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Patterns of kinship in groups of free-living sperm whales (*Physeter macrocephalus*) revealed by multiple molecular genetic analyses

K. R. RICHARD, M. C. DILLON, H. WHITEHEAD*, AND J. M. WRIGHT

Biology Department, Dalhousie University, Halifax, NS Canada B3H 4J1

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ABSTRACT **Mature female sperm whales (*Physeter macrocephalus*) live in socially cohesive groups of 10–30, which include immature animals of both sexes, and within which there is communal care of the young. We examined kinship in such groups using analyses of microsatellite DNA, mitochondrial DNA sequence, and sex-linked markers on samples of sloughed skin collected noninvasively from animals in three groups off the coast of Ecuador. Social groups were defined through photographic identification of individuals. Each group contained about 26 members, mostly female (79%). Relatedness was greater within groups, as compared to between groups. Particular mitochondrial haplotypes were characteristic of groups, but all groups contained more than one haplotype. The data are generally consistent with each group being comprised of several matrilines from which males disperse at about the age of 6 years. There are indications of paternal relatedness among grouped individuals with different mitochondrial haplotypes, suggesting long-term associations between different matrilines.**

Since the early days of whaling, observers have remarked on the unusual social organization of sperm whales (*Physeter macrocephalus*) (1). Mature females and immature whales of both sexes are encountered in socially cohesive groups of 10–30 animals (2). Within such groups, the duration of social bonds appears highly variable (3). Studies of marked or photographically identified whales indicate that some associations between individuals persist for at least several years (3, 4), whereas others last only a few days (3). In the most recent work, analysis of photoidentification records from off the Galápagos Islands has shown that a typical sperm whale group is formed by the short-term merging of smaller, but very stable, "units" (3). Each unit contains about 13 members, and Whitehead and colleagues (3) speculate that these units may be matrilines.

Within groups there appears to be both communal suckling (5, 6) and communal protection of the young from predators (7–9). As well, females have frequently been observed to display considerable altruism toward injured group members (8). Mature males are never seen to be long-term members of social groups (10), and they must at some point disperse from their natal groups. The extent of female dispersal has remained unclear (2). The long-term social bonds observed among females suggest female philopatry, but data from whaling indicate that some dispersal may occur (2).

The kinship structure underlying the social behavior observed in sperm whales has long remained conjectural, although it clearly is fundamental to considerations of the evolution of social organization (3) and may have important implications for the management of populations (11).

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The objective of the present study was to examine the genetic structure of sperm whale groups. Skin that was naturally sloughed by free-living whales in three groups off the coast of Ecuador was collected for DNA analyses. We used microsatellite DNA markers, mitochondrial DNA (mtDNA) sequence, and molecular sexing to construct genetic profiles of individuals, compare relatedness within and between groups, and examine the consistency of the results with a matrilineal model.

MATERIALS AND METHODS

Field Methods. Research was conducted from the 13 m auxiliary cutter *Balaena* off the coast of mainland Ecuador (1°N–3°S, 81–82°W). Groups of sperm whales were located and tracked using a directional hydrophone (12). During daylight hours, individual whales at the surface were discreetly approached from behind and followed for several minutes until they submerged. While with a group, we took identification (13) and measurement (14) photographs of as many different individuals as possible. Pieces of sloughed skin floating in the wake of whales were collected and used as a source of DNA (15–17).

Delineation of Social Groups. It is often difficult to identify the membership of particular social groupings of female and immature sperm whales; the whales spend long periods invisible under water, they spread out when foraging, and groups may temporarily coalesce. For the genetic analyses described in this paper, data were collected from three distinct groups, with group being defined as individuals travelling together for periods of hours to days (see ref. 3). Groups A and B were each followed for two consecutive days (A, February 2–3, 1991; B, March 7–8, 1991), during which time neither group appeared to aggregate with other groups. Many identification photographs were taken, with a substantial number common to the two days. This allowed the numbers of individuals in each group to be estimated using mark-recapture methods (Table 1). Data assigned to group C were collected during an 80-min period on February 22, 1991, from 20–40 whales during an intense and exceptionally animated social interaction at the water surface. No identification photographs were taken and no formal size estimate was possible for this group. Thus, social bonds amongst whales in group C were less clear than in the other two groups. No adult males, which are distinctively larger than females and immature individuals, were observed in any of the groups during the period in which samples were collected.

