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Genetic differentiation between sympatric Killer whale populations

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The genetic variation within and between putative Killer whale (*Orcinus orca*) populations was examined by DNA fingerprinting nuclear genomes and sequencing the D-loop region of the mitochondrial genome. Mitochondrial DNA variation indicated that two sympatric populations in the northeastern Pacific were as genetically distinct as North Pacific populations from a South Atlantic population. The two sympatric populations are known to pursue different foraging strategies. DNA fingerprinting showed very low levels of variation within populations relative to comparisons between allopatric populations, suggesting inbreeding. These results are consistent with predictions about the genetic structure of Killer whale populations based on behavioural observations and variation in colour morphology.

Keywords: DNA fingerprinting, Killer whale, mtDNA, PCR, sympatry.

Introduction

Geographical boundaries and behavioural patterns are believed to cause the phylogeographic structuring of species (see Avise *et al.*, 1987). It is common for genetic distance to be roughly correlated with geographical distance, unless local boundaries limit the movement of conspecific individuals, e.g. when a terrestrial species is distributed on a number of small islands. Genetic differentiation without spatial separation could result from a secondary admixture of populations that differentiated allopatrically, or from some intrinsic mechanism that causes reproductive isolation. There are few known cases of the latter situation. Possible mechanisms would include microhabitat specialization and assortative mating (see Otte & Endler, 1989).

We investigated the phylogeographic structuring of Killer whale populations, with an emphasis on sympatric populations in the eastern North Pacific that are known to pursue different foraging strategies. As it was not possible to obtain large sample sizes, we employed molecular genetic techniques that allow a high level of resolution. Eight samples were collected from three local populations off Vancouver Island, British Columbia, Canada (48°N latitude, 123°W longitude). Two samples were known to have been from the same social group. Five samples were collected in Iceland from a region that extends 200 km along the southeastern coast (near 64°N latitude, 15°W longitude). Three samples were from Argentina near 42°S latitude, 63°W longitude, and one was from the North Sea coast of West Germany.

Two of the populations off Vancouver Island (N & S) are parapatric in that they reside in two adjacent non-mixing communities, north and south of a tidal boundary at a major estuary. The third population (T) moves over a broader geographical range, which is sympatric to the ranges of populations N & S. The three populations have been the subject of an extensive observational study for over 15 years (Bigg *et al.*, 1990). All 350 individual whales are recognized by idiosyncratic morphology, and their movements and associations are well documented. There are no observations of individual whales or social groups (pods) from any of these three populations travelling together, although pods from different populations are occasionally sighted within 1 km of each other.

We investigated the mitochondrial D-loop because it is a non-coding region and highly variable in some taxa (although less so for cetaceans; Hoelzel, 1989). The matrilineally transmitted mitochondrial genome has been used extensively for the comparison of populations (Wilson *et al.*, 1985). In order to assess the levels of genetic similarity within populations using an independent molecular marker, we investigated minisatellite DNA banding patterns (DNA fingerprints) from nuclear genomes.

Materials and methods

Whole cell DNA was extracted from the skin or blood samples using phenol/chloroform. The D-loop was amplified by the polymerase chain reaction (Mullus & Faloona, 1987). Approximately 100 ng DNA were washed in equal volumes of 0.1 M NaOH and 0.1 M KH_2PO_4 and cycled 25 times at 93°C for 0.5 min, 50°C for 1 min, and 70°C for 1 min in the following assay conditions: 5 mM KCl; 1 mM Tris/HCl, pH 8.3; 150 μM $MgCl_2$; 0.1 mg ml^{-1} gelatin; 200 μM each of ATP, TTP, GTP and CTP; 1 μM of each primer and 1 unit of Taq polymerase in a total volume of 50 μl. Amplified D-loop DNA was then subcloned into bluescript sk+ vector and grown up in the TG1 recf- *E. coli* strain. At least two subclones from each individual were sequenced by the chain termination method (Sanger *et al.*, 1977) in both directions. Complete sequences (921 basepairs) were compared between the three populations off Vancouver Island, and a 362 bp Hind III/Bam HI subclone from within the D-loop was compared between Argentina, Germany, Iceland, and one additional individual from each of the Vancouver Island populations.

For DNA fingerprinting, 2 or 3 μg of DNA from each whale were restricted with Hinf1 and run on a 0.8 per cent agarose gel. The gel was vacuum-blotted onto nylon membrane. The polycore construct 33.15 described and donated by Alec Jeffreys (Jeffreys *et al.*, 1985b) was used as a probe. Single stranded M13 and an insert were radioactively labelled by primer extension using the following assay conditions: 50 μM dTTP; 50 μM dCTP; 50 μM dGTP; 5 mM DTT; 20–30 μCi 32P labelled dATP and 1 unit Sequenase (DNA polymerase I). The filter was prehybridized and hybridized in the same solution: 1 × SSC (150 mM NaCl; 15 mM sodium citrate, pH 8); 0.1 per cent SDS; 50 mg ml^{-1} PEG; 50 $\mu g/ml^{-1}$ heparin and 50 $\mu g/ml^{-1}$ tRNA at 58°C. Prehybridization was for 1 h, and hybridization was left overnight. Filters were washed in 1 × SSC, 0.2 per cent SDS at 58°C for 0.5–1.0 h.

