Genetic differences between western and eastern gray whales (*Eschrichtius robustus*)

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ABSTRACT

Molecular data were used to examine the differentiation between the western and eastern gray whale (*Eschrichtius robustus*) populations. Control region sequences were generated from samples collected in the western Pacific ($n = 45$) and eastern Pacific ($n = 120$). There were 36 unique haplotypes identified. Ten haplotypes were represented in the western samples, and 33 in the eastern samples. Seven of these haplotypes were shared between populations, leaving three haplotypes that were only seen in the western samples and 26 only in the eastern. Although there were no fixed (diagnostic) differences between the western and eastern groups, they were significantly different in their haplotype frequency distributions and should be considered as separate populations. None of the 33 haplotypes found in the eastern samples had a frequency of over 11%, yielding an estimated haplotypic diversity of 0.95. This finding indicates that the reduction in abundance due to whaling may not have had a great effect on the haplotypic diversity of the eastern population, although the loss of rare haplotypes may still have occurred and would be difficult to detect. In contrast, the western group was dominated by two haplotypes, which represented over 77% of all individuals sampled, resulting in a substantially lower haplotypic diversity of 0.70. The lack of fixed differences between the two populations and frequency of shared haplotypes renders these data inappropriate for forensic applications at the population level.

KEYWORDS: GRAY WHALE; GENETICS; POPULATIONS; CONSERVATION; NORTH PACIFIC

INTRODUCTION

Although the gray whale (*Eschrichtius robustus*) once occurred in both the North Atlantic and North Pacific, it became extinct in the Atlantic several hundred years ago (Mead and Mitchell, 1984), is severely depleted in the western Pacific (e.g. Weller *et al.*, 2002), and was greatly reduced in the eastern Pacific before its recovery (IWC, 1998). Currently, gray whales are considered as two separate management stocks living along the eastern and western boundaries of the North Pacific. While both were reduced by historical whaling, only the eastern gray whale has recovered to near pre-exploitation levels (IWC, 1998). The western gray whale was thought to be extinct as recently as the early 1970s (Bowen, 1974) but is known to survive today as a remnant population (see review in Weller *et al.*, 2002). Although studies of the behaviour and biology of both eastern and western gray whales have been conducted (see Swartz *et al.*, 2000 for review), questions about the level of genetic differentiation between eastern and western gray whales, or how their exploitation may have affected genetic diversity, have remained largely unaddressed. Contemporary gene flow between them is not likely in that the geographic distributions do not overlap, and the migratory routes are disjunct and lead to opposite sides of the North Pacific basin. However, the possibility of dispersal has yet to be tested with genetic data. If gene flow is negligible or non-existent and the stocks have differentiated genetically since becoming allopatric, an additional question is whether they have diverged enough to allow individual whales from unknown localities (e.g. market samples of meat) to be characterised as eastern or western. As part of an ongoing US-Russia research project studying western gray whales in the Okhotsk Sea, biopsy samples have been routinely taken from animals summering off Sakhalin Island, Russia (Weller *et al.*, 2002). In addition, many samples are available from the eastern gray whale population. Together, these datasets provide an opportunity to characterise the genetic makeup of eastern and western gray whales and to quantify their degree of differentiation.

MATERIALS AND METHODS

Samples from the western population were obtained as biopsies from free-ranging animals on their summer feeding grounds off the northeastern coast of Sakhalin Island, Russia, primarily during 1998 and 1999. Since the biopsied animals were photographed at the time of sampling, cross-matching with the photo-identification catalogue (Weller *et al.*, 1999) enabled the removal of duplicate samples prior to sequencing, giving a total of 42 samples. Three biopsy samples from the same study area were collected in 1995 (Brownell *et al.*, 1997). In the absence of identification photographs, these were only added to the western samples after microsatellite analysis (not described) confirmed they were not from individuals sampled in 1998-1999. This resulted in a total of 45 western samples. A total of 120 eastern samples were collected from many localities between southern California and the Chukotka Peninsula in Russia. These samples were taken primarily from strandings, as well as a few from directed subsistence takes, fishery bycatch and biopsies of living whales. A similar check of individual identity was not done for the eastern North Pacific samples due to the lack of a comprehensive photo-identification catalogue. However, given that over 90% of the eastern samples were collected from dead animals, and given an estimated population size of over 26,000 (Rugh *et al.*, 1999), the effect of any possible duplicate sampling is negligible.

