

Genetic diversity and structure in a selected strain of hybrid oysters between *Crassostrea gigas* and *C. angulata* evaluated from microsatellites and mitochondrial COI sequences

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ABSTRACT

Summer mass mortality is a main problem affecting the production of Pacific oyster (*Crassostrea gigas*). Selective breeding represents a promising method to improve the summer survival rate and growth of the oyster. However, the pitfall is that selection may significantly decrease genetic diversity, leading to inbreeding depression. The current study presented an analysis of genetic diversity in one hybrid oyster (GA - *C. gigas* ♀ × *C. angulata* ♂), parental populations (*C. gigas* ♀ × *C. gigas* ♂ and *C. angulata* ♀ × *C. angulata* ♂) and three generations of mass-strain GA strain (GAF1, GAF2 and GAF3) as well as four wild populations of *C. gigas* (RC, QD, LY and ZS) using 18 microsatellite loci and mitochondrial COI sequences. Heterozygosity levels of GA ($H_e = 0.69$) demonstrated an increased genetic diversity in hybrids compared to the parental populations. Heterozygosity (H_e : 0.64–0.67) and COI haplotypes (H_d : 0.195–0.279) revealed no significant ($P > 0.05$) loss of genetic diversity in selected strains over three generations. However, the average number of alleles (N_a ranging from 8.06 to 7.61), polymorphic information content (I ranging from 1.53 to 1.43), heterozygosity (H_o ranging from 0.72 to 0.55) and effective population size (N_{e-lin} ranging from 144.1 to 51.2) exhibited a tendency to decrease with selection. Moderate genetic structuring (F_{ST} : 0.059–0.124; Nei 's D : 0.140–0.326) was found among the selected strains and wild populations (QD, LY and ZS). These results indicate that breeders need to be cautious about artificial selection beyond the third generation and work to improve effective population sizes to maintain genetic gains. The results obtained in this study is that it provides important inference and guidance concerning future genetic improvement project.

1. Introduction

Selective breeding is a major conventional breeding method with an aim to improve one or more traits of commercial importance. As a result of decades, selective breeding of domestic livestock has resulted in significant improvements in growth rate, productivity, forage transformation efficiency and reproductive characteristics (Mignon-Grasteau et al., 2005; Lind et al., 2012). In contrast, aquaculture species have undergone a limited amount of genetic improvement (Hillen et al., 2017). Nonetheless, its high fecundity, short generation interval and rich phenotypic variation among other attributes, make it ideally suitable for intensive genetic improvement, thus achieving faster genetic gains (Gjedrem, 2012; Saura et al., 2021). With the development of

artificial cultivation techniques, some selection programs aimed at improving economically important traits of aquatic animals have achieved encouraging results. For example, the genetically improved farmed tilapia, GIFT strain, showed 60% increase in weight at harvest compared to previous tilapia strain, and has become a globally important freshwater aquaculture commodity (Lal et al., 2021). Selective breeding in oysters has mainly aimed at improving growth rate, survival, environmental fitness, disease resistance, shell shape and color (see Sheridan, 1997). In China, mass selection was initiated to improve the growth performance of *C. gigas*, resulting in an average growth rate of 10% per generation (Li et al., 2011); a study based on 33 months of MSX exposure suggested that the survival rate of selected *C. virginica* increased from 28% in the F1 generation to 60% in F5 generation (Ford

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and Haskin, 1987); Dégremont et al. (2015) reported that the survival of the selected *C. gigas* increased by 61.8% compared to the control after four-generation mass selection for survival and OsHV-1 resistance.

Recent researches have emphasized the balance among the short- and long-term genetic gains for maintaining the viability of the genetic improvement scheme (Ponzoni et al., 2010; Yáñez et al., 2014; Hillen et al., 2017). High selection intensity can contribute to a significant decrease in genetic variability of selected populations (Felsenstein, 1965; D'Ambrosio et al., 2019). The genetic diversity decides long-term survival and evolutionary potential of species, and is, in turn, the result of its selective and demographic past (Mackintosh et al., 2019). Inbreeding depression by loss of genetic diversity is consistent with deterioration in adaptive capacities of species, which could eventually restrict future genetic improvement by artificial selection (Bentsen and Olesen, 2002; Evans et al., 2004). Genetic loss among selected strains has been documented in various aquaculture species including red sea bream (Sawayama and Takagi, 2016), tilapia (Lal et al., 2021), banana shrimp (Knibb et al., 2014), pearl oysters (Lind et al., 2009), abalone (Evans et al., 2004) and Pacific oyster (Appleyard and Ward, 2006), etc. Therefore, the genetic structure and genetic diversity of selected strains must be characterized in order to maximize the genetic gains while preventing potential inbreeding and random genetic drift (Mickett et al., 2003).

The Pacific oyster *Crassostrea gigas*, which is native to the coast of East Asia, is one of the main marine bivalves with a high aquaculture interest and is mainly cultured in China (FAO, 2020). *C. gigas* contributes over 1.58 million metric tons to oyster production in China, accounting for approximately 27% of oyster aquaculture production (BOF, 2022). Presently, one of the most serious problems in the *C. gigas* industry is the epidemic of mass summer mortalities (Mao et al., 2005; Lian et al., 2010; Jiang et al., 2022a). To restore the viability in summer and increase the growth rate of oyster, a genetic improvement project was initiated in China in 2019. The core of the project is divided into two steps: 1) obtaining a hybrid oyster with thermal tolerance of *C. angulata* and fast growth of *C. gigas* through hybridization; 2) breeding a new strain with stable characteristics in rapid growth and thermotolerance by continuous mass selection of the hybrids. Field- and laboratory-based tests showed that the hybrid GA exhibited faster growth rates, higher survival rates and better heat tolerance than the two parental species. Still missing is the genetic information about these populations.

