Scientific Research and Essays Vol. 5(13), pp. 1649-1654, 4 July, 2010
Available online at http://www.academicjournals.org/SRE
ISSN 1992-2248 ©2010 Academic Journals

Full Length Research Paper

Genetic population structure of *Ovalipes punctatus* revealed by AFLP markers

Cun Gen Yu[1], Zhi Qiang Han[1, 3*], Ji Zheng[1], Li Jian Xue[2] and Tian Xiang Gao[1,3]

[1]Fishery College, Zhejiang Ocean University, Zhoushan, 316004 P. R. China.
[2]Marine Fisheries Research Institute of Zhejiang Province, Zhoushan, 316100 P. R. China.
[3]The Key Laboratory of Mariculture, Ministry of Education, Ocean University of China, Qingdao 266003 P. R. China.

Accepted 3 June, 2010

The population genetic structure of sand crab, *Ovalipes punctatus* in East China and Yellow Sea was analyzed using amplified fragment length polymorphism (AFLP). A total of 196 loci were detected by four primer combinations among 95 individuals collected from five locations, 117 of which were polymorphic (59.69%). The proportion of polymorphic loci and Nei's genetic distances for five populations ranged from 23.15 - 53.49%, and from 0.0029 - 0.0138, respectively. AMOVA analysis and pairwise F_{ST} revealed significant genetic differentiation among five samples, supporting that there are separate populations of this species. The pattern of isolation by distance indicated that the significant genetic differentiation observed between localities of *O. punctatus* was mainly due to the geographic distance.

Key words: *Ovalipes punctatus*, AFLP, genetic structure.

INTRODUCTION

The sand crab, *Ovalipes punctatus* (De Haan, 1833) (Crustacea: Brachyura: Portunidae) belongs to a genus that is distributed worldwide along sandy coastlines of subtropical and temperate waters. The sand crab is common in the coastal waters of China and Japan, supporting an important commercial fishery in the East China Sea (Yu et al., 2004). It is found on sandy bottoms below the intertidal zone, but peak abundance is at 5 - 60 m. *O. punctatus* is extremely well adapted to life on sand, with its strong swimming and burrowing abilities (Takahashi and Kawaguchi, 2001). It is voracious carnivore and significant predators of mollusks on sandy beaches (Zhang et al., 1991).

Female sand crabs spawn from mid-September until mid-November in offshore water 40 - 60 m deep (Sasaki and Kawasaki, 1980). The incubation period of the eggs is about 20 days. After a 3 - 4 months planktonic phase, the larvae settle on sandy bottoms in March and April. On the basis of long planktonic larval stages and high potential mobility during the crab phase, a high gene flow level is expected in this species.

Previous studies mainly focused on the biological characteristics of this species (Yu et al., 2004, 2005; Takahashi and Kawaguchi, 2001; Zhang et al., 1991; Sasaki and Kawasaki, 1980), and there have been no publications concerning genetic diversity and population subdivisions of *O. punctatus*. The recognition of genetically differentiated populations within a species is of importance for fishery management (Klinbunga et al., 2007), and can provide essential information on resource recovery and to aid in delineating and monitoring stocks for fishery management. Failure to detect population units can lead to local over-fishing and ultimately to severe stock declines (Zhang et al., 2006).

Amplified fragment length polymorphism (AFLP) analysis (Vos et al., 1995) is a PCR-based (Polymerase chain reaction) multilocus fingerprinting technique that combines the strengths and overcomes the weaknesses of PCR-RFLP (Restriction fragment length polymorphism) and RAPD (Random Amplification of Polymorphic DNA). AFLP analysis has been used for indirect examination of levels of genetic diversity in several crab species (Klinbunga et al., 2007; Huang et al., 2008; Liu et al., 2008). The major strengths of the AFLP method include

*Corresponding author. E-mail: d6339124@163.com.

