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Genetic structure of wild brown sole inferred from mitochondrial DNA analysis

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The population structure of brown sole was examined in a total of 308 samples collected from three geographical groups: one locality (Gangneung) on the east side of the Korean Peninsula, two localities (Erimo and Tomakomai) on the southeastern side and four localities (Onishika, Teshio, Tomamae and Yoichi) on the northwestern side of Hokkaido Island, Japan, by using sequences of 484 bp from the 5′ end of the control region of mtDNA. We detected 225 haplotypes, but 183 of them were unique to an individual. A total of 116 nucleotide sites were variable. Haplotype diversity (h) was very high, ranging from 0.989 to 1.000, and nucleotide diversity (π) was detected between 0.015 and 0.022. Genetic distances (Φ_{ST}) within populations, among populations and among geographical groups were low (0.0002 to 0.0014). No significant difference was detected by the AMOVA test ($P < 0.05$). Pairwise F_{ST} values between sampling localities were also low and not significant. Genetic differentiation was not detected among sampling localities.

Keywords: *Pleuronectes herzensteini*; brown sole; genetic structure; mitochondrial DNA; control region

Introduction

Brown sole, *Pleuronectes herzensteini*, is an important species as an inshore fishery resource in Japan. Stocking of artificial brown sole seeds has been performed since 1998 at the Hidaka district of Hokkaido, Japan (Anonymous 2000). Brood stocks are usually taken from wild populations and the genetic influence of stocking on wild populations has not been sufficiently considered in most ranching species. The genetic diversity of artificial seeds is generally lower than that of wild populations due to unconscious selection and limited numbers of parental brood stock in the hatchery. Thus large-scale stocking may cause a reduction of genetic diversity in wild populations. To minimize the genetic difference between hatchery and wild populations, the genetic structure of wild populations should be clarified before the initiation of stocking in each target species to monitor the effect of the stocked hatchery population on the wild population.

Mitochondrial DNA (mtDNA) has been actively used in genetic studies on population structure because of its compactness, maternal inheritance and relatively high mutation rate (Avise 1998). In artificial stocking, understanding the population structure is required for the preservation of the genetic diversity of the wild population. In flatfish species, high genetic variation in wild populations and reduced variation in hatchery populations were reported (Fujii and Nishida 1997; Asahida et al. 2003; Sekino et al. 2003). The genetic differentiation between hatchery and wild populations should be minimized by an appropriate management procedure based on genetic structure. The stocking of a hatchery population produced from parental fish collected from genetically different populations may damage the indigenous structure of the wild population in which artificial seeds are released. Genetic management must be carried out in each hatchery unit.

In the present study, the genetic diversity in wild populations of brown sole was examined for samples from one locality in Korea and six localities in Hokkaido, Japan, using a sequence of 484 bp from the 5′ end of the control region of mtDNA. Sequence data were compared among samples from different localities to clarify genetic structure of brown sole populations.

Materials and methods

Fish samples

A total of 308 brown sole were collected from six localities in Hokkaido, Japan, and one locality in the Republic of Korea (Figure 1), and fin-clips were fixed in 99.9% ethanol. These localities can be categorized into three geographical groups: the east side of the Korean Peninsula, and the southeastern side and the northwestern side of Hokkaido Island. Twenty-eight individuals were caught off the coast of Gangneung (GAN) in 2004, Republic of Korea. Forty-eight

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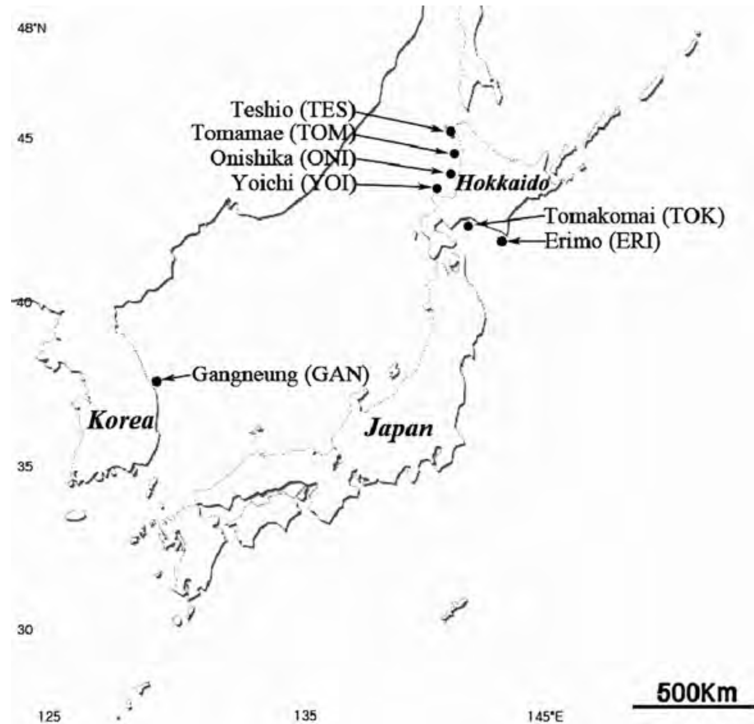

Figure 1. Sampling locations for brown sole, *Pleuronectes herzensteini*, off the coast of Japan and Korea.

individuals and 44 individuals were caught off the coasts of Erimo (ERI) in 2003 and Tomakomai (TOK) district in 2006, respectively, on the southeastern side of Hokkaido. Forty-six, 48, 46 and 48 individuals were caught off the coasts of Onishika (ONI), Teshio (TES), Tomamae (TOM) and Yoichi (YOI) district in 2006, respectively, on the northwestern side of Hokkaido.