As part of the genetic improvement project, this study investigated the consequences of continuous mass selection on genetic diversity and structure in a selected strain of hybrid oysters using 18 microsatellite loci and mitochondrial COI sequences. The objectives of this study were 1) to assess the level of genetic diversity of hybrids (base population) relative to their parents; 2) to evaluate whether the genetic diversity was preserved in selected strain over three selection generations; 3) to evaluate the degree of genetic differentiation between selected strains and wild populations.

2. Materials and methods

2.1. Selection program

The selectively breeding strains of *C. gigas* (Fig. 1) was established at a hatchery in Laizhou, Shandong Province, China (Fig. 2). In short, *C. gigas* was derived from a strain (Zhang et al., 2018) selected for growth over twelve generations in Rongcheng, Shandong Province, China while *C. angulata* was obtained from a commercial oyster farm in Zhangzhou, Fujian Province (Fig. 2). In June 2019, two reciprocal hybrids were produced by a complete diallel cross between *C. gigas* and *C. angulata* (Jiang et al., 2021a). Given its superior performance in growth, survival and thermotolerance (Jiang et al., 2021b), hybrid *C. gigas* ♀ × *C. angulata* ♂ (GA) was selected as the base population to establish the first-generation mass-selected strain (GAF1) in the next year. Six hundred adult oysters were selected from the top end of shell-

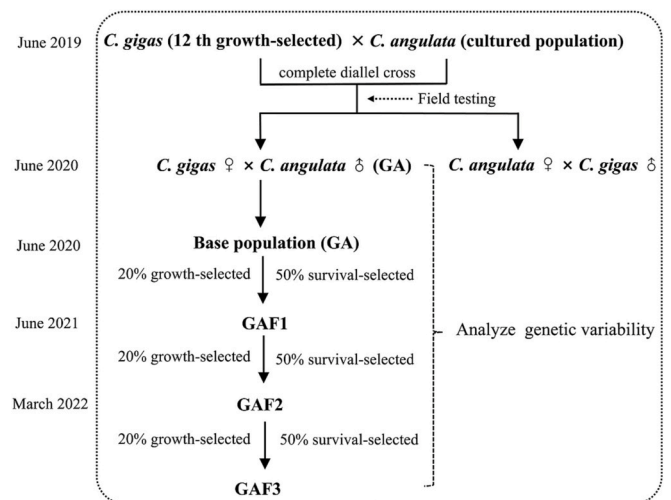


Fig. 1. Diagram of the selection design and genetic analysis used in breeding oysters for resistance to mortality.

height distribution of GA at a 20% selected rate. We then heat-shocked these oysters to induce a 50% mortality. The survivors were dissected and mated to produce GAF1 strain. To provide equal mating opportunities for both parents, equal numbers of eggs from per female were pooled together and fertilized with a mixture of sperm from each male. With the identical criteria, selections were performed for the next two successive generations of mass selection (GAF2 and GAF3) in 2021 and 2022. Details of broodstocks and selection rate for each generation are given in Table 1. All these strains were transferred to Rongcheng for cultivation.

2.2. Sample and DNA isolation

The breeding populations consisted of two cultured populations (GG and AA), one base population (GA) and three selected populations (GAF1, GAF2 and GAF3). A wild population of *C. gigas* came from Rongcheng (RC) (Fig. 2). A total of 336 individual adductor muscles samples were collected with 48 individuals sampled per population between years 2019 and 2022. Sample size and sampling date of all the ten populations are described in Table 1. Adductor muscles were stored in absolute ethanol at -30°C . Genomic DNA was extracted according to the traditional phenol/chloroform extraction procedure (Li et al., 2006). The concentration of DNA templates was measured by NanoDrop 2000 spectrophotometer (Thermo Scientific) and diluted to $100\text{ ng }\mu\text{l}^{-1}$ for further PCR analysis.

2.3. Microsatellite analysis

Eighteen microsatellite loci previously developed for *C. gigas* (Li et al., 2003; Sekino et al., 2003; Yamtich et al., 2005; Qi et al., 2009; Sauvage et al., 2009) were amplified in six multiplex PCRs. The universal tailed primers were synthesized with the 5' end of each forward primer labeled with a FAM, VIC or Hex fluorescent dyes. PCR were optimized and characterized as described previously (Zhang et al., 2023). The effectiveness of these microsatellite loci in all populations was verified by PCR amplification. Each multiplex PCR mixture consisted of 50 ng template DNA; 0.25 U 2× PCR Master Mix, 0.15 $\mu\text{mol/L}$ forward primer, 0.06 $\mu\text{mol/L}$ reverse primer, 0.15 $\mu\text{mol/L}$ universal tailed primer and then sterile water added to a final volume of 10 μl . The PCR profile was programmed for 3 min for initial denaturation at 95°C , followed by 35 cycles of denaturation at 95°C for 15 s and an additional 40 s at the optimal annealing temperature for each primer pair, extension at 72°C for 60 s; and finally, 8 cycles of 15 s at 93°C , 60s at 72°C , with a final at 72°C for 10 min. The amplified products were checked in