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In addition to these samples, sequences of a 361 base pair (bp) segment of the mitochondrial control region from two gray whales that were sampled in Japan (M. Goto and L. Pastene, pers. comm.) are used here in our discussion of the gray whale market samples sequenced by Baker *et al.* (2002). One whale stranded on the eastern side of Hokkaido in 1995 (Anon., 1997), and the other was an animal harpooned in the Sea of Japan off western Hokkaido in 1996 (Brownell and Kasuya, 1999). These sequences were not used in the population genetic analyses.

Using standard protocols, DNA was extracted from each sample, and a 523 bp region of the 5' end of the mitochondrial control region was amplified and sequenced. The primers used for amplification and sequencing were 5'-TACCAAATGTATGAAACCTCAG-3' (Rosel *et al.*, 1995) and 5'-CCTCCCTAAGACTCAAGGAAG-3' (designed at SWFSC). Haplotypic diversity was calculated using the computer program Arlequin (Schneider *et al.*, 2000), which was also used to calculate the divergence between populations with F_{ST}, ϕ_{ST} (an F_{ST} analogue) and χ^2, as well as to create a minimum spanning tree based on the number of differences between haplotypes. Haplotypic diversity (h) is calculated by the formula $h = 1 - \Sigma p_I^2$, where p_I is the frequency of the I^{th} haplotype (Nei, 1987).

RESULTS

A total of 36 haplotypes defined by 37 variable sites were found among the 165 samples examined. Thirty-three of these haplotypes occurred in eastern samples and 10 in the western samples; seven haplotypes were shared between the two samples. Fig. 1 shows the minimum spanning network of the 36 haplotypes. Fig. 2 shows the frequencies of the different haplotypes in the eastern and western samples. Haplotypic diversity differed greatly with the eastern samples showing a diversity of 0.95 +/- 0.01 and the western samples having a value of 0.70 +/- 0.05. The average percent difference (i.e. nucleotide diversity) between individuals differed little, with the eastern samples averaging 1.6% sequence difference from each other and the western samples averaging 1.7%. The average percent difference for between-population pairwise comparisons was 1.9%. In genetic studies, one must be cautious that some results, such as the much lower level of diversity found in the western gray whale population, are not caused by inadequate or biased sampling. However, it is doubtful that this is the case here. The 120 eastern samples actually represent a much lower overall proportion of the eastern gray whale population than the 45 samples do from the western population, which may number less than 100 animals (e.g. see Weller *et al.*, 2002). Therefore, the probability of there being appreciable amounts of unsampled variation in the western population is relatively low, despite the smaller number of samples. Finally, examination of the degree of genetic sub-division between the eastern and western samples indicated that they are significantly different from each other ($\phi_{ST} = 0.117$, $p < 0.001$; $\chi^2 = 65.9$, $p < 0.001$; $F_{st} = 0.087$, $p < 0.001$).

