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Global matrilineal population structure in sperm whales as indicated by mitochondrial DNA sequences

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The genetic variability and population structure of worldwide populations of the sperm whale was investigated by sequence analysis of the first 5′L 330 base pairs in the mitochondrial DNA (mtDNA) control region. The study included a total of 231 individuals from three major oceanic regions, the North Atlantic, the North Pacific and the Southern Hemisphere. Fifteen segregating nucleotide sites defined 16 mtDNA haplotypes (lineages). The most common mtDNA types were present in more than one oceanic region, whereas ocean-specific types were rare. Analyses of heterogeneity of mtDNA type frequencies between oceans indicated moderate ($G_{ST}=0.03$) but statistically significant ($p=0.0007$) genetic differentiation on a global scale. In addition, strong genetic differentiation was found between potential social groups ($G_{ST}=0.3$–0.6), indicating matrilineal relatedness within groups. The global nucleotide diversity was quite low ($\pi=0.004$), implying a recent common mtDNA ancestry ($<100\,000$ years ago) and a young global population structure. However, within this time period, female dispersal has apparently been limited enough to allow the development of global mtDNA differentiation. The results are consistent with those from observational studies and whaling data indicating stable social affiliations, some degree of area fidelity and latitudinal range limitations in groups of females and juveniles.

Keywords: sperm whale; mtDNA; population structure; dispersal; differentiation; kin groups

1. INTRODUCTION

The sperm whale, *Physeter macrocephalus*, is the largest toothed whale, and the most sexually dimorphic whale, with female and male maximum lengths of about 12 and 18 m, respectively, and an approximate threefold difference in mass (Best 1979; Rice 1989). With its exceptional diving abilities, normally to 200–600 m, sometimes reaching 1000–2000 m (Lockyer 1977; Papastavrou *et al*. 1989; Watkins *et al*. 1993), the sperm whale feeds mainly on meso- and bathypelagic cephalopods (Kawakami 1980), a specialization that has enabled the species to successfully colonize every ocean of the world. In most baleen whales, both sexes migrate between summer feeding areas in high latitudes and winter breeding areas in low latitudes, with the Northern and Southern Hemisphere populations seasonally out of phase. However, sperm whale movement patterns are quite different. Groups of females and immatures ('mixed schools') are normally restricted in their movements to tropical and subtropical waters, whereas males leave their natal groups at an age of about four to five years or older, join all-male groups ('bachelor schools'), and show decreasing sociality and increasing migration range with age (Best 1979; Rice 1989). Thus, males eventually reach as far as polar waters, migrating to warmer waters to breed after having attained breeding size and age (Best 1979; Rice 1989).

Commercial exploitation of sperm whales, which has been extensive, started in the 18th century (Rice 1989), but ended in 1988 as a result of international regulations. Although there are no reliable population estimates, present-day populations are considered depleted compared to those preceding exploitation (Klinowska 1991; Whitehead 1995). Despite the long history of exploitation, very little is known about the extent of dispersal and reproductive exchange throughout the oceans. The tremendous dispersal potential of the species is borne out by its global distribution, but the apparent latitudinal limits of females and immatures and some observations of local site fidelity in these schools (Gordon 1987; Whitehead *et al*. 1992; Dufault & Whitehead 1995) suggest that the possibility for genetic differentiation between populations may nevertheless exist.

To investigate whether there is differentiation between oceanic populations of sperm whales, we analysed sequence variability in the mitochondrial DNA (mtDNA) control region, the most variable part of the mtDNA molecule. If female dispersal is limited, mtDNA would potentially be a particularly useful tool to study population differentiation owing to its maternal, haploid mode of inheritance, high substitution rate and apparent absence of recombination (Wilson *et al*. 1985; Avise 1986). In a previous study, Lyrholm *et al*. (1996) examined

