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Population Structure of Minke Whales (*Balaenoptera acutorostrata*) in the Korean Waters Based upon Mitochondrial DNA Polymorphism

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Abstract: The Minke whale, *Balaenoptera acutorostrata*, is the smallest baleen whale in the suborder Mysticeti. Because this species inhabits coastal areas, it became a main target species of coastal small-type whaling in the North Atlantic and the Northwest Pacific Oceans, and the species' population size dramatically decreased because of over-exploitation. As a result, the International Whaling Commission declared a global moratorium on whaling and launched the development of a management procedure for protecting the whales. Morphological studies, whaling history analysis, and genetic studies conducted mainly by Japanese scientists showed the existence of one unique "E" stock that inhabits the waters around the Korean peninsula and mixes with the "O" stock in the southern part of the Sea of Okhotsk. We used the mitochondrial DNA control region polymorphism of 348 Minke whales bycaught or stranded in Korean waters from 30 October 1998 to 25 June 2005 to assess the whale population structure by year. The frequency of the 10 major haplotypes from the 40 identified haplotypes was not significantly different among groups, suggesting that a sub-population was not present. A comparison of the genetic distances calculated with Tamura-Nei's method showed that the distances between groups were lower than those within groups, which suggests that there was no genetic difference in the Minke whale populations. The Fst comparison between groups and the phylogenetic tree constructed using the unweighted pair group method with arithmetic mean (UPGMA) and Neighbor Joining (NJ) method also detected no obvious sub-stock structure.

Key words: minke whales, mitochondrial DNA, polymorphism, haplotype, population structure

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INTRODUCTION

Mitochondrial DNA (mtDNA) does not have homologous chromosomes or the enzymes that are necessary for the recovery of DNA replication errors (Clayton, 1982), so the mtDNA evolutionary rate is reported to be 5-10 times faster than that of nuclear DNA (Brown et al., 1979).

These characteristics of mtDNA make it a suitable indicator to assess genetic diversity in many species, including whales (Baker et al., 1990; Meyer et al., 1990; Hoelzel and Dover, 1991; Kraus and Miyamoto, 1991; Schaeff et al., 1991; Gatesy et al., 1992; Baker et al., 1994; Milinkovichi et al., 1994; Rosel et al., 1994; Taberlet and Bouvet, 1994; Koh et al., 2004).

Although the mtDNA control region of whales has a slower evolutionary rate than that of other mammalian species (Hoelzel et al., 1991), these genetic markers have enough discriminative power to investigate the population structures of the Pacific and Atlantic Ocean humpback whale and the Common Dolphin (Baker et al., 1993; Palsboll et al., 1995; Rosel et al., 1994). Based on these results, the International Whaling Commission (IWC) is encouraging the introduction of mtDNA for stock assessment in Minke whales (Baker et al., 2000; Goto and Pastene, 1997, 1999; Goto et al., 2000, 2001).

Since 1983, the IWC has classified the northwest Pacific Minke whale resources into three populations for the purpose of protection: 1. East Sea/Sea of Japan-Yellow Sea-East China Sea Stock, "E" Stock; 2. Okhotsk Sea-West Pacific Stock, "O" Stock; 3. Remainder Stock (Ohsumi, 1983; IWC, 1983; Horwood, 1990).

A genetic analysis of the population structure of Minke

whales in the oceanic regions of Korea and Japan is currently underway. According to this analysis, the E stock population structure is more complex than expected and consists of more than two sub-structures (Baker et al., 1999, 2000; Dalebout et al., 2000; Lavery et al., 2001, 2002, 2003, 2004). Subsequently, to allow better stock management of the Minke whales in the northwest Pacific, the IWC recommended an in-depth assessment of the population structure (IWC, 2004). As part of that effort, this study intends to identify the population structure of Minke whales in Korean waters, using bycaught or stranded Minke whales.

MATERIALS AND METHODS

Samples

To define the genetic population structure of Minke whales, we used 348 bycaught and stranded whales sampled from 30 October 1998 to 25 June 2005.

Of the 348 whales, 277 were taken off the East Sea, 31 off the West, and 40 off the South; 298 of the whales were taken from the sea along the coast and 51 were taken from offshore (Fig. 1).

DNA extraction and amplification

Mitochondrial DNA was extracted from frozen muscle tissue using an automated DNA extraction system (Mag Extractor MFX-6100; TOYOBO). Approximately 10 mg of muscle were suspended in 900 μL of lysis buffer (MFX-2000, TOYOBO) with 5 μL of proteinase K at 37°C overnight. Total genomic DNA was isolated with a Mag Extractor genome DNA purification kit (TOYOBO). The DNA integrity was visually inspected on 1.8% agarose gels.

