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Genetic Variation in the Asian Shore Crab *Hemigrapsus sanguineus* in Korean Coastal Waters as Inferred from Mitochondrial DNA Sequences

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Abstract

Genetic variation in the Asian shore crab *Hemigrapsus sanguineus* was determined from partial mitochondrial DNA (mtDNA) sequences of the cytochrome *b* (*Cyt*b) gene. Samples included 143 crabs from six localities along three coastlines in South Korea. A nucleotide sequence analysis revealed 38 variable sites in a 470-bp sequence, which defined 37 haplotypes. The haplotypes were not associated geographically and had a shallow genealogy. Pairwise F_{ST} tests and a two-dimensional scaling analysis revealed no significant genetic differentiation among most of the populations. The low pairwise comparison values, but significant genetic differentiation of a northeastern population from all other populations, might have been influenced by a restriction in gene flow caused by hydrographic conditions such as ocean boundaries. The high haplotype diversity, low nucleotide diversity, and time since *H. sanguineus* expansion in Korean coastal waters indicate rapid population growth and a recent, sudden expansion in the Late Pleistocene.

Key words: Cytochrome *b*, Genetic differentiation, Genetic variation, *Hemigrapsus sanguineus*

Introduction

The Asian shore crab *Hemigrapsus sanguineus* is distributed widely in the western Pacific, including Hong Kong, Taiwan, Korean, Chinese, and Japanese coastal waters, and as far north as Sakhalin Island, Russia (Sakai, 1976; Fukui, 1988; Dai and Yang, 1991; Hwang et al., 1993). It is abundant in rocky intertidal habitats and is an ecologically important predator in coastal ecosystems, as are other shore crab species (Kikuchi et al., 1981; Takada and Kikuchi, 1991; McDermott, 1998a, 1998b; Lohrer et al., 2000). In Japan, the breeding season of *H. sanguineus* is March-October, with a main peak May-June (Fukui, 1988). It has a planktonic larval stage of more than 1 month before developing into the juvenile crab (Fukui, 1988). The larval dispersal pattern and preferred habitat might have caused geographically distinct regional populations to become homogeneous.

Estimating genetic structure among populations using molecular markers has become a common approach to determining sustainable yields and genetic diversity (Dunham, 2004). The population genetic structures of some marine species are influenced by their larval dispersal pattern and behavior after

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spawning, which are determined by oceanographic features including sea currents, hydrological conditions, and physical barriers (Doyle et al., 1993; Hsieh et al., 2010). In general, most marine species have limited population substructures and high levels of gene flow because of the effect of sea currents. Ocean structure and dynamics, including current boundaries and hydrographic conditions, have caused reproductive and partial genetic isolation in geographically distinct regional populations (Wares et al., 2001; Bilton et al., 2002).

Although DNA markers are expected to overcome deficiencies in allozyme analysis by increasing the accuracy and resolution of population-structure assessments in crab species (McMillen-Jackson et al., 1994; Creasey et al., 1997), there are few reports on the use of mitochondrial DNA (mtDNA) to measure genetic variation in shore crabs (Cassone and Boulding, 2006). Maternally inherited mtDNA has greater sequence variability than do most nuclear genes (Brown et al., 1979). Moreover, it has a compact genome size in both conserved and variable regions and a conserved gene content and arrangement (Anderson et al., 1981). Therefore, mtDNA analysis has become a key method in evolutionary and ecological studies of crabs (Pfeiler et al., 2005; Cassone and Boulding, 2006; Azuma et al., 2008; Wang et al., 2008).

This study investigated the genetic variation and population structure of *H. sanguineus* in Korean coastal waters using mtDNA cytochrome *b* (*Cyt*b) sequences to assess phylogeographic and demographic patterns.

