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Population Characteristics of DNA Fingerprints in Humpback Whales (*Megaptera novaeangliae*)

C. S. Baker, D. A. Gilbert, M. T. Weinrich, R. Lambertsen, J. Calambokidis, B. McArdle, G. K. Chambers, and S. J. O'Brien

Humpback whales exhibit a remarkable social organization that is characterized by seasonal long-distance migration (>10,000 km/year) between summer feeding grounds in high latitudes and winter calving and breeding grounds in tropical or near-tropical waters. All populations are currently considered endangered as a result of intensive commercial exploitation during the last 200 years. Using three hypervariable minisatellite DNA probes (33.15, 3'HVR, and M13) originally developed for studies of human genetic variation, we examined genetic variation within and among three regional subpopulations of humpback whales from the North Pacific and one from the North Atlantic oceans. Analysis of DNA extracted from skin tissues collected by biopsy darting from free-ranging whales revealed considerable variation in each subpopulation. The extent of this variation argues against a recent history of inbreeding among humpback whales as a result of nineteenth- and twentieth-century hunting. A canonical variate analysis suggested a relationship between scaled genetic distance, based on similarities of DNA fingerprints, and geographic distance (i.e., longitude of regional subpopulation). Significant categorical differences were found between the two oceanic populations using a multivariate analysis of variance (MANOVA) with a modification of the Mantel nonparametric permutation test. The relationship between DNA fingerprint similarities and geographic distance suggests that nuclear gene flow between regional subpopulations within the North Pacific is restricted by relatively low rates of migratory interchange between breeding grounds or assortative mating on common wintering grounds.

From the Laboratory of Viral Carcinogenesis, National Cancer Institute, Frederick, MD 21701-1013 (Baker, Gilbert, and O'Brien), the Biological Carcinogenesis and Development Program, Program Resources, Inc., National Cancer Institute, Frederick Cancer Research Facility, Frederick, Maryland (Baker and Gilbert), the Cetacean Research Unit, Gloucester, Massachusetts (Weinrich), Ecosystems Technology Transfer, Inc., University City Science Center, Philadelphia, Pennsylvania (Lambertsen), the Cascadia Research Collective, Olympia, Washington (Calambokidis), the School of Biological Sciences, University of Auckland, Auckland, New Zealand (McArdle), and the School of Biological Sciences, Victoria University, Wellington, New Zealand (Chambers). Dr. Baker is now at the Pacific Biomedical Research Center, Kewalo Marine Laboratory, University of Hawaii, 41 Ahui Street, Honolulu, Hawaii 96813, USA. We thank Drs. M. Bush, M. Dean, and N. Yuhki, and J. Martenson, I. Hermans and M. J. MacCarthy for discussions and technical assistance, and A. Perry, J. Straley, and D. Matkin for their assistance in the field. We thank A. Jeffreys for generously providing his clonal probes 33.15 and 33.6. This research was funded in part with federal funds from the Department of Health and Human Services under contract with Program Resources, Inc., and by grants from the National Geographic Society and the U.S. National Science Foundation. C. S. Baker was supported by a postdoctoral fellowship from the National Zoological Park, Smithsonian Institution, and the University Governors Council, Victoria University, Wellington, New Zealand. The content of this publication does not necessarily reflect the views or policies of these agencies. Biopsy samples were collected in territorial waters of the United States, in full compliance with a permit issued to R. H. Lambertsen and to C. S. Baker by the National Marine Fisheries Service, Department of Commerce, USA. Address reprint requests to Dr. O'Brien at the address above.

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The worldwide distribution of humpback whales is divided into three major oceanic populations by continental landmasses and the seasonal opposition of the hemispheres. Oceanic populations are further subdivided into relatively discrete subpopulations or seasonal subpopulations that are not separated by obvious geographic barriers (Mackintosh 1965). In the western North Atlantic (Katona et al. 1979; Katona and Beard 1990; Martin et al. 1984; Whitehead 1982) and the central and eastern North Pacific (Baker et al. 1986; Darling and McSweeney 1985; Perry et al. 1990), individual whales consistently migrate to one of several discrete coastal regions where they feed during summer and fall (Figure 1). The continued seasonal return of individual whales identified during their first year of life suggests that fidelity to a specific feeding ground is the result of a calf's early migratory experience (Baker et al. 1987; Clapham and Mayo 1987; Martin et al. 1984). The prolonged suckling period experienced by humpback whale calves, relative to other balaenopterid whales (Brown and Lockyer 1984), may help reinforce this maternal inheritance of migratory destinations.

During winter months humpback whales congregate to give birth and presumably to mate in shallow waters near islands or continental coastlines. In the North Atlantic, individuals from all known feeding grounds have been observed to intermingle on a single large wintering ground in the West Indies (Katona and Beard 1990; Martin et al. 1984; Mattila et al. 1989). In the central North Pacific, individuals from Alaskan feeding grounds migrate primarily to wintering grounds around the windward islands of Hawaii (Baker et al. 1986; Darling and Jurasz 1983; Darling and McSweeney 1985). In the eastern North Pacific, individuals from the central California feeding ground migrate primarily to wintering grounds along the coast of Mexico (Calambokidis et al. 1990; Urban and Aguayo 1987). Although the majority of data on migratory movement of naturally marked individuals suggests a demographic division between the central and

