

The phylogeographic pattern of mitochondrial DNA variation in the Dall's porpoise *Phocoenoides dalli*

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Abstract

We used 11 restriction endonucleases to study mtDNA variation in 101 Dall's porpoises *Phocoenoides dalli* from the Bering Sea and western North Pacific. There was little phylogeographic patterning among the 34 mtDNA haplotypes identified in this analysis, suggesting a strong historical connection among populations across this region. Nonetheless, mtDNA variation does not appear to be randomly distributed in this species. Both G_{ST} and AMOVA uncovered significant differences in the distribution of mtDNA variation between the Bering Sea and western North Pacific populations. These mtDNA results, coupled with differences in allozyme variation and parasite infestation, support the demographic distinctiveness of Bering Sea and western North Pacific stocks of Dall's porpoise. The lack of a strong phylogeographic orientation of mtDNA haplotypes within the Dall's porpoise is similar to the pattern reported in other vertebrates such as coyotes, blackbirds, chickadees, marine catfish, and catadromous eels. Like Dall's porpoise, these species are broadly distributed, and have large populations linked by moderate to high levels of gene flow. However, the more complex, deeply branched phylogenetic network of mtDNA haplotypes within Dall's porpoise, relative to these other vertebrates, suggests important differences between these species in the forces shaping mtDNA variation. One such force is the effective size of female populations, which appears to have been comparatively large and stable in Dall's porpoise.

Keywords: cetaceans, equilibrium populations, genetics, intraspecific gene genealogies, management

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Introduction

Intensive, long-term study of a species is often initially motivated by management concerns. In many cases, knowledge gained from these studies also provides valuable insights into the evolutionary process (Avice 1994; Moritz 1994). A case in point is the North Pacific Dall's porpoise, *Phocoenoides dalli*. Despite its high population numbers, estimated to be between 1.44 and 2.81 million (Jones *et al.* 1987), the Dall's porpoise is becoming increasingly threatened. Resource managers' concern for the increasing number of porpoises killed accidentally in salmon and squid drift nets or purposefully by a growing harpoon fishery has prompted research examining the

population structure of this species. The mitochondrial DNA (mtDNA) data reported here compliment earlier ecological and biochemical studies used to support management decisions concerning the Dall's porpoise (Jones *et al.* 1987; Winans & Jones 1988). Furthermore, our results provide useful comparative data for exploring, more generally, the evolutionary forces shaping the architecture of intraspecific mtDNA polymorphism.

The geographical range of *P. dalli*, a small, typically black and white porpoise, extends in a broad arc from Ballenas Bay, California, north and westward across the North Pacific and Bering sea to Honshu, Japan (Fig. 1). Two Dall's porpoises colour morphs, so-called *truci*- and *dalli*-types, are recognized and differ in the amount of white on the ventral surface and their geographical range. In the *truci*-type, the prominent white ventral patch characteristic of this species extends across the thorax between the base of the flippers, much further than in the *dalli*-type. In addition to these two colour forms, all black or all grey

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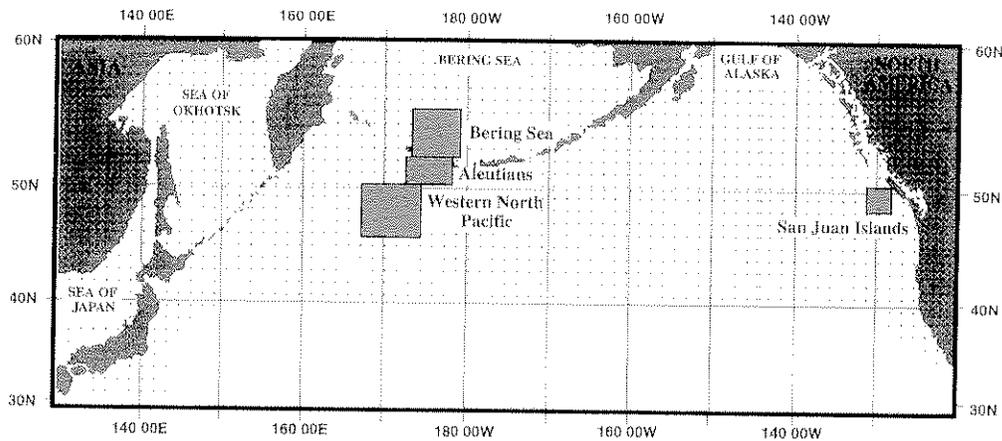


Fig. 1 Geographical range of *Phocoenoides dalli*. Collection areas are presented as shaded boxes. Collection numbers are as follows: for the Bering Sea, $n = 28$; for the Aleutian Islands, $n = 49$; for the western North Pacific, $n = 24$; for the San Juan Islands, $n = 2$.

individuals are occasionally sighted. *Truei*-type individuals predominate in the coastal Japanese population, while *dalli*-type characterize the Sea of Japan and Okhotsk Sea population and offshore North Pacific and Bering sea population (Kasuya & Jones 1984).

In recent years, the United States National Marine Fishery Service (NMFS) has been concerned with the management of the offshore North Pacific Dall's porpoise population. As well as encompassing much of the total geographical range of this species, a significant portion of this population falls within the United States 200 mile Fishery Conservation Zone and under the mandate of the Marine Mammal Protection Act. Salmon and squid drift net fisheries operating within this region lay down approximately 2000 000 km of fishing nets in which as many as 12 000 Dall's porpoises become tangled and drown annually (Jones *et al.* 1987). NMFS was charged with establishing limits on the number of porpoises that could be killed annually and had to determine whether incidental kill limits should be calculated for single or multiple populations of the Dall's porpoise in the North Pacific and Bering Sea. Dall's porpoises are fast, powerful swimmers capable of traversing great distances, but it was unknown if this potential for long distance dispersal would be reflected in extensive gene flow across their distribution. Structured populations of other social cetaceans with high dispersal potential had been observed (Baker *et al.* 1990; Hoelzel & Dover 1991) suggesting the possibility that the Dall's porpoise should be managed as a subdivided population.

