UPDATE ANALYSES OF THE GENETIC STRUTCTURE OF WESTERN NORTH PACIFIC MINKE WHALES

Oscar E. Gaggiotti and Eric Durand

Laboratoire d'Ecologie Alpine, UMR CNRS 5553, BP 53, 38041 Grenoble cedex 9, France

ABSTRACT

Several population genetic studies of the western North Pacific minke whales have been carried out in the past but we do not yet have a clear picture of the spatial structuring of this species. The most important obstacle faced by these studies is a lack of knowledge of the location of the breeding grounds and the fact that available samples correspond to migrating individuals. In principle genetic clustering algorithms should be able to identify population boundaries but the degree of genetic differentiation falls below the minimum required to obtain reliable results. In this report we carry out a very detailed genetic structure analysis using both mtDNA and microsatellite markers. The results indicate that there is genetic structuring in this species but does not correspond to the baseline C hypothesis. One possible interpretation of the results is that two or more stocks exist and that their limits change through time due to temporal changes in migration routes. We provide suggestions for further analyses that should be carried out to test this hypothesis.

INTRODUCTION

A better understanding of the genetic structure is required for undertaking a full implementation review of WNP minke whales and to examine effects of Scientific Permit catches on stocks. The current management paradigm for WNP minke whales is based in part on studies conducted in 2003 (Taylor and Martien 2004) using mtDNA data available at that time. Recently, Japanese scientists have collected a great deal of new genetic information for WNP minke whales, and it is now possible to carry out a reassessment of the genetic structure in this species based on the new data.

The main obstacle to arriving at a clear understanding of the genetic structuring in this species in the western North Pacific is that the breeding grounds have not been identified and all genetic samples were obtained from migrating individuals. Furthermore, previous studies (e.g. Kanda et al. 2009) suggest that available samples represent a genetic mixture of two stocks, the so-called J stock that occurs mainly in the Sea of Japan, and the O stock with a distribution that covers the western North Pacific.

Three alternative population structure scenarios have been proposed, baselines A, B, and C (e.g. see Taylor and Martien 2003). Baseline A contends that most whales to the East of Japan are O stock but that there are occasional intrusions of individuals from a third stock, W, that would appear in area 9W in 60% of the years. Baseline B contends that there is a single stock East of Japan. Baseline C proposes the existence of three stocks, Ow, Oe and W. The boundary between Ow and Oe would be located at 147°E while that between Oe and W would be situated at 157°E.

The contract stipulated that the new microstellite data would be used to carry out two different analyses: (i) a new simulation study that consider the same demographic scenario studied by Taylor and Martien (2004) with the objective of estimating migration rates between the Oe and Ow stocks. The results of this study are presented in the appendix. (ii) A thorough analyses of the genetic structure of minke whales using the Boundary Rank analysis proposed by Martien and Taylor (2001). This study forms the main body of this report. Additionally, we carried out an analysis using a new method that instead of using a contingency test of genotype frequencies used a test of panmixia. The results of this analysis are presented in the appendix.

MATERIAL AND METHODS

Data

We used both mitochondrial DNA and microsatellite data provided by the Institute of Cetacean Research (Japan) corresponding to samples of common minke whales taken by JARPN (1994-99) and JARPNII (2000-07). The samples cover sub-areas 7, 8 and 9 in the western North Pacific and include 1158 offshore and 481 coastal individuals. The mtDNA data consisted of the first half of the control region and covered 487bp (see Goto et al. 2008 for details). The microsatellite data included 16 loci and are described in Kanda et al. (2009)

Preliminary Analyses

We used the method of boundary rank analysis developed by Martien and Taylor (2001) to study the genetic structure of minke whales. The application of this method requires two preliminary steps: (i) defining the initial grouping of samples and (ii) defining the connectivity matrix that determines which groups can be merged together.

In order to obtain the initial configuration we first partitioned the whole area into broad regions based on a spatial Principal Component Analysis (Patterson et al. 2006) of the full mtDNA and microsatellite data sets (no individuals were excluded from this analysis). This is a method that is particularly appropriate for situations where genetic differentiation is very low. PCA does not group individuals into discrete populations being only able to output each individual's coordinates along axes of variation. However, it is possible to represent each individual PCA score for each axis of variation on a map, as it is done in Figure I for PC1 and PC2. The former explains 49.6% of the variance while the latter explains 6.5% of the variance. We carried out the TW test suggested by Patterson et al. (2006) and obtained p-values of 0.023 for PC1 and 0.02 for PC2. All other axes of variation were not significant.

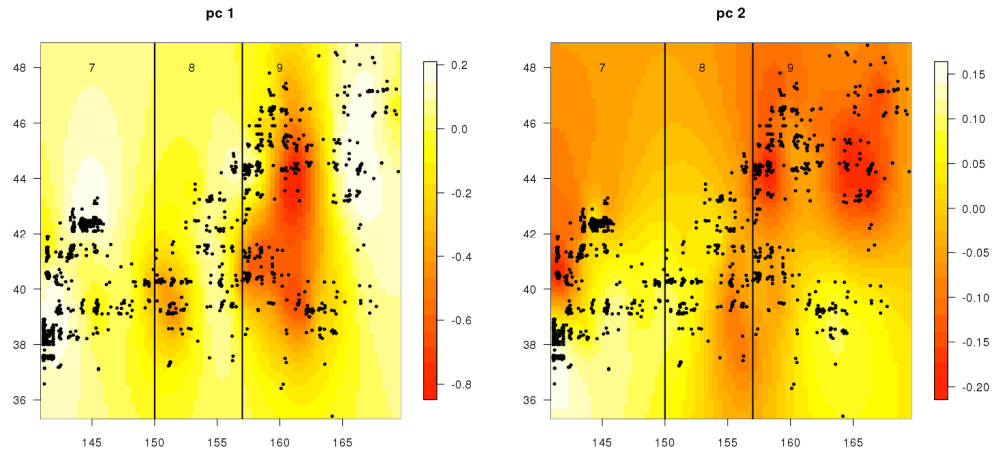

Figure 1: results of the PCA showing the distribution of PCA scores for the first two axis of variation.

A first clustering of individuals is obtained by grouping contiguous samples that have similar scores along the first axis. This initial clustering can be further subdivided by considering the scores obtained along the second axis. Proceeding in this fashion we obtained 4 clusters in area 7, 2 in area 8 and 5 in area 9. Additionally we tried to further subdivide these 11 clusters based on geographic proximity. However, we could only identify spatial discontinuities in area 9, which allowed us to further subdivide one of its clusters into two. Thus, we obtained a total of 12 clusters that are represented in Figure 2.

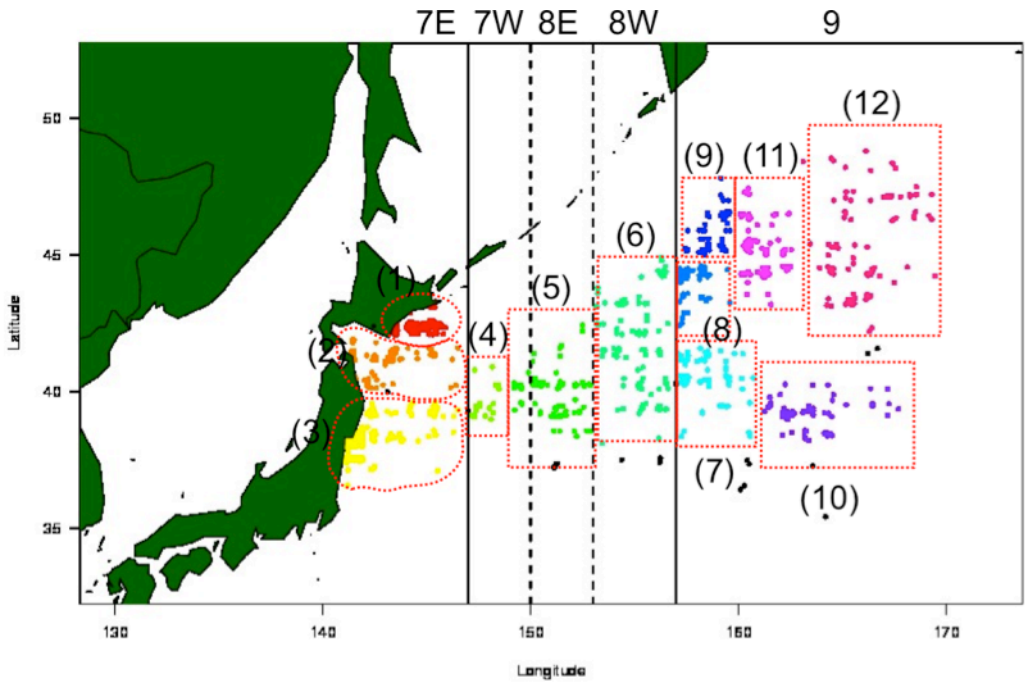

Figure 2: Map with the initial clustering of samples used for the Boundary Rank analyses. Vertical lines indicate the boundaries between the different areas.

