Low Diversity and Biased Substitution Patterns in the Mitochondrial DNA Control Region of Sperm Whales: Implications for Estimates of Time Since Common Ancestry

Thomas Lyrhom,*† Olof Leimar,* and Ulf Gyllensten†

*Department of Zoology, Stockholm University; and †Department of Medical Genetics, Biomedical Center, Uppsala University

The mitochondrial DNA (mtDNA) control region was sequenced in 37 sperm whales from a large part of the global range of the species. Nucleotide diversity was several-fold lower than that reported for control regions of abundant and outbred mammals, but similar to that for populations known to have experienced bottlenecks. Relative rate tests did not suggest that the low diversity is due to a lower substitution rate in sperm whale mtDNA. Rather, it is more likely that demographic factors have reduced diversity. The pattern of nucleotide substitutions was examined by cladistic methods, facilitated by the apparent monophyly of lineages from the Southern Hemisphere, as defined by a single base pair deletion. Substitutions were nonrandom in nature, confined to a few "hot spots," and parallel substitutions constituted a majority of the inferred changes. The substitution pattern fitted a negative binomial distribution better than a Poisson distribution, and the bias in number of substitutions among sites was considerably higher than previously reported for the mtDNA control region of any species. A novel method of estimating time since common ancestry was developed, which utilizes the transition/transversion ratio R and the number of substitutions inferred from a parsimony analysis. Using this method, we estimated the age of sperm whale mtDNA diversity to be about 6,000–25,000 years, and when the uncertainty of R was accounted for, a range of about 1,000–100,000 years was obtained.

Introduction

Mitochondrial DNA (mtDNA) has become one of the most frequently studied regions of the mammalian genome for the purposes of individual identification, reconstruction of phylogenetic relationships, and analyses of geographical patterns of intraspecific genetic variation. The usefulness of mtDNA in evolutionary studies derives from its predominantly maternal inheritance, high substitution rate, and an apparent lack of recombination (Wilson et al. 1985; Avise 1986). Due to a high level of sequence polymorphism, analyses of the mtDNA control region (D-loop) have afforded a particularly high resolution view of intraspecific genetic structure in a variety of taxa, e.g., humans (Vigilant et al. 1991), whales (Baker et al. 1993), and birds (Wenink, Baker, and Titanus 1993).

The sperm whale (Physeter macrocephalus) is one of the most widely distributed mammalian species, occurring in all oceans. Females and immatures range from the tropics to temperate waters, while adult males reach as far as polar waters (Best 1979). The world population numbers are not known with any precision but probably exceed 100,000, and preexploitation numbers seem to have been substantially higher (Klinowska 1991). Due to international regulations, there has been no commercial hunting for sperm whales since 1988.

In order to study the levels of intraspecific mtDNA diversity and the degree of genetic differentiation among populations, we sequenced the mtDNA control region of sperm whales from a wide range of the global distribution of the species. The mtDNA variability was unusually low compared to that normally seen in mammals. Two alternative explanations for the low diversity were investigated: (1) a slower rate of evolution in sperm whale mtDNA and (2) a recent common mitochondrial ancestry. First, using various portions of the mtDNA molecule in addition to the control region, a test of the rates of nucleotide substitution in sperm whales relative to other cetaceans was performed. Second, a parsimony analysis of the substitution patterns in the sperm whale control region sequences, facilitated by the apparent monophyly of Southern Hemisphere lineages, was used together with a novel method of calculating divergence times to estimate the time since common mtDNA ancestry. The results showed that the rates of nucleotide substitution in the sperm whale control region are highly variable between sites and that the low diversity is likely to be related to an evolutionarily recent ancestry of the lineages.

Materials and Methods

Population Samples

Tissue samples (skin, liver, and muscle) from 37 sperm whales collected from the following oceanic areas were used: North Atlantic (n = 10), North Pacific (n = 11), Galapagos Islands (n = 5, included in the North Pacific material in the analyses), and the Southwest Pacific/Antarctic (n = 11). Samples were obtained by skin biopsy methods (Lambertsen 1987), by retrieving sloughed skin found in the water behind whales (Amos et al. 1992), and by consulting tissue archives. The samples were chosen such that they would likely be derived from unrelated whales based on either individual identifications and association data or a large separation in the time and locations of sampling events.