Table 1 Basepair differences (below diagonal) and genetic distance (above diagonal) for interpopulation sequence comparison of 362 bp Hind III/Bam HI subclone from the D-loop region. I = Iceland, G = Germany, A = Argentina, R = populations N & S, T = population T

	I	G	A	R	T
I		0.55	1.38	0.55	0.83
G	2		0.83	0.0	0.55
A	5	3		0.83	1.38
R	2	0	3		0.55
T	3	2	5	2	

Results

D-loop sequences for populations N & S were different by one substitution and one nucleotide deletion. Population N was different from population T by eight substitutions and one deletion. Population S was different from population T by seven substitutions and two deletions. There were no differences between individuals within any of the populations off Vancouver Island, nor between populations N & S for the 362-bp fragment. Basepair differences between the 362 bp sequences for samples from Iceland, Germany, Argentina, populations N & S together (R) and population T are shown in Table 1.

Sequences were compared to estimate the genetic distance by the formula derived by Kimura & Ohta (1972), which corrects percentage sequence difference for chance multiple substitutions (see Table 1). Pairwise comparisons were made between the entire D-loop sequences for the populations off Vancouver Island: the genetic distance between populations N & S was 2.2×10^{-3} substitutions per nucleotide, and the average genetic distance between populations N & S and population T was 9.8×10^{-3} bp. The average from all pairwise comparisons ($n = 36$) of the nine individual Killer whales (one from each of Iceland, the Netherlands and Argentina, and two from each population off Vancouver Island) sequenced for the 362 bp Bam HI/Hind III D-loop fragment was 6.0×10^{-3} bp (s.d. $= 4.0 \times 10^{-3}$ bp).

Minisatellites were visualized after probing with the human multilocus probe 33.15 (Jeffreys *et al.*, 1985b) and compared using a simple bandsharing coefficient (F after Upholt, 1977). The bandsharing coefficient was used as an estimate of the probability of a band in one individual being present in another by chance. This quantity (x in Jeffreys *et al.*, 1985a) is related to the allele frequency (q) by $x = 2q - q^2$. All pairwise comparisons between seven whales from the Vancouver Island populations, the five Icelandic samples, and two Argentine samples for bands between 1 kb and 15 kb are presented in Table 2. The DNA fingerprints compared in this table are shown in Fig. 1. Comparisons were checked by running the same individuals in a different order on another gel and comparing them blind, which gave the same results (data not shown).

The mean number of bands between 1 kb and 15 kb per individual is 33 (s.d. = 4). The average bandsharing coefficient for within population comparisons is 0.64 (s.d. = 0.11, $n = 22$). The average bandsharing coefficient between populations, excluding the comparison between areas N & S, is 0.30 (s.d. = 0.04, $n = 69$). These means are substantially different from one another. Unfortunately, no formal statistical test can be used to test the significance of this difference because

Table 2 Average and 1 s.d. for bandsharing coefficients between and within major areas. A = Argentina, T = population T, S = population S, N = population N, I = Iceland

		A	T	S	N	I
A	*x*	0.77				
	s.d.	—				
T	*x*	0.34	0.68			
	s.d.	0.05	—			
S	*x*	0.27	0.29	0.73		
	s.d.	0.01	0.03	—		
N	*x*	0.30	0.33	0.72	0.72	
	s.d.	0.03	0.02	0.08	0.06	
I	*x*	0.32	0.31	0.27	0.28	0.55
	s.d.	0.03	0.01	0.02	0.04	0.08

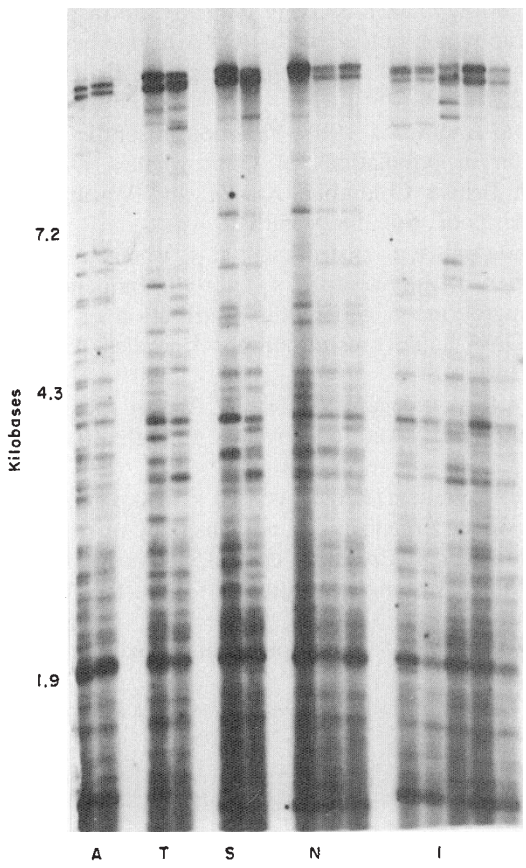

Fig. 1 Fourteen individual killer whales from five putative populations digested with Hinf1 and probed with 33.15. A = Argentine, T = population T, S = population S, N = population N, I = Iceland.

the data are non-independent. However, there is no overlap between the distributions of bandsharing coefficients in the 'within' and 'between' categories, and this suggests a real difference (range within = 0.43–0.81, range between = 0.21–0.38).