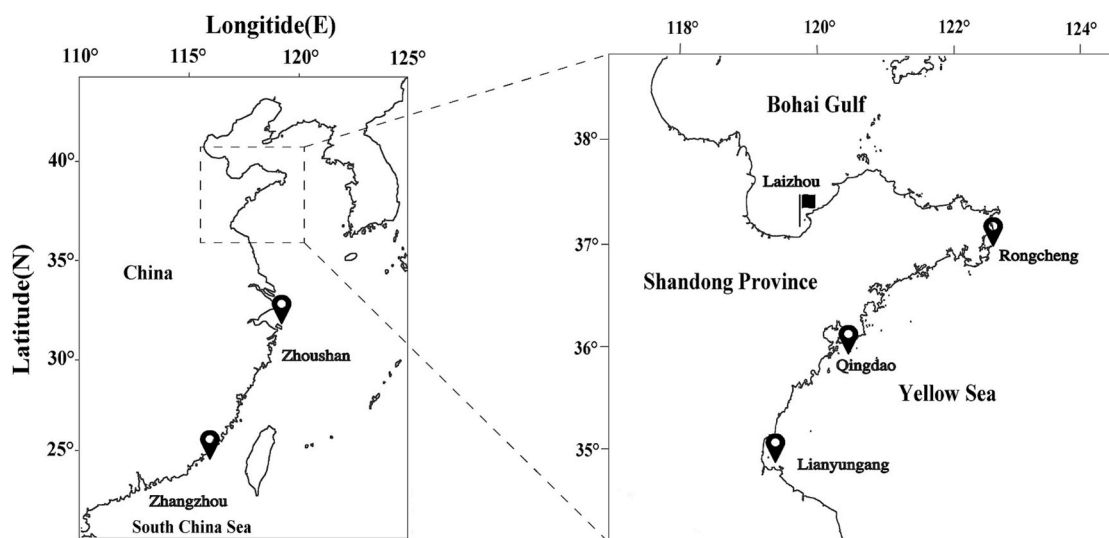


Fig. 2. Location of the broodstocks origin and oyster sampling sites in China.

Table 1

List of broodstocks, selection intensity and sample details for each generation.

Sort	Population	Number of parents		Selection rate	Sampling date	Sample size		Effective population size	
		Dam	Sire			Microsatellite	mtCOI	N_{e-lin}	95% C.I. (lower-upper)
Parental species	GG	50	50	–	2019.06	48	20	152.2	104.8–265.0
	AA	–	–	–	2019.06	48	20	1142.5	288.2 – Infinite
Hybrid F1	GA	40	40	–	2020.06	48	20	98.2	76.4–133.9
Selected stains	GAF1	50	50	10%	2021.06	48	20	144.1	102.1–234.3
	GAF2	40	40	10%	2022.03	48	20	92.8	70.1–132.5
	GAF3	40	40	10%	2022.10	48	20	51.2	43.3–61.7
Wild stocks	RC	–	–	–	2022.10	48	20	400.3	227.0–1468.2
	QD	–	–	–	2020.12	48	20	143.0	110.8–197.7
	LY	–	–	–	2020.12	48	20	673.4	314.0 –Infinite
	ZS	–	–	–	2020.12	48	20	100.0	82.5–125.3

2.0% agarose gels, stained with gel red and visualised under UV light using a 100- DNA ladder. All samples were detected in an ABI PRISM 3130 Automated DNA Sequencer (Applied Biosystems) with the LIZ 500 ladder as a reference. To provide a benchmark for assessment of genetic diversity, genotyping data (Chen et al., 2022) from three additional wild populations in Qingdao (QD), Lianyungang (LY) and Zhoushan (ZS) (Fig. 2) were also added.

Scoring of output data was analyzed using GeneMarker v.2.2.0 (Applied Biosystems). To check for potential genotypic errors caused by stuttering or large- allele dropout, all of the genotype data were tested with Micro-checker v.2.2.3 (Van Oosterhout et al., 2004). A test for conformation to the Hardy-Weinberg equilibrium (HWE) by a Markov chain approximation of the Fisher's exact test was undertaken by Genepop v.4.0 (Rousset, 1995). GenAlix v.6.5 (Peakall and Smouse, 2012) and CERVUS v.3.0 (Kalinowski et al., 2007) were used to estimate the number of alleles (N) and effective alleles (N_e), inbreeding coefficient (F_{IS}), Shannon-Wiener index (H), observed heterozygosity (H_o), expected heterozygosity (H_e) and the average polymorphic information content (PIC). The non-parametric test (Kruskal-Wallis test) was performed to analyze the difference in the above genetic diversity indicators. FreeNA software (Chapuis and Estoup, 2007) was used to examine the frequencies of null alleles and the INA correction (Chapuis et al., 2008) was carried out to decrease the deviations caused by null alleles in genetic diversity.

A hierarchical AMOVA was carried out using ARLEQUIN 3.5 (Excoffier and Lischer, 2010) to estimate how variation was divided within and among stocks. The calculation of F_{ST} was carried out using ARLEQUIN 3.5. A principal coordinates analysis (PCoA) was constructed

using GenAlix v.6.5 to visualize the genetic distances between populations. A neighbor-joining tree was constructed with the $Nei's D$ in Mega v5.0 (Tamura et al., 2011). The effective population size (N_{e-lin}) and 95% confidence intervals (lower-upper) were estimated using the linkage disequilibrium method implemented in NeEstimator v.2.1 (Do et al., 2014).

2.4. Mitochondrial COI analysis

Twenty DNA samples from seven populations (20 samples per population) were amplified at the partial mitochondrial DNA cytochrome c oxidase subunit I (COI) gene using the primers LCO1490 and HCO2198 (Vrijenhoek, 1994). PCR products were sequenced from the forward directions and the raw sequencing files (ab1 files) were used for further analyzed. Meanwhile, the mtCOI sequences of three additional wild populations (QD, LY and ZS) were also added. Multiple COI sequences were aligned and trimmed in DNASTAR v7.1 and MEGA v5.0. The aligned sequences were screened for total number of haplotypes (N_h), haplotype diversity (H_d), percent nucleotide diversity (P_i) and average number of nucleotide differences (k) in DNASP v.5.10 (Librado and Rozas, 2009). A Median-joining (MJ) networks was built using all COI sequences from ten populations (included wild populations QD, LY and ZS) on Popart v1.7 (Leigh and Bryant, 2015).