Key words: sperm whale, mitochondrial DNA, substitution rate, molecular clock, diversity.

Address for correspondence and reprints: Thomas Lyrlholm, Department of Zoology, Stockholm University, S 10691 Stockholm, Sweden. E-mail: thomas.lyrholm@medgen.uu.se.

© 1996 by the Society for Molecular Biology and Evolution. ISSN: 0737-4038
Table 1
Polymorphic Sites Among 37 Sperm Whale Control Region Sequences

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>58</th>
<th>62</th>
<th>121</th>
<th>184</th>
<th>260</th>
<th>272</th>
<th>288</th>
<th>319</th>
<th>324</th>
<th>574</th>
<th>872</th>
<th>947</th>
<th>Area (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>T</td>
<td>C</td>
<td>C</td>
<td>T</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>G</td>
<td>C</td>
<td>G</td>
<td>A</td>
<td>A</td>
<td>NA (4), NP (7)</td>
</tr>
<tr>
<td>B</td>
<td>T</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>G</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>G</td>
<td>.</td>
<td>NA (3)</td>
</tr>
<tr>
<td>C</td>
<td>T</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>NA (2), NP (4)</td>
</tr>
<tr>
<td>D</td>
<td>.</td>
<td>T</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>NA (1)</td>
</tr>
<tr>
<td>E</td>
<td>T</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>NP (2)</td>
</tr>
<tr>
<td>F</td>
<td>C</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>NP (1)</td>
</tr>
<tr>
<td>G</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>G</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>G</td>
<td>.</td>
<td>NP (1)</td>
</tr>
<tr>
<td>H</td>
<td>T</td>
<td>C</td>
<td>G</td>
<td>G</td>
<td>A</td>
<td>T</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>SH (2)</td>
</tr>
<tr>
<td>I</td>
<td>.</td>
<td>T</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>SH (1)</td>
</tr>
<tr>
<td>J</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>SH (1)</td>
</tr>
<tr>
<td>K</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>SH (6)</td>
</tr>
<tr>
<td>L</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>SH (1)</td>
</tr>
<tr>
<td>M</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>.</td>
<td>SH (1)</td>
</tr>
</tbody>
</table>

Note: — The numbers at the top indicate the sequence positions of the sites and the letters in the left column show the names of the haplotypes defined. Oceanic areas are: NA, North Atlantic; NP, North Pacific; SH, Southern Hemisphere. n is sample size. Matches with reference to the top sequence are indicated by “.,” and indels are indicated by “—.”

Laboratory Procedures

Tissue samples were digested with Proteinase K in 50 mM KCl; 10 mM Tris-HCl, pH 8.3; 1.5 mM MgCl₂; and 10% SDS. The DNA was isolated using standard phenol/chloroform extraction and collected by ethanol precipitation. The oligonucleotide primer pair described by Hoelzel, Hancock, and Dover (1991) was used to PCR amplify (Mullis and Faloona 1987; Saiki et al. 1988) the entire mtDNA control region in a 100-μl reaction containing 50 mM KCl; 10 mM Tris-HCl, pH 8.3; 1.5 mM MgCl₂; 0.45% Nonidet P40; 0.45% Tween 20; 200 μM of each dNTP; 1 μM of each primer; and 2.5 units of Taq polymerase (AmpliTaq, Perkin-Elmer). The PCR cycle consisted of 1 min at 94°C, 1 min at 53°C, and 1 min at 72°C. One of the PCR primers in each reaction was labeled in the 5’ end with biotin, enabling the ssDNA to be captured onto streptavidin-magnetic beads for sequencing (Hultman et al. 1989). The ssDNA PCR fragment was sequenced using the chain termination method (Sanger, Nicklen, and Coulson 1977), employing both the PCR primers and additional primers complementary to internal regions of the sequence. Both strands were sequenced.

Sequence Analyses

Sequence alignments were generated using the PileUp program of the GCG sequence analysis package (Genetics Computer Group 1991). Maximum-parsimony analysis was performed using the computer software PAUP (Swofford 1993), applying branch-and-bound searches with the sequence addition options set alternately to furthest or simple, and with indels included as a character. The computer software MEGA (Kumar, Tamura, and Nei 1993) was used to estimate genetic distances.