Partial sequence of the control region was amplified with three primers: MT4-F of the mitochondrial DNA $tRNA^{pro}$ region, Dlp-5R of 5'-CCTCCCTAAGACTCAAGGAAG-3' (Arnason et al., 1993), and the $tRNA^{phe}$ region; 5'-CCATCGAGATGTCTTATT AAGGGGAAC-3' (Baker et al., 1996; Dalebout et al., 1998). The polymerase chain reaction (PCR) was performed in a 10-μL volume containing 1 μL of genomic DNA, 0.8 μL of dNTP mixture (2.5 mM each), 0.4 μL of each primer (10 pM), 1 μL of 10x PCR buffer, and 0.1 μL of Taq polymerase (5 units/μL) (TAKARA). Thirty-five PCR cycles were performed: the denaturation step was at 94°C for 2 min, the annealing step was at 50°C for 1 min, and the polymerization reaction was performed at 72°C for 1.5 min with a final extension at 72°C for 7 min. The PCR products were purified using a QIA quick PCR purification kit (QIAGEN). The purified PCR products were sequenced directly using a Prism Big Dye Terminator Cycle Sequencing kit with AmpliTaq DNA Polymerase FS (Applied Biosystems) and the respective amplification primers. Each reaction contained 1 μL of purified PCR product, 1.6 μL of primer (1 pmol), 1.5 μL of 5x sequencing buffer, 1 μL of Big Dye Terminator, and 4.9 μL of distilled water, and was reacted for 25 cycles of 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min, with a hold at 4°C. The DNA from the reactions was purified by standard ethanol/sodium acetate precipitation (Sambrook *et al.*, 1989). Each purified sample was resuspended in 10-12 μL of Template Suppression Reagent (Hi-Di formamide; Applied Biosystems), denatured at 95°C for 2 min, and chilled on ice for 5 min. Capillary electrophoresis was performed with 10 μL of each sample, using a 3130xl Genetic Analyzer (Applied Biosystems) and DNA sequencing analysis software (Applied Biosystems).

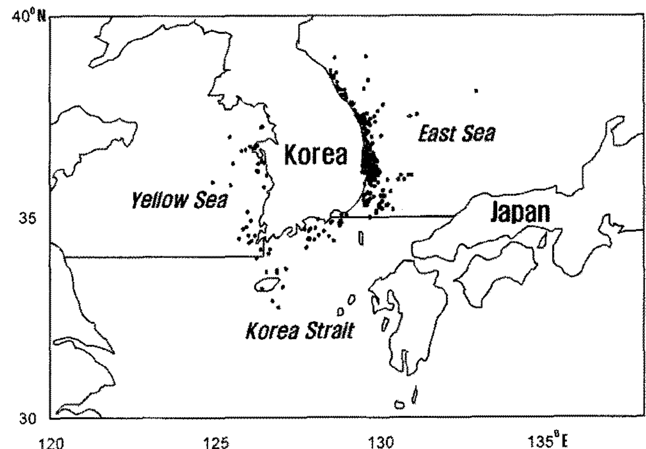

Fig. 1. The locations of the bycaught and stranded minke whales in the Korean waters.

Statistical analysis

The polymerase chain reaction (PCR) products were sequenced with an automated DNA sequencer (ABI 377), and 487 bp among the determined 580 bp were used for additional analysis. The base sequence arrangement was analyzed by DNASIS ver. 2.5 (Hitachi Software Engineering Co., Ltd.).

Individual haplotype was determined, and the individual number was confirmed with the POSTR2 software program. The best-fit model to calculate genetic distance was chosen after considering the AIC value taken from the Posada and Crandall (2001) method which compared, the Jukes-Canter model (Jukes and Canter, 1969), the Kimura's 2-parameter model (Kimura, 1980), the Tamura-Nei model (Tamura and Nei, 1993), and gamma corrected forms of the 3 models.

The Fst for an analysis of genetic distance and genetic population structure was calculated using the method of Excoffier and Smouse (1994), and the significance of the Fst was verified using more than 1,000 permutations (ARLEQUIN ver. 2.0; Schneider et al., 2000).

The unweighted pair group method with arithmetic mean (UPGMA; Sneath and Sokal, 1973) and Neighbor Joining

(NJ; Jin and Nei, 1990) methods, which indicate the genetic relationship of populations, were used to construct a phylogenetic tree using MEGA ver. 3.1 software, and the consensus tree was constructed and verified by a bootstrap analysis with more than 1,000 repetitions (Kumar et al., 2004).

RESULTS

Haplotype diversity

The analysis of the 487-bp mtDNA control region in Minke whales showed that there were 21 variations and 40 haplotypes. The variations in the 6th position and 463 segments were caused by adenine insertion/deletion and transversion, and the others variations were formed by transition (Table 1).

Analysis of the frequency at which the 40 halotypes occurred per year indicated that haplotype 1, which was considered a major haplotype, varied from 13.33% in 2000 to 36.84% in 2001, with an average frequency of 25.57%., The average values for haplotypes 2 and 3 were 14.94 and 9.20%, respectively. The frequency of the top three

Table 1. Aligned control region sequences of 40 haplotypes from 348 minke whales, showing variable sites and insertion-deletion events (-). Variable sites within the 487 bp fragment are numbered and the number of bases are counted

