Molecular Ecology (2000) **9**, 1793–1802

World-wide genetic differentiation of *Eubalaena*: questioning the number of right whale species

H. C. ROSENBAUM,*† R. L. BROWNELL JR,‡ M. W. BROWN,§ C. SCHAEFF,¶ V. PORTWAY,¶** B. N. WHITE,†† S. MALIK,†† L. A. PASTENE,‡‡ N. J. PATENAUDE,§§ C. S. BAKER,§§ M. GOTO,‡‡ P. B. BEST,¶¶ P. J. CLAPHAM,*** P. HAMILTON,††† M. MOORE,‡‡‡ R. PAYNE,§§§ V. ROWNTREE,§§§ C. T. TYNAN, ¶¶¶ J. L. BANNISTER**** and R. DeSALLE*

**Molecular Systematics Laboratory, American Museum of Natural History, 79th Street and CPW, New York, NY 10024, USA, †Wildlife Conservation Society, Science Resource Center, 2300 Southern Boulevard, Bronx, NY 10460, USA, ‡National Marine Fisheries Service, South-west Fisheries Science Center, Box 271, La Jolla, CA 92038, USA, §Center for Coastal Studies, PO Box 1036, Provincetown, MA, 02657 USA and East Coast Ecosystems, PO Box 36, Freeport, Nova Scotia BOV IBO Canada, ¶Biology Department, American University, 4400 Mass. Ave, N.W. Washington D.C. 20016–8007, USA, **Molecular Genetics Laboratory, National Zoological Park, Smithsonian Institution, Washington D.C. 20560, USA, ††Biology Department, McMaster University, Hamilton, Ontario, Canada L8S 4K1, ‡‡The Institute of Cetacean Research, 4–18 Toyomi-cho, Chuo-ku, Tokyo 104–0055, Japan, §§Ecology and Evolution Group, School of Biological Sciences, University of Auckland, Private Bag 92019, Auckland, New Zealand, ¶¶Mammal Research Institute, University of Pretoria, c/o South African Museum, PO Box 61, Cape Town, 8000 South Africa, ***National Marine Fisheries Service, NEFSC, 166 Water Street, Woods Hole, MA 02543, USA, †††New England Aquarium, Central Wharf, Boston, MA 02110, USA, ‡‡‡Woods Hole Oceanographic Institution, Woods Hole, MA 02543, USA, §§§Whale Conservation Institute, 191 Weston Road Lincoln, MA 01773, USA, ¶¶¶ Joint Institute for the Study of the Atmosphere and Ocean, University of Washington, c/o National Marine Mammal Laboratory, NOAA, 7600 Sand Point Way NE, Seattle, Washington 98115, USA, ****c/o The Western Australian Museum, Francis Street, Perth WA 6000, Australia*

Abstract

Few studies have examined systematic relationships of right whales (*Eubalaena* spp.) since the original species descriptions, even though they are one of the most endangered large whales. Little morphological evidence exists to support the current species designations for *Eubalaena glacialis* in the northern hemisphere and *E. australis* in the southern hemisphere. Differences in migratory behaviour or antitropical distribution between right whales in each hemisphere are considered a barrier to gene flow and maintain the current species distinctions and geographical populations. However, these distinctions between populations have remained controversial and no study has included an analysis of all right whales from the three major ocean basins. To address issues of genetic differentiation and relationships among right whales, we have compiled a database of mitochondrial DNA control region sequences from right whales representing populations in all three ocean basins that consist of: western North Atlantic *E. glacialis*, multiple geographically distributed populations of *E. australis* and the first molecular analysis of historical and recent samples of *E. glacialis* from the western and eastern North Pacific Ocean. Diagnostic characters, as well as phylogenetic and phylogeographic analyses, support the possibility that three distinct maternal lineages exist in right whales, with North Pacific *E. glacialis* being more closely related to *E. australis* than to North Atlantic *E. glacialis*. Our genetic results provide unequivocal character support for the two usually recognized species and a third distinct genetic lineage in the North Pacific under the Phylogenetic Species Concept, as well as levels of genetic diversity among right whales world-wide.

Keywords: cetaceans, conservation, phylogeny, right whales, taxonomy

Received 30 April 2000; revision received 14 June 2000; accepted 14 June 2000

Introduction

Right whales are among the most endangered of large cetaceans, with all northern populations listed as a top priority for marine mammal conservation efforts (International Whaling Commission 1998; Clapham *et al*. 1999). All right whale populations are listed as either endangered or vulnerable by the IUCN (Reeves & Leatherwood 1994). One of the first steps in the conservation of any endangered species from a genetic perspective is the determination of the biological entities being conserved (Baker & Palumbi 1994; Moritz 1994; Vogler & DeSalle 1994). Unfortunately, the taxonomic structure of right whales has not been examined in a comprehensive framework for the purposes of conservation and systematics, which would help guide management decisions for these cetaceans.

