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Evaluation of translocation impacts on genetic patterns in farmed and naturalized populations of *Mytilus galloprovincialis* along the China coast: clues from mitochondrial cytochrome c oxidase I sequences

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Abstract

As an introduced species, *Mytilus galloprovincialis* has developed into self-sustaining naturalized populations and has been widely cultivated in northern China. The *M. galloprovincialis* aquaculture industry wholly depends on the movement of naturalized juveniles onto farms. It is, therefore, necessary to understand the genetic effect of continuous spats' translocation. This study divided 12 localities of *M. galloprovincialis* along the China coast into three types of populations—farmed, naturalized adjacent farmed, and isolated—to investigate the genetic variation and differentiation. The genetic variability is reflected by haplotype diversity, nucleotide diversity, and the mean number of pairwise differences expressed as farmed populations > naturalized adjacent farmed populations > isolated populations. The Hierarchical analyses and Mantel-test indicated slight divergence between farmed and naturalized populations, northern and southern populations. The farmed and naturalized populations clustered into two separate categories in the neighbor-joining tree except two anthropogenically intervened localities. The present results suggest that the translocation practice positively affected genetic variability and played a vital role in shaping genetic composition. The information obtained in this study provides new insights into the impacts of the translocation culture model of marine mollusks.

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Introduction

Moving economically valuable aquaculture species to new regions is typical for conservation management and the commercial industry (Beaumont, 2000). The deliberate movement of populations can be usefully categorized into transfer (movement within a species' range) or introduction (movement outside a species' range) (Beaumont, 2000; Lemer and Planes, 2012). Depending on the particular situation, these changes may range from beneficial or harmful (Gaffney, 2006). When the transferred individuals are hatchery propagations, some genetic impact on the wild population is inevitable if the transferred stocks survive and reproduce (Castro et al., 2017). The primary issue focus on the changes in adequate population size and genetic composition of the wild populations (Gaffney, 2006; Lemer and Planes, 2012). The genetic effect between hatchery-produced and wild stocks has been widely studied in numerous aquaculture species, demonstrating a decrease of genetic variability in wild populations due to interbreeding with hatchery-propagations produced by limited parents (An et al., 2014; Behera et al., 2018; Rahman et al., 2009; Xing et al., 2014; Yu and Li, 2007). Different from the hatchery-propagations transfer, several studies concentrated on the effect of wild individuals transfers, named as translocation (Lemer and Planes, 2012). For commercial purposes between different regions, they recommend that the translocation homogenized the transferred and received populations, both in terms of genetic composition and variability (Arnaud-Haond et al., 2003, 2004). However, few empirical studies have been performed to study the effect of translocation among self-sustaining introduced populations named "naturalized" (Sutherland et al., 2020), like the *Mytilus galloprovincialis* scattered along the China coast. Like the wild populations, the translocation of naturalized populations is less likely to cause a reduction of adequate population size. However, it may increase genetic diversity and homogenization by pooling genetically divergent populations (Lemer and Planes, 2012).

Mussels are economically important bivalves in China aquaculture. The production of cultured mussels in China, primarily from three species—*M. galloprovincialis*, *M. couscous*, and *Perna viridis*—is more than 870 thousand tons (Bureau of Fisheries, 2020). Official statistics do not distinguish the output of individual mussel species. However, experts estimate that *M. galloprovincialis*, known as the Mediterranean mussel or blue mussel, account for about 55% of total production. These three mussels have relatively well-defined cultured regions. *M. couscous* and *P. viridis* are farmed in southern China, with *P. Viridis*, in Fujian and Guangdong provinces further south than those of *M. coruscus*, farmed mainly in Zhejiang province. The chief culture areas of *M. galloprovincialis* are along the coast of northern China, centering in the eastern waters of the Liaodong Peninsula and Haizhou Bay at the Shandong and Jiangsu province junction. Cultured under intertidal zone by the long-line or rope culturing techniques, the *M. galloprovincialis* grows faster, and meets sales specifications after the first growing season and spawns thousands of gametes in the next year (Wang, 1997).

The spread and cultivation of *M. galloprovincialis* in China occupy two distinct characters. Firstly, the wide distribution benefited from anthropogenic farming activities. *M. galloprovincialis* was introduced into China via ballast water from the Mediterranean Sea and initially identified in the 1950s in the northern Yellow Sea (Wang, 1997; Zhang et al., 1955). Due to its rapid growth and high production, combined with the success of natural seed collection, the culture of *M. galloprovincialis* became popular, forming a vast aquaculture industry after the 1970s. Meanwhile, *M. galloprovincialis* was deliberately moved southward to Zhejiang, Fujian, and Guangdong provinces widely from northern China for trial breeding in a short time (Zhang, 1984). As the problems of adaptation and transportation cost, the culture of *M. galloprovincialis* in southern China withered gradually and was replaced by indigenous species. To date, there is no large-scale transfer and culture in southern waters. However, the domestic or naturalized (self-sustaining introduced) populations (Sutherland et al., 2020) had left and isolated from northern populations (Pickett and David, 2018). Secondly, the aquaculture of *M. galloprovincialis* is exclusively based on the naturalized seeds rather than hatchery-produced individuals. Since the spawn time is not synchronized, the transplant of spats among chief culture regions is ubiquitous. For example, due to the higher temperature, the spawn time of *M.*