In order to define the connectivity matrix, we assumed that two clusters, *i* and *j*, were connected only if an individual could go from cluster *i* to cluster *j* without

traversing a third cluster. The connectivity graph we obtained is presented in Figure 3.

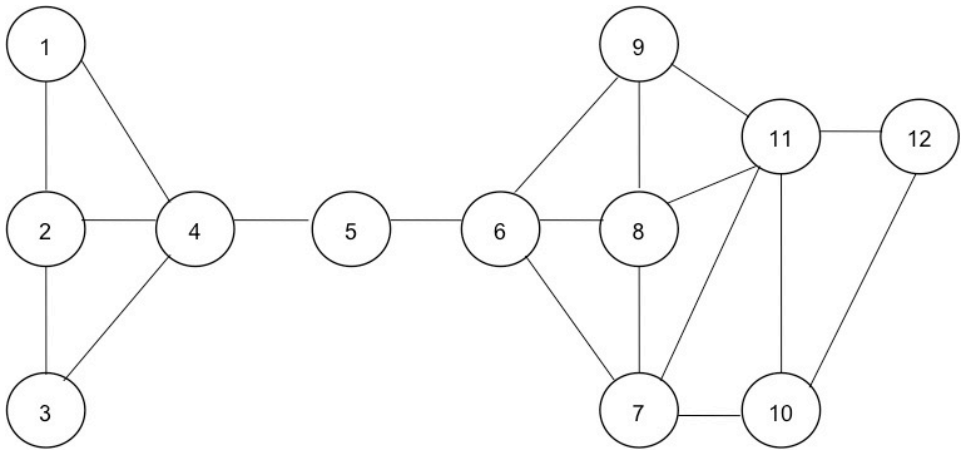

Figure 3: Connectivity graph corresponding to the initial configuration shown in figure 2.

One important problem that needed to be addressed is the effect that the choice of individuals to be included in the analysis could have on the results. The data set available can potentially contain a mixture of different stocks. More precisely, individuals could be classified as belonging to the J or O stock. Since the objective is to try to determine if there is heterogeneity within the O stock, we would like to avoid the inclusion of J-stock individuals. However, there is little agreement on how to assign individuals to each one of these stocks. Therefore, removal of J-stock or J-stock-like individuals should be implemented with caution, as it is important to try to find a balance between two types of potential biases: a) Inclusion of too many 'J' individuals could mask a true signal of heterogeneity in offshore whales, or could create a false signal that is interpreted as heterogeneity in the O stock; b) Aggressive purging of 'J' like individuals could eliminate a true signal due to additional offshore stocks that happen to be genetically similar to J stock. Given this potential biases we carried out a sensitivity analysis focused on the effect of the exclusion of J-stock individuals (see below).

There are two genetic criteria that could be used. One, based on mtDNA , is the so called "2 site mtDNA CR criterion" used by Baker et al. (2000): combinations of bases "A" and "G" in positions 298 and 463. Combination "AG" identifies O stock animals while the remaining combinations ("AA", "GA" and "GG") identify J stock animals. The individuals included in the data set were labelled with a "*" if they were identified as belonging to stock J using this criterion.

A second criterion is based on microsatellite data and a STRUCTURE analysis. In this case, it is necessary to choose a threshold for the membership probabilities. Individuals included in the microsatellite data set are labelled according to their assignment probabilities using the following rules:

O= membership probability for O stock of over 90%
J= membership probability for J stock of over 90%
O?= membership probability for O stock between 75-89.9%

J?= membership probability for J stock between 75-89.9%
??= unknown (probability between 50 and 74.9%)

Note that STRUCTURE does not identify individuals as O or J; it simply finds two different gene pools. Then each one is labelled based on the geographic location of individuals.

Given the many alternative ways of classifying individuals as belonging to the J-stock we decided to carry out a thorough sensitivity analyses that considered a wide range of alternative ways of purging the data set from J-stock individuals. More precisely, we considered the following alternative scenarios:

Scenario 1: all individuals were included regardless of their potential origin.
Scenario 2: only individuals identified as belonging to the J stock using mtDNA data (those labelled "*" in the data base) were excluded.
Scenario 3: only individuals identified as belonging to the J stock with a probability larger than 90% using the microsatellite criterion (those labelled "J" in the data base) were excluded.
Scenario 4: only individuals belonging to the O stock with a probability larger than 90% using the microsatellite criterion (those labelled "O" in the data base) were included in the analysis.

Scenario 1 considers no purging, scenario 3 represents mild purging and scenario 4 represents aggressive purging. The inclusion of scenario 2 allows us to investigate the extent to which mtDNA and microsatellite criteria differ.

The four scenarios described above were investigated using mtDNA and microsatellite data separately. The consideration of the mtDNA criterion could allow us to make comparisons with the results of previous Boundary Rank analyses. However, it was not possible to obtain enough details about the initial clustering used in these studies. Therefore, we carried out additional BR analyses that only considered the 559 individuals included in previous studies. Here we also considered all four scenarios described above.

Boundary Rank Analysis

Boundary rank analysis (henceforth called BR) implements a hierarchical clustering algorithm that aggregates genetic samples on the basis of a measure of their genetic similarity. It starts from an initial partition of samples into clusters. At each step, the algorithm merges the two most similar clusters until all clusters are joined together.

In order to perform the BR analysis, it is necessary to define a measure of genetic similarity between pairs of clusters. A natural way to measure genetic similarity would be to test for statistical independence of genotype counts per pair of clusters, using Pearson's chi-square test. However, if the expected values within cells of the contingency table are very small, the calculated statistic may be inflated upwards (Cochran 1954). This is likely to happen for genotype counts in pairs of clusters, where rare genotypes can be in very low frequencies (or even absent) in at least one of the two clusters. Thus, BR analysis uses the p-value from a $\chi 2$ test based on Monte-Carlo simulations (Roff and Bentzen 1989) as the measure of genetic similarity. It tests for statistical independence between two clusters as follows. First, we build the contingency table of genotype counts in the

two clusters, and we compute the observed $\chi 2$ statistic, denoted X0. Then, we simulate a large number of randomized contingency tables, with the constraint that simulated tables have the same marginal counts as the observed table. We compute the $\chi 2$ statistic, denoted Xr, for each simulated contigency table. The p-value is then computed as the number of times that $Xr > X0$ divided by the total number of simulations.

Our implementation of the algorithm proceeds as follows. First, we define an initial clustering of samples. We also define a neighbourhood between the initial clusters, so that only neighbouring units are compared. Note that we could allow all clusters to be neighbours. Then, we compute the p-value from the $\chi 2$ test of Roff and Bentzen (1989) as described above for all pairs of neighbouring clusters. We aggregate the pair that has the highest p-value. We update the clustering of samples and the neighbourhood graph. The process is repeated until all units are put into one cluster. The output of the algorithm is a limited set of population structures, each one containing one cluster less than the previous.

Although it is not possible to define a rigorous way of interpreting the output of a BR analysis because of the problem of multiple tests, it is possible to use them in order to propose possible population structures that could be further investigated using other methods. In what follows we use an ad-hoc rule whereby we deem that two clusters are distinct if their associated p-value is less than 0.10. As stated before, this is not a statistically rigorous approach but it is justifiable because we are using BR only as an exploratory technique.

We carried out separate BR analyses with mtDNA and microsatellite data. BR was developed for mtDNA-sequence data, in which case the data consists of haplotypes counts. However, there are no specific guidelines for its use with microsatellite data. Thus, we considered two different approaches for the formatting of the microsatellite data. First, we formatted the microsatellite data into individual genotypes. Thus, we defined single locus genotypes based on the two observed alleles and then we obtained individual multilocus-genotypes by concatenating locus specific genotypes. We considered that data were not phased and thus we did not distinguish between the two possible states of heterozygous genotypes. The second approach formatted the microsatellite data into allele counts per sampling site. To do so, we computed the number of distinct allele for all loci in all clusters. We then applied the same BR algorithm, with the exception that contingency tables where built on allele counts rather than genotype counts. The results obtained by the two approaches were almost identical the only difference being a reversal of the order of the two first clustering steps. In other words, the first step in the genotype-based analyses corresponded to the second step in the allele-based analysis and vice versa. Thus, we only present the results obtained with the first approach.

RESULTS

Mitocondrial DNA data – New data set

The results of scenarios 2 and 3 are very similar (see Tables 1 and 2). The first three clustering steps group the same three pairs of initial sampling groups (2 with 3, 7 with 8 and 10 with 11). Then clustering proceeds progressively from West to East by adding individual groups or pair of groups to the pair formed by

groups 2 and 3. This suggests that there is no clear boundary separating sampling groups in the West-East direction. Interestingly, the last three clustering steps consist in the addition of the same individual groups (9, 12 and 1) to the large cluster obtained after the eighth step. Although the order in which groups 9 and 12 are added differ between scenarios 2 and 3, the very last sampling group to be added is group 1 for both scenarios.

