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Population genetic structure of North Atlantic, Mediterranean Sea and Sea of Cortez fin whales, *Balaenoptera physalus* (Linnaeus 1758): analysis of mitochondrial and nuclear loci

M. BÉRUBÉ,*† A. AGUILAR,‡ D. DENDANTO,§ F. LARSEN,¶ G. NOTARBARTOLO DI SCIARA,** R. SEARS,†† J. SIGURJÓNSSON,‡‡ J. URBAN-R.,§§ and P. J. PALSBØLL*¶¶

**Institute of Zoology, Department of Population Biology, University of Copenhagen, DK-2100, Copenhagen, Denmark, †Department of Natural Resource Sciences, Campus Macdonald, University McGill, Ste-Anne de Bellevue, Quebec, H9X 3V9, Canada, ‡Department of Animal Biology, Faculty of Biology, University of Barcelona, 08071 Barcelona, Spain, §Allied Whale, College of the Atlantic, Bar Harbor, Maine, 04609 USA, ¶Greenlandic Fisheries Research Institute, Nuuk, Greenland, **Tethys Research Institute, Acquario Civico, 20121 Milano, Italy, ††Mingan Island Cetacean Study Inc., St-Lambert, Quebec, J4P 1T3 Canada, ‡‡Program for Whale Research, Marine Research Institute, 121 Reykjavik, Iceland, §§Department of de Biologia Marina, Universidad Autonoma de Baja California Sur, La Paz, 23080 B.C.S., Mexico, ¶¶Ecology and Evolutionary Biology, University of California, Irvine, CA 92697–2525, USA*

Abstract

Samples were collected from 407 fin whales, *Balaenoptera physalus*, at four North Atlantic and one Mediterranean Sea summer feeding area as well as the Sea of Cortez in the Pacific Ocean. For each sample, the sex, the sequence of the first 288 nucleotides of the mitochondrial (mt) control region and the genotype at six microsatellite loci were determined. A significant degree of divergence was detected at all nuclear and mt loci between North Atlantic/Mediterranean Sea and the Sea of Cortez. However, the divergence time estimated from the mt sequences was substantially lower than the time elapsed since the rise of the Panama Isthmus, suggesting occasional gene flow between the North Pacific and North Atlantic ocean after the separation of the two oceans. Within the North Atlantic and Mediterranean Sea, significant levels of heterogeneity were observed in the mtDNA between the Mediterranean Sea, the eastern (Spain) and the western (the Gulf of Maine and the Gulf of St Lawrence) North Atlantic. Samples collected off West Greenland and Iceland could not be unequivocally assigned to either of the two areas. The homogeneity tests performed using the nuclear data revealed significant levels of divergence only between the Mediterranean Sea and the Gulf of St Lawrence or West Greenland. In conclusion, our results suggest the existence of several recently diverged populations in the North Atlantic and Mediterranean Sea, possibly with some limited gene flow between adjacent populations, a population structure which is consistent with earlier population models proposed by Kellogg, Ingebrigtsen, and Sergeant.

Keywords: fin whale, gene flow, microsatellites, mitochondrial control region, mtDNA, population identity

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Introduction

The subcutaneous layer of adipose tissue, referred to as blubber, found in all extant mysticetes (baleen whales), has provided this group of mammals with the physiological means for extensive ranges of movement. The blubber acts not only as a highly efficient insulating barrier, but also for storage of excess energy (Brodie 1975), facilitating semiannual migrations of more than 8000 km (Stone *et al*. 1990).

Given these characteristics, it is not surprising that spatial as well as temporal separation of feeding and

Correspondence: M. Bérubé. Unit of Evolutionary Genetics, Department of Biology, Free University of Brussels, cp 244, Boulevard de Triomphe, B-1050, Brussels, Belgium. E-mail mberube@ulb.ac.be.

breeding ranges has been observed in many mysticetes. Most mysticetes summer in high latitudes in cold water where the primary production is high. The blubber acquired during the summer months provides sufficient energy reserves for the often extensive semiannual migrations to lower latitudes where the primary production is low but the water temperature adequate for giving birth to insufficiently insulated calves. Mating also takes place in low latitudes, usually immediately following parturition.

'Typical' examples of species following the above migration pattern are the humpback whale, *Megaptera novaeangliae*, (Katona & Beard 1990) and the North Atlantic right whale, *Eubalaena glacialis*, (Knowlton *et al*. 1992) for which the winter and summer ranges are relatively well documented. For many mysticetes, however, the winter range has not yet been identified. Some species such as the Bryde's whale, *Balaenoptera edeni*, have a year-round tropical distribution (Best 1977), whereas other species, such as the minke whale, *B. acutorostrata*, and the fin whale, *B. physalus*, are observed in temperate waters during the summer as well as the winter months (Ingebrigtsen 1929; Kellogg 1929; Sergeant 1977; IWC 1992).

The fin whale is a cosmopolitan mysticete currently found in all major oceans. Being the second largest of all whales, the fin whale has been the target of commercial whaling operations which have severely depleted their abundance in some oceans. The abundance of fin whales in the North Pacific Ocean has been reduced from an estimated 42 000–45 000 to perhaps only 16 000 individuals (Barlow *et al*. 1995), whereas fin whales are still relatively numerous in the North Atlantic Ocean (≈ 56 000 individuals, Buckland *et al*. 1992a,b; IWC 1992).

While the distribution of fin whales in the North Atlantic has been described in general terms (Ingebrigtsen 1929; Kellogg 1929; Jonsgård 1966; Sergeant 1977; IWC 1992), little information is available regarding the population identity and population structure (IWC 1992). Ingebrigtsen (1929) discarded the hypothesis of a single common breeding ground based on the fact that fin whales are simultaneously observed in all regions of the North Atlantic and during the entire year. The movements of fin whales appeared to be mainly dictated by the availability of prey and the extent of ice (Ingebrigtsen 1929). Newborn calves were observed during the winter months in high latitudes off northern Norway, indicating that warm and protected tropical waters are not a requirement for calving (Ingebrigtsen 1929) as presumed for, e.g. the humpback whale (Mitchell & Reeves 1983). Kellogg (1929) proposed that separate 'stocks' of fin whales had overlapping ranges, i.e. that the summer range for one stock of fin whales may constitute the winter grounds for another. Later, Sergeant (1977) postulated another pattern of distribution described as a 'patchy continuum', mainly defined by areas of high primary production, and with a considerable degree of migration between adjacent areas. The distribution of mark–recaptures of tags, reported later (IWC 1992), was consistent with the above description of relatively limited ranges of movements. Of a total of 685 marked fin whales, 97 tags were later recovered. Only three of these 97 tags were recovered outside the area in which the whale were tagged: two animals marked in Nova Scotia were recovered in Newfoundland and one animal marked in Newfoundland turned up in Iceland 9 years later (Sigurjónsson *et al*. 1991). The limited range of individual fin whales observed in the mark–recapture studies above was later confirmed by re-sightings of individual fin whales identified by their natural markings at summer feeding areas off eastern North America (Agler *et al*. 1990; Clapham & Seipt 1991).