Results

mtDNA Diversity

Twelve nucleotide sites out of 954 were variable among the 37 sperm whale control region sequences, identifying 13 mtDNA types (table 1). Eleven of the polymorphisms were single-nucleotide transition substitutions, consistent with the high transition bias reported for mtDNA (Brown 1985), and one single-base-pair insertion/deletion was detected in a stretch of G’s at sequence position 574. The worldwide nucleotide diversity, π (average number of pairwise nucleotide differences per site), and haplotype diversity (Nei 1987, pp. 256, 260) for the whole control region were estimated to be 0.0020 (range 0.001–0.0084, SE = 0.00028 due to haplotype sampling) and 0.86, respectively. Since most of the population studies carried out to date have been confined to roughly 300 bp within the most variable 5’ light chain (5’L) part of the control region, we also estimated diversity for the corresponding part in the sperm whale. The π for the 5’L 300 bp was 0.0038 (range 0.0033–0.017, SE = 0.00059) and the haplotype diversity was 0.74. The nucleotide diversity estimate for sperm whales is considerably lower than most of those reported for the control regions of other mammals (examples in fig. 1). A lower diversity is not a general feature of cetaceans, since the estimate of π for humpback whales, Megaptera novaeangliae, over their worldwide range is roughly six-fold higher than for sperm whales (Baker et al. 1993), and that for the harbor porpoise, Phocoena phocoena, is about seven-fold higher (Rosel, Dizon, and Haygood 1995). A study on killer whales, Orcinus orca, from the Pacific and Atlantic Oceans also indicated low control region diversity (Hoelzel, Hancock, and Dover 1991), but, due to the limited sampling strategy, the comparison might not be appropriate. Harbor seals, Phoca vitulina, have a π roughly four times higher than that of sperm whales (Stanley et al. 1996). The π for the southern elephant seal, Mirounga leonina, population at South Georgia (SGI) is about five-fold higher than that for sperm whales, whereas populations of the same species in Argentina (PVA) and of the northern elephant seal, M. angustirostris (NES), have a diversity roughly equal to that of the sperm whale (Hoelzel et al. 1993). Regarding hap-
lophotype diversity, the estimate for sperm whales (for the 5'L 300 bp) was most similar to those of the PVA elephant seal (0.70) and the harbor seal (0.78). The northern elephant seal had substantially lower diversity (0.41, only two haplotypes present) and the SGI seal population, as well as humpback whales and harbor porpoises, had higher diversities (0.98, 0.94, and 0.94, respectively, i.e., almost every individual unique).

Relative Rate Test

In order to determine whether the low π in sperm whales could be attributed to a lower mtDNA substitution rate compared to that of other cetaceans, a test of the relative rates of nucleotide substitution (Wu and Li 1985) was performed. No indications of a lower substitution rate in the sperm whale were found for any of the analyzed regions (the control region, cytochrome b, 12sRNA, and 16sRNA) (table 2). Since transitions are likely to have saturated at rapidly evolving sites, the most informative comparisons might be those excluding transitions (i.e., B13 - B23) and, for cytochrome b, those excluding third codon positions (Irwin, Kocher, and Wilson 1991). A significant rate variation is suggested for the control region only, where the sperm whale seems

![Graph showing control region diversity percentage for some marine mammal species.](image)

**Figure 1.**—Control region diversity (%) for some marine mammal species. Sperm 954 = sperm whale, entire control region; sperm 300 = sperm whale, the first 5'L 300 bp. Abbreviations and data sources for other species are: humpback whale (Baker et al. 1993); porpoise = harbor porpoise (Rosel, Dizon, and Haygood 1995); orca = killer whale (Hoelzel and Dover 1991); H. SEAL = harbour seal (Stanley et al. 1996); N. E.SEAL = northern elephant seal; PVA E.SEAL = southern elephant seal, Peninsula Valdez population; and SGI E.SEAL = southern elephant seal, South Georgia population (Hoelzel et al. 1993).

