Acta Oceanol. Sin., 2025, Vol. 44, No. 1, P. 104–112
https://doi.org/10.1007/s13131-024-2383-y

Deep gene exchange break among *Konosirus punctatus* populations across the northwestern Pacific inferred from AFLP and ISSR markers

Na Song[1], Yiping Ying[2], Yang Zhang[3], Tianxiang Gao[4*]

[1] The Key Laboratory of Mariculture (Ocean University of China), Ministry of Education, Qingdao 266003, China
[2] Key Laboratory of Sustainable Development of Polar Fishery of Ministry of Agriculture and Rural Affairs, Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Qingdao 266071, China
[3] Agricultural Product Quality Safety Inspection and Testing Center of Shenzhen, Shenzhen 518101, China
[4] Fishery College, Zhejiang Ocean University, Zhoushan 316022, China

Received 18 March 2024; accepted 26 August 2024

Abstract

The correct understanding of fish population structure plays a positive role in their fishery management. The dotted gizzard shad, *Konosirus punctatus*, is widely distributed in the coastal waters of the northwestern Pacific. With the over-exploitation of economically important fishes, its importance is increasingly prominent. To further examine the population genetic structure of *K. punctatus* across the northwestern Pacific, the amplified fragment length polymorphism (AFLP) and the inter-simple sequence repeats (ISSRs) were employed to perform genetic variation analysis. The results showed that the combination of polyacrylamide gel electrophoresis and silver staining can effectively detect genetic variation for *K. punctatus* populations. The average proportions of polymorphic loci were 46.26% and 87.13% for AFLP and ISSR markers, respectively, and the genetic diversity parameters showed no obvious differences among populations. Both analysis molecular variance (AMOVA) and pairwise F_{st} suggested that there was significant genetic differentiation between Chinese and Japanese populations. All samples also clustered into two clades based on the unweighted pair-group method analysis (UPGMA) tree by two markers, which indicated significant genetic differentiation among populations. Consistent with the previous studies, there are two highly differentiated groups at the nuclear gene level and they were suggested to be treated as two separate genetic management units. The results of the present study could provide the genetic management strategy for this important economic species.

Key words *Konosirus punctatus*, population genetic differentiation, AFLP, ISSR

Citation Song Na, Ying Yiping, Zhang Yang, Gao Tianxiang. 2025. Deep gene exchange break among *Konosirus punctatus* populations across the northwestern Pacific inferred from AFLP and ISSR markers. Acta Oceanologica Sinica, 44(1): 104–112, doi: 10.1007/s13131-024-2383-y

1 Introduction

The dotted gizzard shad, *Konosirus punctatus*, belongs to the family Clupeidae, Clupeiformes, which is widely distributed in the coastal waters of the northwestern Pacific (Whitehead, 1985; Zhang, 2001). It is a euryhalinous species and can tolerate a wide range of salinity, even surviving in freshwater (Kuroda et al., 2002). *Konosirus punctatus* has commercial value and many studies focused on its growth, feeding habit, and biology (Kuroda et al., 2002; Kawasaki et al., 2006; Choi et al., 2015; Ping et al., 2019). The larval pelagic duration of *K. punctatus* is about 24−28 d, and the adults of *K. punctatus* mainly feed on plankton and organic debris, which makes it to be an excellent mixed-culture species for aquaculture (Song et al., 2017; Shan et al., 2020a). With the over-exploitation of economically important fishes, the proportion of small pelagic fishes gradually increased (Bian et al., 2022). However, although *K. punctatus* has been reported to be the dominant species in some coastal

Foundation item: The National Key Research and Development Program of China under contract No. 2023YFD2401903.
*Corresponding author, E-mail: gaotianxiang0611@163.com

waters (Zhang et al., 2022), the total market landings showed an obvious decline trend. The global capture production of *K. punctatus* declined from 23 707 t in 1995 to 4 300 t in 2016 (Liu et al., 2020). Moreover, *K. punctatus* is constantly taken as by-catch, putting it at the crisis of overfishing. Since the 1960s, harbor breeding has started in the coastal areas of China (Ping et al., 2024). With the development of artificial breeding technology, the stock enhancement of *K. punctatus* was conducted to repair the marine ecosystem due to its low nutritional filter feeding habits (Shan et al., 2020b). Thus, *K. punctatus* is not only an important fishing object but also plays an important role in aquaculture and marine ecosystems.

The correct understanding of fish population structure could provide an important reference basis for the evolutionary mechanism, and also play a positive role in their fishery management (Ying et al., 2011). Although marine fish were considered to lack obvious genetic differentiation due to the absence of obvious geographical isolation in the marine environment (Grant and Bowen, 1998; Zhang et al., 2020a), significant population genetic structure has been detected in many marine fish with the development of molecular biotechnology (Liu et al., 2007; Song et al., 2019). Until now there were several population genetic studies of *K. punctatus* (Myoung and Kim, 2014; Gwak et al., 2015; Li et al., 2016; Song et al., 2017; Liu et al., 2020), and its complete mitochondrial genome, the whole transcriptome, and the chromosome-level genome were also reported (Li et al., 2016; Zhang et al., 2020b; Lou et al., 2021; Liu et al., 2022). We used the first hypervariable region of mitochondrial DNA marker to evaluate the population genetic divergence of *K. punctatus* across the northwestern Pacific, and significant genetic differentiation was detected between Chinese and Japanese clades (Song et al., 2017). Moreover, there existed low genetic differentiation among populations of *K. punctatus* along the Chinese coast, which was also supported by another study using COI and Cyt *b* genes as molecular makers (Liu et al., 2020). The strong dispersal ability of the larvae and adults may play vital roles in gene homogeneity and the ocean currents may greatly promote the gene exchange of *K. punctatus* populations (Song et al., 2017; Liu et al., 2020). However, until now no population genetic studies based on nuclear markers have been conducted.