Table 1 : Clustering steps under scenario 2 using the new mt-DNA data set.

Clustering steps	p-values	# of clusters
(7) and (8)	1.00	11
(10) and (11)	0.99	10
(2) and (3)	0.89	9
(2, 3) and (4)	0.87	8
((2, 3), 4) and (5)	0.82	7
(((2, 3), 4), 5) and (6)	0.46	6
((((2, 3), 4), 5), 6) and (7, 8)	0.72	5
(((((2, 3), 4), 5), 6), (7, 8)) and (10, 11)	0.77	4
((((((2, 3), 4), 5), 6), (7, 8)), (10, 11)) and (9)	0.20	3
(((((((2, 3), 4), 5), 6), (7, 8)), (10, 11)), 9) and (12)	0.13	2
((((((((2, 3), 4), 5), 6), (7, 8)), (10, 11)), 9), 12) and (1)	0.04	1

Table 2 : Clustering steps under scenario 3 using the new mt-DNA data set.

Clustering steps	p-values	# of clusters
(2) and (3)	0.99	11
(10) and (11)	0.98	10
(7) and (8)	0.95	9
(2, 3) and (4)	0.61	8
((2, 3), 4) and (5)	0.91	7
(((2,3), 4), 5) and (6)	0.48	6
((((2,3), 4), 5), 6) and (7,8)	0.86	5
(((((2,3), 4), 5), 6), (7,8)) and (10, 11)	0.78	4
((((((2,3), 4), 5), 6), (7,8)), (10, 11)) and (12)	0.25	3
(((((((2,3), 4), 5), 6), (7,8)), (10, 11)), 12) and (9)	0.17	2
(1) and ((((((((2,3), 4), 5), 6), (7,8)), (10, 11)), 12), 9)	0.00	1

Rather surprisingly the results of scenarios 1 (including all individuals) and 4 (including only individuals identified as pertaining to the O stock with more than 90% probability) were somewhat similar (see Tables 3 and 4) and very different from those obtained for scenarios 2 and 3. Here there is no progressive

clustering from West to East. The first three steps include the same three pairs of groups as before but the clustering steps that follow show two different clusters that are formed progressively, one to the West (1 through 5) and the other to the East (6 to 12). The main difference between scenarios 1 and 4 is that in the former sampling group 1 is the last to be added to the western cluster while in the latter it is added very early on. Thus, aggressive purging of individuals makes the samples from the Northwest (population 1) more similar to samples from area 7E.

There is one similarity between scenarios 1, 2 and 3 that is worth mentioning. In all of them, groups 1, 9 and 12 are the last to be added to the existing cluster(s) obtained after the ninth or tenth steps. Group 1 seems to be the most distinct since it is always the last to be joined. This is not true o scenario 4, presumably because aggressive purging may have eliminated the genetic signal responsible for its uniqueness.

Table 3 : Clustering steps Under scenario 1 using the new mt-DNA data set.

Clustering steps	p-values	# of clusters
(7) and (8)	1.00	11
(10) and (11)	0.98	10
(2) and (3)	0.97	9
(2, 3) and (4)	0.81	8
((2, 3), 4) and (5)	0.76	7
(6) and (7, 8)	0.36	6
(6, (7, 8)) and (10, 11)	0.77	5
((6, (7, 8)), (10, 11)) and (12)	0.40	4
(((6, (7, 8)), (10, 11)), 12) and (9)	0.15	3
(1) and (((2,3), 4), 5)	0.02	2
(1, (((2,3), 4), 5)) and ((((6, (7, 8)), 10), 12), 9)	0.00	1

Table 4 : Clustering steps under scenario 4 using the new mt-DNA data set.

Clustering steps	p-values	# of clusters
(10) and (11)	0.99	11
(7) and (8)	0.97	10
(2) and (3)	0.90	9
(1) and (4)	0.56	8
(1,4) and (5)	0.69	7
(6) and (7, 8)	0.57	6
(6, (7,8)) and (10, 11)	0.44	5
((6, (7, 8)), (10, 11)) and (12)	0.54	4
((1,4), 5) and (2, 3)	0.21	3
(((1,4), 5), (2, 3)) and (((6, (7, 8)), (10, 11)), 12)	0.11	2
((((1,4), 5), (2, 3)), (((6, (7, 8)), (10, 11)), 12)) and (9)	0.22	1

This sensitivity analysis shows that excluding individuals that may belong to the J stock can have an important effect on the results. However, none of the analyses identify a clear population boundary between individuals sampled in areas 7W and 7E. There is also no clear boundary between samples from areas 8 and 9. Only scenarios 1 (all individuals) and 4 (O-stock individuals only) can distinguish a clear clustering of samples, with two groups, one on the West (populations 1 to 5) and another one on the East (populations 6 to 12). It should be noted, however, that results also suggest that genetic differences between population 1 and the four other populations of the eastern cluster seem to be important. Thus, it could be possible to consider a scenario with three putative populations, one in the northwest, one in the southwest and a third one in the East.

Mitocondrial DNA data – Old data set

In order to evaluate how the increase of the sample size influences the results of the BR analyses we considered the same four scenarios described above and used the 559 samples that were used in previous studies. The overall result is that there is a big change in the clustering of samples.

When all 559 individuals are considered regardless of their putative origin (scenario 1, Table 5), it is possible to distinguish up to three clusters (1 to 4, 5 to 8, and 9 to 12). This pattern can also be discerned in the results of all other three scenarios (see Tables 6-8) although the third westernmost cluster is less evident under scenarios 2 (without J-stock individuals using the mtDNA criterion, Table 6) and 4 (O-stock individuals only, Table 8).

Table 5 : Clustering steps under scenario 1 using old mt-DNA data set.

Clustering steps	p-values	# of clusters
(3) and (4)	0.96	11
(10) and (11)	0.93	10
(2) and (3, 4)	0.75	9
(5) and (6)	0.75	8
(5, 6) and (7)	0.78	7
(9) and (10, 11)	0.58	6
((5, 6), 7) and (8)	0.19	5
(((5, 6), 7), 8) and (9, (10, 11))	0.16	4
(1) and (2, (3, 4))	0.16	3
((((5, 6), 7), 8), (9, (10, 11))) and (12)	0.05	2
(1, (2, (3, 4))) and (((((5, 6), 7), 8), (9, (10, 11))), 12)	0.01	1

Table 6 : Clustering steps under scenario 2 using old mt-DNA data set.

Clustering steps	p-values	# of clusters
(10) and (11)	0.93	11
(3) and (4)	0.91	10
(6) and (7)	0.80	9
(2) and (3, 4)	0.85	8
(6, 7) and (9)	0.57	7
(5) and ((6, 7), 9)	0.35	6
(5, ((6,7), 9)) and (10, 11)	0.20	5
((5, ((6,7), 9)), (10, 11)) and (12)	0.13	4
(2, (3, 4)) and (((5, ((6,7), 9)), (10, 11)), 12)	0.19	3
(1) and ((2, (3, 4)), (((5, ((6,7), 9)), (10, 11)), 12))	0.10	2
(1, ((2, (3, 4)), (((5, ((6,7), 9)), (10, 11)), 12))) and (8)	0.04	1

Table 7 : Clustering steps under scenario 3 using old mt-DNA data set.

Clustering steps	p-	# of
(10) and (11)	0.96	11
(3) and (4)	0.88	10
(2) and (3, 4)	0.72	9
(5) and (6)	0.73	8
(5, 6) and (7)	0.72	7
(9) and (10, 11)	0.66	6
((5, 6), 7) and (8)	0.17	5
(1) and (2, (3, 4))	0.19	4
(((5, 6), 7), 8) and (9, (10, 11))	0.06	3
((((5, 6), 7), 8), (9, (10, 11))) and (12)	0.10	2
(1, (2, (3, 4)) and (((((5, 6), 7), 8), (9, (10, 11))), 12)	0.03	1

Table 8 : Clustering steps under scenario 4 using old mt-DNA data set.

Clustering steps	p-	# of
(10) and (11)	0.91	11
(3) and (4)	0.90	10
(1) and (3, 4)	0.85	9
(1, (3, 4)) and (2)	0.82	8
(6) and (8)	0.77	7
(6, 8) and (7)	0.55	6
((6,8), 7) and (9)	0.47	5
(5) and (((6, 8), 7), 9)	0.22	4
(5, (((6, 8), 7), 9) and (10, 11)	0.20	3
((5, (((6, 8), 7), 9), (10, 11)) and (12)	0.09	2
((1, (3, 4)), 2) and (((5, (((6, 8), 7), 9), (10, 11)), 12)	0.00	1

These results are consistent with the baseline C hypothesis. The only difference is that the Eastern boundaries for the population O_E and O_W are shifted eastwards. This difference may be due to the fact that the initial configuration used in the present analyses didn't group together samples from areas 7E and 8, while previous analyses may have done so (cf. unofficial small genetic meeting report provided by Luis Pastene).

