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Genomic selection accelerates genetic improvement of resistance to Vibriosis in the Pacific oyster, Crassostrea gigas

Ben Yang^a, Chengjun Zhi^a, Pengfei Li^a, Chengxun Xu^a, Qi Li^{a,b}, Shikai Liu^{a,b,*}

a Key Laboratory of Mariculture (Ocean University of China). Ministry of Education, and College of Fisheries. Ocean University of China. Oingdao 266003. China ^b Laboratory for Marine Fisheries Science and Food Production Processes, Laoshan Laboratory, Qingdao 266237, China

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Keywords: Genomic selection Resistance Crassostrea gigas Precision breeding	Due to lack of acquired immune system, the oysters cultured along coasts are subject to frequent pathogen threats, which leads to severe disease outbreaks around the world. It's well recognized that the selection breeding of aquatic animals can be accelerated via the harnessing of genomic tools to increase genetic gain and shorten the breeding time. In this work, we carried out genomic selection breeding in the Pacific oysters (<i>Crassostrea gigas</i>) for genetic improvement of resistance to <i>Vibriosis</i> . The genome-wide variations were geno- typed by ddRAD-seq from 295 oysters with contrasted resistance to <i>Vibrio</i> infection. Based on genome-wide SNPs, we performed an estimation of genomic heritability and prediction accuracy for resistance to <i>Vibrio alginolyticus</i> in <i>C. gigas</i> . The genomic heritability of resistance to <i>V. alginolyticus</i> was low to moderate, ranging from 0.1405 to 0.2730. Four genomic selection models including rrBLUP, Bayes A, Bayes B and Bayesian Lasso were evaluated, of which Bayes A showed superior prediction accuracy and computational speed. The genomic estimated breeding value (GEBV) calculated by genomic selection model can effectively distinguish the resistance or sus- ceptibility of oysters to <i>Vibriosis</i> . Selection of individuals with high GEBV as broodstock greatly improved the resistance to <i>Vibriosis</i> of their progeny, resulting in 18.42% increase in relative survival rate and 12.73% increase in relative survival time compared to the control population. For the first time, this work reported the efficiency of genomic selection breeding for genetic improvement for resistance trait to <i>Vibriosis</i> in the <i>C. gigas</i> , which would greatly accelerate the cultivation of <i>Vibriosis</i> resistant oyster strains to support the healthy and sustainable development of aquaculture			

1. Introduction

It is predicted that the global population will be increasing to over 10 billion by the end of this century (Lee, 2011). Meanwhile, meeting the protein needs of such a large population has become an important issue. Aquaculture has been considered an important alternative to meet the future protein supply of human beings, due to its rapid growth, and huge production and trading volume (Little et al., 2016). The Pacific oyster (Crassostrea gigas), commonly known as "milk of the sea" for its high level of protein and glycogen, is an important aquaculture species (Meng et al., 2019). This species has been cultivated over the world with its global annual production of 5.85 million tons by 2021 (FAO, 2023). However, in recent years, a widely occurred disease outbreak called "summer mortality syndrome" resulted in serious economic losses and hindered the sustainable development of oyster industry (Friedman et al., 2005; Garnier et al., 2007; Yang et al., 2021a). As a widely distributed bacterium in the ocean, Vibrio has been reported to have pathogenic effects on various aquatic organisms (Sanches-Fernandes et al., 2022). Various Vibrio species such as V. splendidus (Le Roux et al., 2002), V. aestuarianus (Garnier et al., 2008), V. alginolyticus (Yang et al., 2021a) and V. crassostreae (Bruto et al., 2017) have been associated with oyster mass mortality events worldwide. As a major oyster farming country, China's oyster industry is currently affected by mass summer mortality caused by Vibrio (Chi et al., 2021; Wang et al., 2021; Yang et al., 2021a; Zhang et al., 2023).

Various genetic breeding programs have been performed in C. gigas around the world with a focus on the selection of traits of interest such as fast-growth (Gutierrez et al., 2018; Zhang et al., 2019), shell color (Han and Li, 2020; Han et al., 2019), nutrient composition (Wan et al., 2020), glycogen content (Liu et al., 2019) and thermal tolerance (Ding et al.,

E-mail address: liushk@ouc.edu.cn (S. Liu).

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^{*} Correspondence author at: Key Laboratory of Mariculture (Ocean University of China), Ministry of Education, and College of Fisheries, Ocean University of China, China.

2020). Moreover, genetic improvement of disease resistance was reported in *C. gigas* with studies focusing on resistance to *Vibrio aestuarianus* and *Ostreid herpesvirus* 1 (Azéma et al., 2017; Dégremont et al., 2020). In the previous study, we identified a pathogenic *V. alginolyticus* strain as the causative pathogen associated with the summer mortality of *C. gigas* cultured in northern China (Yang et al., 2021a). Thus, to enhance the survival rate of *C. gigas* for aquaculture, we performed a genetic breeding program to improve the resistance to *Vibriosis* using *V. alginolyticus* as a reprehensive pathogen. The estimated low-level heritability for *Vibriosis* resistance traits suggested that traditional selection would not be efficient for the genetic improvement of the trait (Zhai et al., 2021). The genomics tools should be utilized to accelerate the genetic breeding of disease-resistant oyster strains (Gutierrez et al., 2020).