The population genetic structure of the Dall's porpoise was also interesting in the light of published, mtDNA-based studies of other moderate and high dispersal vertebrates. Although there have been other studies of mtDNA variation in cetaceans (e.g. Baker *et al.* 1990; Wada *et al.* 1991), this was one of the first large-scale restriction fragment length polymorphism (RFLP) investigation is of a cetacean that had not been severely exploited by humans. Thus, we reasoned that the pattern of mtDNA variation would reflect historical demographic forces unlinked to

recent increases in human exploitation of Dall's porpoises. Other large scale surveys of population heterogeneity in cetaceans, such as Dizon and co-workers' (1991) research on spinner porpoises, used only restriction enzymes known to cut polymorphic sites in the assayed species. Estimates of mtDNA divergence and nucleotide diversity resulting from such studies are 'biased' and make comparisons across taxa difficult.

The homology of animal mtDNA and the phylogenetic information carried by the molecule make comparisons across taxa particularly useful for insights into evolutionary and demographic processes. In this respect, the large number of 'unbiased' mtDNA-based RFLP studies provides fertile opportunity for exploring the forces which shape mtDNA variation in natural populations. Here we compare the pattern of mtDNA haplotype variation observed in Dall's porpoises to that observed in coyotes *Canis latrans* (Lehman & Wayne 1991), blackbirds *Agelaius phoeniceus* (Ball *et al.* 1988), black-capped chickadees *Parus atricapillus* (Gill *et al.* 1993), hardhead catfish *Arius felis* (Avise *et al.* 1987), and American eels *Anguilla rostrata* (Avise *et al.* 1986). All these species share broad population and life history characteristics that make such a comparison powerful, including high-dispersal potential, expansive distributions and large current-day population size. Additionally, these species show similar geographical patterns of mtDNA variation. Identical or very closely related mtDNA haplotypes are distributed widely suggesting a strong evolutionary linkage among populations across the range of each species. Thus, we have attempted to control for extrinsic or intrinsic differences in dispersal in order to focus on other historical and ecological factors that can shape mtDNA variation in natural populations.

Materials and methods

This study examined 103 Dall's porpoises and two harbour porpoises (*Phocoena phocoena*). The subsequent analysis focused on 101 Dall's porpoises collected from the

Bering Sea and North Pacific Ocean within a region bounded by 169°E and 179°W longitude and 46°N and 56°N latitude (see Fig. 1). These 101 porpoise were incidental casualties of the salmon and squid drift net fisheries operating within this region. Tissue samples were collected by US foreign fishery observers, immediately frozen at -20 °C, and stored at -70 °C. The majority of the porpoises examined were collected in 1983 ($n=38$) and in 1986 ($n=58$) with the remaining five collected in the intervening years. This sample contained 96 *dalli*-type individuals, two *truei*-type individuals, two all black individuals and one black-cross individual (an offspring of a all black by *truei*-type mating). The additional four porpoises studied, two Dall's and two harbour, were found stranded on the San Juan Islands, in Puget Sound, Washington State. Precise sampling locales and collection dates for all 105 porpoises are available upon request.

DNA extraction, restriction digests, electrophoresis and Southern blotting

Whole genomic/mtDNA preparations were obtained from heart or liver tissue. Approximately 0.5 g of tissue was homogenized in 5 mL of grinding buffer (100-mM Tris-HCl, pH 8.0; 100-mM NaCl; 50-mM EDTA, pH 9.1; 200-mM Sucrose; 0.5% SDS). The homogenate was then filtered through cheesecloth and 1.23 mL of 5-M KOAc was added. This solution was incubated on ice for \approx 30 min and spun at 10 000 r.p.m. for 10 min in a Sorval RB-5C centrifuge to pellet cellular debris. The supernatant was extracted once with an equal volume of phenol, followed by a second extraction with phenol/chloroform/isoamyl alcohol (25:24:1), and a third extraction with chloroform/isoamyl alcohol solution (24:1). DNA was recovered by cold-ethanol precipitation (Sambrook *et al.* 1989). The pellet was dissolved in 200 μ L of 1 \times TE, and dialysed overnight against 1 \times TE with one buffer change after 8 h.

The following restriction enzymes ($r=6$; Nei 1987) were used separately to digest a 10- μ L aliquot of DNA prepared from each individual porpoise: *Asp718*, *Bam*HI, *Bcl*I, *Bst*EII, *Dra*I, *Eco*RI, *Eco*RV, *Hind*III, *Hpa*I, *Stu*I, *Xba*I. Restriction endonuclease digestions were carried out overnight under assay conditions recommended by the vendor. DNA fragments were separated electrophoretically in 0.9–1.2% agarose gels. After in-gel denaturation, the size-fractionated DNA samples were transferred onto a nylon membrane (Zetabind®). SCP buffer (10 \times : 1-M NaCl, 0.3-M Na₂HPO₄, 10-mM EDTA, pH 7.0–7.5) was used for both transfers and post transfer washing of the membranes. Three to five blots were prehybridized overnight in 25–40 mL of prehybridization solution (10% dextran sulphate, 0.5 M NaCl, 1% SDS). Following prehybridization, blots were probed with ultra-pure mtDNA that was either random-primed or nick-translated in the presence of [α -

³²P]dCTP. Probe was prepared from caesium gradient-purified mitochondrial DNA (see procedure outlined in Lansman *et al.* 1981) extracted from fresh Dall's porpoise liver. Both prehybridization and hybridization were performed at 65 °C in sealed bags with constant agitation. Following hybridization, blots were washed in three 15-min baths of increasing stringency at 65 °C (2 \times SCP, 1% SDS; 0.2 \times SCP, 0.1% SDS; 0.1 \times SCP, 0.05% SDS). The resulting banding patterns were visualized on autoradiographs following overnight exposures.

Restriction enzymes generally cleaved an individual porpoise's mitochondrial genome into several fragments (Appendix 1). In cases where more than one fragment pattern was generated, the most common pattern observed was chosen as the reference and designated by the letter code 'C'. Less common fragment patterns were coded with other letters. In all cases, the different patterns produced by a particular restriction enzyme could be related to one another by a series of single restriction site changes. Thus, it was possible to infer restriction site changes from the fragment pattern data (Bermingham 1990). For all Dall's porpoises examined we recorded the presence or absence of each restriction site (Appendix 2). The letter codes for each of the 11 restriction enzyme patterns were combined to define each porpoise's composite mtDNA haplotype (Appendix 2). Individuals possessing the same composite genotype were considered to have identical mtDNA haplotypes.

mtDNA sequence divergence analyses

The extent of sequence divergence between individuals was calculated using the maximum likelihood estimate described in Nei & Tajima (1983). Average mtDNA divergence within (π) and between (d_{xy}) sampling localities of Dall's porpoises were estimated by averaging pairwise comparisons of sequence divergence across all individuals.