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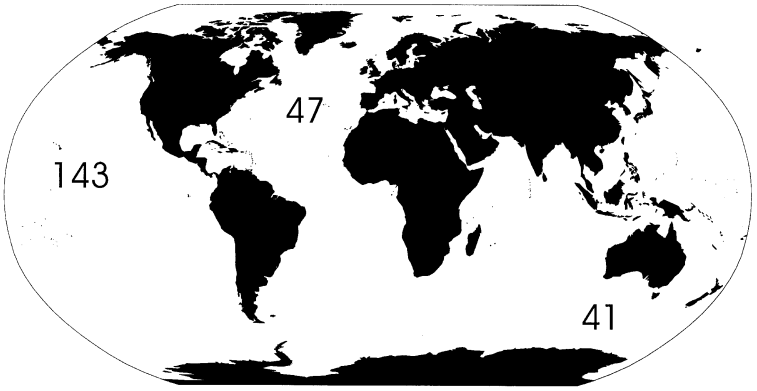

Figure 1. Locations and sample sizes of the samples used in the analyses.

sequence variation and substitution patterns in the entire sperm whale control region from a limited number of individuals sampled worldwide. The results suggested an unusually low diversity. In addition, sequence-based phylogenetic methods were found to be of little utility in investigations of geographic differentiation, owing to extensive parallelisms and reversals of nucleotide substitutions. However, the results also indicated that analyses of haplotype frequency variation could be more informative about population differentiation. In this study, we therefore investigated the degree of mtDNA differentiation between global populations of sperm whales based on a larger sample (231 whales) from the North Atlantic, North Pacific and Southern Hemisphere, using modern statistical techniques to test for haplotype frequency differences.

2. MATERIALS AND METHODS

(a) *Samples*

The samples were collected using skin biopsy techniques (Lambertsen 1987), retrieval of sloughed skin (Amos *et al*. 1992), and from tissue archives. The locations of the sampling areas are given in figure 1. Sample sizes were: North Atlantic (NA) $n=47$ (Azores $n=13$, Denmark $n=15$, Norway $n=8$, Iceland $n=8$, Sweden $n=1$, Florida $n=1$ and Dominican Republic $n=1$); North Pacific (NP) $n=143$ (areas between *ca*. 24° and 34°N latitudes: off the Japanese coast $n=29$, western NP (161°–171°E) $n=34$, central NP (150°–170°W) $n=11$, eastern NP (120°–130°W) $n=31$; and Galápagos (at the equator) $n=38$); and Southern Hemisphere (SH) $n=41$ (south of Fiji $n=20$, southeast of Australia $n=14$, southern Indian Ocean $n=3$, and Antarctica (south of Indian Ocean) $n=4$).

(b) *Laboratory procedures*

Samples were digested with Proteinase K and DNA isolated using standard techniques (Sambrook *et al*. 1989). The mtDNA control region was PCR amplified, as described by Lyrholm *et al*. (1996), except that a new primer (TL12R) for the heavy strand was used (sequencing tail in brackets): 5′-AAACTGACTAGCAGGACG(CAGGAAACAGCTATGACC)-3′. Fluorescent cycle sequencing was performed directly on the PCR product using the light strand PCR primer with dye terminators and a dye-labelled M13 primer complementary to the tail in TL12R (AmpliTaq FS, Dye Terminator and Dye Primer kits, Perkin-Elmer, Inc.). Where unknown, sex was determined using the technique of Bérubé & Palsbøll (1996).

(c) *Data analysis*

Nucleotide and haplotype diversities were estimated as defined by Nei (1987). Exact tests of heterogeneity were performed by a Markov chain permutation approach (Raymond & Rousset 1995) using the software Arlequin (Schneider *et al*. 1997). The amount of differentiation, G_{ST}, was estimated by the procedure of Nei & Chesser (1983).