Genomic selection is an effective approach for selective breeding based on genomic estimated breeding value (GEBV), which used to evaluate the breeding potential of candidate individuals through genome-wide genetic markers (Meuwissen et al., 2001). The GEBV of candidates can be estimated with high precision from the reference population, in which the phenotype and genotype data are available. Then, the GEBV of candidate individuals from the breeding population can be estimated from genome-wide markers without phenotypic data (Xu et al., 2020). Therefore, genomic selection is an efficient approach for traits that are difficult to precisely phenotype, such as disease resistance, fish fillet yield, and reproductive capacity (Bhat et al., 2016). Because of its accurate assessment of breeding potential, genomic selection has been applied to numerous aquatic animals to accelerate the genetic gain of selection breeding (Liu et al., 2018; Zhao et al., 2021).

In this study, we performed genomic selection for the improvement of *Vibrio* resistance in *C. gigas*. Four genomic selection models were constructed to estimate the GEBV of oysters, and the optimal model was evaluated using cross-validation to estimate the breeding value of candidate population. The progeny produced from the selected individuals with high GEBV and control population were infected by *V. alginolyticus* to verify the effectiveness of genomic selection. This work, for the first time, showed the efficiency of genomic selection breeding for genetic improvement of resistance to *Vibriosis* in the *C. gigas*, which would greatly accelerate the cultivation of *Vibriosis*resistant oyster strains to support the healthy and sustainable development of aquaculture.

2. Materials and methods

2.1. Construction of breeding population

The origin of the oyster population has been described previously (Zhai et al., 2021). Briefly, eighty-six oysters (*C. gigas*, mean shell height 80.6 mm, 43 dams and 43 sires) from different geographical distributions were collected as broodstocks in 2019. When the gonads developed well, sperm and eggs were excised by gonad dissection, and then evenly stirred in a beaker for fertilization. The culture of larvae was carried out as routine. When half of the larvae appeared eyespots, a bunch of scallop shells was put into the culture barrel for oyster larvae to attach. After all the larvae completed the metamorphosis and became spat, they were artificially reared for one week in an outdoor nursery tank and then transferred to the raft in the open ocean for culture in Rongcheng, Shandong province, which was the major area for *C. gigas* culture in China.

2.2. Challenge trial

The experimental oysters were collected from the farm in the abovementioned area and transported to the laboratory, where they were cleaned from algae and dirt on the surface using a brush. Thereafter, a total of 1402 oysters were randomly placed in water tanks containing UV sterilized seawater for acclimatization for two weeks at 22 °C. Before the experiment, all instruments were bathed in 10 ppm potassium permanganate (KMnO4) solution for four hours to ensure that there were no other pathogens (Mohammed and Arias, 2015).

After anesthesia by 50 g/L magnesium chloride solution, each oyster was injected with 96 h-LD₅₀ doses (5×10^7 CFU) of *V. alginolyticus* via intramuscular injection, the injection dose was determined based on the pilot experiment. The mortality was counted every two hours and recorded the unique ID and the time of death. The phenotype of *C. gigas* to *Vibrio* infection was defined as survival status (death versus survival) and survival time (time to death). The oysters that survived at the end of the experiment and those with longer survival time were considered as resistance to *Vibriosis*. During the experiment, the water quality was monitored every day. The experiment was terminated when the daily mortality was lower than 1% for 3 consecutive days. Susceptible individuals (earliest dead oysters) and resistant individuals (survivors) were sampled and stored in a commercial DNA preservation solution (Sangon Biotech Co., Ltd., China, CAT. NO. B644771) at room temperature for DNA extraction.

2.3. Genotyping by ddRAD sequencing

A total of 100 mg muscle tissue was cut into pieces with surgical scissors and digested overnight in Proteinase K buffer system (pH 8.1) at 37 °C, and then DNA was extracted by phenol-chloroform method and dissolved in double distilled water. The concentration and quality of DNA were detected by Qubit fluorescence quantitative analyzer and 1% agarose gel electrophoresis. Samples with clear gel electrophoresis bands and DNA concentrations above 100 ng/µL were used for further analysis. The double digest restriction-site associated sequencing library was constructed according to the protocol of Peterson et al. (Peterson et al., 2012) and outsourced to Novogene Technology Co. (Tianjin, China) for pair-end sequencing at Illumina HiSeq 2500 platform.

After extracting the sequencing data of different individuals from the library by index and barcode and the low-quality reads were removed using Stacks 2.0 (Rochette et al., 2019). High quality clean reads were mapped to the reference genome of *C. gigas* (GCA_902806645.1) (Peñaloza et al., 2021) using BWA with algorithm mem (Li and Durbin, 2009). After sorting the mapped BAM files, SNP identification was performed by the Populations parameter of Stacks software. High quality SNP sets were filtered by the following parameters: call rate > 0.90, MAF > 0.05. The imputation of missing genotypes was performed by Beagle (Browning and Browning, 2016). Then, according to the reading requirements of the software, it was made into genotype files encoded by 0, 1, 2 using Plink.

2.4. Estimation of heritability

The genotype and phenotype were used to evaluate the resistance heritability of the two traits. The heritability was calculated according to the following formula:

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_e^2}$$

Wherein, the additive genetic variance is denoted by σ_a^2 and residual variance denoted by σ_e^2 .

