growth, calmodulin (CaM) (*26*), an intracellular Ca^{2+} receptor protein; calcineurin (*19*), a CaM-dependent protein phosphatase; and Ca^{2+}-CaM–dependent protein kinase II (*27*) regulate nerve growth. Double immunostaining of IP_3R1 and microtubules revealed that the distribution of IP_3R1 was associated with that of microtubules such as tubulin in the growth cone (*28*). Thus, IICR through IP_3R1 in the growth cone and its downstream effectors might act locally to regulate microtubule assembly and promote neurite extension. This notion is also supported by our findings that IP_3R1 appears not to be distributed in filopodia and that inactivation of this molecule does not affect filopodial motility. $[Ca^{2+}]_i$ mobilization by IICR could modulate Ca^{2+} influxes through Ca^{2+} channels. Therefore, IICR from internal stores and Ca^{2+} influx may act together to direct nerve growth.

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11. DRG neurons from ED11 chick embryos were dissociated by trypsinization and cultured on poly-L-lysine– and laminin (Gibco-BRL)-coated cover slips with nerve growth factor–containing L-15 medium (Gibco-BRL) (*21*). The cells were cultured for 2 hours and fixed with 4% paraformaldehyde in phosphate-buffered saline for 30 min. Indirect immunocytochemistry was done as modified from P. C. Letourneau and T. A. Shattuck [*Development* **105**, 505 (1989)].
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14. Heparin or de-*N*-sulfated heparin (Sigma) dissolved in culture medium (1.6 mg/ml) was loaded into DRG neurons by trituration. The trituration loading was confirmed by observing fluorescence of FITC-conjugated dextran (molecular size: 150 kD; Sigma) coincidentally loaded with the test reagents.
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Cultural Selection and Genetic Diversity in Matrilineal Whales

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Low diversities of mitochondrial DNA (mtDNA) have recently been found in four species of matrilineal whale. No satisfactory explanation for this apparent anomaly has been previously suggested. Culture seems to be an important part of the lives of matrilineal whales. The selection of matrilineally transmitted cultural traits, upon which neutral mtDNA alleles "hitchhike," has the potential to strongly reduce genetic variation. Thus, in contrast to other nonhuman mammals, culture may be an important evolutionary force for the matrilineal whales.

Most female pilot whales (*Globicephala melas* and *G. macrorhynchus*), sperm whales (*Physeter macrocephalus*), and killer whales (*Orcinus orca*) spend their entire lives with close female relatives, form new groups primarily by group fission, and so have a social structure that may be called matrilineal (*1*). These toothed whale (suborder Odontoceti) species have mtDNA nucleotide diversities about tenfold lower than is estimated for other whales and dolphins, with the exception of the narwhal (*Monodon monoceros*) (Table

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1). The social system of the narwhal is poorly known but has been described as "matrifocal" (*2*), so this may also be a matrilineal species. mtDNA diversity in these matrilineal species, all of which have large, geographically widespread populations, is exceeded even by that of Hector's dolphin (*Cephalorhynchus hectori*), which is confined to New Zealand waters and numbers in the low thousands (Table 1). The reduced mtDNA diversities of the matrilineal whales do not seem to be methodological artifacts. All studies examined the same "control" region of the mitochondrial genome, studies of matrilineal cetaceans covered generally larger geographic areas than those of nonmatrilineal species (*3*), and nucleotide diversities of the matrilineal whales were little changed when only one animal per group was used in the calculations (Table 1).

Low genetic diversity may result from a low mutation rate. However, control region mutation rates are no lower in the matrilineal whales than in other cetaceans (*4*). Generation time and population size may also affect genetic diversity (*5*). I converted mtDNA nucleotide diversities into approximate effective population sizes (the size of a population of females that has an expected equilibrium nucleotide diversity equal to that measured from the species) using known generation times and the relation between mutation rate and body size. Matrilineal cetaceans have considerably reduced effective population sizes, and there is no relation between effective population size and the order of magnitude estimated current population size (Table 1).

Population bottlenecks might have reduced genetic diversity (*6*). However, to reduce nucleotide diversity by a factor of 10, the bottleneck would have had to have been extremely severe or prolonged [about 100 animals for 100 generations or 1000 animals for 1000 generations, from the methodology of Amos (*7*)]. Such scenarios seem unlikely,

Table 1. Estimates of nucleotide diversity of control region mtDNA, approximate effective population size, and order of magnitude estimate of population size for cetacean species and subspecies. Diversities are as given by authors or as calculated from presented data (calculated values are given when discrepancies were found). Estimates with restriction fragment length polymorphisms are not presented because they are not comparable to those from sequencing data. Only estimates for the largest available geographic range of a species or subspecies are presented. Where two studies have similar geographic ranges, both are presented.