DNA extraction and PCR amplification

Genomic DNA were fixed in 99.9% ethanol, stored in 4 M urea buffer (Asahida et al. 1996) and then subjected to extraction of DNA from the fin-clip using the standard phenol-chloroform method (Morishima et al. 2001). The primer sets were designed for an approximately 484 bp portion from the 5′ end according to the control region sequence excluding tandem repeats of barfin flounder deposited in the NCBI Genbank (accession number AB207249; Ortega-Villaizan Romo et al. 2006). PCR was performed in a 15 µl reaction mixture containing a 50–100 ng DNA template. The reaction mixture contained 0.075 µl *r*Taq polymerase (5 U/µl, Takara, Japan), 1.5 µl 10 × PCR buffer, 1.2 µl dNTPs (200 µM), 1.5 µl forward primer (10 pmol/µl, 5′-CCAAAGCTAGGATTCTAGCA-3′) and 1.5 µl reverse primer (10 pmol/µl, 5′-TGCTGGGTAACGAGTCGTAT-3′). The PCR condition included 40 cycles of denaturing for 15 sec at 96°C, annealing for 15 sec at 54°C and extension for 30 s at 72°C, with post-cycling extension for 5 min at 72°C.

Sequence analysis

The PCR products were purified (AMPure® PCR PURIFICATION Kit, Agencourt) and the cycle-sequencing using the PCR primer sets was performed by dideoxynucleotide chain termination (BigDye® Terminator v3.1 Cycle Sequencing Kit, Applied Biosystems). After cycle-sequencing reactions, the products were purified by Agencourt® CleansSEQ® (BECKMAN COULTER, Agencourt Bioscience Corp., USA). The sample was resuspended in 40 µl 0.1 mM EDTA buffer and loaded with an automated sequencer (ABI PRISM™ 3130).

Nucleotide sequence data were analyzed after multiple alignments using the Clustal X for defining haplotypes (Thompson et al. 1997). Using ARLEQUIN ver 2.000 software (Schneider et al. 2000), genetic variability was estimated by the number of polymorphic sites, haplotype and nucleotide diversities. Using the same software, genetic differentiation within the population from each sampling locality, among populations within the same geographical group and among geographical groups (east coast of Korea, southeastern side and northwestern side of Hokkaido Island) was evaluated by the analysis of molecular variance (AMOVA). Genetic differentiation between

sampling localities was also evaluated by the pairwise F_{ST}. Genetic distance matrices of pairwise divergences were used to infer phylogenetic relationships among individuals following the UPGMA method by MEGA 4.0 software.

Results

The sequences of control region gave 225 haplotypes in 308 individuals examined, but each of 183 haplotypes was unique to only one individual (Table 1). The frequency of haplotype H38 (11/308) was the highest among all the haplotypes examined (Table 1). The haplotype H210 (7/308) was the second highest. Two haplotypes, H2 and H102 (6/308), were the third highest. The number of haplotypes ranged between 28 and 45 among sampling localities (Tables 1 and 3). Sequence data were deposited in the DDBJ/ENBL/GenBank under accession no. AB447559.

In approximately 484 bp, a total of 116 nucleotide positions were variable; 52 sites (10.7%; transitions, 41; transversions, 12; indels, 4) in GAN, 60 sites (12.4%; transitions, 53; transversions, 8; indels, 5) in ERI, 71 sites (14.7%, transitions: 63, transversions: 9, indels: 7) in TOK, 57 sites (11.8%; transitions, 49; transversions, 7; indels, 5) in ONI, 67 sites (13.8%; transitions, 59; transversions, 11; indels, 4) in TES, 64 sites (13.2%; transitions, 57; transversions, 11; indels, 5) in TOM, and 56 sites (11.6%; transitions, 52; transversions, 8; indels, 4) in YOI (Tables 2 and 3). Thus, a total of 135 base substitutions (109 transitions and 26 transversions) with eight indels (single base pair insertion/deletion) were found (Tables 2 and 3). Both transitions and transversions occurred in the 20 nucleotide sites (sequence Nos. 17, 39, 159, 173, 182, 185, 186, 187, 204, 205, 214, 227, 247, 249, 254, 287, 288, 291, 295 and 333). More indels appeared in A–G transition sites except for sequence Nos. 290 and 297. Sequence Nos.11, 32 and 195 were characteristic to the samples collected in GAN. Sequence Nos. 35, 195 and 289 were also characteristic to ERI, TES and TOM, respectively.

Haplotype diversity (h) was very high, ranging from 0.989 to 1.000 among samples from different localities (Table 3). Nucleotide diversity (π) was detected between 0.015 and 0.022 (Table 3). Genetic distances (Φ_{ST}) among the three geographical groups (east side of Korean Peninsula, southeastern and northwestern side of Hokkaido Island), among populations within groups and within populations were very low (0.0002 to 0.0014) and significant difference ($P < 0.05$) was not detected by the AMOVA test (Table 4). Pairwise F_{ST} values between sampling localities were also low and not significant (Table 5). Thus, genetic differentiation was not detected among sampling localities.

Geographic characteristics were examined in haplotypic divergence by genetic distance and UPGMA analysis for 225 haplotypes. However, the phylogenetic tree did not show any associations with geographic localities among any ofl the populations, even between Korea and Japan (Figure 2).