Recently, population genetic studies of North Atlantic fin whales based upon analyses of isozymes (Arnason *et al*. 1991; Danielsdottir *et al*. 1991, 1993) and mitochondrial (mt) DNA restriction fragment length polymorphisms (Danielsdottir *et al*. 1992) have been undertaken in an attempt to gain further insight into the population structure of North Atlantic fin whales. The above mentioned studies revealed significant differences in isozyme allele and mtDNA haplotype frequencies between samples from different geographical regions, as well as between years, off Iceland (Danielsdottir *et al*. 1991). Furthermore, significant intra-annual deviations from Hardy–Weinberg (HW) proportions of genotype frequencies were observed in samples of fin whales taken off Iceland. Hence, the results of the genetic analyses undertaken so far indicate mixing of fin whales from different populations on at least one summer feeding ground (off Iceland).

In this study, we present the results of a population genetic study based upon the variation in the nucleotide sequence of the mt control region and allele frequencies at six nuclear microsatellite loci in 407 fin whale samples. The samples were collected at six different summer feeding areas in the North Atlantic and Mediterranean Sea. In addition, we included samples, as a reference population, from the Sea of Cortez in the North Pacific, where fin whales are observed throughout the year.

Considering the previous models regarding the population structure of the North Atlantic fin whales proposed by Kellogg, Ingebrigtsen, and Sergeant, we wanted to test the hypothesis that fin whales from different areas in the North Atlantic and Mediterranean Sea correspond to subpopulations between which there are limited amounts of gene flow. Accordingly, this should be evident as a positive correlation between the net interpopulation genetic and geographical distances. Our study differs from pre-

vious analyses in its extensive geographical coverage as well as the use of hypervariable nuclear and mt loci in each sample. Parallel analyses of Mendelian and maternally inherited loci are particularly important in species, such as marine mammals, which may display maternally directed philopatry (Palumbi & Baker 1994; Larsen *et al*. 1996).

Materials and methods

Sample collection

Samples were collected from five summer feeding areas in the North Atlantic Ocean: the Gulf of St Lawrence, the Gulf of Maine, West Greenland, Iceland and the western coast of Spain. In addition, samples were obtained from fin whales in the Ligurian Sea (the Mediterranean Sea) and the Sea of Cortez (the North Pacific Ocean) (see Fig. 1). The fin whales in the Sea of Cortez are assumed to represent a resident year-round population that is isolated from populations in the North Pacific (Gambell 1985; Leatherwood *et al*. 1988). All samples, except those from Iceland and Spain, were obtained as skin biopsies taken from free-ranging whales. The Icelandic and Spanish samples were collected from fin whales taken during whaling operations prior to the international moratorium of commercial whaling. A few of the West Greenland samples were obtained from aboriginal catches.

Skin biopsies from free-ranging fin whales were collected using a crossbow and a bolt with a modified stainless steel tip and a float molded to the bolt (Palsbøll *et al*. 1991). The entire biopsy or, in some instances, only the skin section was conserved in a saturated NaCl solution with 20% dimethylsulphoxide (Amos & Hoelzel 1991). All samples were stored at either – 20 °C or – 80 °C pending analysis.

Laboratory analysis

DNA extraction and sex determination

Total-cell DNA was extracted using standard procedures (Sambrook *et al*. 1989) with cell lysis caused by addition of sodium dodecyllauryl sulphate, proteinase K digestion, followed by phenol/chloroform/isoamyl alcohol extractions and finally precipitation with ethanol. The sex of each sample was determined as described by Bérubé & Palsbøll (1996a,b).

Microsatellite loci

Five microsatellite loci (TAA023, GATA028, GATA053, GATA098, GGAA520) with either tri- or tetramer repeat motifs were amplified as described by Palsbøll *et al*. (1997a). The letters in the microsatellite names identify the nucleotide sequence of the repeat motif followed by a serial number denoting the clone. The sixth loci, GT011, with a dimer repeat motif, was isolated from total-cell DNA extracted from a humpback whale. GT011 was amplified under similar conditions as the other loci using the oligonucleotide primers GT011F 5'-CATTTTGGGTTGGATCATTC-3' and GT011R 5'-GTGGAGACCAGGGATATTG-3'. The annealing temperature for locus GT011

Fig. 1 Sampling areas.

was set at 62 °C. The amplification products were electrophoresed through a standard 5% denaturing polyacrylamide gel and visualized by autoradiography. The sequences of M13 mp18, a blank reaction (all reagents except genomic DNA), as well as five control samples were included with each set of 43 samples to permit accurate sizing of the alleles, detection of contamination as well as loading errors.

MtDNA control region sequences

The nucleotide sequence of the 5' end of the mt control region was determined for all samples by direct sequencing (Saiki *et al*. 1988). Symmetric double-stranded and subsequent asymmetric amplifications of the control region were performed as described in Palsbøll (1995). Blank reactions were added to detect possible contamination. Sequencing was performed following the manufacturer's instructions (Sequenase Ver. 2, US Biochemicals Inc.), using Bp15851F (Larsen *et al*. 1996) as sequencing primer.

Data analysis

Sex ratios

We used the χ^2 test, (Lindgren 1975) to evaluate the significance of deviations from parity in the sex ratio within sampling areas.

Analysis of microsatellite loci

Level of polymorphism. The level of variation at nuclear loci was estimated as the number of alleles per locus, the expected heterozygosity, and the probability of identity, *I* (Paetkau & Strobeck 1994).

Testing for deviations from HW genotypic proportions and linkage disequilibrium. Evaluations of possible deviations from the expected HW genotypic frequencies and linkage disequilibrium were performed using Fisher's exact test and the Markov chain method implemented in GENEPOP version 1.2 (Raymond & Rousset 1995). Tests for HW genotypic proportions were conducted for different partitionings stratified by sex, year, sampling area or oceanic origin. To correct for multiple simultaneous comparisons in the tests of HW genotypic frequencies and linkage disequilibrium, sequential Bonferroni corrections were applied (Rice 1989) using a global significance level of 0.05.

Homogeneity tests and levels of differentiation. Several statistics were employed based upon either allele frequencies (GENEPOP Ver. 1.2, Raymond & Rousset 1995) or microsatellite-specific estimators of the variance of allele lengths, R_{ST} (Slatkin 1995) and differences in mean allele length, $(\delta\mu)^2$ (Goldstein *et al*. 1995). The statistical significance of the observed values of R_{ST} and $(\delta\mu)^2$ was evaluated by comparison with 1000 Monte Carlo simulations. As reported previously by Valsecchi *et al*. (1997) 'traditional' homogeneity tests based upon allele frequencies proved more powerful than R_{ST} or $(\delta\mu)^2$. Consequently, we employed the homogeneity test implemented in GENEPOP version 1.2 which uses a Markov Chain method to estimate the values of Fisher's exact test (Raymond & Rousset 1995). Homogeneity tests between partitionings were performed as pairwise comparisons in a hierarchical manner starting within areas comparing (i) different months, (ii) males to females, (iii) different years, and (iv) sampling areas, for each locus independently. Partitionings between which no heterogeneity was detected were pooled in subsequent tests. The overall level of significance for all six loci was evaluated as suggested by Sokal & Rohlf (1995). Sequential Bonferroni corrections were applied when performing multiple pairwise comparisons using a table-wide significance level of 0.05 (Rice 1989).

