

Molecular analysis of paternity shows promiscuous mating in female humpback whales (*Megaptera novaeangliae*, Borowski)

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SUMMARY

It is widely assumed that the mating system of the humpback whale, *Megaptera novaeangliae*, is similar to that of most mammals in that it represents some form of polygyny or promiscuity, but this cannot be tested without observations of copulation or data on paternity of offspring. Microsatellite DNA markers were used to examine the paternity of calves born to individually identified mature female humpback whales from the Gulf of Maine. Skin biopsies were obtained from three females, and several (range: three to five) of their known offspring. Multiple paternity of offspring, indicated by the presence of at least three different paternal alleles, was evident in all three females at either three or four of the six microsatellite loci surveyed. Such promiscuous mating is expected given current knowledge of the social ecology of this species. It is also consistent with resightings of individually identified female humpbacks with different male associates during two or more breeding seasons.

1. INTRODUCTION

In the last two decades, observations of living humpback whales (*Megaptera novaeangliae*, Borowski) in numerous locations have led to a better understanding of the mating system of this species. During the winter, humpbacks migrate from summer feeding areas in high latitudes to traditional breeding grounds in tropical waters (Chittleborough 1965; Dawbin 1966). There, males sing long, complex songs (Payne & McVay 1971; Tyack 1981) the primary function of which is probably as a reproductive display to attract potential mates. In addition, males engage in often aggressive intrasexual competition for females (Tyack & Whitehead 1983; Baker & Herman 1984), perhaps sometimes in coalitions (Clapham *et al.* 1992; Brown & Corkeron 1995). Female humpbacks invariably give birth to a single calf, which remains with its mother for a year (Clapham & Mayo 1990). There is no paternal investment in the rearing of offspring.

Despite our broadening knowledge of humpback whale behaviour in winter, we currently know little about individual mate choice, or about the role played by females in the mating system. Overall, it is widely assumed that the humpback's mating system is similar to that of most mammals in that it represents some form of polygyny or promiscuity (reviewed by

Clapham 1996). However, this idea cannot be tested without observations of copulation (an act that has yet to be witnessed in this species), or data on the paternity of offspring.

Here, we report the results of a molecular analysis of the paternity of calves born to individually identified female humpback whales from the Gulf of Maine. The Gulf is one of several feeding grounds to which North Atlantic humpback whales return each spring from winter breeding areas in the West Indies (Katona & Beard 1990); identified individuals within the population of humpbacks that summers in this region have been the subject of a long-term study since the 1970s (Clapham *et al.* 1993*a*). Many individuals have been observed for up to 20 years, and detailed reproductive histories are available for mature females and their offspring (Clapham & Mayo 1990). Females give birth on average every 2 or 3 years, and many females in this population have been documented with successive calves over the study period. Knowledge of these relationships provides an opportunity to establish whether calves born to the same mother were fathered by different males.

2. MATERIALS AND METHODS

Between 1990 and 1993, we obtained skin biopsies from humpback whales in various locations in the Gulf of Maine, and (in a few cases) on this population's breeding ground in

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Table 1. *Alleles at six microsatellite loci in three mature females (Cardhu, Rune and Salt) and their offspring*

(In each case, the mother's name is given first (in upper case). This is followed by the names of her calves, with the year of birth in parentheses. Paternal alleles are underlined; cases where both are underlined represent instances in which the paternal allele could be either of the pair. Informative loci, in which the presence of three or more different paternal alleles indicates more than one father among the offspring, are shown in bold.)

whale	GATA28	TAA31	GATA53	GATA98	GATA417	GGAA520
CARDHU	110–117	65–65	141–169	51–87	94–109	154–198
Ember (1982)	<u>106</u> –110	<u>59–65</u>	<u>141–141</u>	<u>51–87</u>	<u>94–117</u>	<u>198–198</u>
Slope (1987)	<u>106</u> –117	<u>65–65</u>	<u>141–157</u>	<u>51–67</u>	<u>106–109</u>	<u>154–198</u>
Treasure (1989)	<u>106</u> –110	<u>65–68</u>	<u>153–169</u>	<u>51–51</u>	<u>94–121</u>	<u>154–198</u>
RUNE	106–114	62–65	141–153	51–51	109–109	198–218
Alphorn (1983)	106– <u>106</u>	<u>65–71</u>	<u>141–157</u>	<u>51–67</u>	109– <u>121</u>	<u>170–218</u>
Nine (1989)	<u>110</u> –114	<u>59–62</u>	<u>141–153</u>	<u>51–79</u>	<u>109–109</u>	<u>198–218</u>
Tripod (1992)	<u>106</u> – <u>114</u>	<u>62–68</u>	<u>141–165</u>	<u>51–51</u>	<u>109–109</u>	<u>154–198</u>
SALT	106–114	57–59	137–153	67–67	94–106	154–170
Crystal (1980)	<u>106</u> –106	<u>57–68</u>	<u>137–161</u>	<u>67–83</u>	<u>106–117</u>	<u>154–154</u>
Thalassa (1985)	<u>106</u> –106	<u>59–71</u>	<u>137–149</u>	<u>51–67</u>	<u>106–113</u>	<u>162–170</u>
Brine (1987)	<u>114</u> –114	<u>57–59</u>	<u>137–153</u>	<u>67–83</u>	<u>106–109</u>	<u>170–309</u>
Bittern (1989)	<u>114</u> –114	<u>59–68</u>	<u>137–153</u>	<u>51–67</u>	<u>98–106</u>	<u>154–170</u>
Salsa (1991)	<u>106</u> –106	<u>59–59</u>	<u>137–149</u>	<u>51–67</u>	<u>94–125</u>	<u>154–194</u>

the West Indies. Biopsies were taken using a 68 kg draw crossbow and sampling dart (modified from Lambertsen 1987). Additional samples from unbiopsied animals were collected in the form of sloughed skin found at the water surface following high-energy behaviour such as breaching (Clapham *et al.* 1993*b*). All samples were stored in a solution of saturated sodium chloride and dimethyl sulfoxide (Amos & Hoelzel 1991), or in liquid nitrogen.

