Genetic population structure in the western North Pacific minke whale: an analysis of mtDNA data

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Abstract

Data gathered during scientific whaling (JARPN) to investigate whether there is population structure among minke whales to the east and north of Japan are re-analyzed here using a randomization version of χ^2. Comparisons of sub-areas 7 and 8 to sub-area 9 result in $p \approx 0.06$-0.07 whether 7 and 8 are compared separately or pooled together. The power to detect population subdivision is also likely to be compromised by initial boundary placement because the boundaries between these sub-areas clearly cut through high densities of samples. The two primary conclusions are: there is no justification for asserting that these data are consistent with no population subdivision within these areas, and it is likely that analysis of population structure has been compromised by poor initial boundary placement. Given that statistical power is likely to be low for dispersal rates of interest, a p-value of 0.06 likely indicates that population subdivision is present. The differences between stocks are not fixed differences, but rather small differences in haplotype frequencies. These small frequency differences will make it impossible to identify most individuals to their stock-origin.

Introduction

Since 1994 Japan has been conducting scientific whaling (JARPN) for minke whales (*Balaenoptera acutorostrata*) in the North Pacific with a primary objective of defining population structure. Although there has long been clear evidence for a separate stock in the Sea of Japan (J-stock) (for a review see Pastene et al. SC/F2K/J1), stock structure to the east and north of Japan was and is not clear. This paper deals only with the contested stock structure in sub-areas 7, 8 and 9 where recent samples have been obtained during the JARPN surveys. These data were analyzed in Goto & Pastene (1999) and summarized in Pastene et al. (SC/F2K/J1). These papers concluded that no significant heterogeneity was found in these areas. The analysis used sequence statistics (H_{ST}, K^*_{ST}) and found p-values for differences between the coastal sub-area 7 (n = 89) and the far offshore sub-area 9 (n = 188) of 0.0979 and 0.1186 for H_{ST} and K^*_{ST} respectively. Because abundance in these areas is relatively high from the perspective of the amount of expected genetic drift, statistical power is expected to be low (SC/F2K/J4). Two studies that compared the power of different statistics of population differentiation revealed that the statistics used by Goto & Pastene (1999) were not the most powerful statistics (Hudson et al. 1992, Taylor & Chivers SC/F2K/J5). Therefore, I re-analyzed the data in Goto & Pastene (1999) using the most powerful statistic: a randomization version of χ^2 (Roff & Bentzen 1989).

Methods

I used data from Table 2 of Goto & Pastene (1999). Looking at this table reveals that while sub-areas 7 and 8 are similar, some frequencies appear to be higher in sub-area 9 (n = 188) than in either 7 (n = 89) or 8 (n = 91). I therefore did pairwise comparisons between all the sub-areas, plus I pooled sub-areas 7 and 8 and compared this pooled population with sub-area 9. The randomization technique to calculate p-values for χ^2 (Roff & Bentzen 1989 Hudson et al. 1992) is designed to solve the problem of comparing frequencies of cells that have fewer than five samples. Instead of using the standard statistical tables, which assume generous representation in each cell to get the p-value, a unique null distribution is created from the data. The assumption for the null distribution is that the partitioning of individuals into strata was completely arbitrary. Therefore, the data can be repartitioned many times by randomly assigning individuals to strata. Thus, for samples of n1 = 89 and n2 = 188, the procedure would randomly assign 89 of the total 277 samples to strata #1 and the remainder would be assigned to strata #2. A χ^2-value would be calculated and saved as part of the null distribution. I performed 5,000 of these randomizations to obtain the null distribution. The p-value is the proportion of the null distribution that is equal to or more extreme than the observed χ^2 obtained from the actual sampled individuals.

Results

As expected, the use of χ^2 resulted in decreased p-values (Table 1).

	sub-area 7 (n = 89)	sub-area 8 (n = 91)
sub-area 8 (n = 91)	0.704	
sub-area 9 (n = 188)	0.066	0.068

Table 1. P-values for population subdivision among sub-areas for North Pacific minke whales using the randomization χ^2.

Pooling sub-areas 7 and 8 (n = 180) and comparing this unit to sub-area 9 results in p = 0.062.