The degree of genetic differentiation between partitionings of samples was estimated as either F_{ST} (Weir 1990) or Nei's *D* (Nei 1987). Estimates of F_{ST} and confidence intervals across all six loci were obtained by bootstrapping over loci (Weir 1990).

Analysis of mtDNA

Levels of polymorphism, homogeneity tests and levels of differentiation. The degree of variation within samples was estimated as the nucleotide diversity (Nei 1987). Homogeneity tests between partitionings were conducted as described by Hudson *et al*. (1992), using the χ^2 statistic (which proved more powerful than either K_{ST} or H_{ST}). The level of statistical significance was estimated from 1000 Monte Carlo simulations as the proportion of simulations in which a similar or more extreme value of χ^2 was observed. Pairwise comparisons were performed in the same order and manner as described above for the microsatellite analysis. As in the microsatellite analysis, sequential Bonferroni corrections were applied when performing multiple pairwise comparisons (Rice 1989) using 0.05 as the table-wide level of significance.

The degree of differentiation between partitionings was estimated as either F_{ST} (with frequencies only using GENEPOP, Raymond & Rousset 1995) or Nei's *D* (Nei 1987).

MtDNA genealogy. Genealogies were estimated from the mt haplotypes employing the PHYLIP 3.5c computer package by Felsenstein (1993). The phylogenies were rooted using the homologous sequence from a North Atlantic humpback whale (Palsbøll *et al*. 1995) as an outgroup. First, a phylogeny was estimated using DNAML

with multiple jumbles (n = 10), in order to assess the phylogeny with the highest maximum likelihood value. Second, bootstrap values were obtained by generating 500 random samples (SEQBOOT), for which distance matrices were also computed (Kimura's 2-parameter model, DNADIST) and a genealogy was estimated using the neighbour-joining method (Felsenstein 1993). Finally, a majority-rule consensus genealogy was calculated from the resultant 500 genealogies.

Frequency distribution of pairwise genetic distances among individuals. The distribution of the number of nucleotide substitutions between all pairs of individuals within sampling localities was computed (MEGA version 1.01, Kumar *et al*. 1993) and compared to the Poisson distribution (Slatkin & Hudson 1991) expected if the population had undergone exponential growth. The agreement of the observed distribution with the expected distribution was evaluated using the χ^2 test (Lindgren 1975).

Time since divergence of North Atlantic and North Pacific fin whale populations. The level of differentiation between the two oceans was estimated as Kimura's net interpopulation genetic distances, d_A (Nei 1987). The time since divergence (T) was calculated as $T = d_A/2\lambda$ (Nei 1987). We used an overall nucleotide divergence rate (λ) of 0.5–1.0% per Myr suggested by Hoelzel *et al*. (1991) and Baker *et al*. (1993) specifically for cetaceans. It should be noted, however, that this rate is probably an underestimate (see Lyrholm *et al*. 1996), which implies that T was likely to be an overestimate.

Test of isolation-by-distance

Geographical distances between sampling localities were measured using the Geod computer program. The natural logarithm (ln) of the geographical distances (in kilometers) was plotted against Nei's D (Nei 1987), estimated from the mt (denoted D_{MT}) as well as the microsatellite allele frequencies (denoted D_{MS}). To test the degree of correlation between D_{MT} or D_{MS} and the geographical distances, the Mantel test was used (Mantel 1967). The statistical significance of the observed test statistic was found by comparison with 1000 random permutations of the geographical distance matrix, with a new test statistic estimated each time. The significance level was defined as the proportion of permutations in which the test statistic was equal to or more extreme than the observed value.

Phylogenetic relationship between areas

Nei's standard genetic distances for the mtDNA and microsatellite loci (denoted D_{MT} and D_{MS}, see above) between sampling areas were employed to estimate a phylogeny of the populations. The phylogeny was estimated using the Fitch–Margoliash program in the PHYLIP 3.5c package (Felsenstein 1993), with global rearrangements and multiple jumbles (n = 10). A majority-rule consensus phylogeny was estimated from 500 bootstrap replicates, by resampling either loci (microsatellite only) or haplotypes (e.g. microsatellite alleles or mtDNA haplotypes) within each population.

Results

Sex ratio

Two instances of statistically significant deviations from parity were observed; in the samples collected in the Gulf of St Lawrence (2.4 males:1 female; χ^2 (1 d.f.) = 16.33, $P < 0.0001$, n = 98) and off Iceland (0.4 male:1 female, χ^2 (1 d.f.) = 6.82, $P < 0.01$, n = 33) (Table 1). While the female-biased sex ratio detected in the Icelandic samples might be due to whalers' preference (females are larger than males), the overrepresentation of males in the Gulf of St Lawrence was detected only in large groups (M. Bérubé *et al*. unpublished).

Microsatellite loci

Level of polymorphism

The total number of alleles per microsatellite locus ranged from eight to 19 with an average of 10.7. In the North Atlantic/Mediterranean Sea the mean expected heterozygosity was estimated at 0.81 (range: 0.67–0.90) which was significantly higher than the estimate of 0.42 for the Sea of Cortez (range: 0.12–0.68) (Table 2). More than one copy of private alleles (an allele detected only in one area) was found only in the Sea of Cortez.

The overall probability of identity (across all loci), in the North Atlantic Ocean was estimated at 1.14×10^{-8} yielding an expectation of 4×10^{-4} samples having identical genotypes due to chance alone [$(N \times N - 1)/2$ pairwise comparisons among sampled whales (N) multiplied by 1.14×10^{-8}]. The expected number of matches in genotype due to chance for the Mediterranean Sea was estimated at 2.7×10^{-5}. Among the North Atlantic and Mediterranean Sea samples a total of 21 pairs as well as a single incidence of three samples had identical sex, nuclear genotype and mt haplotype, all observed within the same sampling area. These were inferred to be duplicate samples collected from the same individual, because of the low expectation of a match by chance. Only in the Gulf of St Lawrence and in the Gulf of Maine did we detect matches between years. All samples except one with identical genotypes were removed if present in the same partitioning, during the subsequent data analyses. As the expected number of pairs of samples with identical genotypes due to chance in the Sea of Cortez was estimated at 7.2, the

Table 1 Number of samples (N) and individuals (n) per year and sex ratio for the Gulf of St Lawrence (GSL), the Gulf of Maine (GM), West Greenland (WG), Iceland (IL), Spain (SP), the Ligurian Sea (IT), and the Sea of Cortez (SC) fin whales

	Number of individuals (*n*)												
Areas	Sample sizes (*N*)	1982	1984	1987	1988	1989	1990	1991	1992	1993	1994	Sex ratio (M/F)	Total *n*
GSL	109						10	29	40	14	5	69/29	98
WG	46				5		5	6	5	7	11	19/20	39
IL	33			9		24						9/24	33
SP	39	25	14									12/13*	39
GM	31							20	8			17/11	28
IT	74							1	16	21	34	37/33†	72
SC	75									19	42	38/23	61

The underlined values indicate significant differences between the proportion of males and females. *Only the 1982 samples from Spain were used for the estimation of the sex ratio as only female samples were analysed from the 1984 season.
†The ZFX–ZFY locus did not amplify in two individuals.