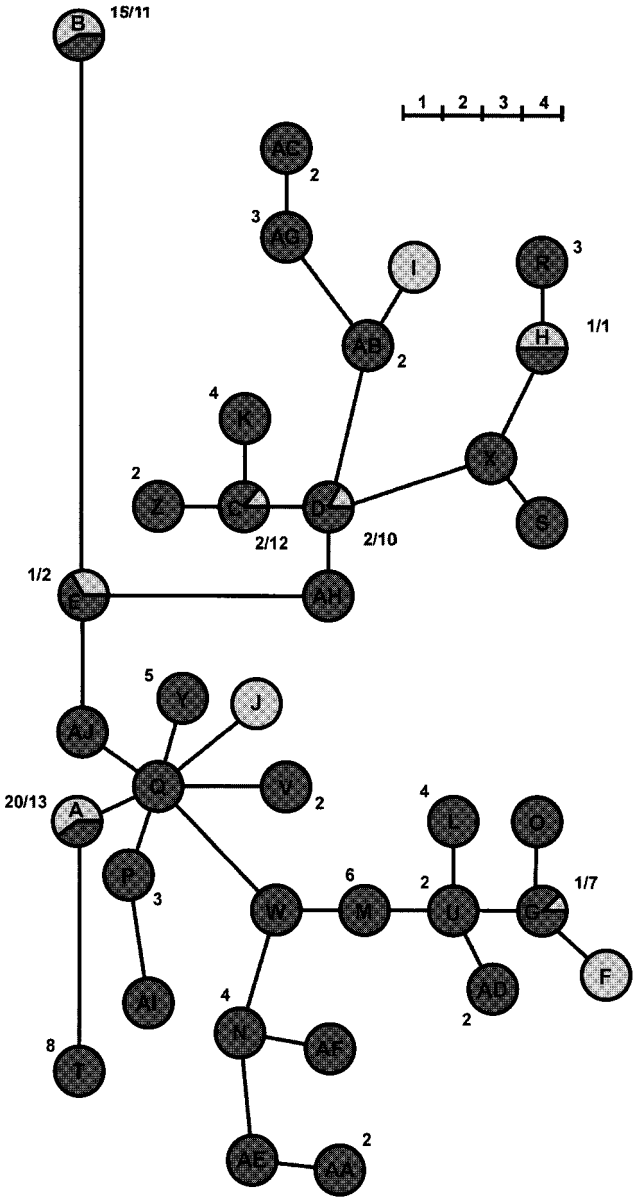

Fig. 1. Minimum spanning tree of the 36 haplotypes from this study. Numbers beside circles indicate the number of individuals having that haplotype in western (light gray) and eastern (dark gray) sample sets. Circles without numbers indicate haplotypes only represented by single individuals. The scale gives number of changes along connecting branches.

DISCUSSION

The results presented here show that the eastern and western gray whales are genetically differentiated at the population level. The significant difference found between the two populations and the negligible levels of gene flow that it implies, agrees well with their very different recovery histories; dispersal that is significant in any management sense should not be expected to occur. However, the statistical population differentiation arises primarily from differences in haplotypic frequencies (Fig. 2) and reflected in their respective haplotypic diversity indices. The populations have apparently not been isolated for a sufficiently long period of time for the shared haplotypes to be removed via genetic drift, and therefore no diagnostic character or characters within the 523 bp region can be reliably used to distinguish one population from another, or to determine the source of a gray whale of unknown affinity (e.g. a forensic analysis of market meat). The case could be made that if a test animal has a haplotype unique to the eastern samples, then it probably arose from there, since the absence of that haplotype in the western population is based on a fairly thorough sampling scheme (perhaps 50% of the population sampled so far). However, the converse (a test animal having a haplotype unique to the western sample set being from the Okhotsk Sea population) is more difficult to

