FOR CONSIDERATION BY THE SCIENTIFIC COMMITTEE OF
THE INTERNATIONAL WHALING COMMISSION
TROMSO, NORWAY, JUNE 2011

Investigation of Hardy-Weinberg equilibria and population differentiation in North Pacific minke whales, based on microsatellite genotypes of Japanese 'bycatch' and scientific whaling

Beth Slikas and C. Scott Baker
1) Marine Mammal Institute, Oregon State University, Newport, Oregon

Abstract

Here we update initial analyses reported in SC/D10/NPM4 by investigating differences among western North Pacific minke whales using microsatellite genotypes from samples collected from Japanese 'bycatch' and scientific whaling. These data were made available in three datasets, courtesy of the Institute for Cetacean Research of Tokyo (ICR) through the IWC Data Availability Group (with correction in versions 2.0 and 3.0). The dataset included microsatellite genotypes at 16 loci for 2546 samples. The analyses addressed the relative plausibility of different stock structure hypotheses, as outlined in discussions of the in-depth assessment of western North Pacific common minke whales. Overall results are consistent with analyses for mitochondrial haplogroups presented in SC/62/NPM20, for mitochondrial haplotypes reported in SC/D10/NPM3 and SC/63/RMP16, and for initial analyses of microsatellite data reported in SC/D10/NPM4. Tests of genetic differentiation and calculation of F_{ST} values based on the Japanese microsatellite dataset show significant differentiation for almost all comparisons of subareas and strata. However, tests for deviation from Hardy Weinberg equilibria, using Bonferroni corrections, do not show a pattern consistent with the mixing of two distinct stocks. A Mantel test of genetic distance versus geographic distance for samples from Pacific coastal and offshore whaling (e.g., subareas 7 and 8, excluding bycatch), showed a significant relationship, supporting an isolation-by-distance population structure along an east/west gradient. Together, these results indicate the existence of multiple stocks of minke whales in the North Pacific, with a gradient of genetic differentiation along an east/west axis, as predicted in Stock Structure Hypothesis III, rather than a mixing of two stocks as predicted in Stock Structure Hypotheses I and II (SC/63/RMP8).

Introduction

Here we evaluate plausible stock hypotheses, as outlined in discussions of the in-depth assessment of western North Pacific common minke whales. Such 'hypothesis testing' is considered the most sensitive approach to identifying populations characterized by moderate differences in genotype frequencies (i.e., moderate levels of gene flow, Waples and Gaggiotti 2006). Analyses were performed with a dataset of microsatellite genotypes for 2546 individuals from several subareas in the North Pacific (with corrections received in versions 2.0 and 3.0 of the dataset), made available from the Institute for Cetacean Research of Tokyo (ICR) through the Data Availability Group of the International Whaling Commission (IWC). We present results from tests of population differentiation and Hardy-Weinberg equilibrium in various subareas and geographic strata and in simulated mixed stock populations, to test the hypothesis that certain subareas include a mix of individuals from two differentiated stocks. We also present results from a Mantel test of geographic distance versus genetic distance, to test for an isolation-by-distance population structure. These analyses add to previous analyses by Kanda et al. (2009, 2010) and Park et al. (2010).

Methods

Dataset history. Information from Japanese 'bycatch' and scientific whaling was made available in three datasets, courtesy of the Institute for Cetacean Research of Tokyo (ICR), through the IWC Data Availability Group: Japanese 'bycatch' (n=832), 'coastal whaling' (n=481) and 'offshore whaling' (n=1238). Following quality control review of mtDNA sequences and communication with ICR, a revised dataset with corrected haplotypes was received on 13 April 2010 (version 2.0) and further corrections for 'distance from shore' on 30 August 2010 (ver 3.0). Following a second data

availability request for access to microsatellite genotypes (16 loci), the microsatellite data and additional information on position of bycatch and distance from shore of coastal and offshore whaling were received on 23 October 2010 (version 3.0). The microsatellite dataset received on 23 October 2010 did not include associated sex, mtDNA haplotype or collection data (e.g., no date, subarea, latitude or longitude). For most analyses, we collated information from the microsatellite genotypes with the previous corrected file for mtDNA (NP minke mtDNA-data ver 3.0 30 August 2010.xls).

