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Full Length Research Paper

Genetic diversity and historical demography of Chinese shrimp *Feneropenaeus chinensis* in Yellow Sea and Bohai Sea based on mitochondrial DNA analysis

Yu Long Li[1,2], Xiao Yu Kong[2]*, Zi Niu Yu[2], Jie Kong[3], Shen Ma[1] and Li Mei Chen[1]

[1]Mariculture Research laboratory, Education Ministry of China, Ocean University of China, Qingdao 266003, China.
[2]Laboratory of Marine Bio-resource Sustainable Utilization, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou 510301, China.
[3]Yellow Sea Institute of Fisheries Research, Chinese Academy of Fisheries Science, Qingdao 266071, China.

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Genetic structure and demographic history of shrimp *Feneropenaeus chinensis* were examined using sequence data from portions of the mitochondrial cytochrome *c* oxidase I (COI) gene. Samples of 91 individuals were collected from 5 localities over most of the species' range in the Yellow Sea and Bohai Sea. Variability was detected at a total of 20 nucleotide sites, defining 20 haplotypes of COI in this species. The detected level of variation was low, with H = 0.4816 ± 0.0639 and π = 0.0846 ± 0.0711% respectively. Analysis of molecular variance and the conventional population statistic F_{ST} revealed no significant genetic structure throughout the range of *F. chinensis*. Both mismatch distribution analyses and neutrality tests suggested a late Pleistocene or Holocene population expansion (12,100 – 28,500 years ago) for the species, which was consistent with the geological period of formation of the Yellow Sea and Bohai Sea. These results indicated that *F. chinensis* in the Yellow Sea and Bohai Sea might have undergone a genetic bottleneck or founder event during the late Pleistocene globle glacial period.

Key words: Cytochrome *c* oxidase subunit I gene, *Feneropenaeus chinensis*, historical demography, mtDNA, population expansion.

INTRODUCTION

Mainly occurring in coasts of northern China and Korea, Chinese shrimp *Fenneropenaeus chinensis* (Osbeck, 1765, previously called *Penaeus orientalis*, Kishinouye, 1918) has some unique biological characteristics among nearly 29 species in the family Penaeidae, including high latitude distribution, high rates of migration and dense schooling behavior (Kim, 1973; Deng et al., 1983). With high market values and abundance, it has been one of the most important commercial shrimp species for marine fisheries and aquaculture industries along the coasts of the Yellow Sea and Bohai Sea in northern China, Comprising approximately 80% of total shrimp landings per year before its aquaculture collapsed (Liu, 1990).

Most previous researches focused on geographical distribution, habitats, and behavior ecology of *F. chinensis* (Deng et al., 1983, 1990; Liu,1990, 1955, 1959; Liu and zhong, 1988; Ye et al., 1980; Zhang and Deng, 1965). According to Liu (1959), the range of *F. chinensis* is primarily restricted to the inshore areas of China. A large quantity of this species was fished every year from the Yellow Sea and Bohai Sea, with an annual catch of about 10, 000 tons; fewer shrimp were taken from the East China Sea and the South China Sea. *F. chinensis* also can be found along the west coast of the Korean Peninsula and western Japan, where the quantity is very small. Based on recapture rates after artificial stocking, *F. chinensis* in the Yellow Sea and Bohai Sea can be divided into two independent populations. One is Yellow Sea and Bohai Sea (YB) coast population with a larger population size and bigger body sizes, and the other is the smaller western Korean peninsula (KW) coast population, with smaller body sizes (Deng et al., 1990). Mature individuals of the two populations spawn separately in

*Corresponding author. E-mail: xykong@scsio.ac.cn. Tel./ Fax: +86 20 8910 2507.

inshore waters and shallow estuaries of the Yellow Sea and Bohai Sea, from May to late June, and then juveniles feed in October. After reaching maturation, they copulate between October and November. In late November, these two populations begin to migrate to wintering grounds in the central and southern part of the Yellow Sea. It is noteworthy that the wintering grounds of the two stocks are slightly different, the former being eastward, and the latter, westward (Deng and Zhao, 1991). These two populations are considered to be geographically isolated from each other.

Recently, a new population of *F. chinensis* was found near the Jeju Island near southern Korea, called the southern coast population of the Korean peninsula (KS) (Liu et al., 2004). Its spawning location and migration routes are different from the others. Given the differences in spawning, migrating time and wintering places, *F. chinensis* may have three geographic populations: the Yellow Sea and Bohai Sea (YB) coast population, the western Korean peninsula (KW) coast population, and the southern coast of Korean Peninsula (KS).

So far, population genetic variation and structure of *F. chinensis* have been examined based on different molecular markers, such as the random amplification polymorphisms of DNA (RAPD, Liu et al., 2000a, b; Meng et al., 2004; Shi et al., 1999, 2001; Zhuang et al. 2001), isozyme analysis (Wang et al., 2001), and microsatellite analysis (Liu et al., 2004, 2006). The genetic diversity of this species was much lower than that of other arthropods and other penaeids (e.g. *Litopenaeus setiferus, Marsupenaeus japonicus,* Hualkasin et al., 2003; McMillen-Jackson and Bert, 2003, 2004; Tzeng et al., 2004).