The amplified fragment length polymorphism (AFLP), is a multi-locus fingerprinting technique, which has the advantages of high repeatability and polymorphic sites (Vos et al., 1995). It combines the advantages of RFLP and RAPD and overcomes their instability and constraints, and has been widely used in studies on the population genetics of marine organisms (Ferreira et al., 2015; Da Silva et al., 2016). The inter-simple sequence repeats (ISSRs), are also powerful molecular tools for population genetic studies, which have the advantages of being fast, stable, low cost and does not require a priori genome sequence information (Yang et al., 2011; Kamangar and Rostamzadeh, 2015). Although the simple sequence repeats (SSRs) are more commonly used in population genetic studies, they often do not transfer well from one species to another, and we often need to isolate and develop new microsatellite primers for each taxon (Castoe et al., 2010). And then compared with SSRs, ISSRs could be amplified without knowing the sequence information in advance and have better versatility.

In this study, the population genetic structure of *K. punctatus* was firstly evaluated by the combination of AFLP and ISSRs. We want to check the genetic variations from the perspective of nuclear gene level, and provide the genetic management strategy for this important economic species.

2 Materials and methods

2.1 *Sample collection*

Samples of *K. punctatus* were collected from nine localities along the Chinese and Japanese coasts between 2006 and 2007 (Table 1, Fig. 1). Six populations were collected from Chinese coastal waters and three populations were collected from Japan. All individuals were identified on the basis of morphology (Zhang, 2001), and a piece of muscle was preserved in 95% ethanol. All nine populations were used in the AFLP analysis and five of them (QD, ZS, DB, AM and NG) were chosen for ISSR analysis (Table 1).

Genomic DNA was isolated from muscle by proteinase K digestion followed by a standard phenol-chloroform method (Sambrock and Russel, 2001). The procedures of AFLP were essentially based on Vos et al. (1995) and Wang et al. (2000). Five selective primer combinations were tested and used in the present study, including E-ACC/M-CTA, E-AGA/M-CTG, E-AGG/M-CTT, E-ACG/M-CTC, and E-AGA/M-CTA. Procedures of ISSRs were essentially the same of Yang et al. (2011). ISSR primers used in this study were according to the primer set published by Yang et al. (2008) and University of British Columbia (UBC) (http://www.michaelsmith.ubc.ca/services/NAPS/Primer_Sets/Primers_Oct2006.pdf). A total of 12 ISSR primers were assessed, and four highly polymorphic primers (UBC834, ISSR4, UBC841, and ISSR62) were chosen for the following analysis.

All the PCR products of AFLP and ISSR were run on 6.0% denaturing polyacrylamide gel electrophoresis (PAGE) for 2.5 h at 50℃ on the Sequi-Gen GT Sequencing Cell (Bio-Rad, USA), and finally detected using the silver staining technique modified from Merril et al. (1979).

2.2 *Data analysis*

All clear and unambiguous AFLP and ISSR bands were recorded as binary data (1 = presence, 0 = absence), and transformed into a 0/1 binary character matrix in Mi-

Table 1. Sample information and genetic diversity information of *K. punctatus*

Population	ID	Time of collection	*n*	Number of loci	Number of polymorphic loci	Proportion of polymorphic loci/%	Nei's genetic diversity	Shannon's diversity index
AFLP								
Aomori	AM	2006-05	17	192	62	32.29	0.091 8	0.140 1
Tokyo Bay	TB	2007-06	27	196	67	34.18	0.080 1	0.126 8
Nagasaki	NG	2007-06	27	191	66	34.55	0.077 1	0.123 1
Qingdao	QD	2006-04	17	198	70	35.35	0.079 7	0.128 6
Huanghe River Estuary	YR	2007-04	14	195	62	31.79	0.069 5	0.112 8
Zhoushan	ZS	2006-05	14	196	65	33.16	0.091 8	0.141 9
Chengshantou	CS	2007-05	19	196	74	37.75	0.089 1	0.142 0
Kongdong Island	KI	2007-05	16	195	62	31.79	0.061 7	0.102 4
Daya Bay	DB	2006-04	15	182	50	27.47	0.062 6	0.099 7
Total			166	214	99	46.26		
ISSR								
Aomori	AM	2006-05	18	168	120	71.42	0.179 1	0.277 0
Nagasaki	NG	2007-06	17	167	126	75.44	0.191 1	0.292 3
Qingdao	QD	2006-04	18	168	128	76.19	0.182 3	0.284 0
Zhoushan	ZS	2006-05	17	163	118	72.39	0.182 7	0.280 8
Daya Bay	DB	2006-04	18	165	127	76.96	0.184 5	0.287 7
Total			88	202	176	87.13		

