Paternity analysis on Antarctic minke whales using JARPA and JARPAII samples

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ABSTRACT
Applications of paternity analysis are very wide from documentation of pedigrees based on genetic relationships of individuals, exploration of migration patterns based on position data, to estimation of stock sizes by mark-recapture approach. Paternity analysis in a wild stock is however challenging because stock size and geographic distribution of the wild animals tend to be too large and wide to obtain an appropriate sample. This study is the first attempt to conduct the paternity analysis in a large stock of Antarctic minke whales using JARPA and JARPAII samples. Genotypic data of a maximum of 12 microsatellite DNA loci was prepared from 137 fetuses that were collected from pregnant females captured during 2003/04 JARPA, and their potential fathers were sought among 1,779 males collected from 2001/02 JARPA to 2010/11 JARPAII. One matching case of a fetus-mother-father trio was found. Although its statistical significance was varied depending on the level of uncertainty considered, other information such as the area they collected (Area IIIE), and father's body length (8.66m) and estimated age (12 years) did not necessarily deny the match.

Detection of at least one match of a fetus-mother-father trio in the partial, yet relatively large, samples of JARPA and JARPAII increases the potential for paternity analysis in our future research as an additional approach to estimate the stock size as well as infer the stock distribution. For large stock like Antarctic minke whales, the effectiveness of this approach depends on size and randomness of samples, and our samples from the comprehensive large-scale JARPA and JARPAII surveys should meet the requirements.

KEYWORDS: ANTARCTIC, FEEDING GROUNDS, GENETICS, ANTARCTIC MINKE WHALE

INTRODUCTION

Applications of paternity analysis are very wide, for instance, from documentation of pedigrees based on genetic relationships of individuals, exploration of migration patterns based on position data, to estimation of stock sizes by mark-recapture approach. In addition, once fetus-mother-father trios are identified, information of their sampling locations allows us to determine stock boundaries, and the stock origins of the parents tell us the level on genetic exchange within or between stocks.

Recently, the genetic mark-recapture approach has been used in whale studies to estimate stock size and examine the migration pattern (Palsbøll, 1999; Pearse *et al.*, 2001). Use of biopsy samples may be popular for this purpose, but it is not practical especially for the large stocks because the amount of effort involved to obtain the large sample size is excessive. Paternity testing in catches appears to be an alternative way to compensate for such difficulty. For the paternity analysis, DNA profiles, usually analyzed by a set of microsatellite loci, of the mother-fetus pairs are used to look for potential fathers of the fetuses in the sample. If their fathers are found, the number of the matches can be applied to the traditional mark-recapture analysis such as the Petersen method for estimation of stock size (e.g., Skaug and Øien, 2004).

Paternity analysis in a wild stock is however challenging because the stock size and the geographic distribution of the wild animals tends to be large and wide. For such large stocks, a small sample from a narrow scale sampling may not contain the potential fathers by chance. Antarctic minke whale samples obtained from our comprehensive large-scale JARPA and JARPAII provide us a good opportunity to conduct the paternity analysis in the large stock. This study is the first attempt to conduct the paternity analysis in the large stock of Antarctic minke whales using JARPA and JARPAII samples.

MATERIALS AND METHODS

Samples

Mother-fetus pairs of 2003/04 JARPA survey were used in this study, and potential fathers of the fetus were sought in the male samples of JARPA and JARPAII from 2002/03 to 2010/11 (Table 1). The males from the 2002/03 JARPA sample were used because considering the pregnancy period of females (Kato, 1990), some of them could have been fathers of fetuses collected in 2003/04. The mothers of the mother-fetus pairs were collected from Areas IIIE and IV, and all other whales in the samples were collected from Areas IIIE to VI.

Microsatellite DNA data

The JARPA samples (2002/03 to 2004/05) were analyzed with six microsatellite loci: EV1, EV104 (Valsecchi and Amos, 1996), GT023, GT195, GT211 (Bérubé *et al.*, 2000) and DlrFCB14 (Buchanan *et al*., 1996), whereas the JARPAII samples were analyzed with 12 loci: old set + AC045, AC082, AC087, AC137, CA234, and GT129 (Bérubé *et al*., 2005). Details of laboratory work can be found in Kanda *et al*. (2014). The power to detect the potential fathers depends on the number of loci used for the paternity analysis, so we recognized the importance of analyzing the JARPA samples with the additional six loci. Because of a time constraint, however, it was difficult for us to genotype all of the 2002/03-2004/05 JARPA samples with the additional six loci. We, therefore, conducted an initial screening with the first six microsatellite loci to select whales from the JARPA samples that would require an increased data set. Only these screened individuals from the JARPA samples were analyzed with the additional six loci. It is important to note that because the condition of extracted DNA of some individuals was not very good these individuals had the genotypic data obtained from less than six loci in the JARPA samples and 12 in the JARPAII samples. Those individuals that had the data from at least three loci were used. MICRO-CHECKER (van Oosterhout *et al*., 2004) was used to check for null alleles and reading/typing errors.

Paternity analysis

The Paternity analysis was conducted using CERVUS (Marshall *et al*., 1998), a computer program for assignment of parents to their offspring using genetic markers. Because this was a preliminary analysis, we looked for the mother-fetus-father trio that showed complete matches over the loci in Mendelian fashion. This means we ignored the effects of null alleles, typing error, and point mutation that could show allelic disagreement even between mother and fetus. This treatment could have underestimated the number of matches.