Haplotypes	6	17	80	90	115	132	191	204	209	218	272	290	298	311	314	341	373	381	406	429	463
1	A	A	T	C	A	C	A	G	C	A	T	T	G	T	T	G	C	A	C	T	A
2	.	G	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	G
3	-	G	.	.	.	.	.	A	.	.	.	C	.	C	.	.	.	.	.	.	G
4	.	G	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.
5	-	G	.	.	.	.	.	A	.	.	.	C	A	C	.	.	.	.	.	.	G
6	-	G	.	.	.	.	.	A	.	.	.	C	A	C	.	.	.	.	.	.	.
7	-	G	.	.	.	.	.	A	.	.	.	C	.	C	.	.	.	.	.	.	.
8	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G
9	-	G	.	T	.	.	.	A	.	.	.	C	A	C	.	.	.	.	.	.	.
10	.	G	.	T	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.
11	.	G	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
12	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.
13	-	G	.	.	.	.	.	A	.	.	.	.	A	C	.	A	.	.	.	.	G
14	-	G	.	T	.	.	.	A	.	G	.	C	.	C	.	.	.	.	.	.	.
15	-	G	.	T	.	.	.	A	.	.	.	C	A	.	.	.	.	.	T	.	G
16	.	G	.	.	.	.	.	A	.	.	.	C	.	C	.	.	.	.	.	.	G
17	-	G	.	.	.	.	.	A	.	.	.	C	A	C	.	.	.	.	T	.	.
18	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.
19	.	G	.	.	.	.	.	.	T	.	.	C	.	.	.	.	.	.	.	.	G
20	.	G	.	.	.	.	.	A	.	.	.	C	.	C	.	.	.	.	.	.	.
21	.	G	.	.	.	.	.	A	.	.	.	C	A	C	.	.	.	.	.	.	G
22	-	G	.	.	.	.	.	A	.	.	.	C	A	C	.	A	.	.	.	.	G
23	-	G	.	.	.	.	.	A	.	.	.	C	.	C	.	.	.	.	.	G	G
24	-	G	.	T	.	.	.	A	.	.	.	C	.	C	.	.	.	.	.	.	.
25	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
26	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G
27	.	G	.	.	.	.	.	A	.	.	.	C	A	C	.	.	.	.	.	.	.
28	.	G	.	T	.	.	.	A	.	G	.	C	.	C	.	.	.	.	.	.	.
29	-	G	.	.	.	.	.	A	.	.	.	C	A	C	.	.	.	.	T	.	G
30	.	G	C	.	.	.	.	A	.	.	C	C	A	C	.	.	.	.	T	.	G
31	.	G	.	.	.	.	.	A	.	.	C	C	A	C	.	.	.	.	.	.	.
32	.	G	.	.	.	.	.	.	.	.	.	C	.	C	.	.	.	.	.	.	.
33	-	G	.	.	.	.	.	A	.	.	.	C	.	.	.	.	.	.	.	.	.
34	-	G	.	T	.	.	.	A	.	.	.	C	.	C	.	.	.	G	.	.	.
35	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.
36	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.
37	-	G	.	T	.	.	.	A	.	.	.	C	A	.	.	.	.	.	T	.	.
38	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T
39	-	G	.	.	.	.	G	A	.	.	.	C	.	C	.	.	.	.	.	.	.
40	-	G	.	.	.	.	.	A	.	.	C	C	A	C	.	.	.	.	.	.	.
Number of bases	1	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3

Table 2. Estimated individual number and percent (%) frequency (In parentheses) of mitochondrial DNA haplotypes from each year

Haplotype	Year								
	Total	1998	1999	2000	2001	2002	2003	2004	2005
1	89(25.57)	1(33.33)	9(19.15)	2(13.33)	7(36.85)	19(33.33)	20(22.48)	14(23.33)	17(29.32)
2	52(14.94)		10(21.28)	3(20.00)	1(5.26)	4(7.02)	12(13.48)	12(20.00)	10(17.24)
3	32(9.20)	1(33.33)	2(4.26)	3(20.00)	4(21.06)	4(7.02)	3(3.37)	4(6.67)	11(18.98)
4	22(6.32)		6(12.77)	2(13.33)	1(5.26)	4(7.02)	4(4.49)	4(6.67)	1(1.72)
5	21(6.03)		2(4.26)		1(5.26)	2(3.51)	7(7.87)	3(5.00)	6(10.34)
6	15(4.31)					5(8.77)	7(7.87)	2(3.33)	1(1.72)
7	14(4.02)		1(2.13)		1(5.26)	2(3.51)	4(4.49)	5(8.33)	1(1.72)
8	13(3.74)		4(8.51)			1(1.75)	3(3.37)	4(6.67)	1(1.72)
9	13(3.74)					5(8.77)	1(1.12)	2(3.33)	5(8.62)
10	10(2.87)		5(10.64)				3(3.37)	1(1.67)	1(1.72)
11-40	67(19.26)	1(33.33)	8(17.00)	5(33.34)	4(21.05)	11(19.30)	25(28.09)	9(15.00)	4(6.90)
Total	348(100)	3(100)	47(100)	15(100)	19(100)	57(100)	89(100)	60(100)	58(100)

Table 3. Calculated haplotype diversity (*H*) and nucleotide diversity (π) from each year