Efforts to differentiate the shrimp populations based on molecular markers have produced discordant results. The studies utilizing allozyme and microsatellite analysis revealed little genetic structure among Chinese shrimp samples. In contrast, the *G*st values calculated from RAPD data revealed significant genetic differentiation between pairs of samples (*G*st = 0.032 - 0.233, Meng et al.,-2004). Cui et al. (2007) examined variation of *F. chinensis* mtDNA control region using PCR followed by RFLP analysis and found that only six haplotypes were identified in 122 *F. chinensis* individuals from six sampling localities. Of those individuals, 86.9% shared one haplotype, which constituted 75.0 - 93.1% of each of the samples. The haplotype diversity of all individuals was only 0.24. Furthermore, the authors sequenced the complete control region from 14 individuals. Both RFLP and sequence data suggest that *F. chinensis* distributed along the coasts of northern China and the Korean Peninsula are not genetically differentiated.

Up to now, most studies have been focused on genetic diversity and population genetic structure of *F. Chinensis*. Little is known about the historical population dynamics. AnimalmtDNA genes such as cyt b (cytochrome b), CR (control region), COI (cytochrome oxidase I) have been widely used as genetic markers to assess intraspecific genetic diversity, phylogeography, and phylogeny in numerous species and genera, owing to a higher mutation rate found of this maternally transmitted genome (Avise et al., 1987; Baldwin et al., 1998; Funk, 1999; Walton et al., 2000). The amount and pattern of polymorphism in DNA sequences can be used to infer the history of populations as well as the mechanism responsible for generating and maintaining the polymorphisms (Li, 1997). In this study, COI sequence data from samples of *F. chinensis* were used to examine genetic variability and geographic structure of this species, and particularly to infer the population history, in order to improve the knowledge of the origin and evolution of this important species and to provide scientific information for its brood stock management.

MATERIALS AND METHODS

Sample Collection

A total of 91 individuals of *F. chinensis* from five localities were collected over its range during 1997 - 2001 (Table 1, Figure 1). Fresh muscle tissue from samples was stored at -70°C before DNA extraction.

DNA extraction, amplification and sequencing

Genomic DNA was isolated from muscle tissue using a standard phenol-chloroform method (Sambrook et al., 1989). DNA was subsequently re-suspended in 50 µl TE buffer. The COI gene fragments were amplified using primers COI f: 5'-cctgcaggaggaggagaycc-3' and TL2-N: 5'-atgcatatctatctgccattttag-3' (Palumbi and Benzie, 1991).PCR amplification was carried out in an Eppendorf authorized thermal cycler. Approximately 30 ng of DNA was utilized as template, reactions were conducted in 25 µl volumes containing 1 U *Taq* DNA polymerase (Takara, China), 0.1 mM of each primers, 2.0 mM $MgCl_2$, 0.1 mM dNTPs, and 2.5 µl 10× PCR buffer. The cycling conditions were the following: a single cycle of 3 min at 95℃, followed by 33 cycles of 45 s at 94℃, 45 s at 50℃, and 90 s at 72℃, with a final cycle of 5 min at 72℃. The PCR products were visualized on 1% agarose gels, and purified using Takara Agarose Gel DNA Purification Kit (Takara, China), then sequenced in both directions using an ABI 3730 automated sequencer (ABI). Primers used in sequencing were the same as those for PCR.

Data analysis

Sequences from both strands in each specimen were aligned with CLUSTAL X1.81 (Thompson et al., 1997) and individual consensus sequences were retrieved with both alignment and manual check. The accuracy of COI sequences was confirmed by translating the nucleotide data to amino acid sequences. Molecular diversity indices such as number of haplotypes, polymorphic sites, transitions, transversions, and indels were obtained using Arlequin (Ver. 2.000, Schneider et al., 2000). Haplotype diversity (H), nucleotidediversity (π), and their corresponding variances were calculated following Nei (1987) as implemented in Arlequin. Implemented with Modeltest 3.06 (Posada and Crandall, 1998), hierarchical series of likelihood ratio tests (Huelsenbeck and Rannala, 1997) were used to identify the appropriate nucleotide substitution models.

An analysis of molecular variation (AMOVA, Excoffier et al., 1992) was used to examine significant population structure in *F. chinensis*. AMOVA and bootstrap analysis with 5,000 replicates

Table 1. The source of *F. chinensis* samples.