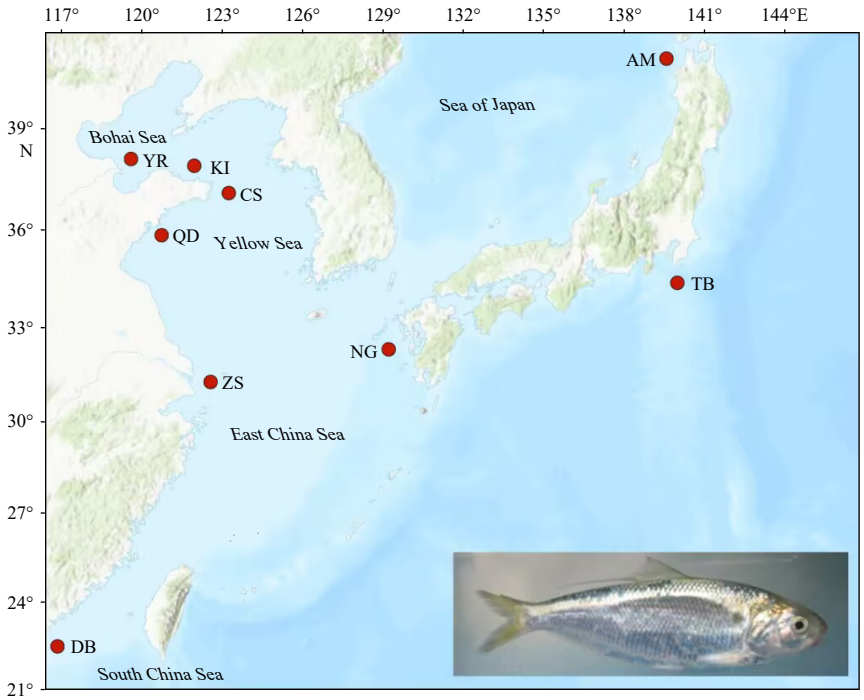

Fig. 1. Sample locations of *K. punctatus* in the present study.

crosoft Office Excel 2010. POPGENE 1.3.1 software was used to calculate the genetic diversity index such as the percentages of polymorphic loci, Nei's genetic diversity and Shannon diversity (Nei and Li, 1979). The UPGMA (unweighted pair-group method analysis) tree of individuals was constructed by software MEGA7.0 based on Nei's genetic distance (Kumar et al., 2016). ARLEQUIN 3.5 was used to calculate pairwise fixation index F_{st} between pairs of population samples and the significance of the F_{st} was tested by 10 000 permutations for each pairwise comparison (Excoffier and Lischer, 2010). When multiple comparisons were performed, P values were adjusted using the sequential Bonferroni procedure (Rice, 1989). To further examine hierarchical population structure as well as the geographical pattern of population subdivision, we used analysis of molecular variance (AMOVA) (Excoffier et al., 1992). Chinese group (population QD, ZS, YR, KI, CS and DB) and Japanese group (population AM, TB and NG) were defined for AMOVA and each group was also analyzed separately.

3 Results

3.1 *Population genetic diversity*

A total of 214 clear and unambiguous bands were amplified by 5 AFLP selective primers for 166 *K. punctatus* individuals (Table 1). The total number of polymorphic loci was 99 (46.26%) and varied for each primer combination (12−31) (Table 2). The number of polymorphic loci per population ranged from 50 (DB) to 74 (CS), and the corresponding percentage was 27.47% to 37.75%. Population ZS and AM showed the highest Nei's genetic diversity and population CS showed the highest Shannon's diversity index, while population KI showed the lowest Nei's genetic diversity and population DB showed the lowest Shannon's diversity index (Table 1).

A total of 202 clear and unambiguous bands were amplified by 4 ISSR primers for 88 *K. punctatus* individuals (Table 1). The total number of polymorphic loci was 176 (87.13%) and varied for each primer combination (33−54) (Table 2). The number of polymorphic loci per population ranged from 118 (ZS) to 128 (QD). Population AM showed the lowest Nei's genetic diversity and Shannon's diversity index, and population CS showed the highest Shannon's information index, while population NG showed the highest Nei's genetic diversity and Shannon's diversity index (Table 1).

3.2 *Population genetic structure*

Two distinct clades were identified for *K. punctatus* based on Nei's genetic distance among 166 individuals by AFLP markers (Fig. 2). Individuals from Chinese populations clustered together and the Japanese clade contained all the individuals from three Japanese populations. Samples also clustered into two clades based on UPGMA tree by ISSR markers, which indicated significant genetic differentiation among Chinese and Japanese populations (Fig. 2).

The analysis of pairwise F_{st} values among populations showed that there was significant genetic differentiation between Chinese and Japanese populations (Tables 3 and 4). To detect the variance components, the AMOVA analysis was conducted by two gene pools (Chinese group and Japanese group). The results showed that differentiation between the two groups was very strong and statistically significant (F_{st} = 0.364 3, P < 0.01) (Table 5). In the Japanese group, 98.37% of the genetic variation existed within populations. The genetic differentiation between population TB and NG was weak, while population AM showed significant genetic difference with these two populations. In the Chinese group, 98.71% of the genetic variation existed within populations. Most pairwise F_{st} values were low and not significant after sequential Bonferroni correction except the comparisons between population DB with other populations (Table 5). The AMOVA analysis by ISSR markers also revealed significant genetic differentiation between the two groups, which suggested limited gene flow among these populations. By contrast, the genetic differentiation between population AM and NG was low and not significant.

