Rep. Res. Edu. Ctr. Inlandwat. Environ., 6 : 115-123 (2010)

Population genetic structure of Yamato-shijimi clam in Lake Shinji, Japan

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Abstract: Yamato-shijimi clam *Corbicula japonica* is the best-known bivalve inhabiting in brackish estuaries and lakes around Japan and one of the most commercially important species in inland fisheries. Although the amount of *C. japonica* from Lake Shinji has accounted for the large part of the domestic catch, its amount has dramatically fallen in recent years. This study was conducted to verify the genetic structure and reproduction mechanism of *C. japonica*, both of which are essential to its stock management in Lake Shinji. We analyzed sequence polymorphism of the 588 bp portion of the mitochondrial DNA cytochrome oxidase subunit I (COI) gene to determine the population genetic structure of *C. japonica* in Lake Shinji. Among a total of 177 *C. japonica* specimens collected from 4 colonies, 37 haplotypes were obtained, and 2 major haplotypes were apparent with relatively high abundance in all colonies. Well corresponding mismatch distributions along the COI gene were determined for the data sets of the individual colonies, and the pairwise population estimates F_{ST} among the individual colonies were also generally low. Such small genetic differentiation of *C. japonica* is derived from high gene flow in Lake Shinji, and this could be caused by a lake-wide dispersion of its larvae mediated by the water movements.

Key words: yamato-shijimi clam, *Corbicula japonica*, Lake Shinji, population genetic structure, gene flow

Introduction

Several species have been recognized in the genus *Corbicula* that inhabits in estuaries, lakes, and rivers widely around East Asia, though *Corbicula* is a morphologically variable and taxonomically complicated group of bivalves (Park and Kim 2003). *Corbicula* is the best-known infaunal

suspension-feeding bivalve that plays an important role in the ecosystem via feeding and nutrient excretion activities, because it often dominates the macrobenthic community in waters (Nakamura et al. 1988; Yamamuro and Koike 1993). In recent years, illegal ingressions of the other exotic *Corbicula* species from East Asian areas frequently occurred in Japan, and then not only ecological but also genetic disturbances have been seriously worried (Suzuki et al. 2006). Otherwise, *Corbicula* is one of the most commercially important resources in Japan, because its fisheries catch reached approximately 17,000 metric tons corresponding to 28% of the total domestic catch of inland fisheries in 2001 (Kasai et al. 2006). Among 3 dominant *Corbicula* species in Japan, yamato-shijimi clam *C. japonica* is most widely distributed from cold Hokkaido through warm Kyushu and often dominates in estuaries and brackish lakes (Kasai and Nakata 2000).

Lake Shinji with the surface area of 79.2 km^2 and adjacent Nakaumi Lagoon with that of 86.8 km^2 are the Hii River estuary draining into the Sea of Japan (Uye et al. 2000) and the second largest brackish- water ecosystem in Japan (Fig. 1). Lake Shinji is one of the best-known fishing grounds of *C. japonica*, but its annual catch in Lake Shinji gradually decreased from approximately 18,000 metric tons in the 1960s to 9,000 metric tons in 1990 and had been on the lowest level of 8,000 metric tons a year on average during 1991 and 2000 (Oshima et al. 2004; Nanbu et al. 2008). In order to verify the current reproduction mechanism and design the stock management of *C. japonica* in Lake Shinji, this study was conducted to determine its population genetic structure based on sequence polymorphisms of the mitochondrial DNA cytochrome oxidase subunit I (COI) gene.

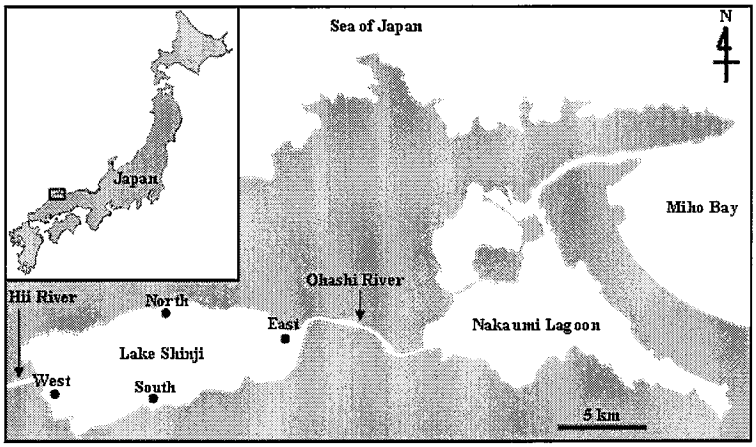

Fig. 1. Maps of Lake Shinji, Japan, showing north, south, east, and west colonies at which a total of 177 *Corbicula* specimens were collected.