	Years							
	1998	1999	2000	2001	2002	2003	2004	2005
No. of samples	3	47	15	19	57	89	60	58
No. of haplotypes	3	14	8	9	19	23	17	14
haplotype diversity	1.00000	0.89269	0.91429	0.83626	0.86717	0.91241	0.89096	0.84211
nucleotide diversity	0.07381	0.05122	0.06112	0.04913	0.05740	0.05976	0.05677	0.05061
Mean (*H*)	0.89448							
Mean (π)	0.05747							

Table 4. AIC values of the genetic distance calculating models estimated by FindModel (http://hcv.lanl.gov/content/hcv-db/findmodel/findmodel.html)

Model	AIC Value	References
Jukes-Cantor	1937.779	Jukes and Canter (1969)
Jukes-Cantor, Gamma*	1896.490	
Kimura2-parameter	1891.879	Kimura (1980)
Kimura2-parameter, Gamma*	1850.570	
Tamura-Nei	1857.672	Tamura and Nei (1993)
Tamura-Nei, Gamma*	1816.132	

*Gamma corrected model's average ratio of transition/transversion is 7.5604 and Gamma model shape parameter, is 0.040.

haplotypes represented 50% of the total frequency. No key markers were found in the frequency of the major haplotypes (haplotypes 1-10; Table 2).

The average haplotype diversity of the Minke whales was 0.89448, and the average nucleotide diversity was 0.05747. The haplotype comparison by year showed that, except for 1998, Of individuals sampled was small, haplotype diversity highest in 2000, at 0.91241, and lowest in 2001, at 0.83626. Nucleotide diversity was highest in 2000 (0.06112) and lowest in 2001 (0.04913). However, there were no significant differences in genetic variations by year (Table 3).

Population analysis

Genetic distance based on the best-fit model was used to estimate genetic difference by year among the Minke whale populations; the Tamura-Nei model adjusted by the gamma distribution had the lowest AIC value (Table 4).

The genetic distance within the populations was the highest in 1998, at 0.01156, but the individual number was small. When 1998 was excluded, the genetic distance within the populations was the highest in 2003 (0.01036) and lowest in 2001 (0.00782). The average genetic distance during the 8 years was 0.0092. The genetic distance between the populations was the highest between 2000 and

Table 5. Pairwise genetic distances estimated by Tamura-Nei's (1993) model between years. Overall average distance is 0.00932

	1998	1999	2000	2001	2002	2003	2004	2005
1998	**0.01156**	0.00926	0.01006	0.01013	0.00887	0.00912	0.01066	0.00917
1999	0.00115	**0.00824**	0.00892	0.00919	0.00807	0.00856	0.00860	0.00810
2000	0.00089	0.00005	**0.00841**	0.01008	0.00929	0.00957	0.01085	0.00931
2001	0.00074	0.00010	-0.00006	**0.00782**	0.00942	0.00968	0.00807	0.00938
2002	0.00042	-0.00009	0.00009	0.00000	**0.00993**	0.00869	0.01082	0.00828
2003	0.00037	0.00010	0.00006	-0.00006	-0.00016	**0.01036**	0.00949	0.00870
2004	0.00067	-0.00109	0.00011	-0.00014	0.00053	-0.00054	**0.00849**	0.00926
2005	0.00084	0.00007	0.00023	0.00007	0.00008	0.00003	-0.00064	**0.00910**

Diagonal: Average distance within population=dii
Lower left: Corrected average distance=dij-(dii+djj)/2
Upper right: Average distance between populations=dij

Table 6. Pairwise Fst (lower left) calculated from genetic distances estimated by Tamura-Nei's method (1993) and *P* value (upper right, *P*<0.05) calculated 3,024 permutations inter-year matrix (overall Fst=0.00707). Bold figures indicate that the two groups are significantly different at 5% level

Year	1998	1999	2000	2001	2002	2003	2004	2005
1998		0.24893	0.55702	0.65917	0.36826	0.24231	0.29157	0.23372
1999	0.06549		0.51372	**0.01917***	0.25521	0.42545	0.43901	0.45388
2000	-0.09295	-0.01558		0.27074	0.77620	0.42380	0.59008	0.51207
2001	-0.11916	**0.10565**	0.01162		0.06909	**0.00992***	**0.02777***	**0.02909***
2002	-0.00904	0.00449	-0.02802	0.05464		0.39570	0.75107	0.47603
2003	0.05241	-0.00323	-0.00711	**0.11005**	-0.00184		0.68529	0.43603
2004	0.03230	-0.00452	-0.01859	**0.08875**	-0.01064	-0.00771		0.66512
2005	0.06438	-0.00604	-0.01582	**0.09429**	-0.00614	-0.00367	-0.00943	

2004, at 0.01085, and the lowest between 1999 and 2002, at 0.00807. Interestingly, the 2004 population had a greater genetic distance than the average, compared to the other populations, indicating that the 2004 population was likely distinct in genetic composition from the other populations. Nevertheless, the genetic distance between 2004 and other population years was not large, so this may represent a probabilistic event (Table 5).