Materials and Methods

Sampling

Muscle samples were taken from 143 live crabs collected from six localities along three coastlines of Korea from 2009 to 2010 (Table 1, Fig. 1). The collected samples were stored at

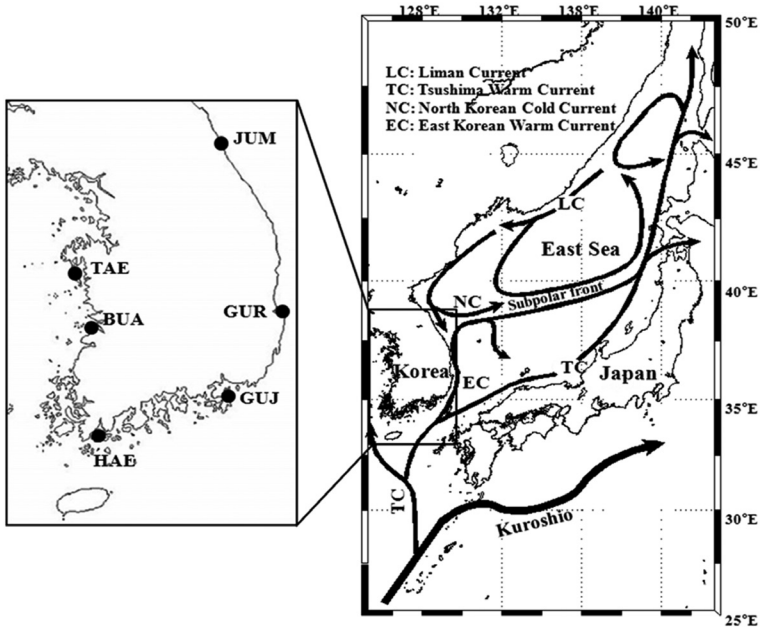

Fig. 1. Sampling locations of six Asian shore crab *Hemigrapsus sanguineus* populations and ocean current flow pattern in the Korean waters. BUA, Buan; GUJ, Geoje; GUR, Guryonpo; HAE, Haenam; JUM, Jumunjin; TAE, Taean.

Table 1. Sampling sites, and dates, geographical coordinates, number of individuals examined (*n*), haplotype and nucleotide diversity (π) of Asian shore crab *Hemigrapsus sanguineus* populations

Sampling site	Abbreviation	Date of collection	Geographical co-ordinates		*n*	No. of haplotypes	Haplotype diversity (*h*, ±SD)	Nucleotide diversity (π)
			Latitude	Longitude				
Taean	TAE	June, 2009	36°28′10.40" N	126°26′07.19" E	24	6	0.616 ± 0.0911	0.0017
Buan	BUA	June, 2009	35°44′21.83" N	126°35′49.50" E	24	9	0.696 ± 0.0962	0.0021
Haenam	HAE	June, 2009	34°20′21.05" N	126°29′28.47" E	24	11	0.779 ± 0.0815	0.0028
Geoje	GUJ	April, 2010	34°54′18.50" N	128°44′52.55" E	24	7	0.634 ± 0.0973	0.0019
Guryonpo	GUR	April, 2010	36°03′33.00" N	129°31′17.12" E	24	13	0.880 ± 0.0556	0.0034
Jumunjin	JUM	April, 2009	37°53′38.53" N	128°50′06.77" E	23	11	0.778 ± 0.0901	0.0027

-20°C or kept in 100% ethanol at room temperature until used.

PCR amplification and sequence analysis

Genomic DNA was extracted from about 20 mg of each specimen by a PUREGENE DNA isolation kit (Gentra Systems, Minneapolis, MN, USA) following the manufacturer's instructions. The purified DNA was dried at room temperature and dissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA; pH 8.0). To amplify the *Cyt*b gene, a pair of degenerate primers (Cyt9237 5′-GTWGCHCAYATTTGYCGAGA-3′; Cyt10050 5′-ACWGGKCGWGCWCCAATTCA-3′) with expected amplicon sizes of 850 bp was designed based on mtDNA sequences of the closely related crab species available in GenBank (AB093006, AY659990, AY562127, FJ797435, FJ827758-827761). The PCR amplification was performed with a thermocycler DNA Engine (MJ Research, Tokyo, Japan). in a 20-μL reaction volume containing 1-2 μL of genomic DNA, 2 μM of each primer, 0.25 mM of each dNTP, 1 unit of Takara *LA Taq* DNA polymerase (Takara Shuzo, Kyoto, Japan), and 2 μL of 10× *LA Taq* reaction buffer (Takara Shuzo). The PCR conditions consisted of preheating at 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s, with final extensions at 72°C for 5 min. The fragment size of the PCR product was verified using 2% agarose gel electrophoresis after ethidium bromide staining. The PCR product was purified with the AccuPrep PCR Purification Kit (Bioneer, Daejeon, Korea). After cycle sequencing with the ABI PRISM BigDye Terminator v3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA), the purified PCR product was sequenced directly on an ABI 3730xl DNA Analyzer (Applied Biosystems) with two newly designed internal primers, HsCytF (5′-GGGGTCAAATATCATTCTGG-3′) and HsCytR (5′-GCCTTTGGAATTTTGAAGAG-3′).