Historically, two species of right whales are recognized: the northern right whale *Eubalaena glacialis*, which is currently classified as *E. glacialis* regardless of Atlantic or Pacific Ocean origin (Rice 1998) and the southern right whale *E. australis*, which has multiple populations with a circumpolar distribution (Rice 1998). The distinction of *E. glacialis* and *E. australis* is based upon a single morphological character (the alisphenoid bone) in the orbital region of the skull (Müller 1954). The character is ontogenetic, and the limited number of specimens analysed possibly represent different developmental stages from each of the species (Müller 1954). Historically, North Pacific right whales were given full species status as *E. japonica* (Lacépède 1818) or were classified as a subspecies of *E. glacialis* (*E. g. sieboldii* Gray 1864). Currently, right whales from the North Pacific Ocean are classified only as a separate intraspecific population of northern right whales (Rice 1998).

Limited morphological and physiological differences have been found among pairwise comparisons of right whales from the three different ocean basins. These comparisons have included: skeletal differences (Omura 1958; Omura *et al*. 1969), body measurement variation (Ivanova 1961a,b; Best 1987), frequency differences in appearance of callosities (patches of thickened epidermis that occur on the heads of right whales; Best 1970; Kraus *et al*. 1986), association of parasites (Scarff 1986) and dorsal and ventral coloration patches (Schaeff & Hamilton 1999; Schaeff *et al*. 1999). Restriction fragment length polymorphisms (RFLPs) and mitochondrial sequence haplotypes have been examined for North and South Atlantic right whale populations with no shared haplotypes found between them (Schaeff *et al*. 1991; Malik *et al*. 2000). No study to date has used any source of character information (morphological, physiological, or molecular) in a phylogenetic context to examine the distinctiveness of right whales from all three different ocean basins.

For lack of concrete evidence, the status and validity of species within *Eubalaena* (and *Eubalaena* itself) has been questioned. Recently, it has been suggested that the genus *Eubalaena* be subsumed into *Balaena* (Arnason & Gullberg 1994; Rice 1998). Rice (1998) questions a putative subspecific classification for *E. australis* and *E. glacialis*, specifying that an evaluation of right whale populations must consider that, 'the validity of any subspecies will remain dubious until adequate series of specimens from both sides of the North Atlantic and North Pacific and from different sectors of the southern hemisphere have been critically compared'.

Obtaining the necessary information from all right whales, particularly those from the North Pacific ocean basin, has been difficult. The North Pacific right whale is one of the most depleted populations of large whales (Brownell *et al*., in press). The eastern population is suspected to be a small number of individuals (Brownell *et al*., in press) of which a remnant population can still be found in the south-eastern Bering Sea (Goddard & Rugh 1998; Tynan 1999; Brownell *et al*. unpublished data). In the eastern North Pacific, whaling by American, French and British vessels began in 1835 (Scarff 1986). This was followed by a period of exploitation so intense that within 14 years the whalers were forced to turn their attention to bowhead whales. Additional unrestricted whaling in the Okhotsk Sea and Kuril Islands in the western North Pacific further reduced the populations and by 1900 right whales were considered rare (Scarff 1991). Whales from the eastern North Pacific population, perhaps irreversibly damaged by recent illegal Soviet catches (Brownell *et al*., in press), have such a small population size that a single sighting has merited publication (e.g. Rowntree *et al*. 1980; Carretta *et al*. 1994; Gendron *et al*. 1999). No calf has been observed this century in the eastern North Pacific, and the mating and calving grounds for the eastern and western ranges still remain largely unknown. The western range of the population is more numerous, particularly in the Kuril Islands and Okhotsk Sea region (Brownell *et al*., in press), where limited quantitative and systematically collected data suggest a population in the high hundreds (Miyashita & Kato, in press).