In SC/D10/NPM4, we presented results from initial analyses of these data, including tests of Hardy Weinberg equilibrium and population differentiation among various geographic strata/subareas. Here, we extend those preliminary analyses. We repeat tests of Hardy Weinberg equilibrium for a set of 8 geographical strata: subarea 6 (6Ebc), subarea 2 (2bc), 4 strata representing subarea 7, subarea 8 (8), and subarea 9 (9). The four strata from subarea 7 were separated based on geographic location and source (hunting, bycatch) and were found to be significantly differentiated from each other (SC/D10/NPM4): bycatch (7bc), Sanriku and 'offshore' 7W samples collected <60 nm from shore, south of 41 degrees latitude (7CS), Kushiro and 'offshore' 7W samples collected < 60nm from shore, at or north of 41 degrees latitude (7CN), and 7E and 7W samples collected > 60 nm from shore (7E+7W>60).

Simulated mixed stocks. In addition, we created a simulated mixed-stock dataset by combining bycatch samples from subarea 6E (n=411), representing pure JW stock, and offshore hunting samples from subarea 9 (n=466), representing pure OE stock. A summary of the rationale for designation of proxies for 'pure' stock samples is presented in Wade and Baker (2011). We tested for Hardy-Weinberg equilibrium in each of the 8 geographical strata and in the simulated mixed stock, using a two-tailed probability test in Genepop version 4 (Rousset 2008, option 1.3). We selected the option to use complete enumeration whenever possible. For the Markov chain, we ran analyses with dememorisation=10000, BatchNumber=1000, and BatchLength=5000. We also calculated F_{ST} values (Genepop, option 6.2) and conducted G tests (Genepop, option 3.4) to assess population differentiation.

Isolation by distance. To test for isolation by distance among putative O stock samples (as defined in stock structure Hypotheses I and II), we performed a Mantel test of genetic distance versus geographic distance using the software Genalex (version 6, Peakall and Smouse 2006). To represent the O stock, we used genotypes from subarea 8 (n=224) and coastal and offshore hunting genotypes from subarea 7 (n=943). We calculated both genetic and geographic distance in Genalex. For genetic distance, we used the option for codominant data. For geographic distance, we performed two separate calculations, using latitude and longitude data. Given that sampled individuals might be migrating along a north/south axis, we performed a one-dimensional distance calculation, in which we assigned a fixed latitude value to all samples (42 degree N), paired with the actual longitude value. We also performed a second, two-dimensional distance calculation, using the actual latitude and longitude values for each sample. We ran a Mantel test for each of the distance calculations (one-dimensional, two-dimensional) versus genetic distance. The Mantel tests were run with 999 permutations, to obtain a null distribution for the test statistic (Rxy).

We performed a principal component analysis (PCA) based on allele frequencies to visualize the distribution of 5 subareas/populations (subarea 6, subarea 2, the coastal hunting samples from subarea 7, subarea 8, subarea9) along the first two PCA axes.

Results

Sample strata and test of differentiation. The revised ICR datasets (Japanese Coastal, Offshore and Bycatch) totaled 2,551 samples, of which 2,546 included microsatellite genotype data for 16 loci. We divided the samples into 8 strata, including 4 strata representing subarea 7, based on previously-reported analyses showing significant differentiation among these strata (SC/D10/NPM4). The 8 strata include the following: subarea 6 bycatch (6Ebc), subarea 2 bycatch (2bc), subarea 7 bycatch (7bc), Sanriku and "offshore" 7W samples collected <60 nm from shore, below 41°N (7CS), Kushiro and "offshore" 7W samples collected < 60 nm from shore, at or above 41°N (7CN), 7E and 7W samples > 60 nm from shore (7E + 7W>60), subarea 8 (8), and subarea 9 (9). Tests of genetic differentiation showed significant differentiation in almost all comparisons among the 8 geographic strata/subareas across the Pacific (Table 3).

Hardy-Weinberg equilibrium – simulated mixed stock JW+OE. We simulated a mixed stock of pure JW and pure OE individuals using samples from subareas 6 and 9 and examined HWE in the simulated mixed stock, as well as in eight geographical strata/subareas across the Pacific. In the simulated mixed stock, 5 of 16 microsatellite loci were out of HWE, as expected for a Wahlund effect due to a mixed sample from two stocks with distinct differences in allele frequencies (Table 1). Among the 8 geographic strata/subareas defined in SC/D10/NPM4, however, we observed far less deviation from HWE: three strata were out of HWE at one of 16 loci, and five strata were out of HWE at zero of 16 loci (Table 2).