Sample	Captive location	Data of collection	Sample size
KW	West coast of Korean Peninsular (126ºE, 35ºN)	May 1997	19
KS	Nan Hai along shore of Korea (34º30'N, 127º30'E)	Sept. 2005	19
RS	Rushan Bay of Yellow Sea (122º E, 37º N)	Aug. 2001	17
LD	Liaodong Bay of Bohai Sea (40º30'N, 121º30'E)	Sept. 2001	18
ZW	Haizhou Bay of Yellow Sea (35º N, 120º E)	Sept. 2001	18

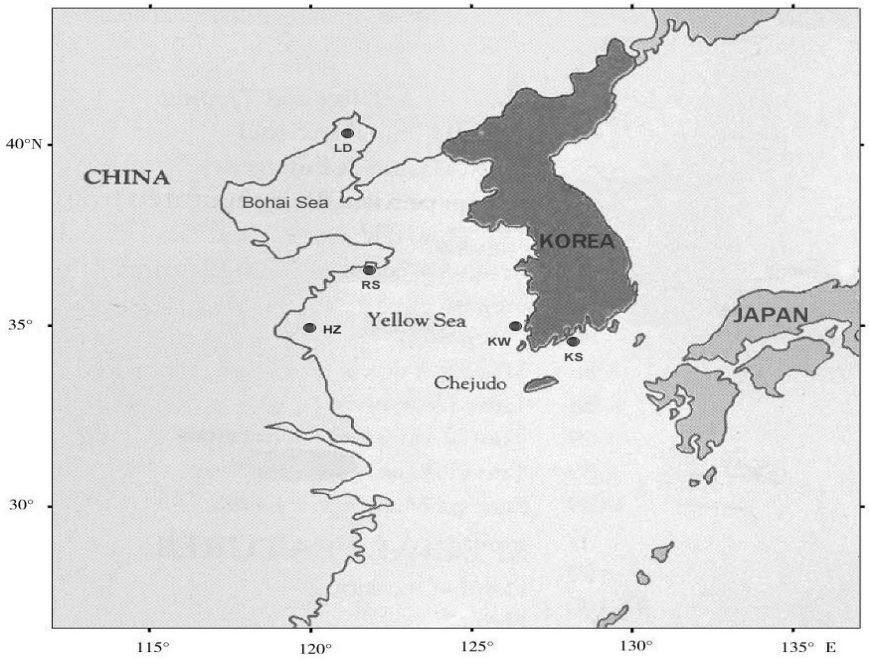

Figure 1. A map showing sampling locations of *F. chinensis*, samples are marked by abbreviations that correspond to those in Table 1.

were performed in Arlequin. For COI data, the appropriate model of nucleotide substitution was the HKY85 model (Hasegawa et al., 1985) with no invariable sites and equal mutation rate. Because the HKY model was not available in Arlequin, the more inclusive Tamura-Nei (TrN) (Tamura and Nei, 1993) modelwas used to calculate the genetic pairwise distances between haplotypes. F_{ST} statistics were calculated between pairs of populations. The significance (5% level) of the F_{ST} was tested by 1,000 permutations for each pairwise comparison.

Phylogenetic trees of the haplotypes were constructed using PAUP (Swofford, 2002) and MEGA 4.0 (Tamura et al., 2007). The neighbour-joining (NJ) algorithm (Saitou and Nei, 1987) was implemented to construct a phylogenetic tree from the maximum likelihood (ML) distances estimated under the selected models. Relationships between haplotypes were also determined with the Kimura two-parameter distance model by using the neighbour-joining method in MEGA 4.0. Corresponding sequence data from *Penaeus monodon* (GenBank accession No. AF217843) was used as the outgroup for the analysis. Bootstrap analysis with 1,000 replicates was used to evaluate reliability of phylogynetic relationships (Felsenstein, 1985). In addition, genealogical relationships were examined by constructing haplotype networks using median-network approach (Bandelt et al., 1995, 2000).

Both mismatch analysis and neutrality tests were performed in Arlequin. The historical demographic expansions were examined by two different approaches. First, we evaluated whether the COI gene evolved under strict neutrality. We tested the following hypotheses: (1) all mutations are selectively neutral, and (2) the population has evolved according to the Wright-Fisher model with a constant effective population size. Two widely used statistical tests were employed: Tajima's *D* (Tajima, 1989) and Fu's *F*s (Fu, 1997), both were performed in Arlequin. Tajima's *D* test compares two estimators of the mutation parameter θ, Watterson's estimator θ_s and Tajima's estimator θ_π, significant *D* values can be estimated due to factors such as selection, population expansion, and bottleneck (Tajima, 1989). As *S* depends more on the present population size and *k* on the size of the original population, a history of population growth can inflate *S* significantly compared with *k* and generate a negative value of Tajima's *D* (Tajima, 1989). Fu's *F*s test is constructed based on selective neutrality using the probability of the number of alleles in a sample. Fu (1997) found that the *F*s are sensitive to population demographic expansions, which generally lead to large negative *F*s values. The significance of the neutrality statistics were tested by generating random samples under the hypothesis of selective neutrality and population equilibrium, using a coalescent simulation algorithm adapted from Hudson (1990).

Historic demographic expansions were also investigated by examination of frequency distributions of pairwise differences betw-

Table 2. The variable sites and haplotype frequencies of COI gene fragments of *F. chinensis*.