As groups of females and immatures partly contain whales 'permanently' associated (Whitehead *et al*. 1991), which appear to be related (Richard *et al*. 1996), it was necessary to investigate whether group structure was present in the material. Such structure could cause inflated statistical significances in geographical comparisons. Thus, all tests and calculations were performed on two sets of data: 'all the material' and a 'restricted material', the latter including only one, randomly chosen whale from each potential social group. Whales sampled on the same day, or, in the case of samples from living whales, in the same period of days during which continuous contact with whales was maintained, were considered to potentially be part of the same group. In the Galápagos material, photographic identification (Arnbom 1987) was also used to link individuals to potential groups. All males known to be longer than 11 m were considered likely to have dispersed from their natal group (Best 1979) and thus were not excluded.

Where the identity of the sampled whales was unknown, samples with identical microsatellite (T. Lyrholm and U. Gyllensten, unpublished data) and mtDNA genotype combinations were considered to potentially be the same individual and only included once.

Time since common mtDNA ancestry of sperm whale lineages was estimated by the method in Lyrholm *et al*. (1996). This uses an interspecific estimate of the transition/transversion ratio in the cetacean control region and the number of transition substitutions from root to terminal taxa in a phylogeny of the sperm whale lineages, inferred by maximum parsimony. The parsimony analysis was performed using the software PAUP (Swofford 1993) with the options TBR Mulpars, stepwise addition and random addition sequence.

3. RESULTS

(a) *Sequence variation and diversity*

Sequence variation was investigated in the first 330 base pairs (bp) of the control region (referring to the light chain), as Lyrholm *et al*. (1996) found that most of the nucleotide substitutions were concentrated to this sequence portion. In addition, we examined a tract of guanines (G)

	1 1 1 2 2 2 2 2 2 2 2 3 3
	5 6 0 2 8 0 1 6 7 7 8 8 8 1 2
	8 2 5 1 4 0 1 0 2 3 6 7 8 9 4
1	T C C C T T C A A C A A A G C
2	. T . . . . G . .
3	. T . . . .
4	. . . T . . . .
5	C . . . .
6	. T . . C . . G G . . . . A T
7	. . . . G . .
8	. T . . . . A .
9	. T . . C . . G . . . . . A T
10	. . . . T . G . . .
11	. T . . . . G . . . .
12	. T . . . . T . . G . T
13	. . . . . C T . . . .
14	. T . . . . . G . . . . . A T
15	. T T . C . . G G . . . . A T
16	. T . . . . T . . . . .

Figure 2. Segregating sites (sequence positions identified on the top rows i.e. from 58 to 324) in the first 330 bp of the control region and the lineages (identified in the leftmost column) they define. Sequence lineage no. 1 is written in its entirety and any subsequent matching nucleotides are indicated by dots.

between 574 and 581 bp, previously shown to harbour an indel polymorphism (Lyrholm *et al*. 1996). Among the first 330 bp, we found 15 segregating sites, six of which were new compared with those reported by Lyrholm *et al*. (1996), defining a total of 16 lineages (or haplotypes) (figure 2). All nucleotide substitutions were transitions.

The nucleotide diversity (π) and haplotype diversity are given for each oceanic region and globally in table 1. As suggested by Lyrholm *et al*. (1996), an unusually low diversity is indicated in this species.

The indel polymorphism showed a complex pattern of variation. A minority of individuals was apparently heteroplasmic for seven and eight G in varying proportions. Consequently, we did not use this character to further define lineages, but limited the analysis to the frequency of occurrence of the indel among oceans.

(b) *Genetic differentiation between and within oceans*

The frequency distributions of the various haplotypes in each ocean are shown in table 2. There was a highly significant heterogeneity in the distribution of haplotypes between oceans (table 3). The amount of differentiation, G_{ST}, was estimated as 0.048 and 0.030 based on all material and the restricted material, respectively.