2.5. Genomic selection models

Four genomic selection models were used in this study, including rrBLUP and three Bayesian methods (Bayes A, Bayes B and Bayesian Lasso), they can be described as:

$y=\mu+Zu+e$

where y is the phenotypic value, μ is the population mean, Z is SNP marker matrix (with values 0, 1, or 2 denoted major homozygotes AA,

heterozygotes Aa and minor homozygotes aa), u is marker effect value vector, e is residual. There are differences in the contribution of SNPs to traits among these four models, where rrBLUP assumes that all SNPs have the same effect on the trait. Bayes A assumes that each SNP has effect on the trait, and the effect values follow a scaled-t distribution, while the effect variance follows scaled-inverse Chi-squared distribution. Bayes B assumes that a small number of SNPs have an effect, and the effect variance follows an inverse Chi-squared distribution, while the majority of SNPs have no effect. Bayesian Lasso assumes each SNP has effect, and the SNP effect follows Laplace distribution, which allows for a higher probability of the maximum or minimum value occurring. The parameters of all Bayesian models were set as follows: a total of 50,000 iterations were performed, the first 10,000 iterations were discarded as burn-in, and the thickness was 5 (Pérez and de los Campos, 2014).

2.6. Prediction metrics for V. alginolyticus resistance traits

The entire data set was split into a training set (90%) and a test set (10%) by random sampling to perform cross validation. The training set estimated the effect value of each marker using phenotype and genotype data. The GEBV of the test set was calculated by the effect value matrix estimated by the training set and the marker matrix of the test set. The prediction accuracy was measured by the Pearson correlation coefficient between EBV and phenotype divided by the square root of the heritability:

$$r_{(EBV,TBV)} = \frac{Cor(EBV, y)}{h}$$

where EBV was the estimated breeding value in genomic selection models, TBV was the true breeding value, and y was the phenotypic value observed, h was the square root of the heritability.

In order to avoid sampling errors, all prediction accuracy evaluations were repeated 50 times.

2.7. Prediction metrics on SNP panels with different density

Although different genomic selection models may be sensitive to marker density, previous research results showed that using 500 SNPs can achieve the same prediction accuracy as using all 18 K SNPs in the genomic selection of OsHV-1 resistance in *C. gigas* (Gutierrez et al., 2020). Therefore, SNP panels with eight densities were constructed to evaluate *Vibrio* resistance genomic selection at different densities, in order to use the least markers to achieve the same prediction accuracy.

2.8. Validation of selective breeding

A total of 120 candidate oysters selected from representative families were genotyped and used as broodstocks. The Bayes A model was used to estimate GEBV. The oysters with the top 20% GEBV were used as resistant broodstock, and the others as control broodstock. Oyster breeding and offspring management were carried out as previously described (Zhai et al., 2021), and the offspring were challenged with *V. alginolyticus* to evaluate the effectiveness of genomic selection.

2.9. Statistic analysis

The difference in breeding values between the resistant candidate population and the control candidate population was analyzed by *t*-test, and the survival time between the resistant offspring population and the control offspring population was analyzed by t-test, the *P*-value <0.05 was considered significant. The survival curve was analyzed by the Logrank (Mantel-Cox) test.

3. Results

3.1. Resistant phenotype of challenge experiment

The experiment lasted 12 days, the cumulative mortality began to be lower than 1% on day 10 post challenge. At the end of the experiment, a total of 420 oysters survived from *V. alginolyticus* infection, with a survival rate of 29.96% (Fig. 1). In order to ensure that oysters died because of *V. alginolyticus* infection, we conducted bacterial culture experiments with the randomly selected dead oysters. The bacteria isolated from the dead oysters were identified as *V. alginolyticus* by Gram staining and 16S rRNA sequencing. After tissue homogenization and plate coating, *Vibrio* was not detected in the surviving oysters.

3.2. Generation of high-quality SNP sets and genomic relationship matrix

A total of 136.02 Gb cleaned sequencing data were generated for 295 individuals from the constructed RAD library. The base quality evaluation showed that Q20 was 97.76%, and Q30 was 93.73%, respectively. The 90.17% of the clean data were aligned to the *C. gigas* genome. After data statistics, the average sequencing depth of individuals through quality control reached 17.5 ×. Because of the high polymorphism of the *C. gigas* genome, we used more strict filtering parameters to obtain a final set of 48,099 high quality SNPs with MAF >0.05 and missing rate <90% for downstream analysis.

Based on these 48 K SNP, the genomic relationship matrix was constructed for reference population as shown in Fig. 2, with a gradient from blue to red indicating increasing kinship. Kinship analysis indicated a relationship of -0.169 between the furthest individuals, and 1.301 for the closest individuals.

3.3. Heritability of the resistance against Vibrio

The heritability of resistance traits estimated by four methods was shown in Table 1, among which the estimated heritability of survival status was 0.1310–0.1722, while that of survival time was 0.1405–0.2732. Although the estimated heritability of different models was different, all showed the resistance heritability of low to a moderate level in the two traits.

3.4. Consistency of breeding value calculation in genomic selection model

The breeding values of two resistance traits in different genomic selection models were calculated, and the Pearson correlation coefficient was used to measure the correlation as shown in Table 2. The correlation between GEBV estimated by different models ranged from 0.940 to 0.987 for survival status and from 0.974 to 0.995 for survival time. These models have different theoretical assumptions for GEBV calculation, but they are all adhere to linear models grounded in additive effects, so the estimated GEBV has high similarity.