Discussion

The mtDNA sequencing analysis in the mtDNA control region revealed high genetic variation including 225 haplotypes (308 individuals) for wild populations of brown sole. Such a high genetic variation has been demonstrated among populations of marine fish species including flatfishes by genetic studies using mtDNA markers. Fujii and Nishida (1997) revealed 54 haplotypes from 55 individuals in mtDNA of olive flounder (*Paralichthys olivaceus*). In summer flounder (*Paralichthys dentatus*; Jones and Quattro 1999) and European plaice (*Pleuronectes platessa*; Hoarau et al. 2004) also show high genetic diversity. Ortega-Villaizan Romo et al. (2006) compared mtDNA diversity among various fish species, including barfin flounder (*Verasper moseri*), spotted halibut (*Verasper variegatus*), olive flounder, plaice, red sea bream (*Pagrus major*) and ayu (*Plecoglossus altivelis*), and concluded that marine fish, except for spotted halibut, showed higher haplotype diversity. Asahida et al. (2003) found 50 different haplotypes among 60 individuals collected from the wild population of olive flounder. They also observed that hatchery populations gave lower genetic variation when compared with wild population.

The frequency of variable sites, which is often used in estimates of nucleotide and haplotypic diversities, in the mtDNA control region in brown sole (about 24%) was lower than that of olive flounder (36%) reported by Fujii and Nishida (1997), and that of summer flounder (34%) reported by Jones and Quattro (1999), but greater than that of barfin flounder (1.3%) reported by Ortega-Villaizan Romo et al. (2006). Although the result by Ortega-Villaizan Romo et al. (2006) was obtained from a small sample size ($n = 22$), the level of variation at 10 sites out of 765 bp of the mtDNA control region was extremely low.

The high variation of mtDNA in olive flounder makes it possible to identify individuals, strains and/or parentage assignment using haplotypes as genetic tags (Sekino et al. 2005). In brown sole, mtDNA sequences can also be used as molecular genetic tags of individuals, because of the abundance of private haplotypes within a species. Therefore, such individual-specific haplotypes make it possible to use them for parentage assignment in hatchery-produced brown sole. In brown sole, assignment of parentage has already been carried out in a hatchery population produced by natural

Table 1. Haploytpe distribution in sampling localities.

	Haplotype																								
Locality	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15	H16	H17	H18	H19	H20	H21	H22	H23	H24	H25
GAN			1	1							1					1									1
ERI		2				1		1			1	1					1						1		
TOK		2			1																				
ONI	1																		1						1
TES							1						1	1		1				1					
TOM										1					1							1		1	
YOI		2							1									1			1				

Locality	H26	H27	H28	H29	H30	H31	H32	H33	H34	H35	H36	H37	H38	H39	H40	H41	H42	H43	H44	H45	H46	H47	H48	H49	H50
GAN																					1				
ERI													4							1					1
TOK	1								1				2	1	1		1						1	1	
ONI		1		1			1				1		2			1									
TES										1			2					1							
TOM					1	1		1											1						
YOI			1									1	1									1			

Locality	H51	H52	H53	H54	H55	H56	H57	H58	H59	H60	H61	H62	H63	H64	H65	H66	H67	H68	H69	H70	H71	H72	H73	H74	H75
GAN				1		1	1														1				
ERI			1						1	1		1					2				1				
TOK					1				1																1
ONI								1	1																
TES	1	2									1			1				1							
TOM									1				1									1	1		
YOI		1													1	1			1	1	1		1	1	

Locality	H76	H77	H78	H79	H80	H81	H82	H83	H84	H85	H86	H87	H88	H89	H90	H91	H92	H93	H94	H95	H96	H97	H98	H99	H100
GAN	1		1														1								1
ERI								1		1												1			
TOK										1		1	1						1	1			1		
ONI				1	1																	1			
TES					1						1														
TOM	1					1						1		1	1			1				1		1	
YOI		1					1		1							1	1				1				

Locality	H101	H102	H103	H104	H105	H106	H107	H108	H109	H110	H111	H112	H113	H114	H115	H116	H117	H118	H119	H120	H121	H122	H123	H124	H125
GAN				1								1													
ERI																									
TOK		2			1				1													1			
ONI			1					1					1	1	1										
TES		2								1								1			1		1	1	
TOM		1				1						1							1	1					
YOI	1	1				1	2				1	1				1	1								1

Locality	H126	H127	H128	H129	H130	H131	H132	H133	H134	H135	H136	H137	H138	H139	H140	H141	H142	H143	H144	H145	H146	H147	H148	H149	H150
GAN							1	1											1	1					
ERI			2			1					1				1						1		1	1	
TOK													1	1											
ONI			1	1					1																
TES			1		1					2		1					1								
TOM									3							1						1			
YOI	1	1																1							1

	H151	H152	H153	H154	H155	H156	H157	H158	H159	H160	H161	H162	H163	H164	H165	H166	H167	H168	H169	H170	H171	H172	H173	H174	H175
GAN						1										1	1					1			
ERI												1			1					1					
TOK							1											1					1		1
ONI		2	1	1					1				1	1					1	1					
TES		1	1		1						1										1				1
TOM	1													1										1	
YOI								1		1						2									

	H176	H177	H178	H179	H180	H181	H182	H183	H184	H185	H186	H187	H188	H189	H190	H191	H192	H193	H194	H195	H196	H197	H198	H199	H200
GAN	1								1													1			1
ERI			2			1	2					1						1			1			2	
TOK		1																	1		1			1	
ONI							1			1	1		1										1		
TES			1	1										1			1								
TOM				1	1		2								1	1				1					
YOI					1			1																	1

	H201	H202	H203	H204	H205	H206	H207	H208	H209	H210	H211	H212	H213	H214	H215	H216	H217	H218	H219	H220	H221	H222	H223	H224	H225
GAN																									
ERI			1										1									1			
TOK					1			1		2	1				1				1				1		
ONI							1			4					1	1					1				1
TES	1		2	1					1			1					1					1			
TOM		1				1								1								1	1	1	
YOI								1		1								1		1					

Table 2. Variable nucleotide sites in the 484 bp sequences from the 5′ end of the mtDNA control region in brown sole.