We investigated the geographical distribution of the indel polymorphism as follows. Apart from the clear, homoplasmic cases, we classified individuals that were heteroplasmic as having either mainly seven or mainly eight G depending on the relative peak heights in the sequence chromatograms (the criterion for heteroplasmy was arbitrarily set to the lower peak height being at least 25% of the higher). Based on these criteria, there were highly significant differences in frequency distribution between oceans ($p<0.00001$); all cases of the deletion (i.e. 7 G), except one, occurred in the Southern Hemisphere

Table 1. *MtDNA control region sequence diversity*

			diversity	
oceans	material	*n*	nucleotide	haplotype
North Atlantic	all	47	0.0033±0.0002	
	restricted	42	0.0033±0.0002	0.71
North Pacific	all	143	0.0036±0.0003	
	restricted	71	0.0039±0.0006	0.70
Southern Hemisphere	all	41	0.0055±0.0010	
	restricted	23	0.0055±0.0015	0.75
total	all	231	0.0039±0.0003	
	restricted	136	0.0041±0.0004	0.73

(table 4). The differences remained highly significant if the heteroplasmic cases were removed ($p<0.00001$).

Next, we investigated the within-ocean differentiation in the North Pacific (the only ocean from which we had a sufficient sample size). Although there was highly significant heterogeneity among all individuals, we did not detect any significant structure in the restricted material (table 3). Thus, the heterogeneity found in the whole North Pacific sample could be due to the effect of kin groups.

(c) *Group structure*

To examine whether the whales in mixed schools identified as having been sampled in close temporal and spatial proximity could represent related individuals, we performed the same tests of heterogeneity among females from nine potential social groups in the northern NP areas (six groups of $n=4$, one each of $n=5$, 6 and 7), three in Galápagos ($n=4$, 6 and 7) and four in the SH ($n=4$, 5, 5 and 7). These tests were highly significant ($p<0.001$) within each area. Furthermore, G_{ST} between the groups was estimated to be 0.48, 0.34 and 0.61 in the NP, Galápagos and SH areas, respectively, an order of magnitude higher than that between oceans. Thus, the results indicated the presence of groups of matrilineally related individuals.

(d) *Age of common ancestor*

In the phylogenetic analysis, a total of 92 equally parsimonious trees were obtained, containing 19 character changes. However, few branches were supported in bootstrap and consensus analysis. This is likely to be due partly to the existence of several mutational 'hot spots' in the mtDNA control region of sperm whales, in which multiple substitutions cause considerable homoplasy (Lyrholm *et al*. 1996). However, the most important property of the tree used in estimating the time since common ancestry is not the topology but the length (i.e. the number of character changes). Because all the equally parsimonious trees have the same length they will give similar results. We arbitrarily chose one tree that was used to estimate the average number of transition substitutions from root to tip in the sperm whale lineages to 1.9. Thus, with 330 sites, the average number of substitutions per site, *s*, was 0.00576. Phylogenies of humpback whales (Baker *et al*. 1993), North Atlantic fin

Table 2. *Frequencies of haplotypes in the various areas*

(NA=North Atlantic, NP=North Pacific, SH=Southern Hemisphere, G=Galápagos Islands, JC=Japanese coastal, NPW=North Pacific west, NPC=North Pacific central, NPE=North Pacific east. The first frequency is based on all individuals, whereas the second is based on the restricted material.)

	Oceans			NP areas				
types	NA	NP	SH	G	JC	NPW	NPC	NPE
1	0.38/0.33	0.46/0.48	0.29/0.48	0.39/0.40	0.66/0.65	0.32/0.27	0.45/0.57	0.48/0.33
2	0.36/0.38	0.13/0.13	—	0.21/0.20	0.070/0.10	0.088/0.091	0.45/0.29	0.032/0.067
3	0.19/0.21	0.27/0.25	0.29/0.17	0.37/0.40	0.070/0.050	0.35/0.32	0.091/0.14	0.29/0.40
4	0.021/0.024	—	—	—	—	—	—	—
5	—	0.056/0.028	0.024/0.044	—	0.035/0.050	0.12/—	—	0.097/0.067
6	—	0.0070/0.014	—	—	0.035/0.050	—	—	—
7	—	—	0.22/0.087	—	—	—	—	—
8	0.043/0.048	0.035/0.028	0.049/0.087	—	0.14/0.10	—	—	0.032/—
9	—	—	0.049/0.039	—	—	—	—	—
10	—	0.014/0.014	—	0.026/—	—	0.029/0.046	—	—
11	—	0.0070/—	—	—	—	0.029/—	—	—
12	—	0.014/0.028	—	—	—	0.059/0.091	—	—
13	—	0.014/0.028	—	—	—	—	—	0.065/0.13
14	—	—	0.024/—	—	—	—	—	—
15	—	—	0.024/0.044	—	—	—	—	—
16	—	—	0.024/0.044	—	—	—	—	—
n	47/42	143/71	41/23	38/15	29/20	34/14	11/7	31/15