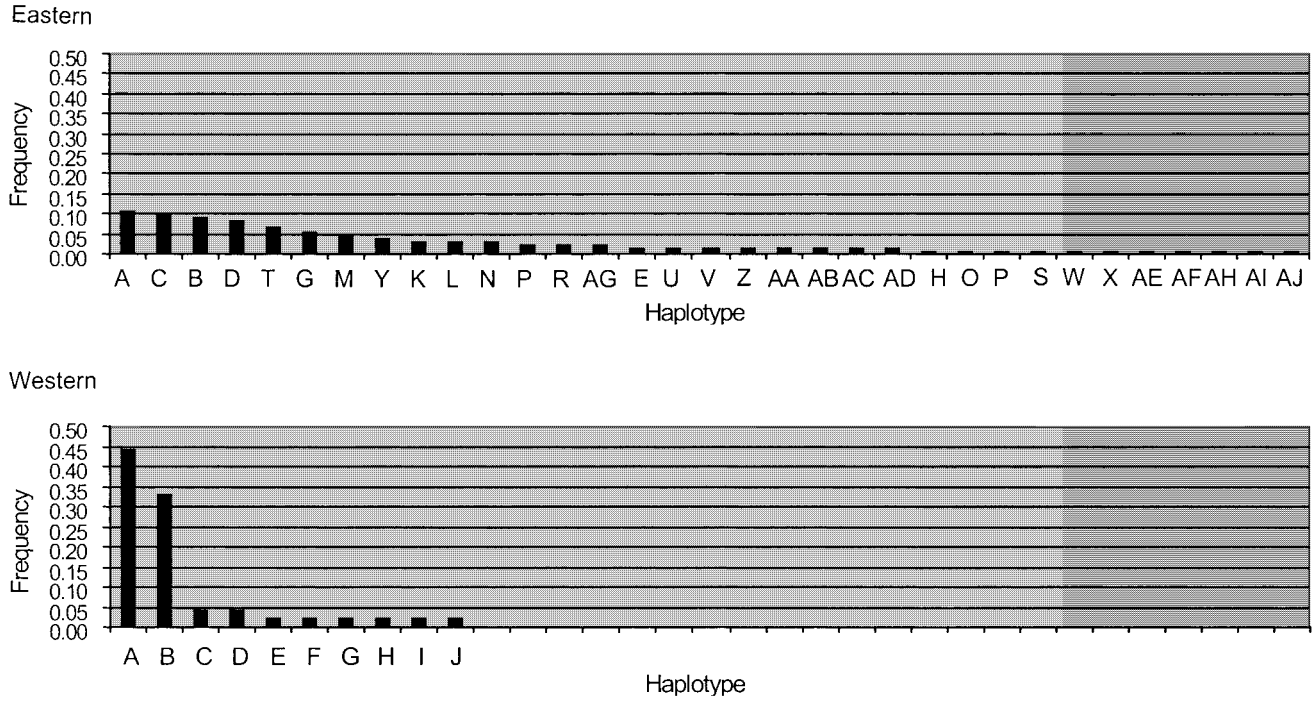

Fig. 2. Frequency distributions of haplotypes in each of the gray whale populations.

argue, since the very high diversity in the eastern population allows for the presence of many unsampled haplotypes. Although the genetic differences between the populations are modal rather than absolute, the differentiation is large, and demographically they should be treated as isolated population units, especially for management purposes as they have been and are by the International Whaling Commission (e.g. IWC, 1998).

The differences between the two populations in their haplotypic diversities may reflect differences in their past levels of abundance and effects of exploitation. Haplotypic diversity (*h*) is affected by both the numbers of haplotypes present in the population and their relative frequencies, with numerous haplotypes and equal frequencies both serving to increase the value of *h*. In the present dataset, the differing values of *h* arise primarily from the differences in haplotype frequency distributions, which differed dramatically between the two populations (Fig. 2). In the eastern samples, the most common haplotype was only represented in 10.8% of the individuals, and seven of the 33 haplotypes were represented in over 5% of the samples. The overall effect is one of a fairly even frequency distribution of haplotypes. In contrast, the two most common haplotypes in the 45 western samples were represented by 20 (44.4%) and 15 individuals (33.3%), with the remaining eight haplotypes appearing in single animals or in pairs. If the 10 western haplotypes were evenly distributed in the sample set, h_{west} would increase from 0.70-0.90, but an equivalent redistribution of the 33 eastern haplotypes would only increase h_{east} from 0.95-0.97. In other words, given the differences in sample sizes, the western and eastern sample sets contained comparable numbers of haplotypes, albeit with very different frequency distributions.

The haplotypic diversity value (0.95) calculated for the eastern samples in the present dataset is similar to the value (0.94) found by Steeves *et al.* (2001) in their study of 57 samples of eastern gray whales. These relatively high values for contemporary eastern gray whales seem to indicate that there was little loss of genetic variation in this population due to historical whaling. However, it is possible that some loss of diversity due to whaling may have occurred without a reduction in haplotypic diversity. In the calculation of haplotypic diversity, the frequencies of haplotypes are squared, so that the resulting value of *h* is not greatly affected by the occurrence or number of rare haplotypes, those contained in the population in very low frequencies. Therefore, although the eastern sample set contained 11 haplotypes only represented by single samples, the loss of other rare haplotypes cannot be ruled out.

In the western population, the lower haplotypic diversity value may be, but is not necessarily, a result of whaling. The lower value for the western animals may be related to their history of overexploitation, but it is also consistent with a smaller long-term effective population size (N_e). The amount of diversity that a population can maintain is directly determined by its N_e, which for the haplotypic and uniparentally-inherited mitochondrial genes, is approximately one-quarter of the N_e of nuclear genes. Although there are no reliable estimates for the pre-exploitation size of the western gray whale population, it was very likely to have been smaller than the eastern population (Weller *et al.*, 2002). Furthermore, the ten haplotypes found in the western sample set are not closely related to each other. Indeed, the two dominant western haplotypes (A and B) are very different from each other (Fig. 1). In a statistical sense, the occurrence of relatively few, but quite divergent, haplotypes explains why the western population exhibits an equal level of average sequence divergence when compared to the eastern population, despite its lower haplotypic diversity. Biologically, this pattern is consistent with either a loss of haplotypes due to long-term genetic drift or a whaling-induced bottleneck.