3.5. Comparison of different models

Cross validation was used to evaluate the prediction accuracy of the four models based on 48 K SNPs. The results showed that for the same trait, the prediction accuracy of the four genomic selection models was different, and all of Bayesian models performed better than the traditional rrBLUP model in survival status trait (0.341–0.388 versus 0.262) (Fig. 3). Among the three Bayesian models, Bayes A showed better prediction performance, both in survival status (0.388) and survival time (0.364) traits (Fig. 3A and Fig. 3B).

In large-scale breeding projects, the computational resource consumption of genomic selection model is a cost factor worthy of consideration. Therefore, we evaluated the prediction time of four genomic selection models (Fig. 4). Among the four models, rrBLUP showed the lowest computational time and Bayesian Lasso showed highest



Fig. 1. Survival curve of C. gigas challenged by V. alginolyticus for 12 days.

computational time. In the two traits, rrBLUP model only needs five seconds to complete a 10-fold validation, while Bayes method needs 8–12 min to complete the same calculation. (Fig. 4). Among the three Bayesian models, Bayes A model required the least computing time.

3.6. Prediction accuracy of different SNP panels

Different numbers of SNP panels were constructed to establish the genomic selection model and evaluate its prediction accuracy. As shown in Fig. 5, the use of a low-density SNP panel can maintain a high level of prediction accuracy. When the number of SNPs used to construct the genomic selection models decreased from 48 K to 1 K, a slight reduction occurred in prediction accuracy of survival status trait and moderate reduction in prediction accuracy of survival time trait. In contrast, when the number of SNPs decreased from 1 K to 200, the prediction accuracy of the genomic selection model showed significant decrease.

3.7. Effectiveness of genomic selection breeding

Based on the principal component analysis, the reference and candidate populations showed a high degree of similarity in population structure (Fig. 6A). The GEBV of the resistant population was 25.35% higher than that of the control population (Fig. 6B). In order to validate the effectiveness of the selection, a total of 1312 progenies (shell height 61.67 mm) were challenged with *V. alginolyticus*. The results showed that the survival rate of resistant progeny was 85.34%, and was significantly higher than that of the control group (72.06%), and the relative survival rate increased by 18.42% (Fig. 6C). In addition, the survival time of the resistant progeny against the infection of *V. alginolyticus* was also longer than that of the control, which increased from 9.58 days to 10.80 days, and the relative increase was 12.73% (Fig. 6D).

4. Discussion

As an invertebrate, oysters lack acquired immunity and can not produce antibodies for pathogens, so it is not possible to use vaccines for immune protection (Wang and He, 2019). The semi-open circulatory system makes oysters suffering from the invasion of pathogens all the time (Schmitt et al., 2012). Moreover, the intertidal habitat is changeable, which aggravates the environmental burden of oysters (Green et al., 2016). Due to the above reasons, oyster diseases caused by pathogen infection occur frequently and threaten the oyster industry in various countries in the past two decades (Alfaro et al., 2019). The main pathogens of oysters affect the oyster industry are reported as various *Vibrios* and OsHV-1 (Petton et al., 2021). Therefore, it is urgent to generate new strains with disease resistance through the genetic improvement. In the previous studies, we investigated the pathogenic factors of mass mortality of *C. gigas* in China and identified a highly virulent strain which is *V. alginolyticus* Cg5, as a pathogen (Yang et al., 2021a). Genetic breeding toward enhancement of resistance to *Vibriosis* was carried out to cultivate *Vibrio* resistant strains for the sustainable and healthy development of the oyster industry.

The first step of a breeding program is to evaluate the heritability of the target traits. We first evaluated the heritability of resistance traits with four genomic selection models based on genome-wide 48,099 highquality SNPs. The results showed that the genomic heritability of resistance traits was a low to moderate level, which was consistent with that obtained by pedigree information evaluation as previously reported (Zhai et al., 2021). The results of many previous studies were consistent with our results, indicating that the heritability of Vibrio resistance in aquatic animals is low to moderate level, such as in oyster (0.09–0.33) (Azéma et al., 2017), Pacific white shrimp (0.15-0.26) (Wang et al., 2019), Atlantic cod (0.08-0.17) (Kettunen et al., 2007) and turbot (0.110-0.296) (Wang and Ma, 2019). Different selection methods should be performed according to the heritability of different traits. In general, mass selection based on phenotype is suitable for traits with high heritability, BLUP method based on family should be used for moderate heritability, and genomic selection should be used for traits with low heritability (Calus et al., 2008). The rationale behind this lies in the heightened precision with which genomic selection can estimate the breeding value of candidate individuals. This enhanced accuracy facilitates more precise and efficient breeding practices, thereby accelerating the attainment of genetic gains. (Tessema et al., 2020; Xu et al., 2020). Therefore, genomic selection should be used for genetic enhancement of resistance to Vibriosis in C. gigas in the breeding process (Klápště et al., 2020).



Fig. 2. Heatmap of genomic kinship relationship matrix for reference population.

Table 1

Estimates of heritability for survival status and survival time against *V. alginolyticus* in *C. gigas* using different models.