galloprovincialis in Haizhou Bay is earlier than that in the Liaodong Peninsula. The farmers in the Liaodong Peninsula transplant spats from Haizhou Bay for earlier harvest and sale. Correspondingly, the farmers in Haizhou Bay supplement the seed from other later-spawning places for the seed shortage caused by disease, natural disaster, or the scaled-culture demand. In addition, the farmers reflected that there is an advantage for non-local individuals in growth and survival for unknown reasons.

Several studies have investigated the genetic relationship of different *M. galloprovincialis* populations along the China coast, such as among natural populations (Han et al., 2017; Shen et al., 2011), cultured populations (Guo et al., 2012; Zhou et al., 2015), or between them (Pang et al., 2012). Evidence has confirmed the genetic homogeneity of the naturalized populations of *M. galloprovincialis* in China (Han et al., 2017; Shen et al., 2011). However, the conclusions of the other two aspects were unconvincing following the reasons as i: no species confirmation, especially for the southern populations due to the sympatry of *M. coruscus* with similar shell shape and possible hybridization (Chang et al., 2008; Shen et al., 2006; Yang et al., 2019), ii: minimal sampling sites that could not reflect the whole scenario between naturalized and farmed populations. Furthermore, no single study has considered the wild juvenile collection and artificial migrations. The genetic effect of translocation of *M. galloprovincialis* along the China coast requires further investigation to verify if the ongoing spats translocation of *M. galloprovincialis* increases gene flow among populations, leading to an increase in genetic diversity and decrease of genetic divergence among populations.

The capacity of the mitochondrial DNA (mtDNA) *cytochrome c oxidase I* (*COI*) gene to reveal ongoing genetic interactions between populations has been confirmed in extensive marine mollusks (Guo et al., 2015; Mao et al., 2011; Ni et al., 2012a, 2012b; Wang et al., 2017), including *M. galloprovincialis* (Gerard et al., 2008; Han et al., 2017; Pickett and David, 2018). The present study compared three populations from intensive sampling along the China coast: farmed, naturalized adjacent to the farm, and isolated naturalized populations by amplifying *COI* sequences. In addition, the levels of genetic diversity and population differentiation were evaluated, which provided a reference for the genetic evaluation of *M. galloprovincialis* or other commercially naturalized species relevant to the translocation culture model.

Materials and Methods

sample collection

A comprehensive survey of the *M. galloprovincialis* farming industry in China was conducted. We sampled i: individuals in *M. galloprovincialis* farms referred to as "farmed populations"; ii: naturalized individuals in regions absent from *M. galloprovincialis* farming in northern China, referred to as "naturalized adjacent farmed populations"; iii: naturalized individuals from southern China, referred to as "isolated naturalized populations" (**Figure 1A**, **Table 1**). Farmed mussels were mainly collected from chief culture regions: Dalian (DL), Zhuanghe (ZH) in the east of the Liaodong Peninsula, and Ganyu (GY), Jiaonan (JN), surrounding the Haizhou Bay. Individuals from Aoshan (AS) were also collected and classified into the farmed population as small-scale farming in this locality. To avoid the interference of current-year-transplanting seedlings, we did not collect the farmed individuals directly. Still, we sampled from the reefs near farms or the breeding facilities, like floats, ropes, or cultivating boats. Naturalized adjacent to farmed individuals were collected from farming cages of oyster or scallop in Qinhuangdao (QHD), Changdao (CD), Kongtongdao (KTD), Rongcheng (RC), Rushan (RS). In these regions, the mussel is a kind of fouling organism for the other shellfish aquaculture. Isolated naturalized mussels were collected from offshore islands of Gouqidao (GQD) and Pingtan (PT) in southern China. The mantle was incised and frozen in liquid nitrogen and stored at -80 ℃ for DNA extraction.