Microatellite data – New data set

As it was the case for the mtDNA data, the results of scenarios 2 and 3 are very similar (see Tables 9 and 10). Although there are some small differences for the first few clustering steps, the last 5 steps include the same populations. In both cases we obtain two main clusters, one to the East including populations 1 through 4 and another one to the west including populations 5 through 12. The

overall clustering pattern is different from that obtained with mtDNA but it also suggest that there is no clear boundary between areas 7E and 7W.

Table 9 : Clustering steps under scenario 2 using new microsatellite data set.

Clustering steps	p-values	# of clusters
(7) and (8)	1.00	11
(6) and (7, 8)	1.00	10
(9) and (11)	0.95	9
(5) and (6, (7, 8))	0.95	8
(3) and (4)	0.93	7
(5, (6, (7, 8))) and (9, 11)	0.80	6
((5, (6, (7, 8))), (9, 11)) and (12)	0.92	5
(1) and (3, 4)	0.48	4
(((5, (6, (7, 8))), (9, 11)), 12) and (10)	0.33	3
(1, (3, 4)) and (2)	0.22	2
((1, (3, 4)), 2) and ((((5, (6, (7, 8))), (9, 11)), 12), 10)	0.67	1

Table 10 : Clustering steps under scenario 3 using new microsatellite data set.

Clustering steps	p-values	# of clusters
(7) and (8)	1.00	11
(5) and (6)	0.99	10
(5, 6) and (7, 8)	0.99	9
(3) and (4)	0.97	8
((5, 6), (7, 8)) and (11)	0.93	7
(((5, 6), (7, 8)), 11) and (12)	0.92	6
((((5, 6), (7, 8)), 11), 12) and (9)	0.66	5
(1) and (3, 4)	0.56	4
(((((5, 6), (7, 8)), 11), 12), 9) and (10)	0.28	3
(1, (3, 4)) and (2)	0.20	2
((1, (3, 4)), 2) and ((((((5, 6), (7, 8)), 11), 12), 9), 10)	0.59	1

Scenario 4 (Table 11) leads to results that resemble those obtained for scenarios 2 and 3 in the sense that it is possible to distinguish two main clusters, one on the West (populations 1, 3, and 4) and another one on the East (populations 5 to 12). However, population 2 does not cluster with the other three eastern populations, being the last to be added to the overall cluster.

Table 11 : Clustering steps under scenario 4 using new microsatellite data set.

Clustering steps	p-values	# of clusters
(7) and (8)	1.00	11
(5) and (6)	1.00	10
(5, 6) and (7, 8)	0.99	9
(3) and (4)	0.98	8
((5, 6), (7, 8)) and (11)	0.92	7
(10) and (12)	0.90	6
(1) and (3, 4)	0.78	5
(((5, 6), (7, 8)), 11) and (9)	0.65	4
((((5, 6), (7, 8)), 11), 9) and (10, 12)	0.22	3
(1, (3, 4)) and (((((5, 6), (7, 8)), 11), 9), (10, 12))	0.31	2
((1, (3, 4)), (((((5, 6), (7, 8)), 11), 9), (10, 12))) and (2)	0.61	1

The results of scenario 1 fail to uncover the existence of clear clusters (Table 12) and they are very different from all the results obtained with the mtDNA data. In the present case, clustering proceeds from the center (area 8 and western boundary of area 9) towards the East and West. The only similarity is that population 1 is one of the last to be added to the final cluster. Note that although no clear clustering of samples is uncovered, there is evidence for genetic structuring since the p-values of the last two clustering steps are very small.

Table 12 : Clustering steps under scenario 1 using new microsatellite data set.

Clustering steps	p-values	# of clusters
(7) and (8)	1.00	11
(5) and (6)	1.00	10
(5, 6) and (7, 8)	0.99	9
((5, 6), (7, 8)) and (11)	0.93	8
(((5, 6), (7, 8)), 11) and (12)	0.93	7
(2) and (4)	0.89	6
(2, 4) and ((((5, 6), (7, 8)), 11), 12)	0.71	5
((2, 4), ((((5, 6), (7, 8)), 11), 12)) and (9)	0.62	4
(((2, 4), ((((5, 6), (7, 8)), 11), 12)), 9) and (10)	0.23	3
(1) and ((((2, 4), ((((5, 6), (7, 8)), 11), 12)), 9), 10)	0.01	2
(1, ((((2, 4), ((((5, 6), (7, 8)), 11), 12)), 9), 10)) and (3)	0.00	1

Microsatellite data – Restricted data set

For the sake of completeness we also carried out BR analyses of the four scenarios using microsatellite data corresponding to the 559 individuals that were used in previous mtDNA-based studies. All results (Tables 13 to 16) suggest the existence of three clusters, one to the east (populations 2 to 4), a large cluster

in the center (populations 5-9 and 11) and a third cluster to the west (populations 10 and 12). These results resemble the baseline C hypothesis but population 1 cannot be assigned to any particular cluster being the last one to be joined to the final cluster containing all populations.

Table 13 : Clustering steps under scenario 1 using the reduced microsatellite data set.

Clustering steps	p-values	# of clusters
(7) and (8)	1.00	11
(5) and (6)	0.97	10
(7, 8) and (11)	0.97	9
(10) and (12)	0.95	8
(5, 6) and ((7, 8), 11)	0.79	7
((5, 6), ((7, 8), 11)) and (9)	0.62	6
(2) and (3)	0.61	5
(2, 3) and (4)	0.60	4
((2, 3), 4) and (((5, 6), ((7, 8), 11)), 9)	0.68	3
(((2, 3), 4), (((5, 6), ((7, 8), 11)), 9)) and (10, 12)	0.12	2
(1) and ((((2, 3), 4), (((5, 6), ((7, 8), 11)), 9)), (10, 12))	0.23	1

Table 14 : Clustering steps under scenario 1 using reduced microsatellite data set.

Clustering steps	p-values	# of clusters
(7) and (8)	1.00	11
(5) and (6)	0.99	10
(7, 8) and (11)	0.96	9
(10) and (12)	0.83	8
(5, 6) and ((7, 8), 11)	0.76	7
(3) and (4)	0.66	6
((5, 6), ((7, 8), 11)) and (9)	0.50	5
(3, 4) and (((5, 6), ((7, 8), 11)), 9)	0.53	4
(2) and ((3, 4), (((5, 6), ((7, 8), 11)), 9))	0.86	3
(2, ((3, 4), (((5, 6), ((7, 8), 11)), 9))) and (10, 12)	0.16	2
(1) and ((2, ((3, 4), (((5, 6), ((7, 8), 11)), 9))), (10, 12))	0.25	1

Table 15 : Clustering steps under scenario 3 using reduced microsatellite data set.

Clustering steps	p-values	# of clusters
(7) and (8)	1.00	11
(7, 8) and (11)	0.98	10
(5) and (6)	0.98	9
(10) and (12)	0.95	8
(5, 6) and ((7, 8), 11)	0.79	7
(2) and (3)	0.65	6
(2, 3) and (4)	0.65	5
((2, 3), 4) and ((5, 6), ((7, 8), 11))	0.73	4
(((2, 3), 4), ((5, 6), ((7, 8), 11))) and (9)	0.58	3
(1) and ((((2, 3), 4), ((5, 6), ((7, 8), 11))), 9)	0.17	2
(1, ((((2, 3), 4), ((5, 6), ((7, 8), 11))), 9)) and (10, 12)	0.22	1

Table 16 : Clustering steps under scenario 4 using reduced microsatellite data set.

Clustering steps	p-values	# of clusters
(7) and (8)	1.00	11
(5) and (6)	0.99	10
(7, 8) and (11)	0.98	9
(10) and (12)	0.96	8
(5, 6) and ((7, 8), 11)	0.80	7
(3) and (4)	0.67	6
((5, 6), ((7, 8), 11)) and (9)	0.59	5
(2) and (3, 4)	0.48	4
(2, (3, 4)) and (((5, 6), ((7, 8), 11)) ,9)	0.78	3
((2, (3, 4)), (((5, 6), ((7, 8), 11)) ,9)) and (10, 12)	0.09	2
(1) and (((2, (3, 4)), (((5, 6), ((7, 8), 11)) ,9)), (10, 12))	0.17	1

<u>The effect of changes in the initial clustering</u>

In order to determine to what extent the results were dependent on the initial clustering used, we run additional BR analyses, the first of which shifted the Western boundary of all clusters by 1° towards the East and only considered scenario 1 using mtDNA data. The clustering obtained (Table 13) greatly differed from that obtained previously for the same scenario (Table 3). In the present case it is possible to distinguish two main clusters, one to the West (populations 1-4) and another one to the East (populations 5-12). It is worth noting that one of the effects of the eastward shift is that individuals from area 7E cluster with individuals from area 7W. Interestingly, this is similar to the pattern obtained for the scenarios 2 and 3 using microsatellite data (Tables 9 and 10).