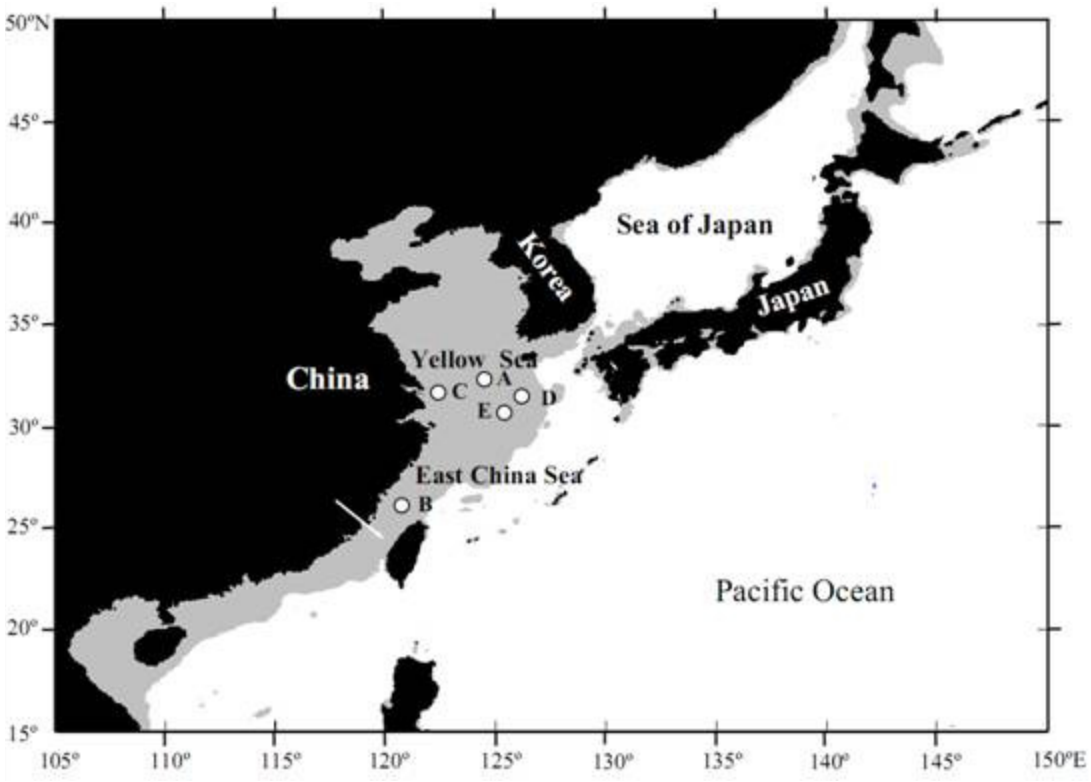

Figure 1. Sample sites for *O. punctatus*

simultaneous screening of a large number of polymorphic loci, high reproducibility, and relative cost effectiveness (Liu and Cordes, 2004). Moreover, it does not require any prior molecular information about sequences under investigation and is thus especially applicable to species for which the genome sequences are not well characterized, such as *O. punctatus*.

Because *O. punctatus* is an important commercial crab species, confirmation of whether or not it is a single population in the East China Sea and Yellow Sea or whether different genetic populations occur over its distribution would represent a useful contribution to conservation policies and resource management. The objective of this study was to determine the degree of genetic diversity and intraspecific population differentiation of *O. punctatus* in the East China Sea and Yellow Sea using AFLP analysis.

MATERIALS AND METHODS

Sample collection

Ninety five individuals of *O. punctatus* were collected by local fishermen from five geographic locations (A to E) from the East China Sea and Yellow Sea during January 2009 to March 2009 (Figure 1 and Table 1). The whole crab was frozen and shipped to Zhejiang Ocean University. Muscle samples were obtained and preserved in 95% ethanol or frozen for subsequent DNA extraction.

AFLP analysis

Genomic DNA was isolated from muscle tissue by proteinase K digestion followed by a standard phenol–chloroform method. DNA was subsequently resuspended in 100 µl of TE buffer (10 mmol/L Tris-Cl, 1 mmol/L EDTA, PH = 8.0). Procedures of AFLP were essentially based on Vos et al. (1995) and Wang et al. (2000). Selective PCR products 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). Sequences of AFLP adapters and primers are listed in Table 2. Four primer combinations (E-ACA/M-CAC, E-AAC/M-CAC, E-AAC/M-CAG and E-AGA/M-CAG) were chosen for AFLP analysis (Table 2).