Table 2 Polymorphism at every locus for all sampling localities

		Microsatellite loci						
Areas		GT011	TAA023	GATA098	GATA028	GATA053	GGAA520	All loci
North Atlantic Ocean*								
Gulf of St. Lawrence*	No. of alleles	8	7	8	15	10	14	
	H	0.83	0.85	0.80	0.85	0.74	0.89	0.83
	I	0.05	0.11	0.07	0.02	0.04	0.02	7.32×10^{-9}
Gulf of Maine	No. of alleles	7	7	6	13	9	11	
	H	0.82	0.80	0.78	0.83	0.73	0.87	0.81
	I	0.05	0.11	0.08	0.04	0.07	0.03	4.70×10^{-8}
West Greenland	No. of alleles	7	7	6	13	9	14	
	H	0.83	0.77	0.75	0.87	0.72	0.89	0.81
	I	0.05	0.12	0.10	0.03	0.08	0.02	3.15×10^{-8}
Iceland*	No. of alleles	8	6	6	12	9	14	
	H	0.84	0.76	0.78	0.87	0.67	0.89	0.80
	I	0.04	0.16	0.08	0.03	0.08	0.02	3.04×10^{-8}
Spain	No. of alleles	7	7	6	14	11	13	
	H	0.82	0.81	0.78	0.89	0.70	0.88	0.81
	I	0.06	0.14	0.08	0.02	0.06	0.03	2.17×10^{-8}
Mediterranean Sea								
Ligurian Sea	No. of alleles	7	6	7	15	10	13	
	H	0.84	0.74	0.82	0.89	0.75	0.90	0.82
	I	0.05	0.10	0.06	0.02	0.09	0.02	1.00×10^{-8}
North Pacific Ocean								
Sea of Cortez*	No. of alleles	6	4	2	9	5	5	
	H	0.52	0.12	0.46	0.68	0.52	0.25	0.42
	I	0.31	0.33	0.40	0.13	0.79	0.58	2.60×10^{-3}

The abbreviations for the areas are as described in Table 1.
H, expected heterozygosity; *I*, the probability of identity.
Seven individuals from IT and one from GSL did not amplify successfully at all loci; therefore they were not included in the microsatellite analysis.
*denotes populations which deviate from the Hardy–Weinberg genotypic proportions.

observed 10 pairs and two trios with identical genotypes were not necessarily duplicate samples from the same individual. Indeed, in two cases, samples with identical nuclear genotypes had a different sex or haplotype. As it was impossible to discern true matches from false matches, only one sample from each match with identical genotype, sex and mt haplotype was kept in the subsequent analysis. Thus, the 407 samples analysed were inferred to represent 370 individual fin whales (Table 1).

Tests of HW genotypic proportions and linkage disequilibrium. The combined samples from the North Atlantic and the Mediterranean Sea as well as the combined samples from the North Atlantic deviated significantly from HW genotypic proportions (χ^2 (12 d.f.) = infinity, $P < 0.0001$ in both cases). In addition, significant deviations from HW genotypic proportions were detected in the 1989 Icelandic samples ($n = 24$, χ^2 (12 d.f.) = 30.0, $P = 0.0028$) and in the Sea of Cortez, among the 1994 samples (χ^2 (12 d.f.) = 23.0, $P = 0.0274$, $n = 42$). Although not statistically significant after applying the sequential Bonferroni test, deviations were also observed among the samples collected in the Gulf of St Lawrence (χ^2 (12 d.f.) = 24.8, $P = 0.0156$, $n = 97$) and off Iceland (χ^2 (12 d.f.) = 25.8, $P = 0.0116$, $n = 33$). In the Sea of Cortez, no statistically significant deviations from HW genotypic proportions were detected after exclusion of the samples with the two rare mt haplotypes (Bp50 and Bp51, Fig. 2). The above deviations from the HW genotypic proportions were all due to an excess of homozygotes and observed at two loci in the Gulf of St Lawrence (GT011 and GGAA520) and at a single locus in the Sea of Cortez and Iceland (locus GATA028).

Of 45 tests for linkage disequilibrium, six loci tested in each of the three locations (the North Atlantic Ocean, the Mediterranean Sea and the Sea of Cortez), only six pairwise comparisons yielded statistical significant P values after application of sequential Bonferroni corrections. No single pair of loci in linkage disequilibrium occurred in more than one of the three locations, suggesting the absence of physical linkage between loci.

Homogeneity tests. Homogeneity tests between sampling localities in the North Atlantic and Mediterranean Sea against the Sea of Cortez yielded significant differences across all loci (range of F_{ST}: 0.234–0.280, Table 3). Only the two most northwestern sampling areas in the North

