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Abstract:

Our objective was to further address the recommended ‘analysis 2’ from the report of the workshop on Western North Pacific common minke whale stock structure (SC_67B_REP-05) using the microsatellite DNA database provided by the Institute of Cetacean Research, supplementing our main report (SC/F19/WNPM/02). We test the assignment of putative populations using Geneland. After identifying a core of consistently assigned individuals from repeat analyses, we plot RHO_{ST} against RHO_{IS} and find that artificially mixed ‘populations’ show significant regressions, while ‘pure’ putative population samples do not.

Introduction:

Extensive investigations have assessed the stock structure of common minke whales in the western North Pacific, including a study based on single-locus Wahlund effects that suggested the possibility of more than a simple mixture of J and O stocks in the sub-area designated 7C (though more mixture models were needed for greater certainty; Waples SC63/RMP7). In our study (de Jong & Hoelzel SC/F19/WNPM/02) we assigned putative populations using Geneland (version 4.0.8; Guillot et al. 2005) and the dataset based on 16 microsatellite DNA loci provided by collaborators at the Institute of Cetacean Research, together with their data on the GPS location of each sample included in the database. Here we attempt to distinguish between two alternative hypotheses, on the one hand the hypothesis that the Geneland analyses identified populations on independent evolutionary trajectories, and on the other that putative coastal populations in areas 2C, 7C and 11 reflect instead mixtures of J-stock and O-stock whales.

Methods:

We ran the analysis in Geneland twice using 16 loci, and once using 8 loci (in each case using 4656 putative unrelated samples and assuming correlated allele frequencies, allowing the program to determine the best K, setting delta-coord to zero, using a burn-in of 200,000 iterations, and a full run of 1M iterations). The outcome was 4 putative populations in each case (see de Jong & Hoelzel SC/F19/WNPM/02). We then compared assignments to the four populations from each separate run, retaining as assigned to a given population only those samples that assigned with a probability of 1 for all three runs (rounding 0.995 and higher up to 1), designating these as ‘pure assigned’ samples (see below).

When assessing the allele frequency size distributions of the 16 microsatellite DNA loci used, although there is some indication of indels, in most cases the distribution of allele sizes is consistent with a stepwise process of evolution (see Figure 4 in de Jong & Hoelzel SC/F19/WNPM/02). We therefore used RHO_{ST} and its derivations (calculated in Genepop; Raymond & Rousset 1995), an unbiased version of R_{ST} (Slatkin 1995) based on the stepwise

mutation model and for which allele sizes are transformed into standardised variances (see Rousset 1996).

Assignment to populations in Geneland was broadly consistent among runs (80-90% overlap), however, the best assigned population was not always with a probability of 1, shared by all independent runs. To provide a core sample set with the best assignment likelihood to a given putative population, we compared three runs all with the same starting conditions and identified concordant assignment for 1306 samples to the 'green' population (J-stock), 766 to the 'orange' population (O-stock), 244 to the 'red' population, and 820 to the 'blue' population (see Figure 5 in de Jong & Hoelzel SC/F19/WNPM/02). Two of these runs were based on all 16 loci, while one was based on loci 1-8, included on the assumption that only the samples that assign most strongly would be identified with the lower level of power provided by half the number of loci.

The full 'pure assigned' datasets for green (J-stock) and orange (O-stock) putative populations were used to generate RHO_{ST} values in Genepop (on the web version). All plots of RHO_{IS} against RHO_{ST} used this same set of values (which provides a reflection of the maximum discriminating power of each locus for this dataset). Test comparisons of mixtures used equal numbers from green and orange putative populations (766 or 125 each), 70% green and 30% orange, or 90% green and 10% orange. The strength of correlations were assessed using a linear regression implemented using GraphPad (<https://www.graphpad.com>).

Results and Discussion:

A 50:50 mixture of 'pure' green (J-stock) and orange (O-stock) produced RHO_{IS} values with a significant linear relationship to the RHO_{ST} values comparing populations green and orange (Figure 1). This remained true when a subsample of 250 samples was analysed (125 from each population), and when either 70:30 or 90:10 (green: orange) mixtures were assessed (Figure 1). Each of the putative populations identified by Geneland (red and blue in the coastal waters of sub-area 2C, 7C & 11, green representing J-stock and orange representing O-stock) were then compared individually, plotting RHO_{IS} against RHO_{ST} , and none of these comparisons showed a significant regression (Figure 2). Among these the largest R^2 value was for the orange population (O-stock), though it was not significant ($R^2 = 0.16$, $p = 0.125$; Figure 2). The smallest was from the red population, found mostly in sub-areas 7CN and 7CS ($R^2 = 0.009$, $p = 0.727$; Figure 2). The results confirm that the sample identified to represent J-stock (green) is not likely to be a mixture, and the same is true for that identified as O-stock (orange), though in the latter case it is possible that greater power may have suggested mixing, given the magnitude of the R^2 value found. For the two putative coastal populations found mostly in sub-areas 7C and 11 neither show evidence for being mixtures of J-stock and O-stock, based on these data. Note that a more extensive assessment of mixture proportions between 'pure' green (J-stock) and orange (O-stock) may have revealed mixture scenarios that also showed no significant regressions. Greater power could be achieved by the inclusion of a larger number of loci.