Table 3. *Geographic differentiation of mtDNA haplotypes*

(The comparisons over oceans are between North Atlantic, North Pacific and Southern Hemisphere, and those within the North Pacific are between the Galápagos Islands, the Japanese coast, and the western, central and eastern areas. 'All' means all the individuals were used, whereas 'restricted' means that only one individual was used from each potential social group.)

	between oceans		within North Pacific	
	all	restricted	all	restricted
G_{ST}[a]	0.048	0.030	0.052	0.009
p[b]	<0.00001	0.0007	<0.00001	0.12

[a]Wright's fixation index calculated according to Nei & Chesser (1983).
[b] p values are from an exact test of population differentiation (Raymond & Rousset 1995).

Table 4. *Geographic distribution of indel polymorphism*

(The occurrence of a deletion leading to seven as opposed to eight G in a tract beginning at sequence position 574. Heteroplasmic individuals were scored as having mainly seven (7>8) or mainly eight (7<8) G (see text for details). Highly significant heterogeneity (exact test, $p<0.00001$) was found, whether the heteroplasmic individuals were included or not.)

	oceans		
no. of G	NA	NP	SH
7	1	0	5
8	43	66	1
7>8	0	0	14
7<8	0	0	2

whales (Bérubé *et al*. 1998), and North Atlantic and Black Sea harbour porpoises (Rosel *et al*. 1995) were added to the sperm whale data to give an estimate of the transition/transversion ratio, R, in the cetacean control region of 55. Using the same species in the calibration of transversion substitution rates as in Lyrholm *et al*. (1996), two estimates of sperm whale common ancestry of $t=24\,000$ years and $t=92\,000$ years were obtained.

4. DISCUSSION

Our results provide evidence of mitochondrial genetic differentiation in sperm whale populations on a worldwide scale, consistent with the indications of the smaller study by Lyrholm *et al*. (1996). This suggests that interoceanic dispersal of female lineages is limited. Observational studies have resulted in substantial between-year resightings of female/juvenile groups in the study areas (Gordon 1987; Whitehead *et al*. 1992; Dufault & Whitehead 1995) and little dispersal between geographically distant regions, such as southeast and southwest Pacific (Dufault & Whitehead 1995). Recaptures of marked females caught in whaling operations have involved cases of both site fidelity and long-distance movements (up to *ca*. 1500–4000 km) within oceans (Best 1979; Brown 1981; Ivashin 1981; Kasuya & Miyashita 1988). However, in all these cases, the distribution of effort in space and time has been a limitation of the likelihood of finding long-distance dispersal. Nevertheless, so far there have been no reports of between-ocean female dispersal, indicating that it may be rare. This might be

expected, given the latitudinal range limitations of females and juveniles (Best 1979; Rice 1989).

We did not find any evidence of genetic differentiation within the North Pacific. However, within the oceans it is probably particularly important to take into account the differential dispersal patterns of the sexes and the age categories of males. Thus, we also tested heterogeneity after having removed all males that were probably sexually mature (over 13 m in length; this also led to the removal of the NPC area sample), which were likely to have the most wide-ranging dispersal patterns (Best 1979). There was a tendency of more structure in this test, but the result was not significant ($p=0.081$). Further studies based on larger sample sizes are needed to investigate within-ocean population structure, taking account of the sex and age differences in dispersal patterns. Based on a variety of whaling-related data (including mark–recaptures), Kasuya & Miyashita (1988) proposed at least three distinct populations of sperm whales in the North Pacific, a hypothesis we were unfortunately not able to test with the present data.