To rigorously examine the taxonomic status of *Eubalaena*, as well as to understand the phylogenetic relationships among members of this group, we have compiled an extensive mitochondrial DNA (mtDNA) sequence database that includes the first analysis of North Pacific right whale specimens at the molecular level. These sequences were generated from DNA isolated from skin tissue biopsy, stranded animals and historical whaling samples from 385 right whales throughout the northern and southern hemispheres. These molecular data allow us to examine

Correspondence: H.C. Rosenbaum. Fax: 1–212–769–5277; E-mail: hcr@amnh.org

the following: (i) the support for the distinction between *E. glacialis* and *E. australis*; (ii) the detection of any genetic differences between North Pacific and North Atlantic *E. glacialis*, and (iii) the phylogenetic relationships among right whales of the North Atlantic, North Pacific and southern hemisphere.

Materials and methods

Samples

Tissue samples were obtained from nearly all geographical regions where right whales are known to exist. In the North Atlantic Ocean, sequence information is based on 269 of the approximately 320 individuals remaining in the western population (Malik *et al*. 1999). North Atlantic right whale samples are from the Bay of Fundy, Cape Cod Bay and Roseway Basin ($n = 180$ plus 89 haplotypes inferred from known maternal offspring relationships). Haplotypes were assigned to the same matriline of a sampled whale based on confirmation from photographic identification studies (e.g. calves of a sampled female, a mother of a sampled calf or the calf's half-siblings). Since inference of haplotypes occurred across a maximum of three generations within a matriline, we feel the assumption that there were no mutations in the mtDNA between direct descendants and strict maternal inheritance is justified for the study. The importance of confirming 269 haplotypes from the remaining extant population illustrates with confidence that a majority (84%) of the extant population had been surveyed genetically (Malik *et al*. 1999). Furthermore, no new maternal lineage haplotypes were found in historical sampling of North Atlantic right whales (Rosenbaum *et al*., in press).

The samples from the North Pacific (historical baleen and skin tissue biopsy samples, $n = 12$) represent the only genetic survey of right whales for the entire ocean basin. North Pacific tissue biopsy samples ($n = 5$) are from the Bering Sea; North Pacific historical samples ranged from baleen samples from specimens collected 32–44 years ago to specimens collected by Charles Scammon dating back to 1862. Samples are from the Bering Sea and Pacific Coast of Japan ($n = 2$), south of Kodiak Island ($n = 2$), the Okhotsk Sea ($n = 2$), and Monterey, CA ($n = 1$). The sampling of *Eubalaena australis* populations ($n = 99$) is the most representative to date, covering two feeding and four known major breeding grounds throughout the southern hemisphere (Payne 1986; Baker *et al*. 1999; Portway *et al*., submitted). Southern hemisphere *E. australis* samples from tissue biopsy or stranded animals are from South Georgia ($n = 8$), Argentina ($n = 20$), South Africa ($n = 21$), Auckland Islands ($n = 20$), and Western Australia ($n = 20$). Biopsy samples from the Antarctic Area IV ($n = 10$) were collected during the summer feeding seasons by the Japanese Whale Research Programme (Table 1).

Table 1 Sampling of right whales world-wide

Species	Region	Number of samples	mtDNA sequence	Reference or Source information
Eubalaena australis	SW Australia	20	289	Baker *et al*. 1999
	Auckland Islands	20	289	Baker *et al*. 1999
Eubalaena australis	Argentina	20	294	Portway *et al*., submitted
	South Africa	21	294	Portway *et al*., submitted
	South Georgia	8	294	Portway *et al*., submitted
Eubalaena australis	Antarctic Area IV	10	540	this study Genbank Accession nos. AF275358–AF275363
Eubalaena glacialis	western North Atlantic Ocean	269*	500	Malik *et al*. 1999
Eubalaena glacialis	Bering Sea	5	540	this study
	Okhotsk Sea, Bering Sea and Pacific Coast of Japan	3	540	this study (1)
	Kodiak Island	2	540	this study (1)
	Okhotsk Sea	1	540	this study (2)
	Monterey, California	1	540	this study (3) GenBank Accession nos. AF275349–AF275357

The number of samples available and obtained (total of 380) is shown by region. *Indicates that maternal haplotype designations were inferred for 89 samples (see text for full description). *Italics* show historical samples analysed in the study. (1) Samples collected between 1956 and 1968 from Table 1 of Omura *et al*. 1969; (2) Baleen from National Museum of Natural History, A0013986, collected in 1862 by Charles Scammon; (3) Baleen from National Museum of Natural History, 12050, collected by Charles Scammon, late 19th century (exact year unknown).