These data are consistent with the analyses from de Jong & Hoelzel (SC/F19/WNPM/02) that suggest that one or two populations identified using Geneland are distinct (weakly differentiated) from J&O stocks, and reside in the coastal waters of eastern Japan and off Hokkaido Island (sub-areas 7CS, 7CN, 11 and to a lesser extent, 2C). This in turn is consistent with some earlier reports suggesting further stock structure in this region (e.g. Wade & Baker SC64/NPM11), including one study based on similar methods (Waples SC63/RMP7), though contradicted by others (e.g. Pastene et al. SC/F16/JR/40).

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Figure 1: Single-locus Wahlund effect for green (J-stock) vs orange (O-stock) – a) 767 pure assigned samples mixed from each stock (N=1534); b) 125 samples from each stock; c) 70% green, 30% orange (N=1698); d) 90% green, 10% orange (N=1437).

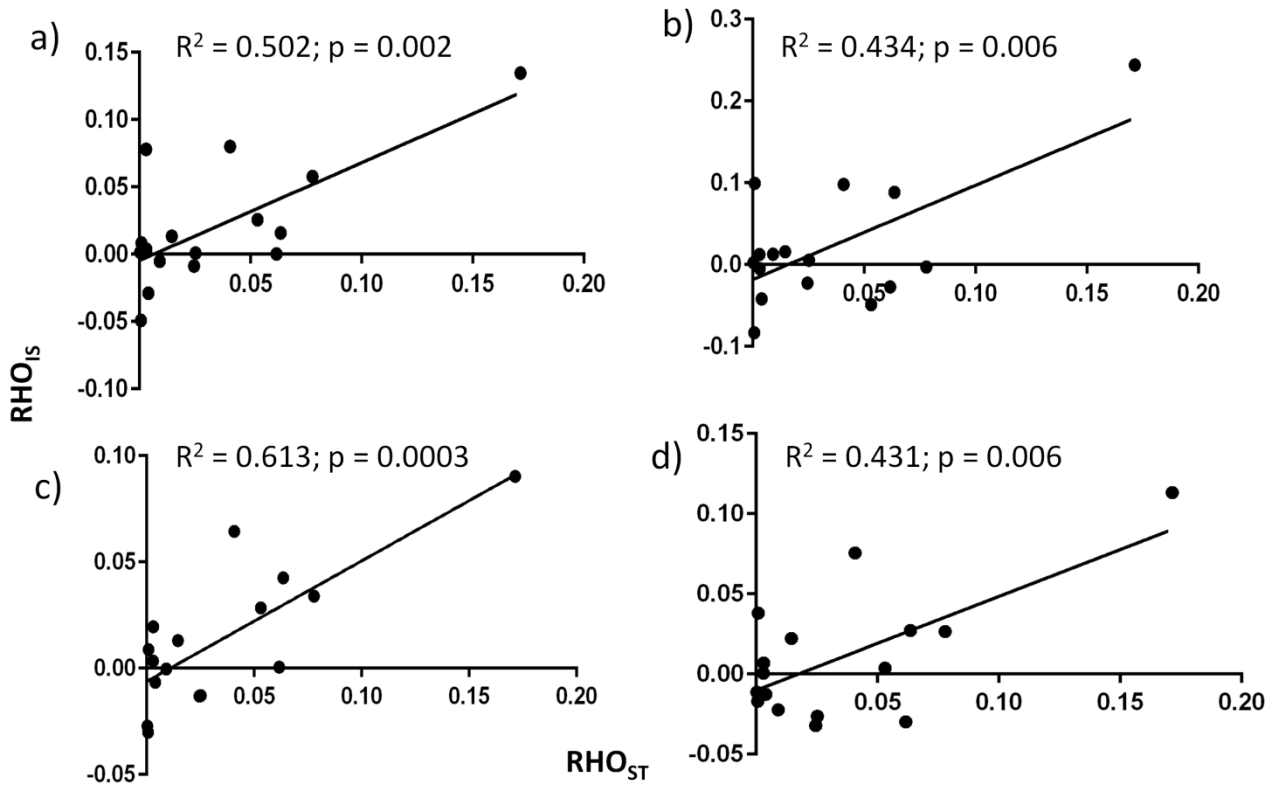


Figure 2: Test of pure assigned populations derived from analysis using Geneland.