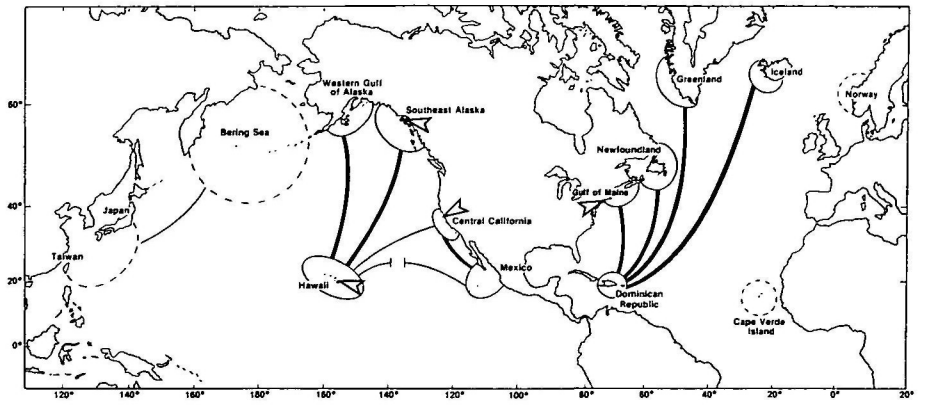

Figure 1. The migratory destinations and population structure of humpback whales in the North Pacific and western North Atlantic oceans, based on observations of marked individuals (adapted from Baker et al. 1986; Darling and Jurasz 1983; Darling and McSweeney 1985; Katona and Beard 1990; Martin et al. 1984; Nishiwaki 1967). Locations of biopsy sample collection are indicated by open arrows. Regions encircled by solid lines are defined by current observations of seasonal return by naturally marked individuals. Regions encircled by broken lines are defined by historical patterns of distribution during periods of commercial whaling. Lines connect seasonal habitats visited by individually identified whales but do not indicate migratory routes: thick lines connect regions with known strong migratory exchange, and thin lines connect regions with weak migratory exchange. The broken line connecting Hawaii and Mexico indicates the probable presence of an intervening seasonal migration by individuals sighted on both winter grounds in alternate years.

eastern components of the North Pacific population (Perry et al. 1990), important exceptions have been noted. A few whales from Alaska have been observed in Mexico, and at least one whale from central California has been observed in Hawaii (Baker et al. 1986; Perry et al. 1990). Sightings of the same individual whale on both wintering grounds have also been documented, although these occurrences are infrequent (Baker et al. 1986; Darling and Jurasz 1983; Darling and McSweeney 1985).

Humpback whales once numbered more than 125,000 individuals worldwide. Before protection by international agreement in 1966, two centuries of hunting had reduced this worldwide population to less than 5% of its estimated preexploitation size, and some regional populations of humpback whales were considered nearly extinct (Johnson and Wolman 1985). On the central California feeding grounds, for example, catch-per-unit-effort (a relative measure of abundance) declined nearly 100-fold from 1956 to 1965 (Rice 1974). Nearly 10 years after the full protection of humpback whales from hunting, Rice (1974, p. 187) concluded that "the entire eastern North Pacific stock now numbers only a few hundred individuals." After nearly 30 years of protection in most oceans, some regional populations of humpback whales have yet to show clear evidence of significant recovery in numbers.

Large stable populations that are reduced to low numbers may suffer from inbreeding depression as a result of mating between closely related individuals and subsequent loss of genetic variation (Franklin 1980; Lande 1991; Ralls et al. 1979). A slow rate of recovery will compound this loss by extending the number of generations over which genetic drift operates in small populations, although long generation times and age-structured populations can mitigate this "bottleneck" effect (Dinerstein and McCraken 1990; Nei et al. 1975). In other species of large mammals, similar population bottlenecks have been implicated in loss of heterozygosity, reduced reproductive success, and increased susceptibility to disease (Bonnell and Selander 1974; O'Brien and Evermann 1988; O'Brien et al. 1983, 1985; Packer et al. 1991b; Ralls et al. 1979). The severe depletion of humpback whales, their apparently slow rate of recovery, and uncertainties concerning other of their life history parameters are cause to consider the possible loss of genetic variation in these populations.

Here we provide an estimate of relative nuclear genetic variation in four regional subpopulations of humpback whales using hypervariable minisatellite probes that produce individual-specific patterns referred to as DNA fingerprints (Jeffreys et al. 1985a; Vassart et al. 1987). Our present study complements and extends a previous survey of geographic variation of these subpopulations using the maternally inherited genetic marker, mitochondrial (mt) DNA (Baker et al. 1990). The mtDNA survey revealed comparatively high levels of within-population nucleotide diversity and distinct nonoverlapping distributions of haplotypes between oceanic populations, as well as between two feeding grounds within the North Pacific, supporting the

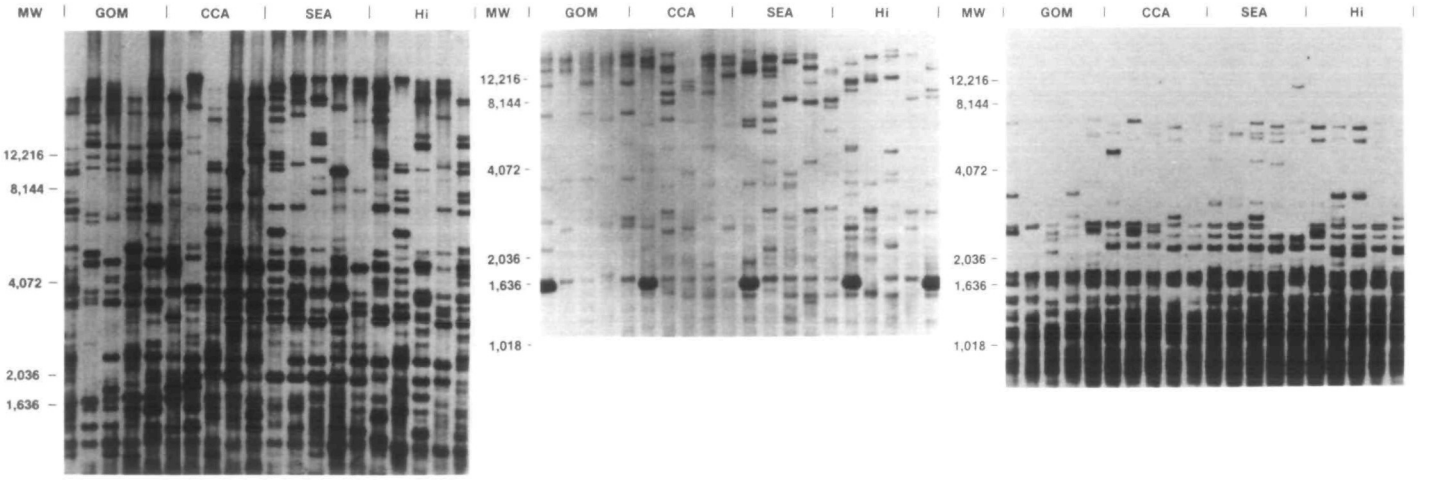

Figure 2. DNA fingerprints of five humpback whales from the Gulf of Maine (GOM), southeastern Alaska (SEA), central California (CCA), and Hawaii (HI) revealed by three molecular probes: 33.15, left panel; 3'HVR, middle panel; and M13, right panel. Position of molecular weight (MW) standards shown in left-most column of each panel. Scale of molecular weight given in number of base pairs (bp).

