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A note with an estimate of microsatellite genotyping error rate for common minke whales

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ABSTRACT

The JARPNII review workshop provided three short-term analytical recommendations on the genetic analyses of stock structure. This paper responds to the recommendation of estimating genotyping error rates. For this aim, a total of 200 common minke whales from JARPN/JARPNII (approximately 8% of the total available samples) were randomly selected and newly genotyped at the same16 loci (repeat-genotyping). The genotyping error rate combined over all loci and all samples was low, 0.0044 per reaction or 0.0025 per allele. These rates were similar to the rates estimated for fur seals and lower than the rates estimated for bowhead whales

KEYWORDS: PACIFIC OCEAN; COMMON MINKE WHALE; GENETICS

INTRODUCTION

The procedures to ensure data quality at the genetic laboratory of the Institute of Cetacean Research (ICR), in accordance with IWC guidelines for DNA quality, was presented and discussed at the IWC SC in 2014 (Kanda *et al*., 2014). The IWC SC welcomed this document and agreed that it responded appropriately to previous recommendations from the JARPAII review workshop (IWC, 2015: p. 59).

The JARPNII review workshop noted that information on genotyping and DNA sequencing errors rates were not provided in Kanda *et al*. (2014) and consequently recommended that 'more explicit information on quality checks be provided in each study as well as study-specific estimates of genotyping and DNA sequencing error rates' (IWC, 2016).

The authors consider that providing information on quality check in each study is not necessary as the laboratory and analytical procedures at ICR are very similar and consistent through different case studies. Here the authors respond to the recommendation from the JARPNII review workshop by providing estimates of genotyping error based on a sub-sample of common minke whales from the western North Pacific.

MATERIAL AND METHOD

A total of 200 common minke whale individuals sampled by JARPN/JARPNII in coastal and offshore waters off Japan were randomly selected from the total samples. These samples were genotyped with the same 16 microsatellite loci employed for common minke whales in previous study (repeat-genotyping) (Pastene *et al*., 2016). Discrepancies between the two sets of genotypes were then examined based on the following classification: (i) adjacent allele heterozygote scoring error, resulting from confusion between homozygote and adjacent allele heterozygote genotypes; (ii) miss-scoring in either set; (iii) data input, where mistake was made in transcribing genotypes into the excel spread sheet; and (iv) unknown, where the error could not be attributed to any of the above cases.

To enable comparison with other studies (Hoffman and Amos, 2005; Morin *et al*., 2007), error rates were expressed wherever possible as both the number of errors per allele and per reaction, and summarized for each locus individually and across all loci. Consequently, error rate per reaction was calculated as the number of incorrect genotypes divided by the total number of reactions used for comparison. Similarly, error rate per allele was calculated as the number of incorrect alleles divided by the total number of alleles.

RESULTS AND DISCUSSION

A total of 200 common minke whales were re-genotyped successfully at all 16 loci. The genotyping error rate combined over all loci and all samples was low, 0.0044 per reaction or 0.0025 per allele. The breakdown being 28 reactions mistyped, accounting for 32 incorrect alleles out of 12,800 alleles (Table 1). Seven (21.9%) were categorized as adjacent allele heterozygote scoring error, 12 (37.5%) were miss-scoring, nine (28.1%) were data input error, and the remaining four (12.5%) were found as unknown.

The genotyping error rates estimated in this study were similar to those estimated for fur seals of 0.0038 per reaction or 0.0022 per allele (Hoffman and Amos, 2005), and lower than those estimated for bowhead whales of 0.010 per allele (Morin *et al*., 2007).

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Table 1. Locus-specific and overall genotyping error rates estimated from concordance among duplicate genotypes.

Locus	Number of reactions	Number of mistyped reactions	Number of mistyped alleles	Error rate per reaction	Error rate per allele
EV37	400	1	1	0.0025	0.0013
EV1	400	1	1	0.0025	0.0013
GT310	400	0	0	0	0
GATA28	400	12	14	0.0300	0.0175
GT575	400	1	1	0.0025	0.0013
EV94	400	2	3	0.0050	0.0038
GT23	400	0	0	0	0
GT509	400	1	1	0.0025	0.0013
GATA98	400	2	2	0.0050	0.0025
GATA417	400	5	5	0.0125	0.0063
GT211	400	0	0	0	0
EV21	400	1	1	0.0025	0.0013
DlrFB14	400	0	0	0	0
EV14	400	0	0	0	0
GT195	400	1	2	0.0025	0.0025
TAA31	400	1	1	0.0025	0.0013
Overall	6400	28	32	0.0044	0.0025