### Table 2

Differences in the Number of Substitutions per Site in mtDNA Between Sperm Whales and Other Cetaceans

<table>
<thead>
<tr>
<th>Sequences</th>
<th>Control Region</th>
<th>Cytochrome b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sperm - humpback</td>
<td>0.25* ± 0.04</td>
<td>0.12* ± 0.03</td>
</tr>
<tr>
<td>Sperm - fin</td>
<td>0.27* ± 0.04</td>
<td>0.12* ± 0.03</td>
</tr>
<tr>
<td>Sperm - orca</td>
<td>0.23* ± 0.04</td>
<td>0.07* ± 0.03</td>
</tr>
<tr>
<td>Sperm - spotted dolphin</td>
<td>— —</td>
<td>— —</td>
</tr>
<tr>
<td>Sperm - harbor porpoise</td>
<td>— —</td>
<td>— —</td>
</tr>
<tr>
<td>Sperm - common dolphin</td>
<td>— —</td>
<td>— —</td>
</tr>
</tbody>
</table>

**Note.**—The relative rate test was done following Wu and Li (1985). Data are from this study and Anderson et al. (1982), Árnason, Gullberg, and Widegren (1991), Hoelzel, Hancock, and Dover (1991), Irwin, Kocher, and Wilson (1991), Milinkovitch, Ortí, and Meyer (1993), and Árnason and Gullberg (1994, 1996). K13 - K23 and B13 - B23 are the number of total and transversion substitutions per site, respectively, between species 1 (the sperm whale) and species 2 (the other cetacean).
to have evolved faster, rather than slower, compared to the other whales.

Population Structure

The nucleotide diversity was similar for the different oceans (North Atlantic [NA], $\pi_{NA} = 0.0018 \pm 0.00032$; North Pacific [NP], $\pi_{NP} = 0.0018 \pm 0.00068$; and Southern Hemisphere [SH], $\pi_{SH} = 0.0016 \pm 0.00062$), and not significantly different from the total diversity $\pi = 0.0020 \pm 0.00028$. An analysis of haplotype frequencies indicated the presence of genetic structure. The most common haplotypes (A and C) were shared between the NA and NP while the remaining haplotypes were ocean-specific (table 1). Using the procedure of Nei and Chesser (1983), we estimated $G_{ST}$ (Nei's measure of population differentiation) to be 0.13. Pooling all but the most common haplotype (to avoid small expectations) yielded significant haplotype frequency differences between the three oceans ($\chi^2 = 6.78$, df = 2, $P < 0.05$). Finally, the deletion occurred in all Southern Hemisphere but in no Northern Hemisphere lineages, a highly significant difference in distribution (Fisher’s exact test, df = 1, $P = 1.2 \times 10^{-9}$). We can thus conclude that the populations are structured on an ocean and hemisphere scale.

Substitution Processes

The pattern of nucleotide substitution was studied by a cladistic analysis largely following the approach of Tamura and Nei (1993) and Wakeley (1995). This method is based on the reconstruction of a phylogeny of the sequences and the use of parsimony to estimate the number and nature of inferred substitutions at each site. A parsimony phylogeny of the sequences in table 1 was obtained with the constraining assumption that the Southern Hemisphere lineages had a monophyletic origin. The rationale for this contraint was (1) the observation that the deletion at nucleotide position 574 occurred in all SH lineages but only there and (2) the possibility that indels are less likely to occur than are nucleotide substitutions (as suggested by the much lower frequency of the former in the data), and should therefore be given higher weight. Fifteen equally parsimonious trees were obtained, each requiring 18 nucleotide substitutions and one indel (defining the SH/NH dichotomy) (fig. 2a). If substitutions occur at random, we expect only a small overlap of substitution sites and nature between the clade with and the clade without the deletion. Of the seven phylogenetically informative sites (excluding the indel), six (85%) showed parallel substitutions in the two groups, and the homoplasgy index (HI) was 0.467 (estimated in PAUP by HI = $1 - CI$, where CI is the consistency index [Swofford 1993]). Thus, most of the inferred nucleotide substitutions in the sperm whale control region were confined to a few substitution “hot spots” and restricted in nature. A parsimony analysis without any assumption of monophyly of the SH lineages (and thus giving equal weight to the indel and substitutions) was also performed. This resulted in six equally parsimonious trees which also showed considerable homoplasy (HI = 0.333) and, thus, several multiple substitutions (including multiple indel events) (fig. 2b).