Data analysis

AFLP bands were scored for presence (1) or absence (0), and transformed into 0/1 binary character matrix. Proportion of polymerphic loci, Nei's genetic diversity and Nei's standard genetic distance (Nei, 1972) were calculated by POPGEN. Genetic relationships among populations were estimated by constructing UPGMA tree based on Nei's standard genetic distance in Mega 3.0. Population structure of *O. punctatus* was investigated using the molecular variance software package (AMOVA) and F-statistics in ARLEQUIN 2.000. To test for isolation by distance (Slatkin, 1993), pairwise values of genetic distance were plotted against geographical distance (one-dimensional stepping-stone model) between sample sites of *O. punctatus*. The strength and significance of the relationship between genetic distances and geographic distances was assessed using reduced major axis regression and Mantel tests

Table 1. Sample information for populations of *O. punctatus*.

Populations	n	Date of collection	Coordinates
A	20	Mar 2009	124.50°E 32.50 °N
B	13	Mar 2009	121.00 °E 26.00 °N
C	24	Mar 2009	122.50°E 32.00°N
D	14	Mar 2009	126.50°E 32°N
E	24	Jan 2009	126.00°E 31.50°N

Table 2. Adaptor and primer sequences used in AFLP analysis

Primer	Sequence
Adapters	
*Eco*RI-adapter	5'-CTCGTAGACTGCGTACC-3' 5'-AATTGGTACGCAGTCTAC-3'
*Mse*I-adapter	5'-GACGTGAGTCCTGAG-3' 5'-TACTCAGGACTCAT-3'
Pre-amplification primer	
*Eco*RI	5'-GACTGCGTACCAATTC-3'
*Mse*I	5'-GATGAGTCCTGAGTAA-3'
Selective amplification primer	
E-ACA/M-CAC	5'-GACTGCGTACCAATTCACA-3' 5'-GATGAGTCCTGAGTAACAC-3'
E-AAC/M-CAC	5'-GACTGCGTACCAATTCAAC-3' 5'-GATGAGTCCTGAGTAACTC-3'
E-AAC/M-CAG	5'-GACTGCGTACCAATTCAAC-3' 5'-GATGAGTCCTGAGTAACAG-3'
E-AGA/M-CAG	5'-GACTGCGTACCAATTCAGA-3' 5'-GATGAGTCCTGAGTAACAG-3'

using RMA.

RESULTS

Among 95 individuals, a total of 196 loci were detected by the four primer combinations, ranging between 50 and 500bp, 117 of which were polymorphic (59.69%, Table 3). The average number of bands scored per primer pair was 49, ranging from 40 - 60. The number of polymorphic loci amplified by each primer combination over all populations ranged from 18 - 34, with the average of 29.25 polymorphic loci per prime combination (Table 3).

Low genetic diversity was observed in all geographic samples. The population with the highest proportion of polymorphic loci (53.49%) was population A, whereas, that with the lowest value was population D, in which the proportion of polymorphic loci and number of polymorphic loci was 23.15% and 25, respectively. The population with the highest Nei's genetic diversity was also A population, with a value of 0.0631, the lowest Nei's genetic diversity was also found in D population, only with a value of 0.0217(Table 4).

Genetic differentiation among *O. punctatus* from five populations was high and significant (AMOVA, F_{ST} = 0.0995, $p < 0.001$), suggesting significant genetic differentiation among localities. Significant geographic heterogeneity was observed between all pairwise comparisons of samples analyzed. Pairwise F_{ST} values among populations were significant ($p < 0.05$), ranging from 0.0385 - 0.2616 (Table 4). These analyses indicated that several distinct populations of *O. punctatus* existed in the East China Sea and Yellow Sea. Additionally pairwise F_{ST} analysis indicated the largest genetic difference among populations existed in locations B and D (F_{ST} = 0.2616, $p < 0.01$), whereas, the difference between D and E was the smallest (F_{ST} = 0.0385, $p < 0.01$) (Table 5). The Nei's genetic distance between pairs of geographic samples ranged from 0.0029 - 0.0138, which suggested that populations A and B were the most different genetically (*D*

Table 3. Number of bands generated by primer combinations.