Data analysis

The sequence fragments obtained in this study were aligned with GENETIX-WIN ver. 4.0.1 (Software Development, Tokyo, Japan) to identify sequence variants. The integrated software package DnaSP version 5 (Librado and Rozas, 2009) was used to determine the genotypes or haplotypes. A parsimony network connecting the observed haplotypes to resolve the genealogy was plotted with TCS version 1.21 (Clement et al., 2000). Genetic variation within the populations, expressed as haplotype diversity (h) and nucleotide diversity (π), was estimated according to Nei (1987) based on Kimura's two-parameter distance method using K and DA in the REAP program (McElroy et al., 1992). Pairwise population F_{ST} values were calculated to estimate the genetic differentiation among the populations according to Slatkin and Hudson (1991) using ARLEQUIN version 3.1 (Excoffier et al., 2005). The significance of each F_{ST} value was tested using 10,000 random permutations. Gene flow among populations was estimated with N_em, the number of migrations per generation between population pairs (Slatkin, 1993) using the following equation:

$$N_e m = (1/F_{ST} - 1)/4,$$

where N is the effective population size, m is the effective proportion of immigrants, and F_{ST} is the fixation index. To examine the relationships among populations visually, we used a two-dimensional scaling (TDS) analysis based on the matrix of pairwise F_{ST} values from the *Cyt*b sequence data calculated by SPSS version 14.0K (SPSS, Inc., Chicago, IL, USA).

Neutral expectation and historic demographic expansions were investigated by examining Fu's F_S and Tajima's D and mismatch distributions with the sudden expansion model (Rogers and Harpending, 1992). A goodness-of-fit test was used to test the validity of the sudden-expansion model using a parametric bootstrap approach based on the sum of squared deviations (SSD) to compare the observed and estimated mismatch distributions (Schneider and Excoffier, 1999). Both the neutrality test and mismatch distribution analysis were performed in ARLEQUIN version 2000 (Schneider et al., 2000). Since the mutation rate of the *H. sanguineus Cyt*b gene over the estimated time since expansion was unknown, a molecular clock was calculated using the sequence-divergence rates of mitochondrial protein coding regions for other marine crustaceans; rates ranged from 2.2 to 3.1% per million years (Knowlton and Weigt, 1998; Schubart et al., 1998).

Results

*Cyt*b variation in H. *sanguineus*

The degenerate primers newly designed in this study (Cyt9237 and Cyt10050) successfully amplified the mtDNA *Cyt*b region of the 143 *H. sanguineus* individuals. Direct sequencing of the PCR products with two internal primers (HsCytF and HsCytR) yielded a fragment with an amplicon size of 470 bp, which revealed 38 variable nucleotide sites defining 37 haplotypes among the populations (Table 2). The variable nucleotide sites observed consisted of 33 transitions and four transversions. All substitutions were biallelic except one, which was triallelic, suggesting the occurrence of a single base substitution among sequences. The nucleotide sequences of the 37 haplotypes have been deposited in the DDBJ/GenBank database under accession numbers AB570203-AB570239.