The differential female and male dispersal patterns could result in contrasting mitochondrial and nuclear genetic structure of populations, where the former would be expected to show more differentiation owing to maternal inheritance, whereas male breeding dispersal may homogenize allele frequencies at the nuclear loci (Avise 1994). Thus, it would be interesting to compare the present results with allelic variation in nuclear markers.

Because only the first 5′L 330 bp were investigated in the present study, the amount of geographic structure of mtDNA lineages was probably underestimated. It has been shown that the 3′ end of the control region also contains variation, which would divide the material into additional haplotypes, as would the indel polymorphism in the polyG region (Lyrholm *et al*. 1996). For example, some haplotypes that were ocean-specific in the latter study would be undetected in the present analysis. Furthermore, substitutional 'hot-spots' have been found in the sperm whale control region, which lead to parallelisms and reversals (Lyrholm *et al*. 1996). It is unclear how seriously this will affect the observed frequencies of haplotypes, but it is conceivable that frequent reversals to ancestral types could lead to underestimates of the haplotype frequency differences. This problem warrants further investigation.

The low diversity and the estimates of common ancestry of 24 000–92 000 years are consistent with the suggestion by Lyrholm *et al*. (1996) that worldwide sperm whale population structure is relatively young. The young estimated age could perhaps be due to population bottlenecks and/or other demographic factors, or a selective sweep (Lyrholm *et al*. 1996).

Taken together, our results could be interpreted as an evolutionarily recent global range expansion, after which inter-oceanic dispersal of females has been limited enough for some differentiation to develop. The low estimate of G_{ST} could be indicative of gene flow, or a consequence of the recent estimated common ancestry.

The analysis of potential social groups of females supported the conclusions of Richard *et al*. (1996), who studied the genetic composition of groups off the coast of Ecuador, showed that groups of females and immatures contain genetically related whales, although more than one matriline may be present. From their table 1, we estimated a G_{ST} of 0.32 between groups, consistent with our estimate from the Galápagos groups. Our higher estimates of G_{ST} from the North Pacific and Southern Hemisphere groups may include a geographic component, as these groups were sampled over larger areas. In general, our selection of potential groups was rather crude, since we were not able to conduct a proper association analysis of long-term bonds (Whitehead *et al*. 1991) of whales in the present study. Observational and marking data indicate long-term stable associations between some females within mixed schools (Ohsumi 1971; Gordon 1987; Whitehead *et al*. 1991). Relatedness and stable membership within groups could provide opportunity for the evolution of cooperation through kin selection (Hamilton 1964) or reciprocity (Trivers 1971), and there are some observations of such behaviour. For example, different females have been seen taking care of calves that are unable to accompany the adults during foraging dives (Gordon 1987; Arnbom & Whitehead 1989; Whitehead 1996), care-giving behaviour towards whales that were harpooned has also been seen (Caldwell & Caldwell 1966; Best 1979), as has cooperative protection against predators (Arnbom *et al*. 1987). Finally, social bonds have apparently facilitated the evolution of group-specific dialects (Weilgart & Whitehead 1997). Geographic vocal variation, with little similarity in repertoires in different oceans, was also found (Weilgart & Whitehead 1997), an observation lending some support to the indications of limited inter-oceanic dispersal of female/immature social groups suggested in the present study.

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REFERENCES

Amos, W., Whitehead, H., Ferrari, M. J., Glockner-Ferrari, D. A., Payne, R. & Gordon, J. 1992 Restrictable DNA from sloughed cetacean skin; its potential for use in population analysis. *Mar. Mamm. Sci*. **8**, 275–283.

Arnbom, T. 1987 Individual identification of sperm whales. *Rep. Int. Whal. Commn* **37**, 201–204.

Arnbom, T. & Whitehead, H. 1989 Observations on the composition and behaviour of groups of female sperm whales near the Galápagos Islands. *Can. J. Zool*. **67**, 1–7.