	E-ACA/ M-CAC	E-AAC/ M-CAC	E-AAC/ M-CAG	E-AGA/ M-CAG	Total
Number of loci	60	40	40	56	196
Number of polymorphic loci	39	18	26	34	117
Proportion of polymorphic loci (%)	65.00	45.00	65.00	60.71	59.69

Table 4. Parameters of genetic diversity for populations of *O. punctatus.*

Populations	Number of loci	Number of polymorphic loci	Proportion of polymorphic loci (%)	Nei's genetic diversity
A	172	92	53.49	0.0631
B	120	35	29.17	0.0341
C	157	75	47.77	0.0454
D	108	25	23.15	0.0217
E	144	63	43.75	0.0405

Table 5. Nei's genetic distance (above) and pairwise F_{ST} (below) between populations.

	A	B	C	D	E
A	-	0.0138	0.0051	0.0040	0.0043
B	0.1098*	-	0.0065	0.0134	0.0163
C	0.0552*	0.0728*	-	0.0053	0.0063
D	0.0416*	0.2616*	0.1233*	-	0.0029
E	0.0492*	0.2382*	0.1131*	0.0385*	-

*significant $p < 0.05$

= 0.0138), whereas, the most similar populations were D and E (D = 0.0029) (Table 5). Based on the UPGMA tree (Figure 2), the five populations were clustered into two groups. The north group, included populations A, C, D, and E, was from the northern East China Sea and southern Yellow Sea. The population B from the central East China Sea was clustered as the south group. Among sample sites for *O. punctatus*, a Mantel test indicated a significant relationship ($p = 0.008$, $r = 0.90$) between genetic distance and geographic distance indicating isolation by distance (Figure 3), with geographic distance explaining 90% of the variation in genetic differentiation for species.

DISCUSSION

Marine organisms generally show low levels of genetic differentiation among geographic regions due to higher dispersal potential during planktonic egg, larval, or adult history stages coupled with an absence of physical barriers to movement between ocean basins or adjacent continental margins (Hewitt, 2000; Liu et al., 2007). Adult of *O. punctatus* have strong swimming and burrowing ability (Zhang et al., 1991). The duration of the larval stage in *O punctatus* was two to three months (Sasaki and Kawasaki, 1980). *O. punctatus* with long planktonic larval stage and strong swimming ability for adult might confirm to this pattern. However, contradicting to this hypothesis, the present study indicated that the gene pool of *O. punctatus* was not homogeneous but was micro geographically fragmented intraspecifically. The plot of Nei's genetic distance and geographic distance revealed a strong pattern of isolation by distance in this species (< 855 km), indicating that population subdivision among localities of *O. punctatus* was mainly due to the geographic distance (90%). Moreover, the results of the phylogenetic analysis also revealed clear correlation between the clustering of populations and their geographic origins.

Genetic isolation by distance has rarely been described in marine species with high potential for dispersal at both the larval and adult life-history stages. The significant association between gene flow and distance detected in *O. punctatus*, particularly at small geographic scales, is unexpected and appears difficult to reconcile with many aspects of its biology. Isolation by distance occurs within a continuously distributed population, when dispersal of gametes and/or zygotes is spatially restricted. For this crab, isolation by distance showed evidence that eggs and larvae must be retained in spawning grounds by behavioral or physical oceanographic mechanisms.

Larval and eggs retention may be important processes in preventing high levels of gene flow between different populations in a species with a long larval dispersal phase. Retention of egg and larvae was considered as one reason for genetic isolation by distance in highly mobile Atlantic cod, *Gadus morhua* (Pogson et al., 2001). Similar result for local retention of pelagic larvae was reported in

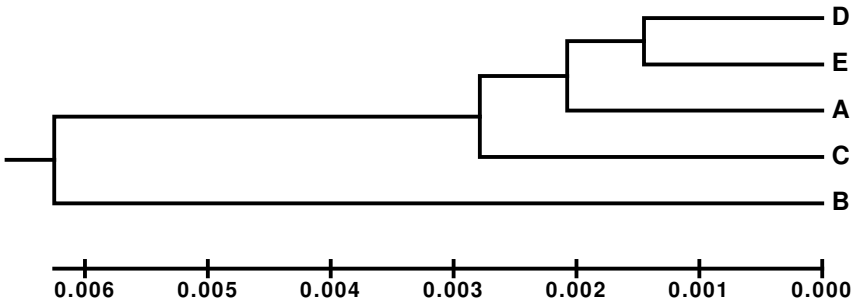

Figure 2. UPGMA cluster analysis based on Nei's genetic distances among five populations.