The parsimony network of the *Cyt*b haplotypes in *H. sanguineus* did not provide evidence of geographical association (Fig. 2). In the network, two focal haplotypes, HeS1 and HeS12, were abundant, whereas the others, including singletons, were rare and radiated from these focal haplotypes. The distribution of the 37 haplotypes among the six *H. sanguineus* populations is presented in Table 3. Among them, 28 were found at single localities, and nine (HeS1, HeS4, HeS9,

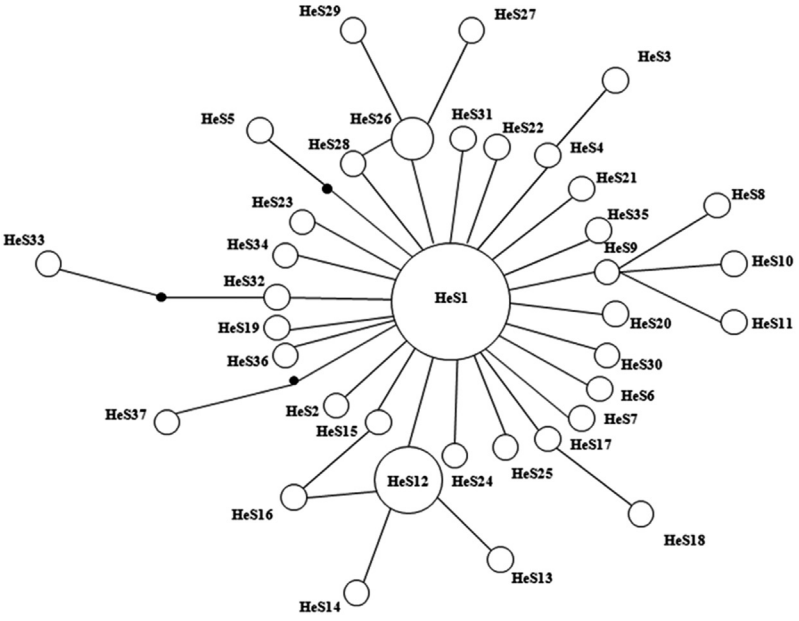

Fig. 2. A single minimum spanning tree for the 38 mitochondrial cytochrome *b* region haplotypes of Asian shore crab *Hemigrapsus sanguineus* (Table 2). Circle sizes reflect haplotype abundance..

Table 2. Variable nucleotide sites in 470 bp sequences of mitochondrial cytochrome *b* gene partial sequences in Asian shore crab *Hemigrapsus sanguineus*

Haplotype	Variable nucleotide sites																																				
	2	17	35	38	47	56	89	95	107	137	159	168	179	188	197	212	215	227	236	263	278	281	285	293	308	320	323	341	350	353	365	368	371	386	428	437	455	470
HeS1	A	A	C	T	G	A	T	T	G	T	A	C	T	G	C	T	A	A	C	T	T	G	A	C	A	C	T	T	C	A	G	C	A	A	T	A	T	A
HeS2	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
HeS3	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	C	·	·	·	·	·	·	·	·	·	·
HeS4	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	·	·	·	·	·	·	·	·	·	·	·
HeS5	·	·	·	·	·	·	·	C	·	·	·	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
HeS6	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	·	·	·	·	G	·	·	·	·
HeS7	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
HeS8	·	·	·	·	·	·	·	·	·	·	G	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
HeS9	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
HeS10	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·
HeS11	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·
HeS12	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·
HeS13	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·
HeS14	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	A	T	·	·	·	·	·	·
HeS15	·	·	·	C	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
HeS16	·	·	·	C	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·
HeS17	·	·	·	·	·	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
HeS18	·	·	·	·	·	·	·	·	·	·	·	·	C	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
HeS19	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	·	·	·	·	·	·
HeS20	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
HeS21	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	C	·
HeS22	·	·	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
HeS23	G	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
HeS24	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
HeS25	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G
HeS26	·	·	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
HeS27	·	G	·	·	·	·	·	·	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
HeS28	·	·	·	·	·	·	·	·	C	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
HeS29	·	·	·	·	·	G	·	·	A	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
HeS30	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	·	·	·	·	·
HeS31	·	·	·	·	·	·	·	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
HeS32	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·
HeS33	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	G	T	·	T	·	·	·	·	·	·	·	·	·	·	·	·
HeS34	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
HeS35	·	·	·	·	·	·	C	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·
HeS36	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	C	·	·	·
HeS37	·	·	·	·	·	·	·	·	·	C	·	T	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·	·

