Genetic analyses reveal promiscuous mating in female common minke whales, *Balaenoptera acutorostrata*

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ABSTRACT

Based on 25 microsatellites, first order relatedness was established for three dyads of individuals contained in the Norwegian minke whale DNA-register. One large female minke whale was a member of all three dyads. Two competing genealogies were considered and under both of these the quartet contained siblings that with high probability must be half-siblings, as opposed to being full siblings.

KEYWORDS: COMMON MINKE WHALE; DNA FINGERPRINTING; NORTH ATLANTIC OCEAN; REPRODUCTION; GENETICS; NORTHERN HEMISPHERE

INTRODUCTION

Our current knowledge regarding the breeding biology of baleen whales in general and of common minke whales, *Balaenoptera acutorostrata*, in particular is very limited. For many species (or populations) specific breeding ground locations are not known and thus direct observations of mating behaviour are not feasible. For those species for which the breeding grounds are known, behavioural observations thus far strongly indicate the absence of mate-fidelity in either sex. Female right (*Eubalaena spp.*) and gray whales (*Eschrichtius robustus*) have been observed to copulate with multiple males during the course of one bout of mating (Payne *et al.*, 1986; Stone *et al.*, 1988; Swartz, 1986). For humpback whales, *Megaptera novaeangliae*, behavioural observations of actual mating success are lacking, but observed behaviours involving competitive groups support a mating system that is likely to be promiscuous (Clapham, 1996 and references therein).

Genetic analyses have been employed to investigate the mating system of humpback whales (Cerchio *et al.*, 2005; Clapham and Palsbøll, 1997; Nielsen *et al.*, 2001). In an analysis of known mothers where samples were available from two or more calves, multi-annual sighting records of individually identified humpback whales were used to confirm that female humpback whales indeed mated promiscuously across seasons (Clapham and Palsbøll, 1997). For males, Nielsen *et al.* (2001) used genetics to determine that some have higher reproductive success than others, and Cerchio *et al.* (2005) showed evidence of polygyny (males mating with multiple females). In species without the extensive multi-year sighting records of individuals that are available for the humpback whale, both maternity and paternity may need to be inferred indirectly. The latter approach is appealing for minke whales, as they occur almost exclusively as solitary individuals. Skaug and Øien (2005) used DNA-profiles from mother-foetus pairs to establish paternity in a database of male minke whales.

Skaug *et al.* (2005) used statistical methods to identify pairs of related individuals in the Norwegian minke whale DNA-register, which contains DNA-profiles from nearly all animals caught by Norway since 1997. Among their findings were three dyads of 1st order relatives having one female individual in common. Two possible genealogies for the four individuals involved in these three dyads are considered in this paper. Given the genealogy and additional biological information, the probability that certain members of the quartet were full siblings, as opposed to being half-siblings was calculated. These findings yield information about minke whale breeding biology that is otherwise difficult to obtain.

MATERIAL AND METHODS

The establishment of the Norwegian minke whale DNA-register ensures that samples (muscle tissue) are taken from each animal caught under the Norwegian catch quota and that a DNA-profile is established from each individual whale and stored in a database (Olaisen, 1997). The DNA-profile consists of 10 microsatellites, mitochondrial (mt) DNA and a sex-marker (Dupuy and Olaisen, 1998). In addition, for each animal the register contains information about the time and geographical location of capture, as well as some biological parameters (length, etc). For the period 1997-2002, the DNA-register contains DNA-profiles for 3,301 individuals. These samples were geographically spread according to the IWC's Small Area delineation of the Northeastern Atlantic (Fig. 1). The best estimate of population size for minke whales in the region is 107,000 animals with a Coefficient of Variation (CV) of 0.14 (Skaug *et al.*, 2004).