4 Discussion

4.1 *Population genetic diversity*

In the present study, each primer produced an average of 42.8 and 50.5 bands for AFLP and ISSR markers, respectively, which showed their high amplified efficiency. The combination of polyacrylamide gel electrophoresis and silver staining could greatly improve the detection rate of bands and the proportion of polymorphic sites, which was much higher than those by agarose electrophoresis (Yang et al., 2008; Liu et al., 2009). The average proportion of polymorphic loci was 46.26% and 87.13% for two markers, and high polymorphism can effectively detect genetic differentiation among populations. ISSR or AFLP markers were thought to be more suitable for estimating the genetic diversity of germplasm resources than SSR markers because of their higher marker indices (Sabir et al., 2014). Moreover, the proportion of polymorphic loci was higher in ISSR markers than in AFLP in this study. Meng and Chen (2001) found that ISSR markers were more effective and economical than AFLP markers in detecting genetic variation in *Phialophora gregata*, which was consistent with the results of this study.

The genetic diversity parameters for all populations, including the proportion of polymorphic loci, Nei's and

Table 2. Polymorphism information of primers based on two markers

	AFLP primers					
	E-ACC/ M-CTA	E-AGA/ M-CTG	E-AGG/ M-CTT	E-ACG/ M-CTC	E-AGA/ M-CTA	Total
Number of loci	42	71	39	23	39	214
Polymorphic loci	19	31	17	12	20	99
Proportion of polymorphic loci/%	45.23	43.66	43.59	52.17	51.28	46.26
	ISSR primers					
	UBC834	ISSR4	UBC841	ISSR62		Total
Number of loci	41	58	62	41	−	202
Number of polymorphic loci	39	54	50	33	−	176
Proportion of polymorphic loci/%	95.12	93.10	80.65	80.49	−	87.13

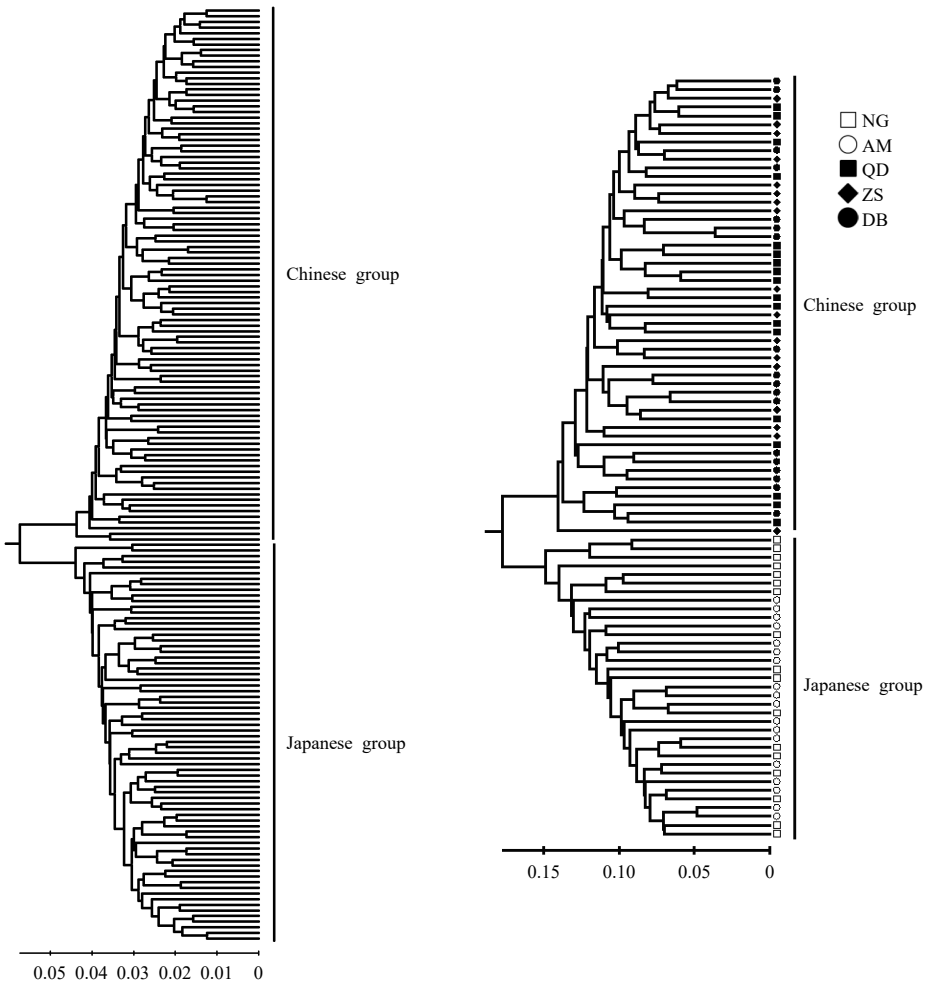

Fig. 2. UPGMA tree based on genetic distance among individuals of *K. punctatus* by AFLP (left) and ISSR (right) markers.