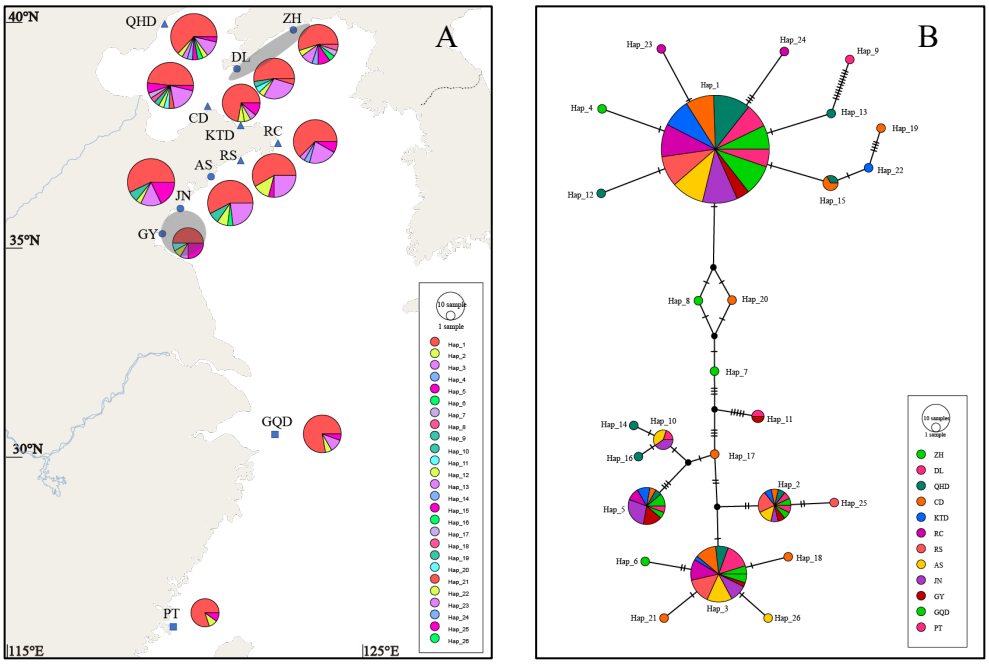

Figure 1 Spatial distribution (A) and network (B) of *M. galloprovincialis* haplotypes along the China coast. The size of circles is proportional to haplotype frequencies. A: variable symbols represent different types of populations: circle, farmed populations; triangle, naturalized adjacent farmed populations; square, isolated naturalized populations. Gray shading indicates the two main aquaculture waters: Haizhou Bay (round) and the eastern area of Liaodong Peninsula (oval). B: the haplotypes are separated by short segments as one mutation step in networks. Locality abbreviation is shown in **Table 1**.

Table 1 Sampling information and diversity indices of 12 localities along China coast

Region	Population	Locality	Locality abbreviation	Coordinates	*N*	*n*	*h*	*π*	*k*
Northern	Farmed populations	Zhuanghe	ZH	39.49°N, 123.04°E	20	8	0.7116	0.01159	7.1157
		Dalian	DL	38.86°N, 121.55°E	21	6	0.6667	0.01356	8.3285
		Aoshan	AS	36.37°N, 120.73°E	26	5	0.6246	0.01156	7.0953
		Jiaonan	JN	35.69°N, 119.93°E	28	5	0.6376	0.01294	7.9471
		Ganyu	GY	34.97°N, 119.49°E	12	5	0.7273	0.01542	9.4697
			Total		107	12	0.6556	0.01255	7.7055
	Naturalized adjacent to farmed populations	Qinhuangdao	QHD	39.63°N, 119.36°E	27	9	0.6040	0.00963	5.9145
		Changdao	CD	37.92°N, 120.69°E	27	10	0.7464	0.01141	7.3162
		Kongtongdao	KTD	37.55°N, 121.52°E	18	5	0.4837	0.00951	5.8366
		Rongcheng	RC	37.08°N, 122.50°E	24	5	0.5797	0.01069	6.5616
		Rushan	RS	36.73°N, 121.49°E	24	4	0.6051	0.01177	7.2246
			Total		120	18	0.6108	0.01072	6.5811
Southern	Isolated naturalized populations	Gouqidao	GQD	30.68°N, 122.69°E	18	4	0.3987	0.00864	5.3072
		Pingtan	PT	26.68°N, 120.35°E	10	3	0.3778	0.00898	5.5111
			Total		28	4	0.3810	0.00846	5.1958

N: number of individuals, *n*: number of haplotypes, *h*: haplotype diversity, *π*: Nucleotide diversity, *k*: mean number of pairwise difference

Sequence acquisition and species confirmation

Total genomic DNA was extracted from the mantle using the DNeasy tissue kit (Qiagen) accordingly. The sequence of the *COI* gene was amplified with the previously described primers (Folmer et al., 1994). The polymerase chain reaction (PCR) reaction mixture (10 ul) contained 0.8ul 2.5mM dNTPs, 1 ul 10 × reaction buffer (Mg^{2+} plus), 1 ul genomic DNA, 1 ul of 10 uM each primer and 0.05 ul Taq polymerase (Takara). The PCR procedure was performed under the following conditions: initial denaturation at 94℃ for 3 min, 35 cycles

at 94℃ for 1 min, 52℃ for 1 min, and 72℃ for 1 min, followed by a final extension at 72℃ for 10 min. The products of PCR were sequenced by Sangon Biotech (Shanghai) Co., Ltd.