In order to further explore how sensitive the results of BR analyses are to the location of the limit between populations 4 and 5 we shifted this limit eastwards so that it coincided with the boundary between areas 7E and 8. In this case we considered scenarios 2 and 3, which seem the most conservative approaches to the purging of J-stock individuals. The results are presented in Tables 14 and 15, respectively. If the criterion for purging is based on mtDNA (scenario 2), the results do not differ at all from those obtained previously. However, if the criterion is based on microsatellite data (scenario 3), the results change to a large extent but there is still an overall pattern in which clustering proceeds from the East to the West without uncovering any clear boundary between areas 7E and 7W or between areas 8 and 9.

Table 17 : Clustering steps obtained when the eastern limits of all initial clusters are shifted eastwards by 1°. Results were obtained under scenario 1 using the new mtDNA data set.

Clustering steps	p-values	# of clusters
(2) and (3)	0.99	11
(6) and (8)	0.96	10
(6, 8) and (11)	1.00	9
(10) and (12)	0.83	8
((6, 8), 11) and (10, 12)	0.60	7
(((6, 8), 11), (10, 12)) and (7)	0.59	6
(5) and ((((6, 8), 11), (10, 12)), 7)	0.37	5
(2, 3) and (4)	0.30	4
(1) and ((2, 3), 4)	0.06	3
(5, ((((6, 8), 11), (10, 12)), 7)) and (9)	0.07	2
(1, ((2, 3), 4)) and ((5, ((((6, 8), 11), (10, 12)), 7)), 9))	0.00	1

Table 18 : Clustering steps obtained when the borders between populations 4 and 5 are shifted eastwardly so that it coincides with the limit between areas 7 and 8. Results were obtained under scenario 2 using the new mtDNA data set.

Clustering steps	p-values	# of clusters
(7) and (8)	0.99	11
(10) and (11)	0.97	10
(2) and (3)	0.93	9
(2, 3) and (4)	0.58	8
((2, 3), 4) and (5)	0.88	7
(((2, 3), 4), 5) and (6)	0.43	6
(((2, 3), 4), 5), 6) and (7, 8)	0.74	5
(((2, 3), 4), 5), 6, (7, 8)) and (10, 11)	0.78	4
((((2, 3), 4), 5), 6), (10, 11)) and (9)	0.22	3
(((((2, 3), 4), 5), 6), (10, 11)), 9) and (12)	0.15	2
(1) and ((((((2, 3), 4), 5), 6), (10, 11)), 9), 12)	0.09	1

Table 19 : Clustering steps obtained when the borders between populations 4 and 5 are shifted eastwardly so that it coincides with the limit between areas 7 and 8. Results were obtained under scenario 3 using the new mtDNA data set.

Clustering steps	p-values	# of clusters
(10) and (11)	0.99	11
(7) and (8)	0.98	10
(2) and (3)	0.97	9
(10, 11) and (12)	0.32	8
(7, 8) and (9)	0.29	7
(1) and (4)	0.25	6
(1, 4) and (5)	0.81	5
((1, 4), 5) and (6)	0.28	4
(((1, 4), 5), 6) and ((7, 8), 9)	0.26	3
((((1, 4), 5), 6), ((7, 8), 9)) and ((10, 11), 12)	0.62	2
(((((1, 4), 5), 6), ((7, 8), 9)), ((10, 11), 12)) and (2, 3)	0.26	1

<u>The effect of removing the geographical adjacency constraint</u>

The PCA results (Figure 1) suggest that geographically disjoint groups of samples are genetically similar. For example initial clusters 6 and 12 (c.f. Figure 2) are genetically similar (see Figure 1). Something similar happens with clusters 2 and 9. Therefore, we decided to explore to what extent the removal of the geographical adjacency constraint modified the results.

Tables 20-23 present these results for analyses based on mtDNA. As expected, the clustering steps involve radically different combinations of populations. The results are much more difficult to visualise but the most interesting comparison is between the clusters involved in the two last clustering steps, which are those that consider the most genetically dissimilar clusters. An important consequence of removing the constraint is that the differences between the results obtained under the different purging scenarios increase. Additionally, sampling group 12 that was one of the last to be clustered when using spatial constraints now clusters early on with group 6, and therefore can no longer be considered as genetically distinct. The distinctiveness of group 1 also decreases, being now clustered somewhat earlier with groups 10 and 11, which are much further to the East.

Table 20: Clustering steps under scenario 1 when the geographical adjacency constraint is removed. BR analysis based on mtDNA.

Clustering steps	p-values	# of clusters
(5) and (8)	0.99	11
(10) and (11)	0.98	10
(5, 8) and (7)	0.96	9
(2) and (3)	0.96	8
(6) and (12)	0.89	7
(2, 3) and (4)	0.82	6
(6, 12) and (10, 11)	0.50	5
((2, 3), 4) and (9)	0.49	4
(((2, 3), 4), 9) and ((5, 8), 7)	0.13	3
((((2, 3), 4), 9), ((5, 8), 7)) and ((6, 12), (10, 11))	0.03	2
(1) and (((((2, 3), 4), 9), ((5, 8), 7)), ((6, 12), (10, 11)))	0.00	1

Table 21: Clustering steps under scenario 2 when the geographical adjacency constraint is removed. BR analysis based on mtDNA.

Clustering steps	p-values	# of clusters
(3) and (8)	0.97	11
(10) and (11)	0.88	10
(3, 8) and (5)	0.77	9
((3, 8), 5) and (7)	0.83	8
(6) and (12)	0.72	7
(4) and (6, 12)	0.58	6
(2) and (9)	0.38	5
(1) and (10, 11)	0.18	4
(2, 9) and (((3, 8), 5), 7)	0.16	3
((2, 9), (((3, 8), 5), 7)) and (4, (6, 12))	0.13	2
(1, (10, 11)) and (((2, 9), (((3, 8), 5), 7)), (4, (6, 12)))	0.07	1

Table 22: Clustering steps under scenario 3 when the geographical adjacency constraint is removed. BR analysis based on mtDNA.

Clustering steps	p-values	# of clusters
(3) and (8)	1.00	11
(10) and (11)	0.99	10
(2) and (3, 8)	0.96	9
(2, (3, 8)) and (7)	0.89	8
(6) and (12)	0.89	7
((2, (3, 8)), 7) and (5)	0.83	6
(1) and (10, 11)	0.69	5
(((2, (3, 8)), 7), 5) and (4)	0.62	4
(1, (10, 11)) and (9)	0.34	3
((((2, (3, 8)), 7), 5), 4)) and (6, 12)	0.21	2
((1, (10, 11)), 9) and (((((2, (3, 8)), 7), 5), 4)), (6, 12))	0.02	1

Table 23: Clustering steps under scenario 4 when the geographical adjacency constraint is removed. BR analysis based on mtDNA.

Clustering steps	p-values	# of clusters
(10) and (11)	0.99	11
(3) and (8)	0.99	10
(2) and (3, 8)	0.92	9
(6) and (12)	0.87	8
(2, (3, 8)) and (7)	0.86	7
(1) and (5)	0.80	6
(4) and (6, 12)	0.55	5
(4, (6, 12)) and (10, 11)	0.50	4
((2, (3, 8)), 7) and (9)	0.14	3
(1, 5) and (((2, (3, 8)), 7), 9)	0.05	2
((1, 5), (((2, (3, 8)), 7), 9) and ((4, (6, 12)), (10, 11))	0.04	1

Tables 24-27 show the results of the unconstrained BR analyses for microsatellite data. As it was the case for the mtDNA unconstrained analyses, the results differ radically from those obtained with the constrained BR analyses. Sampling groups 1, 9, and 12, previously identified as genetically distinct are now grouped with other samples very early on. The effect of different purging strategies is much stronger than in the case of the unconstrained BR based on mtDNA.

Table 24: Clustering steps under scenario 1 when the geographical adjacency constraint is removed. BR analysis based on microsatellite data.

Clustering steps	p-values	# of clusters
7 and 8	1.00	11
4 and 6	0.99	10
5 and (7, 8)	0.99	9
2 and 11	0.98	8
(4, 6) and (5, (7, 8))	0.93	7
1 and 9	0.83	6
10 and 12	0.68	5
(2, 11) and ((4, 6), (5, (7, 8)))	0.47	4
(1, 9) and 3	0.29	3
((1, 9), 3) and ((2, 11), ((4, 6), (5, (7, 8))))	0.00	2
(((1, 9), 3), ((2, 11), ((4, 6), (5, (7, 8))))) and (10, 12)	0.00	1

Table 25: Clustering steps under scenario 2 when the geographical adjacency constraint is removed. BR analysis based on microsatellite data.