Subpopulation structure

To identify the existence of different subpopulations in the Minke whales bycaught in Korea, Fst was calculated using haplotype frequency and genetic distance. Based on the haplotype frequency, the 1999 population was different from the 2001, 2002, and 2005 populations (*P*<0.05). Considering genetic distance, the 2001 population was different from the 1999, 2003, 2004 and 2005 populations (*P*<0.05). The genetic distances in the 2001 and 2003 populations were different based on 99% confidence limits, whereas the remaining population years were different with 95–99% confidence limits (Table 6).

Population relationships

A phylogenetic tree was constructed using the UPGMA and NJ methods to confirm the relationship between the Minke whale populations by year. The UPGMA method revealed a close relationship between the 2001 and 2004 populations, and showed that the relationship between 2001 and 1999 population was more distant than between other years. The NJ method divided Minke whales into two major subpopulations. One group included 1998, 1999, 2001 and the other included 2000 and 2002–2005, although no clear cluster was formed when all of the populations were analyzed (Fig. 2).

The maximum likelihood method (ML) was used to construct the genetic relationships among the 40 haplotypes to identify occurrence by year (Fig. 3). No difference was found in the genetic relationship of the populations by year, indicating that there are no subpopulations of Korean Minke whales.

Discussion

There were 40 mtDNA haplotypes in the Minke whales bycaught in Korean waters, fewer than the number of haplotypes found in Atlantic Minke whales (51) and in the northwest Pacific ‘O’ population (83). Based on habitat size, the 40 haplotypes of the Korean ‘E’ population was

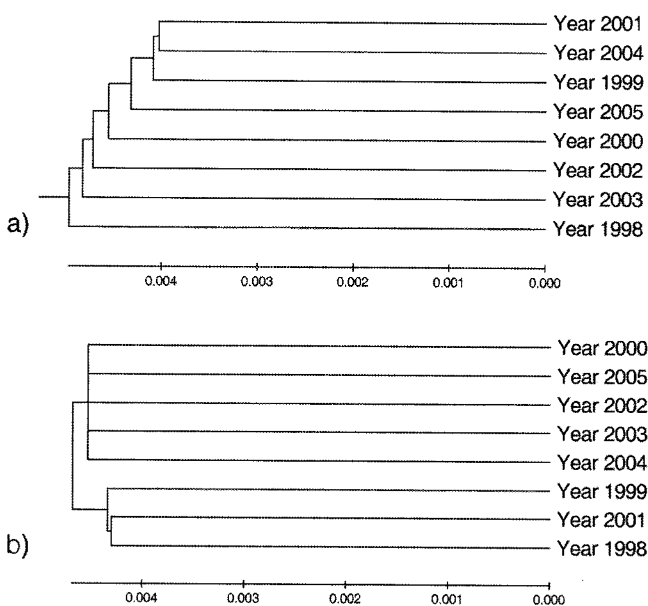

Fig. 2. UPGMA (a) and Neighbor Joining (b) consensus trees based on pair-wise genetic distance estimated by Tamura-Nei's method among years.

rather large.

The distributions of haplotypes 1-10 were similar among years, and this trend occurred in many individuals. Considering this data, it is unlikely that a subpopulation of Minke whales exists in Korean waters. A new haplotype occurred in every year, although this was caused by an increase in the number of total samples over time and did not represent a change in the haplotype distribution (Table 2).

Nucleotide diversity increased by year according to sample variance, with no significant difference (Table 3). Natoli et al. (2004) and Rosel et al. (1994) showed that the haplotype distribution is different even for species with a wide geographical distance, which allows haplotype distribution comparison to be used to identify population structure. The consistency of the distribution of haplotypes 1-10 over all of the groups, therefore, indicates that there is no subpopulation of Minke whales in Korea.

The genetic distance between the populations, which indicates the genetic difference, normally must be estimated. However, recently developed models weigh each calculation by classifying DNA changes into transition, conversion, and insertion/deletion. Because of this, these models are more sensitive to base changes and produce a larger genetic distance value, which makes genetic differences clear. Also, because the model optimizes the use of genetic information, it better reflects the degree of interpopulation change.

We compared models that estimate genetic distance by comparing DNA (base) sequences. The Tamura-Nei method, which produced the minimum AIC value among the models, was adapted for estimating genetic distance. This method produced larger values than those of the conventionally used Kimura's 2-parameter method (Table 5). For this reason, the Tamura-Nei method produced more exact estimates of the Minke whale subpopulation in Korean waters. However, the genetic distances within Minke whale populations produced by the Tamura-Nei method were often larger than those found between populations, which makes it unlikely that there is a Minke whale subpopulation in Korean waters.

The pair-wise Fst, which indicates the degree of genetic population differentiation, was calculated using the allele DNA and haplotype frequencies. Various differences were found, based on the models used, but the variability in the individual population was often larger, suggesting that there is no evidence for a subpopulation (Table 6 and 7).

A phylogenetic tree was constructed using the UPGMA and NJ methods to identify the genetic relationship between the populations. The phylogenetic tree did not change significantly, regardless of the method used, when there was a clear genetic difference between the populations. The population structures produced by the UPGMA and NJ methods were significantly different, although in nearly all of the populations, no distinct group was found (Fig. 2).