Molecular data collection

Molecular analysis of samples from the North Atlantic and southern hemisphere was carried out according to the protocols detailed in Baker *et al.* (1999), Malik *et al.* (1999), or Portway *et al.* (submitted). For historical specimens of baleen from the North Pacific, DNA extraction, polymerase chain reaction (PCR) amplification and fluorescent dideoxy cycle sequencing were performed according to the strict handling and control procedures detailed in Rosenbaum *et al.* (1997). The variable portion of the mtDNA control region was primarily chosen because of its utility in genealogical and systematic studies, and because it is a common marker being analysed by all the coauthors for their own studies. Consensus sequences from both strands were generated for all samples. A 292-base pair (bp) consensus region was constructed from all sequences analysed in Sequencher. Because sequences from published reports were of varying lengths (Table 1), the 292-bp consensus region represents the maximum number of bp from the mtDNA control region common to all samples analysed.

Geographic structure

Diversity measures (nucleotide diversity, haplotype diversity, and associated standard deviations) were estimated using the computer program ARLEQUIN VERSION 1.1 (Nei 1987; Schneider *et al.* 1997). Nucleotide diversities (π) are calculated from pairwise differences between mtDNA sequences; haplotype diversities (h) are calculated without regard to genetic distance (i.e. no consideration for number of nucleotide polymorphisms) between mtDNA sequences (Nei 1987). Geographic differentiation for the mtDNA sequences of right whales from the North Atlantic, North Pacific and southern hemisphere was assessed using two methods: an analysis of molecular variance (AMOVA, where the test statistic, ϕ_{ST}, includes the variance attributable to molecular distances between haplotypes) and by conventional F_{ST} statistics using only haplotype frequencies (Wright 1951; Takahata & Palumbi 1985; Excoffier & Smouse 1992; Schneider *et al.* 1997). Significance of the observed ϕ– or F-values was determined by comparison to a null distribution of these values based on 10 000 random permutations of the data matrix using ARLEQUIN VERSION 1.1 (Schneider *et al.* 1997).

Population aggregation analysis (PAA) and phylogenetic analysis

All sequences of right whales were aligned to the putative sister taxon, *Balaena mysticetus* (Arnason *et al.* 1993). Alignment was trivial as there were only single bp insertions detected. PAA was performed according to the diagnostic character framework in Davis & Nixon (1992). Fixed nucleotide characters are the unit for which aggregation of Operational Taxonomic Units (OTUs) occur. For diagnosis, attributes whose fixed unique states unite a group (populations, species) to the exclusion of other groups are considered characters (Davis & Nixon 1992). Polymorphic attributes, or traits, are indicative of population frequency differences. The strength of this method is that it provides 'a consistent, definable scheme that avoids both practical and logical shortcomings of other approaches and species concepts' (Goldstein *et al.* 2000).

Phylogenetic analysis using parsimony methods was conducted using the stepwise-addition option of the heuristic search in PAUP 4.0 using 10 random addition heuristic searches with tree bisection reconnection (TBR) branch swapping (Swofford 1999) and all trees were rooted with sequence from *B. mysticetus*. Additionally, the effects of character polarity were tested by exploring variation in the outgroup taxa ($n = 56$, Dizon & Brownell, unpublished data, and GenBank Accession nos X72197 and L35609) in the phylogenetic analysis. Clade stability was evaluated using jackknife parsimony (Farris *et al.* 1996) and Bremer support analysis (Bremer 1994). Jackknife values were generated in PAUP 4.0 using 10 000 replicates for each analysis (Swofford 1999). Bremer support measures the number of extra steps in tree length that occur before a node collapses (Bremer 1994). Comparisons are made between the length of the most parsimonious tree(s) and the length of the shortest tree(s) not containing a given node. The magnitude of the value is indicative of the support for a given node. The higher the Bremer support, the greater the character support for the node. Bremer supports were generated with AUTODECAY (Eriksson 1998) using five random addition heuristic searches for each node.

Results

A total of 65 variable nucleotide positions were detected (including those that separate *Balaena mysticetus* from *Eubalaena*), among the 380 individuals defining 46 unique mtDNA control region haplotypes world-wide. From a phylogeographic perspective, all haplotypes were geographically concordant with the three ocean basins; no haplotypes were shared between right whales from the North Atlantic, North Pacific and southern hemisphere populations. Haplotype (h) and nucleotide (π) diversities were lower in North Atlantic *Eubalaena glacialis* than among North Pacific *E. glacialis* and *E. australis* (23–24% and 66–72% lower for North Atlantic *E. glacialis* h and π, respectively; Table 2). North Pacific *E. glacialis* samples showed high haplotype and nucleotide diversity (Table 2). However, haplotype diversity was lowest among the five sampled extant individuals. Historical samples from the