	Variable sites and positions																				Samples				
	0	0	0	1	1	1	2	3	3	4	5	5	5	5	6	7	7	7	8	8					
	3	3	5	2	3	7	0	6	8	7	2	5	5	7	9	1	5	5	0	1					
Haplotype	3	6	5	6	5	7	1	3	7	7	8	3	5	9	8	1	0	4	8	3	**RS**	**KS**	**ZW**	**KW**	**LD**
Hap_1	T	G	C	G	A	A	A	C	T	A	A	G	A	A	T	T	C	G	A	A	14	16	11	14	13
Hap_2	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	1				
Hap_3	.	.	.	.	.	.	.	.	.	.	.	.	T	.	C	.	.	.	.	.	1				
Hap_4	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	1				
Hap_5	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		1			
Hap_6	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.		1			
Hap_7	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		1		2	
Hap_8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.			1		
Hap_9	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.			1		
Hap_10	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G			1		
Hap_11	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.			1		
Hap_12	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.			2		
Hap_13	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.			1		
Hap_14	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.				1	
Hap_15	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.				1	
Hap_16	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.				1	1
Hap_17	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.					1
Hap_18	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.					1
Hap_19	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.					1
Hap_20	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.					1
Total																					17	19	18	19	18

een sequences (mismatch distribution), which was based on three parameters: θ_0, θ_1 (before and after the population growth) and τ (time since expansion expressed in units of mutational time (Rogers and Harpending, 1992; Rogers, 1995). The mismatch distribution is usually multimodal in samples drawn from populations at demographic equilibrium, but it is usually unimodal in populations following a recent population demographic expansion and range expansion (Rogers and Harpending, 1992; Slatkin and Hudson, 1991; Ray et al., 2003; Excoffier, 2004). The parameters of the demographic expansion τ, θ_0 and θ_1 are estimated by a generalized non-linear least-square approach, and confidence intervals of the parameters are computed using a parametric bootstrap approach (Schneider and Excoffier, 1999). The values of τ were transformed to estimate of real time since expansion with the equation $\tau = 2ut$, where u is the mutation rate per generation for the whole sequence under study and t is the time measured in years since expansion.

Baldwin (1998) studied the relationship of the *Penaeus* among thirteen species in six subgenera, and estimated the average pairwise sequence divergence rate at about 3% per million years in COI gene with the fossil evidence as references. Unless stated otherwise, we used this rate for our analysis (i.e. the estimated single-lineage value for μ was 1.5×10^{-8}).compares two estimators of the mutation parameter θ, Watterson's estimator θ_s and Tajima's estimator θ_π, significant *D* values can be estimated due to factors such as selection, population expansion, and bottleneck (Tajima, 1989). As *S* depends more on the present population size and *k* on the size of the original population, a history of population growth can inflate *S* significantly compared with *k* and generate a negative value of Tajima's *D* (Tajima, 1989). Fu's *F*s test is constructed based on selective neutrality using the probability of the number of alleles in a sample. Fu (1997) found that the *F*s are sensitive to population demographic expansions, which generally

RESULTS

Sequence variation

A 850 bp fragment of mitochondrial COI gene was amplified. The average base composition of the COI gene fragment in *F. chinensis* was as follows, A: 27.99, T: 36.59, G: 16.36 and C: 19.06%. The high AT content (65%) of this sequence was consistent with descriptions of counterpart sequences from other arthropods (Spicer, 1995) as well as other *Penaeus* species (Garcia-Machado et al., 1996). A total of 20 variable sites were detected from 91 individuals, including 18 transitions and 2 transversions (Table 2). The majority of variable sites (75%) occurred in the third position of codons and are, therefore, silent; there were 4 variable sites at the first position of codons and only one at the second position of codon. In total, there were 4 variable sites which caused amino acid substitutions (Val replaced by Met, Val by Ala, Ala by Ser, and Asn by Asp). Twenty haplotypes were defined from all individuals sequenced (designated as Hap1-20, GenBank accession nos. EU366231-EU366250). Sequence differences of the twenty haplotypes ranged from 0.12 - 0.36%. Haplotype 1 was the

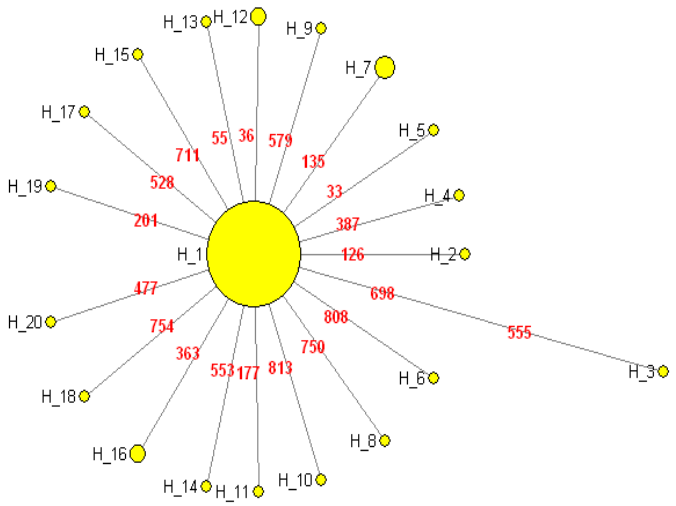

Figure 2. Median-network showing phylogenetic relationships among mtDNA COI gene haplotypes of *F. chinensis*. Numbers cross the lines represent the sites of nucleotide substitutions, circle areas depict proportions of haplotypes.