All biopsied animals were photographed with a 35 mm camera, equipped with a 300 mm telephoto lens, power winder and ISO 400 black and white print film. Individuals were identified from these photographs by using variations in the pattern on the ventral surface of the tail flukes, as well as in the shape and scarring of the dorsal fin (Katona & Whitehead 1981). Each animal's identity was subsequently confirmed by matching photographs to a master catalogue of known individuals. The names of all identified whales from the Gulf of Maine are based upon prominent field marks, and names are used to refer to specific individuals in this report. All field work was conducted from a 14 m auxiliary ketch, or from a 5 m inflatable boat powered by a 25 hp outboard engine.

In the laboratory, DNA was extracted from the skin samples using standard protocols, with cell lysis followed by phenol/chloroform extraction and precipitation with ethanol (Maniatis *et al.* 1982). Six tri- and tetramer microsatellite loci, isolated from humpback and fin whales, *Balaenoptera physalus* (Palsbøll, submitted), were each amplified by the polymerase chain reaction (Saiki *et al.* 1988) under standard conditions (Palsbøll *et al.* 1995), except that the end-labelled primers were added in a 0.04 mM concentration. Annealing temperatures varied between 49 °C and 55 °C depending on the locus. For each amplification one primer was end-labelled with ³²P ATP using T4 kinase (Maniatis *et al.* 1982). Electrophoresis of the amplification products was done using a standard 6% polyacrylamide denaturing matrix. Three M13 sequences, as well as six samples of known allelic composition, were included on each gel. The alleles were visualized by overnight autoradiography.

Because inheritance of autosomal microsatellite loci is biparental, one of the two alleles at any locus will come from the animal's mother, the other from its father. Thus, identification of the paternal allele is relatively simple, since

it involves eliminating the known maternal allele from the offspring's pair. In some instances, mother and offspring have identical alleles, in which case the paternal allele could be either of the pair. Multiple paternity is indicated when more than two different paternal alleles are found among the offspring.

3. RESULTS

Skin samples were obtained from 225 individual humpback whales. In order to test for multiple paternity among calves born to the same mother, we required samples from any mature female and at least three of her offspring. Three individually identified mature females met this criterion: 'Cardhu' (biopsied together with three of her offspring), 'Rune' (also biopsied with three of her offspring) and 'Salt' (biopsied with five of her offspring). Alleles at the six microsatellite loci for each female and her calves are shown in table 1. Multiple paternity of offspring, indicated by the presence of at least three different paternal alleles at any locus, is evident in three of six loci among the calves of Cardhu, and in four of six loci among the calves of both Rune and Salt.

4. DISCUSSION

The results reported here indicate unequivocally that the calves of all three mature females had been fathered by more than a single male. Since a father can contribute to a calf either one of two alleles at any locus, it is not possible to determine whether more than two males are represented unless more than four different paternal alleles are present at any single locus. With samples from only three (or four) offspring, this is impossible, as is the case here with the three calves of Cardhu and Rune. However, five paternal alleles were

documented at locus GATA417 among the calves of Salt; therefore, her five offspring were fathered by at least three (and possibly up to five) different males.

Individually identified humpback whales have been resighted with different associates of the opposite sex over two or more winters (Glockner-Ferrari & Ferrari 1990). While the nature of such interactions is unclear, this pattern of association is consistent with the finding of promiscuous mating reported here. Promiscuity is also to be expected given the absence of paternal care in humpback whales, and the distribution and social organization of this species. Humpback whales do not live in stable groups (Mobley & Herman 1985; Clapham 1996), and they are widely scattered throughout an extensive breeding range in winter (Winn *et al.* 1975; Baker & Herman 1981; Whitehead & Moore 1982). Thus, the rates of re-association between specific individuals across years are probably low relative to those of species that are more social (e.g. killer whales, *Orcinus orca*; Bigg *et al.* 1990), or that are forced by resource-dependence to cluster in confined areas during the breeding season (e.g. grey seals, *Halichoerus grypus*; Amos *et al.* 1995).

Known mature female humpbacks have also been observed with different associates over periods of up to several weeks during a single winter (Mobley & Herman 1985; Clapham 1996). In some cases, the female concerned was the so-called 'nuclear animal' at the centre of two or more 'competitive groups', in which males fight to secure an apparently key position next to the female (Tyack & Whitehead 1983). However, the frequency with which females copulate with the winners of such contests is unknown. Given the considerable difficulty of observing actual sexual contact between humpback whales, it is unlikely that we will soon resolve the question of whether a female mates with more than one male during a single breeding season.

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