The mitochondrial genomes of the two harbour porpoises were also examined with the same 11 restriction enzymes used to cut the Dall's porpoise DNA. *Phocoena phocoena* and *Phocoenoides dalli* differed to such an extent that most enzyme patterns could not be readily related to each other by changes in a few restriction sites. As a result, we could not use *Phocoena phocoena* as an outgroup in the phylogenetic analyses discussed below. For estimating sequence divergence between these two species, we used the proportion of shared fragments (Engles 1981).

Phylogenetic and population genetic analyses

Unrooted mtDNA haplotype phylogenies for Dall's porpoises were constructed by PAUP using the heuristic search setting (Swofford 1993). The tree bisection and

reconnection (TBR) branch swapping algorithm was chosen to search for optimal trees within this search framework. In order to examine the possibility that our single heuristic search became stranded at a 'local' rather than 'global' optimum, we performed 100 'abbreviated' heuristic searches. In each of the replicate searches, taxa were added randomly and the search was terminated after 20 trees were found. In our analysis, restriction enzyme site changes were treated as relaxed Dollo characters as suggested by Swofford & Olsen (1990). Under our weighting scheme, restriction site gains were given two times the weight of restriction site losses. This criterion was based on previous observations suggesting that (1) polymorphic restriction sites were more likely to fall within protein coding regions (Cann *et al.* 1984) and (2) most substitutions within protein coding regions were transitions at the third position of a codon (McMillan & Palumbi 1995). Thus it seemed justified to assume that most substitutions causing a restriction site to be polymorphic were transitions at the third position of a codon (see also Lehman & Wayne, 1991). Six base restriction enzyme sites contained two such positions. A restriction site was lost if either of these positions changed. On the other hand, there was only one way to regain a previously lost site. Likewise, there was only one way to gain a 'one-off' site (Templeton 1983; Swofford & Olson, 1990).

For population genetic analysis, porpoises were grouped into three broad geographical regions: the Bering Sea ($n = 28$), the Aleutians (Aleutian Islands south to 50°N ; $n = 49$), and the western North Pacific (south of 50°N and west of 180°W ; $n = 24$) (Fig. 1). The two Dall's porpoises collected off the coast of the San Juan Islands, WA were not used in the analysis of population structure. Although no obvious physical barrier partitions the range of the Dall's porpoise into these units, these divisions were defined by previous stock distinctions based on differences in parasite abundance and distribution (Walker 1991), reproductive timing (Kasuya & Jones 1984; Jones *et al.* 1987), and the distribution of isozyme variation (Winans & Jones 1988).

We explored the spatial distribution of mitochondrial variation using analysis of molecular variance (AMOVA) (Excoffier *et al.* 1992) and G_{ST} (Nei 1973; Takahata & Palumbi 1985). In our AMOVA analysis we used the number of restriction site differences between haplotypes as a Euclidean distance. Populations were considered to be significantly different from one another if the observed within-population variance was less than 95% of the within-population variances produced by 500 random permutations of the squared Euclidean distance matrix. Similarly, in the G_{ST} analysis significant population heterogeneity was assumed if the observed G_{ST} value was larger than 95% of the G_{ST} values generated by randomly assigning individuals into identical sized demes (see Palumbi & Wilson 1990; Palumbi *et al.* 1991).

Census estimation of effective female population size in the Dall's porpoise

The Dall's porpoise is one of the most abundant marine cetaceans. Current estimates of total population size fall between 1.44 and 2.81 million (Jones *et al.* 1987). We use the average of estimates, or 2.1 million individuals, as a reasonable approximation of the total population size for this species. Of these 2.1 million individuals approximately half are female (Kasuya 1978). Incidental catch data suggest that 58% of all females caught are sexually mature (data averaged over seven years; Jones *et al.* 1987) suggesting a total adult female population size of 609 000 individ-

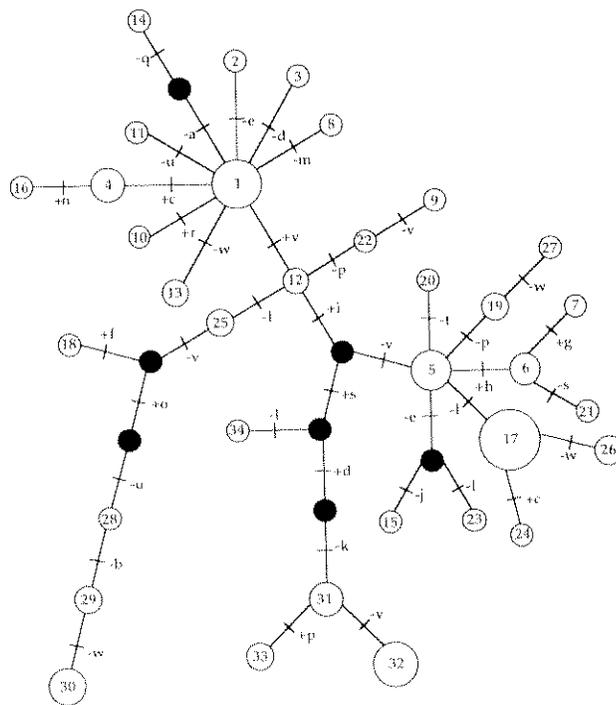


Fig. 2 One of the many possible maximum parsimony networks detailing the evolutionary relationship among mitochondrial genotypes observed in *Phocoenoides dalli*. Trees were generated by a heuristic search with the phylogenetic package PAUP (Swofford 1991) using the assumption that a restriction site loss is twice as likely as a restriction site gain (see Methods). The open circles represent the mtDNA haplotypes uncovered in this analysis. The number in the centre refers to the haplotype identification number. The restriction site presence/absence array for each mtDNA haplotype is given in Appendix 2. The area of each circle represents the proportion of individuals possessing each genotype. Black circles are hypothetical genotypes. Each radial arm represents a single restriction site change. Tick marks along the arm designate the changes (see Appendix 2 for site codes). + represents a restriction site gain (i.e. a change from 0 to 1 in the site matrix) and - a site loss. Tree length was 55 steps with a CI=0.56 and RI=0.68. Because of the small number of polymorphic restriction sites and low level of sequence difference between haplotypes, few nodes within most parsimonious trees were strongly supported by bootstrap analysis.

uals. Correcting for overlapping generations by dividing by the generation time of 4.5 years suggests an effective female population size of ≈ 135333 individuals (see Nei 1987).

