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Further microsatellite analysis on North Pacific minke whales including JARPN and JARPNII samples from 1994 to 2001

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ABSTRACT

We used seven microsatellite loci to examine genetic stock structure of minke whales collected from sub-areas 7, 8, 9, and 11 in the western North Pacific during JARPN and JARPNII from 1994 to 2001.
No evidence of genetic differences was detected among the samples from different sub-areas, indicating existence of a single stock in the study area. No evidence of genetic differences was also detected from all comparisons conducted between different combinations of the samples from different sub-areas: SA (7+11) vs SA8, SA (7+11) vs SA9, and SA (7+8+11) vs SA9. No evidence of genetic difference was detected between samples collected from east and west of longitude 153 degree. Although the possibility that the members of a different stock might occasionally enter west of the sub-area 9 cannot be excluded, our results did not support additional boundaries in the western North Pacific.

INTRODUCTION

Genetic variations at seven microsatellite loci were examined on samples of minke whales taken from sub-areas 7, 8, 9, and 11 during JARPN and JARPNII in 1994-2001 to describe stock structure in the western North Pacific. In this paper, we reanalyzed our data following the last year's IWC/SC recommendation that J-stock whales should be removed for all comparisons meant to address O-W stock issues (IWC, 2002). We also conduced heterogeneity tests between the samples from different combinations of these sub-areas on the basis of the requests made by a member of the North Pacific Minke Whale RMP IST Steering Group.

MATERIALS AND METHODS

Samples and localities

Samples used for the analysis was taken from sub-areas 7, 8, 9, and 11 during JARPN and JARPNII in 1994-2001 (Fig. 1). Suspected J-type individuals were excluded from the analysis based on the criterion proposed by Goto *et al.* (SC/J02/NP10). The number of samples for each sub-area and collection dates were in Table 1.

Microsatellites

Microsatellite polymorphisms were analyzed from seven loci. The details of the seven loci used and all the procedures for DNA extraction, PCR amplification, and electrophoresis were described in our previous papers (e.g., Goto *et al.*, 2001).

Data analysis

All statistical tests were conducted using the computer program GENEPOP (Raymond and Rousset, 1995). Decision of statistically significance on hypothesis testing was made using the chi-square value obtained from summing the negative logarithm of P-values over the seven loci (Fisher 1950).

RESULTS AND DISCUSSION

We first tested if there was any evidence of genetic differences between samples collected from each sub-area in different years. If no genetic difference exists, then we combine the samples from the same sub-areas into one in the following analysis. Contingency table chi-square analysis for heterogeneity of allele frequencies indicated statistical heterogeneity among the samples within the sub-area 9 (P=0.030), but no heterogeneity in the sub-areas 7 (P=0.106) and 8 (P=0.234). Pair-wise comparisons between the samples within the sub-area 9 suggest the heterogeneity observed appeared to have derived from the allele frequencies of the sample collected in 2001 (data not shown). We, therefore, conducted further analysis using the samples with and without the 2001 sample from the sub-area 9.

The next issue to address was whether there was any evidence of genetic differences among samples collected from different sub-areas in the western North Pacific (Table 2). If genetic differences exist, then this would indicate the existence of multiple stocks in the study area. Contingency table chi-square analysis for heterogeneity of allele frequencies indicated that the chi-square value combined for the seven loci was not statistically significant for both cases with and without the 2001 sub-area 9 sample, indicating the members of same single stock of minke whales inhabit the sub-areas 7, 8, 9, and 11.

We also conducted the heterogeneity tests for comparisons between samples from sub-areas (7+11) and 8, (7+11) and 9, (7+8+11) and 9, and west and east of longitude 153 degree as requested by a member of the North Pacific Minke Whale RMP IST Steering Group (Table 3). All comparisons did not show evidence of heterogeneity for both cases with and without the 2001 sub-area 9 sample, supporting again the existence of only the single stock in the study area.

It is important to note, however, that the genetic difference we observed among the samples collected from the sub-area 9 in different years could indicate that some members of the different stock of minke whales might occasionally enter west side of the sub-area 9.

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International Whaling Commission. 2002. Report of the Scientific Committee. Annex D. Report of the sub-committee on the Revised Management Procedure. *J. Cetacean Res. Manage. (Suppl.)* 4:00–00.

Table 1. Samples of North Pacific minke whales taken from sub- areas 7, 8, 9, and 11 during JARPN (1994- 1999) and JARPNII (2000- 2001) used for the current study.

Sample	Collected years	J- type excluded
Within SA7	1996, 1997, 1998, 1999, 2000, 2001	173
Within SA8	1996, 1997, 1998, 2001	96
Within SA9	1994, 1995, 1997, 2000, 2001	192
Within SA11	1996, 1999	50

Table 2. Results (P- values) of heterogeneity test for the allele frequencies at seven microsatellite loci among the samples of minke whales collected from sub- areas 7, 8, 9 and 11. P- value for All was calculated from summation of - 2ln(P).

	Microsatellite locus							
Sample	EV104	GT211	GATA28	EV1	GATA417	GATA98	GT509	All
SA7x8x9x11 *1	0.251	0.742	0.053	0.038	0.956	0.758	0.486	0.212
SA7x8x9x11 *2	0.249	0.633	0.052	0.071	0.980	0.571	0.420	0.217

*1 includes the sample collected from SA9 in 2001.