3. Results

3.1. Genetic diversity

Genotyping errors caused by stuttering errors or large allelic dropout were not detected in ten populations. Only 12 of the 180 locus-population groups had >0.20 null allele frequencies (ranging from 0.21 to 0.36), which had no qualitative effect on the results after validation.

Considering all 18 microsatellite loci, the number of alleles varied between 130 and 210 alleles per population, with an average value (N_a) ranging from 7.22 to 11.67 per locus (Table 2). Average numbers of alleles per locus do not differ ($P > 0.05$) between the selected strains (GAF1: 7.67; GAF2: 7.39; GAF3: 7.61), base population (GA: 8.06) and parental species (GG: 7.22; AA: 8.11). However, significantly fewer ($P < 0.05$) alleles were found in selected strains than wild population LY (11.67). The lower number of effective alleles (A_e) indicated that several alleles were plentiful at one locus. Lower genetic diversity among the selected strains (1.41–1.47) compared to wild populations (1.66–1.78) was also observed in Shannon Wiener index (I).

Within GAF1 – GAF3, no significant ($P > 0.05$) difference in H_o (0.55–0.61) and H_e (0.64–0.67) was observed, however H_o , H_e and PIC gradually decreased with the continuous mass selection (Table 2). In particular, the H_o was the highest in GA (0.72) among the 10 populations and significantly higher than that in GAF3. Heterozygosity ($H_e > H_o$) and inbreeding coefficient (F_{is} : 0.17–0.28) both highlight heterozygote deficiencies in wild populations, while heterozygote excesses were observed in GA ($H_e > H_o$; $F_{is} = -0.04$). All of the populations exhibited significant deviations from Hardy–Weinberg equilibrium (HWE), whereas deviation degree was relatively modest in the selected strains.

A total of 595-bp sequences of mtCOI gene derived from 200 individuals were utilized for genetic diversity assessment. 29 haplotypes were obtained from ten populations, of which 22 were unique haplotypes and 7 were shared haplotypes (Table 3). The remarkable high-frequency haplotypes 4 was observed in all nine populations (74% prevalence in 480 individuals), with the exception of the AA population, which had seven unique haplotypes (Fig. 3). With the continuous selection, the number (N_h) and diversity (H_d) of haplotypes increased slightly in the selected lines (N_h : 2–3; H_d : 0.100–0.279), despite being lower than in the wild population (N_h : 3–9; H_d : 0.279–0.705). The average number of nucleotide differences (k) lay in the range from 0.000 (GG) to 41.416 (RC).

3.2. Effective population size (N_{e-lin})

The effective population sizes (N_{e-lin}) for all selected strains ranged from 51.2 to 144.1, and were lower overall than values obtained for both parental species (152.2 and 1142.5 for GG and AA, respectively) and wild populations (100.0–673.4) (Table 1). N_{e-lin} of 51.2 (95% C.I. =

43.3–61.7) for GAF3 was relatively low and smaller than the actual number of parents (80 oysters).

3.3. Genetic differentiation

Analysis of molecular variance (AMOVA) of microsatellites displayed an overall F_{ST} of 0.02 ($P < 0.01$) for the selected strains versus 0.07 ($P < 0.01$) for all 10 populations (Table 4). Pairwise F_{ST} estimates were statistically significant ($P < 0.05$) for all population comparisons (Table 5). The wild population QD and ZS exhibited relatively high levels of divergence from the hatchery-produced populations (F_{ST} : 0.093–0.143; $Nei's D$: 0.228–0.326). Large genetic divergence was also found between parental species, GG and AA ($F_{ST} = 0.137$; $Nei's D = 0.332$). Besides, among six pairs of selected strains (including base population GA), the average F_{ST} and $Nei's D$ were 0.022 and 0.045, respectively, reflecting high genetic similarity among the selected generations. With the continuous selection, pairwise F_{ST} and $Nei's D$ values between base population and selected strains were increasing gradually.

Consistent with these results, the PCoA graph showed three main clusters, i. e. GG, AA and wild population (Fig. 4). In contrast, individuals derived from GA and selected strains formed a tight cluster that was distributed between two parental species. Overall, 16.89% of the total genetic variation was explained by the first two PCoA axes. For all ten populations, Neighbor-joining tree indicated that hatchery-produced populations grouped together and separated from wild populations that also grouped together (Fig. 5).

4. Discussion

In aquaculture, there are major questions as to whether genetic diversity is lost in selected strains after long-term or intensive selective breeding. We evaluated the genetic impact of mass-selected hybrid oyster strain derived from *C. gigas* and *C. angulata* for enhanced performance in growth and survival. Our main results suggested that there was no significant loss in genetic diversity of the selected strains after three generations of mass selection in spite of the lower haplotype diversity compared with wild oyster. Nevertheless, the genetic diversity revealed a general trend of gradual decline over artificial selection.

Aquaculture practices have been reported to reduce genetic variation in hatchery-produced cohorts or strains, which could lead to a decline in adaptive capacity (Allendorf and Phelps, 1980; Lind et al., 2009). Crossbreeding is essential for broadening the genetic base and creating new phenotypes in breeding programs (Bartley et al., 2001). The increase of genetic variation by hybridization is possibly associated with heterosis (Ferdinandez and Coulmam, 2002). Here, we found a clear elevation in heterozygosity levels of GA ($H_o = 0.72$; $H_e = 0.69$) compared to the parental populations, indicating increased genetic diversity of hybrids. That matters because genetic diversity lays the foundation for aquatic animals to survive and adapt to changing

Table 2
Genetic parameters for all ten populations based on 18 microsatellite loci.

