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Article *in* Nature · April 1990

DOI: 10.1038/344238a0 · Source: PubMed

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Influence of seasonal migration on geographic distribution of mitochondrial DNA haplotypes in humpback whales

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HUMPBACK whales (*Megaptera novaeangliae*) migrate nearly 10,000 km each year between summer feeding grounds in temperate or near-polar waters and winter breeding grounds in shallow tropical waters[1]. Observations of marked individuals suggest that major oceanic populations of humpback whales are divided into a number of distinct seasonal subpopulations which are not separated by obvious geographic barriers[2,3]. To test whether these observed patterns of distribution and migration are reflected in the genetic structure of populations, we looked for variation in the mitochondrial DNA of 84 individual humpback whales on different feeding and wintering grounds of the North Pacific and western North Atlantic oceans. On the basis of restriction-fragment analysis, we now report a marked segregation of mitochondrial DNA haplotypes among subpopulations as well as between the two oceans. We interpret this segregation to be the consequence of maternally directed fidelity to migratory destinations.

Using a small biopsy dart[4], skin samples were collected from free-ranging humpback whales on two feeding grounds, southeastern Alaska ($n = 20$) and central California ($n = 20$), and on a single wintering ground, Hawaii ($n = 16$) in the North Pacific, and on a single feeding ground, the Gulf of Maine ($n = 28$) in the North Atlantic (Fig. 1). Total cellular DNA was extracted from the skin samples and used for restriction-fragment analysis following Southern blotting and hybridization with a radioactively labelled molecular clone of porpoise mitochondrial (mt) DNA (Fig. 2). We scored a total of 81 to 85 restriction fragments per individual, surveying an average of 468 nucleotides, or 2.84% of the ~16,500 base pairs in the humpback whale mtDNA. The simple structure of the restriction-fragment length patterns allowed us to infer the presence of 88 independent restriction sites, of which 9 were polymorphic. The sex of each individual was identified by Southern hybridization of *Eco*RI-restricted total cellular DNA with the clone pDP1007, which is derived from the human Y chromosome[5].

TABLE 1 Nucleotide divergence (%) of mtDNA within and between subpopulations of humpback whales

	SEA	HI	CCA	GOM
Southeastern Alaska (SEA)	**0.000**	0.000	0.176	0.282
Hawaii (HI)	0.014	**0.028**	0.156	0.262
Central California (CCA)	0.274	0.267	**0.195**	0.112
Gulf of Main (GOM)	0.380	0.374	0.308	**0.196**

Diagonal shows nucleotide diversity within a geographic region. Values below the diagonal are genetic distances between subpopulations uncorrected for within-region divergence. Values above the diagonal show genetic distances between regions after correcting for within-region divergence. All values were derived using the computer program MAXLIKE, courtesy of M. Nei and L. Jin, Center for Demographic and Population Genetics, University of Texas.

Among the 84 individuals surveyed, we found 12 mtDNA haplotypes that differed from one another by between 1 and 7 restriction sites. The average nucleotide divergence (that is, π, nucleotide diversity[6]) among all individuals was 0.248% (s.d. = 0.0151%) and ranged from 0.00% to 0.196% within each geographic subpopulation (Table 1). Genetic distance (d, the divergence between subpopulations after adjustment for within-subpopulation diversity[6]) between subpopulations indicated that southeastern Alaska and Hawaii are essentially identical to one another and diverged from the Gulf of Maine by 0.26-0.28%; California is intermediate between the Gulf of Maine and Hawaii/southeastern Alaska, diverging from each by 0.11-0.18%. There were no significant biases in the geographic distribution or haplotype frequencies of the sexes (C.S.B. *et al.*, manuscript submitted).

A topological network of restriction-site changes generated by maximum parsimony showed a striking agreement between the geographic distribution of haplotypes and previously reported patterns of migratory movement (Fig. 3). There was no overlap in haplotypes from the two oceanic populations, or

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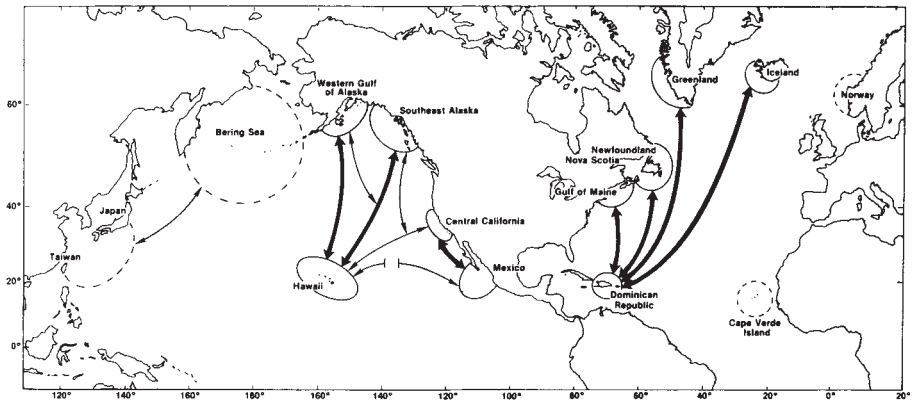