Species	Region	Haplotypes	h ± SD	π ± SD
E. australis	All southern hemisphere	32	0.913 ± 0.018	2.68 ± 1.40
E. glacialis	western North Atlantic Ocean	5	0.698 ± 0.016	0.60 ± 0.30
E. glacialis	North Pacific Ocean	9	0.923 ± 0.057	2.10 ± 1.20
	tissue samples	2	0.600 ± 0.129	1.89 ± 1.22
	historical samples	7	1.000 ± 0.076	1.94 ± 1.22

Table 2 Diversity of mtDNA control region sequences for right whales world-wide. Haplotype (h) and per cent nucleotide (π) diversity are shown

Table 3 ϕ_{ST} and F_{ST} estimates below and above the diagonal, respectively. *P*-values from 10 000 permutations were all <0.00000 except for *E. australis* and North Pacific *E. glacialis* comparisons ($P < 0.00001$) with sequential Bonferroni correction

	NA *E. glacialis*	NP *E. glacialis*	*E. australis*
NA *E. glacialis*	—	0.219	0.205
NP *E. glacialis*	0.817	—	0.083
E. australis	0.754	0.568	—

NA is North Atlantic, NP is North Pacific.

North Pacific were each determined to belong to a unique maternal lineage (Table 2). A total of six haplotypes were found among *E. australis* samples from Antarctic Area IV. Three of these haplotypes were the same as those reported by Baker *et al.* (1999), while one haplotype was shared with a lineage reported by Portway *et al.* (submitted). Additionally, one haplotype was common to both studies and another was only found in the sample from Antarctic Area IV. A more detailed analysis of population structure among southern right whales is currently in preparation (Patenaude *et al.* in preparation; Portway *et al.* in preparation).

There was significant differentiation of mtDNA lineages from North Atlantic *E. glacialis*, North Pacific *E. glacialis* and southern hemisphere *E. australis* using both AMOVA ($\phi_{ST} = 0.747$, $P < 0.0000$) and conventional *F*-statistics ($F_{ST} = 0.200$, $P < 0.0000$). All pairwise comparisons between ocean basins were highly significant for both ϕ_{ST} and F_{ST} (Table 3). The ϕ_{ST} demonstrated that nearly 75% of the molecular variance was attributed to the different ocean basins, while the traditional F_{ST} using haplotype frequencies only showed 80% of the variance occurred within ocean regions.

Four diagnostic nucleotide characters (shared among all 269 individuals including 180 individuals directly analysed and 89 individuals whose haplotypes were inferred through known maternal relationships) distinguish North Atlantic right whales from all other right whales (Fig. 1). The sample of North Pacific *E. glacialis* shows three fixed, and therefore diagnostic, nucleotide characters distinguishing them from all other right whales. Three additional positions diagnose all sampled members of *E. australis* from both North Atlantic and North Pacific *E. glacialis*. There are no diagnostic sites among the different populations of *E. australis* in the southern hemisphere. *B. mysticetus* is diagnosable and different from all right whales at seven nucleotide sites.

A strict consensus of 985 equally parsimonious trees from the parsimony analysis of all the mtDNA control region sequences is shown in Fig. 1 (length 112, CI = 0.46, RI = 0.84). Phylogenetic analysis resulted in three well-resolved clades with all the North Atlantic Ocean haplotypes forming the most basal monophyletic group. Two additional resolved sister clades exist, one containing North Pacific *E. glacialis* lineages and the other possessing all lineages of southern hemisphere *E. australis* (Fig. 1). Jackknife values and Bremer supports were high for each of these clades (Fig. 1). *Eubalaena* monophyly was supported and confirmed by the addition of representative haplotypes from the outgroup taxon, *B. mysticetus*.

Discussion

Our data demonstrate that there are three diagnosably distinct maternal lineages of right whales world-wide geographically concordant with their distribution in the corresponding oceanic basins. The diagnostic molecular characters provide the first unequivocal character support for *Eubalaena glacialis* and *E. australis*. Historically, these morphologically based classifications have remained controversial (Rice 1998). Without additional character support, the evidence that has been used to uphold the present species classifications results from a diverse geographical distribution and migratory behaviour of right whales world-wide. Significant differentiation and diagnostic characters also distinguish North Atlantic and North Pacific *E. glacialis* populations.