Comparisons across taxa

Mitochondrial DNA haplotype diversity in the Dall's porpoise was compared to published studies of mtDNA diversity in coyotes (Lehman & Wayne 1991), blackbirds (Ball *et al.* 1988), chickadees (Cill *et al.* 1993), marine catfish (Awise *et al.* 1987), and catadramous eels (Awise *et al.* 1986). Each of the above data sets was reanalysed using the restriction site weighting scheme used for Dall's porpoise. For the coyote data set, our analyses excluded restriction enzymes with *r*-values of 4 and 5.3. As with Dall's porpoise, minimum-length networks were drawn from the shortest length tree suggested by a heuristic search of the computer program PAUP. In cases where more than one most parsimonious tree was found, minimum-length networks were drawn from a single, randomly chosen tree.

Results

In the 103 Dall's porpoises collected from the Bering Sea (*n* = 28), Aleutians (*n* = 49), western North Pacific (*n* = 24), and San Juan Islands, WA (*n* = 2) we assayed 50–56 restriction enzyme sites per individual, representing between 300 and 336 base pairs, of the ≈ 16.4 kb mitochondrial genome. Thirty-nine restriction sites were monomorphic and 23 were polymorphic (see Appendix 2). No mtDNA heteroplasmy or size heterogeneity was observed.

Phylogenetic analyses

There were 34 mitochondrial haplotypes identified from the 103 individuals of Dall's porpoise surveyed. Our heuristic search strategy on PAUP yielded many equally most parsimonious trees. An unrooted parsimony network (Fig. 2) shows the changes in each polymorphic site along one of the most parsimonious arrangements. This network is presented to detail the complexity of the evolutionary relationships among mitochondrial haplotypes within Dall's porpoise. No single genotype, or cluster of closely related haplotypes dominated this network. Although five of the 34 haplotypes (1, 5, 17, 30 and 32) were represented in seven or more individuals, these haplotypes differed from each other by an average of six restriction site changes. Within this network, mtDNA haplotypes differed by as many as 11 restriction sites, corresponding to an estimated 1.9% mtDNA sequence divergence. Within any of the shortest-length trees, many restriction sites changed more than once. For example, within the network presented in Fig. 2, nine of the 23 poly-

morphic restriction enzyme sites are hypothesized to have changed more than once. Some are envisioned to have changed many times. Restriction sites 'l' and 'w' were lost independently four times and site 'v' five times along this particular network. This extensive amount of parallel change in these data made determination of the true evolutionary relationships among mitochondrial haplotypes impossible.

Population genetic analyses

For the examination of population heterogeneity, we assumed that there was no temporal variation in mtDNA haplotypes across the three year period of this study. To test this assumption we compared the composition of mtDNA haplotypes found in porpoises collected in 1983 (*n* = 36) to the composition of mtDNA haplotypes found in porpoises collected in 1986 (*n* = 58) using the G_{ST} analysis. No temporal structuring of mtDNA genotypes was evident over the entire collection, or within any of the three hypothesized geographical regions.

Additionally, we assumed that our collection of porpoises represented a random sample of the population.

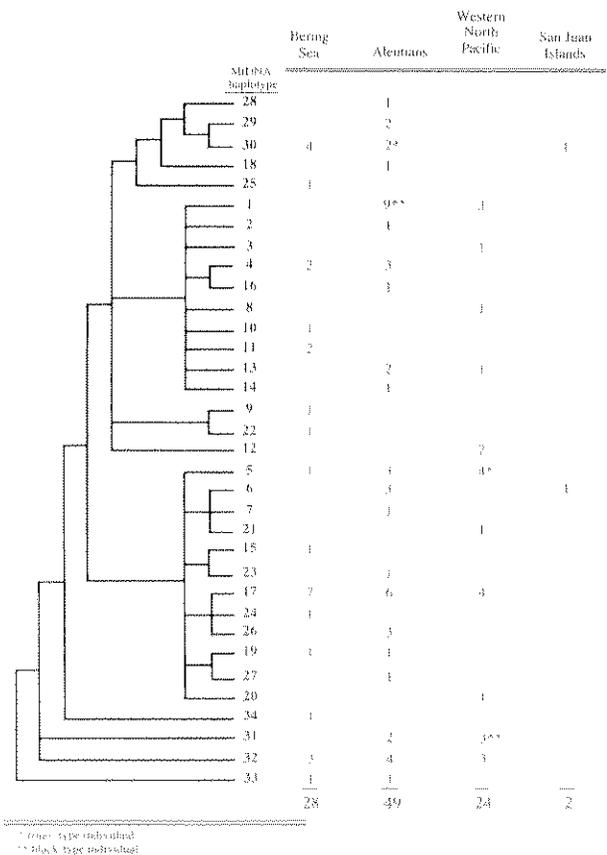


Fig. 3 Geographical distributions of the 34 mtDNA haplotypes observed in the 103 *Phocoenoides dalli* surveyed in this study. The mtDNA branching network is the same as depicted in Fig. 2.

Table 1 mtDNA genetic diversity (π) (Nei 1973) within and between each of the three hypothesized demes

Location	Bering Sea	Aleutians	Western N. Pacific
Bering Sea	0.0064		
Aleutians	0.0057	0.0056	
Western N. Pacific	0.0058	0.0059	0.0045

Behavioural observations of some cetaceans suggest that associations of closely related individuals often move together and are stable over time (Wells *et al.* 1980; Bigg *et al.* 1990; Wells & Scott 1990; Ford 1991; Amos *et al.* 1991). In our sample of incidental kills, some individuals were collected on the same day within the same area. This fact raised the possibility that we may have sampled porpoises from the same family group. We examined how this possibility would effect our interpretation of the distribution of genetic variation by comparing the gene identity probabilities of groups of four or more porpoises collected on the same day from the same area to same sized groups drawn at random from the entire data set. Individuals collected from the same day in the same locale were no more related to one another than were individuals composing random groups.