		Control region																												
		Sequence No.																												
Locality	Nucleotides	11	16	17	20	21	22	24	25	28	32	35	37	38	39	47	73	78	85	88	101	108	109	113	131	133	134	135	136	138
GAN	A	–	–	–	28	–	28	–	2	–	–	28	1	–	–	–	–	–	–	–	27	–	–	–	–	–	–	28	–	23
(n = 28)	C	28	6	28	–	–	–	–	–	–	28	–	–	–	–	–	–	–	26	–	–	27	–	–	4	–	3	–	1	–
	G	–	–	–	–	–	–	–	26	–	–	–	27	28	1	–	–	28	–	–	1	–	–	–		–	–	–	–	5
	T	–	22	–	–	28	–	28	–	28	–	–	–	–	27	28	28	–	2	28	–	1	28	28	24	28	25	–	27	–
	indels	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
ERI	A	–	–	–	48	–	48	–	–	–	–	–	–	1	–	–	–	–	–	–	47	–	–	–	–	–	–	48	–	27
(n = 48)	C	48	11	48	–	1	–	–	–	–	48	–	–	–	1	–	–	–	46	–	–	44	4	–	9	1	6	–	1	–
	G	–	–	–	–	–	–	–	48	–	–	48	48	47	–	–	–	48	–	–	1	–	–	–	–	–	–	–	–	21
	T	–	37	–	–	47	–	48	–	48	–	–	–	–	47	48	48	–	2	48	–	4	44	48	39	47	42	–	48	–
	indels	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
TOK	A	–	–	–	44	–	43	–	–	–	–	44	–	–	–	–	–	1	–	–	44	–	–	–	–	–	–	42	–	27
(n = 44)	C	44	4	44	–	–	–	–	–	–	44	–	–	–	–	–	1	–	40	1	–	40	4	–	4	–	1	–	3	–
	G	–	–	–	–	–	1	–	44	–	–	–	44	44	–	–	–	43	–	–	–	–	–	–	–	–	–	2	–	17
	T	–	40	–	–	44	–	44	–	44	–	–	–	–	44	44	43	–	4	43	–	4	40	44	40	44	43	–	41	–
	indels	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
ONI	A	–	–	–	45	–	46	–	–	–	–	45	–	–	–	–	–	–	–	–	46	–	–	–	–	–	–	45	–	25
(n = 46)	C	46	8	46	–	–	–	–	–	1	46	–	–	–	–	–	–	–	40	–	–	44	3	–	7	–	3	–	3	–
	G	–	–	–	1	–	–	–	46	–	–	1	46	46	1	–	–	46	–	–	–	–	–	–	–	–	–	1	–	21
	T	–	38	–	–	46	–	46	–	45	–	–	–	–	45	46	46	–	6	46	–	2	43	46	39	46	43	–	43	–
	indels	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
TES	A	–	–	–	48	–	48	–	1	–	–	48	1	1	–	–	–	–	–	–	48	–	–	–	–	–	–	46	–	31
(n = 48)	C	48	8	48	–	–	–	–	–	–	48	–	–	–	–	–	–	–	47	–	–	46	2	1	10	–	8	–	2	–
	G	–	–	–	–	–	–	–	47	–	–	–	47	47	–	1	–	48	–	–	–	–	–	–	–	–	–	2	–	17
	T	–	40	–	–	48	–	48	–	48	–	–	–	–	48	47	48	–	1	48	–	2	46	47	38	48	40	–	46	–
	indels	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
TOM	A	–	–	–	46	–	46	–	–	–	–	46	1	–	–	–	–	–	–	–	46	–	–	–	–	–	–	45	–	28
(n = 46)	C	44	8	44	–	–	–	1	–	–	46	–	–	–	–	–	–	–	44	–	–	46	–	–	6	–	4	–	4	–
	G	–	–	1	–	–	–		46	–	–	–	45	46	–	–	–	46	–	–	–	–	–	–	–	–	–	1	–	18
	T	2	38	1	–	46	–	45	–	46	–	–	–	–	46	46	46	–	2	46	–	–	46	46	40	46	42	–	42	–
	indels	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Locality	Nucleotides																													
YOI	A	–	–	–	48	–	48	–	–	–	–	48	1	–	–	–	–	–	–	–	48	–	–	–	–	–	–	47		37
(n = 48)	C	48	5	48	–	–	–	–	–	–	47	–	–	–	–	–	–	–	46	–	–	47	1	–	2	–	–	–	2	–
	G	–	–	–	–	–	–	–	48	–	–	–	47	48	–	–	–	48	–	–	–	–	–	–	–	–	–	1	–	11
	T	–	43	–	–	48	–	48	–	48	1	–	–	–	48	48	48	–	2	48	–	1	47	48	46	48	48	–	46	–
	indels	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