The present study involves three dyads of individuals, which among other dyads were identified in Skaug *et al.* (2005) as likely relatives based on the 10 microsatellites constituting the DNA-register. These three dyads, all being consistent with a parent-offspring relationship, were brought to special attention due to the fact that they shared a common individual. All four individuals involved were females and the fact that they shared mtDNA haplotype suggested that all three dyads were mother-daughter pairs. To ascertain the estimated degree of relatedness the four individuals were typed at 15 additional microsatellite loci (Table 1). As part of a larger study 439 additional individuals were typed at the same 15 loci, allowing estimates of population allele frequencies to be established.

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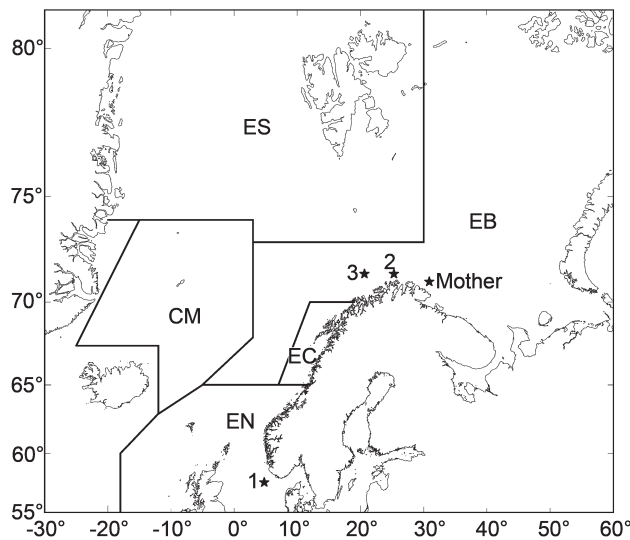
Fig. 1. Capture locations (stars) of the individuals in the quartet of individuals (Table 2). Also shown are (pre-2003) Small Areas to which the minke whale catches were allocated. The 3,301 genetic samples used in the present study were distributed among Small Areas as follows, EN: 634, EC: 69, EB: 1626, ES: 719, CM: 253.

Table 1

Multilocus genotypes for the four individuals involved in the present study. The genotypes consist of 25 microsatellite loci (Tautz, 1989), out of which 10 loci comprise the Norwegian minke whale DNA-register loci (Dupuy and Olaisen, 1999). The 15 additional loci are described in Palsbøll *et al.* (1997) and Bérubé *et al.* (2005). The three 1st order dyads are: (Mother, 1), (Mother, 2), (Mother, 3).

10 original loci comprising the DNA-register				
	GATA098	**GT509**	**EV1**	**EV37**	**GT310**
Mother	91/95	193/211	153/161	197/201	115/117
1	91/95	193/211	149/161	197/207	115/117
2	91/95	193/193	153/171	199/201	115/117
3	91/95	193/207	155/161	197/199	111/115
	GT211	**GT575**	**GT023**	**GATA028**	**GATA417**
Mother	106/108	162/164	97/105	161/207	213/220
1	102/106	154/164	105/105	161/211	220/228
2	106/106	162/164	97/99	207/211	213/220
3	102/108	154/162	99/105	161/207	217/220
15 additional loci				
	AC045	**AC087**	**AC137**	**ACCC392**	**CA128**
Mother	182/190	163/167	109/119	246/246	137/139
1	170/182	163/167	117/119	246/246	137/139
2	182/190	163/167	109/121	246/246	137/139
3	182/188	167/167	109/119	246/254	139/143
	CA232	**EV094**	**EV096**	**GT122**	**GT129**
Mother	148/148	212/212	242/252	140/142	103/103
1	148/152	212/212	252/252	138/140	103/103
2	148/152	212/212	242/250	140/142	103/103
3	148/152	212/212	242/252	140/140	103/105
	GT195	**GT307**	**GT541**	**RW26**	**RW4-10**
Mother	162/168	136/140	102/102	169/171	200/204
1	164/168	140/140	102/102	167/169	198/200
2	162/168	136/140	102/102	171/171	204/204
3	162/168	136/136	102/102	167/171	200/204

For each of the three dyads, the computer program *Familias* (Egeland *et al*., 2000) was used to calculate the posterior probability of a parent-offspring relationship, versus unrelatedness. In these calculations, only the 15 new loci were used, to avoid any ascertainment bias that would arise from including the ten original loci. Originally developed for forensic science, *Familias* uses a Bayesian framework to calculate the probability of each candidate pedigree, given DNA-profiles for some or all individuals in the pedigree. In the present application of *Familias* all candidate pedigrees were assumed *a priori* equally likely.