Next, we investigated the fit of the inferred number of substitutions per site to a Poisson distribution. As is evident from table 3, which shows the results from the constrained parsimony analysis, the fit to a Poisson distribution was poor. In humans, the pattern of nucleotide substitution in the mtDNA control region has been shown to fit a negative binomial distribution better than a Poisson distribution (Kocher and Wilson 1991; Tamura and Nei 1993; Wakeley 1993). The number of substitutions will follow a negative binomial distribution when the substitution rate, $\lambda$, varies among sites according to a gamma distribution (Tamura and Nei 1993). The shape of the distribution is commonly described by the parameter $a = \lambda^2/\lambda(\lambda)$, where $\lambda$ and $\lambda(\lambda)$ are the mean and variance over sites of the rate of substitution, respectively (the Poisson distribution corresponds to $V(\lambda) = 0$, i.e., $a = \infty$). We used the maximum-likelihood method to estimate and fit a negative binomial distribution to the data and obtained a much better fit than to a Poisson distribution (table 3). The parameter $a$ was estimated with the maximum-likelihood method at 0.013 ± 0.007, using data from the entire sperm whale control region. Restricting the analysis to the first 5'L 300 bp yielded $a = 0.024 \pm 0.016$. If the unconstrained parsimony analysis is used, $a$ for the whole control region becomes 0.024 ± 0.015. For human sequences, Tamura

Table 2

<table>
<thead>
<tr>
<th>Sequences</th>
<th>12sRNA</th>
<th>16sRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$K_{13} - K_{23}$</td>
<td>$B_{13} - B_{23}$</td>
</tr>
<tr>
<td>Sperm-humpback</td>
<td>$-0.012 \pm 0.016$</td>
<td>$0.009 \pm 0.007$</td>
</tr>
<tr>
<td>Sperm-fin</td>
<td>$-0.029 \pm 0.017$</td>
<td>$-0.003 \pm 0.009$</td>
</tr>
<tr>
<td>Sperm-orca</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sperm-spotted dolphin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sperm-harbor porpoise</td>
<td>$-0.005 \pm 0.019$</td>
<td>$-0.007 \pm 0.010$</td>
</tr>
<tr>
<td>Sperm-common dolphin</td>
<td>$-0.004 \pm 0.020$</td>
<td>$-0.009 \pm 0.009$</td>
</tr>
</tbody>
</table>

* The values for cytochrome $b$ indicated by "*" are based on all codon positions, and those indicated by "t" are based on first and second positions only.
FIG. 2.—a. Maximum-parsimony tree of the 13 sperm whale haplotypes shown in table 1. This was one of 15 trees all showing 18 nucleotide substitutions which were obtained in branch-and-bound runs in PAUP by constraining the Southern Hemisphere types I, J, K, L, and M to be monophyletic. As a consequence, the deletion at position 574 (see arrow) thus had a higher weight than substitutions and occurred only once. The characters shown on the tree are numbered as in table 1. The homoplasy index, excluding noninformative characters, was 0.467. b. One of the six equally parsimonious trees each requiring 16 character changes, which resulted from an analysis similar to that in a, except without any topological constraints. Homoplasy index, excluding noninformative characters, was 0.333. None of the branches were supported in bootstrap analyses or in a strict consensus tree.

Table 3
Observed and Expected Distributions of the Number of Nucleotide Substitutions per Site in the Control Region of Sperm Whales

<table>
<thead>
<tr>
<th>SEQUENCE PORTION</th>
<th>NO. OF SUBSTITUTIONS PER SITE</th>
<th>NO. OF SITES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Observed</td>
<td>Poisson</td>
</tr>
<tr>
<td>954 bp. . . .</td>
<td>0 942</td>
<td>935.2</td>
</tr>
<tr>
<td></td>
<td>1 5</td>
<td>17.7</td>
</tr>
<tr>
<td></td>
<td>2 5</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>3 1</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>≥4 0</td>
<td>0</td>
</tr>
<tr>
<td>300 bp. . . .</td>
<td>0 293</td>
<td>288.2</td>
</tr>
<tr>
<td></td>
<td>1 3</td>
<td>11.5</td>
</tr>
<tr>
<td></td>
<td>2 3</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>3 1</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>≥4 0</td>
<td>0</td>
</tr>
</tbody>
</table>

Note.—The observed number of substitutions per site was estimated by parsimony analysis and the expectations under negative binomial distribution were obtained by maximum likelihood.