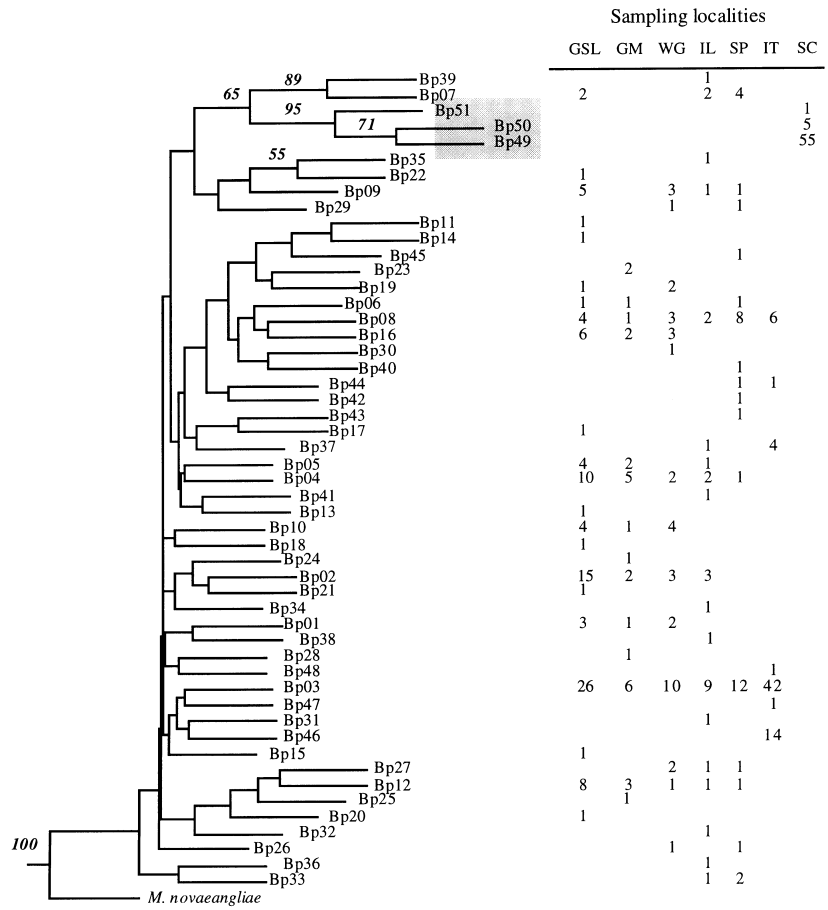

Fig. 2 Majority rule consensus genealogy estimated from the mt haplotypes. The number at the branches denotes the mt haplotype number. Only bootstrap values above 50% are shown. The abbreviations used in the sampling area are described in Table 1. Sequences have been submitted to GenBank.

Atlantic (the Gulf of St Lawrence and West Greenland) differed significantly in allele frequencies from the Mediterranean Sea (Table 3). Estimates of R_{ST} differed from zero only between the North Atlantic/Mediterranean Sea and the Sea of Cortez (range: 0.021–0.069, data not shown). The $(\delta\mu)^2$ estimates ranged from 0.345 to 13.669 with seemingly little correlation to the geographical distances (e.g. Gulf of Maine – Iceland: 4.477 and Gulf of Maine – Sea of Cortez: 3.983) (data not shown).

MtDNA

Levels of polymorphism. The first 288 bp at the 5' end of the mt control region was sequenced in 402 samples. It was impossible to obtain unambiguous mtDNA sequences for two Icelandic and three Mediterranean Sea samples. Thirty polymorphic sites were detected of which 29 were transitions and one a transversion. No insertion/deletion events were observed. The 30 polymorphic sites defined 51 unique haplotypes (Fig. 2). The majority of the samples collected in the North Atlantic and the Mediterranean Sea had mt sequences of haplotype number Bp02, Bp03, Bp04 or Bp08, accounting for 23 (6.3%), 104 (28.4%), 20 (5.4%) and 24 (6.6%) individuals, respectively. The mt haplotypes Bp03 and Bp08 were found in all North Atlantic/Mediterranean Sea sampling localities. A total of 90% of the individuals from the Sea of Cortez were of haplotype number Bp49. In the North Atlantic Ocean private haplotypes (haplotypes unique to a specific sampling locality) found in more than one individual were only observed in the Gulf of Maine (haplotype Bp23). In the Mediterranean Sea three private haplotypes were observed whereas all Sea of Cortez haplotypes were private (Fig. 2).

In the North Atlantic, the overall nucleotide diversity was estimated at 0.0113 (SE = 0.0006). The estimates of the nucleotide diversity at all North Atlantic sampling localities were all within the same range. The nucleotide diversity of 0.0057 (SE = 0.0009) observed in the Mediterranean Sea samples was significantly lower than any of the observed values at North Atlantic sampling localities. The nucleotide diversity of 0.0007 (SE = 0.0002) estimated in the samples from the Sea of Cortez was exceptionally low, and significantly lower than in any other sampling localities.

Homogeneity tests

As noted above, the χ^2 statistic proposed by Hudson *et al.* (1992) detected more incidences of heterogeneity than either the sequence-based statistic K_{ST}, or the frequency-based H_{ST}. No statistically significant levels of heterogeneity were observed between sexes and years within any sampling area. However, significant levels of heterogeneity were detected between the Sea of Cortez and all the North Atlantic/Mediterranean Sea sampling localities. Similarly, statistically significant levels of heterogeneity were detected between the Mediterranean Sea and all North Atlantic samples. Within the North Atlantic, a significant degree of differentiation was observed between the western (defined as the Gulf of St Lawrence and the Gulf of Maine) and the eastern (defined as Spain) North Atlantic. Neither Iceland nor West Greenland could be assigned unambiguously to either the eastern or the western North Atlantic 'population'. The average level of differentiation (estimated as F_{ST}) between the Sea of Cortez and the remaining sampling areas was estimated at 0.51 (SE = 0.058). Similarly, the mean degree of divergence between North Atlantic and Mediterranean Sea sampling areas was found to be 0.12 (SE = 0.022). Within the North Atlantic, F_{ST} values ranged from zero to 0.036 (Table 3).

MtDNA haplotype genealogy. The two genealogies estimated by either the maximum likelihood or the neighbour-joining method, respectively, yielded similar topologies. For this reason, only the majority-rule con-

Areas	GSL	GM	WG	IL	SP	IT	SC
GSL		_	_	_	0.001	0.007*	0.234*
GM	_		_	_	_	_	0.269*
WG	_	0.002		_	_	0.006*	0.258*
IL	_	_	_		_	_	0.280*
SP	0.033*	0.036*	0.015	_		_	0.270*
IT	0.119*	0.150*	0.116*	0.094*	0.097*		0.254*
SC	0.422*	0.516*	0.486*	0.509*	0.519*	0.601*	

Table 3 Degree of differentiation (F_{ST}) between areas and their significance for both the microsatellite (above diagonal), and mtDNA (below diagonal) allele frequencies

The abbreviations for the areas are as described in Table 1. The F_{ST} values were estimated using GENEPOP (Raymond & Rousset 1995) for the microsatellite and the mtDNA alleles (haplotypes) frequencies. *, significant at the $P < 0.001$ level.

sensus tree with bootstrap values above 50% was presented (Fig. 2). No correspondence between the topology and geographical origin of the samples was observed except for the three haplotypes found in the Sea of Cortez, which was supported by a bootstrap value of 95%. Surprisingly, two North Atlantic haplotypes (Bp07 and Bp39 representing nine individuals) were clustered (bootstrap value of 62%) with the three Sea of Cortez haplotypes (Fig. 2).

Frequency distribution of pairwise genetic distances. All observed distributions were significantly different than the expected Poisson distribution ($P < 0.001$) (Fig. 3). Nonetheless, Slatkin & Hudson (1991) found, by computer simulation experiments, that the distribution of pairwise differences will almost never show a statistically rigorous fit to a Poisson distribution even for populations (simulated) that have undergone exponential growth (Slatkin & Hudson 1991). However, unimodality (as opposed to 2+ modes) of the frequency distribution is indicative of exponential population growth (Slatkin & Hudson 1991). The distributions plotted in Fig. 3 reveal a cline from the western to the eastern part of the North Atlantic in which the frequencies of pairwise differences gradually change from predominantly unimodal (the Gulf of Maine and the Gulf of St Lawrence) to multimodal (Spain).