The genetic differentiation among all three lineages is demonstrated by the AMOVA results with significant partitioning of molecular variance accounted for by the division of the ocean basins. The discrepancy between ϕ_{ST} and traditional F_{ST} statistics using haplotype frequencies only (no molecular information) clearly illustrates the relatively deep historical separation of these three lineages. Thus, the more coalescent time for accumulation of mutations in each of these lineages, the greater the difference between the estimates. The high genetic variance detected

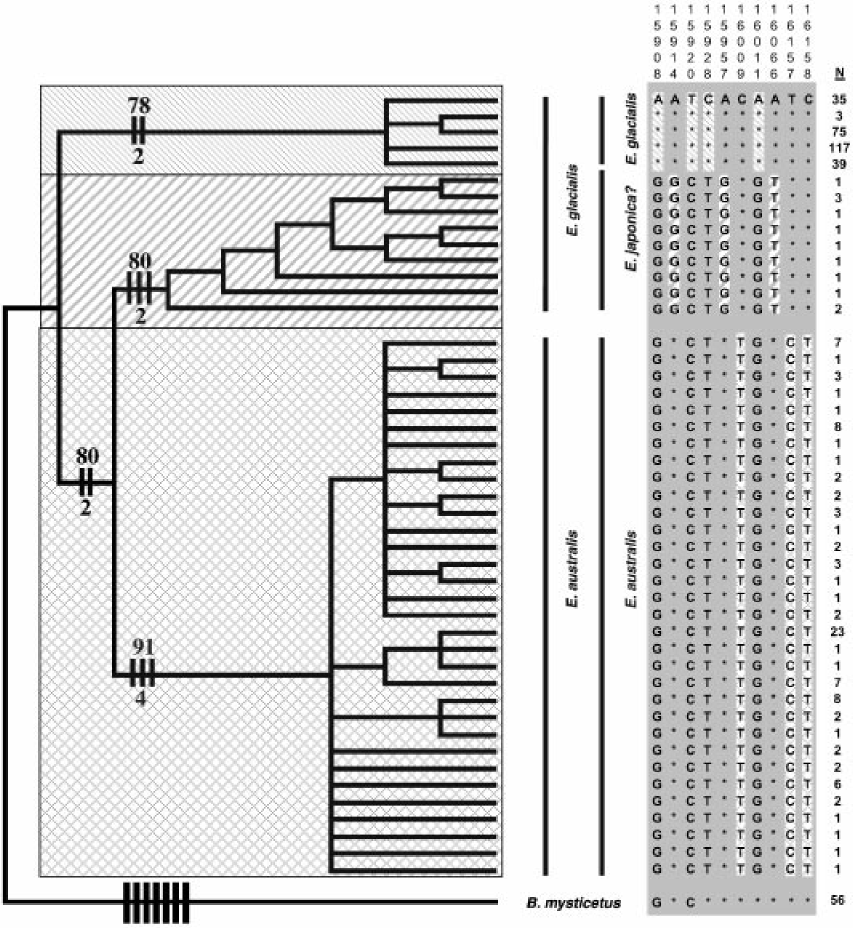

Fig. 1 Diagnostic nucleotide positions and phylogenetic relationships of right whales from three ocean basins. Nucleotide positions correspond to the fin whale (*Balaenoptera physalus*) mitochondrial genome positions 15897–16189 (Arnason *et al.* 1991). Positions 15908, 15920, 15928 and 16011 diagnose North Atlantic right whales (top) from all other right whales. Positions 15914, 15957 and 16066 diagnose the North Pacific right whales (middle). Southern hemisphere right whales are diagnosed by positions 16009, 16157 and 16158 (bottom). An asterisk indicates an identical nucleotide position to the North Atlantic right whale sequence shown at the top. Jackknife and Bremer support values are shown above and below nodes, respectively. The cross-hatch bars on the strict consensus parsimony tree trace the diagnostic characters determined by PAA. The set of bars immediately next to the cladogram illustrates current species definitions based for northern and southern right whales, while the next set of bars corresponds to the three genetically distinct lineages of *Eubalaena* as determined from this analysis. *Eubalaena japonica* was the original taxon name for North Pacific right whales. N is the number of individuals found for each mtDNA control region haplotype.

is attributed to within-ocean differences because no haplotypes are shared among the different ocean basins. The phylogenetic analyses further show that North Pacific *E. glacialis* are distinct and more closely related to *E. australis* than to North Atlantic *E. glacialis* (Fig. 1). The combined phylogeographic, phylogenetic and population aggregation analyses all clearly demonstrate the differentiation of maternal lineages among right whales world-wide. The existence of three distinct genetic lineages among right whales does not, however, imply or preclude the existence of other management units for conservation among right whale populations within each ocean basin determined by other genetic or nongenetic factors, as has been reported elsewhere (Baker *et al.* 1999).