Although the haplotypic diversity is lower in the western population, the fact that 10 haplotypes still remain in a population this small is encouraging. It may indicate that a considerable amount of variation is still contained within the gene pool. In comparison, only five haplotypes have been observed in the western North Atlantic population of right whales, currently estimated at approximately 300 individuals (Malik *et al.*, 2000). However, the retention of 10

haplotypes in the western gray whale population has some relevance to another important issue, namely whether or not the western population is recovering. Weller *et al.* (2002) estimated that less than 50 of the western gray whales are mature, and that the current sex ratio of this population is approximately 60% male:40% female. This translates into an estimate of approximately 19 reproductive females; probably even less according to Weller *et al.* (2002). The recovering eastern population has been estimated to have had a maximum growth rate of 3.3% per year (for the interval 1967/68 to 1987/88), even higher if the aboriginal take of approximately 180/yr was taken into account (IWC, 1998). Applying a 3.3% recovery rate, and assuming that there are 19 reproductive females today in the western population, there would have been only about six reproductive females in the western population when whaling ended in 1966. That is an extremely unlikely scenario considering that there were still 10 extant western haplotypes in 1999, two of which are now in very high frequency. Even if there had been ten reproductive females extant in 1966, each with a different haplotype, reproductive success would have had to be extremely skewed towards two of those matrilines.

There are a number of possible explanations for this many haplotypes persisting in such a small population. First, the current abundance estimate could be low. However, photographic identification data (Weller *et al.*, 1999; 2002 do not support the existence of an appreciably greater abundance off Sakhalin Island, although a still undiscovered feeding area cannot be ruled out. A second possibility is that the population has grown much more slowly than 3.3% since 1966 (i.e. the bottleneck was not as severe as six, or even ten, reproductive females). Although this scenario would bode well for the level of genetic diversity still contained in the population, it would nonetheless have serious implications for their viability. If the population in 1966 contained much more than six adult females, it raises the possibility that the population has only been holding steady or even continuing to decline since then rather than recovering. In other words, a population the size of the western gray whales that has been growing since 1966 would not be expected to contain as many as 10 haplotypes. These sub-optimal population trajectories suggest the existence of some yet to be determined source of mortality (e.g. bycatch in fisheries, direct kills, vessel strikes, etc.) or other impediment to recovery (e.g. habitat degradation as reviewed in Weller *et al.*, 2002).

Another possibility is that there is some dispersal from the eastern stock. In general, the gene pool of a small population is strongly influenced by even trivial amounts of gene flow from a larger neighbour, and the significant differences found between these populations would seem to contradict this possibility. However, given the maternal inheritance of the mitochondrial data examined here, male dispersal could still occur but would have little or no long-term effect on haplotype distributions (and mitochondrial differentiation). Indeed, of the eight western haplotypes represented by only one or two individuals, only two (*E* and *H*) came from females, with the remaining six only represented by males. Future work using microsatellite data may be able to test hypotheses of male dispersal. Because of the higher diversity and number of haplotypes in the eastern population, animals dispersing into the western population are most likely to carry haplotypes considered rare in the west (i.e. ones other than 'A' or 'B'). Animals with these rare haplotypes could be the focus of microsatellite-based assignment tests (e.g. Paetkau *et al.*, 1995), to see if they show greater affinity to the eastern population than do the rest of the western animals. However, since it is the number of females that seems to have dropped to critical levels at present (Weller *et al.*, 2002), any influx of males that may occur would not be of immediate benefit to the western population, although it would mitigate any effects of inbreeding and loss of diversity in the nuclear genome. Overall, the present findings that the mitochondrial differentiation between eastern and western gray whales is large and female dispersal is negligible at best, coupled with the paucity of females in the western population (Weller *et al.*, 2002), underscores the critical status of the western gray whales (e.g. see IWC, 2002).