Mantel test of geographic distance versus genetic distance. Mantel tests of geographic versus genetic distance showed a significant relationship for both 2 dimensions (latitude and longitude) and 1 dimension (longitude only) across the coastal and offshore whaling samples of the Pacific: one dimensional $Rxy = 0.052$, $p=0.001$ and two-dimensional $Rxy=0.057$, $p=0.001$. These results support an isolation-by-distance pattern of genetic divergence, primarily along an east/west axis across the range of the putative O stock, as assumed in Hypothesis I and II, or across the OW and OE stocks of Hypothesis III (Figure 1).

Principal Component Analysis (PCA): The PCA analysis based on all samples (e.g., bycatch, coastal and offshore whaling) showed clear separation of the 5 subareas/population strata, along both PCA axes, supporting the existence of more than 2 stocks in the Pacific (Figure 2).

Conclusions

The analyses presented here are consistent with the predictions of multiple stocks of minke whales in the Pacific coastal and offshore waters of Japan, as described in Stock Structure Hypothesis III, rather than a complex mixing of two stocks, as described in Stock Structure Hypotheses I and II (see SC/63/RMP8).

1) Analyses of microsatellite allele frequencies (and mtDNA haplotype frequencies) showed significant differentiation in almost all comparisons among the eight geographic strata in the North Pacific dataset (Table 3).
2) Analyses of Hardy-Weinberg equilibrium are not consistent with mixing of two distinct stocks in any subarea. *Evidence:* We simulated a mixed stock of pure J and pure O individuals, and examined HWE in the simulated mixed stock, as well as in eight geographical strata/subareas across the Pacific. In the simulated mixed stock, 5 of 16 microsatellite loci were out of HWE, as expected in a mixed population (Table 2). Among the 8 geographic strata/subareas defined in SC/D10/NPM4, far fewer loci were out of HWE: three strata were out of HWE at one of 16 loci, and five strata were out of HWE at zero of 16 loci (Table 1).
3) A Mantel test of geographic versus genetic distance showed a significant relationship for the combined samples from coastal and offshore whaling in subarea 7 and 8, suggested a pattern of isolation-by-distance along an east/west axis, more consistent with the presence of an OW and OE stock (Figure 1).
4) A PCA analysis based on allele frequencies showed separation of 5 subareas in the Pacific, supporting the existence of more than 2 stocks (Figure 2).

Acknowledgements

Literature Cited

Baker, CS, RM Hamner, RL Brownell Jr., and PR Wade. 2010. Stock structure of western North Pacific minke whales based on mtDNA haplogroups from 'bycatch' and scientific whaling. Report (SC/62/NPM20) to the Scientific Committee of the International Whaling Commission.

Baker, CS, B Slikas, RL Brownell Jr., and PR Wade. 2011. Stock structure of western North Pacific minke whales based on mtDNA haplotypes from Korean 'bycatch' and Japanese 'bycatch' and scientific whaling, in relation to Hypothesis III. Report (SC/63/RMP16) to the Scientific Committee of the International Whaling Commission.

Baker, CS, B Slikas, RL Brownell Jr., and PR Wade. 2010. Stock structure of western North Pacific minke whales based on mtDNA haplotypes from Korean 'bycatch' and Japanese 'bycatch' and scientific whaling. SC/D10/NPM3.

Slikas, B, and Baker, CS. 2010. Preliminary investigation of Hardy-Weinberg equilibria and population differentiation in NP minke whales, based on microsatellite data. Report (SC/D10/NPM4) to the Scientific Committee of the International Whaling Commission..

Kanda, N., M. Goto, S. Nagatsuka, H. Kato, L.A. Pastene, and H. Hatanaka. 2009. Analyses of genetic and non-genetic data do not support the hypothesis of an intermediate stock in sub-area 7. Report (SC/S10/NPM9) to the Scientific Committee of the International Whaling Commission.

Kanda, N., J.Y. Park, M. Goto, Y.-R. An, S.-G. Chou, D.-Y. Moon, T. Kishiro, H. Yoshida, H. Kato and L.A. Pastene. 2010. Genetic analysis of western North Pacifc minke whales from Korea and Japan based on microsatellite DNA. Report (SC/62/NPM11) to the Scientific Committee of the International Whaling Commission.

Park, JY, M Goto, N Kanda, T Kishiro, H Yoshida, H Kato, and LA Pastene. 2010. Mitochondrial DNA analyses of J and O stocks common minke whale in the western North Pacific. Report (SC/62/NPM21) to the Scientific Committee of the International Whaling Commission.

Peakall, R. and P. Smouse. 2006. Genetic analysis in Excel: Population genetic software for teaching and research. Molecular Ecology Notes 6:288-295.

Rousset, F. 2008. Genepop,'007: a complete re-implementation of the Genepop software for Windows and Linux. Molecular Ecology Resources 8:103-106.