	Models	rrBLUP	Bayes A	Bayes B	Bayesian Lasso
Traits					
Survival s Survival t	tatus ime	0.1639 0.1776	0.1696 0.2732	0.1310 0.2128	0.1722 0.1405

Table 2

Pearson correlation coefficients of genomic estimated breeding value (GEBV) estimated by different genomic selection models.

tus rrBLUP	Bayes A	Bayes B	Bayesian Lasso
1	0.96943	0.987448	0.986082
0.988501	1	0.951203	0.940768
0.995359	0.993755	1	0.986754
0.992831	0.974032	0.985895	1
	tus rrBLUP 1 0.988501 0.995359 0.992831	tus rrBLUP Bayes A 1 0.96943 0.988501 1 0.995359 0.993755 0.992831 0.974032	tus rrBLUP Bayes A Bayes B 1 0.96943 0.987448 0.988501 1 0.951203 0.995359 0.993755 1 0 0.992831 0.974032 0.985895

The upper right of the table is the correlation coefficient between survival status, The bottom left of the table is the correlation coefficient between survival time.

Genomic selection breeding has been widely used to accelerate the genetic improvement of aquatic animals (Chang et al., 2018; Dong et al., 2016; Tsairidou et al., 2020; Vallejo et al., 2017). With the development of genomics tools, aquatic animal breeding is moving in a more efficient direction. Our previous studies revealed that the resistance to Vibrio infection is a polygenic genetic basis (Yang et al., 2021b). The performance of the selection method using high-density genome-wide molecular markers to calculate GEBV has been reported in a variety of aquatic animals (Liu et al., 2018; Toro et al., 2017; Vallejo et al., 2017; Zhao et al., 2021). However, the performance of genomic selection of Vibrio resistance in C. gigas is not clear, so we performed cross validation to evaluate the prediction accuracy of genomic selection using different models. Cost is a key factor that breeding programs need to focus on (Gorjanc et al., 2017). Through the construction and evaluation of the genomic selection model built by different numbers of SNPs, we showed that thousands of SNPs can obtain high accuracy for GEBV estimation, which makes the genotyping cost for genomic selection greatly reduced (Zhao et al., 2021). The calculation time of breeding values for selecting a candidate population also affects the breeding project. Therefore, we evaluated the prediction time of different genomic selection models. Similar to the previous results, Bayesian model is superior to the traditional genomic selection model, which has the characteristics of high



Fig. 3. (A) The prediction accuracy of the survival status in different models, (B) The prediction accuracy of the survival time in different models. The four genomic selection models were built based on all SNPs. The error bars represent the standard error of 50 repeats.



Fig. 4. The calculation time of four genomic selection models in two traits. This is the time to calculate the GEBV of 10% of individuals without phenotype in the whole data set by 10-fold cross validation.

accuracy and high computational resource consumption (Luo et al., 2021; Shan et al., 2021). Among the three Bayesian models, Bayes A had higher prediction accuracy and less computational resource consumption.

After establishing the optimal genomic selection model, we carried out genomic selection breeding to assess the effectiveness of genomic selection. The subsequent selection breeding showed the high efficiency of genomic selection breeding and revealed the bright prospect of genomic selection for *Vibrio* resistance breeding. A crucial aspect of constructing a candidate population for breeding is ensuring a close genetic relationship with the reference population. Failing to do so could impact the accuracy of breeding value estimation. Principal component analysis revealed that the candidate population in our study maintained a close genetic relationship with the reference population, ensuring the accuracy of breeding value estimation and the effectiveness of selection. The breeding value calculated based on the genomic selection model can effectively distinguish whether oysters are resistant or susceptible to *Vibrio* infection. Selection of individuals with high GEBV as broodstocks significantly enhanced the *Vibrio* resistance of progeny, which was consistent with the observation as reported in large yellow croaker (Zhao et al., 2021). Previous studies have shown that the genetic gain of disease resistance traits in mollusks was 15.7% per generation (Hollenbeck and Johnston, 2018). Selective breeding of OsHV-1 resistance in oysters yielded an averaged genetic gain of 10% (Kube et al., 2018) and 10.6% (Divilov et al., 2021) per generation. In the present study, a genetic gain of 18.42% in survival rate and 12.73% in survival time was achieved, suggesting the great potential of genomic selection to enhance breeding efficiency. This work will provide valuable information for the development of accurate breeding of aquatic animals and promote its application in aquatic animals.

5. Conclusion

In this study, we estimated the genomic heritability of resistance to



Fig. 5. (A) The prediction accuracy of the survival status in four genomic selection models under different marker density, (B) The prediction accuracy of the survival time in four genomic selection models under different marker density.



Fig. 6. The progeny test revealed the high survival rate and survival time of resistant progeny bred from broodstocks with high GEBV. (A) The population structure of candidate and reference populations revealed by principal component analysis. (B) The GEBV of the resistant population and the control population were calculated by genomic selection model constructed by the survival status traits. (C) The survival rate of resistant progeny was significantly higher than that of control progeny. (D) The survival time of resistant progeny was significantly longer than that of control progeny.

Vibrio infection in *C. gigas* using genome-wide SNP markers. The results showed that the heritability was low to moderate level, implying the potential of genetic improvement. We, for the first time, evaluated the prediction accuracy of genomic selection for *Vibrio* resistance in *C. gigas* under different marker densities, suggesting the potential of utilizing low-density panels in genomic selection breeding. Furthermore, GEBV estimated by genomic selection model can effectively distinguish the resistance or susceptibility of oysters to *Vibrio*, and the selection of individuals with high GEBV can effectively improve the *Vibrio* resistance.