Baker et al. (2000) compared the mtDNA haplotype sequences in Minke whale populations in Korean waters using samples collected from market. Their results are

Table 7. Pairwise Fst (lower left) calculated from haplotype frequencies and P value (upper right, P<0.05) calculated 3,024 permutations inter-year matrix (overall Fst=0.00857). Bold figures indicate that the two groups are significantly different at 5% level

Year	1998	1999	2000	2001	2002	2003	2004	2005
1998		0.48033	0.65091	0.76264	0.82380	0.66347	0.72992	0.75041
1999	-0.00817		0.40165	**0.03471***	**0.01322***	0.10479	0.51074	**0.02380***
2000	-0.06458	0.00062		0.20198	0.06777	0.10810	0.30876	0.25157
2001	-0.11360	**0.03716**	0.01863		0.45124	0.09785	0.15603	0.53322
2002	-0.05667	**0.02759**	0.03157	-0.00108		0.18281	0.12893	0.14645
2003	-0.03169	0.00817	0.01833	0.01732	0.00469		0.50281	0.05091
2004	-0.03223	-0.00197	0.00515	0.01413	0.00876	-0.00152		0.21785
2005	-0.07156	**0.02432**	0.01134	-0.0064	0.00828	0.01286	0.00541	

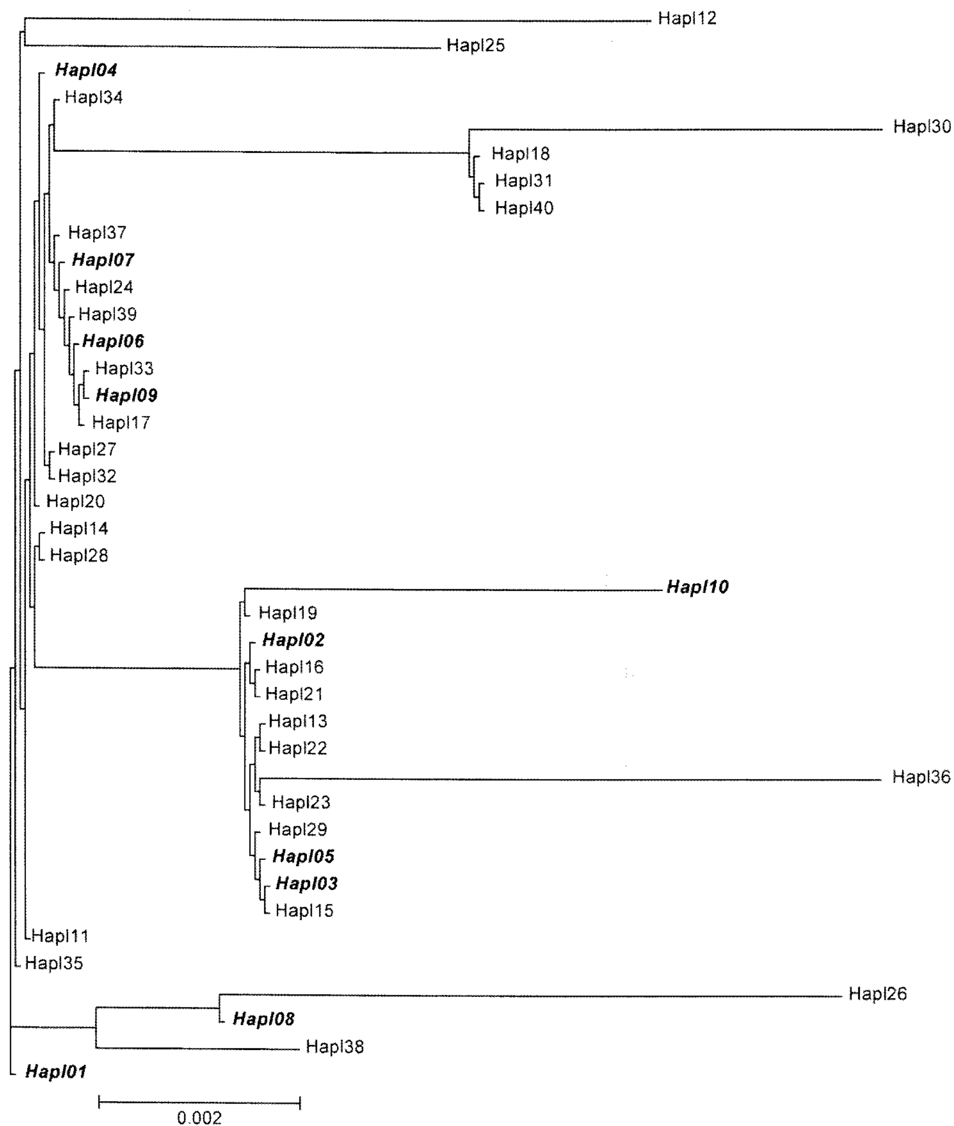

Fig. 3. Phylogenetic tree of 40 haplotypes appeared in Korean minke whales constructed by maximum likelyhood method (jumbled 10 times) with PHYLIP program.

questionable because the origin of the Minke whale samples was unknown and the ecological characteristics were not considered.