Divergence time. Kimura's net interpopulation distance between the Sea of Cortez and the North Atlantic/Mediterranean Sea sampling localities ranged from 0.021 to 0.027, with a mean of 0.023. Assuming two rates of sequence divergence of 0.5% and 1.0% per Mya (Hoelzel *et al*. 1991; Baker *et al*. 1993), the approximate time of divergence was estimated at 1.15 Ma (range: 1.05–1.35 Ma) and 2.30 Ma (range: 2.10–2.70 Ma), respectively.

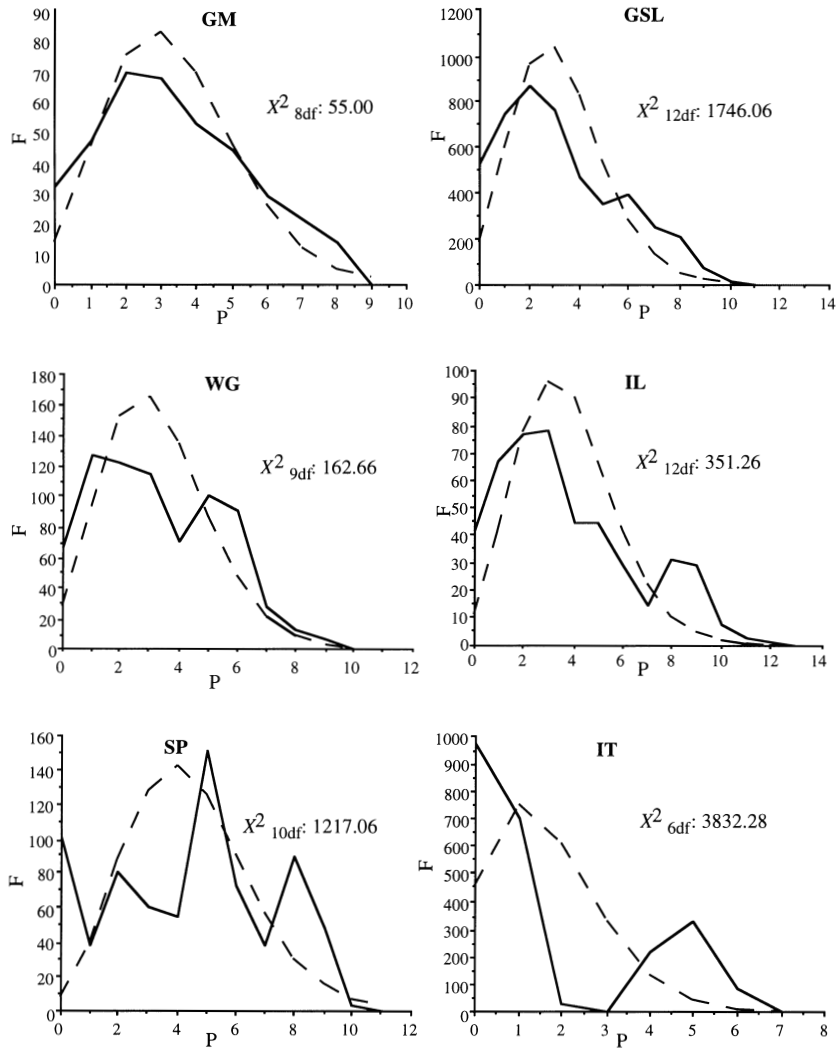

Fig. 3 Observed distributions of pairwise difference (plain line) compared with the number of expected distributions assuming a Poisson distribution (dashed line) for each of the North Atlantic areas and the Mediterranean Sea. The abbreviations used in the sampling area are described in Table 1. The *x* axis (*P*) is the number of substitutions, the *y* axis (*F*), the frequencies of the pairwise comparisons.

Isolation-by-distance

Figure 4 shows plots of Nei standard genetic distances (D_{MT} and D_{MS}, see above) against geographical distances (ln of kilometres) between the North Atlantic and Mediterranean Sea sampling localities. The Mantel tests revealed a statistically significant correlation between D_{MT} as well as D_{MS} and geographical distance when the Mediterranean Sea samples were included ($PD_{MT} = 0.008$, estimated coefficient of correlation (r) = 0.55 and $PD_{MS} = 0.031$, $r = 0.41$), and only between D_{MT} and the geographical distance when the Mediterranean Sea was excluded ($PD_{MT} = 0.014$, $r = 0.70$).

Phylogenetic relationship between sampling areas

As the homogeneity tests as well as the genealogy of the mt haplotypes clearly distinguished the Sea of Cortez as separate from the remaining sampling localities, we used the Sea of Cortez as an outgroup for the estimation of the phylogenetic relationship between the sampling areas in the North Atlantic and Mediterranean Sea (Fig. 5). None of the nodes were supported by bootstrap values above 50% (by resampling of nuclear alleles or mt haplotypes within each population). However, the mt and the nuclear phylogeny both had similar topologies and were consistent with the results obtained from the mt homogeneity tests. Bootstrap values for all nodes above 50% were obtained for the nuclear-based phylogeny (when bootstrapping over loci), demonstrating that the topology was independent of the choice of locus.

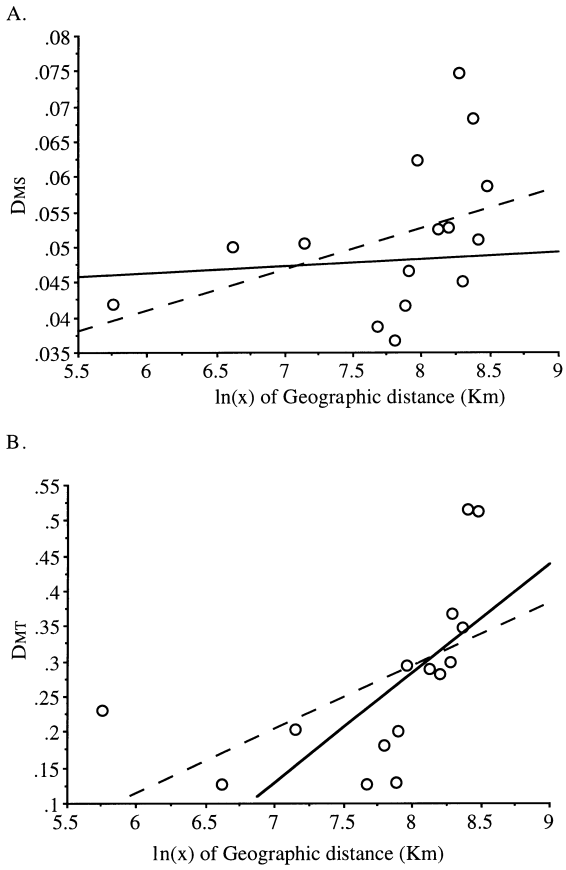

Fig. 4 Plot of the Nei's standard genetic distances calculated for (A) the microsatellite allele (D_{MS}) and (B) the mtDNA haplotypes frequencies (D_{MT}) against the natural logarithm (ln) of the geographical distance between the North Atlantic sampling localities. The regression was performed between the geographical distance (ln km) and all the North Atlantic and Mediterranean Sea localities (dashed regression line) as well as between the North Atlantic localities exclusively (solid regression line).