Arnbom, T., Papastavrou, V., Weilgart, L. S. & Whitehead, H. 1987 Sperm whales react to an attack by killer whales. *J. Mamm*. **68**, 450–453.

Avise, J. C. 1986 Mitochondrial DNA and the evolutionary genetics of higher animals. *Phil. Trans. R. Soc. Lond.* B **312**, 325–342.

Avise, J. C. 1994 *Molecular markers, natural history and evolution*. New York: Chapman & Hall.

Baker, C. S., Perry, A., Bannister, J. L., Weinrich, M. T., Abernethy, R. B., Calambokidis, J., Lien, J., Lambertsen, R. H., Ramirez, J. U. & Et, A. 1993 Abundant mitochondrial DNA variation and world-wide population structure in humpback whales. *Proc. Natn. Acad. Sci. USA* **90**, 8239–8243.

Bérubé, M. & Palsbøll, P. 1996 Identification of sex in cetaceans by multiplexing using ZFX and ZFY specific primers. *Molec. Ecol.* **5**, 283–287.

Bérubé, M., Aguilar, A., Dendanto, D., Larsen, F., Notarbartolo di Sciara, G., Sears, R., Sigurjónsson, J., Urban-Ramirez, J. & Palsbøll, P. 1998 Population genetic structure of North Atlantic and Mediterranean Sea fin whales, *Balaenoptera physalus* (Linnaeus 1758): analysis of mitochondrial and nuclear loci, and comparison with the Sea of Cortez fin whale population. *Molec. Ecol.* **7**, 585–600.

Best, P. B. 1979 Social organization in sperm whales, *Physeter macrocephalus*. In *Behavior of marine animals*, vol. 3 (ed. H. E. Winn. & B. L. Olla), pp. 227–289. New York: Plenum Press.

Brown, S. G. 1981 Movements of marked sperm whales in the Southern Hemisphere. *Rep. Int. Whal. Commn* **31**, 835–837.

Caldwell, M. C. & Caldwell, D. K. 1966 Epimeletic (caregiving) behavior in Cetacea. In *Whales, dolphins and porpoises* (ed. K. S. Norris), pp. 755–788. Berkeley, CA: University of California Press.

Dufault, S. & Whitehead, H. 1995 The geographic stock structure of female and immature sperm whales in the South Pacific. *Rep. Int. Whal. Commn* **43**, 401–405.

Gordon, J. C. D. 1987 Sperm whale groups and social behaviour observed off Sri Lanka. *Rep. Int. Whal. Commn* **37**, 205–218.

Hamilton, W. D. 1964 The genetical evolution of social behavior, I and II. *J. Theor. Biol.* **7**, 1–52.

Ivashin, M. V. 1981 Some results of the marking of sperm whales (*Physeter macrocephalus*) in the Southern Hemisphere under the Soviet marking programme. *Rep. Int. Whal. Commn* **31**, 707–718.

Kasuya, T. & Miyashita, T. 1988 Distribution of sperm whale stocks in the North Pacific. *Sci. Rep. Whales Res. Inst. Tokyo* **39**, 31–75.

Kawakami, T. 1980 A review of sperm whale food. *Sci. Rep. Whales Res. Inst. Tokyo* **32**, 199–218.

Klinowska, M. 1991 *Dolphins, porpoises and whales of the world. The IUCN red data book*. Gland and Cambridge: IUCN.

Kumar, S., Tamura, K. & Nei, M. 1993 *MEGA: molecular evolutionary genetics analysis, version 1.0*. The Pennsylvania State University, University Park, PA 16802, USA.

Lambertsen, R. H. 1987 A biopsy system for large whales and its use in cytogenetics. *J. Mammal.* **68**, 443–445.

Lockyer, C. 1977 Observations on diving behaviour of the sperm whale, *Physeter catodon*. In *A voyage of discovery* (ed. M. Angel), pp. 591–609. Oxford and New York: Pergamon Press.

