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

HANS J. SKAUG[1], MARTINE BÉRUBÉ[2], MARY BETH REW[2], AND PER J. PALSBØLL[2]

[1]*Institute of Marine Research, P.O. 1870, N-5817 Bergen, Norway*

[2]*Department of Environmental Science, Policy and Management, University of California Berkeley, 137Mulford Hall, Berkeley, California 94720, USA*

Contact e-mail: Hans.Skaug@imr.no

Our current knowledge regarding the breeding biology of baleen whales in general and of minke whales, *Balaenoptera acutorostrata*, in particular is very limited. In many species (or populations) specific breeding ground locations are not known and thus direct observations of mating behaviour are not feasible. In those species where the breeding grounds are known, the behavioral observations made so far strongly indicate the absence of mate-fidelity in either sex. In right whales (*Eubalaena spp*.) and gray whales (*Eschrichtius robustus*), females have been observed to copulate with multiple males during the course of one bout of mating (Payne 1986, Stone et al 1988, Swartz 1986). In humpback whales, *Megaptera novaeangliae*, behavioral observations of actual mating success are lacking, but observed behaviors 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 (Clapham and Palsbøll 1997, Nielsen et al 2001, Cerchio et al 2005). 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). From the male perspective, Nielsen et al. (2001) used genetics to determine that some males 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. This latter approach was employed in the present study where dyads of 1st order relatives among 3,301 sampled minke whales were identified by analysis of 25 microsatellite loci. We found that one large female minke whale was a member of three such dyads, in all cases paired with

other female minke whales. Other 1st order dyads were found as well, but as non of these involved overlapping individuals, they were not relevant for the present study.

Putative dyads of 1st order relatives were selected based upon data from 10 microsatellite loci (Table 1) among 3,301 minke whale tissue samples available from minke whales caught under the Norwegian catch quota during 1997-2002. These individual DNA-profiles are part of the Norwegian minke whale DNA register, which was established to track the individual identities of whales caught by whaling vessels (Olaisen, 1997). In addition to the genotype at the aforementioned 10 microsatellite loci, the sex and the nucleotide sequence at mitochondrial control region (mtDNA) was also determined (Dupuy and Olaisen, 1999). The register also contains data on the time and geographical location of each minke whale sampling event, and biological parameters, such as zoological length. An initial likelihood-based screening of the DNA-register revealed the presence of the aforementioned three dyads of putative 1st order relatives. All four involved individuals shared the same mtDNA haplotype.

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. 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.

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 (i) a mother and her three female offspring; or, (ii) a grandmother, her daughter and her two granddaughters. In case (i) the question of interest is whether any of the three

offspring were sired by the same male. In case (ii) 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 the program Familias (Egeland et al. 2000) which uses a Bayesian framework to calculate the probabilities of candidate pedigrees, based on genotype data from all 25 loci.

The 15 loci confirmed 1st order relatedness (probability 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 (i) the probability that three different males sired the three offspring was found to be .980. Under genealogy (ii) the probability that two different males sired the two offspring was .9998. Even if the grandmother in reality should be #3 (and not #2 as assumed by us), the calculations done under genealogy (i) show that .980 is a lower bound on this probability.

The results presented here constitute the first indication of the occurrence of promiscuous mating of female minke whales. Our results 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. In many cetacean species this may be the only viable option for collecting such data.

ACKNOWLEDGEMENTS

This study was funded by the Norwegian Research Council.

LITERATURE CITED

Berube M., Rew M.B., Skaug H., et al. 2005. Polymorphic microsatellite loci isolated from humpback whale, *Megaptera Novaeangliae* and fin whale, *Balaenoptera*

Physalus. Conservation Genetics 6: 631-636.

Cerchio, S., J.K. Jacobsen, D.M. Cholewiak, et al. 2005. Paternity in humpback whales, Megaptera novaeangliae: assessing polygyny and skew in male reproductive success. Animal Behaviour 70: 267-277.

Clapham, P. J. 1996. The social and reproductive biology of Humpback Whales: An ecological perspective. Mammal Review 26: 27-49.

Clapham, P. J. and P. J. Palsbøll. 1997. Molecular Analysis of Paternity Shows Promiscuous Mating in Female Humpback Whales (Megaptera Novaenagliae, Borowski). Proceedings of the Royal Society of London Series B-Biological Sciences 264: 95-98.

Dupuy, B. and B. Olaisen. 1999. Typing procedure for the Norwegian minke whale DNA register. Institute of Forensic Medicine, University of Oslo.

Egeland, T., P. Mostad, B. Mevaag and M. Stenersen. 2000. Beyond traditional paternity and identification cases. Selecting the most probable pedigree. Forensic Science International 110: 47-59.

Olaisen, B. 1997. Proposed specifications for a Norwegian DNA database register for minke whales. Unpublished report to the Scientific Committee of the International Whaling Commission (Available upon request from the IWC secretariat) SC/49/NA1.

Palsbøll, P. J. 1999. Genetic Tagging: Contemporary Molecular Ecology. Biological Journal of the Linnean Society 68: 3-22.

Payne P.M., J.R. Nicolas, L. Obrien, et al. 1986. The distribution of the humpback whale, Megaptera-Novaeangliae, on Georges bank and in the gulf of Maine in relation

to densities of the sand eel, Ammodytes-Americanus. Fishery Bulletin 84: 271-277.

Skaug, H. J., N. Øien, T. Schweder and G. Bøthun, G. 2004. Abundance of Minke Whales (Balaenoptera Acutorostrata) in the Northeast Atlantic: Variability in Time and Space. Canadian Journal of Fisheries and Aquatic Sciences 61: 870-886.

Stone, G.S., S.D. Kraus, et al. 1988. Significant aggregations of the endangered right whale, Eubalaena glacialis, on the continental shelf of Nova Scotia (Canada). Canadian Field-Naturalist 102: 471-474.

Tautz D. 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Research 17: 6463-6471.

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 Berube 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

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.

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