most common and was observed in all samples, and its frequency was 75% in the total samples. Haplotype 7 was shared by the KW and KS samples, Haplotype 16 was observed in both the KW and LD samples. All others were sample-specific haplotypes. Haplotype frequencies of COI fragment and their distributions in the five samples are shown in Table 2.

Haplotype diversity (H), nucleotide diversity (π), and other sample-specific diversity indices are presented in Table 3. The genetic variation level was low, whether measured as haplotype diversity (0.30 ± 0.13 to 0.63 ± 0.13) or nucleotide diversity (0.0372 ± 0.0443% to 0.0901 ± 0.0774%).

Genetic relationships among Haplotypes

The topology of the neighbor-joining tree was shallow, and there were no significant genealogical branches or clusters of samples corresponding to sampling locality (not shown). Typically, haplotypes in a sample were scattered throughout the tree.

A median network based on nucleotide divergences among the haplotypes detected in this study indicated that most haplotypes were closely related, with the dominant haplotype (haplotype 1) as the center of radiation (Figure 2). Haplotype 1 was also the dominant haplotype of these five populations. The proportion of haplotype 1 in these five samples ranged from 72 to 84%, with an overall proportion of 75% in the total samples. Many adjacent haplotypes differed from each other by one nucleotide, and only one haplotype was two mutational steps apart from haplotype 1; this star-like network suggests a signature of population expansion.

Whether based on phylogynetic analyses or median network, both the results indicated a very likely existence of recent historic population expansion, and the expansion might consist of range expansion and demographic expansion.

Population genetic structure

Genetic differentiation among Chinese shrimp populations was assessed using F_{ST} pairwise comparisons. F_{ST} values were low in general (-0.01270 ~ 0.01978) and none were statistically significant (P > 0.05). In the 10 possible comparisons, two of the pairwise F_{ST} estimates were negative (Table 4), indicating that the variation within samples was greater than variation between samples.

AMOVA analysis showed that 99.36% of the total molecular variance was distributed within samples (Table 5). Fixation index over all samples (F_{ST}) was 0.00635, and showed no significant differences (P > 0.05). These results suggest no significant population differentiation throughout the range of *F. chinensis*.

Historical demography

Fu's *F*s and Tajima's *D* were used to test for neutrality. Fu's *F*s test showed significantly negative values (P < 0.02) for all samples. However, when the samples were pooled together, a very large *F*s index gave significantly negative values. As mentioned by Fu (1997), the *F*s statistic is very sensitive to demographic expansion. All D-values obtained from Tajima's *D*-tests were negative and ranged from -1.62 (P = 0.025) for KW to –2.54 (P = 0.00) for the total sample (Table 3). These results were consistent with other evidence for recent population expansion of *F. chinensis*.

Mismatch distribution was used to explore the historical population dynamics of Chinese shrimp. The result was unimodal, closely matching the expected distributions under the sudden expansion modal (Figure 3). The tau value (τ), which reflects the location of the mismatch distribution crest, provided a rough estimation of the time when rapid population expansion started. The observed value of the age expansion parameter (τ) was 0.59 (95% CI: 0.309 - 0.727). The estimation of time of expansion for *F. chinensis,* based on the mutation rate of 1.5%/MY for COI gene and the equation $\tau = 2ut$, was 23,000 (12,100 - 28,500) years ago.

The network is dominated by a 'star-like' pattern indicative of a population or range expansion. When haplotype 1 was defined as the ancestral type in this expansion and all others associated with the expansion derived from haplotype 1, a rho-value (*r*) of 0.264 ± 0.064 (standard deviation) was obtained. Using a mutation rate of 1.5%/MY, we estimated a substitution in the mtDNA COI gene occurring approximately every 80,000 yBP. This placed the time of major expansion of Chinese shrimp at 21,099 ± 5126 years before present (yBP).

Table 3. Summary of molecular diversity for *F. chinensis*. Tajima's *D* and Fu's *F*s, corresponding P values, and mismatch distribution parameter estimates were also indicated.