Considering these problems, this study used 40 haplotypes and the ML method to construct the genetic relationship and indentify subpopulation structure (Fig. 3). The haplotype comparison identified the existence of several groups. However, no group property was found in the relationship between the groups, as classified by the phylogenetic tree and the haplotype by year. Although the groups classified by the phylogenetic tree formed around the major group of haplotypes 1-10, and a haplotype was found among the populations, it was difficult to genetically divide these into several groups. Therefore, the mtDNA analysis suggested that there is no Minke whale subpopulation in Korean waters. Further investigation is needed to clarify this result using more refined genetic techniques, such as microsatellite DNA and single nucleotide polymorphism analysis.

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REFERENCES

Akaike H (1985) Prediction and entropy, In: A Celebration of Statistics, Atkinson, AC, and SE Feinberg (eds), Springer-Verlag, Berlin, 1-24.

Andersen LW, Born EW, Dietz R, Haug T, Oien N, and Bendixen

C (2003) Genetic population structure of minke whales Balaenoptera acutorostrata from Greenland, the North East Atlantic and the North Sea probably reflects different ecological regions. *Mar Ecol Prog Ser* 247: 263-280.

Arnason U, Gullberg A, and Widegren B (1993) Cetacean mitochondrial DNA control region: sequences of all extant baleen whale and two sperm whale species. *Mol Biol Evol* 10: 960-970.

Baker CS, Perry A, Bannister JL, Weinrich MT, Abernethy RB, Clambokidis J, Lien J, Lambertsen RH, Urban Ramirez J, Vasquez O, Clapham PJ, Alling A, O'Brien SJ, and Palumbi SR (1993) Abundant mitochondrial DNA variation and world-wide population structure in humpback whales. *Proc Natl Acad Sci USA* 90: 8239-8243.

Baker CS, Cipriano F, and Palumbi SR (1996) Molecular identification of whale and dolphin products from commercial markets in Korea and Japan. *Mol Ecol* 5: 671-685.

Baker CS, Lento GM, Cipriano F, and Palumbi S (2000) Predicted decline of protected whales based on molecular genetic monitoring of Japanese and Korean markets. *Proc Roy Soc London* B267: 1191-1199.

Baker CS, Dalebout ML, Congdon BC, and Lento GM (1999) Molecular genetic identification of whale and dolphin products for sale in Japan, 1998-99. Paper SC/51/O15 presented to the IWC Scientific Committee.

Baker CS, Slade RW, Bannister JL, Abernethy RB, Weinrich MT, Lien J, Urban J, Corkeron P, Clambokidis J, Vasquez O, and Palumbi SR (1994) Hierarchical structure of mitochondrial DNA gene flow among humpback whales Megaptera novaeangliae, world-wide. *Mol Ecol* 3: 313-327.

Baker CS, Palumbi SR, Lambertsen RH, Weinrich MT, Calambokidis J, and O'Brien SJ (1990) Influence of seasonal migration on geographic distribution of mitochodrial DNA haplotypes in humpback whales. *Nature* 344: 238-240.

Brown WM, George M, and Wilson AC (1979) Rapid evolution of animal mitochondrial DNA. *Proc Natl Acad Sci USA* 76: 1967-1971.

Clayton DA (1982) Replication of animal mitochondrial DNA. *Cell* 28: 693-705.

Dalebout ML, Lento GM, Cipriano F, and Baker CS (2000) Microsatellite profiling of North Pacific minke whales: stock differentiation and bycatch estimation. Paper SC/52/RMP19 presented to the IWC Scientific Committee.

Excoffier L and Smouse PE (1994) Using allele frequencies and subdivision to reconstruct gene trees within a species-molecular variance parsimony. *Genetics* 136: 343-359.

Gatesy J, Yelon D, DeSalle R, and Vrba ES (1992) Phylogeny of the Bovidae (Artiodactyla, Mammalia), based on mitochondrial ribosomal DNA sequences. *Mol Biol Evol* 9: 433-446.

Goto M and Pastene LA (1997) Population structure of the western North Pacific minke whale based on an RFLP analysis of the mtDNA control region. *Rep Int Whal Comm* 47: 531-537.

Goto M and Pastene LA (1999) Genetic population structure in the western North Pacific minke whales examined by mtDNA control region sequencing analysis. Paper SC/51/RMP8 presented to the IWC Scientific Committee.

Goto M, Kim ZG, and Pastene LA (2001) Genetic analysis of additional samples from the 'J' stock and implications for the estimation of mixing proportion of 'J' and 'O' stocks in sub-area 11. Paper SC/53/RMP12 presented to the IWC Scientific Committee.

Goto M, Kim ZG, Abe H, and Pastene LA (2000) A note on the possibility of identifying individual 'J' stock animals from a mixed assemblage based on mitochondrial DNA analysis. Paper SC/52/F2K/J28 presented to the IWC Scientific Committee Workshop to Review the Japanese Whale Research Programme under Special Permit for North Pacific Minke Whales (JARPN), Tokyo, 7-10 February 2000 (unpublished), pp. 9.

Hoelzel AR and Dover GA (1991) Genetic differentiation between sympatric killer whale populations. *Heredity* 66: 191-195.