Our evaluation of right whale genetic differentiation based on molecular characters shows that diagnostic characters exist for members of this group, despite the paucity of obvious morphological differences for these allopatric populations. No other populations of cosmopolitan large whales surveyed as extensively exhibit the same type of fixed molecular character states as those observed among *Eubalaena*. Among northern and southern hemisphere populations of humpback whales (*Megaptera novaeangliae*) and northern hemisphere fin whales (*Balaenoptera physalus*), there are no diagnostic molecular characters in the mtDNA control region that identify whales by a region or geographical area (Baker *et al.* 1993; Bérubé *et al.* 1998; Baker & Medrano-Gonzalez, in press). Only in the case of minke whales (*Balaenoptera acutorostrata* and *B. bonaerensis*) is a taxonomic distinction proposed in different ocean basins (Arnason *et al.* 1993; Rice 1998), and recently supported by several diagnostic nucleotide positions in the mitochondrial control region (Bakke *et al.* 1996). The combination of the 10 diagnostic sites among right whales world-wide would permit accurate geographical assignment of any sample from one of these right whale populations for forensic application or phylogeographic origin (Baker & Palumbi 1994).

An isolation phenomenon among certain marine taxa, known as antitropical distribution, is a plausible explanation that could have led to the differentiation and discreteness of the three maternal lineages of right whales detected in our study. The antitropical distribution of extant northern and southern hemisphere populations of right whales has been well-documented (Davies 1963), which has eventually led to reproductive isolation and absence of maternal gene flow. The differences in migratory behaviour for right whales within each hemisphere, along with the antitropical distribution, maintain and promote these boundaries to maternal gene flow. Our results strongly support this scenario and suggest that such isolation has resulted in the existence of distinct genetic lineages. The evolutionary origin of these discontinuous, antitropically distributed southern and northern hemisphere species has been suggested to occur during cooler Pleistocene glacial episodes (Davies 1963; McLeod *et al*. 1993). During these cooler periods, right whale populations may have expanded their range and eventually crossed the Equator. In subsequent warmer interglacial stages, right whale ranges may have receded to higher latitudes, splitting the two groups antitropically. The continual reoccurrence of glacial and interglacial periods would have caused the retraction of these species to higher or more temperate latitudes (McLeod *et al*. 1993).

The genetic data also suggest that no exchange has occurred between North Atlantic and North Pacific right whales, as has been proposed for the bowhead whale populations in the two ocean basins, either via the Arctic Basin or Central American Seaway (Barnes 1984; McLeod *et al*. 1993). The presence of the isthmus of Panama, emerging from the Late Miocene through the Late Pleistocene (≈ 3.5 million years ago), ultimately closed a route for dispersal and gene flow for right whales between the Atlantic and Pacific (Jones & Hasson 1985). The long periods of glacial–interglacial fluctuations during the Pleistocene, resulting in range expansion and subsequent antitropical distribution could have been one of the phenomena that have led to the close relationship between maternal lineages from North Pacific *E. glacialis* and *E. australis*.

The monophyly of *Eubalaena* maternal lineages, as well as the number of fixed differences between *Eubalaena* and *Balaena*, show a clear distinction between maternal lineages of the two genera. Morphological and fossil evidence (though incomplete) also show support for the distinction of the extant genus *Eubalaena* as separate from *Balaena* (McLeod *et al*. 1993). Some authors do not recognize the genus *Eubalaena*, and it has receently been suggested that *Eubalaena* be subsumed back into *Balaena* (Rice 1998). While there are no benchmarks for measuring differences among genera, we feel it is better to conserve the generic name *Eubalaena* for the present time because of the existence of diagnosably distinct characters between the two.

It is alarming that among *Eubalaena*, the two genetic lineages of right whales existing in the northern hemisphere have not experienced significant recovery since extensive historical whaling or recent exploitation. In the North Pacific, this lack of recovery is at least partly due to Soviet pelagic whaling in the 1960s even though right whales had been officially protected throughout their range since 1935 (Yablokov 1994; Brownell *et al*., in press). While haplotype diversity appears to be relatively high in the North Pacific, a more detailed analysis reveals that only two haplotypes were found among five extant animals. Each of the historical specimens carried a unique haplotype. The high mtDNA diversity results from the historical samples, where proper treatment and analysis of historical specimens was undertaken to ensure the authenticity of the sequences (Rosenbaum *et al*. 1997). The scarcity of these animals in the North Pacific and the observed reduction in genetic diversity between the historical samples and those from extant specimens (both with small sample sizes) may be due to extensive whaling that caused extreme losses in genetic lineages. However, a reduction in genetic variation in the North Pacific population cannot be adequately assessed without further samples, both from the historical and extant populations.