Population	No. of haplotype	*S*	*h*	π (%)	Tajima's *D*		Fu's *F*s		Mismatch distribution		
					D	P	*F*s	P	τ	θ_0	θ_1
RS	4	4	0.33±0.14	0.0901±0.0774	-1.84	0.02	-1.86	0.016	3	0	0.504
KS	4	3	0.30±0.13	0.0372±0.0443	-1.72	0.02	-2.68	0.003	3	0	0.445
ZW	7	6	0.63±0.13	0.0901±0.0774	-1.85	0.02	-1.88	0.000	0.959	0	99999
KW	5	4	0.46±0.14	0.0606±0.0596	-1.62	0.03	-2.98	0.001	0.625	0	99999
LD	6	5	0.49±0.14	0.0654±0.0628	-1.96	0.01	-4.43	0.000	0.672	0	99999
Total	20	20	0.48±0.06	0.0846±0.0711	-2.54	0.00	-∞	0.000	0.590	0	99999

S, number of segregating site; *h*, haplotype diversity; π, nucleotide diversity.

Table 4. Genetic differentiations in five samples of *F. chinensis*.

Sample	RS	KS	ZW	KW	LD
RS		n.s	n.s	n.s	n.s
KS	0.00135		n.s	n.s	n.s
ZW	0.00957	0.01332		n.s	n.s
KW	0.01154	-0.01270	0.01978		n.s
LD	-0.00024	0.00092	0.00990	0.00024	

n.s, not significant; F_{ST} values are below the diagonal and P values are above the diagonal.

Table 5. Analysis of molecular variation for samples of *F. chinensis*.

	Degree of freedom	Summation of mean square	Contribution of variation	Percentage of variation	F_{ST}	P
Among population	4	1.170	0.00169	0.64	0.00635	0.12414
Within population	86	22.524	0.26191	99.36		
Total	90	23.695	0.26360	100.00		

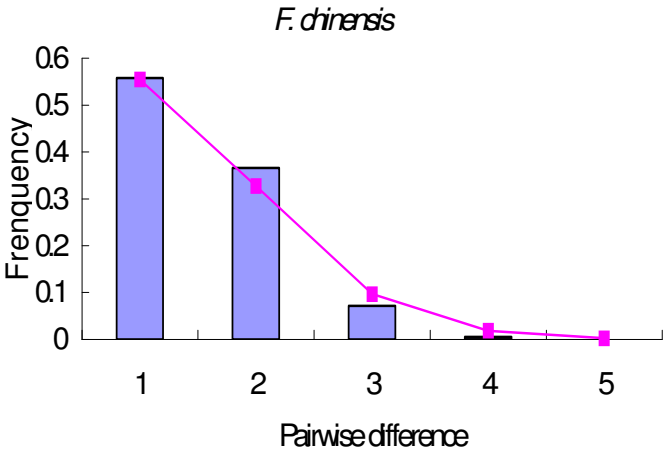

Figure 3. The observed pairwise difference (bars) and the expected mismatch distributions (line) under the sudden expansion model of COI gene haplotypes in *F. chinensis*.

DISCUSSION

In recent years, cytochrome c oxidase subunit I (COI) sequence data have been widely used for inferring population structure and molecular phylogeography in decapod crustaceans (Roman and Palumbi, 2004; Pfeiler et al., 2005). Pfeiler (2005) analyzed sequences of COI and cyt b gene segments to assess the population structure and historical demography of the swimming crab *Callinectes bellicosus* from the eastern Pacific, and showed that the results were similar, but the genetic diversity from COI sequences was higher than that from cyt b. Roman (2004) analyzed COI gene sequences of *Carcinus maenas* in 217 and 13 specimens collected from the North Atlantic and the Mediterranean, respectively, and found that there was considerable genetic diversity and that COI was a suitable marker for population genetic studies. All these results confirmed the applicability of the COI gene for population genetic studies in

crustaceans.

Our results based on COI sequence data indicate that the genetic diversity of *F.chinensis* is rather low, revealing a likely recent population expansion of this species during the end of late Pleistocene or early Holocene. During this period (about more than 10,000 - 20,000 years ago), the sea level was low, the east Yellow Sea was ever an amplitude of plain, and old Yellow Sea was only a narrow waterway close to the Korean side of modern Yellow Sea depression (Chen, 1991). During the last deglaciation, the coastline migrated about 1200 km landwards from the western border of the Okinawa Trough to the western coast of the modern Bohai Gulf (Wang, 1999; Xu and Oda, 1999), resulting in the formation of modern Bohai Sea and the Yellow Sea.

Genetic diversity and Pleistocene ice ages

Obviously, a low level of genetic diversity of *F. chinensis* in our study is consistent with the results of others (Shi, 1999; Song, 1999; Wang, 2001; Liu et al., 2004). When compared with similar studies, our measured level of genetic diversity is the lowest among *Penaeus* species studied so far (Hualkasin et al., 2003; McMillen-Jackson and Bert, 2003, 2004; Tzeng et al., 2004; Table 3). Though the estimated mutation rate was different when using different mtDNA markers, the level of variation in Chinese shrimp was still low. For this reason, it is likely that the populations have been strongly affected by the most recent glacial maximum (LGM). Within glacial periods, the displaced populations had to survive along the glacial refugium in a compressed biome, inevitably leading to reduced genetic diversity (Hewitt, 1996, 2000). So, we could presume that *F. chinensis,* almost completely eradicated during last glacial maximum in its present distribution range, should have been most severely impacted by the past glaciations. This inevitably led to reduced genetic diversity. Similar phenomena have been found in coral reef fish species with different habitat preferences (lagoon or outer slope) in French Polynesia; species inhabiting lagoons demonstrate reduced mtDNA diversity when compared with species inhabiting stable environments (the outer slope) due to the fact that lagoons dried out during the Holocene era sea-level regression (Fauvelot et al., 2003).