Species confirmation was performed by comparing sequence similarity to the reference data set. Each distinct sequence was compared with available sequences in the GenBank database using the Basic Local Alignment Search Tool (BLAST) and rechecked on the BOLD (http://www.boldsystems.org/).

Data analysis

All sequences were assembled and modified by DNASTAR software (DNASTAR, Inc.). The package of Biopython 1.7 was used to treat sequences in Python 3.8, including sequence alignment, clipping, and format conversion. The number of haplotypes (*n*), Haplotype diversity (*h*), nucleotide diversity (π), and the average number of nucleotide differences (*k*) were calculated for each locality with DnaSP 6.1 (Rozas et al., 2017). Networks of haplotypes were estimated using Popart 1.7 (Clement et al., 2000). Finally, the mean distance and pairwise *Fst* among 12 localities were measured with MEGA X (Kumar et al., 2018) and Arlequin 3.5 (Excoffier and Lischer, 2010), respectively.

Hierarchical analyses of molecular variation (AMOVA) were performed in Arlequin 3.5 (Excoffier and Lischer, 2010) to estimate the population genetic structure following three grouped strategies as i: Three groups: farmed populations, naturalized adjacent farmed populations, isolated naturalized populations; ii: Two groups A: farmed populations, naturalized populations including naturalized adjacent farmed populations and isolated naturalized populations; iii: Two groups B: northern populations including farmed populations and naturalized populations adjacent farmed populations, southern populations equivalent to isolated naturalized populations. The variance components, the sum of squares, and Φ statistics were calculated between groups, among populations within groups, and within populations, respectively. The significance of Φ-Statistics analogs (Φ_{CT}, Φ_{SC}, and Φ_{ST}) was evaluated with 10^5random permutations. To verify the influence of spats exchange on the genetic distance between locations, Mantel-test was performed for all localities (northern localities and farmed localities) to examine the association between the *Fst* and geographical distance (log-transformed). An unrooted neighbor-joining tree (NJ tree) based on the genetic distance was constructed to evaluate the genetic relationships using the software PHYLIP 3.698 (Felsenstein, 2009) **Figure 3**.

Results

Genetic variability

Twenty-six haplotypes with 49 polymorphic sites (29 parsimony informative sites) were revealed according to the 614 bp *COI* segment in 12 localities. The sequences of haplotypes were deposited in the GenBank database with accession numbers MT581451 to MT581476. The genetic variation expressed by haplotype diversity, nucleotide diversity, and average number of nucleotide differences decreased from the farmed populations (*h*: 0.6556, π: 0.01255, *k*: 7.7055) to the naturalized adjacent farmed populations (*h*: 0.6108, π: 0.01072, *k*: 6.5811) and then the isolated populations (*h*: 0.3810, π: 0.00846, *k*: 5.1958) (**Table 1**). Two haplogroups were formed in the TCS network with no correspondent relationship with the geographical distribution of 12 localities (**Figure 1B**). Hap_1 was the sole haplotype shared in all 12 localities with an average frequency of 60%, varying from 48% to 80% in each locality (**Table 2**). The CD belonging to the naturalized adjacent farmed population exhibited the highest haplotype diversity (*h*: 0.7467) and the largest haplotype number (n = 10) than all other localities. The isolated naturalized populations showed the lowest haplotype number (GQD: 4, PT: 3) and the highest frequency of Hap_1 (GQD: 78%, PT: 80%).

Table 2 Haplotype distribution among localities

	ZH	DL	QHD	CD	KTD	RC	RS	AS	JN	GY	GQD	PT	N
Hap_1	11	11	17	13	13	15	14	15	16	6	14	8	153
Hap_2	1	1	1	1	1		3	2	1	1	1	1	14
Hap_3	2	6	3	5	1	5	6	6	4	1	2		41
Hap_4	1												1
Hap_5	2		1	1	2	2			5	3	1	1	18
Hap_6	1												1
Hap_7	1												1
Hap_8	1												1
Hap_9		1											1
Hap_10		1						2	2				5
Hap_11		1								1			2
Hap_12			1										1
Hap_13			1										1
Hap_14			1										1
Hap_15			1	2									3
Hap_16			1										1
Hap_17				1									1
Hap_18				1									1
Hap_19				1									1
Hap_20				1									1
Hap_21				1									1
Hap_22					1								1
Hap_23						1							1
Hap_24						1							1
Hap_25							1						1
Hap_26								1					1

Hap = Haplotype; N = total number

Genetic differentiation

Genetic distance and pairwise *Fst* values ranged from 0.00844 to 0.01464 and -0.08037~0.06312 with no static significance, respectively (**Table 3)**. In addition, most of the pairwise comparisons of *Fst* values were negative. AMOVA was conducted for all the 12 sampling sites in three patterns (**Table 4**). All grouped strategies revealed significant variation among groups ($p < 0.05$) with low variance components (1.57%, 1.74%, and 3.15%, respectively).