This work will provide valuable information to accelerate the genetic breeding of *Vibrio* resistant varieties of oysters.

CRediT authorship contribution statement

Ben Yang: Formal analysis, Investigation, Writing – original draft. Chengjun Zhi: Investigation. Pengfei Li: Investigation. Chengxun Xu: Investigation. Qi Li: Resources, Supervision. Shikai Liu: Conceptualization, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

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References

- Alfaro, A.C., Nguyen, T.V., Merien, F., 2019. The complex interactions of Ostreid herpesvirus 1, Vibrio bacteria, environment and host factors in mass mortality outbreaks of Crassostrea gigas. Rev. Aquac. 11, 1148–1168.
- Azéma, P., Lamy, J.-B., Boudry, P., Renault, T., Travers, M.-A., Dégremont, L., 2017. Genetic parameters of resistance to *Vibrio aestuarianus*, and OsHV-1 infections in the Pacific oyster, *Crassostrea gigas*, at three different life stages. Genet. Sel. Evol. 49, 23.
- Bhat, J.A., Ali, S., Salgotra, R.K., Mir, Z.A., Dutta, S., Jadon, V., Tyagi, A., Mushtaq, M., Jain, N., Singh, P.K., Singh, G.P., Prabhu, K.V., 2016. Genomic selection in the era of next generation sequencing for complex traits in plant breeding. Front. Genet. 7, 221.
- Browning, Brian L., Browning, Sharon R., 2016. Genotype imputation with millions of reference samples. Am. J. Hum. Genet. 98, 116–126.
- Bruto, M., James, A., Petton, B., Labreuche, Y., Chenivesse, S., Alunno-Bruscia, M., Polz, M.F., Le Roux, F., 2017. *Vibrio crassostreae*, a benign oyster colonizer turned into a pathogen after plasmid acquisition. ISME J 11, 1043–1052.
- Calus, M.P., Meuwissen, T.H., de Roos, A.P., Veerkamp, R.F., 2008. Accuracy of genomic selection using different methods to define haplotypes. Genetics. 178, 553–561. Chang, L.-Y., Toghiani, S., Ling, A., Aggrey, S.E., Rekaya, R., 2018. High density marker
- panels, SNPs prioritizing and accuracy of genomic selection. BMC Genet. 19, 4. Chi, Y., Li, Q., Liu, S., Kong, L., 2021. Genetic parameters of growth and survival in the
- Pacific oyster Crassostrea gigas. Aquac. Res. 52, 282–290.
 Dégremont, L., Azéma, P., Maurouard, E., Travers, M.-A., 2020. Enhancing resistance to Vibrio aestuarianus in Crassostrea gigas by selection. Aquaculture. 526, 735429.
- Ding, F., Li, A., Cong, R., Wang, X., Wang, W., Que, H., Zhang, G., Li, L., 2020. The phenotypic and the genetic response to the extreme high temperature provides new insight into thermal tolerance for the Pacific oyster *Crassostrea gigas*. Front. Mar. Sci. 7, 399.
- Divilov, K., Schoolfield, B., Mancilla Cortez, D., Wang, X., Fleener, G.B., Jin, L., Dumbauld, B.R., Langdon, C., 2021. Genetic improvement of survival in Pacific oysters to the Tomales Bay strain of OsHV-1 over two cycles of selection. Aquaculture. 543, 737020.
- Dong, L., Xiao, S., Chen, J., Wan, L., Wang, Z., 2016. Genomic selection using extreme phenotypes and pre-selection of SNPs in large yellow croaker (*Larimichthys crocea*). Mar. Biotechnol. 18, 575–583.
- FAO, 2023. Fisheries and aquaculture software. In: FishStatJ Software for Fishery Statistical Time Series. FAO Fisheries and Aquaculture Department Rome. https ://www.fao.org/fishery/en/statistics/software/fishstatj.
- Friedman, C.S., Estes, R.M., Stokes, N.A., Burge, C.A., Hargove, J.S., Barber, B.J., Elston, R.A., Burreson, E.M., Reece, K.S., 2005. Herpes virus in juvenile Pacific oysters *Crassostrea gigas* from Tomales Bay, California, coincides with summer mortality episodes. Dis. Aquat. Org. 63, 33–41.
- Garnier, M., Labreuche, Y., Garcia, C., Robert, M., Nicolas, J.L., 2007. Evidence for the involvement of pathogenic bacteria in summer mortalities of the Pacific oyster *Crassostrea gigas*. Microb. Ecol. 53, 187–196.
- Garnier, M., Labreuche, Y., Nicolas, J.-L., 2008. Molecular and phenotypic characterization of Vibrio aestuarianus subsp. francensis subsp. nov., a pathogen of the oyster Crassostrea gigas. Syst. Appl. Microbiol. 31, 358–365.
- Gorjanc, G., Battagin, M., Dumasy, J.-F., Antolin, R., Gaynor, R.C., Hickey, J.M., 2017. Prospects for cost-effective genomic selection via accurate within-family imputation. Crop Sci. 57, 216–228.
- Green, T.J., Vergnes, A., Montagnani, C., de Lorgeril, J., 2016. Distinct immune responses of juvenile and adult oysters (*Crassostrea gigas*) to viral and bacterial infections. Vet. Res. 47, 72.
- Gutierrez, A.P., Matika, O., Bean, T.P., Houston, R.D., 2018. Genomic selection for growth traits in Pacific oyster (*Crassostrea gigas*): potential of low-density marker panels for breeding value prediction. Front. Genet. 9, 391.
- Gutierrez, A.P., Symonds, J., King, N., Steiner, K., Bean, T.P., Houston, R.D., 2020. Potential of genomic selection for improvement of resistance to ostreid herpesvirus in Pacific oyster (*Crassostrea gigas*). Anim. Genet. 51, 249–257.
- Han, Z., Li, Q., 2020. Mendelian inheritance of orange shell color in the Pacific oyster Crassostrea gigas. Aquaculture. 516, 734616.