Indeed, individuals with identical haplotypes were generally found in more than one of the geographical regions. Three of the four most abundant haplotypes were found across the entire sampling area and in the San Juan Islands (Fig. 3). In addition, π values observed within the three regions ranged between 0.0045 and 0.0064 and were similar to levels of divergence between regions which ranged between 0.0057 and 0.0059 (Table 1). There was no obvious phylogeographic orientation of mitochondrial haplotypes nor any significant relationship between mtDNA haplotype genetic distance and geographical dis-

tance (Mantel matrix correlation; $r=0.074$, $P=0.08$ based on 500 random permutations).

Both the G_{ST} and AMOVA analyses suggested that a large proportion of the genetic variation was partitioned within rather than between the three geographical locales. For example, the AMOVA analysis partitioned 96.9% of the total variance within populations and only 3.1% between populations (Table 3). However, mtDNA variation was not partitioned randomly. Although neither pairwise analysis distinguished between Bering Sea and Aleutian populations, the western North Pacific population was identified as distinct from both. This distinction is evident whether these latter two groups were considered as a single population or as two distinct populations (Table 3).

mtDNA divergence between *Phocoenoides dalli* and *Phocoena phocoena*

The two *Phocoena phocoena* examined had the same mtDNA haplotype. This haplotype differed from the mtDNA haplotypes of *Phocoenoides dalli* by estimated sequence differences that ranged between 0.054 and 0.062. We used the weighted average of these values, 0.057, as our estimate of the sequence divergence between the mitochondrial genome of these two species.

Discussion

The Dall's porpoise provided an interesting genetic case study for at least two reasons. First, increases in mortality from growing drift-net fisheries and harpooning necessitated a detailed examination of population heterogeneity in this species. Genetic information on population subdivision and the phylogenetic relationships between regional populations had practical applications for establishing short- and long-term management strategies for this marine mammal (Dizon *et al.* 1992; Avise 1994; Moritz 1994). Secondly, our mtDNA analysis of this porpoise pro-

	Phylogenetic method†	Non-phylogenetic methods	
		AMOVA‡	G_{ST}
Among all populations	22	3.1*	0.1632***
Bering Sea/Aleutians	16	-1.6	0.1037
Bering Sea/Western N. Pacific	18	3.5*	0.1262*
Aleutians/Western N. Pacific	11	3.5*	0.1311*
Bering Sea and Aleutians/Western N. Pacific	7	8.8***	0.1432**

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

†Minimum number of migration events: our estimate was determined by comparing the observed value to values obtained from 500 random trees (Slatkin 1989).

‡Percentage variation between populations.

Table 2 Summary of results for the geographical structuring of populations. See methods for both a brief description of each analysis and the statistical test of significance

Table 3 Comparison of the patterning of mtDNA diversity within several high dispersal vertebrate species.

Species	Average number of nucleotides examined	Within species nucleotide diversity	Maximum % sequence difference between haplotypes	% of Individuals 0 or 1 site removed from most common haplotype	Genotype diversity ($1 - \sum f_i^2$)	Reference
Dall's Porpoise	314	0.006	1.9	28	0.93	this study
Blackbirds	378	0.002	0.8	74	0.80	Ball <i>et al.</i> (1988)
Chickadees	240	0.002	0.9	87	0.64	Gill <i>et al.</i> (1992)
Coyotes	330	0.005	2.1	63	0.75	Lehman & Wayne (1991)
Marine catfish	342	0.002	0.8	83	0.30	Avise <i>et al.</i> (1987)
American eel	331	0.001	0.6	95	0.53	Avise <i>et al.</i> (1986)

vided an opportunity for cross-taxa comparisons of mtDNA phylogenetic networks with other abundant, broadly distributed vertebrates with moderate to high dispersal. By focusing on these groups, we reasoned that we could gain insights into the historical and ecological factors shaping the architecture of mtDNA variation that would not be strongly linked to extrinsic or intrinsic differences in dispersal.

Management implications of mtDNA variation in Dall's porpoise

Managers charged with developing and implementing short- and long-term management strategies require information on the genetic structure of populations across the range of a species. RFLP analyses of mtDNA provide such information and permit a phylogenetically based assessment of the historical relationship of conspecific populations. Thus, it is possible to determine if populations across the range of a species are evolving along separate evolutionary trajectories and ought to be considered as evolutionarily significant units (ESUs: Ryder 1986; Waples 1991; Dizon *et al.* 1992; Moritz 1994). When measures of population differentiation (genetic or otherwise) fail to discriminate distinct phylogenetic entities, but are able to statistically distinguish regional populations or stocks, the evolutionary significance of these populations becomes exceedingly difficult, if not impossible, to assess. This owes to the fact that the time between demographic change and the compensatory genetic change can be long (Palumbi *et al.* 1991; McMillan *et al.* 1992). Higher resolution genetic markers such as microsatellites may decrease the lag time between genetic and demographic change but will not eliminate it (Bruford & Wayne 1993). Thus, populations may behave as demographically significant units long before, if ever, they become evolutionary significant units (see Moritz 1994). This is a conundrum currently plaguing resource managers and can be distilled into the following question: Do we manage our natural resources

to preserve potential or realized ESUs or both?

This question will have to be answered by the resource managers responsible for the Dall's porpoise in the North Pacific as our analyses suggest that porpoise populations in this area are demographically but not evolutionarily distinct. The spatial distribution of Dall's porpoise mtDNA variation suggested a very tight evolutionary linkage among populations in the central North Pacific. Identical mtDNA haplotypes were distributed widely, with no obvious phylogeographic structuring. Indeed, nearly 97% of the observed mtDNA variation was found within demes (Table 3). In addition, both East Pacific individuals, sampled over 6000 km away, had mtDNA haplotypes identical to ones found within our primary study area suggesting that the close evolutionary relationship among populations extended across the range of Dall's porpoise. Despite this observation, mtDNA variation was not distributed randomly across the central north Pacific. Statistically significant differences in the distribution of mtDNA variation between the Bering Sea and near Aleutian populations and the western North Pacific population argued against Dall's porpoise panmixis and for the demographic distinction of Bering Sea porpoise (*sensu* Moritz 1994).