Table 1. Population characteristics of humpback whale DNA fingerprints based on three molecular probes

Probe (restriction enzyme)	Average no. of bands/ individual (SD)	APD[a] (SD)	Average band frequency (SD)	Average heterozygosity
North Pacific and Western North Atlantic regions ($n = 20$)[b]				
33.15 (*Hae* III)	10.6 (2.1)	84.6 (14.2)	0.15 (0.09)	0.89
3'HVR (*Alu* I)	9.8 (3.1)	83.0 (13.0)	0.14 (0.10)	0.88
M13 (*Alu* I)	7.3 (1.8)	64.5 (16.6)	0.18 (0.20)	0.72
North Pacific regions ($n = 15$)[b]				
33.15 (*Hae* III)	10.2 (2.0)	84.0 (16.0)	0.14 (0.11)	0.88
3'HVR (*Alu* I)	10.7 (2.9)	79.0 (10.0)	0.15 (0.13)	0.84
M13 (*Alu* I)	8.0 (1.4)	54.0 (10.2)	0.19 (0.25)	0.54

[a] APD = average percentage of dissimilarity (and standard deviation) for all $[n(n-1)/2]$ pairwise comparisons of individual fingerprints.

[b] n = no. of individual humpback whales sampled.

hypothesis that site fidelity is maternally directed. The extent of sexual isolation between regional supopulations, however, could not be determined from the distribution of maternal lineages alone or from available demographic data. Our present survey of multilocus DNA fingerprints confirms the presence of extensive nuclear genetic variation within subpopulations, despite their histories of near extinction, and suggests an effect of geographic distance on genetic distance between regional populations.

Table 2. Within-region variability of DNA fingerprints based on clonal probe 33.15

Region (no. of individuals)	Average band/ individual (SD)	APD (SD)	Average band frequency (SD)	Heterozygosity	mtDNA diversity[a] (%)
Gulf of Maine ($n = 8$)	14.8 (4.1)	65.0 (16.2)	0.32 (0.20)	0.71	0.196
Central California ($n = 11$)	13.2 (2.6)	72.1 (20.0)	0.24 (0.16)	0.79	0.195
Southeastern Alaska ($n = 10$)	11.1 (3.7)	76.9 (13.3)	0.22 (0.14)	0.81	0.000
Hawaii ($n = 16$)	10.3 (2.9)	83.0 (16.0)	0.17 (0.10)	0.88	0.028

[a] See Baker et al. (1990).

Methods

Biopsy Collection and DNA Extraction

We collected samples of skin tissues from free-ranging humpback whales using a crossbow and stainless steel biopsy dart (Lambertsen 1987). Samples were collected on two feeding grounds, southeastern Alaska and central California, and one wintering ground, Hawaii, in the North Pacific, and from one feeding ground in the North Atlantic, the Gulf of Maine (Figure 1). Total cellular DNA was isolated from skin tissue by homogenization in RSB buffer and digestion with proteinase K (Davis et al. 1987). The homogenate was extracted twice with neutralized phenol-chloroform-isoamyl alcohol (24:24:1) and twice with chloroform-isoamyl alcohol (24:1), then precipitated with ethanol and dissolved in TE. [Abbreviations and formulae for standard molecular reagents follow Maniatis et al. (1982) unless otherwise noted.]. The concentrations of dissolved DNA were determined from their optical density at 260 nm.

DNA Electrophoresis and Transfer

Approximately 10 μg of DNA was digested in a five- to tenfold excess of restriction enzyme (50–100 units) in a total volume of 200 μl using standard buffers provided by the manufacturers (BRL) with the addition of 1/10 volume 40 mM spermidine. A variety of 4-base cutters, including *Alu*

Table 3. The APDs (and SD) of five individuals from the Gulf of Maine (GOM), central California (CCA), southeastern Alaska (SEA), and Hawaii (HI) based on DNA fingerprints revealed by hybridization with the clonal probes 33.15, 3'HVR, M13, and all probes combined

	Region			
Region	GOM	CCA	SEA	HI
33.15				
GOM	71.3 (18.9)			
CCA	85.9 (11.8)	81.5 (17.4)		
SEA	94.6 (7.0)	78.4 (15.2)	82.3 (11.6)	
HI	84.5 (13.9	86.2 (13.7)	84.7 (14.5)	85.0 (10.8)
3'HVR				
GOM	69.6 (22.2)			
CCA	88.1 (14.4)	82.5 (13.3)		
SEA	91.8 (6.7)	80.9 (9.5)	78.0 (9.5)	
HI	89.65 (9.5)	79.1 (9.4)	77.8 (11.6)	74.6 (10.9)
M13				
GOM	71.6 (15.3)			
CCA	75.2 (12.4)	56.6 (6.9)		
SEA	81.1 (9.6)	54.4 (12.4)	51.5 (6.9)	
HI	80.5 (6.7)	54.2 (10.8)	52.7 (12.1)	49.3 (13.8)
All probes combined				
GOM	70.4 (13.3)			
CCA	83.2 (6.9)	74.7 (11.6)		
SEA	89.7 (5.1)	73.0 (8.8)	73.7 (4.7)	
HI	83.7 (5.7)	73.8 (7.9)	72.9 (6.9)	70.3 (3.6)