Materials and methods

Sample collection

A total of 177 *Corbicula* specimens were collected from 4 colonies of Lake Shinji; north (N=44), south (N=45), east (N=45), and west (N=43); in April 2009 (Fig. 1). All specimens were boiled, and adductor muscle was excised from soft tissue followed by stored in a laboratory freezer at –20 °C until DNA preparation.

DNA preparation

High quality total genomic DNA was prepared from small scraps of frozen adductor muscle according to the modified urea-SDS-Proteinase K method (Aranishi and Okimoto 2004, 2005; Aranishi 2006). Samples were incubated in the extraction buffer (10 mM Tris-HCl, pH 7.5, 20 mM EDTA, pH 8.0, 1 % SDS, and 4 M urea) containing 25 μg Proteinase K at 55 °C, and then 5 M NaCl was added and mixed. DNA was isolated with phenol-chloroform-isoamyl alcohol and subsequent chloroform-isoamyl alcohol followed by precipitation with ethanol. DNA pellet was washed with ethanol, dried, and resuspended in 10T0.1E (10 mM Tris-HCl, pH 7.5, 0.1 mM EDTA, pH 8.0).

PCR amplification

PCR amplification of an apparent 630 bp fragment encoding the partial COI gene was performed in GoTaq Green PCR Master Mix (Promega) containing 2 mM $MgCl_2$, 0.5 μM each primer, and template DNA in a Techgene thermal cycler (Techne). The primers used were LCO1490 5'-GGTCA ACAAA TCATA AAGAT ATTGG-3' and LCO2198 5'-TAAAC TTCAG GGTGA CCAAA AAATC A-3' (Folmer et al. 1994). PCR protocol consisted of an initial denaturation at 94 °C for 2 min, followed by 35 cycles of 10 sec at 94 °C, 20 sec at 54 °C and 40 sec at 72 °C, and a final extension at 72 °C for 5 min. The PCR products were analyzed using a DNA-1000 Reagent Kit (Shimadzu) containing a SYBR Gold Nucleic Acid Gel Stain (Invitrogen) in a MCE-202 MuitiNA microchip electrophoresis system (Shimadzu).

Nucleotide sequencing

Nucleotide sequencing of double strands of PCR products was accomplished using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) in an automated 3730xl DNA Analyzer (Applied Biosystems). Although the amplified 630 bp fragment of the partial COI gene was directly

sequenced using the LCO1490 primer, only the 588 bp portion was selected as the dataset for the following analysis to avoid erroneous sequence determination at the 5' and 3'-end regions.

Data analysis

Multiple sequence alignments were performed to define haplotypes based on nucleotide polymorphisms using MEGA 4.0 (Tamura et al. 2007). Phylogenetic analysis was conducted by the neighbor-joining (NJ) methods based on genetic distances estimated by Kimura's two-parameter model (K2P, Kimura 1980), together with reference sequences of *C. japonica* (GenBank Accession numbers AF196271, AF367440 and AF367441) and *C. fluminea* (ibid., AF120666 and U47647) as an outgroup. Haplotype genealogy was resolved by haplotype network using TCS Network version 1.21 (Clement et al. 2000). Arlequin version 3.11 (Excoffier et al. 2005) was used for estimation of the haplotype and nucleotide diversities within samples and pairwise F_{ST} values between samples (Reynolds et al. 1983) and for exact tests of population differentiation between colonies (Raymond and Rousset 1995). Significance of the variance components and F_{ST} values was tested via the permutation method.

Results

Table 1. Variable nucleotide positions of *C. japonica* haplotypes based the mitochondrial DNA COI gene by north, south, east, and west colonies of Lake Shinji