The mtDNA evidence for the distinction of Bering Sea porpoise resonates across studies of parasite loads, reproductive timing, and isozyme variation. Comparisons between populations of the Dall's porpoise from the Bering Sea and western North Pacific identified differences in both the presence and intensity of infection of two common cetacean parasites, *Phyllobothrium* sp. (Cestoda) and *Crassicauda* sp. (Nemotoda) (Walker 1987). Moreover, the reproductive cycles of porpoises captured in these two regions were out of phase. Calving was displaced by over a month in the Bering Sea relative to the western North Pacific suggesting differences in the breeding cycle that may translate into reproductive barriers between the regional populations (Jones *et al.* 1987). Lastly, although genotype proportions at allozyme loci were similar, heterozygote deficiencies at two loci in the pooled sample

suggested significant population heterogeneity across this area. These anomalies led Winans & Jones (1988) to support the distinctiveness of the Bering Sea populations and to suggest that the area south of the Aleutian Islands (our Aleutian sample) represented a zone of mixing.

mtDNA haplotype variation in the Dall's porpoise compared to other high dispersal vertebrates

Coyotes (*Canis latrans*), blackbirds (*Agelaius phoeniceus*), black-capped chickadees (*Parus atricapillus*), hardhead catfishes (*Arius felis*), and American eels (*Anguilla rostrata*) share many population and life history attributes with the Dall's porpoise that make cross-taxa comparisons of the architecture of extant mtDNA variation valuable. Like Dall's porpoise, these north temperate species are all capable of moderate to high dispersal as adults (blackbirds, chickadees, coyotes, marine catfish), or larvae and adults (eels), have broad geographical distributions, and large current-day populations. Additional characteristics of these species are provided in Table 5. Moreover, the geographical patterning of mtDNA variation in these five species was similar to Dall's porpoise. Identical or closely related mtDNA genotypes were widely distributed, suggesting a tight evolutionary linkage among populations across the range of each species (Avisé *et al.* 1986; Avisé *et al.* 1987; Ball *et al.* 1988; Lehman & Wayne 1991; Gill *et al.* 1993).

Although mtDNA variation in all six vertebrates shows little geographical structure, there were marked differences in the phylogenetic pattern of this variation among the six species. Two simple measures helped highlight differences in complexity: (1) the percentage of individuals 0 or one restriction site change removed from the most common genotype; (2) the genotype diversity index ($1 - \sum f_i^2$).

The higher the percentage of individuals 0 or 1 change removed from the most common genotype the shallower and more star-like the mtDNA phylogeny. The lower the genotype diversity index, the more a single genotype dominated a population. Among our sample of vertebrates, Dall's porpoises possessed both the lowest percentage of individuals 0 or 1 site removed from the most common genotype and the highest genotype diversity index (Table 3).

Differences in these measures of complexity were reflected in differences in the 'shape' of the parsimony networks of mtDNA variation (Fig. 4). Here we use 'shape' to refer both to the depth of bifurcating networks, or the point where all mtDNA lineages coalesced, and to the actual pattern of branching and extinction events within them. The mtDNA networks of blackbirds, chickadees, hardhead catfish, and American eels and to a lesser extent coyotes tended to be shallow and star-like and were dominated by a single mtDNA haplotype or a cluster of closely related haplotypes. As an example, in blackbirds, assayed with 18 restriction enzymes (16 $r=6$, 2 $r=5.3$ enzymes), 74% of the individuals analysed carried the common haplotype or a haplotype one restriction site removed from the common haplotype (Ball *et al.* 1988). The two most divergent blackbird mtDNAs differed by six restriction sites. In contrast, the mtDNA network resulting from an analysis of the Dall's porpoise using only 11 enzymes (all $r=6$) was more complex and deeply branched. The common haplotype and its nearest neighbours (haplotypes one restriction site removed) accounted for only 28% of the 103 Dall's porpoises analysed and the most dissimilar haplotypes differed at 11 assayed sites (Table 3).

The mtDNA network of coyotes was most similar to that of Dall's porpoise. However, even in coyotes 63% of

Table 5 Some life-history parameters for several high dispersal marine and terrestrial vertebrates

Species	Age at first reproduction	Average number of offspring/year*	Mortality in first year†	Approximate geographical area (km ²)‡	Reference
Dall's porpoise	4.5	low	low	15 000 000	Kasuya 1978
Coyotes	2	moderate	moderate	8000 000	Bekoff & Wells (1986), Berg & Chesness (1987)
Chickadees	1	moderate	moderate-high	10 000 000	Dhondt (1989), McCleery & Perrins (1989)
Blackbirds	1	moderate	moderate-high	9000 000	Orians & Beletsky (1989)
Hardhead Catfish	1	high	high	7000	Avisé <i>et al.</i> (1988)
American eel	10	high	high	10 500	Avisé <i>et al.</i> (1988)

*Low, 1–2 offspring per year; moderate, 4–15 offspring per year; high, >> 15 offspring per year.

†Low, < 30% per year; moderate, > 30% but < 80% per year; high > 80% per year.

‡Because no species is continuously distributed our estimates should be viewed as extremely crude. This is especially true for the hardhead catfish and the American eel. Both species are distributed along the Atlantic and Gulf coast of North America. For these two species the geographical 'area' is given by the linear coastal distance (k) that their range encompassed.

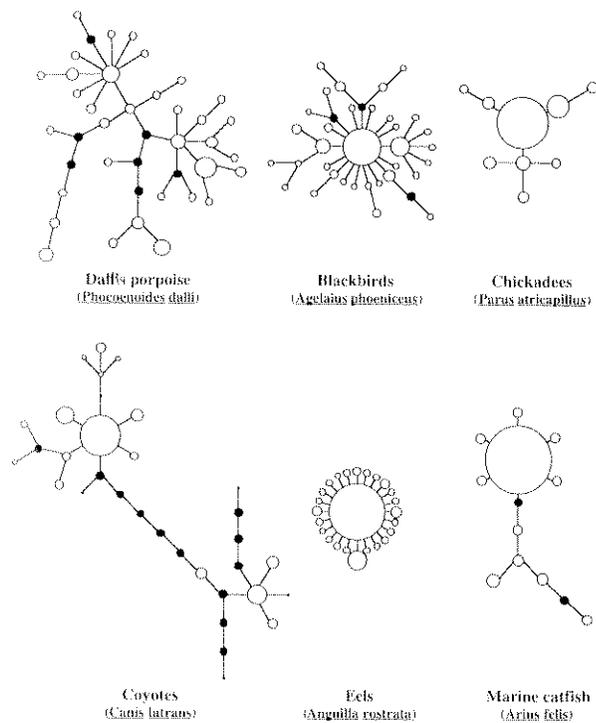


Fig. 4 Comparison of the patterning of mtDNA variation among several high dispersal vertebrate species. As in Fig. 2, parsimony networks were generated by a heuristic search by the phylogenetic package PAUP (Swofford 1991) using the assumption that a restriction site loss is twice as likely as a restriction site gain. Open circles represent observed mtDNA genotypes, the area of which corresponding to the proportion of individuals with that genotype. Each radial arm represents a single restriction site change. References for each study are cited in Table 4.

