on 9th month. We noticed that these oysters presented three predominant shell color patterns. Fifteen (25%) in 60 oysters displayed solid orange color on both left and right shells. Fourteen (approximately 25%) of in the 60 oysters exhibited purple coloration on both two shells. The remaining 31 (approximately 50%) oysters had no specific color and the left shell was presented (or not) with radial stripes. Furthermore, 30 purple individuals were randomly selected from the crossed F2 hybrid families, and another 30 oysters were also sampled from WP to calculate their phenotypic traits (shell height, shell length, shell width and wet weight). Significant difference in growth metrics were visible between the two groups ( $P < 0.05$ ) (Table 4). In addition, a systematic comparison was also made between the orange individuals in F2 hybrid families and OO for growth traits. The shell height and wet weight of the orange individuals in F2 hybrids was increased by  $8.39\%$  and  $45.67\%$  compared to the Orange-shell line (Table 4).

**Table 4**  
Phenotypic traits for the purple-shell and orange-shell variants of the two F2 hybrids, Orange-shell line and wild population of the Pacific oyster *C. gigas*.

Lines	Shell height (mm)	Shell length (mm)	Shell width (mm)	Wet weight (g)
Orange-shell line	63.21 ± 10.61 <sup>b</sup>	33.60 ± 6.26 <sup>a</sup>	21.10 ± 4.71 <sup>a</sup>	56.69 ± 6.34 <sup>b</sup>
Orange mutants	68.51 ± 8.90 <sup>a</sup>	36.12 ± 15.10 <sup>a</sup>	20.98 ± 4.49 <sup>a</sup>	82.58 ± 8.54 <sup>a</sup>
Purple mutants	79.71 ± 8.95 <sup>a</sup>	35.24 ± 7.76 <sup>a</sup>	20.48 ± 4.95 <sup>a</sup>	53.81 ± 10.81 <sup>a</sup>
Wild population	64.73 ± 10.11 <sup>b</sup>	25.58 ± 5.17 <sup>b</sup>	16.44 ± 4.10 <sup>b</sup>	47.44 ± 12.21 <sup>b</sup>

The phenotypic traits of Orange-shell line were compared with orange variants in F2 hybrids, and the traits of purple variants were compared with wild population. Different superscript letters between the two populations in the same column indicated significant differences ( $P < 0.05$ ).



**Fig. 3.** The survival rates for six groups at larval and grow-out stage. A: the survival rates of six groups at larval stage; B: the survival rates of six groups during the grow-out stage. Different superscript letters in the same day indicate significant difference ( $P < 0.05$ ) among six groups.

## 4. Discussion

### 4.1. Heterosis in F1 hybrid families

Theoretically, F1 demonstrated a remarkable growth advantage probably because the cross increased the heterozygosity of growth-related loci (Lu et al., 2012). Mitton and Grant (1984) have also documented that the successful utilization of heterosis is attributed to an increase in heterozygosity. In this study, the considerable heterosis in shell height and wet weight were manifested in two F1 hybrids compared to the two purebreds throughout the cultivating period. Additionally, crosses in other aquatic organisms were also identified to substantially improve growth-related traits in the offspring, including scallop (Wang and Li, 2010; Zhang et al., 2007), oyster (Ma et al., 2022; Zhang et al., 2017a, 2017b; Liang et al., 2022a, 2022b; Hedgecock and Davis, 2007) and giant clam (Zhang et al., 2020). Therefore, significantly growth advantage expressed in F1 hybrids in this study perhaps owing to higher heterozygosity existed in F1 hybrids than in two parental lines (Liang et al., 2023). The survival in F1 hybrids has also been improved compared to the parental lines. On the one hand, this might be attributed to crosses potentially obscure the deleterious or even lethal recessive viability-associated genes in the parental lines (Burke and Arnold, 2001). On the other hand, reciprocal hybrids may be more well adapted to a given environment than their purebred counterparts. However, the magnitude of heterosis in reciprocal hybrids appears to be affected by environmental factors, maternal effects and extra-nuclear effects (Zhang et al., 2017b). At one extremity, when hybrids succeed incompatible genes from their parents, it is likely to entail a decrease in the ability to adapt to the environment, which is commonly labeled as outbreeding depression or hybrid breakdown (Stelkens and Seehausen, 2009).