Microsatellite DNA Profiling. Multilocus genetic profiles were constructed for each sample with five microsatellite markers isolated from sperm whales, using methods presented elsewhere (19). One locus (SW15) was X-linked. Six individuals could not be typed at locus SW19; however, there was no evidence for null alleles at this locus, nor at any of the other loci (19).

The analysis first sought to determine which samples were collected from the same whales, since we had collected about twice as many samples as there were individuals in the groups (Table 1). The genetic variation displayed by the suite of

Abbreviation: mtDNA, mitochondrial DNA.

*To whom reprint requests should be addressed.

Table 1. Data collected, estimated group size, sex ratio, and distribution of mtDNA haplotypes in three groups of sperm whales

	Group		
	A	B	C
ID photographs taken	87	113	0
Estimated numbers (SE)*	28.1 (1.5)	24.3 (0.8)	–
Skin samples	48	56	33
Genetically identified individuals	18 (17)†	20 (18)†	18 (15)†
Sex ratio	12♀, 6♂	16♀, 4♂	16♀, 2♂
mtDNA haplotypes:			
1	1♀, 2♂	1♀	7♀, 1♂
2	1♂	14♀, 3♂	3♀
3	9♀, 3♂	1♀, 1♂	
4			1♀
5			3♀
6	1♀		2♀, 1♂
Unable to type	1♀		

*Population estimates calculated using unbiased Petersen mark-recapture methods (18) on photographs of moderate to high quality: Arnbom's $Q \geq 3$ (13).
†Numbers of individuals typed at all five microsatellite loci are given in parentheses.

microsatellite markers appeared sufficient to distinguish between most, and probably all, individuals (19). Duplicate samples were removed from the analysis of kinship. False exclusion of samples would have made the analysis more conservative by reducing the observed similarity within groups, since all samples with identical multilocus profiles were collected from within the same group.

Molecular Sexing. Sex was determined by polymerase chain reaction-amplification of part of the male-specific *SRY* gene (20).

Mitochondrial DNA Sequencing. The sperm whale mitochondrial control region was amplified as described elsewhere (21). Prior to sequencing, PCR products were digested with exonuclease I and shrimp alkaline phosphatase (22). Dideoxy sequencing (23) was carried out using the T7 enzyme (Pharmacia). Sequencing primers were $tRNA^{Thr}$ (one of the PCR primers) and an internal primer that anneals to "block a" of the sperm whale control region (21). With these primers, ≈400 base pairs of the sperm whale control region were sequenced for each sample. Sequences were aligned, and variable nucleotide positions were identified. All sequence differences were transition substitutions, making sequence alignment straightforward. Based on the sequence at these positions, mtDNA haplotypes were defined. We are confident of our mtDNA sequences for several reasons, including the fact that we reamplified and resequenced in excess of 8000 base pairs, with no discrepancies observed. The set of resequenced samples included all individuals with uncommon haplotypes (i.e., those haplotypes observed in fewer than three individuals within a group).

Number of Individuals in Group with Particular mtDNA Haplotype. For a randomly chosen individual, the mean number of individuals within its group that shared its mtDNA haplotype was estimated from

$$\sum_i n_i \times n_i \times \left(T / \sum_i n_i \right) / \left(\sum_i n_i \right), \qquad [1]$$

where n_i is the number of animals in the group known to have mtDNA haplotype i, and T is the total number of animals in the group (known only for groups A and B for which we have good photographic identification records).

Analysis of Kinship. To investigate patterns of kinship in sperm whales, the number of shared microsatellite alleles was compiled for every pairwise comparison amongst all individuals typed at all five loci. A maximum of one shared allele was counted at the X-linked SW15 locus. The mean number of shared alleles was calculated for all pairs of whales in the same group with the same mtDNA haplotype, and for all pairs in the same group with different mtDNA haplotypes. Similar comparisons were made for pairs of whales in different groups. Significant differences between the set means were examined by using the jackknife procedure (in which each whale is omitted from the analysis in turn) and approximation to the t-distribution (24).