Table 3. Distribution of mitochondrial cytochrome *b* haplotypes among 6 populations of Asian shore crab *Hemigrapsus sanguineus*. Sampling site abbreviations (first row) are listed in Table 1

	TAE	BUA	HAE	GUJ	GUR	JUM	Total
HeS1	14	43	11	14	8	11	101
HeS2						1	1
HeS3	1						1
HeS4		1			1		2
HeS5					1		1
HeS6				1			1
HeS7			1				1
HeS8						2	2
HeS9			1		1		2
HeS10		1					1
HeS11						1	1
HeS12	6	4	4	5	3		22
HeS13		1					1
HeS14					1		1
HeS15			1				1
HeS16			1				1
HeS17						1	1
HeS18			1				1
HeS19					1		1
HeS20		1					1
HeS21			1		1		2
HeS22						2	2
HeS23						1	1
HeS24						1	1
HeS25						1	1
HeS26	1	1	1	1	2		6
HeS27				1			1
HeS28				1			1
HeS29					1		1
HeS30		1		1	1		3
HeS31		1					1
HeS32			1			1	2
HeS33			1				1
HeS34						1	1
HeS35	1				2		3
HeS36	1						1
HeS37					1		1

TAE, Taean; BUA, Buan; HAE, Haenam; GUJ, Geoje; GUR, Guryonpo; JUM, Jumunjin.

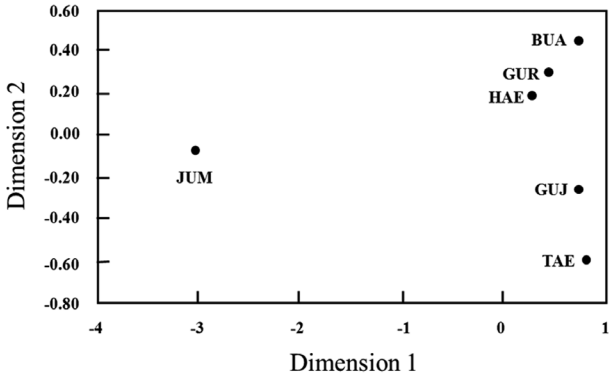

Fig. 3. Two-dimensional scaling analysis based on the matrix of pairwise F_{ST} values from the mitochondrial cytochrome *b* sequence data for Asian shore crab *Hemigrapsus sanguineus*. BUA, Buan; GUJ, Geoje; GUR, Guryonpo; HAE, Haenam; JUM, Jumunjin; TAE, Taean.

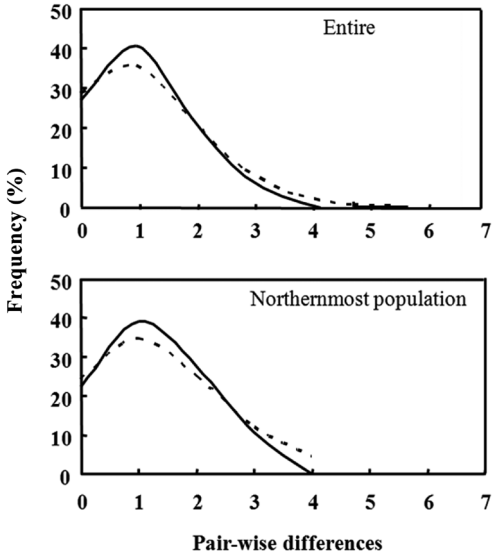

Fig. 4. Mismatch distribution constructed using pairwise differences among mitochondrial DNA (mtDNA) haplotypes of Asian shore crab *Hemigrapsus sanguineus* from the pooled populations and from a single northeastern population (Jumunjin [JUM]). Solid lines, observed frequency; dashed lines, frequency distribution expected from a sudden expansion model.

HeS12, HeS21, HeS26, HeS30, HeS32, and HeS35) were observed in two or more localities. A number of individuals from the populations examined had the HeS12 and HeS26 haplotypes, although these were not found in JUM. HeS1 occurred in all populations.

The haplotype and nucleotide diversities of *H. sanguineus* demonstrated varying levels of genetic variation among populations. Variation was higher in the Guryonpo (GUR), Haenam (HAE), and Jumunjin (JUM) populations (Table 1). Reduced haplotype diversity was observed in the Taean (TAE), Buan (BUA), and Geoje (GUJ) populations.