Population genetic structure

AMOVA did not detected significant differences at all hierarchical levels, and all the conventional population F_{ST} statistics were not significant, indicating that no significant population structure exists throughout the range of *F. chinensis*, consistent with findings of previous research. Using microsatellite DNA analyses, no genetic differentiation among three populations collected from the Yellow Sea and Bohai Sea was found (Liu et al., 2004). However, genetic differentiation between samples from different geographic populations was detected by using RAPD (Meng et al., 2004; Shi et al., 1999).

The RAPD analysis (Shi et al., 1999) indicated that the variation among populations was larger than the variation within populations, and it was consistent with the opinion that there were two geographic populations of *F. chinensis* in the Yellow Sea and Bohai Sea from recapture data (Deng et al., 1990). Meng et al. (2004) studied the genetic diversity among six geographic samples of Chinese shrimp from the Yellow Sea and Bohai Sea using RAPD (four of these samples LD, HZ, RS, and KW were from the same locations as ours). Their results revealed that over three quarters of variation occurred within samples, and that differentiation had taken place to some extent among *F. chinensis*. The differentiation between RS and HZ was little, the differentiation was moderate or large among other samples (Gst = 0.032 - 0.233). One of the reasons for the discordant results may be that different types of genetic markers often mutate at different rates (Freeland, 2005).

Instances of uniform marine populations are regarded to be due either to non-equilibrium populations or to a truly high degree of larval dispersal (Palumbi, 2003). The stocking and recapture data indicated that the reproductive migration routes and directions of migration of the two populations have been separated since their wintering migration, therefore, they are actually geographically isolated from each other (Deng et al., 1983, 1990). In this study, haplotype 1 was found in all samples, indicating a common source of origin for these populations. Haplotype 7 was shared by the KW and KS populations and haplotype 16 was observed in the KW and LD populations. Except for these three haplotypes, all other haplotypes were populations specific. This indicated that gene flow was likely limited to a low degree between samples due to actual barriers among them, though the shrimp are supposed to have high dispersal ability. Cui et al. (2007) suggested that *F. chinensis* populations show little genetic differentiation because of extensive gene flow. Obviously, this view conflicts with the opinion of Deng (1990). With regard to the south coast of Korean Peninsula population, its origin/formation and genetic background were still unknown. We considered that it was close to the west coast population of Korean Peninsula according to morphology characters, isozyme and microsatellite analyses (J. Kong, Yellow Sea Institute of Fisheries Research, personal communication).

In our opinion, the lack of genetic differentiation among samples of *F. chinensis* could reflect how recent a population is or range expansion and insufficient time to attain migration-drift equilibrium. It would be difficult to achieve differentiation either by accumulating an important number of mutational differentiation in the COI gene or through genetic drift during only 20,000 - 30,000 years (Slatkin, 1993).

Indeed, our results clearly demonstrate that the mutation-genetic drift equilibrium has not yet been reached for this species and that genetic patterning (the lack of genetic differentiation among samples of *F. chinensis*) cannot be interpreted as a result of present gene flow. This conflicts with distribution status in its reproductive ecology. Our study underlines the necessity of consider-ing the demographic history of a species when looking at its genetic structure in terms of the interpretation of pre-sent population interconnections.

Population history-crashes and expansion

Both the neutrality tests and the mismatch distribution analysis 'were indicative of recent' population expansion of *F. chinensis*. Population demographic expansion usually leads to star-like genealogies (Slatkin and Hudson, 1991), an excess of rare mutations (Harpending and Rogers, 2000) and unimodal mismatch distribution (Rogers and Harpending, 1992). However, population range expansion can also lead to the same molecular signature as population demographic expansion, if the migration rate between demes is large (Ray et al., 2003; Excoffier, 2004). In this study, both population range expansion and demographic expansion might have had an effect on the pattern of genetic diversity for *F. chinensis*. Furthermore, the star-like median network was also consistent with existence of an evolutionarily recent population expansion. Based on the tau value (τ), the estimated expansion time for Chinese shrimp was 12, 100-28, 500 years ago. Similarly, the estimation based on rho (r) value was approximately 21,098 ± 5,126 years. A study based on PCR-RFLP data suggested a rapid expansion of *F. chinensis* in the Yellow Sea and Bohai Sea during the period of rapid rise of sea level after the last glacial maximum (Cui et al., 2007). The estimated expansion time was approximately 14,000 (1,800 - 20,000) years ago, which was roughly consistent with our estimate. This timescale coincides with the events happening in the late Pleistocene global glacial period (about 10,000-20,000 years) while the world climate was frigid and arid (Zhu et al., 1998), and also with the formation of the Yellow Sea and Bohai Sea, which was related to the late Pleistocene marine transgression (Wang, 1980).