Table 3 Genetic distance and pairwise *F*st among 12 populations of the China Dataset.
Data below the diagonal show the genetic distance, data on the opposite side show the *F*st value.

	ZH	DL	QHD	CD	KTD	RC	RS	AS	JN	GY	GQD	PT
ZH	0	-0.01943	-0.02661	-0.03044	-0.0275	-0.03923	-0.01113	-0.01899	-0.01842	-0.01084	-0.02828	-0.04019
DL	0.012700	0	0.01656	-0.03051	0.03137	-0.00857	-0.03495	-0.03743	-0.00839	-0.0177	0.02243	0.02297
QHD	0.010630	0.012110	0	-0.002	-0.03959	-0.03024	0.03134	0.02023	0.02438	0.0513	-0.0422	-0.05799
CD	0.011740	0.012720	0.011070	0	0.0073	-0.02187	-0.0251	-0.02984	-0.0059	0.0001	0.00103	0.00136
KTD	0.010580	0.012280	0.009480	0.011140	0	-0.03219	0.0461	0.0377	0.02594	0.04748	-0.05326	-0.08037
RC	0.011040	0.012380	0.010160	0.011400	0.010090	0	0.0044	-0.00507	0.00087	0.01704	-0.03642	-0.04467
RS	0.011890	0.012600	0.011360	0.011890	0.011510	0.011620	0	-0.03516	0.00483	0.00082	0.0346	0.03767
AS	0.011690	0.012460	0.011130	0.011730	0.011300	0.011400	0.011600	0	-0.01	-0.00871	0.02882	0.03239
JN	0.012420	0.013530	0.011930	0.012730	0.011930	0.012200	0.012790	0.012490	0	-0.04895	0.03495	0.01971
GY	0.013710	0.014640	0.013410	0.014000	0.013380	0.013580	0.013930	0.013690	0.013900	0	0.06312	0.03855
GQD	0.010140	0.011720	0.009040	0.010630	0.008890	0.009630	0.010920	0.010750	0.011600	0.013100	0	-0.07651
PT	0.010230	0.012020	0.009070	0.010870	0.008830	0.009740	0.011210	0.011030	0.011680	0.013160	0.008440	0

Table 4 Result of the analysis of molecular variance (AMOVA) testing the genetic structure

Source of variation	D.F.	Sum of squares	Percentage of variation/%	Φ-Statistics	*P*-value
Three groups (ZH, DL, AS, JN, GY), (QHD, CD, KTD, RC, RS), (GQD, PT)					
Among groups	2	13.109	1.57	Φ_{CT} = 0.01569	$p < 0.05$
Among populations within groups	9	21.099	-1.54	Φ_{SC} = -0.01562	0.824
Within populations	243	849.011	99.97	Φ_{ST} = 0.00031	0.584
Two groups A (ZH, DL, AS, JN, GY), (QHD, CD, KTD, RC, RS, GQD, PT)					
Among groups	1	9.928	1.74	Φ_{CT} = 0.01745	$p < 0.05$
Among populations within groups	10	24.28	-1.45	Φ_{SC} = -0.01478	0.802
Within populations	243	849.011	99.71	Φ_{ST} = 0.00293	0.584
Two groups B (ZH, DL, AS, JN, GY, QHD, CD, KTD, RC, RS), (GQD, PT)					
Among groups	1	10.932	3.15	Φ_{CT}=0.03155	$p < 0.05$
Among populations within groups	10	20.626	-2.63	Φ_{SC}=-0.02714	0.936
Within populations	252	1274.987	99.47	Φ_{ST}=0.00526	0.848

The Mantel-test revealed significant correlation between genetic and geographic distances for northern populations including farmed localities and naturalized adjacent farmed localities (r = 0.296, $p < 0.05$). But no significant correlation was observed for all populations (r = 0.091, $p > 0.05$) and farmed populations (r = 0.293, $p > 0.05$) (**Figure 2**).