Table 3. Pairwise F_{st} values (below) and genetic distance (above) among *K. punctatus* populations by AFLP marker

	AM	TB	NG	QD	ZS	YR	KI	CS	DB
AM		0.006 8	0.006 2	0.062 6	0.060 9	0.058 9	0.060 7	0.063 4	0.067 8
TB	0.034 49*		0.001 4	0.067 8	0.069 4	0.068 8	0.068 9	0.070 9	0.074 7
NG	0.032 46*	−0.006 05		0.067 1	0.068 9	0.066 3	0.064 9	0.067 2	0.071 1
QD	0.337 90**	0.352 47**	0.361 87**		0.002 9	0.003 6	0.002 8	0.002 6	0.005 5
ZS	0.338 42**	0.362 06**	0.373 36**	−0.008 73		0.003 2	0.004 8	0.005 5	0.008 0
YR	0.333 09**	0.362 11**	0.366 64**	−0.001 66	−0.007 83		0.004 1	0.003 0	0.008 3
KI	0.351 59**	0.371 70**	0.370 26**	−0.004 98	0.012 15	0.006 18		0.004 4	0.007 0
CS	0.351 59**	0.375 12**	0.374 37**	−0.005 03	0.019 20*	−0.003 76	0.013 26		0.004 8
DB	0.351 59**	0.413 14**	0.415 39**	0.025 72*	0.052 09*	0.056 74*	0.047 01*	0.022 2	

Note: * Significant values after Bonferroni correct at 5% ($P < 0.05$); ** significant values after Bonferroni correct at 1% ($P < 0.01$).

Table 4. Pairwise F_{st} values (below) and genetic distance (above) between *K. punctatus* populations by ISSR marker

	AM	NG	QD	ZS	DB
AM		0.005 6	0.105 6	0.109 6	0.127 9
NG	−0.006 80		0.109 0	0.116 0	0.129 6
QD	0.284 66*	0.273 59*		0.004 7	0.014 9
ZS	0.291 24*	0.284 81*	−0.010 00		0.015 7
DB	0.323 67*	0.307 88*	0.032 21*	0.034 13*	

Note: * Significant values after Bonferroni correct at 5% ($P < 0.05$); ** significant values after Bonferroni correct at 1% ($P < 0.01$).

Table 5. AMOVA of *K. punctatus* by AFLP and ISSR markers

Source of variation	AFLP				ISSR			
	Df	Sum of squares	Variance components	Percentage of variation/%	Df	Sum of squares	Variance components	Percentage of variation/%
All populations								
Among groups	1	492.437	5.898 97	36.42	1	368.878	8.188 28	36.42
Among populations within groups	7	88.605	0.139 72	0.86	3	71.015	0.212 04	0.86
Within populations	157	1 594.434	10.155 63	62.71	83	1 654.971	19.939 40	62.71
Total	165	2 175.476	16.194 02	−	87	2 094.864	28.339 73	−
Japanese group								
Among populations	2	29.287	0.175 5	1.63	1	18.024	−0.138 11	−0.68
Within populations	68	718.911	10.572 22	98.37	33	674.490	20.439 10	100.68
Total	70	748.197	10.747 71	−	34	692.514	20.300 98	−
Chinese group								
Among populations	5	59.319	0.128 31	1.29	2	52.991	0.389 91	1.95
Within populations	89	875.523	9.837 34	98.71	50	980.480	19.609 61	−
Total	94	934.842	9.965 65	−	52	1 033.472	19.999 52	−

Note: Df represents degree of freedom. − represents no data.

Shannon's diversity indices, showed no obvious population differences. According to the previous results based on the mitochondrial DNA marker, Japanese populations exhibited higher nucleotide diversity than did Chinese populations (Song et al., 2017). Two subclades were detected in the Japanese clade, which may lead to high nucleotide diversity. The fluctuation of Pleistocene glacial climate led to the isolation and the secondary connection after glacial time may increase the nucleotide diversity (Fields et al., 2016). Therefore, higher nucleotide diversity for Japanese populations was detected by mitochondrial DNA marker.

4.2 *Population genetic structure*

The UPGMA tree based on Nei's genetic distance showed that two distinct clades were detected for *K. punctatus* by two markers, which suggested strictly limited gene exchange between the Chinese and Japanese populations. The AMOVA and pairwise F_{st} analysis also confirmed this deduction. This phylogenetic pattern was highly consistent with previous studies by mitochondrial DNA (Song et al., 2017). The population genetic structure of marine organisms in the northwestern Pacific had been proven to be strongly affected by the temperature changes of the Pleistocene ice age (Liu et al., 2007; Han et al., 2012). The drastic changes in sea level led to population contraction and expansion or range shifts, which can leave marks on the population's genetic structure (Cheang et al., 2012; Yan et al., 2015). The survivals of *K. punctatus* in the different glacial refugia may re-colonize to the new habitat and produce the secondary connect event. The survival isolation in different historic refugia may be one of the factors responsible for two clades (Song et al., 2017). Similar conclusions have been reported in other studies. For example, the allopatric speciation for *Lateolabrax maculatus* and *L. japonicus* was speculated to be the long-time isolation during the Pleistocene ice age (Liu et al., 2006). The significant genetic differentiation for *Pennahia argentata* (Han et al., 2012), *Penaeus japonicus* (Tzeng et al., 2004), and *Scomber japonicus* (Yan et al., 2015) between Chinese and Japanese populations was also detected, and the land bridge formed due to the decline of sea level may block the gene exchange among them.