Discussion

There are two major conclusions to be drawn from this re-analysis. First, there is no basis for maintaining that the data do not indicate any heterogeneity in these areas (Goto & Pastene 1999, SC/F2K/J1). Using $\alpha = 0.05$ as a criterion for significance is completely arbitrary and other choices, such as equalizing Type 1 and Type 2 errors, have been suggested for applied management cases (Berger and Berry 1988, Bernardo & Smith 1994, Ellison 1996, Hind 1984, Lindley 1972, Taylor & Gerrodette 1993). In a management context, the decision of what critical value should be used for a particular decision should reflect acceptable levels of over- versus

under-protection of the exploited resource. We expect statistical power to be low (Taylor & Chivers SC/F2K/J7). In the case where the dispersal rate of interest is ½%/year (Taylor & Chivers SC/F2K/J7) if we choose to equalize errors we would use a critical value of $\alpha = 0.23$. Thus we would reject the following hypotheses: 7 is panmictic with 9, 8 is panmictic with 9 and 7 and 8 pooled together are panmictic with 9. Not only is the p-value below the critical value, but it is far below that value. Thus, it is possible that even if we had used a lower dispersal rate, we still would have rejected the null hypothesis of panmixia. Note that if we instead elected to use $\alpha = 0.05$ our power would be 0.49 and we would be more than ten times more willing (0.51/0.05) to commit a Type 1 than a Type 2 error.

The surprising strength of the p-value comes despite another potential large flaw in the data analysis: the placement of boundaries. Martien & Taylor (SC/F2K/J3) showed that the initial stratification of samples can strongly influence results. For species that are apparently continuously distributed, placement of boundaries is problematic. Figure 1 of Goto & Pastene (1999) illustrates this problem clearly. The boundary between sub-areas 7 and 8 is clearly misplaced because it cuts through the highest density of sampled whales. Similarly, the boundary placed between sub-areas 8 and 9 appears to capture none of the apparent distributional discontinuities. It is very plausible that the p-value would be altered substantially simply by placing the initial boundaries differently. This is the second major conclusion: results are likely driven in part by initial boundary placement.

How could boundary placement be improved? Martien & Taylor have developed a method of hierarchical clustering that uses the location and genotype of each individual to locate and rank the strength of potential boundaries. Thus, the creation of plausible hypotheses is driven by the data. Unfortunately, this technique has only been developed for non-migratory species (harbor seals and harbor porpoise). Minke whales will require a case specific approach because they are not only migratory but also arrive in the different sub-areas at different times according to age and sex (Patene et al. SC/F2K/J1).

An additional consideration that is important to developing the RMS is what these small haplotype frequency differences mean to monitoring the commercial harvest of North Pacific minke whales. The genotyping of legally harvested whales gives the potential of tracking these individuals on the markets. Thus, the technique of genotyping individuals tells us whether marketing is being done legally. But what about market samples that do not match the library of legally harvested individuals? Genotyping can identify that the product was not from a legally harvested whale. Using mtDNA can identify whether the meat came from the North Pacific and whether it came from J-stock or not. Because the differences between sub-area 7 and sub-area 9 are frequency differences there is little chance of being able to identify the geographic origin of a unknown piece of meat. Thus, even if illegal whaling could be detected, the geographic area of the illegal activity has a low likelihood of being identified. The RMP requires estimates of total human kill by small area. Even if a market sampling system could be created to estimate the number of illegally caught whales, partitioning these whales to small area will be problematic.

References

Berger, J. O. and D. A. Berry. 1988. Statistical analysis and the illusion of objectivity. American Scientist 76:159-165.

Bernardo, J. M. and A. F. M. Smith. 1994. Bayesian Theory. John Wiley and Sons, 586 pp.

Ellison, A. M. 1996. An introduction to Bayesian inference for exological research and environmental decision making. Ecological Applications 6:1036-1046.

Goto, M. and L. A. Pastene. 1999. Genetic population structure in the western North Pacific Minke whale examined by mtDNA control region sequencing analysis. Paper SC/51/RMP7 presented to the IWC Scientific Committee, May 1999 (unpublished). 12pp.

Hind, W. T. 1984. Towards monitoring of long-term trends in terrestrial ecosystems. Environ. Conserv. 11:11-18.

Hudson, R. R., D. D. Boos, and N. L. Kaplan. 1992. A statistical test for detecting geographic subdivision. Mol. Biol. Evol. 9:138-151.

Lindley, D.V. 1972. Bayesian Statistics, A Review. Philadelphia SIAM.

Martien, K. K. and B. L. Taylor. SC/F2K/J3. The limitations of hypothesis testing as a means of delineating demographically independent units.

Pastene, L. A., M. Goto, and Y. Fujise. SC/F2K/J1. Review of the studies on stock identity in the minke whale (*Balaenoptera acutorostrata*) from the North Pacific.

Roff, D. A. and P. Bentzen. 1989. The statistical analysis of mitochondrial DNA polymorphisms: χ^2 and the problem of small samples. Mol. Biol. Evol. 6:539-545.

Taylor, B. L., S. J. Chivers, and A. E. Dizon. SC/F2K/J5 Evaluating the performance of different statistics to detect population subdivision.

Taylor, B. L. and S. J. Chivers. SC/F2K/J7. An example of the calculation of the statistical power to detect population sub-division in North Pacific minke whales.