RESULTS AND DISCUSSION

For the initial screening with the data from the six microsatellite loci, the 137 mother-fetus pairs and the 1,779 males were analyzed using CERVUS (Table 1). The initial run selected 82 mother-fetus pairs and 189 males. Among them, some pairs matched to one male and other pairs to more than two males most likely due to the low resolution power of using the six loci (maximum). After the initial screening, these 82 mother-fetus pairs and 189 males were then genotyped with new six loci (maximum), and after the paternity analysis, one match of a mother-fetus-father trio was found (Table 2). Although some of the individuals in the samples had the incomplete dataset, these three individuals had the genotypic data from all of the 12 loci (Fig. 1). According to Kato (1987), body length and age at sexually maturity for males is 7.9 m and around 7 years old. The biological information of the matched potential male did not exclude the possibility of this male mating with 0304427 female some time in 2003.

It is important to note, however, that the level of statistical significance of this match varies depending on the level of uncertainty considered. For instance, proportions of the loci typed and mistyped per individual are critical especially when the proportion of missing data is high. Considering the effects caused by these factors, the complete matches from only a few loci (others had no data) as well as matches containing one mismatched locus were excluded from the final outcome. This may have caused an underestimate for the number of matches. The case with one mismatched locus occurred even between the mother and fetus in our study probably due to a null allele. In addition to that, because it is impossible to collect all of the males in the stock, the significance is influenced by the average number of potential fathers per offspring present at the time of breeding and the proportion of these potential fathers that are sampled and analyzed. These factors should be estimated from field observations that were not available for Antarctic minke whales at the time. Nevertheless, the detection of at least one match of a fetus-mother-father trio in the partial, yet relatively large, samples of JARPA and JARPAII increased the potential for paternity analysis in our future research as an additional approach to estimate stock size as well as infer stock distribution. For the large stock like Antarctic minke whales, the effectiveness of this approach depends on size and randomness of samples, and our samples from the comprehensive large-scale JARPA and JARPAII surveys should meet the requirements.

This single match was used to tentatively estimate the mature male number (*Nm*) in the I stock using the Petersen mark-recapture method modified by Chapman (referred from Van Den Avyle, 1993):

$$\hat{Nm} = \frac{(M+1)(C+1)}{(R+1)} - 1 \text{ with variance } V(\hat{Nm}) = \frac{(M+1)(C+1)(M-R)(C-R)}{(R+1)^2(R+2)},$$

where *M* is the number of the fetus-mother pairs, *C* is the total number of the mature males, and *R* is the number of the males in *C* that matched to the fetus in *M*. Among all of the male samples, we used only mature males collected from IIIE and IV because the mother-fetus pairs were collected from those areas. This method we used estimates mature male abundance in the stock. We plugged *M*=137, *C*=677, and *R*=1 into the formula and obtained *Nm*=46,782 (CV=0.572).

This method with the paternity analysis yielded the estimate of the mature male number, so that total stock size has to be converted from 46,782. Assume mature/immature ratio and sex ratio is 1:1, respectively, then total stock size is estimated as 187,128. The average abundance estimate of Areas IIIE and IV over all of the JARPA and JARPAII surveys is 51,474 (Hakamada and Matsuoka, 2014) and the average abundance estimate of Areas III and IV from two IDCR surveys was 151,174 (IWC, 2013). Our estimated stock size is larger than what we expected from these two reported estimates. This could be because this paper estimated total stock size, while the other two estimated the number of individuals distributed in the research area based on the sighting survey. The large difference between the two could be real because it has been reported that many whales are distributed outside the research area such as polynea (e.g., Murase *et al*., 2014). Another reason for the difference could be that this paper overestimated the total stock size. Several important assumptions must be met for a valid estimation using the Petersen method. Any reasons for underrepresentation of marked individuals in the second sample will lead to overestimation. Among these factors, because our samples were collected from multiple-year samplings in the open ocean, mortality of the marked individuals, recruitment of new individuals, and migrants from different stocks might not be negligible. At this point, we were not able to determine which factor(s) was responsible for the difference.

It is important to note that we found only one match. As indicated by the large CV, the precision of the estimation was low. Because we used only the mother-fetus pairs from the 2003/04 season among those from several different surveys, the estimate was tentative. We should wait till all of the samples will be analyzed for a better estimate with higher precision.

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Table 1. The number of fetus-mothers and males used in the study.

Sample	Season	1st set (I stock)	2nd set
Fetus/Mother	03/04	137	82
Candidate Father	02/03-10/11	1779 (677)	189

Table 2. Sampling and biological information of matched individuals.

ID	Sampling	Lat.	Long.	Length	Sex	Age
0304 427F						
0304 427	3/1/2004	67.39S	77.00E	8.71	F	x
0708AM120	12/29/2007	64.40S	60.03E	8.66	M	12

ID	Microsatellites																							
	EV1		EV104		GT211		DlrFB14		GT195		GT23		AC045		AC082		AC087		AC137		CA234		GT129	
0304 427F	129	133	141	147	100	112	269	271	153	157	107	107	183	187	127	143	171	173	125	135	188	206	110	112
0304 427	129	129	141	157	100	112	269	269	153	153	105	107	185	187	133	143	173	181	123	125	188	196	104	112
0708AM120	133	137	139	147	100	110	269	271	157	159	107	109	183	183	127	137	169	171	105	135	194	206	110	114

Fig. 1. Genotypic data at 12 microsatellite loci of the mother-fetus-father trio found in this study.