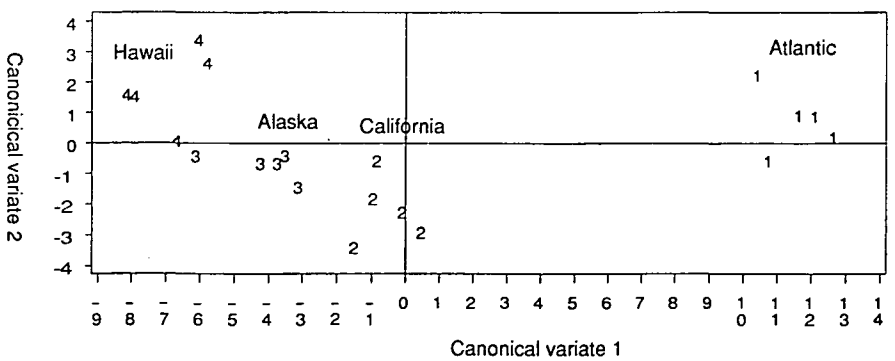

Figure 3. Canonical discriminant analysis on the four populations of humpbacks. The scaled genetic distance, based on a canonical variate analysis, between M13 fingerprints from five humpback whales from the Gulf of Maine (Atlantic), central California, southeastern Alaska, and Hawaii (see Figure 2). The percentage difference matrix of M13 fingerprints was transformed into vectors of principal coordinate scores that defined the position of the individual samples in multivariate space. The multivariate space was then rotated to maximize the between-region variation relative to within-region variation. The relative position of each individual was plotted along the first two canonical variates, and the axes were scaled so that one unit equals the average within-region standard deviation.

I, *Hae* III, *Hinf* I, and *Mbo* I, were tested for optimum resolution of fragments. The most interpretable patterns of restriction fragments were generated by digestion with the restriction enzyme *Hae* III for 33.15 fingerprints and *Alu* I for 3'HVR and M13 fingerprints. Digested DNA was extracted once with neutralized phenol-chloroform-isoamyl alcohol (24:24:1) and once with chloroform-isoamyl alcohol (24:1), then precipitated with ethanol and dissolved in running buffer. This procedure stopped the restriction enzyme activity, removed excess salt, and concentrated the DNA for loading in thin (1 × 16 mm) wells. To accurately measure the size of restriction fragments during autoradiography, 10.0 ng of "cold" molecular weight marker (*Bst*E II cut lambda DNA) was added to each sample before electrophoresis.

We separated restriction enzyme–digested DNA according to molecular weight by electrophoresis in 0.7% to 1.0% agarose gels and TAE buffer. Gels were run at 40–70 volts for 20–36 h with recirculating buffer. Gels were stained with ethidium bromide and photographed under UV light, then depurinated in 0.25 N HCl for 10 min, denatured in 0.5 M NaOH, 3 M NaCl for 30 min, and neutralized in 0.5 M Tris, 3 M NaCl for 20 min. We transferred DNA to nylon membrane (Amersham "Hybond N" or Gelman "Biotrace") by Southern blotting in 10× SSC overnight. DNA was bonded to the nylon membrane by baking at 80°C for 2 h.

DNA Hybridization

We tested three molecular probes known to reveal multiple hypervariable systems in other species on humpback whales: 33.15 (Jeffreys et al. 1985a), 3'HVR (Fowler et al. 1988), and wild-type M13 (Georges et al. 1988). A fourth, 33.6 (Jeffreys et al. 1985a), was tested but produced only a limited number of faint though variable bands. The excised double-stranded insert of each clonal probe was radioactively labeled by primer extension (Amersham) and hybridized to filters in 0.25 M $NaPO_4$, 7% SDS, 10 mM EDTA, and 1% BSA (Church and Gilbert 1984) overnight at 58°C (3'HVR), 62°C (M13), or 64°C (33.15 and 33.6). We washed filters at a final stringency of 0.1× SSC, 0.5% SDS at 50°C, and autoradiographed them for 3–7 days at −70°C with one intensifying screen. Filters were stripped by gentle agitation in 0.4 M NaOH at 42°C for 30 min, followed by neutralization in 0.1× SSC, 0.5% SDS, 0.2 M Tris (pH 7.5). We hybridized stripped filters with alternate fingerprinting probes or with bacteriophage lambda DNA for calibration of band-sharing indices.

Table 4. 33.15 fingerprint

	Region									
	GOM					CCA				
Band	1	2	3	4	5	6	7	8	9	10
1	0	0	0	0	0	0	1	0	0	0
2	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	1	0
5	1	1	0	0	1	0	0	1	0	1
6	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	1	0	0	0	1
9	1	1	0	0	1	0	0	0	0	0
10	0	0	1	1	0	0	0	0	0	0
11	0	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	1	0	0	0
14	0	0	0	0	0	0	0	1	1	0
15	1	0	1	1	1	0	0	0	0	0
16	0	1	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	1	1	0
18	0	1	0	0	0	0	0	0	0	0
19	0	1	0	0	1	1	0	0	1	1
20	0	0	0	0	0	1	0	0	0	1
21	0	0	0	0	0	1	0	0	0	1
22	0	1	0	0	1	0	0	0	0	0
23	0	0	1	0	0	0	0	0	0	0
24	1	0	0	1	0	0	0	0	0	0
25	0	0	0	0	0	0	1	0	1	0
26	0	1	0	0	1	0	0	0	1	0
27	0	0	1	0	0	1	0	0	0	1
28	0	0	0	1	1	0	0	1	1	0
29	0	1	0	0	1	0	0	0	0	0
30	0	0	1	1	0	0	0	1	0	0
31	1	0	0	0	0	0	0	0	1	0
32	0	0	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0	0	0
34	1	0	0	1	0	0	1	1	0	1
35	1	0	0	1	0	0	0	1	0	0
36	0	0	0	0	0	0	0	0	1	0
37	0	0	0	0	1	1	0	1	0	0
38	0	0	0	1	0	0	0	0	0	0
39	1	0	0	0	0	0	0	0	0	0
40	0	1	0	0	1	1	0	0	0	0
41	1	0	1	1	0	0	0	0	0	0
42	0	1	0	0	1	1	0	0	0	0
43	0	0	0	0	0	0	0	1	0	0
44	0	0	0	1	0	0	0	0	0	0
45	0	0	1	0	0	0	0	0	0	0
46	0	0	0	0	0	0	1	0	0	0
47	0	0	0	0	0	0	0	0	1	0
48	0	1	1	0	1	1	0	0	0	0
49	0	0	0	0	0	0	0	0	0	1
50	0	0	0	0	0	1	0	0	0	1
51	1	0	0	0	0	0	1	0	0	0
52	0	0	0	0	0	1	0	0	1	1
53	0	0	0	0	0	0	0	0	0	0
54	1	0	1	0	1	0	0	0	0	0
55	0	0	0	0	0	0	0	0	0	0
56	0	0	0	0	0	1	0	0	0	0
57	0	0	0	0	0	0	0	0	0	0
58	0	0	0	0	0	0	0	0	1	0
59	0	0	0	0	0	0	0	0	0	0
60	0	0	0	0	0	0	0	1	0	0