FIG. 1 The migratory destinations and population structure of humpback whales in the North Pacific and western North Atlantic oceans, based on observations of marked individuals[2,3,7,23-26]. Regions encircled by a solid line are defined by current observations of seasonal return by naturally marked individuals. Regions encircled by a broken line are defined by historical patterns of distribution during periods of commercial whaling. Arrows connect seasonal habitats visited by individually identified whales but do not necessarily indicate migratory routes. Thick arrows connect regions with known strong migratory interchange and thin arrows connect regions with weak migratory interchange. The broken line connecting Hawaii and Mexico indicates the probable presence of an intervening seasonal migration to a feeding ground by individuals sighted on both winter grounds in alternate years.

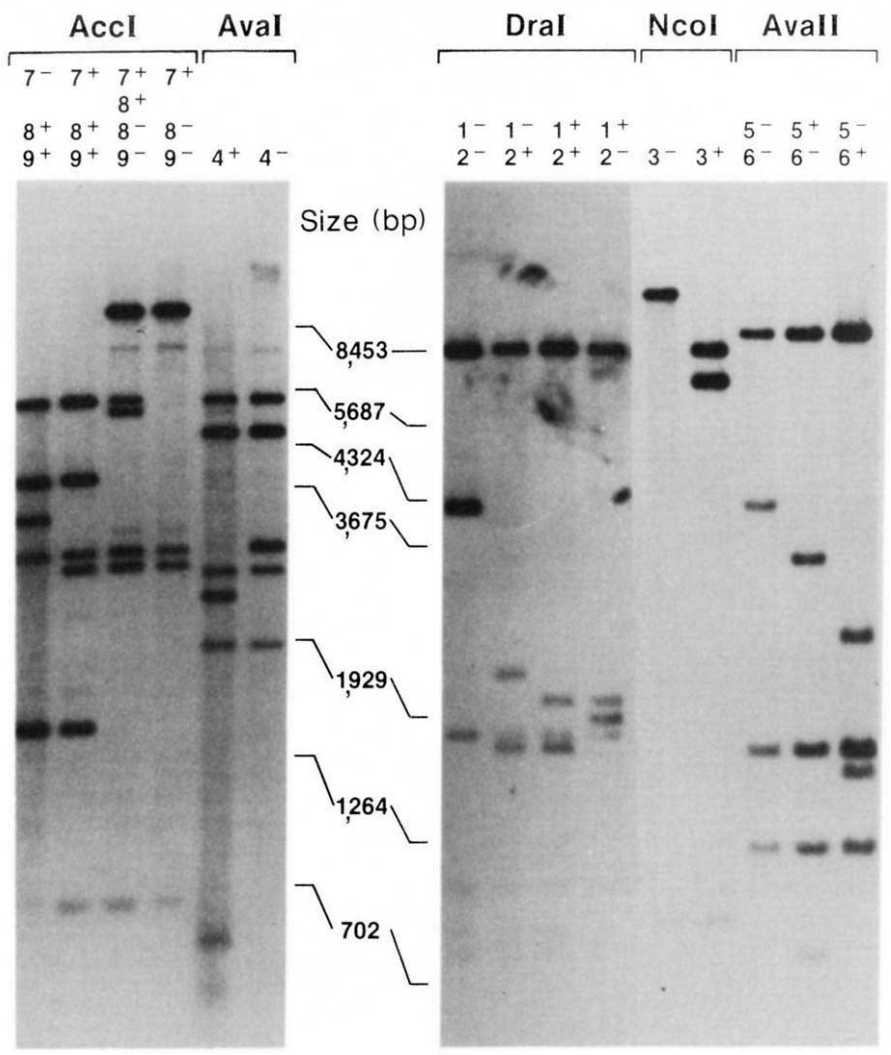

FIG. 2 Polymorphic restriction-fragment sites defining the 12 mtDNA haplotypes of humpback whales. The presence (+) or absence (−) of the 9 restriction sites are noted for each of the 15 unique restriction-fragment patterns. A 0.2-kb fragment in the 1^+, 2^+ pattern of *DraI* is visible on longer autoradiographic exposures. A single individual from Hawaii, referred to as haplotype B in Fig. 3, appeared to be heteroplasmic for restriction site 8 (indicated by 8^+, 8^-)[27]. Migration of calibration fragments is indicated.