Population genetic analysis

The pairwise population F_{ST} estimates and migration rate based on the *Cyt*b sequences are presented in Table 4. Most pairwise values were not significantly different among populations. Although the pairwise F_{ST} estimates showed no clear genetic differentiation (low values among populations), the pairwise population F_{ST} estimates were large when JUM was compared with the other populations. These findings were evident in the TDS analysis and N_em values (Fig. 3). Overall, the TAE, BUA, HAE, GUJ, and GUR populations were genetically close to one another, but distinct from JUM.

Mismatch distributions for all populations pooled and the northernmost population (JUM) are shown in Fig. 4. The pooled populations and JUM had no additional distribution peaks; the highest frequency occurred at one difference. The *D*, Fu's F_S, and SSD (Table 5) values indicated expansion in these populations. Tajima's D and Fu's F_S were significantly negative, with markedly reduced SSD in the pooled popula-

Table 4. Pairwise F_{ST} (below the diagonal) and N_em (above the diagonal) estimates among the six populations of Asian shore crab *Hemigrapsus sanguineus*.

	TAE	BUA	HAE	GUJ	GUR	JUM
TAE		11.1	28.0	14.1	23	3.5
BUA	-0.023		21.1	12.8	18	6.3
HAE	-0.009	-0.012		31.5	50.3	7.8
GUJ	-0.018	-0.020	-0.008		15.9	4.3
GUR	-0.011	-0.014	-0.005	-0.016		8.0
JUM	0.067*	0.038*	0.031*	0.055*	0.030*	

TAE, Taean; BUA, Buan; HAE, Haenam; GUJ, Geoje; GUR, Guryonpo; JUM, Jumunjin.
*Significant differentiation ($P < 0.05$) on the exact test (Raymond and Rousset, 1995).

tion group and JUM population, a parameter combination that strongly supported sudden expansion. Sudden expansion of the pooled population group was estimated to have occurred 0.042-0.060 million years ago (Ma) and that in the JUM population, 0.049-0.069 Ma (Table 5).

Discussion

The genetic variation analysis of the mitochondrial *Cyt*b gene revealed the followings: (i) haplotype and nucleotide diversity occurred at various levels of genetic variation and (ii) there was a low level of genetic differentiation in coastal areas of Korea, but there was some genetic differentiation of the northernmost population (JUM) in the East Sea from the other populations. The observed haplotypes from the *Cyt*b region in *H. sanguineus* were arrayed in star-like genealogies, each with several closely related, low-frequency haplotypes around a central high-frequency haplotype, suggesting a shallow haplotype genealogy. The star-like pattern and shallow genealogy indicate recent appearance and rapid population growth (Slatkin and Hudson, 1991; Rogers and Harpending, 1992). Our estimates showed that *H. sanguineus* began expanding 42,862-60,396 years ago (Table 5). High haplotype diversity (0.616-0.880) but low nucleotide diversity (0.0017-0.0034) within the populations indicates that the populations might have experienced historically rapid population growth from an ancestral population with a small effective population size in the Late Pleistocene (Avise, 2000).

A reduced genetic diversity in the TAE and BUA populations from the west coast in the Yellow Sea compared with the other regional populations, except the GUJ population, was observed from the haplotype and nucleotide diversities. The western Korean populations showed no genetic differentiation from the southern populations as inferred from the pairwise F_{ST} values. Therefore, crabs might have been introduced continuously with high rates of gene flow to the TAE and BUA populations from other sources following a reduction in effective population size.