Fingerprint Analysis

We scored DNA fingerprints for alignment from original autoradiographs and checked them for alignment and relative band intensity using a Molecular Dynamics densitometer. Autoradiographs of probed control lambda DNA were used to evaluate potential differences in migration characteristics of DNA in each lane. A restriction fragment was scored as shared by two individuals if the autoradiographic bands differed by less than 1 mm in alignment and twofold in intensity. Bands less than 2 kb in size were not scored.

A pairwise index of the percentage difference (PD) between DNA fingerprints

Table 4. Extended

Region									
SEA					HI				
11	12	13	14	15	16	17	18	19	20
0	0	0	1	0	0	0	0	0	0
0	1	1	0	0	0	1	0	0	0
0	0	0	0	0	1	0	0	0	0
1	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	1	0
0	0	0	0	1	0	0	0	1	0
1	0	1	0	0	0	0	0	0	0
0	0	0	0	0	0	0	1	0	1
0	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0	0
0	0	0	1	0	0	0	0	0	0
1	0	0	0	0	1	0	0	0	0
0	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	1	0
1	0	0	0	1	1	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	1	0	0	0	0	1	0	1
0	0	1	0	1	0	0	0	0	1
0	0	1	0	0	0	0	0	0	0
0	0	0	0	0	0	0	1	0	1
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	1	0	0	0	0
1	0	0	0	0	1	0	0	0	0
0	0	1	0	0	0	0	0	0	0
1	1	0	0	0	1	1	0	1	1
1	0	0	0	0	0	0	1	0	0
0	0	0	0	0	0	1	0	0	0
0	0	0	1	0	0	0	0	0	0
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1	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	1	0	0	0	0	0	0
0	0	0	0	0	0	0	1	0	0
0	0	0	0	0	0	0	0	0	1
0	0	0	0	1	0	0	0	0	0
0	1	0	1	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	1	0	1	0	0	0	0	0	0
0	0	0	0	0	0	0	1	0	1
0	0	0	0	0	0	0	0	0	0
0	0	0	1	0	0	1	0	0	0
0	0	0	0	0	0	0	0	0	0
1	1	0	0	0	1	0	0	0	0
0	0	1	0	0	0	0	0	0	1
0	0	0	0	0	0	0	0	1	0
0	0	0	0	0	0	0	0	0	0

was calculated as PD = $(F_{ab}/(F_a + F_b) \times 100$, where F_a and F_b are the number of scorable bands observed in the fingerprints of whales *a* and *b*, and F_{ab} is the number of bands that differ between two fingerprints (Gilbert et al. 1990). This index is equivalent to [100(1 − D)] calculated according to Wetton et al. (1987), [100(1 − *x*)] calculated according to Jeffreys et al. (1985b) for unrelated individuals, and [100(1 − S_{xy})] calculated according to Lynch (1990). Population average percentage differences (APD) and standard deviations were used for within- and between-region comparisons of fingerprints (Gilbert et al. 1990). APDs were calculated only from samples run on the same gel, limiting comparisons to a maximum of 20 individuals. Heterozygosity within populations was estimated as

$$H = \frac{\sum_{k=1}^{A} s_k}{A - \sum_{k=1}^{A} \sqrt{1 - s_k}} - 1,$$

using equation 5 from Stephens et al. (1992), where s_k is the frequency of occurrence of the *k*th band and *A* is the total number of scorable bands on the gel. Calculations of APDs and heterozygosity were facilitated by the Macintosh-based computer program THUMBPRINT (Marshall 1992).

The relationship between genetic distance, as measured by the pairwise band-sharing matrix of DNA fingerprints, and geographic distance was described using principal coordinate and canonical variate analyses (e.g., canonical discriminate function analysis, Campbell and Atchley 1981). The principal coordinate analysis transformed the matrix of pairwise differences (PDs) into vectors of principal coordinate scores that defined the position of the individual animals in multivariate space. These vectors were subjected to a canonical variate analysis that rotated the multivariate space to allow the differences between the four subpopulations to be displayed in the minimum number of dimensions (i.e., canonical axes). The canonical axes were rescaled so that one unit was equal to the average within-population standard deviation of the pairwise distances. The relative positions of the four regions were then displayed on the first two canonical axes, and their relationship was examined for concordance with the geographic distance.

We tested the statistical significance of between-region differences in DNA fingerprints by multivariate analysis of variance (MANOVA) using a nonparametric permutation test based on the Mantel procedure (Mantel 1967; McArdle 1990; Smouse et al. 1986). This generates the null distribution of Pillai's trace, a multivariate test statistic (Kshirsagar 1972), from random permutations of the pairwise difference matrix, holding sample size equal and assigning individuals to regional membership at random (McArdle 1990). The null hypothesis of no difference between the regions was rejected if less than 5% of the null distribution was more extreme than the observed value of the Pillai's trace. The MANOVA and permutation procedures are analogous statistically to the analysis of molecular variance (AMOVA) procedure of Excoffier et al. (1992), except that the MANOVA attempts to cor-

rect for the internal correlation structure of the population by multivariate analysis.