Species		Matrilineal?	Location	Number of samples	Base pairs sequenced	Nucleotide diversity	Effective population size*	Approximate population size†	Source
Minke whale	*Balaenoptera acutorostrata acutorostrata*		North Atlantic	87	345	0.0103	61,000	10^4–10^5	(*24*)
Minke whale	*B. acutorostrata bonaerensis*		Antarctic	23	345	0.0161	97,000	10^5–10^6	(*24*)
Humpback whale	*Megaptera novaeangliae*		North Atlantic, North Pacific, Antarctic	90	283	0.0257	245,000	10^3–10^6	(*25*)
Humpback whale	*Megaptera novaeangliae*		North Atlantic, Antarctic	136	288	0.0260	248,000	10^3–10^6	(*26*)
Beluga whale	*Delphinapterus leucas*		North America	628	234	0.0100	36,000	10^4–10^5	(*27*)
Bottlenose dolphin	*Tursiops* spp.		Atlantic, Pacific	11	400	0.0245	56,000	$\geq 10^5$	(*8*)
Northern right whale dolphin	*Lissodelphis borealis*		North Pacific	52	400	0.0210	71,000	$\geq 10^4$	(*28*)
Long-beaked common dolphin	*Delphinus capensis*		North Pacific	11	404	0.0117	39,000	$\geq 10^6$	(*29*)
Short-beaked common dolphin	*Delphinus delphis*		Pacific, Black Sea	18	404	0.0180	61,000	$\geq 10^6$	(*29*)
Harbor porpoise	*Phocoena phocoena*		North Pacific, Black Sea, North Atlantic	105	394	0.0325	155,000	$\geq 10^5$	(*30*)
Hector's dolphin	*Cephalorhynchus hectori*		New Zealand	34	360	0.0060	27,000	10^3–10^4	(*31*)
Pacific white-sided dolphin	*Lagenorhynchus obliquidens*		North Pacific	116	402	0.0211	69,000	$\geq 10^5$	(*32*)
Striped dolphin	*Stenella coeruleoalba*		Eastern Pacific, western Mediterranean, western Atlantic	57	399	0.0210	44,000	$\geq 10^6$	(*33*)
Narwhal	*Monodon monoceros*	?	Greenland area	74	287	0.0017	7,000	10^4–10^5	(*2*)
Sperm whale	*Physeter macrocephalus*	M	North Atlantic, North Pacific, Antarctic	231	330	0.0039 (0.0041)‡	15,000	10^5–10^7	(*6*)
Sperm whale	*Physeter macrocephalus*	M	Pacific, Atlantic, Indian	182	600	0.0028	11,000	10^5–10^7	(*34*)
Killer whale	*Orcinus orca*	M	North Pacific	66	520	0.0054 (0.0067)‡	13,000	10^4–10^5	(*35*)
Short-finned pilot whale	*Globicephala macrorhynchus*	M	Atlantic, Pacific	13	400	0.0011 (0.0014)‡	3,000	$\geq 10^5$	(*8*)
Long-finned pilot whale	*Globicephala melas*	M	Atlantic	59	400	0.0006 (0.0006)‡	2,000	10^5–10^7	(*8*)

*Effective population sizes were calculated as $d/[2\mu(1-d)]$ [from Eq. 25 of (*5*)], where d is the nucleotide diversity and $\mu = 3.42m^{-0.1492}g \times 10^{-8}$ [regression from the homeotherm line in Fig. 2 of (*36*)], where m is the mass of a mature female in kilograms [(*22*), except (*37*), for northern right whale dolphins] and g is the generation length, the mean age at sexual maturity plus interbirth interval (*22*). Unavailable generation lengths were set at 5.5 years for Hector's dolphin and 9 years for northern right whale dolphin and Pacific white-sided dolphin, as approximate medians for animals of similar size and habitat, and unavailable body mass for the short-finned pilot whale was set at 890 kg, as in the long-finned pilot whale. †(*38*). ‡Recalculated with just one randomly chosen animal per matrilineal group (assuming that all separate strandings and by-catch are from separate groups).

especially for long-lived and widespread animals like the matrilineal whales.

If fitness were strongly correlated among females in a matriline, so that they largely reproduce or die together, then this could reduce mtDNA diversity in matrilineal species (*8*). However, no substantial group-specific effects were found in an analysis of killer whale life history parameters (*9*), and a realistic model of group extinction in pilot whales (which could result from mass strandings or drive fisheries) found that mtDNA diversity could be substantially reduced if matrilineal groups of about 20 to 50 females became extinct at a rate of about 1% per year over many generations but that at lower extinction rates (0.1% per year) the effect was very small (*8*). For the more oceanic populations of both pilot whale species, such as those in Antarctic or eastern tropical Pacific waters that contain substantial proportions of the global populations of the two species, group extinction rates of 1% per year seem very unlikely. The scenario is even more improbable for sperm and killer whales, which have smaller matrilineal group sizes and are much more rarely hunted by drive fisheries or observed to mass strand.