Sort	Population	N	N_a	A_e	I	H_o	H_e	PIC	F_{is}	dHW
Parental species	GG	130	7.22 ± 0.81 ^c	3.49 ± 0.35 ^b	1.39 ± 0.13	0.59 ± 0.06 ^{ab}	0.64 ± 0.05 ^a	0.65	0.08	10
	AA	146	8.11 ± 1.91 ^{abc}	3.65 ± 0.53 ^b	1.38 ± 0.15	0.54 ± 0.06 ^b	0.63 ± 0.05 ^a	0.59	0.14	13
Base population	GA	145	8.06 ± 1.03 ^{abc}	4.36 ± 0.60 ^{ab}	1.53 ± 0.13	0.72 ± 0.05 ^a	0.69 ± 0.04 ^a	0.66	-0.04	10
	GAF1	138	7.67 ± 1.20 ^{bc}	4.10 ± 0.58 ^{ab}	1.47 ± 0.14	0.61 ± 0.04 ^{ab}	0.67 ± 0.04 ^a	0.64	0.08	11
Selected strains	GAF2	133	7.39 ± 1.13 ^c	3.64 ± 0.43 ^b	1.41 ± 0.13	0.61 ± 0.05 ^{ab}	0.65 ± 0.05 ^a	0.62	0.06	7
	GAF3	137	7.61 ± 1.02 ^{bc}	4.02 ± 0.58 ^b	1.43 ± 0.15	0.55 ± 0.07 ^b	0.64 ± 0.05 ^a	0.61	0.13	8
	RC	200	11.11 ± 1.51 ^{ab}	5.83 ± 1.10 ^{ab}	1.70 ± 0.19	0.58 ± 0.07 ^{ab}	0.69 ± 0.06 ^a	0.67	0.17	10
Wild stocks	QD	201	11.17 ± 1.76 ^{ab}	5.78 ± 1.24 ^{ab}	1.66 ± 0.21	0.54 ± 0.06 ^b	0.65 ± 0.07 ^a	0.63	0.17	14
	LY	210	11.67 ± 1.64 ^a	6.55 ± 1.29 ^a	1.78 ± 0.20	0.51 ± 0.05 ^b	0.70 ± 0.06 ^a	0.68	0.28	13
	ZS	180	10.00 ± 1.62 ^{abc}	5.93 ± 1.38 ^{ab}	1.68 ± 0.20	0.53 ± 0.06 ^b	0.68 ± 0.04 ^a	0.66	0.22	15

N : number of alleles, N_a : average number of alleles, A_e : number of effective alleles, I : Shannon Wiener index, H_o : observed heterozygosity, H_e : expected heterozygosity, PIC : polymorphic information content, F_{is} : inbreeding coefficient, dHW : number of loci deviating from Hardy-Weinberg equilibrium. Means in the same column superscripted by different letters were significantly different ($P < 0.05$).

Table 3
Genetic diversity and haplotype type of mtDNA sequences for all ten populations.

Haplotype	Parental species		Base population	Selected strains			Wild populations				Total	
	GG	AA	GA	GAF1	GAF2	GAF3	RC	QD	LY	ZS		
1											1	1
2		7										7
3												2
4	20		19	18	17	17	16	11	13		17	148
5								1	1			2
6		3										3
7		1										1
8					2	1						3
9					1	2						3
10								1	1			2
11								1				1
12									1			1
13									2	1		3
14		2										2
15		3										3
16		3										3
17										1		1
18								1				1
19		1										1
20									1			1
21									1			1
22									1			1
23							1					1
24							1					1
25											2	2
26			1				1					2
27							1					1
28				1								1
29				1								1
N_h	1	7	2	3	3	3	5	9	7	3		
H_d	0.000	0.837	0.100	0.195	0.279	0.279	0.368	0.705	0.584	0.279		
P_i	0.000	0.005	0.007	0.001	0.005	0.003	0.070	0.068	0.013	0.001		
k	0.000	3.205	40.500	0.689	2.732	1.568	41.416	40.600	7.958	0.479		

N_h : number of haplotypes, H_d : haplotype diversity, P_i : percent nucleotide diversity, k : average number of nucleotide differences.

environment, and may be positively related to economic characteristics (Hu et al., 2015). It has been shown that crossbred abalone with higher heterozygosity compared with parents exhibited noticeable heterosis in growth and survival traits (Luo et al., 2010). Teng et al. (2005) also attributed the heterosis of the hybrid scallop to its greater genetic diversity. Moreover, heterosis is largely dependent upon the degree of genetic differentiation between parental stocks (Shikano and Taniguchi, 2002). Wachirachaikarn et al. (2009) thus expected that the emergence of beneficial heterosis from a mating between genetically distant catfish stocks. Unsurprisingly, both F_{ST} (0.137) and Nei 's D (0.332) values showed significant inter-population genetic differentiation between GG and AA. Furthermore, based on mitochondrial data, we also found an improvement in percent haplotype diversity in GA, indicating that hybridization may be helpful to improve the diversity of the initial genotype (Evans et al., 2004). The successful implementation of any selection scheme is largely driven by how the foundation stock is established, because the genetic diversity originally available among the founders might influence the genetic progress made in later artificial selection (Holtmark et al., 2008). Our results revealed a high degree of variation in base population GA, which was therefore suitable for further selective breeding.