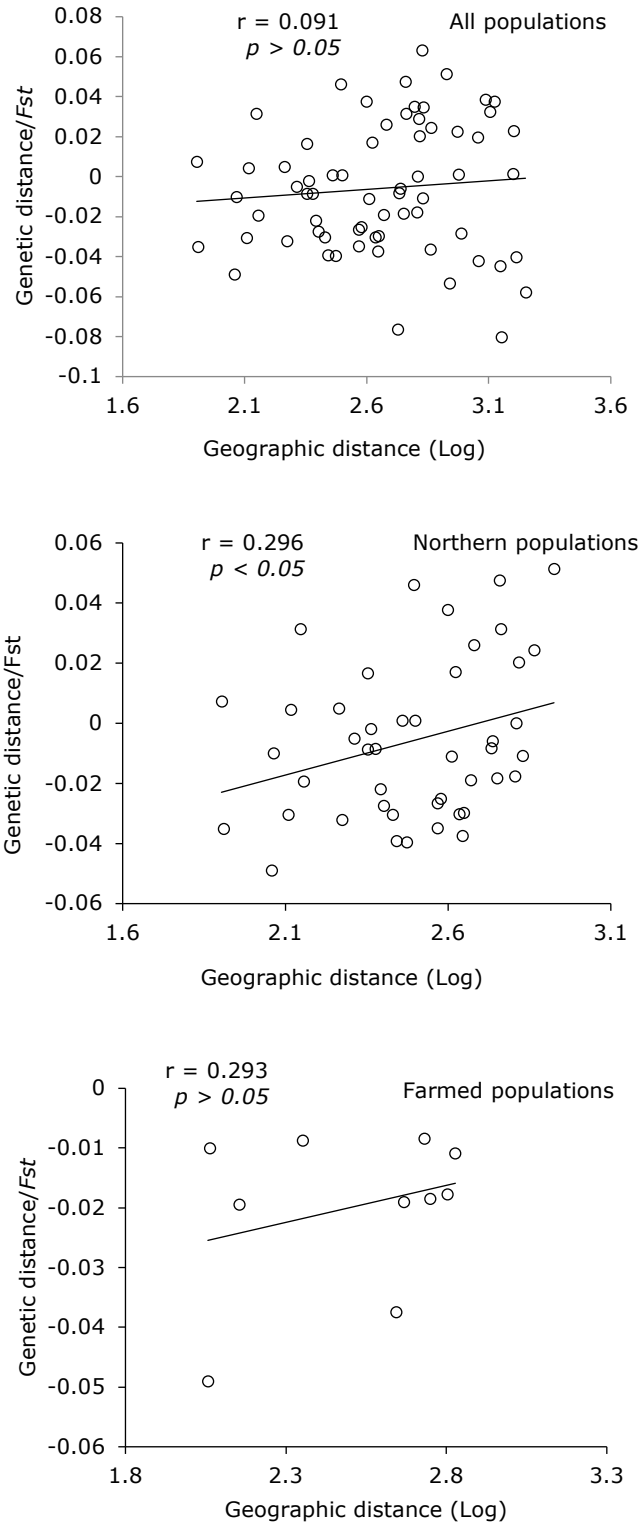

Figure 2 Isolation by distance plots for three grouped strategies of *M. galloprovincialis*. Relationship between genetic distance and geographic distance (log-transferred) for all populations, northern populations, and farmed populations.

The NJ tree constructed based on genetic distances indicated that the 12 localities were allocated into three groups: one group including four naturalized localities (QHD, KTD, PT, and GQD), one group including four farmed localities (JN, GY, AS, and DL) and two naturalized localities (CD, RS), one group containing a separate locality of ZH (**Figure 3**).

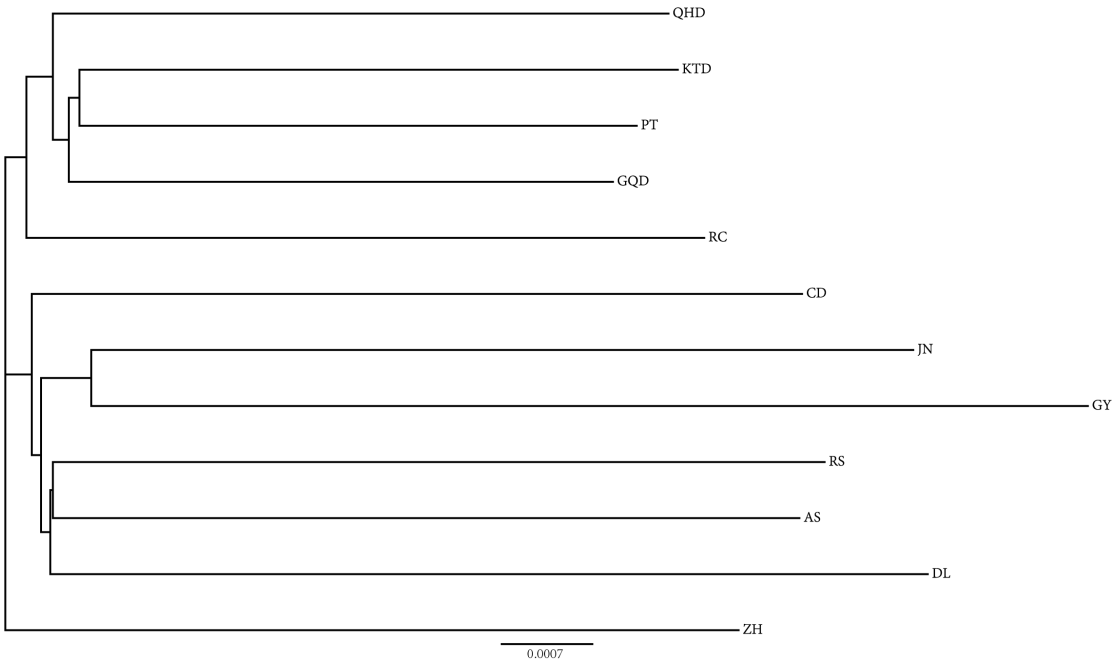

Figure 3 Neighbor-joining tree topology calculated by PHYLIP showing the genetic distance among 12 localities of *M. galloprovincialis*.