Alternatively, selection, the differential survival or reproduction of phenotypes, could be reducing diversity in a number of ways. However, it is not obvious why selection should be particularly prevalent in the mtDNA genome of matrilineal odontocetes, and none of the values of Tajima's "D" statistic (*10*) calculated from the data sets listed in Table 1 showed significant ($P < 0.05$) evidence of selection in the control region. Instead, I suggest that neutral or nearly neutral mtDNA loci hitchhike on selected maternally inherited cultural traits.

Culture can be defined as information that may cause variation in behavior and that is acquired from conspecifics by imitation or learning (*11*). Cultural traits learned, almost without error, from the mother or members of a matrilineal group are analogous to maternally inherited mtDNA loci. So, with the use of a theory developed for molecular hitchhiking in which diversity at a neutral locus is reduced by selection at a linked locus (*12*), beneficial cultural traits that are transmitted maternally (or within matrilineal groups) have the potential to reduce mtDNA diversity. This is shown with simulations in Fig. 1 for the case of a single advantageous (10% fitness advantage) matrilineally transmitted cultural development that sweeps through a population of 200,000 females, devastating mtDNA diversity. The effects are slower but otherwise similar when the fitness advantage is smaller. Nucleotide diversity is still reduced even when the matrilineal transmission is imperfect, either because some daughters do not learn the trait from mothers who have it (Fig. 1C) or because females occasionally learn the trait from outside their matriline (Fig. 1D). However, further simulations showed that if nonmatrilineal transmission is much greater than the 0.5% used to generate Fig. 1D, then mtDNA diversity is little reduced.

Although cetaceans are difficult to study, field research is uncovering many instances of apparent culture. For instance, individuals appear to learn particular feeding techniques from their mothers, members of their matrilineal group, or the general adult population (*13*). Heimlich-Boran and Heimlich-Boran suggest that matrilineally transmitted cultural feeding specializations on fish and marine mammals could have initiated the divergence of the sympatric "resident" and "transient" forms of killer whales off Vancouver Island, which now show genetic and morphological differences (*13*).

The clearest forms of cultural transmission among cetaceans concern vocalizations. Cetaceans are some of the few nonhuman mammals known to have vocal learning (*14*) and thus the potential for culturally acquired acoustic repertoires. In killer and sperm whales, matrilineal groups occupying the same ranges use distinctive, and almost certainly culturally acquired, vocal dialects, a unique finding among nonhuman animals (*15*). In these two species, groups sharing recent common ancestry seem to have similar repertoires, and in sperm whales the vocal repertoire of a group correlates with the types of environmentally acquired markings (such as those from the teeth of predators) on its members (*16*). This suggests that methods of facing environmental challenges (such as predation) are being transmitted culturally and being conserved within matrilineal groups in parallel with vocal dialects.

Simulations show that, to maintain gene-dialect correlations, as found in sperm whales (*16*), rates of nonmatrilineal transmission of dialect must not occur at rates much faster than the genetic mutation rate (*17*). Thus, even though matrilineal groups of whales often meet and merge temporarily (*18*), nonmatrilineal transmission of dialect in these species must occur at a rate below the 0.5% threshold indicated in Fig. 1D for reduction in genetic diversity by cultural hitchhiking. Selectively advantageous cultural traits, which might include migration strategies (*19*), foraging techniques (*13*), and baby-sitting (*1*), may be similarly stable, and so cultural hitchhiking is a feasible explanation for lowered genetic diversity in these species.

If cultural hitchhiking has happened, then the matrilineal odontocetes are exceptions to the general rule that substantial gene-culture coevolution is confined to humans (*20*). Why? They have attributes that favor the evolution of social learning and culture (*21*): long lives (~60 to 90 years), advanced cognitive abilities, prolonged parental care, permanent and cohesive groups, and an environment that varies substantially over large spatial and temporal scales (thus making individual learning costly) (*1*, *22*). Cultural innovations will have most evolutionary importance in populations where ranges overlap, because the innovators will affect many conspecifics and a great deal of habitat. Pilot, killer, and sperm whales have wide ranges (~1000 km) that they share with many hundreds or thousands of conspecifics. Gene-culture coevolution is also more probable when cultural features are transmitted with little error between generations (*20*). Matrilineally based groups provide a suitable environment for stable group-specific cultures to develop through conformist traditions (*11*), and such situations can lead to variation between groups upon which selection may act (*23*).