The estimated pairwise F_{ST} values, N_em rate, and TDS analysis indicate that substantial gene flow has occurred among the populations, suggesting that larval behavior and sea currents are responsible for the high rates of gene flow. The passive dispersal of planktonic larvae and sedentary lifestyle of adult marine invertebrates limit the formation of population substructure (Lessios et al., 2003; Waters and Roy, 2004). Many other marine crab species reportedly have high levels of gene flow between populations or regions (Beckwitt, 1985; Merkouris et al., 1998; Azuma et al., 2008). Hwang et al. (1993) reported that the planktonic larval stage of *H. sanguineus* persists for 25 days until metamorphosis on the sea floor to form the first crab stage. In general, crustacean species with similar persistent pelagic larval stages have a high dispersal potential that produces genetic homogeneity among local populations (Palumbi, 1994). The Tsushima Warm Current (TC) branches off the Kuroshio Current, with part of the TC running into the Yellow Sea and the main part entering the East Sea along the Korean Peninsula (Fig. 1). Therefore, the TC might transport *H. sanguineus* larvae to the western coast of Korea.

Despite the lack of geographically associated haplotypes and genetic structure within and among populations, our analyses indicated a degree of genetic differentiation between the northernmost population (JUM) in the East Sea and the other populations. The sub-polar front in the East Sea is similar to the western boundary current in that a polar front forms at the boundary between the low-temperature, low-salinity waters of the northern region and the high-temperature, high-salinity waters of the southern region (Rhein et al., 1995; Pickart et al., 1997). The sub-polar front, which extends along the coast of Japan before turning abruptly at the Noto Peninsula frontal region toward the center of the East Sea, has a close relationship to the TC and cold-water currents, including the Liman Current (LC) (Senjyu, 1999; Ichikawa and Beardsley, 2002) (Fig. 1). Restricted or se-

Table 5. Asian shore crab *Hemigrapsus sanguineus*: parameters of the sudden expansion model and estimated time since expansion

Populations	Sample size	D (P-value)	F_S (P-value)	SSD (P-value)	τ (95% CI)	θ_0 (95% CI)	θ_1 (95% CI)	Time since expansion (y)
Entire	143	-2.491 (0.001)	-29.249 (0.000)	0.003 (0.059)	1.249 (0.538-1.519)	0.000 (0.000-0.742)	2,071.875 (7.677-5,351.875)	42,862-60,397
JUM	23	-1.970 (0.013)	-8.261 (0.000)	0.004 (0.482)	1.440 (0.184-2.205)	0.000 (0.000-1.493)	1,905.000 (11.560-6,730.000)	49,417-69,633

D, Tajima's D; F_S, Fu's F_S; SSD, sum of squared deviations in the goodness of-fit-test; τ , time since expansion measured in mutational time units; CI, confidence interval; θ_0 and θ_1, population sizes scaled by mutation rate before and after expansion, respectively.

lective gene flow from main distributions can result from such physical barriers, which restrict larval transport (Hedgecock, 1986; Scheltema, 1986; Bowen and Avise, 1990; Palumbi, 1994; Burton, 1998). The JUM population is located at the sub-polar front, where the cold and warm currents of the East Sea meet. Therefore, the balanced effects of the cold (LC) and warm (TC) water currents in the East Sea might explain the genetic differentiation between the JUM population and other wild populations.

The mismatch distribution in our data was unimodal, and the neutrality test gave a significantly negative value, suggesting recent population expansion of *H. sanguineus* in Korea. Estimates of other mismatch distribution parameters corroborate this evidence. Therefore, *H. sanguineus* population expansion in Korean coastal waters resulted from rapid population growth and recent, sudden expansion in the Late Pleistocene. This was corroborated by the star-like genealogy of the haplotypes, high haplotype diversities, close genetic similarities among haplotypes, mismatch distribution pattern supporting a sudden expansion model, and estimated expansion time. However, this perspective remains ambiguous, and further extended sampling from Hong Kong through Japan to Russia is necessary to clarify the historical influences.

Based on our results, the *H. sanguineus* populations might be one large panmictic population in Korean coastal waters, with the exception of the northeastern population. This genetic information about the current condition of *H. sanguineus* will be useful in developing a conservation strategy and subsequent ecological monitoring. *H. sanguineus* has colonized the eastern coast of the USA, Atlantic France, and the Netherlands in recent years. Comparative studies of genetic variation and structure in donor and invader populations using the molecular procedures employed in this study are essential for developing a greater understanding of the spatiotemporal invasion-pattern mechanism. Finally, we demonstrated that genetic variation analysis using mtDNA *Cyt*b sequences is a useful model for research on population-level studies in closely related species.

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