Since the substitutions observed in the sperm whale lineages were all transitions, we needed an estimate of the number of transitional substitutions per unit time in the control region of cetaceans. However, the transition rate cannot be accurately estimated from interspecific comparisons due to the saturation effect mentioned above, which could be particularly prominent in the control region judging by the transition “hot spots” detected in the sperm whale (this saturation effect has recently been illustrated in analyses of the human control region sequences [Wills 1995]). Therefore, we introduced a method in which we first estimated only the transversion rate from interspecific comparisons and then converted this to a transition rate using a separate estimate of the transition/transversion ratio, R. Thus, if T is the divergence time between species, v is the number of transversions per site between species, and s is the number of transitions per site along an average sperm whale lineage, the time t to common ancestry of the lineages will be:

\[ t = \frac{s^2T}{vR} \]

To estimate the transversion rate, v/2T, we need to compare sequences for which divergence times are available. We compared the control regions of the mysticetes blue whale and fin whale (Balaenopteridae, data from Arnason, Gullberg, and Widegren 1993) and the odontocetes killer whale and Commerson’s dolphin (Delphinidae, data from Hoelzel, Hancock, and Dover 1991 and Southern, Southern, and Dizon 1988, respectively). The former species pair diverged about 5–7.5 MYA (Arnason and Gullberg 1993) and the latter diverged about 5–10 MYA (Gaskin 1982; Barnes, Domming, and Ray 1985). The number of transversions per site, v, was estimated as 0.0209 (±0.0060) and 0.0182 (±0.0046) for the blue–fin whale and dolphin–killer whale comparisons, respectively, using the Tamura and Nei (1993) substitution model (which takes into account varying base frequencies), with no correction for variation of substi-
tution rates between sites. Although such rate variation may be present, we have no data to estimate, e.g., the parameter \( a \) for transversions in the cetacean control region, and there is no a priori reason to expect it to be the same as that for transitions. Rate variation among sites will tend to increase the estimate of \( v \). It is noteworthy, however, that in a study of the human control region Wills (1995) found no evidence of substitution rate variation among sites for transversions.

Lacking any transversions between the sperm whale sequences, we needed further data from other species to estimate the transition/transversion ratio, \( R \), in the cetacean control region. We assume here that \( R \) is the same among whales. The cladistic method that we used to estimate the number and types of substitutions for the sperm whale data was applied to an intraspecific phylogenetic tree of humpback whales (Baker et al. 1993), which led to an estimate of 61 transitions and 1 transversion. These were added to the 18 sperm whale transitions, leading to \( R = 79 \). Due to the low number of transversions observed, the proportion of transversions, \( p = 1/80 \), has a wide 95% confidence interval, \( 0.001 < p < 0.067 \) (based on the binomial distribution), corresponding to \( 15 < R < 1,000 \). The average number of transitions from root to terminal taxa over all sperm whale lineages was estimated from the tree in figure 2a to be 1.1 (by rooting at the indel). With 954 sites, \( s \) becomes 0.00115. The estimated time to common ancestry of the sperm whale mtDNA lineages based on the blue-fin comparison and \( T = 5 \) will thus be \( t = 0.00115 \times 5 \times 2/(0.002097) = 0.006965 \) Myr, i.e., about 7,000 years. If the result of the dolphin–killer whale comparison and the divergence time 10 Myr are used, \( t = 0.00115 \times 10/0.018279 = 0.0160 \) Myr, i.e., 16,000 years. These estimates will be somewhat affected whether the sperm whale phylogenetic tree was constrained or not. The unconstrained tree in figure 2b had an average of 0.99 substitutions over all lineages, led to \( R = 77 \), and resulted in coalescence times of about 6,400 and 14,800 years for the blue–fin and dolphin–killer whale calculations, respectively. If this tree is rooted at the midpoint of the longest branches, the average number of substitutions becomes 1.7 and the coalescence times become 11,000 and 25,000 years. Thus, whether we based our calculations on the assumption of SH monophyly or not does not make a major difference in the estimates of the age of the mtDNA diversity. The largest source of error will be the wide 95% confidence interval of the proportion of transversions, which, e.g., translates into a range of \( t \) of 550–37,000 years and 1,300–85,000 years for the 7,000- and 16,000-year estimates, respectively.