Discussion

Since before recorded history, humans have deliberately or accidentally carried biota with them when moving from one place to another (Beaumont, 2000). The failure of natural fisheries and the globalization of the aquaculture industry has resulted in increased pressure to move economically valuable aquaculture species to new regions (Beaumont, 2000; Rosenfield and Mann, 2019). Accidentally introduced into China from the Mediterranean Sea (Daguin and Borsa, 2000; Sanjuan et al., 1997), *M. galloprovincialis* has become an important aquaculture industry in the last 50 years (Zhang, 1984). As expected, the translocation culture model played an essential role in the genetic composition of this recently colonized species.

Different levels of genetic variability and possible explanations

The farmed populations exhibited the highest genetic variability in all three investigated populations measured by haplotype diversity, nucleotide diversity, and the mean number of pairwise differences. It is presumably due to the sizeable adequate population size and random mating within populations (Charlesworth, 2009). In the mussel cultivation industry, seed supply relies on the natural settlement of spats, or/and the spats transferred from other mussel farms, which keep the number of parents involved in reproduction at a high level (Philippe and Le, 2002; Rawson and Hilbish, 1998). Meanwhile, pooling juveniles from different cohorts with different genetic contexts promotes genetic heterogeneity in mussel farms (Lemer and Planes, 2012). Furthermore, commercial rope culturing may enhance gamete mixing and promote random mating within a population (Michalek et al., 2016). It is in line with the finding among Greek *M. galloprovincialis* populations where the genetic diversity of cultured populations was higher than that of wild populations, concluding that the translocation culture model played an important role in shaping patterns of genetic diversity (Giantsis et al., 2012). A similar result was also observed where human cultivation activities of transferring natural spats from the south to the north

had greatly influenced genetic variation of *Ruditapes philippinarum* in China, exhibiting that the human-mediated populations exhibited high genetic diversity and genetic concordance (Hu et al., 2016; Liu et al., 2007).

The isolated naturalized localities, equivalent to southern populations, expressed the lowest genetic diversity. The interaction of several factors may account for this scenario. Firstly, the individuals transferred to the southern China were limited and only partial haplotypes were introduced. Mussel cultivation in southern China lasted briefly in the 1970s, followed by no commercial transfer and culture. Secondly, there was no gene exchange with northern populations. The Yangtze River with massive freshwater outflow acted as a putative physical barrier limiting pelagic larvae migration (Chen et al., 2009; Dong et al., 2012; Wang et al., 2003). Thirdly, un-adaptation to the environment. Random genetic drift and natural selection operated on all populations and tended to reduce genetic variability with decreased or loss of genetic traits, and the change of variants frequencies was faster in small populations (Avise, 1994). The provenance of China *M. galloprovincialis* was the northern basin of the Mediterranean Sea scattering from about 35°N to 45°N (Han et al., 2017; Hilbish et al., 2000), while the latitude of two southern localities was 30.68°N and 26.68°N, respectively. The higher temperature conducted the thermal stress on the *M. galloprovincialis* in southern China. Therefore, the southern populations were in a state of continuous shrinkage, with offspring dramatically declining. Thus, limited individuals were left to propagate, leading to a rapid decreasing of genetic diversity caused by genetic drift (Charlesworth, 2009).

Sandwiched between two main culture areas, naturalized adjacent to farmed populations expressed middle-level genetic variability in all three compared populations. Heterogeneity could be transmitted from farmed populations to naturalized adjacent farmed populations due to interbreeding (Lemer and Planes, 2012). The prolonged planktonic larval stage and ocean currents offer the potential for geographic dispersal over distances, promoting the individuals' infiltration (Gilg and Hilbish, 2003; Liu et al., 2007; Michalek et al., 2016; Riginos et al., 2004). No mussel aquaculture in naturalized adjacent farmed localities but flooded with other shellfish farming like oyster or scallop. Mussels settled on the cages, or the shells of oysters/scallops could be transferred among localities by transporting these commercial species. Both factors increased the chance of individuals and genes exchange, but neither was comparable with artificial translocation within farmed populations.