We wished to examine whether the data on genetic relationships among individuals within a group sharing the same mtDNA haplotype were consistent with those expected from matrilineal groups with male, but no female, dispersal. Therefore, we compared the expected mean number of parent–offspring relationships per individual for a matrilineal group with the mean number in each of our three study groups, estimated as follows.

Individuals were considered potential parent–offspring if they shared mtDNA haplotypes and at least one allele at all microsatellite loci that had been typed in both individuals. (As we did not know the ages of the individuals, we could not distinguish the parent from the offspring.) For each group, the number of such potential parent-offspring relationships, X, was counted. These potential parent–offspring pairs could have consisted of true mother–offspring pairs (male parents were not considered) or "pseudo-parent–offspring" pairs (i.e., pairs of animals whose genetic profiles happened, through chance, to be consistent with mother and offspring). The expected number of pseudo-parent–offspring pairs among the typed animals, Y, was estimated from the frequency distribution of microsatellite alleles at the five loci and the number of individuals with different mtDNA haplotypes within each group. The mean number of (true) parent–offspring relationships per individual, O, was then estimated for each group as follows:

$$O = (X - Y) \times (T/\Sigma n_i)/(\Sigma n_i). \qquad [2]$$

For group C, no size estimate was available, so that only a lower bound for this measure could be calculated (as $T/\Sigma n_i \geq 1$). Standard errors for O were estimated using the uncertainty in estimates of group size T (from the SEs in Table 1) and the jackknife procedure.

These estimates were made separately for the number of female parent–offspring relationships per female, $O(♀,♀)$; the number of male offspring per female, $O(♀,♂)$; and the number of female parents per male, $O(♂,♀)$. Expected numbers were calculated, assuming the population parameters of sperm whales used by the International Whaling Commission (25) and an equilibrium population, as follows:

$$\mathrm{Ex}[O(♀,♀)] = \sum_{a=1}^{\infty} (1-\beta)^a [(1-\beta)^a + \sum_{b=g}^{a-1} p \cdot \sigma \cdot (1-\beta)^{a-b-1}/2] / \sum_{a=1}^{\infty} (1-\beta)^a, \qquad [3]$$

$$\mathrm{Ex}[O(♀,♂)] = \sum_{a=1}^{\infty} (1-\beta)^a \times \left[\sum_{b=\max(g,a-d)}^{a-1} p \cdot \sigma \cdot (1-\alpha)^{a-b-1}/2 \right] / \sum_{a=1}^{\infty} (1-\beta)^a, \qquad [4]$$

$$\mathrm{Ex}[O(♂,♀)] = \sum_{a=1}^{d} (1-\alpha)^a \cdot (1-\beta)^a / \sum_{a=1}^{d} (1-\beta)^a, \qquad [5]$$

where α is the mortality of males over the age of 1 year (0.066 per year), β is the mortality of females over the age of 1 year (0.055 per year), σ is the mortality between birth and age 1 (0.093 per year), p is the birth rate of mature females (0.20 per year), g is the age of first birth for females (10 years), and d is the age of dispersal for males (6 years, see below). It is also assumed that genetic samples were only obtained from animals 1 year of age and older (consistent with our observations in the field).

Age of Male Dispersal. The age of male dispersal from the groups that we studied was estimated from observed sex ratios. Assuming an equilibrium population with equal numbers of each sex at age 1 year, a constant mortality rate of each sex, and that females spend their lives in such groups, then the total number of females in such groups is proportional to $1/\beta$, and the total number of males proportional to $[1 - (1 - \alpha)^d]/\alpha$. If the ratio of males to females is 1:f, then

$$1/f = [(1 - (1 - \alpha)^d)/\alpha]/(1/\beta), \quad \textbf{[6]}$$

and so

$$d = [\log(1 - \alpha/(\beta f))]/[\log(1 - \alpha)]. \quad \textbf{[7]}$$

Confidence intervals for d were calculated using binomial theory.