Lyrholm, T., Leimar, O. & Gyllensten, U. 1996 Low diversity and biased substitution patterns in the mitochondrial DNA control region of sperm whales: implications for estimates of time since common ancestry. *Molec. Biol. Evol.* **13**, 1318–1326.

Nei, M. 1987 *Molecular evolutionary genetics*. New York: Columbia University Press.

Nei, M. & Chesser, R. K. 1983 Estimation of fixation indices and gene diversities. *Ann. Hum. Genet.* **47**, 253–259.

Ohsumi, S. 1971 Some investigations on the school structure of sperm whale. *Sci. Rep. Whales Res. Inst. Tokyo* **23**, 1–25.

Papastavrou, V., Smith, S. & Whitehead, H. 1989 Diving behaviour of the sperm whale, *Physeter macrocephalus*, off the Galapagos Islands. *Can. J. Zool.* **67**, 839–846.

Raymond, M. & Rousset, F. 1995 An exact test for population differentiation. *Evolution* **49**, 1280–1283.

Rice, D. W. 1989 Sperm whale—*Physeter macrocephalus* Linnaeus, 1758. In *Handbook of marine mammals. 4. River dolphins and the larger toothed whales* (ed. S. H. Ridgway & R. Harrison), pp. 177–233. London: Academic Press.

Richard, K. R., Dillon, M. C. & Wright, J. M. 1996 Patterns of kinship in groups of free-living sperm whales (*Physeter macrocephalus*) revealed by multiple molecular genetic analyses. *Proc. Natn. Acad. Sci. USA* **93**, 8792–8795.

Rosel, P. E., Dizon, A. E. & Haygood, M. G. 1995 Variability of the mitochondrial control region in populations of the harbour porpoise, *Phocoena phocoena*, on interoceanic and regional scales. *Can. J. Fish. Aquat. Sci.* **52**, 1210–1219.

Sambrook, J., Fritsch, E. F. & Maniatis, T. 1989 *Molecular cloning. A laboratory manual*, 2nd edn. Cold Spring Harbor Laboratory Press.

Schneider, S., Kueffer, J.-M., Roessli, D. & Excoffier, L. 1997 *Arlequin ver. 1.1: a software for population genetic data analysis*. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Swofford, D. L. 1993 *PAUP: phylogenetic analysis using parsimony, version 3.1.1.* Champaign, IL: Illinois Natural History Survey.

Trivers, R. L. 1971 The evolution of reciprocal altruism. *Q. Rev. Biol.* **46**, 35–57.

Watkins, W. A., Daher, M. A., Fristrup, K. M., Howald, T. J. & Di, S. G. N. 1993 Sperm whales tagged with transponders and tracked underwater by sonar. *Mar. Mamm. Sci.* **9**, 55–67.

Weilgart, L. & Whitehead, H. 1997 Group-specific dialects and geographical variation in coda repertoire in South Pacific sperm whales. *Behav. Ecol. Sociobiol.* **40**, 277–285.

Whitehead, H. 1995 Status of Pacific sperm whale stocks before modern whaling. *Rep. Int. Whal. Commn* **45**, 407–412.

Whitehead, H. 1996 Babysitting, dive synchrony, and indications of alloparental care in sperm whales. *Behav. Ecol. Sociobiol.* **38**, 237–244.

Whitehead, H., Waters, S. & Lyrholm, T. 1991 Social organization of female sperm whales and their offspring: constant companions and casual acquaintances. *Behav. Ecol. Sociobiol.* **29**, 385–389.

Whitehead, H., Waters, S. & Lyrholm, T. 1992 Population structure of female and immature sperm whales (*Physeter macrocephalus*) off the Galápagos Islands. *Can. J. Fish. Aquat. Sci.* **49**, 78–84.

Wilson, A. C. (and 10 others) 1985 Mitochondrial DNA and two perspectives on evolutionary genetics. *Biol. J. Linn. Soc.* **26**, 375–400.