**Discussion**

The most striking results of the present study were the unusually low level of intraspecific mtDNA diversity in sperm whales and the highly biased substitution pattern, with most substitutions occurring as repeated transitions at a few “hot spots.” Possible reasons for the low diversity are a lower substitution rate than in other species or events in the demographic history of sperm whales. The control region is regarded as the fast-evolving part of the mitochondrial genome (Brown 1985), although there are also suggestions of a substitution rate similar to that of other parts of the molecule, e.g., a slower rate of about 0.5%–1% per Myr was estimated for cetaceans (Hoelzel and Dover 1991; Baker et al. 1993). However, the latter estimates are unreliable since they do not adequately take into account the variation of substitution rate among sites and saturation effects. A lower substitution rate in the nuclear DNA of cetaceans compared to other mammals has been suggested (Schlötterer, Amos, and Tantz 1991), as well as in nuclear satellite DNA of sperm whales compared to other cetaceans (Grétarsdóttir and Arnason 1993).

In order to explain the much lower diversity in sperm whales compared to, e.g., humpback whales and harbor porpoises, the substitution rate in sperm whales would have to be about six to seven times lower. The relative rate test provided no indication of a lower substitution rate in the mtDNA of the sperm whale (table 2). This test is independent of paleontological dating, but should nevertheless be interpreted with caution since it rests on the assumption that the substitutions follow a Poisson distribution. We have shown that this assumption does not hold for the sperm whale control region, where a negative binomial distribution provides a better fit to the data, similar to the situation in humans (Kocher and Wilson 1991; Tamura and Nei 1993; Wakeley 1993), but we do not know whether this would also be the case in the other parts of mtDNA. However, it is unlikely that the substitution model used would have a major effect on the relative rates between species, although the estimated absolute number of substitutions would be altered. Another complication is that the control region appears to evolve also by large deletions, rearrangements, and slippage (Hoelzel, Hancock, and Dover 1991; Saccone, Pesole, and Sbisa 1991), such that sudden changes could create episodes of different rates of evolution, which is not taken into account in the tests applied. Nevertheless, the lack of a significant deceleration in substitution rate in the sperm whale in any of the comparisons argues against a rate slowdown as the main reason for the low diversity. Also, in the control region, a faster rather than slower rate was suggested by the relative rate test (table 2), consistent with the divergent nature of the sperm whale sequence when compared to those of other cetaceans (Arnason, Gullberg, and Widegren 1993; Dillon and Wright 1993; unpublished data).

An alternative explanation for the low diversity is that sperm whales have experienced one or more bottlenecks in population size. The estimate of nucleotide diversity in the sperm whale is similar to that of the northern elephant seal, which was reduced to very few individuals by hunting in the 19th century, eliminating most of the genetic variability (Bonnel and Selander 1974; Hoelzel et al. 1993). A bottleneck or a very small founding population is also suggested for the PVA population of southern elephant seals by mtDNA genetic data (Hoelzel et al. 1993). By contrast, the elephant seals of
South Georgia did not get as heavily decimated, in particular not the females (McCann 1985).

Our point estimates of the time since common mtDNA ancestry in the sperm whale, roughly within 6,000–25,000 years ago, suggests that if a bottleneck occurred in the sperm whale it could have coincided with the late Pleistocene glaciations. Perhaps ocean cooling affected sperm whales adversely. Episodes of ocean cooling and warming seem to have influenced the distribution and abundance of many cetacean species (Gaskin 1982) and such events could have led to a restricted distribution of the sperm whale, particularly of females and young, which normally do not range into cold waters (Best 1979, pp. 208–247), and food availability may have been seriously diminished.