the individuals surveyed carried the common haplotype or its nearest neighbour (Lehman & Wayne 1991; Table 4). Perhaps the most interesting aspect of the coyote network was the number of missing intermediates between the two predominant clades (Fig. 4). Owing to the extensive

numerical and geographical sampling of coyotes, one was left with the impression that missing intermediates represented mtDNA lineage extinction events. Although not necessarily the case (see Saunders *et al.* 1986), these extinctions might have resulted if the two major coyote mtDNA clades had been allopatrically separated for some period, a notion reinforced by the frequency distributions of coyote mitochondrial types on either side of the Rocky Mountains.

It is possible that the observed differences in complexity of the mtDNA networks in Fig. 4 have a purely stochastic explanation. Because the mitochondrial genome is a single genetic locus, two populations can have very different genealogical patterns under the same historical demographic conditions (Saunders *et al.* 1986; Slatkin & Hudson 1991). If we assume, however, that the observed differences in complexity reflect real differences in the generation and extinction of mtDNA variation, then how might we explain the differences in the 'shape' of mtDNA genealogies across the six vertebrate species compared here?

MtDNA evolutionary rate may have varied across these species. In this respect, the more complex genealogy in the Dall's porpoise suggests that the rate of mtDNA evolution in this porpoise was high relative to these other species. However, there is no support for accelerated mtDNA evolution in Dall's porpoise. The harbour porpoise, *Phocoena phocoena*, and the Dall's porpoise represent two porpoise subfamilies (Phocoeninae and Phocoenoidinae) which appeared between five and eight million years ago according to fossil evidence (Barnes 1985). Using these fossil dates yielded an estimated porpoise mtDNA evolutionary rate (0.72%–1.15% per million years) that was roughly half the 2% per million year rate frequently proposed for vertebrate mtDNA (Wilson *et al.* 1985; Shields & Wilson 1987). The porpoise fossil record and/or evolutionary relationships may have been misinterpreted; however, it should be noted that depressed

Species	Census estimates*	Expected (generations)	Observed (generations)
Dall's porpoise	138 000	276 000	133 000–298 000 †
Chickadees	500 000	1 000 000	199 000
Coyotes	1 750 000	3 500 000	250 000
Blackbirds	20 000 000	40 000 000	199 000
Hardhead Catfish	10 000 000	20 000 000	98 000
American eel	5 000 000	10 000 000	99 000

* Census female effective population sizes were reported in the references cited in Table 4 for each species. See methods for estimation of the female census size of Dall's porpoise.

† Calculated with the porpoise-specific mtDNA divergence rate of 0.94% per million years.

Table 4 Observed and expected times to common ancestry of mtDNA genes (in generations) in several high dispersal species. Expected times to common ancestry are based on current census estimates of the number of females. Observed times to common ancestry for mtDNA haplotypes were calculated from estimates of historical population sizes generated from observed levels of intraspecific genetic variation and correcting for sample size (Tajima 1983; Wilson *et al.* 1985). For this table the divergence rate for mtDNA was assumed to be 2.0% per million years.

rates of molecular evolution in both mtDNA and nuclear genes have been observed in other cetaceans (see Hoelzel & Dover 1991; Schlötterer *et al.* 1991; Martin & Palumbi 1993). Thus, we do not believe that the 'shape' differences between the mtDNA network of the Dall's porpoise and those of these other species can be explained solely on the basis of different rates of molecular evolution. Nevertheless, mtDNA evolutionary rate differences can influence the measures of shape used here and the very 'shallow' mtDNA networks of the hardhead catfish and American eel can be explained, in part, by relatively slow rates of mtDNA evolution (see Martin & Palumbi 1993).

Our measures of 'shape' will also be influenced by demographic or evolutionary forces buffering mtDNA lineages from widespread extinction. It is obvious, for example, that restrictions in gene flow have led to the maintenance of divergent mtDNA haplotypes (e.g. freshwater fish, Bermingham & Avise 1986; and possibly coyotes, Lehman & Wayne 1991). Less obvious is whether or not the complex social dynamics of many vertebrates might also have a pronounced effect on the 'shape' of a mtDNA network. Many cetaceans form highly social groups (Bigg *et al.* 1990; Wells & Scott 1990; Ford 1991; Amos *et al.* 1991). Long-term observations of these groups have suggested a varying degree of social complexity from strong, long lasting matriarchal associations (e.g. killer whales: Bigg *et al.* 1990; Olesiuk *et al.* 1990) to more fluid and ephemeral associations among individuals (e.g. bottlenose and spinner dolphins: Wursig & Wursig 1977; Norris & Dohl 1980). In cetaceans with strong group affinity, populations might be envisioned as a mosaic of small isolated or semi-isolated, but very mobile, demographic units rather than a group of freely interbreeding individuals. Random genetic drift may act independently in each social unit, both facilitating the fixation of mtDNA lineages and buffering mtDNA lineages from species-wide extinction. This social complexity would both inflate levels of genetic diversity and lead to the complex mtDNA genealogy observed in this study (Slatkin 1987).