Results

General Characteristics

Hybridization of humpback whale DNA with the 33.15, M13, and 3'HVR minisatellite probes revealed complex patterns of restriction fragments similar in number and variability to those reported for humans, birds, and other animals (Figure 2). The positions of scorable bands observed with the 33.15, M13, and 3'HVR fingerprinting probes were nonoverlapping and likely represented independent sets of minisatellite loci since the core sequences of these three probes are distinct (Fowler et al. 1988; Georges et al. 1988; Jeffreys et al. 1985a,b). These characteristics make hypervariable gene families useful for monitoring patterns of nuclear genetic variation in free-ranging populations (Burke and Bruford 1987; Gilbert et al. 1990; Packer et al. 1991a; Triggs et al. 1991; Wetton et al. 1987).

The variability and band-sharing characteristics of 33.15, 3'HVR, and M13 fingerprints were evaluated using a sample of five unrelated whales from each of the three North Pacific regions and the one North Atlantic region (Table 1). Band-sharing characteristics were also calculated for the subset of 15 whales from North Pacific regions only. This allowed an estimate of the variability found within an oceanic population in relation to that found in the combined transoceanic sample. High levels of variability were observed in both 33.15 and 3'HVR fingerprints. With both probes the estimated mean population frequency of resolvable alleles was extremely low (<15%) and corresponding average heterozygosity was high (0.88–0.89). The lowest variability was observed in M13 fingerprints, apparently reflecting the hybridization of this probe with a more conserved set of minisatellite loci than those revealed by the other two probes. Restricting the comparisons to the North Pacific regions resulted in little change to the APD of the 33.15 fingerprints but produced a marked decrease in the APD of the M13 fingerprints.

Within-Region Comparisons

Genetic variability within regional populations was examined further using the 33.15 DNA fingerprint probe and an independent set of gels with additional whales chosen at random from the available samples (Table 2). All regions showed a high degree of variability as indicated by their APDs and average heterozygosity. Individuals from the Gulf of Maine showed the largest number of scorable bands and the lowest variability of 33.15 fingerprints as indicated by the lowest APD. Regional samples from the North Pacific showed slightly fewer scorable bands but more variability than the Gulf of Maine sample. A comparison of within-region APD of 33.15

Table 5. 3'HVR fingerprint

	Region									
	GOM					CCA				
Band	1	2	3	4	5	6	7	8	9	10
1	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	1	0	1	0	0	0
3	1	0	0	0	0	0	0	0	1	0
4	0	1	0	0	0	1	0	0	0	1
5	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	1	0	1	0	0
7	0	0	0	0	1	0	0	0	0	0
8	0	0	0	0	0	1	0	0	0	1
9	1	1	1	0	0	0	1	0	1	0
10	0	0	0	0	0	0	0	0	0	0
11	0	0	0	0	0	0	1	0	0	0
12	0	0	0	0	0	0	0	0	0	0
13	0	0	0	0	0	0	0	0	0	1
14	0	0	0	0	0	0	0	0	0	0
15	0	0	1	0	1	0	0	1	0	0
16	1	0	0	0	0	0	1	0	1	0
17	0	0	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	1	0	0
19	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	1	0	0
21	0	0	0	0	0	0	1	0	1	0
22	0	0	0	0	0	0	1	0	0	0
23	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0	0	0
26	1	0	1	0	1	0	0	0	0	0
27	0	0	0	1	0	0	0	0	0	0
28	0	0	0	0	0	0	1	0	0	0
29	0	0	0	0	0	0	0	1	0	0
30	0	0	0	0	0	1	0	0	0	0
31	0	0	0	0	0	0	0	0	0	1
32	0	0	0	0	0	0	0	0	0	0
33	0	0	0	0	0	0	0	0	0	0
34	0	0	0	0	0	0	0	0	0	0
35	0	1	1	1	1	0	0	0	0	0
36	0	0	0	0	0	0	1	0	1	0
37	0	0	0	1	0	0	0	0	0	0
38	1	0	1	0	0	1	0	0	0	0
39	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0
41	0	0	0	1	0	0	0	0	0	0
42	0	0	0	0	0	1	0	0	0	0
43	0	0	0	0	0	0	0	1	0	0
44	0	1	1	0	1	0	0	0	0	0
45	0	0	0	0	0	0	0	0	0	0
46	1	0	0	0	0	1	0	0	1	0
47	0	0	0	0	0	0	1	0	0	0
48	0	0	0	0	0	0	0	0	0	0
49	0	0	0	0	0	0	0	0	0	0
50	0	0	0	0	0	0	1	0	0	0
51	0	0	0	0	0	0	0	0	0	0
52	0	1	1	0	1	1	1	0	0	0
53	1	0	0	0	0	0	0	0	1	0
54	0	0	0	0	0	0	0	1	0	0
55	0	0	0	0	0	0	0	0	0	0
56	0	0	0	0	0	0	0	0	0	0
57	0	0	0	1	1	0	0	0	0	0
58	0	0	0	0	0	1	0	0	0	0
59	0	1	0	0	0	0	0	0	0	0
60	0	0	0	0	0	0	0	1	0	1
61	0	0	0	0	0	0	1	0	0	0
62	0	0	0	0	0	0	0	0	0	0
63	0	0	0	0	0	0	0	0	0	0
64	1	0	0	0	0	1	0	0	0	0
65	0	0	0	0	0	0	0	0	0	0
66	0	0	0	0	0	0	1	0	0	0
67	0	0	0	0	0	0	0	0	0	0
68	0	0	0	0	0	1	0	1	1	1