METHODS. About 1.5 μg of total cellular DNA from each individual was digested with 18 informative restriction enzymes (*AccI*, *AvaI*, *AvaII*, *Bam*HI, *BclI*, *BglI*, *BglII*, *Bst*EII, *DraI*, *HaeII*, *Hin*cII, *Hin*dIII, *HpaI*, *MspI*, *NcoI*, *SstI*, *Sst*II and *XbaI*). Restricted DNA was separated on the basis of molecular weight by electrophoresis in 0.8% or 1% agarose gels and transferred to nylon filters by Southern blotting in 10×SSC. Filters were hybridized at 65 °C in 0.5 M sodium phosphate, pH 7.2, 7% SDS, 1 mM EDTA, 1% BSA, with radioactively labelled mtDNA from a Dall's porpoise, *Phocoenoides dalli* (gift from E. Bermingham and W. O. McMillan) that was cloned into a λ-phage vector. Filters were washed at a final stringency of 0.1×SSC, 0.5% SDS at 50 °C for 30 min and autoradiographed for 1–3 days at −70 °C.

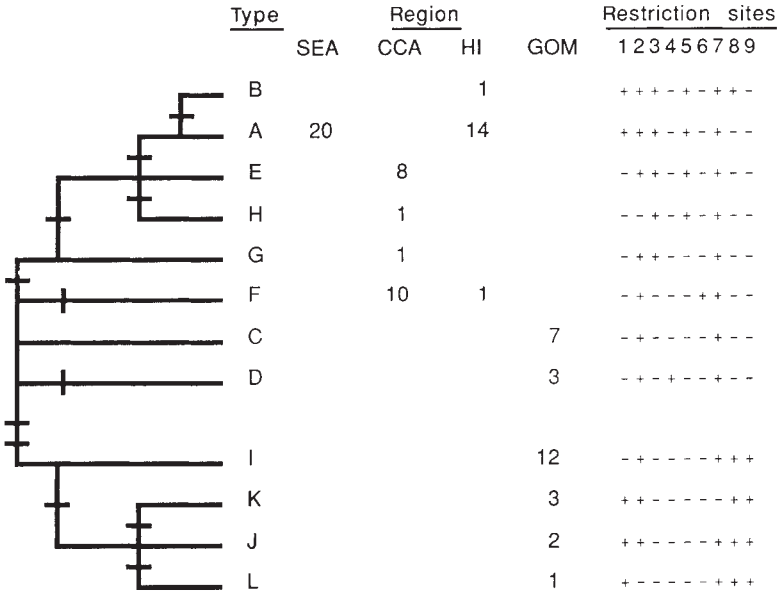

FIG. 3 Topological network of humpback whale mtDNA haplotypes from southeastern Alaska (SEA), central California (CCA), Hawaii (HI) and the Gulf of Maine (GOM). The frequency of haplotypes in each geographic region and the presence (+) or absence (−) of polymorphic restriction sites defining each haplotype are shown adjacent to the branch termini. Polymorphic restriction sites are numbered according to Fig. 2. Restriction site changes are indicated by vertical or horizontal bars along the network branches. This network is in close agreement with the consensus tree of 50 equally parsimonious trees (consistency index of 0.75) generated by the Phylogenetic Analysis using Parsimony computer program (courtesy of D. Swofford, Illinois Natural History Survey, Champaign, Illinois).

between the southeastern Alaska and California feeding grounds in the North Pacific. The Hawaiian wintering ground was dominated by the southeastern Alaska haplotype A, but also contained one individual with an F haplotype, the most common type in the central California sample. This supports earlier observations that individuals from different feeding grounds congregate on a common wintering ground[2,3,7,8]. A computer simulation indicated that such a strong geographic segregation of haplotypes would be expected by chance less than once in 500 samples from a single panmictic population[9].

Given the tremendous mobility of humpback whales and the apparent absence of geographic barriers within oceans, we sug-

gest that the complete segregation of mtDNA types on the North Pacific feeding grounds reflects the result of strong maternal traditions in migratory destinations[10,11], perhaps reinforced by the social or genetic advantages of philopatry[12–14]. These results emphasize the importance of considering behavioural strategies such as migration and natal fidelity[15–17], as well as dispersal[18], in describing the population structure of marine species.