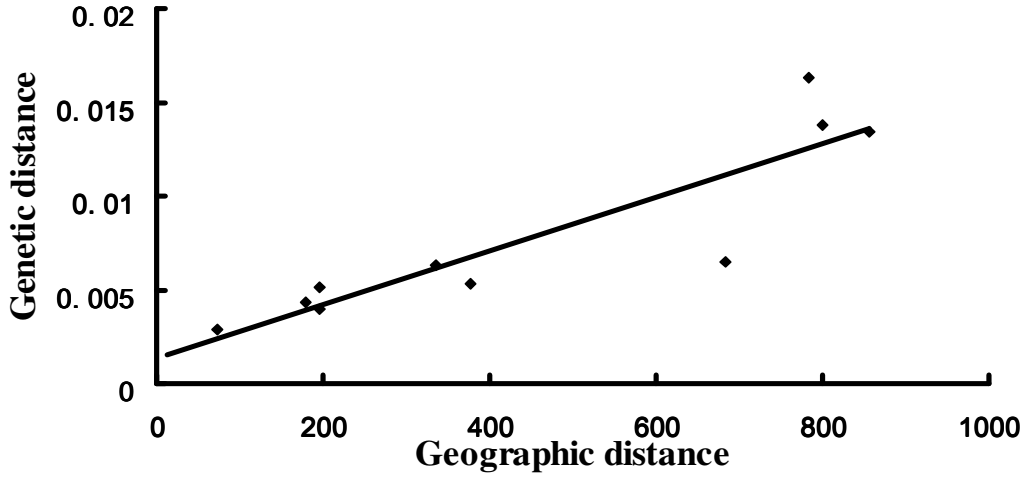

Figure 3. Plot of pairwise estimates of genetic distance and geographic distance between samples of *O. punctatus*

a Caribbean Reef fish *Elacatinus evelynae* (Taylor and Hellberg, 2003). Despite evidence for extended pelagic duration, populations of *E. evelynae* show strong genetic differentiation among island populations. Larval retention has been reported in swimming crab *Callinectes danae* populations in southern Brazil, which prevented panmixia between populations inhabiting different estuaries (Weber and Levy, 2000) *O. punctatus* can adapt to wide temperature and salinity as an eurythermic and euryhaline species, but previous study reported that it tended to distribute low salinity waters (Yu et al., 2005). The salinity in five sample locations varied greatly and was influenced by the outflow of rivers, such as Yangtze River and Mingjiang River. This biological characteristics coupled with different salinity in the study area may limit the distribution of larval and adult of this species, causing local retention of pelagic larvae and eggs.

Although sample size from each geographic site in this study was limited, specimens were collected from different geographic locations. This should be sufficient to generate the preliminary data on genetic diversity and population differentiation of *O. punctatus* in the East China Sea and Yellow Sea. This result indicates the simple assumption that extended pelagic larval duration will result in broad dispersal; is a faulty foundation for the management of fisheries resources and for understanding the geographic context of speciation in the sea. Even in the absence of strong geographic barriers, persistent retention of larvae could allow population differentiation. Moritz (1994) proposed the concept of a management unit (MU), which was defined as a conservation unit that had statistical significant divergence in allele frequencies (nuclear or mitochondrial). Therefore, *O. punctatus* in the Yellow Sea and East China Sea should be considered to be at least two management units, which provides a guideline for further effective conservation and management of the species, and would help greatly in understand it.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (Nos. 30970464), the Key Grant of

Science and Technology Department of Zhejiang Province (2006C23051), and Grant from the Department of Education of Zhejiang Province (Y200803409), was changed to". This work was supported by the National Natural Science Foundation of China (Nos. 30970464), the Key Grant of Science and Technology Department of Zhejiang Province (2006C23051), Grant from the Department of Education of Zhejiang Province (Y200803409) and the Open Foundation from Ocean Fishery Science and Technology in the Most Important Subjects of Zhejiang (20100106).