Wade, P.R. and C.S. Baker. 2011. A summary of the plausibility of western North Pacific minke whale stock structure hypotheses I, II and III. Report (SC/63/RMP8) to the Scientific Committee of the International Whaling Commission.

Waples, R.S. and O. Gaggiotti. 2006. What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. Molecular Ecology 15:1419-1439.

Table 1: Test of Hardy-Weinberg equilibrium for simulated mixed-stock population for 'pure' JW and OE. Significant p values are shown in bold italic; significant values after Bonferroni-correction are indicated with asterisks (* p <0.05, ** p<0.01, after Bonferroni correction).

	combined JW + OE area 6 and 9 (n=877)		JW vs OE area 6 vs area 9
	p-value	Fis - W&C	Fst
EV37	*0.0225*	0.0023	*0.0079* ***
EV1	0.4591	0.0318	0.0308
GT310	*0.0495*	0.0208	0.0657
GATA28	0.0653	0.0109	*0.0128* ***
GT575	0.1561	0.0212	0.0448
EV94	0.9777	-0.0012	0.0229
GT23	*0.0056*	0.0226	*0.0167*
GT509	*0.0000* ***	0.0482	*0.0450*
GATA98	0.9769	0.0162	0.0067
GATA417	0.4206	-0.0161	0.0227
GT211	0.1033	0.0212	0.0513
EV21	0.1979	-0.0455	-0.0010
DlrFB14	*0.0005* **	0.0215	*0.0534* ***
EV14	*0.0000* ***	0.0833	*0.0931* ***
GT195	*0.0000* ***	0.0631	*0.1008* ***
TAA31	*0.0003* **	0.0400	*0.0590* ***

Table 2: Test of Hardy-Weinberg equilibrium for NP minke microsatellite data in 8 geographic strata/subareas, based on 16 loci (test conducted in Genepop), with reported *p* values and results of standard Bonferroni corrections for number of loci with each sample. Significant p values are shown in bold italic; significant values after Bonferroni-correction are indicated with asterisks (* p<0.05, ** p<0.01, after Bonferroni correction).

locus	6Ebc (n=411)		2bc (n=184)		7bc (n=212)		7CS (n=321)		7CN (n=504)		7E+7W>60 (n=118)		8 (n=224)		9 (n=472)		per
	p	Fis	p	Fis	p	Fis	p	Fis	p	Fis	p	Fis	p	Fis	p	Fis	locus
EV37	0.8797	0.0160	*0.0167*	-0.0032	0.6298	0.0319	0.1407	-0.0106	0.6689	-0.0219	0.2871	0.0293	0.6320	0.0638	0.1281	-0.0156	0(1)
EV1	0.7185	0.0169	0.3329	0.0422	0.2945	0.0085	0.1435	0.0317	0.1093	0.0437	0.4117	-0.0086	0.3073	0.0026	0.9656	0.0161	0(0)
GT310	0.2780	-0.0171	0.2800	-0.0164	*0.0221*	0.0779	*0.0187*	0.0665	0.0983	0.0158	0.5752	-0.0035	0.3268	-0.0006	0.9596	-0.0106	0(2)
GATA28	0.3880	-0.0251	0.3592	0.0129	0.0037	0.0110	0.6748	0.0159	0.4942	0.0271	0.4985	-0.0135	0.5581	0.0013	0.6074	0.0285	0(0)
GT575	0.3490	0.0378	0.5628	0.0220	0.3642	0.0312	0.9330	0.0129	0.2179	0.0189	*0.0272*	0.0657	0.3452	0.0600	0.5458	-0.0329	0(1)
EV94	0.3958	0.0005	0.7251	-0.0191	0.3213	0.0681	0.9766	0.0155	0.1862	0.0513	0.8747	-0.0553	0.3493	0.0035	0.6649	-0.0230	0(0)
GT23	0.4237	0.0129	0.2284	0.0410	0.4314	-0.0445	0.2042	0.0252	0.4801	-0.0034	0.2575	0.0958	0.9532	0.0273	0.6843	0.0156	0(0)
GT509	0.6930	0.0297	0.9002	-0.0251	*0.0001* **	0.1227	*0.0208*	-0.0007	*0.0067*	0.0177	0.1085	0.0854	0.4884	-0.0281	0.8260	0.0223	1(3)
GATA98	0.2927	-0.0402	0.8669	-0.0037	0.5393	0.0307	0.5541	-0.0114	0.9173	0.0072	0.3465	0.0043	0.4542	0.0642	0.3575	0.0552	0(0)
GATA417	0.5035	-0.0482	0.0946	0.0097	0.1175	-0.0562	*0.0021* *	0.0055	0.4109	0.0216	*0.0385*	-0.0262	0.4953	-0.0442	0.6362	-0.0120	1(2)
GT211	0.6630	-0.0117	*0.0147*	0.0381	0.4595	0.0167	0.0932	0.0111	0.3414	-0.0029	0.8261	-0.0342	0.1525	-0.0114	0.9328	0.0001	0(1)
EV21	0.4752	-0.0380	0.3895	0.0687	0.6655	0.0286	0.6871	0.0259	0.2245	0.0589	1.0000	-0.0317	0.3156	-0.0748	0.3336	-0.0513	0(0)
DlrFB14	0.0526	0.0122	0.2317	0.1085	0.8002	-0.0677	*0.0102*	-0.0777	0.2619	0.0345	0.6023	-0.0064	0.9990	-0.0271	*0.0318*	-0.0261	0(2)
EV14	0.6390	0.0469	0.0802	0.1338	0.0702	0.0872	*0.0056*	0.0593	0.4577	0.0053	0.9931	-0.0388	0.7784	-0.0257	0.3722	0.0295	0(1)
GT195	0.5362	0.0265	*0.0057*	0.0426	*0.0074*	0.0227	0.1843	0.0018	*0.0131*	0.0295	0.9414	0.0857	0.7919	0.0115	0.8118	-0.0027	0(3)
TAA31	*0.0013* *	0.0379	0.8157	-0.0269	0.2495	0.0911	0.6218	-0.0360	*0.0468*	0.0778	0.1225	-0.0950	0.7410	0.0210	1.0000	-0.0044	1(2)
	6Ebc (n=411)		2bc (n=184)		7bc (n=212)		7CS (n=321)		7CN (n=504)		7E+7W>60 (n=118)		area8 (n=224)		area9 (n=472)		
# loci out of HWE per pop	1(1)		0(3)		1(3)		1(5)		0(3)		0(2)		0(0)		0(1)		
# with Bonferoni_correction (# without Bonferroni-correction)																	