Assuming that all three dyads consist of 1st order relatives, only two individual genealogies were consistent with the data (sex and mtDNA). The four samples are comprised of either: (1) a mother and her three female offspring; or (2) a grandmother, her daughter and her two granddaughters. In case (1) the question of interest is whether any of the three offspring were sired by the same male. In case (2) the two offspring are most likely the two smallest individuals (1 and 3 in Table 1), and the question of interest is whether they have the same father. Both of these hypotheses were tested using *Familias* based on genotype data from all 25 loci.

RESULTS

The 15 loci confirmed 1st order relatedness (probabilities 0.998, 1.000 and 0.999, respectively, for dyads 1, 2 and 3 in Table 2). Additional evidence was provided by the fact that the mtDNA haplotype shared by the four females was rare (population frequency 0.0173). Under genealogy (1) the probability that three different males sired the three offspring was found to be 0.980. It should also be noted that at locus EV1 the three daughters hold as a trio three distinct alleles (149, 155 and 171) not found in the mother (Table 1). This fact excludes the possibility that a single male has sired all three offspring. Under genealogy (2) the probability that two different males sired the two offspring was 0.9998. Even if the grandmother in reality should be 3 (and not 2 as assumed by us), the calculations done under genealogy (1) show that 0.980 is a lower bound on this probability.

Table 2

Summary of non-genetic information about the four individuals (all females) that constitute the three dyads. The column 'ID' gives the internal identification numbers in the DNA-register, and 'length' is the zoological length (cm) measured on board the catching vessel.

Individual	ID	Time of catch			Length (cm)
		Year	Month	Date	
Mother	9802286	1998	June	2	900
1	0104083	2001	May	12	740
2	0201618	2002	June	5	870
3	0003304	2000	July	21	810

DISCUSSION

The results presented here constitute the first indication of the occurrence of promiscuous mating of female common minke whales. They support the expectation from other baleen whale species that female minke whales mate promiscuously across seasons. The genetic analysis employed in this study revealed that even though only 3% of the population was sampled it is possible to obtain insights into parentage and mating strategies.

The 10 microsatellite loci show very little sign of spatial inhomogeneity in allele frequencies (IWC, 2004). It is not straight forward to check if the same holds true for the additional 15 loci, because the 439 individuals used to estimate allele frequencies were not randomly sampled from the DNA-register. The probabilities presented were all calculated under the assumption that the four individuals comes from a single homogeneous population. Violation of

this assumption would make it even more unlikely that the three dyads should match at all 15 loci, and hence is of little concern for the main conclusion regarding relatedness.

The three dyads considered in the present paper are a subset of the dyads detected in Skaug *et al.* (2005). For dyads involving mother and offspring caught in different years, as is the case for the three dyads considered, the capture locations may provide information about maternally-inherited site fidelity. The fact that the mother is caught at the Finnmark coast (Fig. 1), while one daughter (1) was caught in the North Sea, apparently provides evidence against such an hypothesis. This interpretation is however confounded by the fact that minke whales are a migratory species and it is possible that the maternally inherited factor is the whole migratory route and timing. To test this more complicated hypothesis one should also look at date of capture, but a sample of three dyads does not allow such an analysis to be conducted.

Breeding structure is currently not a part of the population dynamics models employed by the IWC, and the findings of the present paper suggest that this practice is appropriate for common minke whales.

ACKNOWLEDGEMENTS

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