The average coefficient between area N & S is 0.72 (s.d. = 0.08, $n = 6$). Note that this is as high as the mean bandsharing coefficient within either of these populations. This genetic similarity and the apparent lack of exchange between populations N & S (Bigg *et al.*, 1990) could be explained if population S had recently been founded by whales from the larger population N.

Discussion

Numerous studies have investigated whole mtDNA variation estimated from restriction fragment length polymorphisms (RFLPs) among conspecific populations (see review by Selander & Whittam, 1983). The average intraspecific, interpopulation genetic distance from four mammalian species (three *Peromyscus* spp. and *Geomys pinetis*) was 2.6×10^{-2} substitutions per nucleotide (Avise *et al.*, 1987). This is about five times the variation seen in the Killer whale D-loop. However, the average genetic distance between whole mtDNA genomes derived from intraspecific, interpopulation comparisons of humpback whales (*Megaptera novaengliae*) (average of 2.6×10^{-3} substitutions per nucleotide; Baker *et al.*, 1990) is closer to the Killer whale genetic distances. These comparisons are relevant because the substitution rate in the cetacean D-loop is roughly equivalent to the whole mtDNA genome rate in cetaceans and other mammalian taxa (Hoelzel, 1989).

The degree of minisatellite DNA bandsharing seen between Killer whale populations was consistent with the level seen between unrelated individuals in various non-human species (e.g. Jeffreys & Morton, 1987; Georges *et al.*, 1988). The level seen within populations, and between population N & S off Vancouver Island, was twice as high. Although various restrictions to the interpretation of minisatellite banding patterns prevent the determination and useful application of Hardy–Weinberg ratios (Lynch, 1988; Hoelzel & Dover, 1989), the pattern described here is strong enough to imply inbreeding within regional populations. Samples were from captured or stranded whales and in most cases within population samples were unlikely to be from first- or second-order relatives due to temporal and spatial differences in sampling. This is known to be true for two important comparisons. First, observational studies indicate that the samples from populations N & S were from individuals that had not shared common ancestors in the recent past (Bigg *et al.*, 1990). Second, the two whales sampled in population T are known to be from different pods, which were not known to interact. However, in each case the bandsharing coefficients were as high as for comparisons of individuals from the same pod. The observa-

tional study off Vancouver Island indicated that for populations N & S, there had at least been no immigration of new individuals and no evidence for dispersal during the course of the 15-year long study (Bigg *et al.*, 1990). This pattern of behaviour would imply inbreeding within small regional populations.

The apparent partitioning of this species into reproductively isolated populations is consistent with earlier speculation on the isolation of orca populations based on colour morphology (Evans *et al.*, 1982; Baird & Stacey, 1988) and acoustical behaviour (Ford & Fisher, 1982; Hoelzel & Osborne, 1986). Strong behavioural differences between 'transient' (population T) and 'resident' (populations N & S) Killer whales off Vancouver Island, especially related to foraging strategy, have indicated some level of isolation between these sympatric populations (Bigg *et al.*, 1990). It is possible that the resident and transient forms off Vancouver Island represent a convergence of populations that become genetically distinct allopatrically. However, a similar division of Killer whale populations by foraging strategy has been reported for several other regions (e.g. Berzin & Vladimirov, 1983). Hoelzel (1989) suggested that differential characteristics of prey dispersion may affect Killer whale social organization and the reproductive strategy of males. This in turn could serve as an ethological isolating mechanism. In general, genetic isolation between sympatric populations within a species is unexpected because very low levels of genetic interchange (roughly one individual per generation in the absence of strong selection; Crow & Kimura, 1970) are sufficient to render populations effectively panmictic. The question of whether differential niche adaptation or assortative mating can lead to sympatric speciation is highly debated (Maynard Smith, 1966; Bush, 1975; Otte & Endler, 1989). We propose that behavioural isolating mechanisms have had the effect of lowering genetic migration between Killer whale populations leading ultimately to genetic differentiation, but that there is sufficient dispersal between populations to maintain some genetic continuity throughout the species.

Our results indicate that genetically distinct Killer whale populations can coexist within a local geographical range. Observational studies have suggested that differences in social and breeding behaviour between Killer whale pods that specialize on marine mammal prey, versus those that specialize on fish prey, may provide a partial barrier to genetic migration between these groups. However, we have no direct proof that this is a causal mechanism. The implication for conservation is that geographical isolation is not the only criterion on which stock divisions should be described. If the populations of Killer whales off Vancouver Island and off Iceland are to be managed as separate stocks, for example, then so should the transient and resident populations off Vancouver Island be considered separate stocks, and this type of genetic division could exist in other regional Killer whale populations.

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