*2 excludes the sample collected from SA9 in 2001.

Table 3. Results (P- values) of heterogeneity tests for the allele frequencies at seven microsatellite loci between samples from sub- areas (7+11) and 8, (7+11) and 9, (7+8+11) and 9, and west and east of longitude 153 degree. P- value for All was calculated from summation of - 2ln(P).

	Microsatellite locus							
Sample	EV104	GT211	GATA28	EV1	GATA417	GATA98	GT509	All
SA (7+11) x 8	0.131	0.411	0.037	0.354	0.774	0.762	0.171	0.162
SA(7+11) x 9 *1	0.362	0.708	0.054	0.127	0.979	0.448	0.844	0.400
SA(7+8+11) x 9 *1	0.721	0.428	0.237	0.055	0.835	0.416	0.654	0.450
west x east *1	0.534	0.857	0.232	0.053	0.838	0.076	0.554	0.253
SA(7+11) x 9 *2	0.285	0.677	0.035	0.202	0.988	0.262	0.844	0.298
SA(7+8+11) x 9 *2	0.698	0.317	0.163	0.134	0.898	0.189	0.750	0.393
west x east *2	0.427	0.813	0.276	0.093	0.937	0.030	0.411	0.191

*1 includes the sample collected from SA9 in 2001.

*2 excludes the sample collected from SA9 in 2001.

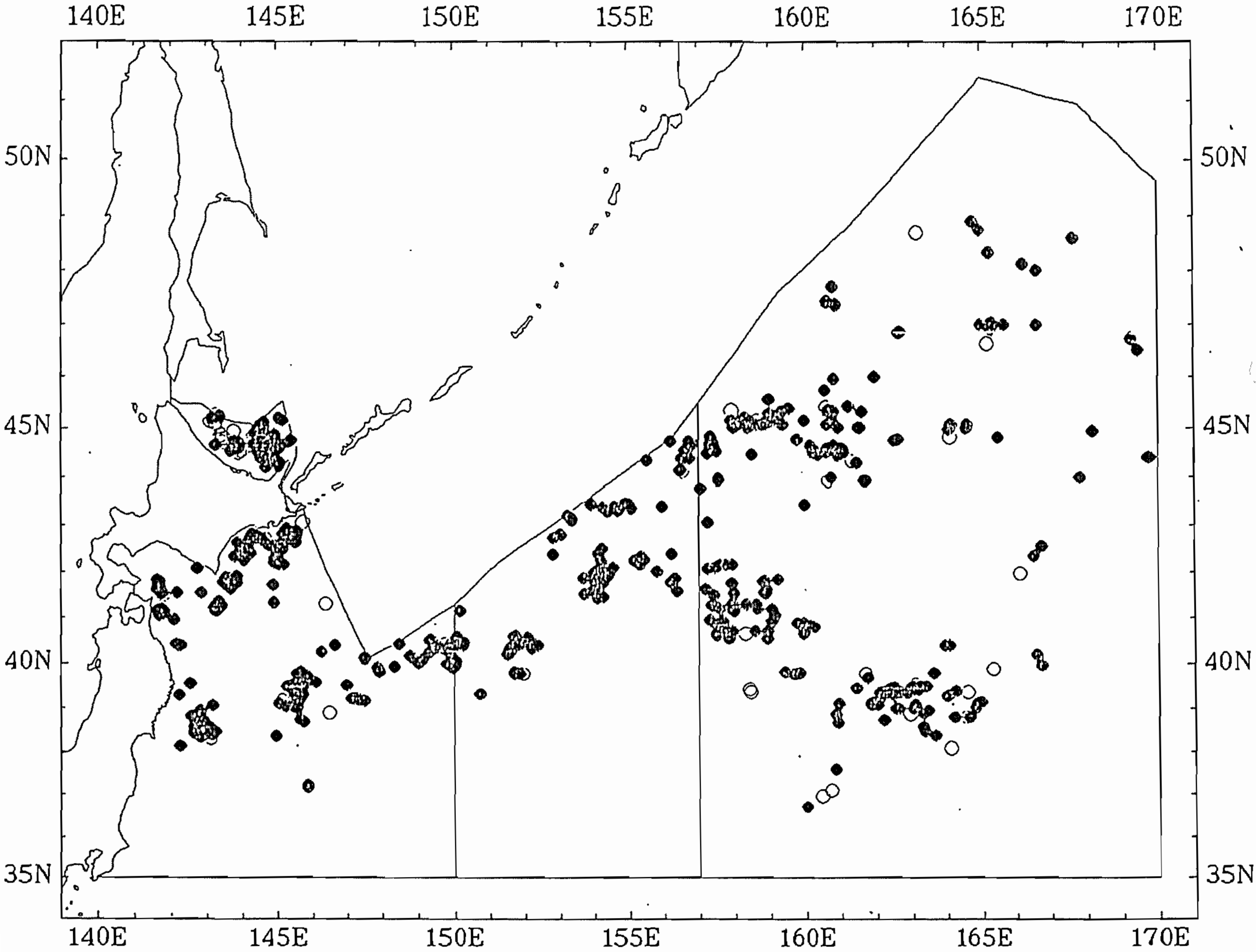

Fig. 1. Geographical positions of North Pacific minke whales sampled in the JARPN and JARPN II surveys from 1994 to 2001. ○: female, ●: male.