Discussion

The absence of a definite, common breeding area for the North Atlantic fin whale (Ingebrigtsen 1929), as well as the population model of multiple subpopulations/stocks suggested by Kellogg (1929), is consistent with the results observed in our study. The existence of separate subpopulations with limited gene flow between adjacent populations should be evident as a positive correlation between the interpopulation genetic and geographical distances. This was indeed the outcome of the Mantel tests. Although a few instances of significant levels of heterogeneity were observed at nuclear loci, we detected higher levels and more incidences of heterogeneity in mtDNA. Such a difference in the level of differentiation between the two genomes is expected either because of the lower divergence rate at nuclear loci relative to mt loci or because of male-mediated gene flow among populations. The difference in divergence rates (all other factors being equal) are due to differences in the effective population size for each of the two genomes, which is four times larger for nuclear loci relative to mt loci. Many of the North Atlantic sampling localities were inaccessible to fin whales during the last glaciation some 18 000 years ago, (Eronen & Olander 1990), and were thus presumably founded relatively recently (in evolutionary terms). From this it follows that the divergence of the North Atlantic populations presumably is in a relatively initial stage and hence we would expect a higher degree of differentiation at mt loci relative to nuclear loci.

The fact that some areas (e.g. Gulf of St Lawrence and Gulf of Maine) only recently became accessible to fin whales was further supported by the distribution of pairwise differences in the mt sequences among individual fin whales. As is evident from the genealogy (Fig. 2), the vast majority of mtDNA haplotypes were separated by only a few substitutions. Hence, most lineages coalesce over a short time span, as expected in an exponentially growing population (Slatkin & Hudson 1991). The distribution of pairwise differences among individual fin whales in the

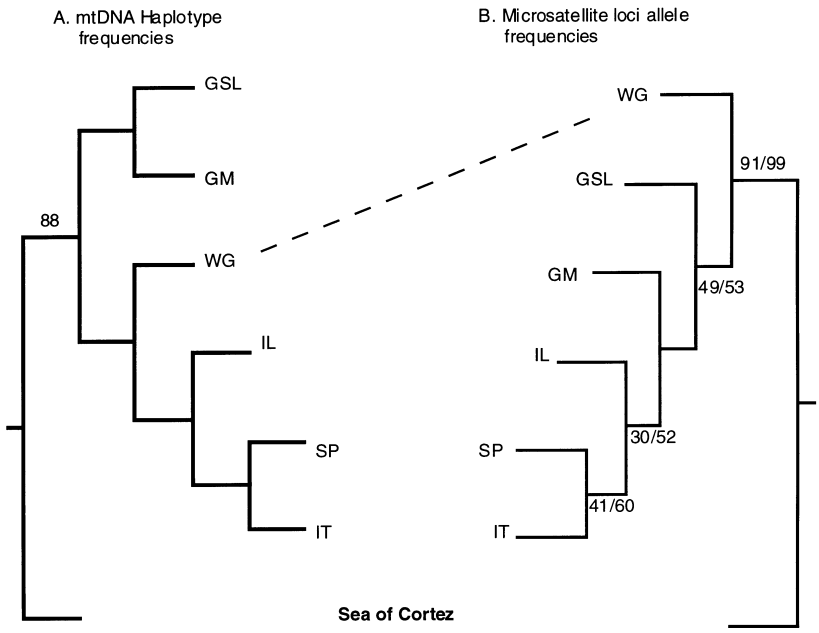

Fig. 5 Majority rule consensus tree estimated from Nei's genetic distances calculated between all localities. The abbreviations used in the sampling area are described in Table 1. Distances were estimated from (A) the mtDNA haplotype frequencies (D_{MT}) and (B) the six microsatellite loci allele frequencies (D_{MS}). Bootstrap values (500 replicates) at the nodes were calculated by resampling alleles (first value in A and B) and then loci (B) within populations.

mt sequences in the western North Atlantic areas (Gulf of Maine and Gulf of St Lawrence) were either unimodal or nearly so (Fig. 3), a finding which is consistent with exponential population growth (Slatkin & Hudson 1991). West Greenland and Iceland, which were not covered by the ice sheet but by continuous sea ice (Eronen & Olander 1990), had intermediate distributions. The more southern areas such as Spain and the Mediterranean Sea, which presumably would have been accessible to fin whales during the last glaciation, had pronounced multimodal distributions of pairwise differences in the mt sequences.

A factor which has to be considered when interpreting our results is that many cetaceans display maternally directed site fidelity (e.g. Baker *et al*. 1986; Clapham & Seipt 1991). Previous humpback whale studies have shown that this behavioural trait can influence the distribution of genetic variation (Palsbøll *et al*. 1995; Larsen *et al*. 1996). Maternally directed site fidelity to the 'summer' areas sampled during this study could generate differentiation at mt loci, even if all sampled whales constitute one panmictic population (Katona & Beard 1990; Clapham *et al*. 1993). However, the significant deviation from HW genotypic proportions observed in the combined North Atlantic samples, in the North Atlantic and Mediterranean Sea sample, as well as the significant level of heterogeneity at the nuclear loci between the Gulf of St Lawrence/West Greenland and the Mediterranean Sea, indicate the existence of nonrandom mating. Hence, a model assuming a panmictic population with maternally directed site fidelity to specific summer feeding areas is not consistent with our findings. Male-mediated gene flow could also contribute to such pattern of differentiation; however, to date there are no observations of sex-specific differences in dispersal for fin whales, as has been observed in, for example North Atlantic minke whales (Larsen & Øien 1988).

Even though neither the mt nor the nuclear phylogeny of populations (Fig. 5) was well supported in terms of bootstrap values, it is noteworthy that the topologies were congruent, especially when one considers the many possible branching patterns. The observed topology agreed with the results of the mt DNA homogeneity tests as well as the predictions from Kellogg's population model.

The level of divergence (estimated as Nei's *D*) at the nuclear loci observed in this study was similar to those reported by Danielsdottir and coworkers (Danielsdottir *et al*. 1991, 1993) who detected statistically significant differences in isozyme allele frequencies between the eastern Canadian coast, Iceland, Spain, and Norway as well as among years in Iceland. The average genetic distance among the North Atlantic sampling localities was, in our study, estimated to be 0.048 (SE = 0.0114), whereas Danielsdottir and coworkers reported a value of 0.013 between Iceland and Spain and 0.060 between Norway and eastern Canada. However, the tests employed in the current study did not detect statistically significant differences in allele frequencies between most areas. This discrepancy in the significance of the results in the two studies may have a statistical rather than a biological origin, i.e. a result of the higher number of alleles detected in the microsatellite analysis relative to studies using isozymes, resulting in a reduced discriminatory power when comparing allele frequencies between areas.