			Site																																			
Sampling colony	Haplotype	N	7	10	15	49	52	58	65	67	130	142	154	157	165	214	217	244	265	271	295	315	327	352	356	362	385	427	430	442	472	484	526	544	550	553	556	580
North	HT-01	19	T	A	A	A	G	A	T	A	C	G	C	A	G	A	A	T	G	A	G	T	C	G	G	C	A	T	G	T	G	G	A	T	G	A	A	G
	HT-02	14	C																		A																	A
	HT-03	2													A																							
	HT-04	3	C												A						A																	A
	HT-05	1	C															C			A																	A
	HT-10	1	C										T								A																	A
	HT-11	1																									G	C										
	HT-12	1	C																	G	A																	A
	HT-13	1			G										A																							A
	HT-14	1													A																					G		
South	HT-01	25																																				
	HT-02	14	C																		A																	A
	HT-03	1													A																							
	HT-07	2																																	A			
	HT-10	1	C										T								A																	A
	HT-15	1	C																A		A																	A
	HT-16	1	C												A						A														A			A
East	HT-01	15																																				
	HT-02	9	C																		A																	A
	HT-03	4													A																							
	HT-04	2	C												A						A																	A
	HT-05	1	C															C			A																	A
	HT-06	2													A																				A			
	HT-17	1	C							G					A						A																	A
	HT-18	1													A								A							C					A			
	HT-19	1													A								A											C				
	HT-20	1													A								A	A	A													A
	HT-21	1													A							C									A							
	HT-22	1				G									A																							
	HT-23	1	C												A			C			A																	A
	HT-24	1													A																	A						A
	HT-25	1	C				A								A						A																	A
	HT-26	1						G	T						A											T			A									A
	HT-30	1													A																							A
	HT-37	1													A	G																						A
West	HT-01	10																																				
	HT-02	12	C																		A																	A
	HT-03	5													A																							
	HT-04	3	C												A						A																	A
	HT-08	2		T											A								A															
	HT-09	2													A		G																					
	HT-27	1													A																			C				
	HT-28	1													A																						G	
	HT-29	1						C							A																							
	HT-31	1		T											A																							
	HT-32	1									T				A																							
	HT-33	1										A			A																							
	HT-34	1	C												A						A								T									A
	HT-35	1												G																								
	HT-36	1	C												A						A												G					A

Nucleotide numbers at sites are given for HT-01. Dot represents the same nucleotide as HT-01

Estimation of the 588 bp sequence encoding the partial COI gene disclosed 36 variable sites in a total of 177 *Corbicula* specimens from 4 colonies of Lake Shinji, defining 37 haplotypes designated as HT-01 to HT-37 (Table 1). Haplotypes HT-01 to HT-10 and HT-11 to HT-37 were identified to be common between more than 2 specimens and unique to specimen, respectively, and all of these haplotypes were verified to be *C. japonica* by the NJ tree analysis (Fig. 2). In haplotype network, focal haplotypes of HT-01, HT-02, HT-03,

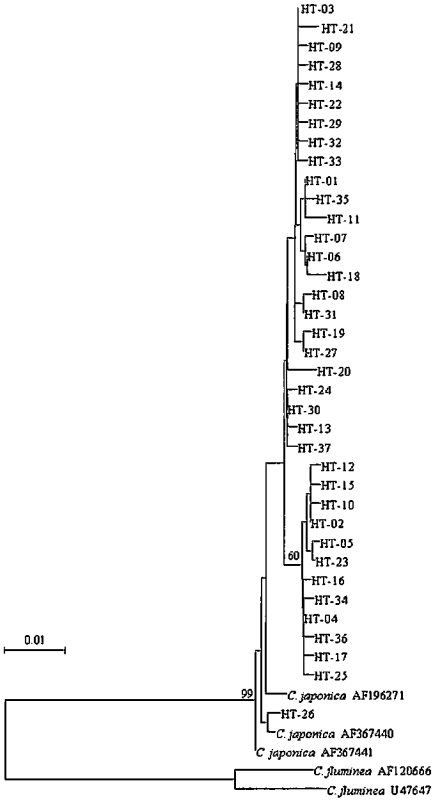

Fig. 2. NJ tree of *C. japonica* haplotypes based on the mitochondrial DNA COI gene by genetic distances estimated according to K2P.