Clustering steps	p-values	# of clusters
7 and 8	1.00	11
5 and 12	1.00	10
6 and (7, 8)	0.99	9
2 and 11	0.99	8
3 and (5, 12)	0.95	7
1 and 9	0.94	6
(3, (5, 12)) and 4	0.90	5
(2, 11) and (6, (7, 8))	0.87	4
(1, 9) and 10	0.52	3
((1, 9), 10) and ((3, (5, 12)), 4)	0.16	2
(((1, 9), 10), ((3, (5, 12)), 4)) and ((2, 11), (6, (7, 8)))	0.00	1

Table 26: Clustering steps under scenario 3 when the geographical adjacency constraint is removed. BR analysis based on microsatellite data.

Clustering steps	p-values	# of clusters
7 and 8	1.00	11
5 and 12	1.00	10
4 and 6	0.99	9
2 and 11	0.99	8
(5, 12) and (7, 8)	0.97	7
1 and 9	0.94	6
(4, 6) and ((5, 12), (7, 8))	0.83	5
(1, 9) and 10	0.67	4
(2, 11) and ((4, 6), ((5, 12), (7, 8)))	0.63	3
((1, 9), 10) and 3	0.40	2
(((1, 9), 10), 3) and ((2, 11), ((4, 6), ((5, 12), (7, 8))))	0.00	1

Table 27: Clustering steps under scenario 4 when the geographical adjacency constraint is removed. BR analysis based on microsatellite data.

Clustering steps	p-values	# of clusters
1 and 8	1.00	11
5 and 7	1.00	10
(5, 7) and 12	1.00	9
4 and 6	0.99	8
(1, 8) and 9	0.98	7
2 and 11	0.97	6
3 and ((5, 7), 12)	0.79	5
(2, 11) and (4, 6)	0.70	4
((1, 8), 9) and 10	0.58	3
(((1, 8), 9), 10) and (3, ((5, 7), 12))	0.28	2
((((1, 8), 9), 10), (3, ((5, 7), 12))) and ((2, 11), (4, 6))	0.05	1

DISCUSSION

The results of the boundary rank analyses carried out using the full data set do not provide evidence for a clear stock boundary between areas 7E and 7W or between areas 8 and 9. Additionally, they indicate that there is some heterogeneity between samples from areas 7E and 8. Thus, these results do not support the baseline C hypothesis according to which we could distinguish three stocks, one to the East corresponding to samples from area 7W, another comprising samples from areas 7E and 8 and finally a stock comprising samples from area 9.

There is little agreement between the results obtained with mtDNA and microsatellite data. The former does not identify well-defined clusters while the latter suggest the existence of two main clusters, one to the West that includes samples from areas 7E and 7W, and another to the East that includes individuals from areas 8 and 9. Note, however, that in this latter case the significance level for the heterogeneity test of the last clustering step is very large, suggesting that there is little evidence supporting the existence of two genetically differentiated stocks.

Most of the BR analyses suggest that population 1 (to the northeast of area 7E) seems to be somewhat distinct being the last to join the final cluster in most of the analyses without aggressive purging. However, when the merging of the groups is carried out without imposing spatial constraints (see below), the apparent distinctiveness of population 1 is greatly reduced.

The reanalysis of the samples used in earlier boundary rank analysis, consisting of 559 individuals, leads to results consistent with the baseline C hypothesis. This suggests that the genetic structuring observed in those earlier analyses might have been an artefact due to a limited sampling coverage.

The effect of removing individuals that could belong to the J stock has an important effect on the results of the boundary rank analysis. However, scenarios 2 (exclusion of individuals based on the mtDNA criterion) and 3 (mild purging of J-stock individuals based on the microsatellites criterion) give very similar results suggesting that these may be the most conservative criteria for purging J-stock individuals. Interestingly, aggressive purging of J-stock individuals (scenario 4) gives results that provide some evidence of genetic structuring. The only homogenising effect of aggressive purging is a decrease in the distinctiveness of population 1.

It is important to note that although the results are not consistent with the C baseline hypothesis, this does not mean that there are no signs of genetic structuring. Indeed, the results of the PC analyses indicate that there is a spatial heterogeneity in the genetic composition of minke whales. This is clearly discernible in the results of a PCA that combines mtDNA and microsatellite data. There are three significant axes of variation; PC1 (49.6% of variation explained and $p = 0.023$) and PC3 (6.5% variation explained and $p = 0.02$) correspond to PC1 and PC2 of the analysis that uses only mtDNA data. PC2 (15% of variation explained and $p=0.001$) corresponds to PC1 of a PCA that only uses microsatellite data. The significance is due to the existence of geographically disjoint cluster of samples that are genetically similar (similar PC scores). Although these results were obtained when all individuals were included in the analysis, we also obtained significant results when using a mild purging of J-stock individuals (scenario 3).

The results of the PCA are confirmed by BR analyses that do not impose any spatial constraint when merging two groups of samples. The spatial structuring detected could be due to temporal changes in the migration patterns, in which case groups with similar PC scores would represent individuals from the same population that used a different migration route in different years. Another explanation could be that there are frequent exchanges between groups of individuals that use different migration routes. The results of our analyses do not allow us to identify the most probable explanation. The use of generalised linear models based on the PC1 loadings and using year of sampling and longitude as explanatory variables may help determine if the heterogeneity is due to temporal changes in migration routes.

Several alternative hypotheses can be drawn from the results of all the analyses that have been carried out until today. Identifying the most probable one is difficult because the genetic samples used in the analyses cover several years during which changes in migration patterns may have occurred. The use of generalized linear models, as proposed above, could provide valuable information to achieve this goal. Nevertheless, in what follows we will provide a general overview of these alternative hypotheses and give an indication of their relative plausibility based on our results.

Hypothesis 1: There is genetic heterogeneity within O-stock like individuals but it is inconsistent with Baseline C. The results of previous analyses could have been an artefact of inadequate sampling. This hypothesis is the one better supported by our results. Note, however, that further analysis should be carried out to explain the observed heterogeneity. One possibility is that there exist two or

more stocks that have changed their migration routes during the time-period covered by the available samples.

Hypothesis 2: No heterogeneity in stock structure exists within O-stock like individuals. Under this hypothesis, the signal consistent with Baseline C in the earlier data could have been an artefact of inadequate sampling. The results indicate the existence of genetic structure so this hypothesis is not plausible.

Hypothesis 3: Heterogeneity seen in the earlier samples was real but not seen in more recent samples because of temporal changes in migration patterns. We think this hypothesis is not very likely because the data used in earlier studies also covered several years (1994-2001) and there is no reason to believe that changes in migration routes were less likely during this period than during the whole period covered by the present study.

Hypothesis 4: Heterogeneity exists within O stock but it was not detected with the full dataset because the differences are small and the analyses do not have adequate power. Although Boundary Rank analysis may not have adequate power to detect genetic structuring, the PCA analysis did detect it. Thus, this hypothesis is not plausible.

Hypothesis 5: The initial configuration of samples obscured the underlying signal. We do not believe this is a plausible hypothesis because the initial configuration of samples was based on a PCA that did detect genetic structure. Furthermore, the sensitivity analysis showed that changing the initial configuration does not have an important effect on the results.

Hypothesis 6: Most of the individuals from a third stock occur in areas that are outside the geographic range of the sampling. This is a rather speculative hypothesis that could only be tested by further increasing the geographic range covered by sampling expeditions.

CONCLUSIONS

Hypothesis 1 should be further explored. In particular, we think that the temporal effects can no longer be ignored. Minke whales carry out extensive migrations and it is difficult to imagine that stock boundaries in the sampled area do not change from year to year. Therefore, it is possible to imagine the existence of two or more stocks whose migration routes have changed during the period covered by the available samples. The use of generalized linear models to identify the temporal effects should allow us to design a connectivity matrix that could be used in future Boundary Rank analyses. We stress the importance of carrying out a thorough power analysis of this method in order to establish the level of genetic divergence that can be detected with the existing data.

ACKNOWLEDGMENTS

We thank scientists from the Institute of Cetacean Research of Japan for sharing unpublished data and the members of the ad-hoc email group for useful information, comments, and suggestions. We thank in particular Luis Pastene for promptly answering to questions about the data and Karen Martien for answering

questions about Boundary Rank analysis. Both of them also provided very useful unpublished documents.

REFERENCES

Baker, CS, GL Lento, F Cipriano and SR Palumbi. 2000. Predicted decline of protected whales based on molecular genetic monitoring of Japanese and Korean markets. Proceedings of the Royal Society of London Series B 267:1191-1199.

Cochran, WG. 1954. Some methods for strengthening the common χ2 tests. Biometrics 10: 417-451.

Goto, M, N Kanda, T Kishiro, H Yoshida, H Kato and LA Pastene. 2009. Further mitochondrial DNA analysis on stock structure in the western North Pacific common minke whales. IWC Scentific Committee Document SC/61/JR7.

Kanda N, M Goto, T Kishiro, H Yoshida, H Kato and LA Pastene. 2009. Update of the analyses of individual identification and mixing of J and O stocks of common minke whale around Japanese waters examined by microsatellite analysis. IWC Scientific Committee Document SC/61/JR5.