RESULTS

Sex Ratio and Age of Male Dispersal. The three groups consisted primarily of females (Table 1). All males must have been juvenile because none of the observed whales were of the distinctively large size (14–18 m) of mature males. Using the pregnancy and mortality rates estimated by the International Whaling Commission, the overall sex ratio (79% female) suggests that males disperse from these groups at the age of 6 years (95% confidence intervals: 2.7–10.9 years).

Genetic Variation Within and Between Groups. Greater genetic relatedness within groups, compared to between groups, is shown by the microsatellite analysis. Pairs of individuals in the same group shared significantly ($P < 0.001$) more alleles than pairs of whales in different groups (Table 2). Each group also had a different dominant mtDNA haplotype, although no group was homogeneous: 71% of group A consisted of mtDNA haplotype no. 3, 85% of group B consisted of haplotype no. 2, and 44% of group C consisted of haplotype no. 1 (Table 1).

Genetic Structure of Groups. Within groups, there was more similarity at the microsatellite loci between individuals with the same mtDNA haplotype than between those with different mtDNA haplotypes. However, the difference was small and far from significant ($P = 0.4$; Table 2).

Table 2. Mean number of shared alleles for individuals in the same and different groups, sharing and not sharing mtDNA haplotypes

	Mean shared alleles*	Tests†		
Within groups, within haplotypes	3.17			
Within groups, between haplotypes	3.10		$P = 0.4$	
Between groups, within haplotypes	3.00	$P < 0.001$		$P = 0.05$
Between groups, between haplotypes	2.83		$P = 0.2$	

*Mean number of shared alleles (out of nine) at five microsatellite loci (including one X-linked locus at which a maximum of only one shared allele was counted).

†One-tailed significance tests for differences between means used the jackknife procedure and t-distribution approximation (49 d.f.).

In groups A and B, the estimated mean numbers of parent–offspring relationships per individual (Table 3) were generally less than expected from perfect matrilines (i.e., if all females and all males less than 6 years of age shared a group with their mother, if living), while group C was more consistent with a set of perfect matrilines. The discrepancies between estimated and expected parent–offspring numbers for groups A and B are likely underestimated in Table 3 since the calculation of the expected number of pseudo-parent–offspring pairs ignores non-parent–offspring relatedness within groups, which is likely to produce additional numbers of pseudo-parent–offspring pairs. The most substantial difference between estimated and expected numbers of parent–offspring relationships is the lack of potential mothers for males in group A; only one of six typed males in this group had a potential mother among the typed females.

The mean number of individuals in a group sharing a mtDNA haplotype with a randomly chosen individual was estimated to be 15.1 for group A and 17.9 for group B. These values would be upper limits on the mean matriline size in which an individual lives, as a mtDNA haplotype could contain two or more matrilines, but no matriline can contain more than one mtDNA haplotype.

There was more similarity at the microsatellite loci among individuals within the same group but with different mtDNA haplotypes, than among individuals in different groups, and the difference was marginally significant ($P = 0.05$; Table 2). This suggests that some individuals within the same group, but characterized by different mtDNA haplotypes, may be paternally related.

DISCUSSION

Our examination of genetic variation at microsatellite and mtDNA markers offers insight into the patterns of kinship in sperm whale groups. Best (2), reviewing knowledge of sperm whale social organization in the late 1970s, concluded that "mixed" groups of sperm whales contained about 78% females (as compared with 79% for groups A, B, and C) and that the age of male dispersal "may be as low as 4–5 years." Although it was known that some females stayed together for periods of years (4), there was no information on genetic relatedness among grouped females from research conducted with the whaling industry. More recent photographic studies, both off the Galápagos Islands and more widely through the South Pacific, have shown that grouped females tend to have the same type of fluke notch, a morphological character thought to be genetically determined, suggesting relatedness within groups (26, 27). In the present study, direct DNA analysis of three groups studied off Ecuador showed that groups contained genetically related animals, but that not all grouped animals were genetic relatives. Genetic structure generally