		Control region																												
		Sequence No.																												
Locality	Nucleotides	145	148	149	152	156	157	158	159	160	164	165	166	167	168	169	170	173	174	175	180	181	182	183	184	185	186	187	188	190
GAN	A	–	26	–	27	–	–	–	22	28	28	6	27	28	28	24	–	–	23	23	–	3	–	–	–	2	26	27	–	–
	C	–	–	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	15	1	–	–	28	28
	G	–	2	–	1	–	–	–	5	–	–	19	1	–	–	–	–	1	5	5	–	25	–	–	–	–	–	–	–	–
	T	28	–	25	–	28	28	28	1	–	–	–	–	–	–	–	28	27	–	–	28	–	28	28	13	25	2	1	–	–
	indels	–	–	–	–	–	–	–	–	–	–	3	–	–	–	4	–	–	–	–	–	–	–	–	–	–	–	–	–	–
ERI	A	–	46	–	48	–	–	–	47	47	48	12	48	48	48	41	–	–	35	34	–	6	–	–	–	3	42	48	–	–
	C	1	–	5	–	1	–	–	–	–	–	–	–	–	–	–	1	1	–	–	–	–	2	–	24	–	–	–	48	48
	G	–	2	–	–	–	–	–	1	1	–	32	–	–	–	2	–	2	13	14	–	42	–	–	–	–	1	–	–	–
	T	47	–	43	–	47	48	48	–	–	–	–	–	–	–	–	47	45	–	–	48	–	46	48	24	45	5	–	–	–
	indels	–	–	–	–	–	–	–	–	–	–	4	–	–	–	5	–	–	–	–	–	–	–	–	–	–	–	–	–	–
TOK	A	–	43	–	41	–	–	–	40	43	44	10	42	44	43	42	–	–	31	35	–	3	1	–	–	2	42	44	–	–
	C	–	–	4	–	–	–	1	–	–	–	–	–	–	–	–	2	–	–	–	1	–	–	–	25	2	–	–	44	44
	G	–	1	–	3	–	–	–	4	1	–	26	2	–	–	1	–	–	13	9	–	41	–	–	–	1	–	–	–	–
	T	43	–	44	–	44	44	43	–	–	–	–	–	–	–	–	42	44	–	–	43	–	43	44	19	39	2	–	–	–
	indels	–	–	–	–	–	–	–	–	–	–	8	–	–	1	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–
ONI	A		44		46	–	–	–	43	42	46	9	46	46	43	46	–	–	31	35	–	3	–	–	–	2	43	44	–	–
	C	–		5	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	1	–	–	–	19	4	–	–	45	46
	G	–	2	–	–	–	–	–	3	4		32	–	–	–	–	–	–	15	11		43	–	–	–	–	–	–	–	–
	T	46	–	41	–	46	46	46	–	–	–	–	–	–	–	–	45	46	–	–	45	–	46	46	27	40	3	2	1	–
	indels	–	–	–	–	–	–	–	–	–	–	5	–	–	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
TES	A	–	48		48	–	–	–	45	47	48	10	48	48	47	38	–	–	35	34	–	6	–	–	–	3	40	47	–	–
	C	–	–	9	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	1	20	4	–	–	47	47
	G	–	–	–	–	–	–	–	3	1	–	33	–	–	1	6	–	2	13	14	–	42	–	–	–	–	3	1	–	–
	T	48	–	39	–	48	48	48	–	–	–	–	–	–	–	–	48	46	–	–	48	–	46	47	28	41	5	–	1	1
	Indels	–	–	–	–	–	–	–	–	–	–	5	–	–	–	4	–	–	–	–	–	–	–	–	–	–	–	–	–	–
TOM	A	–	45	–	46	–	–	–	43	45	45	14	45	45	46	37	–	–	34	35	–	4	–	–	–	2	39	45	–	–
	C	–	–	3	–	–	–	–	–	–	–	–	–	–	–	–	1	1	–	–	–	–	–	–	24	3	–	–	45	46
	G	–	1	–	–	–	–	–	3	1	1	28	1	1	–	7	–	1	12	11	–	42	–	–	–	–	3	–	–	–
	T	46	–	43	–	46	46	46	–	–	–	–	–	–	–	–	45	44	–	–	46	–	46	46	22	41	4	1	1	–
	indels	–	–	–	–	–	–	–	–	–	–	4	–	–	–	2	–	–	–	–	–	–	–	–	–	–	–	–	–	–
YOI	A	–	47	–	47	–	–	–	41	48	47	15	48	48	44	45	–	–	41	41	–	–	–	–	–	–	47	47	–	–
	C	–	–	4	–	–	1	–	–	–	–	–	–	–	–	–	–	1	–	–	2	–	2	–	30	2	–	–	48	47
	G	–	1	–	1	–	–	–	7	–	1	30	–	–	4	2	–	–	7	7	–	48	–	–	–	–	1	–	–	–
	T	48	–	44	–	48	47	48	–	–	–	–	–	–	–	–	48	47	–	–	46	–	46	48	18	46	–	1	–	1
	indels	–	–	–	–	–	–	–	–	–	–	3	–	–	–	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–