### 4.2. Inbreeding depression and superiority in F2 hybrids

In the crossbreeding of plants and animals, the F1 hybrids may not express trait regression during growing because the crosses can combine the superior characteristics of the parents. However, segregation and recombination of the co-adaptive genetic complexes during meiosis may render the heterosis diminished or even inbreeding depression occurred in the F2 generation (Lynch, 1991). Moreover, theory predicts that the dominant fitness breakdown occurs after the F1 hybrid generation, when heterosis ebbs and incompatibility of recessive alleles becomes increasingly pronounced. Trait deterioration in the F2 hybrids has been published in both plants and animals (Li et al., 2021; Argue et al., 2014; Zhang et al., 2016; Šimková et al., 2022; Stelkens et al., 2015). In this study, the growth and survival of the F2 hybrids was superior to that of the purebreds, and significantly outperformed the OO<sub>2</sub> group during the larval and grow-out stages. This result was similar to that of *A. purpuratus* × *A. irradians irradians* and *C. ariakensis* × *C. hongkongensis* (Feng et al., 2012; Qin et al., 2021), while different from that of *I. punctatus* × *I. furcatus* (Argue et al., 2014). The “Haida No.1” line originated from the commercial population in Rushan, Shandong Province, China (Li et al., 2011). While the ancestors of the Orange-shell line were only the four orange mutants in the offspring of purple-black shell color individuals of *C. gigas* (Han et al., 2019). Therefore, the traits segregation in the F2 hybrids may be due to genetic variability among the parents. In addition, such factors as the effect magnitude of trait-related alleles, the environment, the degree of linkage between recombinant genes, and the segregation and recombination of fitness-related loci combinations during meiosis may potentially compromise the degree of decline in heterosis (Benowicz et al., 2020; Emlen, 1991; Lynch, 1991). Furthermore, incompatible alleles inherited from parental populations with different trait-related loci may not be deleterious in the heterozygous form, i.e., in F1, but may be deleterious when homozygous, a situation which should occur more frequently in subsequent multigenerational hybrids. (Templeton et al., 1986). In addition, allele

segregation can destabilize beneficial gene combinations in the F2 hybrids (Turelli and Orr, 2000). Amazingly enough, the F2 hybrids in this study still retained some heterosis in yield traits, probably owing to the fact that the F1 generation was obtained by intra-strain crosses and both parents were cultured in the same area. Furthermore, heterosis has been hypothesized as a mechanism for increased survival of diploid larvae in *C. gigas* (Lannan, 1980) and adult wet weight (Hedgecock et al., 1991). Alternatively, increased heterozygosity in diploid oysters has been coupled with gains in growth and survival in *C. gigas* (Zouros et al., 1988; Mitton and Grant, 1984). Hence it was speculated that the heterozygosity of growth-related loci in the F2 hybrids in this study was somewhat higher than that in the two purebreds.