Table 3. Estimated mean number of parent–offspring relationships per individual from mtDNA and microsatellite data (estimated SE in parentheses), compared with expected mean numbers of true parent–offspring relationships per individual in matrilineal groups

	Estimated mean no. of parent–offspring relationships		
	1 female with		1 male with
	Females	Males	Females
Group A	0.55 (0.92)	−0.01 (0.18)	−0.03 (0.37)
Group B	0.20 (0.28)	0.12 (0.20)	0.49 (0.87)
Group C	>0.74 (0.49)	>0.17 (0.16)	>1.36 (1.00)
Expected	0.94	0.23	0.83
Expected $\beta = 0.11$*	0.61	0.10	0.69

*Expected numbers if the natural mortality of females is doubled to $\beta = 0.11$ per year.

appeared to be matrilineal, though all groups included members of more than one matriline. No adult males were present in the groups, and the sex ratio of females/males was about 4:1, suggesting that males leave their natal groups at about age 6. The patterns of kinship described here could provide suitable conditions for the evolution of the alloparental care observed to occur within sperm whale social groups (6, 8, 9).

Groups A and B possessed fewer parent–offspring combinations than would be expected if all living mothers of all members of the groups were also in the groups (Table 3). This discrepancy could be due to chance (sample sizes were small, standard errors were substantial, and the results for group C seemed consistent with what we would expect from a set of perfect matrilines). Alternatively, the lower than expected numbers of parent–offspring relationships in groups A and B could reflect some dispersal of either females or young males between groups, or errors in the assumed population parameters. For instance, if the assumed female mortality is doubled, perhaps because of the substantial whaling taking place in nearby Peruvian waters 9–30 years previously (28), then expected numbers of parent–offspring relationships are more like those observed in groups A and B (Table 3).

The analysis of microsatellite profiles revealed apparent genetic relatedness among grouped animals that did not share mtDNA haplotypes. Making the reasonable assumption that mitochondrial DNA is maternally inherited in sperm whales, this implies paternal relatedness within groups. None of the groups examined in this paper contained the large males that are thought to be the successful breeders (5). Such males only visit groups briefly (10), and at least 11 of the 12 smaller males that we sampled shared mtDNA haplotypes with females in their groups (Table 1); thus, it seems unlikely that fathers were grouped with their offspring. Alternatively, the large breeding males could inseminate two or more females in the same group, leading to paternally related half-siblings. Some of our knowledge of the mating system of sperm whales seems to oppose much paternal relatedness within groups; the groups are accompanied by a number of large males at different times during the breeding season, a male does not generally stay with a group of females for more than a few hours at a time (10), and the calving rate is sufficiently low [about 0.2–0.25 per mature female per year (5)] that only about 2–3 conceptions would be expected in a group during one breeding season. However, a male may reassociate with a group repeatedly over periods of days (10), females within groups synchronize their oestrous periods (29), and dominance hierarchies and/or female choice could limit the number of males that actually mate.

The results of the molecular analysis have implications for the stability of group membership over time. For groups to contain perfect, or near-perfect, matrilines, members of these matrilines must, by definition, stay together through their lives. In addition, if there is substantial paternal relatedness between individuals of different matrilines, then these separate matrilines must spend at least periods of years together. Long-term associations between individual sperm whales have also been inferred from sighting histories of photographically identified individuals (3). Taken together, the patterns of association and kinship observed in the different studies indicate that female sperm whales form permanent social units based on one or several matrilines. However, the mean number of companions (12.0) in permanent social units off the Galápagos Islands, estimated from the analysis of sighting histories (3), is somewhat lower than the maximum sizes of matrilines (15.1 and 17.9) in groups A and B off Ecuador, estimated from the analysis of mitochondrial DNA. Units often merge with other permanent units for short periods of time, and this could partially confound the analysis of kinship. It is not yet known whether there are preferential associations between particular units.

The results of this study represent significant progress in our understanding of sperm whale social organization. More generally, the results underscore the value of DNA markers for current investigations of wild populations. By combining the use of molecular genetic techniques with innovative field methods, it is now possible to begin detailed social studies of even relatively inaccessible animals, with little impact on their lives.

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