		Control region																												
		Sequence No.																												
Locality	Nucleotides	192	193	195	201	202	203	204	205	206	209	212	214	215	226	227	234	240	246	247	248	249	252	253	254	264	273	276	279	281
GAN	A	27	26	–	1	–	3	2	–	–	24	–	1	28	28	–	1	28	–	6	–	–	–	–	26	–	–	28	–	–
	C	–	–	28	–	1	–	–	–	1	–	–	27	–	–	23	–	–	27	–	28	–	–	28	1	–	–	–	24	2
	G	1	2	–	–	–	–	26	2	–	4	–	–	–	–	5	27	–	–	22	–	–	–	–	1	–	–	–	–	–
	T	–	–	–	26	27	25	–	–	27	–	28	–	–	–	–	–	–	1	–	–	28	28	–	–	28	28	–	4	26
	indels	–	–	–	1	–	–	–	26	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
ERI	A	44	48	–	–	–	12	1	–	–	47	–	–	48	48	–	3	47	–	13	–	–	–	–	46	–	–	48	–	–
	C	–	–	48	–	1	–	1	–	–	–	3	48	–	–	36	–	–	47	–	48	–	–	48	1	–	–	–	42	5
	G	4	–	–	–	–	–	46	1	–	1	–	–	–	–	12	45	1	–	35	–	–	–	–	1	–	–	–	–	–
	T	–	–	–	47	47	35	–	–	48	–	45	–	–	–	–	–	–	1	–	–	48	48	–	–	48	48	–	6	43
	indels	–	–	–	1	–	–	–	47	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
TOK	A	42	43	–	1	–	5	3	–	–	43	–	–	43	44	1	–	43	–	10	–	–	–	–	39	–	–	43	–	–
	C	–	–	44	–	1	–	2	–	–	–	2	44	–	–	35	–	–	44	–	44	–	1	43	–	–	–	–	42	2
	G	2	1	–	–	–	–	38	5	–	1	–	–	1	–	8	44	1	–	34	–	–	–	–	5	–	–	1	–	–
	T	–	–	–	41	43	39	–	–	44	–	42	–	–	–	–	–	–	–	–	–	44	43	1	–	44	44	–	2	42
	indels	–	–	–	2	–	–	1	39	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

ONI	A	44	44	–	–	–	8	3	–	–	45	–	–	46	46	1	3	46	–	12	–	–	–	–	43	–	–	46	–	–
	C	–	–	45	–	2	–	–	1	–	–	–	46	–	–	37	–	–	42	–	46	1	–	46	–	–	–	–	39	3
	G	2	2	–	–	–	–	42	2	–	1	–	–	–	–	8	43	–	–	34	–	–	–	–	3	–	–	–	–	–
	T	–	–	1	45	44	36	–	–	46	–	46	–	–	–	–	–	–	4	–	–	45	46	–	–	46	46	–	7	43
	indels	–	–	–	1	–	–	1	43	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
TES	A	46	47	47	1	–	13	3	1	–	47	–	–	47	48	–	–	48	–	8	–	–	–	–	41	–	–	48	–	–
	C	–	–	–	–	–	–	1	–	–	–	2	47	–	–	41	–	–	45	–	46	2	–	48	1	–	–	–	44	6
	G	2	1	–	–	–	–	44	2	–	1	–	–	1	–	7	48	–	–	40	–	–	–	–	6	–	–	–	–	–
	T	–	–	1	46	48	35	–	–	48	–	46	1	–	–	–	–	–	3	–	2	46	48	–	–	48	48	–	4	42
	indels	–	–	–	1	–	–	–	45	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
TOM	A	43	45	–	–	–	10	1	–	–	41	–	–	46	45	–	2	45	–	5	–	–	–	–	44	–	–	46	–	–
	C	–	–	46	–	2	–	–	–	–	–	1	46	–	–	40	–	–	45	1	46	–	1	46	–	1	1	–	39	4
	G	3	1	–	–	–	–	44	2	–	5	–	–	–	–	6	44	1	–	40	–	–	–	–	2	–	–	–	–	–
	T	–	–	–	45	44	36	–	–	46	–	45	–	–	1	–	–	–	1	–	–	46	45	–	–	45	45	–	7	42
	indels	–	–	–	1	–	–	1	44	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
YOI	A	46	47	–	–	–	6	2	–	–	43	–	–	47	48	1	1	47	–	5	–	–	–	–	46	–	–	48	–	–
	C	–	–	48	–	1	–	–	–	–	–	–	48	–	–	40	–	–	46	–	48	–	1	48	–	–	–	–	45	2
	G	2	1	–	–	–	–	45	1	–	5	–	–	1	–	7	47	1	–	42	–	1	–	–	2	–	–	–	–	–
	T	–	–	–	48	47	42	–	–	48	–	48	–	–	–	–	–	–	2	1	–	47	47	–	–	48	48	–	3	46
	indels	–	–	–	–	–	–	1	47	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