The population structure of North Atlantic fin whales indicated by this study and previous work (Ingebrigtsen 1929; Kellogg 1929; Sergeant 1977; Arnason *et al*. 1991; Danielsdottir *et al*. 1991, 1992) suggests that fin whale populations may be structured differently from those of humpback whales and northern right whales. Indeed, our results suggest substructure over even relatively short distances, and that different subpopulations may utilize the same feeding area, even within the same year, as originally proposed by Kellogg (1929).

The levels of variation in the mtDNA estimated in our study (Table 3) correlated well with the current estimates of abundance. The latest estimate of abundance for the entire North Atlantic Ocean was 56 000 (Buckland *et al*. 1992a,b; IWC 1992), 3583 individuals (95% confidence interval: 2130–6027, (Forcada *et al*. 1996) in the Mediterranean Sea, and 297 (95% confidence interval: 217–376) in the Sea of Cortez (Urban-R. 1996). The nucleotide diversity in the North Atlantic samples was estimated to be 0.0113, which was significantly higher than the estimate of 0.0057 and 0.0007 for the Mediterranean Sea and Sea of Cortez, respectively.

The nucleotide diversity estimated for the samples collected in the Sea of Cortez was among the lowest reported for cetaceans so far (Medrano *et al*. 1995; Rosel *et al*. 1995; Palsbøll *et al*. 1997a) The observed nucleotide diversity was even lower than that of the highly endangered North Atlantic right whale (0.0026 in Schaeff *et al*. 1993).

The significant level of divergence observed at the mt and nuclear loci between the Sea of Cortez and the North Atlantic/Mediterranean Sea is not surprising. Such differences have been reported for the minke whale (*Balaenoptera acutorostrata*) (Van Pijlen *et al*. 1995; Bakke *et al*. 1996) and the humpback whale (Baker *et al*. 1990, 1993, 1994; Valsecchi *et al*. 1997). However, the mt haplotypes unique to the Sea of Cortez clade were separated by only a few nucleotide substitutions from those found in the North Atlantic/Mediterranean Sea. In addition, two of the North Atlantic haplotypes were in the same well-supported clade as those from the Sea of Cortez. These findings were unexpected considering the large geographical distance and physical barriers separating these two oceans. The time of divergence was estimated at 1.2–2.3 Ma (and probably an overestimate of the divergence time, see the Material and methods), which is more recent than the rise of the Panama Isthmus some 3 Ma (Savage 1983). The most probable explanation for the unexpectedly low level of divergence is occasional gene flow between oceans as observed in humpback whales (Baker *et al*. 1990, 1993, 1994; Palsbøll *et al*. 1995; Valsecchi *et al*. 1997). However, the clear separation of the haplotypes found in the Sea of Cortez from the remainder indicates that such gene flow has not occurred recently (on an evolutionary timescale).

The fin whales in the Sea of Cortez probably constitute a year-round resident population (Leatherwood *et al*. 1988). However, the abundance of fin whales increases during the winter and spring, suggesting that fin whales from the Pacific Ocean may visit the Sea of Cortez during part of the year (Tershy *et al*. 1990; Tershy *et al*. 1993). Consistent with this hypothesis, we detected a significant deviation from HW genotypic proportions among the Sea of Cortez samples. If only individuals with the most common mt haplotype in the Sea of Cortez (Bp49, 90% of all the individuals) were considered, no such deviation from HW genotypic proportions was detected. Hence, fin whales which had one of the two rare mt haplotypes could represent migrants from the Pacific Ocean. Nonetheless, the two rare mt haplotypes (Bp50, Bp51) differ from the most common mt haplotype (Bp49) by only one substitution, suggesting that the three mt haplotypes are very closely related. As no information is available concerning the North Pacific Ocean fin whales in general, it is not possible to propose any definite conclusion.

As in the Sea of Cortez, the nucleotide diversity among the Mediterranean Sea samples was significantly lower than that estimated at North Atlantic sampling areas. It is unclear whether the fin whales observed in the Ligurian Sea during the summer are year-round residents of the Mediterranean that may winter in the southern part of this Sea (Duguy 1989) or the eastern part of the North Atlantic. The notion of a year-round resident population was supported by the significant level of differentiation in the mtDNA between the Mediterranean Sea and Spain (Table 3). A similar degree of isolation between the North Atlantic and Mediterranean Sea has also been reported in the striped dolphin (*Stenella coeruleoalba*) (Archer 1996), a species with a migratory potential similar to fin whales. The lack of fin whale sightings in the Strait of Gibraltar (Duguy *et al*. 1988) and the observation of newborns during the summer in the Ligurian Sea (Notarbartolo di Sciara *et al*. 1996), also suggest the absence of major semiannual migration of Mediterranean fin whales into the North Atlantic. In addition, blubber concentrations of persistent organochlorine pollutants are much higher in Mediterranean fin whales than in those from the Atlantic northwest coast of Spain (Marsili & Focardi 1996). However, no significant deviations from HW genotypic proportions were observed in the combined Mediterranean Sea and Spanish samples, nor did we detect any significant levels of heterogeneity at the nuclear loci. Therefore, with the current sample sizes, our study is consistent with the idea of a separate fin whale population in the Mediterranean; however, more data are needed to provide conclusive proof of separation.

In summary, the results from our North Atlantic fin whale study suggests 'isolation-by-distance', which was in general agreement with the population model proposed in

1929 by Kellogg. He suggested that fin whales were subdivided into several 'stocks' with limited but sometimes overlapping ranges. Clear demonstration, from genetic data, of such fine-scaled structure will require a comprehensive collection and analysis of samples well represented in time and space. In addition, the level and distribution of the variation at the mt loci indicated recent population expansion in the western North Atlantic. Our study also indicated that hypervariable microsatellite loci with many alleles may not be the optimal loci for population analyses, and that such studies will probably gain in statistical power if microsatellite loci of intermediate variability are analysed.

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M. Bérubé is a PhD candidate at McGill University studying the molecular ecology of fin whales. M. Bérubé conducted her laboratory work at the Copenhagen University as an exchange student, as part of the Cetacean Genetics Group directed by P. J. Palsbøll and then situated at University of Copenhagen. The Cetacean Genetics Group (currently situated at the Free University of Brussels) focuses on population genetic and evolutionary aspects of North Atlantic cetaceans. A. Aguilar, D. Dendanto, F. Larsen, G. N. di Sciara, R. Sears, J. Sigurjónsson and J. Urban-R. all conduct field studies of various species of cetaceans focusing on aspects such as abundance, behaviour, energetics, population identity, and toxicology.