Pleistocene-era environmental fluctuations provoked range expansions and contractions in species worldwide, directly affecting population distributions and demographics (Hewitt, 1996, 2000). Recurrent fluctuations in sea level and climatic conditions occurred during last glacial period between roughly 12,000 and 75,000 years ago (Chappell, 2002). The sudden population expansion for *F. chinensis* was estimated to have occurred near the end of that glacial period, during the climatic fluctuations associated with the last glacial advance.

Historically, during the most recent Pleistocene-era glacial period, the brown shrimp (*Farfantepenaeus aztecus*) and white shrimp (*L. setiferus*), species sympatric with the pink shrimp (*Farfantepenaeus duorarum*) in the eastern coast waters of the United States, were influenced by historic population expansion (McMillen-Jackson and Bert, 2003). During this period, sea level and climatic conditions fluctuated repeatedly and sometimes drastically (Chappell, 2002), and environmental conditions in the eastern United States would not have been optimal for penaeids (relatively low sea levels, colder waters at lower latitudes, decreased rainfall and increased aridity, and fewer estuaries). As a result, the geographic ranges and population sizes of these penaeids may have fluctuated extensively in magnitude, resulting in the generation of genetic signatures of population expansions (McMillen-Jackson and Bert, 2004). Among these three shrimps, the sudden population expansion of brown shrimp was estimated to have occurred 74,000 years ago, and those of the two white shrimps with different lineages were around 28,000 and 16,000 years ago respectively. A late-Pleistocene era sudden population expansion (approximately 36,000 years ago) was also detected in the pink shrimp *F. duorarum* (McMillen-Jackson and Bert, 2003, 2004).

Geographic distribution, migration, and founder event

F. chinensis is a native species mainly inhabiting the Yellow Sea and Bohai Sea. It is also found near the Shengsi and Zhoushan archipelago in the north part of the East China Sea and the mouth of the Pear River in the South China Sea in fewer quantities. With regard to its distribution range, it is a special species, with the strongest swimming ability. In the Yellow Sea and Bohai Sea, it can swim about 500 - 1,000 km in one month during its breeding migration and wintering migration. At the same time, however, the natural distribution range of this species is confined only in the Bohai Sea, the Yellow Sea, and a small area of the East China Sea and the north part of the South China Sea (Liu and zhong, 1988).

According to previous studies (Liu et al., 1959, 1988), the amount of *F. chinensis* fished in the East China Sea and the South China Sea was very low and usually mixed with *Fenneropenaeus penicillatus* and other *Penaeus* species. Unlike those individuals in the Yellow Sea and Bohai Sea, which usually migrate twice a year for reproduction and wintering during their life cycles, according to a tagging study (Liu and Zhong, 1988), individuals of *F. chinensis* in the South China Sea only move within a small area without long distance migration behavior.

We believe that these unique behaviors of *F. chinensis* in the Yellow Sea and Bohai Sea likely resulted from gradual adaptation during the process of evolution. In late Pleistocene's low sea level stage, the sea level was 130 - 150 m lower than the present level in the East China Sea. Consequently, the entire Bohai Sea and the Yellow Sea were exposed, and the East China Sea was reduced to

an elongated trough (Wang and Sun, 1994). The progenitor population of this species may have lived in this area. With the gradual rising of sea level and warming of climate, this population would have spread gradually over this area, adapted themselves to the waters, and eventually multiplied to modern populations in this area. During this period, they began the behavior of annual long distance wintering migrations to the center-south Yellow Sea back to the coastal areas of the Yellow Sea and Bohai Sea in the following spring for reproduction, probably due to the influence of nutrition, temperature, salinity and extent of stability in the sea.

The origin of this species has not been well understood. Given the formation of the Yellow Sea and Bohai Sea, our results suggest that *F. chinensis* in this area may be derived from one recent maternal ancestor. The reduced diversity, the shallow COI gene genealogies, the star-like shape of haplotype networks, and the significant negative Tajima's *D* value estimated from the data in this study provide evidence that Chinese shrimp have undergone a recent population bottleneck or founder event.

Conclusion

In conclusion, the pattern of genetic variability among samples has been greatly influenced by the history of the Yellow Sea and Bohai Sea. The present results, together with previous data, indicate that *F. chinensis* populations bear low genetic diversity in contrast to other *Penaeus* species, likely due to the reduction of effective population size arising from habitat instability during sea-level variations. Finally, in our study, mtDNA variations of diversity in this species are the legacy of historical events. Further studies using other genetic markers are desirable to enrich our understanding of genetic variation in this species.

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