Martien, KM and B Taylor. 2001. A new method of generating hypothesized population structures for continuously distributed species using genetic data. Paper SC/53/SD7 presented to the IWC Scientific Committee, June 2001. 16pp.

Patterson, N, AL Price, D Reich. 2006. Population Structure and Eigenanalysis. PLoS Genetics 2(12): e190

Roff DA, Bentzen P. 1989. The statistical analysis of mitochondrial DNA polymorphisms : X^2 and the problem of Small samples. Molecular Biology and Evolution 6 :539-545.

Taylor, B. L. and K. Martien. 2003. A summary of data and analyses relating to stock structure of minke whales in the western North Pacific. IWC Scientific Committee Document SC/55/IST9.

Taylor, B. L. and K. Martien. 2004. Dispersal estimâtes for western North Pacific minke whales. Annex C of the Report of the second workshop on North Pacific common minke whale Implementation Simulation Trials. J ; Cetacean Res. Manage. 6 :440-441.

APPENDIX A: SEQUENTIAL CLUSTERING METHOD BASED ON CONTINGENCY TESTS OF GENOTYPE FREQUENCY DATA

In addition to the BR analyses, we used GENEPOP4.0 (Rousset 2008) to test for population differentiation. For any pair of populations, GENEPOP automatically builds microsatellite genotypes for each of the 16 loci. Then, it implements a MCMC algorithm to test the null hypothesis that all genotypes are drawn from a single population. For each locus, the test is performed on a contingency table that counts the number of occurrence of each genotype in each population. The combination of all test p-values across loci is then performed using Fisher's methods.

We proceed in a way similar to the BR analysis. First, all samples are partitioned into initial clusters. In addition, we define a neighbourhood between clusters. Then, we apply GENEPOP4's test for population differentiation for all pairs of neighbouring clusters. The pair with the highest p-value is aggregated into a single cluster, and the neighbourhood is updated. We repeat this process until all clusters are aggregated. In order to obtain results comparable to those obtained with the BR analyses, we only allowed the merging of geographically adjacent sampling groups.

Note that this method differs from both BR, which is based on a contingency test of genotype frequency distribution and RxC (Waples and Gaggiotti , 2006), which is based on contingency tests of allele frequency heterogeneity.

We carried out separate analyses with mtDNA and microsatellite data. In the case of mtDNA data, we used the haplotypes frequencies while in the case of microsatellite data we considered allele frequencies. We restricted the application of this method to analyses that considered the new data set and did not carry out analyses using the 559 individuals that were used in past BR analyses.

RESULTS

We carried analyses separately for mtDNA and microsatellite data but, as opposed to what we did for the BR analyses, we only considered the new data set.

Mitocondrial DNA data

The overall pattern obtained (Tables 28-31) was different from that obtained from the equivalent BR analyses (i.e. based on mtDNA and the new data set; Tables 1-4). Waples and Gaggiotti (2006) approach distinguishes two main clusters composed of sampling groups 1-4 to the West and 5-12 to the East. Interestingly, this overall pattern is similar to that obtained with the BR analyses of microsatellite data.

As opposed to the mtDNA-based BR analyses, sampling groups 1, 9 and 12 were not the last to be clustered. Additionally, the effect of purging putative J-stock individuals is somewhat different from the equivalent BR analyses. In this

case, there are larger differences between the results obtained for scenarios 2 and 3.

Table 28: Clustering steps for the GENEPOP-based method under scenario 1 using mtDNA data.

Clustering steps	p-values	# of clusters
(8) and (11)	0.96	11
(6) and (7)	0.81	10
(8,11) and (9)	0.77	9
((8,11), 9) and (12)	0.89	8
(5) and (6, 7)	0.72	7
(5, (6, 7)) and (((8, 11), 9), 12)	0.65	6
((5, (6, 7)), (((8, 11), 9), 12)) and (10)	0.37	5
(3) and (4)	0.10	4
(1) and (3, 4)	0.06	3
(1, (3,4)) and (2)	0.06	2
((1, (3,4)), 2) and (((5, (6, 7)), (((8, 11), 9), 12)), 10)	0.04	1

Table 29: Clustering steps for the GENEPOP-based method under scenario 2 using mtDNA data.

Clustering steps	p-values	# of clusters
(5) and (6)	0.91	11
(8) and (11)	0.83	10
(5, 6) and (7)	0.81	9
((5, 6), 7) and (10)	0.76	8
(((5, 6), 7), 10) and (12)	0.69	7
(3) and (4)	0.42	6
(((5, 6), 7), 10), 12) and (9)	0.26	5
(1) and (3, 4)	0.19	4
(1, (3, 4)) and (((5, 6), 7), 10), 12), 9)	0.31	3
((1, (3, 4)), (((5, 6), 7), 10), 12), 9)) and (8, 11)	0.24	2
(((1, (3, 4)), (((5, 6), 7), 10), 12), 9)), (8, 11)) and (2)	0.03	1

Table 30: Clustering steps for the GENEPOP-based method of under scenario 3 using mtDNA data.

Clustering steps	p-values	# of clusters
(8) and (11)	0.91	11
(6) and (9)	0.76	10
(5) and (6, 9)	0.76	9
(5,(6, 9)) and (7)	0.82	8
(8, 11) and (12)	0.51	7
(5,(6, 9)), 7) and ((8, 11), 12)	0.54	6
((5,(6, 9)), 7), ((8, 11), 12)) and (10)	0.36	5
(2) and (3)	0.10	4
(1) and (2, 3)	0.46	3
(1, (2,3)) and (4)	0.04	2
((1, (2,3)), 4) and (((5,(6, 9)), 7), ((8, 11), 12)), 10)	0.81	1

Table 31: Clustering steps for the GENEPOP-based method under scenario 4 using mtDNA data.

Clustering steps	p-values	# of clusters
(7) and (8)	0.76	11
(7, 8) and (9)	0.65	10
(5) and (6)	0.52	9
(5, 6) and ((7, 8), 9)	0.54	8
(10) and (11)	0.47	7
(10, 11) and (12)	0.45	6
((5, 6), ((7, 8), 9)) and ((10, 11), 12)	0.43	5
(1) and (2)	0.32	4
(1, 2) and (3)	0.58	3
((1,2), 3) and (4)	0.23	2
(((1,2), 3), 4) and (((5, 6), ((7, 8), 9)), ((10, 11), 12))	0.76	1

Microsatellite data

There are large differences in the results depending on the scenario being considered (Tables 32-35). When all individuals are included (scenario 1) it is possible to distinguish two main clusters, a large one that includes western and central sampling groups (2-6, 8 and 9) and a eastern cluster comprising groups 7 and 10-12. When putative J-stock individuals are purged using the mtDNA criterion (scenario 2), the two clusters change in composition; there is a western cluster comprising groups 1-6 and an eastern cluster comprising groups 7-12.

When there is mild purging based on the microsatellite criterion (scenario 3) the boundary between the two clusters is found between sampling groups 5 and 6. Finally, aggressive purging has a homogenising effect but two clusters are still discernible now with the boundary laying between groups 4 and 5.

Table 32: Clustering steps for the GENEPOP-based method under scenario 1 using microsatellite data.

Clustering steps	p-values	# of clusters
(7) and (11)	0.93	11
(7, 11) and (12)	0.81	10
(8) and (9)	0.79	9
(2) and (3)	0.60	8
(4) and (5)	0.33	7
(6) and (8, 9)	0.30	6
((7, 11), 12) and (10)	0.19	5
(4, 5) and (6, (8, 9))	0.17	4
(2, 3) and ((4, 5), (6, (8, 9)))	0.34	3
((2, 3), ((4, 5), (6, (8, 9)))) and (((7, 11), 12), 10)	0.26	2
(1) and ((2, 3), (((4, 5), (6, (8, 9)))), (((7, 11), 12), 10))	0.00	1

Table 33: Clustering steps for the GENEPOP-based method under scenario 2 using microsatellite data.

Clustering steps	p-values	# of clusters
(8) and (9)	0.89	11
(7) and (8, 9)	0.92	10
(10) and (12)	0.73	9
(2) and (3)	0.50	8
(7, (8, 9)) and (11)	0.45	7
(1) and (4)	0.39	6
((7, (8, 9)), 11) and (10, 12)	0.32	5
(5) and (6)	0.22	4
(1, 4) and (5, 6)	0.13	3
((1, 4), (5, 6)) and (2, 3)	0.32	2
(((1, 4), (5, 6)), (2, 3)) and (((7, (8, 9)), 11), (10, 12))	0.10	1

Table 34: Clustering steps for the GENEPOP-based method under scenario 3 using microsatellite data.