- Han, Z., Li, Q., Liu, S., Yu, H., Kong, L., 2019. Genetic variability of an orange-shell line of the Pacific oyster *Crassostrea gigas* during artificial selection inferred from the second second
- microsatellites and mitochondrial COI sequences. Aquaculture. 508, 159–166. Hollenbeck, C.M., Johnston, I.A., 2018. Genomic tools and selective breeding in molluscs. Front. Genet. 9, 253.
- Kettunen, A., Serenius, T., Fjalestad, K.T., 2007. Three statistical approaches for genetic analysis of disease resistance to vibriosis in Atlantic cod (*Gadus morhua* L.). J. Anim. Sci. 85, 305–313.
- Klápště, J., Dungey, H.S., Telfer, E.J., Suontama, M., Graham, N.J., Li, Y., McKinley, R., 2020. Marker selection in multivariate genomic prediction improves accuracy of low heritability traits. Front. Genet. 11, 499094.
- Kube, P., Dové, M., Cunningham, M., Kirkland, P., Gu, X., Hick, P., O'Connor, W., Elliot, N., 2018. Genetic selection for resistance to Pacific oyster mortality syndrome. In: CSIRO Marine and Atmospheric Research, FRDC and Seafood CRC Project 2012/ 760. Australian, CSIRO Agriculture and Food, NSW Department of Primary Industries, p. 55.
- Le Roux, F., Gay, M., Lambert, C., Waechter, M., Poubalanne, S., Chollet, B., Nicolas, J.-L., Berthe, F., 2002. Comparative analysis of *Vibrio splendidus*-related strains isolated during *Crassostrea gigas* mortality events. Aquat. Living Resour. 15, 251–258.
- Lee, R., 2011. The outlook for population growth. Science. 333, 569–573.
- Li, H., Durbin, R., 2009. Fast and accurate short read alignment with burrows-wheeler transform. Bioinformatics. 25, 1754–1760.
- Little, D.C., Newton, R.W., Beveridge, M.C., 2016. Aquaculture: a rapidly growing and significant source of sustainable food? Status, transitions and potential. Proc. Nutr. Soc. 75, 274–286.
- Liu, Y., Lu, S., Liu, F., Shao, C., Zhou, Q., Wang, N., Li, Y., Yang, Y., Zhang, Y., Sun, H., Zheng, W., Chen, S., 2018. Genomic selection using BayesCπ and GBLUP for resistance against *Edwardsiella tarda* in Japanese flounder (*Paralichthys olivaceus*). Mar. Biotechnol. 20, 559–565.
- Liu, S., Li, L., Meng, J., Song, K., Huang, B., Wang, W., Zhang, G., 2019. Association and functional analyses revealed that PPP1R3B plays an important role in the regulation of glycogen content in the Pacific oyster *Crassostrea gigas*. Front. Genet. 10, 106.
- Luo, Z., Yu, Y., Xiang, J., Li, F., 2021. Genomic selection using a subset of SNPs identified by genome-wide association analysis for disease resistance traits in aquaculture species. Aquaculture. 539, 736620.
- Meng, J., Song, K., Li, C., Liu, S., Shi, R., Li, B., Wang, T., Li, A., Que, H., Li, L., Zhang, G., 2019. Genome-wide association analysis of nutrient traits in the oyster *Crassostrea* gigas: genetic effect and interaction network. BMC Genomics 20, 625.
- Meuwissen, T.H., Hayes, B.J., Goddard, M.E., 2001. Prediction of total genetic value using genome-wide dense marker maps. Genetics. 157, 1819–1829.
- Mohammed, H.H., Arias, C.R., 2015. Potassium permanganate elicits a shift of the external fish microbiome and increases host susceptibility to columnaris disease. Vet. Res. 46, 82.
- Peñaloza, C., Gutierrez, A.P., Eöry, L., Wang, S., Guo, X., Archibald, A.L., Bean, T.P., Houston, R.D., 2021. A chromosome-level genome assembly for the Pacific oyster *Crassostrea gigas*. Gigascience. 10, 1–9.
- Pérez, P., de los Campos, G., 2014. Genome-wide regression and prediction with the BGLR statistical package. Genetics. 198, 483–495.
- Peterson, B.K., Weber, J.N., Kay, E.H., Fisher, H.S., Hoekstra, H.E., 2012. Double digest RADseq: an inexpensive method for de novo SNP discovery and genotyping in model and non-model species. PLoS One 7, e37135.
- Petton, B., Destoumieux-Garzón, D., Pernet, F., Toulza, E., de Lorgeril, J., Degremont, L., Mitta, G., 2021. The Pacific oyster mortality syndrome, a polymicrobial and multifactorial disease: state of knowledge and future directions. Front. Immunol. 12, 630343.
- Rochette, N.C., Rivera-Colón, A.G., Catchen, J.M., 2019. Stacks 2: analytical methods for paired-end sequencing improve RADseq-based population genomics. Mol. Ecol. 28, 4737–4754.
- Sanches-Fernandes, G.M.M., Sá-Correia, I., Costa, R., 2022. Vibriosis outbreaks in aquaculture: addressing environmental and public health concerns and preventive therapies using gilthead seabream farming as a model system. Front. Microbiol. 13, 904815.
- Schmitt, P., Rosa, R.D., Duperthuy, M., de Lorgeril, J., Bachère, E., Destoumieux-Garzón, D., 2012. The antimicrobial defense of the Pacific oyster, *Crassostrea gigas*. How diversity may compensate for scarcity in the regulation of resident/pathogenic microflora. Front. Microbiol. 3, 160.
- Shan, X., Xu, T., Ma, Z., Zhang, X., Ruan, Z., Chen, J., Shi, Q., You, X., 2021. Genomewide association improves genomic selection for ammonia tolerance in the orangespotted grouper (*Epinephelus coioides*). Aquaculture. 533, 736214.
- Tessema, B.B., Liu, H., Sørensen, A.C., Andersen, J.R., Jensen, J., 2020. Strategies using genomic selection to increase genetic gain in breeding programs for wheat. Front. Genet. 11, 578123.
- Toro, M.A., Saura, M., Fernandez, J., Villanueva, B., 2017. Accuracy of genomic withinfamily selection in aquaculture breeding programmes. J. Anim. Breed. Genet. 134, 256–263.
- Tsairidou, S., Hamilton, A., Robledo, D., Bron, J.E., Houston, R.D., 2020. Optimizing low-cost genotyping and imputation strategies for genomic selection in Atlantic salmon. G3-Genes Genom. Genet. 10, 581–590.
- Vallejo, R.L., Leeds, T.D., Gao, G., Parsons, J.E., Martin, K.E., Evenhuis, J.P., Fragomeni, B.O., Wiens, G.D., Palti, Y., 2017. Genomic selection models double the accuracy of predicted breeding values for bacterial cold water disease resistance compared to a traditional pedigree-based model in rainbow trout aquaculture. Genet. Sel. Evol. 49, 17.
- Wan, S., Li, Q., Yu, H., Liu, S., Kong, L., 2020. Estimating heritability for meat composition traits in the golden shell strain of Pacific oyster (*Crassostrea gigas*). Aquaculture. 516, 734532.