4.3 *Gene communication among populations*

Moreover, the results of the present study suggested that the gene communication between the Chinese and Japanese populations was still blocked after the glacial period, indicating that some current isolation mechanisms were maintaining the population genetic differences. Marine currents were known to play an important role in constructing the phylogeographic patterns of marine fishes (Huyghe and Kochzius, 2018). At the mercy of the currents, the marine organisms may be transported for long distance, which can effectively increase the gene exchange among populations (Song et al., 2013). However, external factors may not be always effective for some special cases, such as some species with benthic habits or rockfish (Huyghe and Kochzius, 2018). The high population genetic differentiation between the two groups indicated their limited gene communication. Most marine fishes prefer living in the nearshore waters due to the high primary productivity (Volk et al., 2021). Therefore, despite the long larval dispersal duration and strong swimming ability of adults, the offshore dispersal may not be adverse for their survival, and the Kuroshio Current may have limited functions on the gene flow between the Chinese and Japanese populations.

It's worth nothing that population DB exhibited genetic heterogeneity with other Chinese populations by two markers. According to the results of mitochondrial DNA, this population was inferred to undergo the independent evolution event (Song et al., 2017). The Daya Bay is a semi-closed subtropical embayment in the South China Sea

and the population dispersal may be limited by its special geographical location. The isolation events of different refugia may also be the possible reason for its genetic heterogeneity. Low genetic differentiation among other Chinese populations may be related to the existence of the common wintering ground in the Yellow Sea, and the coastal currents may also promote individual activities. In any case, population DB needs extra attention.

In this study, the phylogeographic pattern of *K. punctatus* was examined by AFLP and ISSR markers, and the results confirmed their effectiveness for population genetic study. These two markers can provide the reliable results and detect genetic differentiation at a low cost. Consistent with previous studies, there are two highly differentiated groups at the nuclear gene level and they should be treated as two separate genetic management units. The climate changes in Pleistocene periods and habits of nearshore life may be the main reasons for the formation and maintenance mechanism, and the coastal currents could benefit the population gene exchange.

Acknowledgements

We thank Takashi Yanagimoto for collecting samples.

References

Bian Xiaodong, Wan Ruijing, Shan Xiujuan, et al. 2022. Preliminary analysis on recruitment variation and the exogenous driving factors to early life stages of small pelagic fishes in the Laizhou Bay. Journal of Fishery Sciences of China (in Chinese), 29(3): 446–468

Castoe T A, Poole A W, Gu W J, et al. 2010. Rapid identification of thousands of copperhead snake (*Agkistrodon contortrix*) microsatellite loci from modest amounts of 454 shotgun genome sequence. Molecular Ecology Resources, 10(2): 341–347, doi: 10.1111/j.1755-0998.2009.02750.x

Cheang C C, Tsang L M, Ng W C, et al. 2012. Phylogeography of the cold-water barnacle *Chthamalus challengeri* in the north-western Pacific: effect of past population expansion and contemporary gene flow. Journal of Biogeography, 39(10): 1819–1835, doi: 10.1111/j.1365-2699.2012.02742.x

Choi H C, Han I S, Suh Y S, et al. 2015. Feeding habits of larval *Konosirus punctatus* from the Nakdong River Estuary, Korea. Korean Journal of Fisheries and Aquatic Sciences, 48(5): 752–759, doi: 10.5657/KFAS.2015.0752

Da Silva L N, Essi L, Welker C A D, et al. 2016. Assessing the genetic diversity and population structure of the endangered *Chascolytrum bulbosum* (Poaceae, Poeae) using AFLP markers. Biochemical Systematics and Ecology, 68: 236–242, doi: 10.1016/j.bse.2016.07.027

Excoffier L, Lischer H E L. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources, 10(3): 564–567, doi: 10.1111/j.1755-0998.2010.02847.x

Excoffier L, Smouse P E, Quattro J M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics, 131(2): 479–491, doi: 10.1093/genetics/131.2.479

Ferreira D G, Galindo B A, Frantine-Silva W, et al. 2015. Genetic structure of a Neotropical sedentary fish revealed by AFLP, microsatellite and mtDNA markers: a case study. Conservation Genetics, 16(1): 151–166, doi: 10.1007/s10592-014-0648-2

Fields A T, Feldheim K A, Gelsleichter J, et al. 2016. Population structure and cryptic speciation in bonnethead sharks *Sphyrna tiburo* in the south-eastern U. S. A. and Caribbean. Journal of Fish Biology, 89(5): 2219–2233, doi: 10.1111/jfb.13025

Gwak W S, Lee Y D, Nakayama K. 2015. Population structure and sequence divergence in the mitochondrial DNA control region of gizzard shad *Konosirus punctatus* in Korea and Japan. Ichthyological Research, 62(3): 379–385, doi: 10.1007/s10228-014-0450-7