Methods to further test this "cultural hitchhiking" hypothesis against feasible alternatives need to be devised. For instance, a comparative analysis of nuclear DNA diversity in the matrilineal and nonmatrilineal

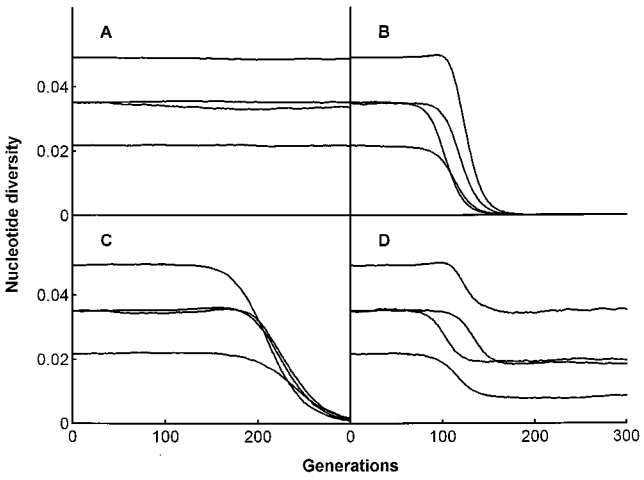

Fig. 1. mtDNA nucleotide diversity trajectories of four simulated populations of 200,000 females over 300 generations. (**A**) Control, (**B**) with a beneficial matrilineally transmitted cultural trait (selective advantage to one female and all her descendants of 10%) introduced at generation 0, (**C**) with 5% of daughters of mothers with the trait not learning it, and (**D**) with 0.5% of transmission of trait going to daughters of mothers lacking it (39).

cetaceans would help distinguish between causes of low mtDNA diversity that operate maternally (such as cultural hitchhiking or selection on the mtDNA genome) or nonmaternally (such as population bottlenecks).

The apparently greater role of cultural inheritance among cetaceans compared with nonhuman terrestrial mammals is likely ultimately linked to environmental differences. Compared with most terrestrial environments, the ocean can support large body sizes, has low travel costs and no barriers, contains dispersed and patchy food, and transmits sound very efficiently. The behavior and social structure of cetaceans seem to have evolved distinctive features in this setting. These features include vocal learning, large home ranges, lack of territoriality, and bisexual group philopatry (*1*). Cultural transmission may be another such feature favored by the environment of the matrilineal whales.

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39. The number of daughters of a female was Poisson distributed with mean Ks/P, where P is the current female population size, s is the selective advantage of the female (1.1 for females with the trait and 1.0 for those without), and K is the carrying capacity (200,000 females). All descendants of females with a particular mtDNA haplotype carried that haplotype, except a proportion μbg {where μ is the mutation rate, set to 7.5×10^{-9}/year [A. R. Hoeltzel, J. M. Hancock, G. A. Dover, *Mol. Biol. Evol.* **8**, 475 (1991)], b is the number of base pairs being considered (300), and g is the generation length (15 years)} carrying novel haplotypes, different from the maternal haplotype at one base pair. The four populations, initially mitochondrially homogeneous, ran for 330,000 generations to stabilize haplotype and nucleotide diversities and then, in each situation (Fig. 1, A to D), for an additional 300 generations to indicate the dynamics of the nucleotide diversity. Runs in which the cultural trait drifted to extinction were discarded.

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Zebrafish *hox* Clusters and Vertebrate Genome Evolution

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HOX **genes specify cell fate in the anterior-posterior axis of animal embryos. Invertebrate chordates have one *HOX* cluster, but mammals have four, suggesting that cluster duplication facilitated the evolution of vertebrate body plans. This report shows that zebrafish have seven *hox* clusters. Phylogenetic analysis and genetic mapping suggest a chromosome doubling event, probably by whole genome duplication, after the divergence of ray-finned and lobe-finned fishes but before the teleost radiation. Thus, teleosts, the most species-rich group of vertebrates, appear to have more copies of these developmental regulatory genes than do mammals, despite less complexity in the anterior-posterior axis.**

HOX cluster genes encode DNA binding proteins that specify fate along the anterior-posterior axis of bilaterian animals (*1*). Remarkably, the order of *HOX* genes along the chromosome reflects the order they act along the body (*2*). Invertebrate chordates have one *HOX* cluster and little axial diversity, but tetrapods have four clusters and substantial axial complexity (*3*). Tetrapod clusters arose by duplications of an ancestral cluster containing 13 genes (*4*). Although it is widely assumed that vertebrates have four *HOX* clusters, initial studies of teleost fish, the most diverse group of vertebrates, revealed unexpected *HOX* genes (*5*–*8*). To understand this problem, we isolated *hox* clusters from the zebrafish *Danio rerio*.

To complement previous surveys of zebrafish *hox* gene fragments (*7, 8*), we identified genomic DNAs in P1 artificial chromosomes (PACs), using degenerate primers to amplify homeoboxes (*9*). We then identified overlap-