The locality of Changdao (CD) showed exceptionally high haplotype diversity in all localities. Changdao is a hub site for the spat transfer between the Liaodong Peninsula and Haizhou Bay. The itinerary between Changdao and localities in the Liaodong Peninsula is by ship across the Bohai Strait. The itinerary between Changdao and Ganyu or Lianyungang in Haizhou Bay was by truck. Individuals may be left in the waters during the loading and unloading on the facility or the period of immerging into seawater when a connection is delayed. These individuals from populations over large geographical distances mate randomly, releasing genetic products and increasing the genetic variation in the new marine waters (Choudhuri, 2014; Giantsis et al., 2012,2014).

Genetic structure

The results of genetic distance, pairwise *Fst*, and Mantel-test for all populations illustrated no genetic differentiation of *M. galloprovinciallis* in China, consistent with previous studies (Han et al., 2017; Shen et al., 2011). No genetic differentiation was common in the recently introduced population (Palumbi, 1994). A homogenous genetic structure was also detected among the *M. galloprovinciallis* populations in South Africa (Zardi et al., 2018) and *Eriocheir sinensis* across the European populations (Hanfling et al., 2002), where introduced species arrived in in the late 1970s (Branch and Nina, 2004) and 1912 (Wang et al., 2008), respectively. The high dispersal and fecundity potential of marine invertebrates are expected to result in high gene flow among populations, leading to little to no signal of genetic differentiation (Gagnaire et al., 2015; Sutherland et al., 2020). The *M. galloprovincialis* was introduced into China over 150 years and rapidly

expanded along the China coast by manual assistance. In China, there is insufficient time for *M. galloprovincialis* to attain migration-drift equilibrium combined with the translocation culture model, increasing the gene exchange among populations (Hossjer et al., 2016).

However, slight genetic divergence was detected with significance among groups when 12 localities were clustered into three or two groups based on the heterogeneity analysis. The strategy of "Two groups B" reflected the divergence between northern and southern populations. The isolation from northern populations and un-adaptation to the environment, combined with the habit competition coming from local species of *M. coruscus* and *P. viridis* (Li et al., 2013; Ye et al., 2012), led the southern populations to be a closed and small size group, where genetic drift is intensified (Hedgecock and Sly, 1990). The lowest haplotype diversity (GQD:0.399, PT: 0.378) and the lowest number of haplotypes (GQD:4, PT: 3) suggested a rapid genetic drift of southern populations. This finding supported the conclusion of a previous study conducted by Pickett and David (2018), who indicated that South China appeared to be a genetically isolated population.

Gene flow within a population can increase the population's genetic variation, whereas gene flow between populations can reduce the genetic difference and homogenized genetic structure (Choudhuri, 2014). Both strategies (Three groups and Two groups A) expressed genetic differentiation between farmed and naturalized populations. It could attribute to the translocation culture model, which increased gene exchange among farmed populations, consequently contributing to the genetic homogeneity and variation (Beaumont, 2000; Benke et al., 2009). It has also been confirmed that anthropogenic activities affected genetic composition in the areas like the Chilean (Toro et al., 2006), northwestern European coasts (Kijewski et al., 2011), and the central-eastern Mediterranean Sea (Giantsis et al., 2012, 2014), where spat transplantation for mussel aquaculture were common. The evidence from the Mantel-test supported the effect of the translocation culture model on the genetic divergence, where a significant correlation between genetic and geographic distance in northern populations was observed, whereas there was no correlation in farmed populations.

The NJ tree topologies yielded a clear separation between the farmed and naturalized populations with some vignettes. On the one hand, the NJ tree expressed that the ZH, located in the northern Yellow Sea, separated with other clusters. It has been proven that the northern Yellow Sea was the first colonized waters of China *M. galloprovincialis* and the original tribe had more variation than other after-diffused populations under the founder event (Han et al., 2017). In contrast, the result that CD and RS were clustered into farmed groups reflected the anthropogenic impacts on genetic patterns of *M. galloprovincialis* in China. As mentioned above, the CD is the intersection of spat transfer among chief farmed localities, and there is little differentiation with farmed populations. In comparison, RS is the Pacific oyster farming and sales center in northern China, where thousands of juvenile or adult oysters were transported from other places for increasing fatness or sale. Thus, the mussel was transplanted along with the transportation of oysters as fouling organisms, reaching the genetic effect like CD or farmed populations.

This study assessed the genetic diversity and differentiation among *M. galloprovincialis* populations along the China coast. Comprehensively comparing the genetic diversity of three groups, the translocation practice seems to affect the genetic variability of the *M. galloprovincialis* positively. Slight but significant divergence was detected between farmed and naturalized populations, suggesting that spat transplantation was essential in shaping genetic composition. However, the genetic structure of a population is generally not static and is prone to change. The degrees of the changes depend on the intensity of interventions. Therefore, the genetic effect of translocation of China *M. galloprovincialis* requires long-term monitoring and in-depth evaluation. And clearly, more comparative investigations are still needed to test and extend the perspective, especially through integrating multiple genetic loci with extensive information like single nucleotide polymorphism.

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