While selective breeding within a closed cohorts allows for rapid improvement of given phenotypes, successive breeding of selected animals regardless of relatedness will lead to loss of genetic variation (Bentsen and Olesen, 2002). Numerous aquatic animals demonstrated a reduced genetic variation in breeding lines relative to wild ancestral stocks (Brown et al., 2000; Dixon et al., 2008; In et al., 2016; Fu et al., 2017). It has been argued that loss of genetic variability can lead to poor characteristics and reduced fitness of aquaculture lines (Evans et al., 2004). Here, we observed no significant sign of genetic variation reduction during three generations of selective breeding as indicated by

heterozygosity and COI haplotypes. In particular, expected heterozygosity and observed heterozygosity, which are common indicators of genetic diversity, were not significantly different between the three selected generations ($P > 0.05$). This high level of diversity indicated the effectiveness of the selective breeding program and the appropriate genetic management of the GA strain. It is speculated that a stable number of effective breeders contributes to the maintenance of high levels of diversity in selected strains (Li et al., 2006; Diyiye et al., 2021). Nevertheless, the number of alleles, polymorphic information content and heterozygosity of microsatellites exhibited a tendency to decrease with selection. It is probable that the loss of genetic variation in the selected strains was a consequence of random genetic drift and intense selection (Romana-Eguia et al., 2004; In et al., 2016). The high fecundity of marine bivalves allows for hard selection, resulting in fast genetic gains (Gjedrem, 2012; Saura et al., 2021). However, the nature of oyster comes with a striking variation in reproductive success, which are often attributed to differences in gamete quality, sperm-egg interactions and viability between genotypes (Boudry et al., 2002). Large differences in reproductive success may have promoted inbreeding and genetic drift in closed stocks (Lallias et al., 2010; Varney and Wilbur, 2020). This could be further aggravated by the limited number of parents in selection (Romana-Eguia et al., 2004; In et al., 2017).

A direct result of inbreeding, referred to as inbreeding depression, is a dramatic decrease in the average phenotype of specific traits as well as an increase in homozygosity in a cohort (Lynch and Walsh, 1998). F_{IS} denotes the inbreeding coefficient of an individual relative to a sub-population, indicating the extent of deviation from random mating. Here, the observed heterozygosity was lower than the expected heterozygosity of selected populations, which may indicate heterozygosity deficiency (i.e., positive F_{IS} value) in selected strains (Luikart et al., 1998), particularly in GAF3 ($F_{IS} = 0.13$). Therefore, the sudden increase

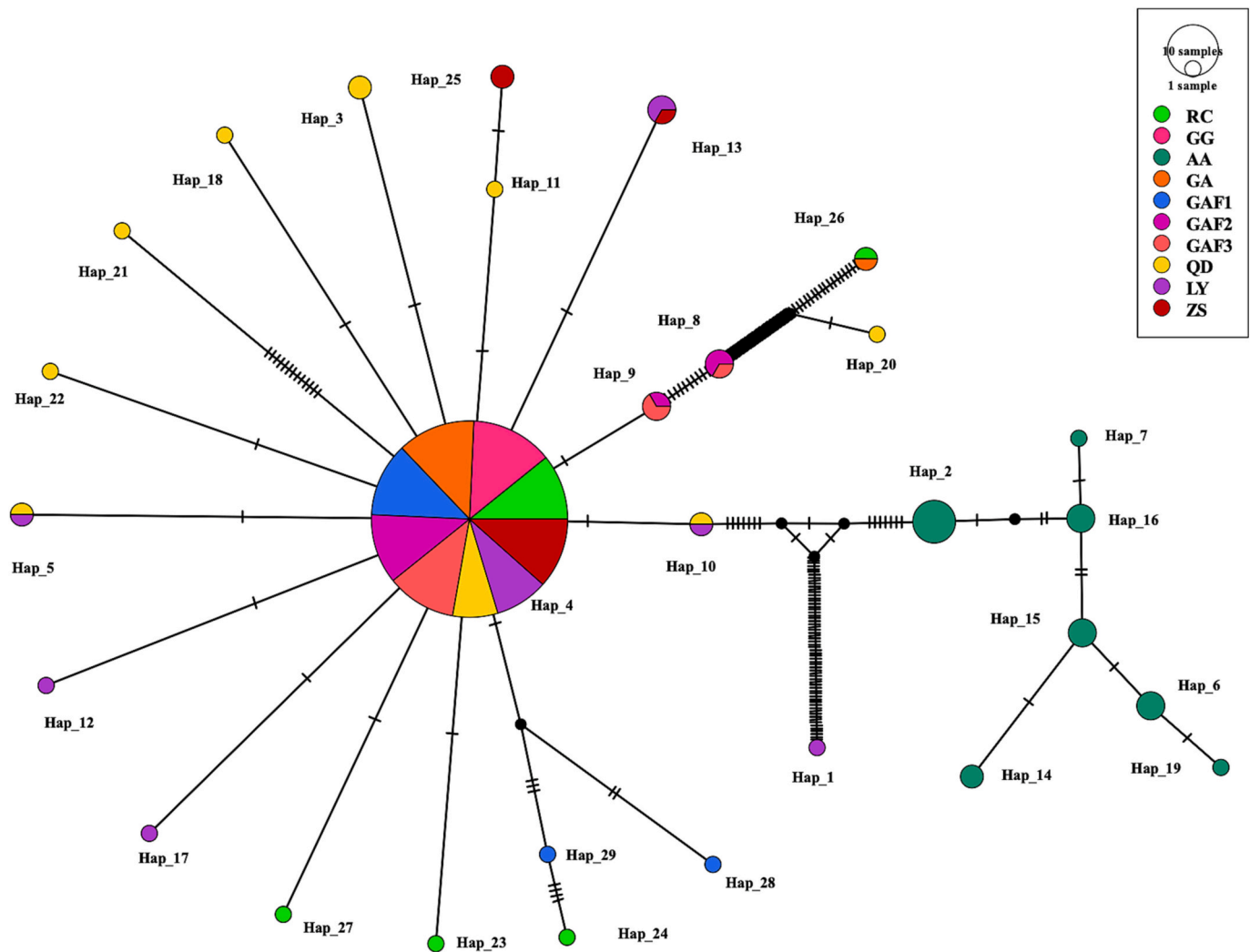


Fig. 3. Median-joining (MJ) networks of 29 COI haplotypes of all ten populations. The number of substitutions separating two haplotypes was indicated by the vertical bars on the line.