Despite the marked segregation of mtDNA haplotypes in populations of humpback whales, average nucleotide diversity was low compared with most other mammals similarly studied[19], and genetic distance between North Pacific and Atlantic populations was small, considering the 3-Myr separation of these oceans by the Isthmus of Panama[20] and assuming a 1–2% Myr^{-1} rate of mtDNA divergence[19,21]. This pattern of low but highly structured variation could be explained by a deceleration of mtDNA evolution in humpback whales, or by periodic gene flow between oceans followed by a rapid assortment of maternal lineages within oceans. A possible avenue for gene flow between oceanic populations is indicated from the logbooks of nineteenth century whaling ships, which report that northern and southern hemisphere populations overlap along the equatorial Pacific coasts of South America[22]. Further analysis of mtDNA from individuals in subpopulations of the southern hemisphere, as well as analysis of nuclear gene markers from various populations, could provide a global description of gene flow in the present populations of humpback whales and allow a reconstruction of the historic processes that preceded the genetic subdivisions of modern populations. □

Received 13 November 1989; accepted 5 February 1990.

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ACKNOWLEDGEMENTS. We thank M. Bush, M. Dean, D. Gilbert, J. Martenson and N. Yuhki for discussions and technical assistance, and C. Belt, A. Frankel, L. Herman, D. Matkin, A. Perry, M. Schilling, J. Sease and J. Straley for their assistance in the field. This research was funded in part by a grant from the National Geographic Society and a postdoctoral fellowship from the National Zoological Park, Smithsonian Institution (to C.S.B.) and a grant from the National Science Foundation (S.R.P.). Biopsy samples were collected in full compliance with a permit issued to R.H.L. by the National Marine Fisheries Service, Department of Commerce, USA.

Muscarinic modulation of a transient K^+ conductance in rat neostriatal neurons

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NEURONS of the neostriatum are richly innervated by cholinergic neurons of intrinsic origin[1–3]. Both pre- and post-synaptic muscarinic receptors mediate the effects of acetylcholine (ACh)[4–6]. Activation of these receptors is functionally significant, particularly in Parkinson's disease[7]. Current-clamp studies indicate that muscarinic receptors serve to decrease the responsiveness of neostriatal neurons to excitatory inputs[8–10]. Here we present evidence that this effect is caused, in part, by the muscarinic modulation of the A-current, a transient outward potassium current. The voltage dependence of this current suggets that normally it enhances spike repolarization and slows discharge rate, but does not affect 'synaptic integration'. We find that under the influence of muscarinic agonists, the voltage dependence of A-current activation and inactivation is shifted towards more negative membrane potentials and the peak conductance is increased. Therefore, at relatively hyperpolarized resting potentials, ACh transiently alters the functional role of the A-current, allowing it to suppress excitatory inputs and further slow the discharge rate. But at relatively depolarized resting potentials, ACh increases excitability by removing the A-current through inactivation.

We studied the effects of muscarinic agonists on the A-current in cultured rat neostriatal neurons using whole-cell voltage-clamp techniques[11]. We maintained neurons from either late embryonic (E17) or postnatal (P1–7) rat pups *in vitro* for 3–21 days[12]. Previous electrophysiological studies of these neurons[13,14] have shown that in the presence of Na^+ and Ca^{2+} channel blockers, the outward K^+ currents activated by depolarization are primarily due to a sustained tetraethylammonium (TEA)-sensitive current and a transient 4-aminopyridine (4-AP)-sensitive current. The transient current has a pharmacology and ionic selectivity similar to those of the A-current in other mammalian brain neurons, but differs in voltage dependence[14]. In most neurons dialysed with 150 mM internal potassium, the half-activation and half-inactivation voltages of the A-current are 20–30 mV more positive than those of hippocampal neurons[15,16] and resemble those of the A-current in mammalian heart cells[17,18] or *Drosophila*[19,20].

The application of the stable nonhydrolyzable cholinergic agonist, carbachol, produced a reversible increase in the amplitude of the transient component of the outward K^+ current (Fig. 1*a*, left). Inactivation of the A-current with a prepulse to −40 mV revealed that the sustained current was unaffected (Fig. 1*a*, right). The response seemed to be relatively slow in onset and offset (Fig. 1, inset). A similar result was obtained in 73% (11 out of 15) of the neurons studied in the absence of external TEA. In 20% (3 out of 15) of this sample, carbachol also increased the amplitude of the sustained outward current. The holding current at −70 mV was not affected. The increase in the transient current induced by carbachol was unaltered by bath application of TEA (20 mM; $n = 20$), but was blocked by 4-AP (5 mM; $n = 3$).

Although carbachol is a mixed cholinergic agonist, its actions were mediated by muscarinic receptors. The enhancement of A-current by carbachol was unaffected by inclusion of the nicotinic antagonist mecamylamine (10 μM) in the bath. By contrast, the muscarinic antagonist atropine (10 μM; $n = 3$) blocked the carbachol response (Fig. 1*b*). We also observed the effect of carbachol on the A-current with the specific muscarinic agonist, muscarine (Fig. 2 and Table 1). Although selective antagonists were not tested, binding studies on intact neostriatal cultures have shown that muscarinic receptor expression is limited to primarily the M1 subtype[21].

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