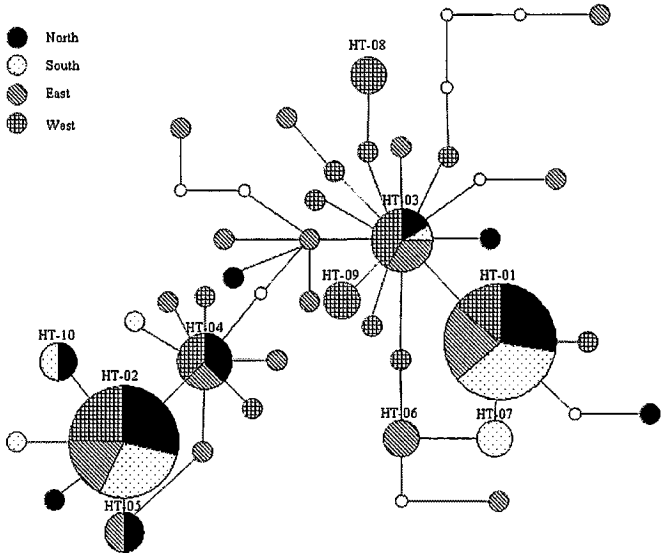

Fig. 3. Haplotype network tree of *C. japonica* haplotypes based on the mitochondrial DNA COI gene by north, south, east, and west colonies of Lake Shinji. White dot represents putative mutational step between haplotypes. Size of circle is proportional to the number of individuals per haplotype.

Table 2. Nucleotide diversity (±SD), haplotype diversity (±SD), and numbers of transition and transversion based on the mitochondrial DNA COI gene of *C. japonica* by north, south, east, and west colonies of Lake Shinji

Sampling colony	Nucleotide diversity (%)	Haplotype diversity	Transition	Transversion
North	0.3622± 0.2281	0.7188± 0.0491	11	0
South	0.2985± 0.1960	0.6040± 0.0566	7	0
East	0.5014± 0.2970	0.8495± 0.0406	22	0
West	0.4559± 0.2749	0.8605± 0.0340	13	2
total	0.4108± 0.2480	0.7649± 0.0240	34	2

and HT-04 were apparent with relatively high abundance, from which other infrequent haplotypes were radiated (Fig. 3). HT-01, HT-02, and HT-03 haplotypes commonly occurred in all colonies, and the frequency of HT-01 was calculated to be higher than 50 % in the south colony. Table 2 shows that both of haplotype and nucleotide diversities were higher in the west and east colonies than in the north and south ones. In addition, the transition: transversion rates suggest that the regional differentiation was mostly ascribed to the divergence among the west and other 3 colonies.

Otherwise, well corresponding mismatch distributions were determined for individual colonies (Fig. 4), and the pairwise population estimates F_{ST} among individual colonies were generally low (Table 3). However, the higher F_{ST} estimates than 0.050 were found between the south and east colonies (0.053) and the south and west colonies (0.063).