Based on molecular identification, Baker *et al.* (2002) determined that seven commercial market products purchased in Wakayama Prefecture, Japan in August and October 1999 were samples of gray whale meat. They noted that all seven products had the same haplotype as a GenBank gray whale sequence (Accession #L35611), from a whale sampled off the coast of Washington, USA. The GenBank sequence and the sequences from the Wakayama gray whale products are all identical to our haplotype 'A' (Figs 1 and 2), the most common haplotype in both the eastern and western sample sets (10.8% and 44.4%, respectively). The sequences are also identical to the sequence provided to us by M. Goto and L. Pastene (pers. comm.) for the whale harpooned off Hokkaido in 1996 (Brownell and Kasuya, 1999), the whale also referred to as the 'Suttsu' whale by Baker *et al.* (2002). This haplotype is shared between the two populations and it is not possible to definitively assign the Wakayama meat samples (or any given gray whale sample) to either population using mitochondrial sequence data. Nevertheless, given the match, and the apparent butchering of the carcass (Brownell and Kasuya, 1999), a reasonable explanation is that the meat from the Wakayama market originated from the whale harpooned off Hokkaido. This explanation can be tested by analysing both samples using microsatellite data, or any other molecular data that allow the genotyping of individual whales. Finally, the sequence sent to us by M. Goto and L. Pastene (pers. comm.) from the 1995 stranding in eastern Hokkaido matched both haplotype 'G' and 'O' of our dataset (the shorter sequence sent by Goto and Pastene did not include the variable sites that distinguish haplotype 'G' from haplotype 'O').

In summary, results presented here show that eastern and western gray whales can be genetically differentiated at the population level, and should be recognised as geographically isolated and demographically closed population units. However, because of shared haplotypes, it is not possible at this time to genetically identify an individual sample to either population. Furthermore, the presence of 10 western haplotypes in a population this small is inconsistent with a population that has undergone any appreciable growth.

ACKNOWLEDGEMENTS

We would like to thank the many organisations and people who provided us with tissue samples from the eastern population. In the NMFS Southwest Regional Stranding Network, the following groups provided tissue: Hubbs-Sea World, The Marine Mammal Center, Moss Landing Marine Laboratory, University of California Berkeley and Humboldt State University. In the Northwest and Alaska Regional Stranding Networks, the following provided samples for this study: Oregon State University, Cascadia Research Cooperative, National Marine Mammal Lab, Alaska Department of Fish and Game, and K. Wynne of the

University of Alaska Fairbanks. We also thank T. Steeves of the National Zoological Park in Washington, D.C. for providing biopsy samples. In addition, we also thank the observers and ship captains in the California gillnet observer programme who collected samples from fishery bycatch. Lastly, we thank S. Blokhin for the samples of eastern gray whales collected in Chukotka, Russia.

Thanks to the following people that helped collect western gray whale samples off Sakhalin Island, Russia: S. Blokhin, A. Bradford, Y. Ivashchenko, R. Pitman, S. Reeve, A. Trukhin and G. Tsidulko. Thanks also to V. Vladimirov for assistance with permits. Funding for the 1995 work was provided by the Bureau of Oceans and International Environmental and Scientific Affairs, US Department of State to the International Whaling Commission; National Marine Fisheries Service, NOAA; and the Humane Society of the United States. We gratefully acknowledge the financial support of Sakhalin Energy Investment Company and Exxon Neftegas Limited to Texas A&M University and the Kamchatka Institute of Ecology and Nature Management for related work on western gray whales between 1997-1999. The Russian State Committee of Environmental Protection in Moscow issued CITES export permits for the Russian samples. This project was conducted as part of the Marine Mammal Project under Area V: Protection of Nature and the Organisation of Reserves within the US-Russia Agreement on Cooperation in the Field of Environmental Protection 1972. We would also like to thank Doug DeMaster at the Alaska Fisheries Science Center for supporting preliminary genetic work on this project. Thanks also to B. Amos and an anonymous reviewer for their helpful comments. Haplotype sequences have been deposited in GenBank, Accession #AF326789-AF326824. This paper represents contribution no. 77 of the Marine Mammal Research Program, Texas A&M University.

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