		Control region																												
		Sequence No.																												
Locality	Nucleotides	286	287	288	289	290	291	293	294	295	296	297	299	324	325	330	333	365	368	373	383	387	391	427	434	436	461	474	475	480
GAN	A	28	1	–	–	–	–	28	28	–	–	–	–	–	–	–	3	28	–	–	–	–	–	28	27	–	–	–	–	28
	C	–	27	26	21	–	28	–	–	–	1	–	–	–	–	27	–	–	1	–	–	–	13	–	–	–	–	–	–	–
	G	–	–	–	–	–	–	–	–	–	–	–	28	28	–	–	25	–	–	28	–	–	–	–	1	–	–	28	–	–
	T	–	–	2	7	–	–	–	–	28	27	–	–	–	28	1	–	–	27	–	28	28	15	–	–	28	28	–	28	–
	indels	–	–	–	–	28	–	–	–	–	–	28	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
ERI	A	48	–	–	–	1	–	48	48	–	–	–	–	2	–	–	4	48	–	–	–	–	–	43	47	–	–	1	3	48
	C	–	48	44	35	–	47	–	–	–	2	–	–	–	–	48	–	–	1	–	–	–	13	–	–	1	–	–	–	–
	G	–	–	–	–	–	–	–	–	–	–	–	48	46	–	–	44	–	–	48	–	–	–	5	1	–	–	47	–	–
	T	–	–	4	13	–	1	–	–	48	46	–	–	–	48	–	–	–	47	–	48	48	35	–	–	47	48	–	45	–
	indels	–	–	–	–	47	–	–	–	–	–	48	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
TOK	A	43	3	–	–	–	–	44	44	–	–	–	–	–	–	–	15	43	–	–	–	1	–	39	44	–	–	1	–	43
	C	–	41	38	31	–	44	–	–	–	7	–	–	–	2	43	–	–	1	–	2	–	14	–	–	1	1	–	–	–
	G	1	–	–	–	–	–	–	–	–	–	–	44	44	–	–	29	1	–	44	–	–	–	5	–	–	–	43	–	1
	T	–	–	6	13	–	–	–	–	44	37	1	–	–	42	1	–	–	43	–	42	43	30	–	–	43	43	–	44	–
	indels	–	–	–	–	44	–	–	–	–	–	43	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
ONI	A	46	–	–	–	–	1	46	46	–	–	–	1	–	–	–	6	46	–	–	–	–	–	44	45	–	–	–	–	46
	C	–	46	39	31	–	45	–	–	–	9	–	–	–	–	46	–	–	1	–	3	–	14	–	–	–	–	–	–	–
	G	–	–	–	–	–	–	–	–	–	–	–	45	46	–	–	40	–	–	46	–	–	–	2	1	–	–	46	–	–
	T	–	–	7	15	–	–	–	–	46	37	–	–	–	46	–	–	–	45	–	43	46	32	–	–	46	46	–	46	–
	indels	–	–	–	–	46	–	–	–	–	–	46	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
TES	A	48	–	–	–	–	–	48	47	1	–	–	–	1	–	–	12	47	–	1	–	–	–	46	48	–	–	–	–	48
	C	–	48	44	34	–	47	–	–	–	3	–	–	–	–	47	–	–	1	–	2	–	14	–	–	1	–	–	–	–
	G	–	–	–	–	–	–	–	–	–	–	–	48	47	–	–	36	1	–	47	–	–	–	2	–	–	–	48	–	–
	T	–	–	4	14	–	1	–	1	47	45	–	–	–	48	1	–	–	47	–	46	48	34	–	–	47	48	–	48	–
	indels	–	–	–	–	48	–	–	–	–	–	48	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
TOM	A	46	4	1	33	–	–	45	46	–	–	–	–	–	–	–	11	46	–	1	–	–	–	46	44	–	–	–	–	46
	C	–	42	40	–	–	46	–	–	1	5	–	–	–	–	46	–	–	1	–	1	–	15	–	–	3	–	–	–	–
	G	–	–	–	–	–	–	1	–	–	–	–	46	46	–	–	35	–	–	45	–	–	–	–	2	–	–	46	–	–
	T	–	–	5	13	–	–	–	–	45	41	–	–	–	46	–	–	–	45	–	45	46	31	–	–	43	46	–	46	–
	indels	–	–	–	–	46	–	–	–	–	–	46	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
YOI	A	48	2	–	–	–	–	48	48	–	–	–	–	–	–	–	12	47	–	–	–	–	–	47	48	–	–	–	–	48
	C	–	45	45	38	–	48	–	–	–	3	–	–	–	–	48	–	–	–	–	1	–	15	–	–	–	–	–	–	–
	G	–	–	–	–	–	–	–	–	–	–	–	48	48	–	–	33	1	–	48	–	–	–	2	–	–	–	48	–	–
	T	–	1	3	10	–	–	–	–	48	45	–	–	–	48	–	1	–	48	–	47	48	33	–	–	48	48	–	48	–
	indels	–	–	–	–	48	–	–	–	–	–	48	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

This table show transitions, transversions and indels of each variable nucleotide site.

Table 3. Genetic diversity of the mtDNA control region in brown sole.

Locality	*N*	Genetic diversity			
		Nh	*Ps* (*ts*/*tv*/*id*)	*h*	π
GAN	28	28	52 (41/12/4)	1	0.019
ERI	48	39	60 (53/8/5)	0.989	0.02
TOK	44	40	71 (63/9/7)	0.996	0.021
ONI	46	41	57 (49/7/5)	0.992	0.02
TES	48	43	67 (59/11/4)	0.996	0.022
TOM	46	43	64 (57/11/5)	0.996	0.02
YOI	48	45	56 (52/8/4)	0.997	0.015
Total	308	225	116(109/26/8)	–	–

N, number of samples; Nh, number of haplotypes; *Ps*, polymorphic sites; *ts*, number of observed transitions; *tv*, number of observed transversions, id, number of observed indels; h, haplotype diversity; π, nucleotide diversity.

Table 4. Hierarchical analysis of molecular variance (AMOVA) within and among the sampling locations. Populations were categorized into three geographical groups: the east side of Korean Peninsula, and the southeastern and northwestern sides of Hokkaido Island.

	%	*P*	Φ
Among groups	0.02	>0.05	0.0002
Among populations within group	0.12	>0.05	0.0012
Within population	99.86	>0.05	0.0014

%, percentage of variation; *P*, probability estimated from permutation; Φ, F-statistics.

spawning in a tank using microsatellite loci (Kim et al. 2007). Genetic variations in mtDNA may assist assignment of the maternal parentage in hatchery stock in the near future.

Phylogenetic divergence of all populations, including the Korean population, was not observed among sampling locations. The failure to detect genetic differentiation and genetic structure in wild populations of brown sole is probably due to the high molecular variability. Pleuronectid species such as plaices, soles and flounders generally have large population sizes and wide distributions (Nielsen et al. 2009). These results suggest that several sea currents are responsible for the high level of gene flow among populations. Generally, ecological characteristics of marine organism with passive dispersal of planktonic larvae, such as brown sole, may be strongly affected by sea currents (Azuma et al. 2007; Saito et al. 2008; Kim et al. 2010). AMOVA showed unusually high variance (99.86%) within populations, probably due to the excess of private (unique) haplotypes (183/225).