Table 3: Fst values and G test of differentiation for 8 geographic strata/subareas based on 16 microsatellite loci.

	6Ebc	2bc	7bc	7CN	7CS	7E+7W>60	8
2bc	0.0008***						
7bc	0.0103***	0.0049***					
7CN	0.0336***	0.0228***	0.0071***				
7CS	0.0313***	0.0209***	0.0049***	0.0008*			
7E+7W>60	0.0500***	0.0355***	0.0142***	0.0013**	0.0026***		
8	0.0415***	0.0286***	0.0105***	0.0005**	0.0014***	-0.0004	
9	0.0406***	0.0284***	0.0104***	0.0002***	0.0013***	0.0001	-0.0005

Figure 1: Mantel test results for a relationship between genetic distance and geographic distance (1-dimensional). The data Rxy (gray) is significantly greater than the values from random permutations (black, 999 permutations) (p=0.001).
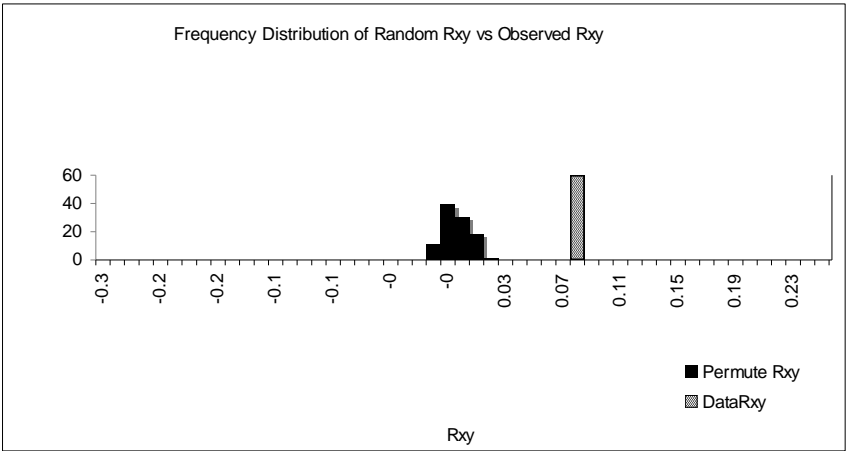

Figure 2: Principal component analysis based on allele frequencies of 16 microsatellite for 5 subareas, performed in Genalex. Coastal whaling samples from Sanriku and Kushiro were combined into 7COw.