Grant W S, Bowen B W. 1998. Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. The Journal of Heredity, 89: 415–426

Han Zhiqiang, Yanagimoto T, Zhang Yaping, et al. 2012. Phylogeography study of *Ammodytes personatus* in northwestern Pacific: Pleistocene isolation, temperature and current conducted secondary contact. PLoS One, 7(5): e37425, doi: 10.1371/journal.pone.0037425

Huyghe F, Kochzius M. 2018. Sea surface currents and geographic isolation shape the genetic population structure of a coral reef fish in the Indian Ocean. PLoS One, 13(3): e0193825, doi: 10.1371/journal.pone.0193825

Kamangar B B, Rostamzadeh J. 2015. Genetic diversity and population genetic structure of Wels (*Silurus glanis* Linnaeus, 1758) in the northwest of Iran. Environmental Biology of Fishes, 98(8): 1927–1934, doi: 10.1007/s10641-015-0411-7

Kawasaki M, Watanabe Y, Shirafuji N, et al. 2006. Larval *Konosirus punctatus* (Clupeidae) in a brackish river mouth on the Pacific coast of central Japan. Journal of Fish Biology, 68(5): 1362–1375, doi: 10.1111/j.0022-1112.2006.01018.x

Kumar S, Stecher G, Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution, 33(7): 1870–1874, doi: 10.1093/molbev/msw054

Kuroda K, Kong L, Kawasaki M, et al. 2002. Long-term fluctuations in the catch data of Konoshiro gizzard shad, *Konosirus punctatus*, around Japan. Bulletin of the Japanese Society of Fisheries Oceanography, 66(4): 239–246

Li Yuan, Zhang Liyan, Wang Liangming. 2016. Population genetics of *Konosirus punctatus* in Taiwan Strait. Journal of Applied Oceanography (in Chinese), 35(4): 522–528

Liu Jinxian, Gao Tianxiang, Wu Shifang, et al. 2007. Pleistocene isolation in the northwestern Pacific marginal seas and limited dispersal in a marine fish, *Chelon haematocheilus* (Temminck & Schlegel, 1845). Molecular Ecology, 16(2): 275–288, doi: 10.1111/j.1365-294X.2006.03140.x

Liu Jinxian, Gao Tianxiang, Yokogawa K, et al. 2006. Differential population structuring and demographic history of two closely related fish species, Japanese sea bass (*Lateolabrax japonicus*) and spotted sea bass (*Lateolabrax maculatus*) in northwestern Pacific. Molecular Phylogenetics and Evolution, 39(3): 799–811, doi: 10.1016/j.ympev.2006.01.009

Liu Jianyong, Lun Zhaorong, Zhang Junbing, et al. 2009. Population genetic structure of striped mullet, *Mugil cephalus*, along the coast of China, inferred by AFLP fingerprinting. Biochemical Systematics and Ecology, 37(4): 266–274, doi: 10.1016/j.bse.2009.04.010

Liu Bingjian, Zhang Kun, Zhang Shufei, et al. 2022. Chromosome-level genome assembly of the dotted gizzard shad (*Konosirus punctatus*) provides insights into its adaptive evolution. Zoological Research, 43(2): 217–220, doi: 10.24272/j.issn.2095-8137.2021.351

Liu Bingjian, Zhang Kun, Zhu Kehua, et al. 2020. Population genetics of *Konosirus punctatus* in Chinese coastal watersinferred from two mtDNA genes (COI and Cytb). Frontiers in Marine Science, 7: 534, doi: 10.3389/fmars.2020.00534

Lou Fangrui, Qiu Shengyao, Tang Yongzheng, et al. 2021. Comprehensive phylogeny of *Konosirus punctatus* (Clupeiformes: Clupeidae) based on transcriptomic data. Bioscience Reports, 41(5): BSR20210455, doi: 10.1042/BSR20210455

Meng Xiangqi, Chen Weidong. 2001. Applications of AFLP and ISSR techniques in detecting genetic diversity in the soybean brown stem rot pathogen *Phialophora gregata*. Mycological Research, 105(8): 936–940, doi: 10.1016/S0953-7562(08)61949-8

Merril C R, Switzer R C, Van Keuren M L. 1979. Trace polypeptides in cellular extracts and human body fluids detected by two-dimensional electrophoresis and a highly sensitive silver stain. Proceedings of the National Academy of Sciences of the United States of America, 76(9): 4335–4339

Myoung S H, Kim J K. 2014. Genetic diversity and population structure of the gizzard shad, *Konosirus punctatus* (Clupeidae, Pisces), in Korean waters based on mitochondrial DNA control region sequences. Genes & Genomics, 36(5): 591–598

Nei M, Li W H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences of the United States of America, 76(10): 5269–5273

Ping Honglin, Yu Fangping, Zhang Tao, et al. 2019. Morphological development and allometric growth pattern of *Konosirus punctatus* at larvae and juvenile stages. Acta Hydrobiologica Sinica (in Chinese), 43(5): 1021–1033

Ping Honglin, Zhang Tao, Shi Huilai, et al. 2024. Principal component and discriminant analyses of morphological traits of cultured *Konosirus punctatus* at different stage. Journal of Fisheries of China (in Chinese), 48(3): 133–142