Differences in demographic parameters, particularly historical swings in effective population size, may underlie the observed differences in 'shape' of the genealogical networks of the Dall's porpoise relative to those of the other high dispersal species. Under neutral theory, the expected time to common ancestry for all mtDNA genes (in generations) is twice the long term female effective population size (Tajima 1983). One of the more remarkable features of mtDNA variation in chickadees, coyotes, blackbirds, catfish, and eels was the one-three order(s) of magnitude discrepancy between the observed time to common ancestry of mtDNA genes and the expected time based on current census estimates (Table 4; Avise *et al.* 1988; Lehman & Wayne 1991). The striking magnitude of this discrepancy has led many researchers to conclude that the popula-

tion sizes of these currently abundant animals were much smaller in the past (Wilson *et al.* 1985; Avise *et al.* 1988; Lehman & Wayne 1991). For coyotes, this has certainly been the case and recent expansions in population sizes are well documented (Lehman & Wayne 1991). For blackbirds and chickadees, it is likely that Pleistocene climatic cycles and the attendant reduction in suitable habitat during glacial epochs depressed population numbers (Ball *et al.* 1988; Gill *et al.* 1993). Likewise, Pleistocene reductions in sea-level led to a decrease in shallow marine habitat and were probably coupled to diminished population sizes for substrate-associated nearshore marine fish species (Shulman & Bermingham 1995). These habitat factors in combination with high variability in female reproductive success driven by high fecundity and high larval mortality probably accounted for the low effective population sizes in marine catfish and eels (Table 4; Avise *et al.* 1988).

Only in the Dall's porpoise were the observed and expected time to common ancestry of mtDNA variation similar (Table 4). This similarity suggests that this porpoise has not undergone the large swings in population size that apparently characterize chickadees, coyotes, blackbirds, catfish, and eels. It seems reasonable to propose that environmental stability may underlie the apparent constancy of population size of Dall's porpoise. Dall's porpoises live along the continental slope and in the deep waters of the North Pacific, an environment that is extremely homogeneous and stable. Day to day, season to season, and year to year variations in environmental conditions are slight, especially when compared to most terrestrial and nearshore marine environments. Moreover, this area was not adversely affected by historical environmental fluctuations. Although sea surface temperatures across this region dropped slightly during periods of global cooling, there was little change in year-round sea ice cover (CLIMAP 1976) and the Dall's porpoise range probably did not change. Thus environmental fluctuations, considered to be one of the most important mechanisms causing variation in population size (DeAngelis & Waterhouse 1987), may not have had a severe impact on Dall's porpoise population numbers. Additionally, this porpoise has several life history characteristics that should make it further resilient to extreme population fluctuations. Variation in female reproductive success is likely low. Dall's porpoises give birth to a single large, well formed progeny, which is nurtured for over a year (Kasuya 1978). As a result, juvenile mortality rates are low (Table 5). Furthermore, they maintain a wide ecological niche feeding on a large variety of meso- and bathy-planktonic vertebrates and invertebrates (Kasuya 1978; Morejohn 1979). This combination of historical and life history parameters may provide the long-term buffering capacity necessary to allow the long-term maintenance of equilibrium population conditions in this species.

Conclusions

The mtDNA data set presented here provided information on the population biology of the Dall's porpoise of interest to both resource managers and students of evolutionary biology. Slight, yet significant differences in mtDNA variation, argued for the regional distinction of Bering Sea and near-Aleutian populations. This conclusion reinforced previous biochemical and ecological studies of the Dall's porpoise across this region. However, there was no strong phylogenetic orientation of mtDNA haplotypes and nearly 97% of the variation in this species is partitioned within regional populations, indicating remarkably tight evolutionary linkage among populations of this species across this region. Similar phylogeographic patterns of extant mtDNA variation were evident in a number of broadly distributed vertebrate species with moderate to high dispersal potential. However, the more complex mtDNA network of the Dall's porpoise relative to these other vertebrates suggested important differences in the historical and ecological factors shaping mtDNA variation. One implication of these differences is that the Dall's porpoise may not have suffered the periodic swings in population size that characterize most natural populations. Thus, the Dall's porpoise may provide a rare snapshot of a gene genealogy of a species maintaining stable effective population size.

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Owen McMillan and Eldredge Bermingham met in Seattle, Washington where they began their study of Dall's porpoise. Though their pathways diverged from there (Eldredge went on to the Smithsonian's Tropical Research Institute in Panama and Owen went to graduate school in Hawaii) they have maintained a close friendship driven by common intellectual and recreational interests. Both continue to utilize molecular approaches to understand the evolutionary and population dynamics of natural populations, and are currently working in the New World tropics on birds, butterflies and fish.

Appendix 2

Polymorphic restriction site matrix for *P. dalli* as deduced from the restriction enzyme fragment patterns (see Appendix 1)

Haplotype	<i>Asp718</i>	<i>BamHI</i>	<i>BclI</i>	<i>BstEII</i>	<i>DraI</i>	<i>EcoRV</i>	<i>HindIII</i>	<i>StuI</i>	<i>XbaI</i>
1	11	011	00011	1	11	001	10	1110	1
2	11	010	00011	1	11	001	10	1110	1
3	11	001	00011	1	11	001	10	1110	1
4	11	111	00011	1	11	001	10	1110	1
5	11	011	00001	1	11	001	10	1110	1
6	11	011	00101	1	11	001	10	1110	1
7	11	011	01101	1	11	001	10	1110	1
8	11	011	00011	1	10	001	10	1110	1
9	11	011	00011	1	11	000	10	1110	1
10	11	011	00011	1	11	001	11	1110	1
11	11	011	00011	1	11	001	10	1100	1
12	11	011	00011	1	11	001	10	1111	1
13	11	011	00011	1	11	001	10	1110	0
14	01	011	00011	1	11	001	00	1110	1
15	11	010	00000	1	11	001	10	1110	1
16	11	111	00011	1	11	101	10	1110	1
17	11	011	00001	1	01	001	10	1110	1
18	11	011	10011	1	01	001	10	1110	1
19	11	011	00001	1	11	000	10	1110	1
20	11	011	00001	1	11	001	10	1010	1
21	11	011	00101	1	11	001	10	0110	1
22	11	011	00011	1	11	000	10	1111	1
23	11	010	00001	1	01	001	10	1110	1
24	11	111	00001	1	01	001	10	1110	1
25	11	011	00011	1	01	001	10	1111	1
26	11	011	00001	1	01	001	10	1110	0
27	11	011	00001	1	11	000	10	1110	0
28	11	011	00011	1	01	011	10	1100	1
29	10	011	00011	1	01	011	10	1100	1
30	10	011	00011	1	01	011	10	1100	0
31	11	001	00001	0	11	001	10	0111	1
32	11	001	00001	0	11	001	10	0110	1
33	11	001	00001	0	11	000	10	0111	1
34	11	011	00001	1	01	001	10	0111	1
Site code	ab	cde	fghij	k	lm	nop	qr	stuv	w