Clustering steps	p-values	# of clusters
(7) and (11)	0.93	11
(10) and (12)	0.83	10
(8) and (9)	0.81	9
(2) and (3)	0.52	8
(2, 3) and (4)	0.34	7
((2, 3), 4) and (5)	0.82	6
(6) and (7, 11)	0.32	5
(6, (7, 11)) and (8, 9)	0.10	4
(((2, 3), 4), 5)) and ((6, (7, 11)), (8, 9))	0.29	3
((((2, 3), 4), 5)), ((6, (7, 11)), (8, 9))) and (10,12)	0.29	2
(1) and (((((2, 3), 4), 5)), ((6, (7, 11)), (8, 9))), (10,12))	0.01	1

Table 35: Clustering steps for the GENEPOP-based method under scenario 4 using microsatellite data.

Clustering steps	p-values	# of clusters
(7) and (8)	0.96	11
(10) and (12)	0.92	10
(7, 8) and (10, 12)	0.82	9
(5) and (6)	0.61	8
(2) and (3)	0.60	7
(5, 6) and ((7, 8), (10, 12))	0.22	6
((5, 6), ((7,8), (10, 12))) and (11)	0.47	5
(2, 3) and (4)	0.12	4
((2, 3), 4) and (((5, 6), ((7,8), (10, 12))), 11))	0.08	3
(((2, 3), 4), (((5, 6), ((7,8), (10, 12))), 11))) and (9)	0.06	2
(1) and ((((2, 3), 4), (((5, 6), ((7,8), (10, 12))), 11))), 9)	0.01	1

CONCLUSIONS

Although we did a very limited use of this method, the results obtained suggest that it has less power than BR analysis to detect genetic structuring. Indeed, we only obtained evidence of clustering when the no J-stock individuals were removed (scenario 1).

REFERENCES

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

Waples, RS, and OE 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.

APPENDIX B : SIMULATION STUDY TO INFER MIGRATION RATES BETWEEN OW AND OE STOCKS USING THE NEWLY AVAILABLE MICROSATELLITE DATA

In order to comply with the terms of the contract we carried out a simulation study similar to that of Taylor and Martien (2004). More specifically, we aimed at inferring the range of migration rates that are consistent with the degree of genetic differentiation observed between the putative stocks Oe, Ow and W. Note, however, that the results obtained using Boundary Rank analysis and PCA do not support this stock structure.

Methods

MS (Hudson, 2002) is a computer program for generating samples under neutral models. The program MS can be used to generate many independent replicate samples under a variety of assumptions about migration and population size to aid in the interpretation of polymorphism studies. The samples are generated using the now standard coalescent approach in which the random genealogy of the sample is first generated and then mutations are randomly place on the genealogy (Hudson, 1990).

We used MS to generate samples for 16 independent loci under a three islands model. We used a mutation rate of 0.0001 for each locus. We assumed that the diploid population sizes in the three islands were equal to 3400 x 2, 1600 x 2 and 5165 x 2, which corresponded to the estimated number of female alleles in the populations Ow, Oe and W respectively (see Taylor and Martien 2004). For each locus, we drew 894 x 2, 270 x 2 and 466 x 2 alleles, which corresponded to the twice the observed number of individuals in the populations Ow, Oe and W respectively. We set the migration rate between Ow and W to zero. We let m, the migration rate per generation between Ow and Oe and between Oe and W vary between 0.001 and 0.05 by increments of 0.001. Thus, we tested 50 migration rates in total. For each migration rate, we performed 1000 independent simulations.

MS outputs the segregating sites, coded as zeros (ancestral state) and 1 (derived state) for each simulated chromosome. In order to simulate microsatellites, we post processed MS output with MS2MS, a perl script that converts MS output into microsatellites. Finally, we used Genepop to estimate the Fst between each pair of islands.

Results

Figure 1A shows the frequency histograms for estimated levels of genetic differentiation for a range of dispersal rates between putative stocks Ow and Oe. The range of migration rates that is consistent with the estimated Fst value (=0.0007; indicated by the red line) is quite large. The observed Fst falls within the 95% confidence interval obtained with the simulations using (per generation) migration rates between 0.033 and 0.05. The migration rate that best fit the observed Fst is m = 0.049 per generation. Note, however, that the results are rather inconclusive due to the very low level of genetic differentiation.

Conclusions

As mentioned before, this analysis was carried out only to comply with the terms of the contract. We do not think these results should be considered as reliable because (1) the stock structure assumed by the simulations is unlikely to be correct and (2) even if the assumed structure is correct, the low genetic differentiation does not allow us to provide reliable estimates of migration rates between putative stocks Oe and Ow.

REFERENCES

Hudson, RR. 2002. Generating samples under a Wright-Fisher neutral model of genetic variation. Bioinformatics 18:337-338.

Taylor, BL and K Martien. 2004. Dispersal estimates for western North Pacific minke whales. Annex C of the Report of the second workshop on North Pacific common minke whale Implementation Simulation Trials. J ; Cetacean Res. Manage. 6:440-441.

Figure Caption
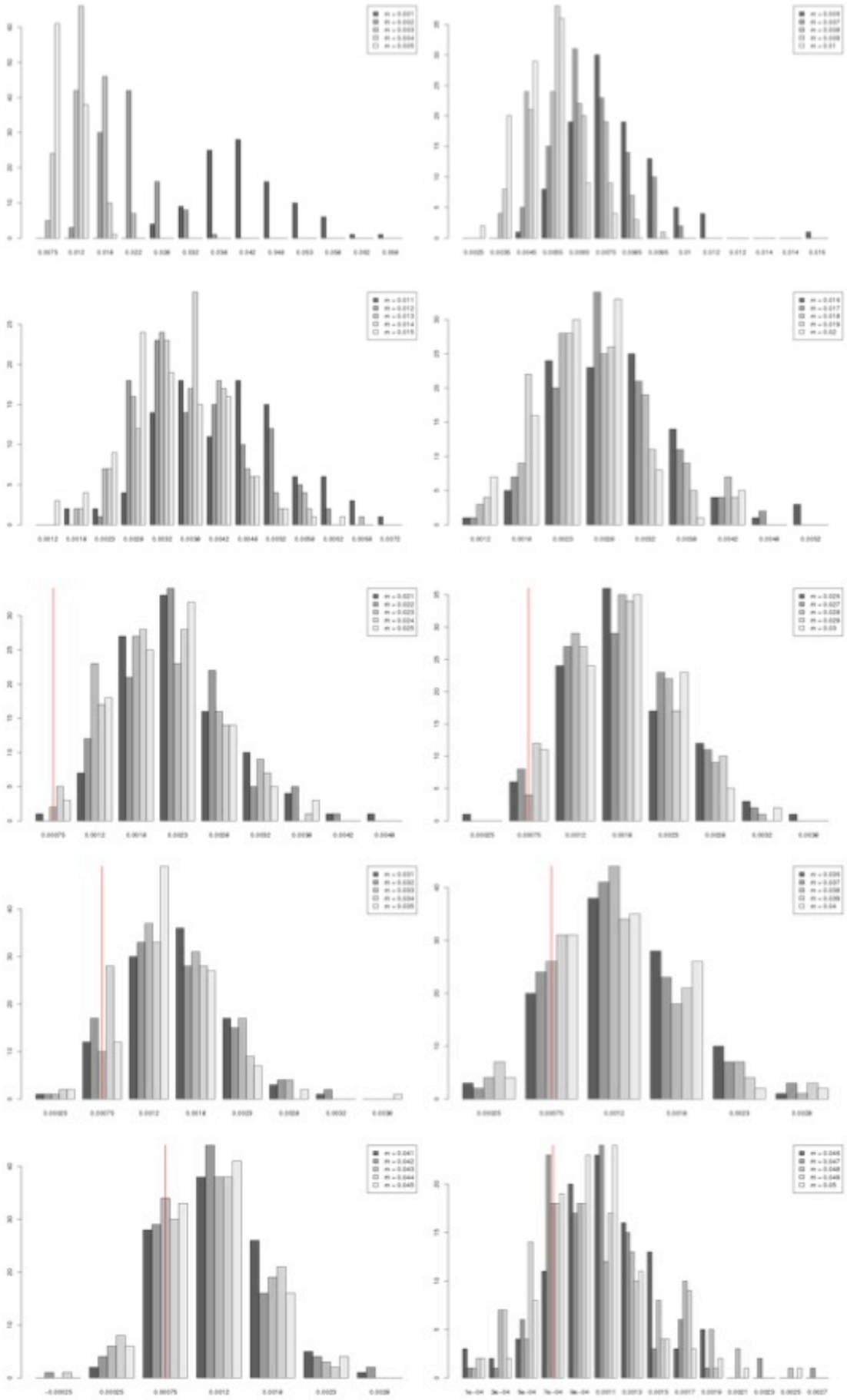
Figure 1A: Frequency histograms for estimated levels of genetic differentiation for a range of dispersal rates between 'OW' and 'OE' with the bin containing the observed Fst value noted with the vertical red line. The absence of the red line indicates that none of the migration rates included in the histogram are consistent with the Fst value observed.