Rice W R. 1989. Analyzing tables of statistical tests. Evolution, 43(1): 223–225, doi: 10.2307/2409177

Sabir J S M, Abo-Aba S, Bafeel S, et al. 2014. Characterization of ten date palm (*Phoenix dactylifera* L.) cultivars from Saudi Arabia using AFLP and ISSR markers. Comptes Rendus Biologies, 337(1): 6–18

Sambrock J, Russel D W. 2001. Molecular Cloning: A Laboratory Manual (3rd edition). New York: Cold Spring Harbor Laboratory Press

Shan Lezhou, Ma Jianzhong, Shao Xinbin, et al. 2020a. Study on the technique of artificial propagation and larva nursery of *Clupanodon punctatus* in Yue Qing Bay. Fisheries Science & Technology Information (in Chinese), 47(3): 130–134

Shan Lezhou, Zhang Lining, Shao Xinbin, et al. 2020b. Artificial breeding and venting operation technology of *Clupanodon punctatus*. Journal of Aquaculture (in Chinese), 41(11): 56–57

Song Na, Gao Tianxiang, Ying Yiping, et al. 2017. Is the Kuroshio current a strong barrier for the dispersal of the gizzard shad (*Konosirus punctatus*) in the East China Sea?. Marine and Freshwater Research, 68(5): 810–820

Song Na, Jia Ning, Yanagimoto T, et al. 2013. Genetic differentiation of *Trachurus japonicus* from the northwestern Pacific based on the mitochondrial DNA control region. Mitochondrial DNA, 24(6): 705–712, doi: 10.3109/19401736.2013.773982

Song Na, Yin Lina, Sun Dianrong, et al. 2019. Fine-scale population structure of *Collichtys lucidus* populations inferred from microsatellite markers. Journal of Applied Ichthyology, 35(3): 709–718, doi: 10.1111/jai.13902

Tzeng T D, Yeh S Y, Hui C F. 2004. Population structure of the kuruma prawn (*Penaeus japonicus*) in East Asia inferred from mitochondrial DNA sequences. ICES Journal of Marine Science, 61(6): 913–920, doi: 10.1016/j.icesjms.2004.06.015

Volk D R, Konvalina J D, Floeter S R, et al. 2021. Going against the flow: barriers to gene flow impact patterns of connectivity in cryptic coral reef gobies throughout the western Atlantic. Journal of Biogeography, 48(2): 427–439, doi: 10.1111/jbi.14010

Vos P, Hogers R, Bleeker M, et al. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Research, 23(21): 4407–4414, doi: 10.1093/nar/23.21.4407

Wang Zhiyong, Jayasankar P, Khoo S K. 2000. AFLP fingerprinting reveals genetic variability in common carp stocks from Indonesia. Asian Fisheries Science, 13(2): 139–147

Whitehead P J P. 1985. FAO Species Catalogue. Vol 7. Clupeoid Fishes of the World (Suborder Clupeoidei): Part 1. Chirocentridae, Clupeidae and Pristigasteridae. Rome: FAO, 305–379

Yan Shuai, Catanese G, Brown C L, et al. 2015. Phylogeographic study on the chub mackerel (*Scomber japonicus*) in the northwestern Pacific indicates the late Pleistocene population isolation. Marine Ecology, 36(3): 753–765, doi: 10.1111/maec.12267

Yang Qiaoli, Gao Tianxiang, Miao Zhenqing. 2011. Differentiation between populations of Japanese grenadier anchovy (*Coilia nasus*) in northwestern Pacific based on ISSR markers: implications for biogeography. Biochemical Systematics and Ecology, 39(4–6): 286–296, doi: 10.1016/j.bse.2011.07.019

Yang Taiyou, Guan Jianyi, Chen Hongxi, et al. 2008. Genetic diversity in PAPD and ISSR on *Squaliobarbus curriculus* in Danjiangkou reservoir. Oceanologia et Limnologia Sinica (in Chinese), 39(2): 157–161

Ying Yiping, Chen Yong, Lin Longshan, et al. 2011. Risks of ignoring fish population spatial structure in fisheries management. Canadian Journal of Fisheries and Aquatic Sciences, 68(12): 2101–2120, doi: 10.1139/f2011-116

Zhang Shiyi. 2001. Fauna Sinica (in Chinese). Beijing: Science Press

Zhang Baidong, Li Yulong, Xue Dongxiu, et al. 2020a. Population genomic evidence for high genetic connectivity among populations of small yellow croaker (*Larimichthys polyactis*) in inshore waters of China. Fisheries Research, 225: 105505, doi: 10.1016/j.fishres.2020.105505

Zhang Kun, Liu Yifan, Yin Xiaolong, et al. 2020b. Characterization of the complete mitochondrial genome of Chinese *Konosirus punctatus* (Clupeiformes, Clupeidae) and phylogenetic studies of Clupeiformes. Mitochondrial DNA Part B, 5(3): 3371–3373, doi: 10.1080/23802359.2020.1823272

Zhang Yunlei, Sun Xiao, Liu Xiaohui, et al. 2022. Impacts of climate changes on the habitat suitability of spawning ground for *Konosirus punctatus* in the central and southern Yellow Sea. Journal of Fisheries of China (in Chinese), 46(2): 215–223