Table 4

Analysis of molecular variances (AMOVA) of microsatellites for generations of the selected strain (including the base population) and all ten populations.

Source of variance	<i>d.f.</i>	Percentage of variation	<i>F</i> -statistic*
Among generations of the selected strain			
Among populations	3	2.11	$F_{ST} = 0.02^*$
Among individuals/within population	188	6.55	$F_{IS} = 0.07^*$
Within individuals	192	91.34	$F_{IT} = 0.09^*$
Among all ten populations			
Among populations	9	6.83	$F_{ST} = 0.07^*$
Among individuals/within population	412	13.00	$F_{IS} = 0.14^*$
Within individuals	432	80.17	$F_{IT} = 0.20^*$

* Significant at $P < 0.01$.

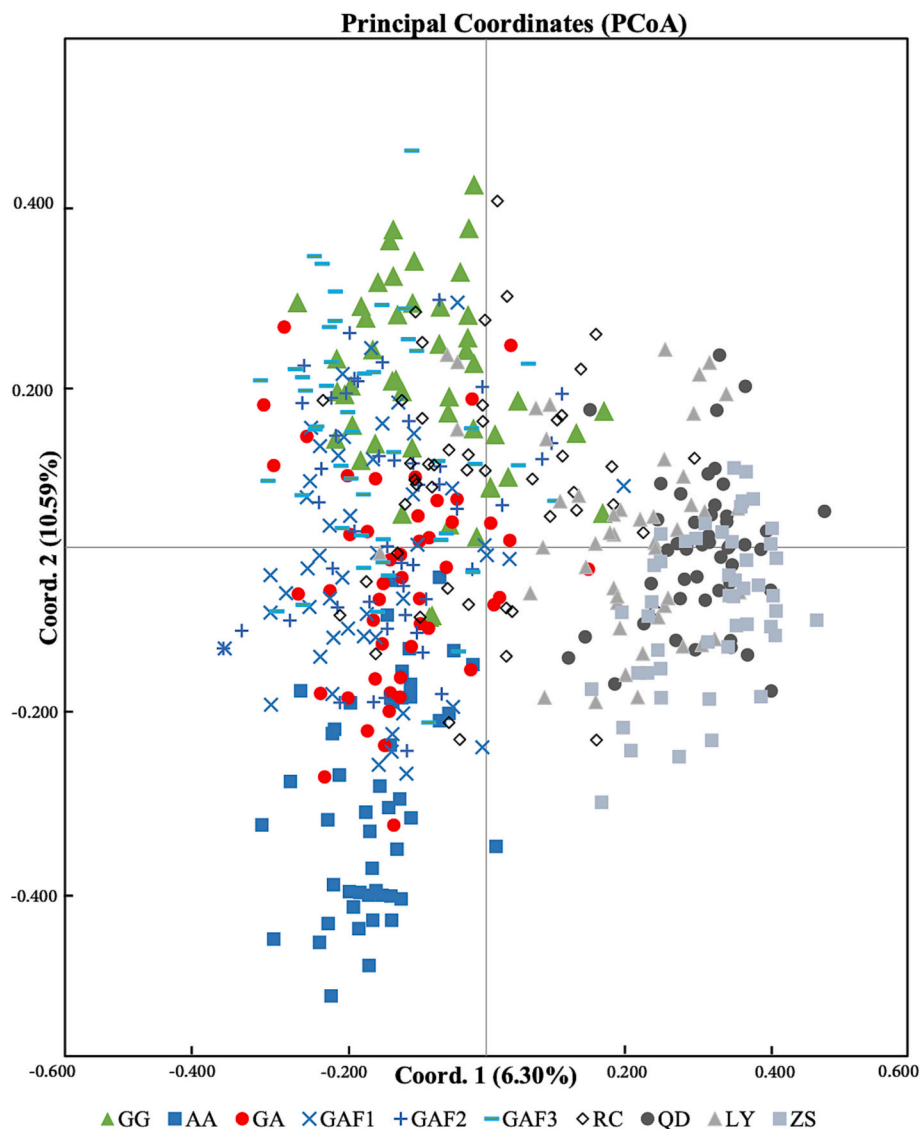
of F_{IS} in GAF3 suggested that non-random mating may have occurred (Li et al., 2006). Moreover, non-random mating may also be a cause of the Hardy-Weinberg disequilibrium observed in this study (Engels, 2009). Increase of F_{IS} has been documented with respect to microsatellite loci in

selected strains of the Pacific oyster (Chen et al., 2022), South African abalone (Rhode et al., 2014) and Black Tiger shrimp (Dixon et al., 2008). However, inbreeding load, the rate at which fitness decreases with rising F_{IS} , is probably greater in bivalves relative to other aquatic organisms (Plough, 2016), which could result in faster inbreeding depression (Launey and Hedgecock, 2001).

The conservation of genetic diversity within a population is accomplished by maximizing effective population size. Franklin and Frankham (1998) suggested a recommended threshold of 500 for N_e to maintain evolutionary potential. However, the effective population sizes N_{e-lin} for the selected strains slipped from 144.1 in GAF1 to 51.2 in GAF3. This means that the estimated N_e of the selected strains were edging closer to the bottom N_e of 50 desired for a sustainable selection scheme (Ponzoni et al., 2010). Effective population size is strongly linked to genetic drift and inbreeding. Except the limited parents and reproductive success differences mentioned previously, variance in contribution of gametes was likely to be a leading cause of reduction of N_e (Li et al., 2007). That's due to inevitable differences in gametic quality, gametic competition, zygotic competition and familial viability, despite attention was paid to balancing the gametic contributions between sires and dams at the fertilization phase (Boudry et al., 2002). Nevertheless, some caution must be exercised in explaining the noted decline as they could also be a result of population admixture (Santiago et al., 2020). Sonesson and Ødegård (2016) noted that increasing the number of parents selected

Table 5Estimated pairwise F_{ST} values (below diagonal) and $Nei's D$ (above diagonal) of all population based on 18 microsatellite makers.