Horwood J (1990) Biology and exploitation of minke whale. CRC Press, Inc., Florida. pp. 238.

International Whaling Commission (1983) Report of the Scientific Committee. *Rep Int Whal Commn* 33: 43-190.

Koh HS, Yoo MH, Lee BG, and Park JG (2004) Molecular DNA Systematic Analyses of East Asian Mammals: Sequence Variation of Cytochrome b Gene and Control Region of Mitochondrial DNA of Common Otter, Lutra lutra lutra L. (Mammalia, Carnivora) from Korea. *Korean J Biol Sci* 8: 231-233.

International Whaling Commission (2004) Report of the scientific committee, Annex D. Report of the sub-committee on the RMP. *J Ceta Res Manage* 6: 75-184.

Jin L and Nei M (1990) Limitations of the evolutionary parsimony method of phylogenetic analysis. *Mol Biol Evol* 7, 82-102. Erratum in: *Mol Biol Evol* 7: 201.

Jukes TH and Cantor CR (1969) Evolution of protein molecules. In: Mammalian Protein Metabolism. Munro HN (ed), Academic Press, New York, pp. 21-132.

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 16: 111-20.

Kraus F and Miyamoto MM (1991) Rapid cladogenesis among the pecoran ruminants: evidence from mitochodrial DNA sequences. *Syst Zool* 40: 117-130.

Kumar S, Tamura K, and Nei M (2004) MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinform* 5: 150-163.

Lavery S, Baker CS, Dalebout ML, Congdon B, Steel D, Lento G, Funahashi N, and Ma YW (2003) Structure of mtDNA diversity among North Pacific minke whales from Japan and Korea. Paper SC/55/RMP7 presented to the IWC Scientific Committee.

Lavery S, Dalebout ML, Lento GM, and Baker CS (2001) Minimum census of minke whale by-catch from Korean waters in 2000/01 using DNA profiling. Paper SC/53/RMP16 presented to the IWC Scientific Committee.

Lavery S, Dalebout ML, Ma YW, Funahashi N, and Baker CS (2002) Market surveys of whales, dolphins and porpoises in Japan and Korea, 2001-2002. Paper SC/54/BC1 presented to the IWC Scientific Committee.

Lavery S, Funahashi N, Ma YW, Dalebout ML, Macinnes H, and Baker CS (2004) Mitochondrial DNA diversity and individual identification of North Pacific minke whales from coastal waters of Japan and Korea. Paper SC/56/BC4 presented to the IWC Scientific Committee.

Meyer A, Kocher TD, Basasibwaki P, and Wilson AC (1990)

Monophyletic origin of Lake Victoria cichlid fishes suggested by mitochondrial DNA sequences. *Nature* 347: 550-553.

Milinkovitch MC, Meyer A, and Powell JR (1994) Phylogeny of all major groups of cetaceans based on DNA sequences from three mitochodrial genes. *Mol Biol Evol* 11: 939-948.

Natoli A, Peddemors VM, and Hoelzel AR (2004) Population structure and speciation in the genus Tursiops based on microsatellite and mitochondrial DNA analyses. *J Evol Biol* 17: 363-375.

Oshumi S (1983) Minke whale in the coastal waters of Japan in 1981, with special reference to their stock boundary. *Rep Int Whal Comm* 33: 365-371.

Palsboll PJ, Clapman PJ, Mattila DK, Larsen F, Sears R, Siegismund HR, Sigurjonsson J, Vasquez O, and Arctander P (1995) Distribution of mtDNA haplotypes in North Atlantic humpback whale: the influence of behaviour on population structure. *Mar Ecol Prog Ser* 116: 1-10.

Posada D and Crandal KA (2001) Selecting the best-fit model of nucleotide substitution. *Syst Biol* 50: 580-601.

Rosel PE, Dizon AE, and Heyning JE (1994) Genetic analysis of sympatric morphotypes of common dolphins (genus Delphinus). *Mar Biol* 119: 159-167.

Schaeff C, Kraus S, Brown M, Perkins J, Payne R, Gaskin D, Boag P, and White B (1991) Preliminary analysis of mitochondrial DNA variation within and between right whale species Eubalaena glacialis and Eubalaena australis. In: Genetic Ecology of Whales and Dolphins (IWC Special Issue 13). Hoelzel, AR (ed), International Whaling Commission, Cambridge, pp. 217-223.

Schneider S, Roessli D, and Excoffier L (2000) Arlequin: A software for population genetics data analysis. Ver 2.000. Genetics and Biometry Lab, Dept. of Anthropology, University of Geneva.

Sneath PHA and Sokal RR (1973) Numerical Taxonomy. Freeman, San Francisco, pp. 573.

Taberlet P and Bouvet j (1994) Mitochondrial DNA polymorphism, phylogeography, and conservation genetics of the brown bear Ursus arctos in Europe. *Proc Roy Soc London* B255: 195-200.

Tamura K and Nei M (1993) Estimating of the number of nucleotide substitution in the control region of mitochondrial DNA in humans and chimpanzees. *Mol Biol Evol* 10: 512-526.

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