REFERENCES

Hewitt GM (2000). The genetic legacy o f the Quaternary ice ages. Nature, 405: 907-913.

Huang L, Wang CH, Li SF (2008). Preliminary study on genetic variability in Chinese mitten crab (*Eriocheir sinensis*) from the Yangtze river by AFLP marker. J. Shanghai Fish. Univ., 4: 385-389.

Klinbunga S, Khetpu K, Khamnamtong B, Menasveta P (2007). Genetic Heterogeneity of the Blue Swimming Crab (*Portunus pelagicus*) in Thailand Determined by AFLP Analysis. Biochem. Genet. 45: 725-736.

Liu JX, Gao TX, Wu SF, Zhang YP (2007). Pleistocene isolation in the Northwestern Pacific marginal seas and limited dispersal in a marine fish, *Chelon haematocheilus* (Temminck and Schlegel, 1845). Mol. Ecol., 16: 275-288.

Liu JZ, Cordes JF (2004). DNA marker technologies and their applications in aquaculture genetic. Aquaculture, 238: 1-37.

Liu S, Xue SX, Sun JS (2008). Genetic diversity of *Portunus triuberbuculatus* revealed by AFLP analysis. Oceanol. et Limnol. Sinica, 2: 152-156.

Merril CR, Switzer RC, Van Keuren ML (1979). Trace polypeptides in cellular extracts and human body fluid detected by two-dimensional electrophoresis and a highly sensitive silver stain. Proc. Natl. Acad. Sci. USA, 76: 4335-4339.

Moritz C (1994). Defining "evolutionary significant units" for conservation. Trends Ecol. Evol., 9: 373-375.

Nei M (1972). Genetic distance between populations. Am. Nat., 106: 283-292.

Pogson GH, Taggart CT, Mesa KA, Boutilier RG (2001). Isolation by distance in the Atlantic cod, Gadus morhua, at large and small geographic scales. Evolution, 55: 131-146.

Sasaki K, Kawasaki T (1980). Some aspects of the reproductive biology of the swimming crab, *Ovalipes punctatus* (De Haan), in Sendai Bay and its adjacent waters. Tohoku. J. Agr. Res. 4:183-194.

Slatkin M (1993). Isolation by distance in equilibrium and non-equilibrium populations. Evolution, 47: 264-279.

Takahashi K, Kawaguchi K (2001). Nocturnal occurrence of the swimming crab *Ovalipes punctatus* in the swash zone of a sandy beach in northeastern Japan. Fish. Bull., 99: 510-515.

Taylor MS, Hellberg ME (2003).Genetic evidence for local retention of pelagic larvae in a Caribbean Reef fish. Sci., 299: 107-109

Vos P, Hogers R, Bleeker M, Reijans M, et al (1995). AFLP: a new technique for DNA fingerprinting. Nucleos. Acids. Res., 23: 4407-4414.

Wang Z, Jayasankar P, Khoo SK (2000). AFLP fingerprinting reveals genetic variability in common carp stocks from Indonesia. Asian Fish. Sci., 13: 139-147.

Weber LI, Levy JA (2000). Genetic population structure of the swimming crab *Callinectes danae* (Crustacea:Decapoda) in Southern Brazil. Hydrobiologia, 420: 203-210.

Yu CG, Song HT, Rao GZ (2004). Biological characteristic of *Ovalipes punctatus* in the East China Sea. J. Fish. China, 6: 657-662.

Yu CG, Song HT, Rao GZ (2005). Study on distribution of *Ovalipes punctatu*s (crab) in the East China Sea. J. Fish. China, 2: 198-204.

Zhang BL, Xiang JH, Wu YQ (1991). The feeding ecology of the two crabs (*Portunus trituberculatus* and *Ovalipes punctatus*) in the estuary and the adjacent sea waters of Changjiang River. Marine Sci., 5: 60-64

Zhang JB, Cai ZP, Huang LM (2006). Population genetic structure of crimson snapper *Lutjanus erythropterus* in East Asia, revealed by analysis of the mitochondrial control region. ICES J. Mar. Sci., 63: 693-704.