	GG	AA	GA	GAF1	GAF2	GAF3	RC	QD	LY	ZS
GG		0.332	0.117	0.081	0.094	0.092	0.092	0.275	0.178	0.288
AA	0.137		0.147	0.119	0.145	0.210	0.213	0.359	0.270	0.350
GA	0.052	0.066		0.011	0.043	0.108	0.104	0.268	0.187	0.267
GAF1	0.039	0.057	0.005		0.013	0.055	0.082	0.265	0.167	0.271
GAF2	0.046	0.070	0.021	0.007		0.038	0.084	0.228	0.140	0.261
GAF3	0.046	0.098	0.049	0.028	0.020		0.087	0.276	0.168	0.326
RC	0.042	0.089	0.041	0.035	0.038	0.041		0.158	0.084	0.190
QD	0.114	0.143	0.101	0.105	0.097	0.116	0.066		0.062	0.089
LY	0.073	0.107	0.067	0.065	0.059	0.071	0.033	0.029		0.091
ZS	0.111	0.134	0.094	0.100	0.102	0.124	0.072	0.041	0.037	

All F_{ST} values were significantly different from zero ($P < 0.05$).**Fig. 4.** Genetic relationships among individuals of ten populations (480 individuals) from principal coordinates analysis (PCoA) using genetic distance matrices. The different populations are indicated by different colours and symbols, respectively.

and single-pair fertilization might contribute to improving N_e . In addition, the mate allocation strategy adopted in Nile Tilapia has also obtained a satisfactory N_e to sustain the selection program (Ponzoni et al., 2005).

Assessing population differentiation helps to understand the origin and mixing of aquaculture populations and to direct the breeding and

application of new varieties. Both F_{ST} analysis (0.007–0.028) and AMOVA ($F_{ST} = 0.02$) revealed little genetic differentiation across generations of the selected strain, which could be explained by the absence of distinct genetic structure or population subdivision, without obvious obstacles for gene flow (Diyie et al., 2021). Meanwhile, the $Nei's D$ values showed a gradual rise in genetic distance from GA to GAF3.

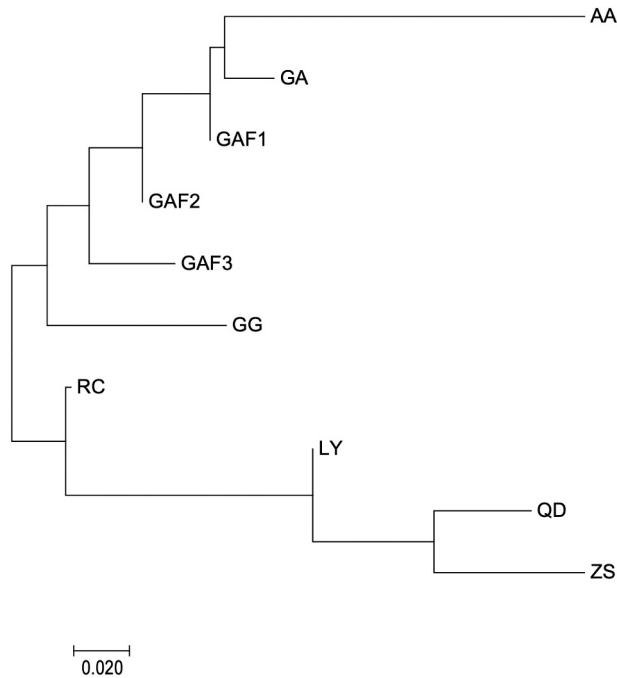


Fig. 5. Neighbor-joining tree of all ten populations based on *Nei's* unbiased genetic distance.

Genetic distance between the base population and the recent generation may be caused by integrated effect of intense mass selection, founder effects and genetic drift (Hillen et al., 2017). In addition, gradual increases in genetic differentiation between selected strains and wild stocks were also observed. In general, the more distinct selected strains are from the local wild stocks, the higher risks are associated with stocking and promotion (Berrebi et al., 2021). Here, three wild stocks (QD, LY and ZS) were moderately differentiated (F_{ST} : 0.059–0.124) from selected strains, with comparatively large genetic distance (*Nei's* D : 0.140–0.326), which probably poses a threat to conservation of native stocks. Therefore, effective precautions such as triploidy techniques, etc., need adoption to avoid potential adverse genetic effects resulting from escapes on wild populations (Jiang et al., 2022b).

Overall, microsatellites and mitochondrial COI revealed no significant loss of genetic diversity in the mass-selected strains of hybrid oyster in three generations. The rich genetic diversity in base population suggests that hybridization can be used to broaden the genetic base of populations. However, the decreased genetic variation and effective population size with selection prompt that measures should be taken to maintain the genetic diversity of strains to ensure stable genetic gains. The data here provide a valuable information for the further genetic improvement of hybrid populations.

CRediT authorship contribution statement

Gaowei Jiang: Investigation, Conceptualization, Formal analysis, Writing – original draft. **Yifei Zhang:** Methodology, Data curation, Formal analysis. **Lijie Du:** Data curation. **Yulu Chen:** Methodology. **Yuanxin Liang:** Methodology. **Yi Yu:** Methodology. **Chengxun Xu:** Supervision, Resources. **Qi Li:** Supervision, Conceptualization, Resources, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

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