In conclusion, the mtDNA control region of brown sole in this study showed a highly polymorphic population structure. We suggest that comparison with the genetic structure of the stocking area population will provide us with new knowledge about the change of the genetic structure during long-term stocking operations.

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Table 5. F_{ST} values (below diagonal) and Kimura 2-parameter distance (above diagonal) between sampling localities of brown sole, *Pleuronectes herzensteini*.

	GAN	ERI	TOK	ONI	TES	TOM	YOI
GAN		0.0182	0.0184	0.0183	0.0186	0.018	0.0155
ERI	0.004(0.099)		0.019	0.0187	0.019	0.0187	0.0165
TOK	0.002(0.135)	−0.001(0.586)		0.019	0.0194	0.0189	0.0164
ONI	0.003(0.072)	0.002(0.144)	−0.001(0.568)		0.0192	0.0187	0.0166
TES	0.002(0.252)	0.001(0.270)	0.000(0.459)	0.002(0.135)		0.019	0.0169
TOM	0.000(0.378)	0.004(0.099)	0.002(0.180)	0.002(0.126)	0.002(0.135)		0.0162
YOI	−0.003(0.937)	0.003(0.090)	−0.002(0.775)	0.003(0.063)	0.001(0.189)	0.001(0.252)	

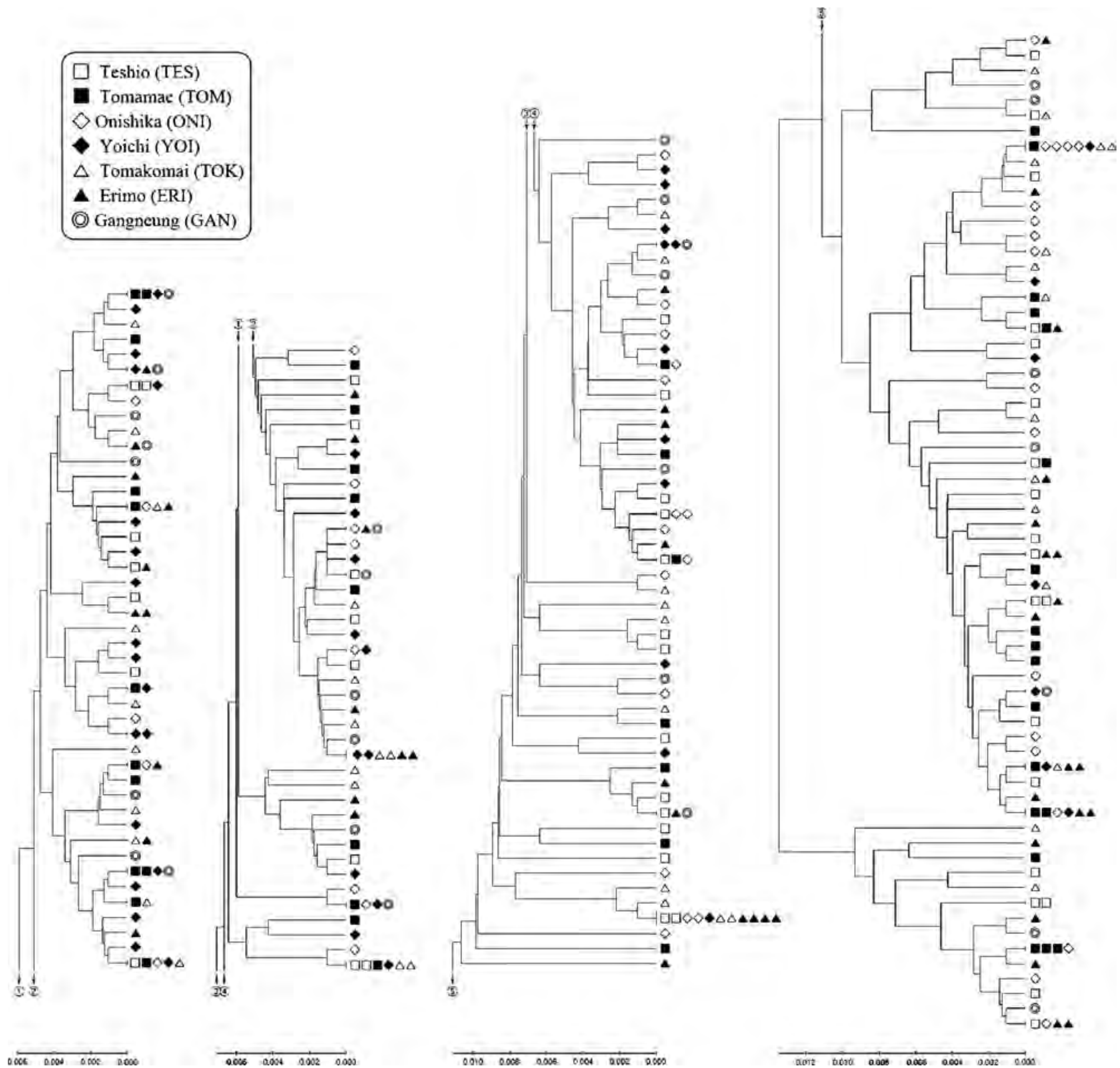

Figure 2. The phylogenic tree constructed by the UPGMA method for the mtDNA control region haplotypes of seven brown sole populations.

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