Table 5. Extended

Region									
SEA					HI				
11	12	13	14	15	16	17	18	19	20
1	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	1	1	0	0	0	1	0	0
0	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0
0	0	0	1	0	0	1	1	1	1
0	1	0	0	0	0	0	0	0	0
1	0	1	0	0	0	0	0	0	0
0	0	0	0	0	1	0	0	0	0
1	0	0	1	0	0	0	0	0	1
0	1	0	0	0	0	0	0	0	0
1	0	0	0	1	0	0	0	0	1
0	0	0	0	1	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	1	0	0	0
0	0	0	0	0	0	0	1	1	0
0	0	0	0	0	1	1	0	0	0
0	0	1	1	0	0	0	0	1	0
0	0	0	0	0	1	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	0	0	0	1
0	1	0	0	1	0	0	0	1	1
0	0	0	1	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0
0	0	0	0	1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
1	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	1	0	0
0	0	0	0	0	0	0	0	0	1
0	1	0	0	1	0	0	0	0	0
0	0	0	1	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	0	0	0	0
0	0	0	0	0	1	0	0	0	0
0	0	0	0	0	0	0	1	0	0
0	0	0	0	1	0	0	0	0	0
0	1	0	1	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	1	0	0	1	0	0	0	0
0	1	0	0	1	0	0	1	0	0
1	1	0	0	1	1	0	1	0	0
0	0	1	0	0	1	1	0	0	0
0	0	1	0	0	0	0	0	0	1
0	0	0	0	0	1	0	0	0	0
0	1	0	0	1	0	0	1	0	0
0	0	0	1	0	0	1	0	0	0
0	0	0	0	0	0	0	0	1	1
0	0	0	0	0	0	0	0	0	0
1	1	1	0	0	1	0	0	0	0
0	0	0	1	1	0	0	0	0	0
0	0	0	0	0	0	0	1	0	0
0	0	0	0	0	0	0	0	0	1
0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0
0	1	1	0	0	0	0	0	0	0
0	0	0	1	1	1	0	1	1	1
1	0	0	0	0	0	1	0	0	0
0	0	0	0	0	1	1	0	0	0
0	0	0	0	0	0	0	0	0	0
1	1	0	0	0	1	0	1	0	0
0	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	1	0	0	0
1	0	1	1	1	1	1	1	0	1

fingerprints and genetic diversity of mtDNA based on RFLP analysis (Baker et al. 1990) suggested no obvious relationship between these measurements of genetic variability. The Gulf of Maine showed the greatest mtDNA diversity and the lowest variability of 33.15 fingerprints, while southeastern Alaska and Hawaii showed low mtDNA diversity but considerable variability in 33.15 fingerprints.

Between-Region Comparisons

The extent of genetic partitioning between regional populations was evaluated initially using within- and between-region APDs calculated from the fingerprints shown in Figure 2 (Table 3). The additional samples from the within-region comparison of 33.15 fingerprints were not included since these were run on independent gels. The highly variable systems of restriction fragments in 33.15 and 3'HVR fingerprints yielded within-region APDs ranging from 70% to 85%, differing only slightly from between-region APDs and from the overall APD of 83%–84.6% calculated for the combined oceanic samples shown in Table 1. Only the APD between the Gulf of Maine and southeastern Alaska yielded a difference that exceeded the standard deviation of the within-region comparisons for both probes.

The more conserved pattern of restriction fragments observed in M13 fingerprints proved more informative to the analysis of geographic variation. Differences between the two oceanic populations were suggested by large APDs and the presence of a band that appeared to be invariant (i.e., fixed) in the North Pacific and absent in the western North Atlantic (Figure 2, approximately 2,200 bp in size). Regional differences within the North Pacific oceanic population were less obvious. Within- and between-region APDs did not vary consistently, and no fixed differences in band frequency were observed.

The relationship between geographic distance and genetic distance as calculated from M13 fingerprints was examined further using a canonical variate analysis. The first two axes of the canonical variate analysis accounted for 77% of the variation between the fingerprints and suggested a striking relationship between geographic distance and the transformed canonical scores of each sample (Figure 3). The Gulf of Maine samples cluster approximately 12 units (i.e., standard deviations) from central California, 15 units from southeastern Alaska, and 19 units from Hawaii, roughly proportional to the longitudinal centers of each region at 68°, 122°, 134°, and 158° west (Figure 1). The relative positions of regional samples along the first canonical variate showed a nearly perfect rank correlation with longitude (Spearman correlation coefficient = $-.897$; $P < .001$).

The significance of the categorical differences between regions was tested by performing a MANOVA on 2,000 random permutations of the PD matrix and com-

puting the resulting Pillai's trace statistic [i.e., a modified Mantel test (McArdle 1990)]. The results indicated that differences between the Gulf of Maine and the three North Pacific regions were unlikely to be attributable to chance alone (P = .0025). Among the North Pacific regions, however, sample size was not sufficient to reject the null hypothesis of random assortment despite the apparent relationship between genetic and geographic differences suggested by the canonical variate analysis.

Discussion

Genetic Variability

The nuclear DNA of humpback whales displays a wealth of variation as revealed by hybridization with available clonal probes from minisatellite regions of human (33.15 and 3'HVR) and M13 phage DNA. The extent of variability observed in 33.15 (Table 4) and 3'HVR (Table 5) fingerprints of humpback whales equals or exceeds that reported previously in humans (Jeffreys et al. 1985b), domestic mammals (Georges et al. 1988), and birds (Burke and Bruford 1987; Hill 1987). These observations contrast with the low levels of variability reported for cetaceans in other genetic systems, including protein allozymes (Sharp 1981; Simonsen et al. 1982; Wada 1983; Wada and Numachi 1991; Winans and Jones 1988), major histocompatibility complex loci (Trowsdale et al. 1989), nuclear regions adjacent to minisatellite repeats (Schlotterer et al. 1991), and mtDNA (Baker et al. 1990, in press; Hoelzel and Dover 1991; Southern et al. 1988). DNA fingerprints of humpback whales also appear more variable than those observed using similar or identical minisatellite probes with other mysticete whales (Amos and Dover 1991; Amos and Hoelzel 1990; van Pijlen et al. 1991). Further characterization of cetacean minisatellite systems is needed to determine if these differences are due to species-specific processes at the molecular level (Dover 1982, 1987; Jarman and Wells 1989) or to population-specific processes resulting from ecological or demographic forces (Gilbert et al. 1990; Packer et al. 1991a).

Despite these whales being reduced to near extinction in many regions, the existing variability of their DNA fingerprints suggests that humpback whale populations have not experienced the prolonged or repeated periods of inbreeding that may have affected geographically isolated populations of terrestrial animals, such as the Channel Island fox (Gilbert et al. 1990), the Gir lion (Gilbert et al. 1991), and the Isle Royale wolf (Wayne et al. 1991), or insular populations of marine mammals such as the Northern elephant seal (Bonnell and Selander 1974). Comparative surveys of DNA fingerprint variation in other mysticete species with different mating systems and different histories of exploitation may provide further insight into the interacting factors that influence the recovery of endangered species.