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- Wang, P.H., He, J.G., 2019. Nucleic acid sensing in invertebrate antiviral immunity. Int. Rev. Cell Mol. Biol. 345, 287–360.
- Wang, X.-A., Ma, A.-J., 2019. Genetic parameters for resistance against Vibrio anguillarum in turbot Scophthalmus maximus. J. Fish Dis. 42, 713–720.
- Wang, Q., Yu, Y., Zhang, Q., Zhang, X., Huang, H., Xiang, J., Li, F., 2019. Evaluation on the genomic selection in *Litopenaeus vannamei* for the resistance against *Vibrio parahaemolyticus*. Aquaculture. 505, 212–216.
- Wang, H., Yang, B., Li, X., Li, Q., Liu, S., 2021. Screening of bacterial pathogens associated with mass summer mortality of the Pacific oyster, *Crassostrea gigas*, in China. Aquac. Rep. 20, 100672.
- Xu, Y., Liu, X., Fu, J., Wang, H., Wang, J., Huang, C., Prasanna, B.M., Olsen, M.S., Wang, G., Zhang, A., 2020. Enhancing genetic gain through genomic selection: from livestock to plants. Plant Commun. 1, 100005.
- Yang, B., Zhai, S., Li, X., Tian, J., Li, Q., Shan, H., Liu, S., 2021a. Identification of Vibrio alginolyticus as a causative pathogen associated with mass summer mortality of the Pacific oyster (*Crassostrea gigas*) in China. Aquaculture. 535, 736363.

- Yang, B., Zhai, S., Zhang, F., Wang, H., Ren, L., Li, Y., Li, Q., Liu, S., 2021b. Genome-wide association study toward efficient selection breeding of resistance to Vibrio alginolyticus in Pacific oyster, Crassostrea gigas. Aquaculture. 548, 737592.
- Zhai, S., Yang, B., Zhang, F., Li, Q., Liu, S., 2021. Estimation of genetic parameters for resistance to *Vibrio alginolyticus* infection in the Pacific oyster (*Crassostrea gigas*). Aquaculture 736545.
- Zhang, J., Li, Q., Xu, C., Han, Z., 2019. Response to selection for growth in three selected strains of the Pacific oyster *Crassostrea gigas*. Aquaculture. 503, 34–39.
- Zhang, X., Huang, B.-W., Zheng, Y.-D., Xin, L.-S., Chen, W.-B., Yu, T., Li, C., Wang, C.-M., Bai, C.-M., 2023. Identification and characterization of infectious pathogens associated with mass mortalities of Pacific oyster (*Crassostrea gigas*) cultured in Northern China. Biology. 12, 759.
- Zhao, J., Bai, H., Ke, Q., Li, B., Zhou, Z., Wang, H., Chen, B., Pu, F., Zhou, T., Xu, P., 2021. Genomic selection for parasitic ciliate *Cryptocaryon irritans* resistance in large yellow croaker. Aquaculture. 531, 735786.