The constrained and unconstrained sperm whale phylogenies resulted in somewhat different estimates of coalescence time and, in our view, given the geographic distribution of the indel, the Southern Hemisphere monophyly assumption of the constrained tree may represent the most plausible scenario. In any case, the differences were minor and the uncertainty in these estimates derives mainly from imprecision in the transition/transversion ratio, \( R \). When this is taken into account, even the upper limit of the ranges suggests a coalescence time of no more than roughly 100,000 years. Is our estimate of \( R \) reasonable? In a study of the human control region diversity, Wills (1995) calculated a theoretical average of \( R = 43 \), while the best empirical estimate (based on the most recent divergences) led to \( R = 64.6 \), suggesting that our estimate of 79 is not unreasonably high.

It is evident that the sperm whale mtDNA diversity is quite young in evolutionary terms and in relation to the estimated age of the oldest sperm whale fossils of about 20 Myr (Barnes, Domming, and Ray 1985). Due to the process of lineage sorting, i.e., random lineage extinction caused by demographic stochasticity, lineages are not expected to trace as far back as the history of the species (Avise, Neigel, and Arnold 1984). However, it seems unlikely that lineage sorting would be sufficient to remove almost all variation, as seen here, unless the populations had experienced dramatic declines. Demographic factors such as long generation time and variance in the number of daughters produced are likely to reduce the female effective population sizes in sperm whales. Also, field studies have suggested that females live in stable social groups (Whitehead, Waters, and Lyrholm 1991) and that they may show fidelity to local areas (Gordon 1987; Whitehead, Waters, and Lyrholm 1992). Consequently, it seems likely that local extinction of lineages and lineage sorting could be accelerated during population declines. Thus, a combination of factors may have been responsible for the low mtDNA diversity in sperm whales. It may be fairly common even among currently very abundant vertebrates that mtDNA lineages have been channeled through relatively small numbers of ancestors by demographic events (Avise, Ball, and Arnold 1988). It is also possible that a selective sweep could have occurred within our estimated coalescence time. It is not necessary for this selection to have acted on the control region directly, since selection anywhere in the mtDNA will affect diversity in the rest of the molecule due to linkage (the "hitchhiking" effect) (Gillespie 1991, pp. 286–287).

The comparison between humans and sperm whales revealed large differences in the estimates of gamma parameter \( \alpha \). The gamma distribution may not be the most appropriate model to describe the substitution rate variation. It is also possible that the estimate of \( \alpha \) varies through time, depending on whether mostly fast- or slow-evolving sites dominate the data. Since the sperm whale lineages appear to be quite young, we examined the substitution process at an early stage of sequence divergence, which may explain the lower \( \alpha \). Our analysis using the deletion specific to the Southern Hemisphere lineages as a natural division of the phylogeny revealed more homoplasy, and thus higher substitution bias, than when the phylogeny was unconstrained. If the diversities in each of the oceanic regions have evolved independently from one lineage that disperses after a putative bottleneck, the homoplasy is even greater. Such a phylogeny required only two more nucleotide substitutions than the tree in figure 2a, at sites that already showed multiple substitutions. Also, since parsimony analysis minimizes the number of character changes, a negative bias in the estimated number of substitutions is expected (Wakeley 1993). In any case, our analyses revealed much more bias in the pattern of nucleotide substitution in the mtDNA control region that has previously been shown.

Acknowledgments

For access to samples and sequences or for the opportunity to collect field samples we are grateful to ÚArnason, J. Gordon (IFAW), H. Kato, Roger Payne (Whale Conservation Institute), Per Palsboll, T. Simila O. Vásquez, S. Wada, Dolphin Research Center, and the Museums of Natural History in Copenhagen and Gothenburg. For cooperation in the field we thank the crew of the vessels "Siben" and "Song of the Whale" and personnel at Hvalsenter, Andenes. The collaboration of the Charles Darwin Research Station and the Galápagos National Parks Service is gratefully acknowledged. For help and advice in the laboratory we thank T. Bergström, R. Hoelzel, and J. Stewart. Funding was provided by WWF Denmark, Nordiskt Kollegium for Marin Biologi, the Swedish Galápagos Foundation, Royal Swedish Academy of Science, the Salén Foundation, the Swedish Natural Sciences Research Council, the Beijer Foundation, P. Singh, and G. Williams. We thank Dr. Richard Harrison and two anonymous reviewers for their constructive comments on the manuscript.

Literature Cited


RICHARD G. HARRISON, reviewing editor

Accepted August 13, 1996