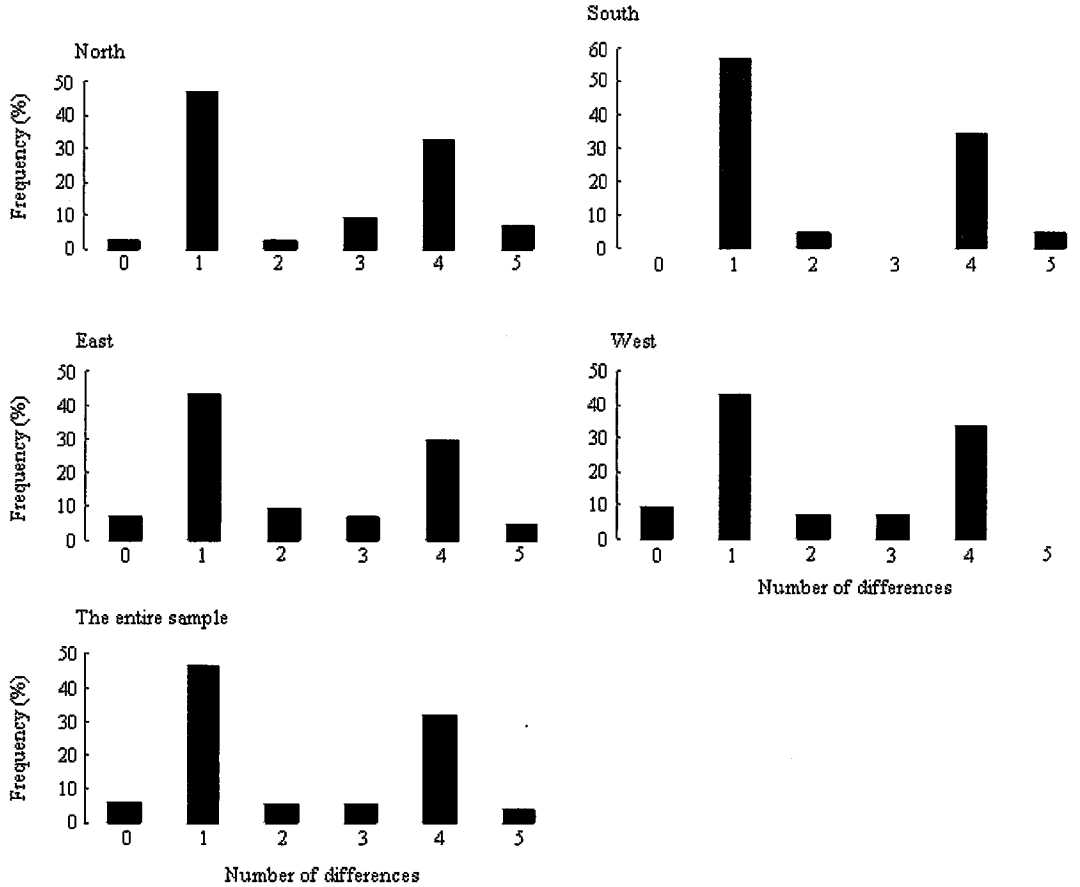

Fig. 4. Mismatch distributions along the mitochondrial DNA COI gene of *C. japonica* by north, south, east, west colonies, and entire samples of Lake Shinji.

Table 3. Pairwise population estimates F_{ST} values based on the mitochondrial DNA COI gene of *C. japonica* (below diagonal) and probability of differentiation with *P* value in F_{ST} estimate (above diagonal) by north, south, east, and west colonies of Lake Shinji

Sampling colony	North	South	East	West
North		-	-	-
South	-0.001		+	+
East	0.029	0.053		-
West	0.028	0.063	-0.011	

Not significant in both estimates (-) and significant in both estimates (+). Significance was tested at the 5 % level with a Bonferroni-corrected *P* for multiple tests.

Discussion

Nucleotide sequences of the mitochondrial DNA COI gene have often been used as a tool for determining population genetic structure of aquatic invertebrates (Azuma et al. 2008). Based on recent phylogenetic study of *C. japonica* using the mitochondrial DNA COI gene, dominant *Corbicula* species in Japan were clearly divided into the estuary group including *C. japonica* and freshwater one including *C. leana* and *C. sandai* (Suzuki et al. 2008). In addition, a robust dichotomy between *C. japonica* and *C. fluminalis*, which is the exotic species widely spreading in Japan, in the estuarine group was evident (Park and Kim 2003). Both of *C. japonica* and *C. fluminalis* were therefore included as reference in phylogenetic analysis of the specimens collected in Lake Shinji, and all of them were determined to be *C. japonica* (Fig. 2).

It is surprising that this study first demonstrated population genetic profile of *C. japonica* in Lake Shinji, whereas the amount of *C. japonica* from Lake Shinji has so far accounted for the major part of its domestic catch (Nanbu et al. 2008). Results obtained in this study suggest the insignificant differentiation among 4 colonies of Lake Shinji (Tables 2 and 3, Fig. 4). In addition, haplotypes analysis showed the radiation of 33 minor haplotypes from 4 focal haplotypes and an association of high frequency of HT-01 and HT-02 with all of 4 colonies (Fig. 3). This haplotype distribution may likely favor post-expansion shuffling rather than recent expansion of the major maternal lineages of *C. japonica* in Lake Shinji. The calculated nucleotide divergences less than 0.7 % among these 4 focal haplotypes also suggest a shallow haplotype genealogy derived from high gene flow (Table 1, Fig. 3).

Passive dispersal of planktonic larvae may be strongly mediated by water movements, and the effect of water movements on the population genetic structure is commonly indicated in aquatic invertebrates (Lessios et al. 2003; Waters and Roy 2004). Adult *C. japonica* is a benthic species inhabiting on brackish grounds, but its planktonic larval stage lasts about 12 days (Kimura et al. 2004). This study genetically demonstrated the low differentiation between the north and south colonies and the marked one between the east and south colonies and between the west and south colonies (Table 3). These results allow us to deduce a possible effect of water movements on a lake-wide larval dispersal of *C. japonica* in Lake Shinji as follows; clockwise circulation of its

larvae from west through north to south at the western half area of the lake, and counterclockwise circulation of its other larvae from east through north to south at the eastern half area of the lake.

Acknowledgement

We thank Professor M. Aizaki and M. Endo, Shimane University, and Shimane Prefectural Fisheries Technology Center for assistance of field sampling of *C. japonica* in Lake Shinji. This study was supported in part by a grant from the Ministry of Land, Infrastructure, Transport and Tourism of Japan.

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