Compared to F1 hybrids, only half as much heterozygosity is produced in F2 hybrids and allelic segregation in F2 generations disrupts beneficial gene combinations (Turelli and Orr, 2000). Thereby, traits degradation occurs in the F2 and subsequent generations. However, because of the gene segregation and recombination, the phenotypic and genetic variation in F2 and Fn generations will be more broad-based compared to the parents (Zhang et al., 2016; Wang et al., 2017; Xu et al., 2019; Chen et al., 2020). In the context of this work, all the F2 hybrid families (HO<sub>2</sub> and OH<sub>2</sub>) in this study demonstrated growth and survival advantages compared to the purebred families (HH and OO). As a consequence, individuals with favorable characteristics in the F2 generations still preserved a potentially high seeding potential. On top of that, some rare mutants with purple shell color and orange shell color were occasionally identified from the descendants. The variation in shell color or mantle color of the hybrid offspring from the parents has been previously recorded in scallop (Wang et al., 2011), manila clam (Yan et al., 2008) and giant clam (Zhou et al., 2020; Zhang et al., 2020). The recombination and segregation of parental genes are quite promising to magnify the genetic and phenotypic diversity present in the hybrid offspring (Edmands, 1999). In this study, the “Haida No. 1” line was selected for growth rate as the dominant breeding interest, the color-associated locus polymorphism may be greater than that of the Orange-shell line. Thereby, the segregation and recombination of parental genes during the self-fertilization of F1 crosses to produce F2 generations gives rise to new genetic features. The inheritance pattern of the purple trait, however, is still lacking and requires deeper investigation. According to Mendel's law of segregation, segregation of phenotypes and genetic traits ordinarily happens in F2 hybrids due to segregation and recombination of parental genes (Mendel, 1941). This rule has been equally validated in the crossbreeding of other marine shellfish, such as *C. gigas* × *C. hongkongensis* (Zhang et al., 2016), *C. ariakensis* × *C. hongkongensis* (Qin et al., 2021) and *Argopecten purpuratus* × *A. irradians irradians* (Feng et al., 2012). In this study, the ratio of orange individuals to non-orange individuals in 60 randomly sampled oysters from F2 showed a ratio of 1:3, further supporting the orange trait as a recessive trait (Han and Li, 2020).

### 4.3. Application prospects of purple and orange individuals in F2 hybrids in aquaculture

In aquaculture, species possessing rare shell coloration are not only of relatively high scientific standing, but are also considered more attractive in the consumer market than species with common shell coloration (Clydesdale, 1993; Alfnes et al., 2006). The comparative results of yield attributes in the context of this study disclosed that the shell height and wet weight of the purple individuals of *C. gigas* were considerably in excess of those of its wild counterparts. Although the pattern of inheritance of the purple trait has not been uncovered yet. But it is anticipated that new variety of *C. gigas* with purple shell color can be established by selecting solid purple shell-colored individuals with optimal production traits as the parents and targeting shell color and growth. Another noteworthy thing is that the orange individuals in the F2 hybrids performed surprisingly in terms of yield traits better than the inbred Orange-shell line, on top of the fact that the orange individuals in

the F2 hybrids also inherited part of the genetic material of the “Haida No. 1” line. Accordingly, the orange individuals found in this study can be introduced into the Orange-shell line to enrich its population genetic diversity. A remark worth making is that in spite of the comparatively inferior trait performance of F2 hybrids with respect to their F1 counterparts, but we opine that selection and breeding for the few most eligible individuals continue to present great promise based on the breadth of variability in phenotypes available in F2 hybrids, which has been attested in scallop (Wang et al., 2017), oyster (Qin et al., 2021) and catfish (Argue et al., 2014).

## 5. Conclusions

In this study, the characteristics (shell height, wet weight, survival rate and shell color) of “Haida No. 1”, the Orange-shell line, their reciprocal F1 hybrid families and F2 hybrid families of *C. gigas* were systematically evaluated through the whole life stage. The two F1 hybrids exhibited significant heterosis in terms of survival and shell height compared to the other four groups during both larval and grow-out stages. Although the phenotypic traits of the F2 hybrid families were inferior to those of the F1 hybrid families, two F2 hybrid families demonstrate considerable advantages in terms of shell height, wet weight and survival rate, with superiority rate at 15.40–20.03%, –24.53–30.36% and 1.55–19.59%, respectively, compared to their purebred counterparts. Furthermore, the orange and purple individuals obtained from F2 hybrid families present great potential for the elaboration of new *C. gigas* strains.

## Credit author statement

Yuanxin Liang: Completion of the experiment, data analysis, and manuscript drafting. Qi Li: Experimental design and coordination and manuscript revision. Chengxun Xu: Oyster farming and data analysis.

## 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.

## Data availability

Data will be made available on request.

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