Table 6. M13 fingerprint

	Region									
	GOM					CCA				
Band	1	2	3	4	5	6	7	8	9	10
1	0	0	0	0	0	0	0	1	0	0
2	0	0	0	0	0	0	0	0	0	1
3	0	0	1	1	1	0	0	0	0	0
4	0	0	0	0	0	0	1	0	0	0
5	1	0	0	0	0	1	0	0	0	0
6	0	0	0	0	0	0	0	1	0	0
7	0	0	0	0	0	0	0	0	1	0
8	0	0	0	0	0	1	0	0	0	0
9	1	0	0	0	0	1	0	1	0	1
10	0	0	0	0	1	0	0	0	0	0
11	1	1	1	0	0	0	0	1	0	0
12	0	0	0	0	0	0	0	0	1	0
13	0	1	1	0	0	0	0	0	0	1
14	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0	0
17	0	0	0	0	0	1	0	0	0	0
18	0	1	0	0	0	1	0	0	0	0
19	0	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	1	0	0	0
23	0	0	0	0	0	0	0	0	0	0
24	0	0	0	0	1	0	0	0	0	0
25	1	0	0	1	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0	0	0
27	0	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	1	0	0	0	1
29	0	0	0	0	0	0	0	0	0	0
30	0	0	0	1	0	0	0	0	1	0
31	1	1	1	0	1	1	1	1	1	1
32	0	0	0	1	0	0	0	0	0	0
33	1	0	0	0	1	0	0	1	1	0
34	0	0	1	0	0	0	0	0	0	0
35	0	0	0	1	0	1	1	0	1	1
36	0	0	1	0	0	0	0	0	0	0
37	0	0	0	0	0	0	1	1	0	0
38	0	0	0	0	0	1	1	1	1	1
39	0	0	0	0	0	0	0	0	0	0
40	0	0	1	0	0	0	0	0	0	0

Population Structure

The analysis of population subdivisions suggested a positive relationship between genetic distance, as measured by the APD of DNA fingerprints, and geographic distance. For 33.15 and 3'HVR fingerprints, this analysis was complicated by the very low levels of band sharing (i.e., high APDs for both within- and between-region comparisons). The more conserved minisatellite loci observed in the M13 (Table 6) fingerprints, however, allowed a significant categorical discrimination between oceanic populations and suggested a relationship between geographic distance and genetic distance among regional populations in the North Pacific based on multivariate analysis. The observed relationship between genetic and geographic distance suggests that the North Pacific population is not a single panmictic unit at the nuclear genetic level despite the interchange of some individuals between breeding grounds (Baker et al. 1986; Darling and McSweeney 1985; Perry et al. 1990). Instead, nuclear gene flow between regional subpopulations may be restricted by relatively low rates of interchange between breeding grounds or assortative mating on common wintering grounds.

The presence of subdivisions in the North Pacific humpback whale population is supported by other genetic and demographic evidence. Maternal lineages, as reflected in mtDNA haplotypes, are strictly segregated between southeastern Alaska

Table 6. Extended

Region									
SEA					HI				
11	12	13	14	15	16	17	18	19	20
0	0	0	1	1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	1	0	0	0	0	0
0	0	0	0	0	0	0	0	1	0
0	0	1	0	0	0	0	0	0	0
1	0	0	1	0	1	1	0	0	0
0	0	0	0	0	0	0	1	0	0
0	0	0	0	0	0	0	0	0	0
1	1	1	1	1	0	0	0	0	1
1	0	0	0	0	0	0	0	1	0
0	0	1	0	0	0	1	0	0	0
1	0	0	0	0	1	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	1	0	0	0	0	0	0
0	0	0	0	0	0	0	0	1	0
0	0	0	0	0	1	0	0	0	1
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0	0
0	0	0	1	0	0	0	0	0	1
0	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0	1
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	1	1	0	0
0	1	0	0	0	0	0	0	0	0
1	0	1	0	1	1	0	1	1	0
0	1	0	0	1	1	1	0	0	1
0	0	1	0	0	0	1	0	0	1
1	1	1	0	0	1	1	1	1	1
0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	1	0	0	0
0	0	0	0	0	0	0	0	0	0
1	1	1	1	1	1	1	1	1	1
0	0	0	0	1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0
1	1	1	1	1	1	1	1	1	1
0	0	0	0	0	0	0	0	0	1
0	0	0	0	0	0	1	1	0	0

and central California feeding grounds (Baker et al. 1990). A low level of mixing between central California and southeastern Alaska mtDNA haplotypes on the Hawaiian wintering ground indicates limited interchange between the eastern and central components of the North Pacific population (Baker et al. 1986). A clinal pattern of variation in the fluke coloration of North Pacific humpback whales is similar to that revealed by the canonical variate analysis of M13 fingerprints. It is also interesting to note that the relative position of the Hawaiian samples on the first canonical variate is not intermediate between the two summering grounds, southeastern Alaska and central California, but appears to be influenced by other unrepresented feeding regions. This interpretation is consistent with the known migration to Hawaii of humpback whales from the Gulf of Alaska and other feeding grounds to the west of southeastern Alaska (Baker et al. 1986, 1990; Darling and McSweeney 1985).

The multivariate analysis of M13 fingerprint similarities suggests a general strategy for testing sociobiological and ecological hypotheses concerning genetic relatedness of individuals or populations. The combined use of principal coordinate analysis to transform the percentage difference matrix from points in multivariate space to principal coordinate scores and the subsequent canonical variate analysis provided a sensitive display of scaled genetic distance between subpopulations. The use of MANOVA and the modified Mantel procedure to derive a null distribution of the test statistic from random permutations of the PD matrix offers a robust nonparametric test of categorical differences in DNA fingerprints (McArdle 1990). The modified Mantel test can be applied to any analysis involving pairwise distance or similarity matrices (e.g., Excoffier et al. 1992; Smouse et al. 1986; Stoneking et al. 1990) and avoids many of the assumptions of both traditional genetic theory and parametric statistics that have confounded previous statistical analyses of DNA fingerprints (e.g., Lynch 1988, 1990).

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