The reduced haplotype and nucleotide diversities detected in North Atlantic *E. glacialis* provide further suggestive evidence of the impact of a millennium of whaling on genetic variation in this species (Malik *et al*. 1999; Rosenbaum *et al*., in press). While these issues remain controversial (Amos 1996), molecular data from historical specimens are helping to determine the extent to which genetic diversity has been lost among cetaceans as a result of intense periods of commercial whaling and exploitation (Rosenbaum *et al*., in press). Because nearly all of the extant individuals have been surveyed genetically and our phylogenetic analysis shows discrete lineages for each ocean basin, the evolutionary potential to increase haplotype diversity and genetic variation for the basal North Atlantic right whales seems unlikely.

The molecular data presented here offer a source of information for refining taxonomy, which is an important aspect in the conservation of these endangered cetaceans. A lack of attention to taxonomic rigor has had severe consequences in other studies of endangered species (Daugherty *et al*. 1990). Historically, the North Pacific right whale was distributed across most of the North Pacific, particularly in the northern Gulf of Alaska and Bering Sea, where they feed in summer months. Their historical winter distribution has been suspected to range south to central Baja California in the eastern Pacific Ocean (Brownell *et al*., in press). There are no reliable estimates of abundance for right whales in the eastern North Pacific, but it is unlikely that their numbers are greater than a few dozen (Brownell *et al*., in press). The few samples obtained from recent sightings may represent the last members of a disappearing population. Although the hunting of right whales has ceased, the right whales in the North Atlantic and North Pacific still face a number of threats. In the western North Atlantic, collisions with ships and entanglements are the most common known cause of right whale mortality. The North Pacific population may encounter similar threats and interactions with certain large-scale fisheries and vessel traffic, but this has yet to be critically evaluated (Kornev 1994; Clapham *et al.* 1999). With the future of eastern North Pacific right whales in question and a high priority for marine mammal conservation (Clapham *et al.* 1999), the genetic data, in addition to the demographic history of this population, provide critical evidence to support necessary conservation of these distinct maternal lineages.

Our genetic results provide unequivocal character support consistent with distinguishing the three right whale lineages as phylogenetic species. Additional evidence from nuclear genetic markers is needed to confirm the present findings, particularly in a group of organisms where fidelity to an area is maternally directed (Baker *et al.* 1990; Palsbøll *et al.* 1995; Malik *et al.* 1999). These isolated populations have been differentiated at various times throughout their history as species, subspecies, or populations. We conclude that these genetic data must be considered in any taxonomic revision of the group, in any attempts to resurrect the North Pacific right whale as a valid taxon (*Eubalaena japonica*), and in the overall context of cetacean systematics and conservation. Failure to do so may result in the lack of identification of a possible third species of right whale.

Acknowledgements

We would like to thank Andy Dizon for providing bowhead whale sequences. Jim Mead and Charlie Potter of the Smithsonian Institution generously gave us access to historical specimens. The collection of biopsy samples from right whales in the southeast Bering Sea was supported by a grant to C. Tynan by the Office of Protected Resources, NOAA, Recover Protected Species Program. For North Atlantic samples, the work was supported by grants from the National Science and Engineering Research Council of Canada to B.N. White and from the National Marine Fisheries Service, United States to M.W. Brown. Financial support and laboratory support for the genetic analyses of South Atlantic samples was provided by The American University, the Pittsburgh Zoo, Rob Fleischer and the Molecular Genetics Laboratory, National Zoological Park, Smithsonian Institution. Some southern right whale biopsy samples were collected by Japanese researchers in the Antarctic Area IV under the Japanese Whale Research Programme. P.B. Best is thankful for the support of Earthwatch and the Foundation for Research Development. D. Reeb, F. Kemp, V. Calder, K.P. Findlay, A. Carribero, R. Benegas, K. Marshall-Tilas, M. Rivarola and M. Sanson assisted in the collection of tissue samples. Western Australian specimens were obtained through funding from the Scott Foundation, with field assistance from Chris Burton, Richard Holst, and Peter Spurr. M. Egan, P. Ersts and R. Baker provided valuable assistance during different stages of the work. We would like to thank L. Bernatchez, G. Amato, P. Doukakis, M. Levesque and three anonymous reviewers for their helpful comments to improve this manuscript. This work was funded in part by grants to HCR from the National Marine Fisheries Service and the Massachusetts Environmental Trust.

References

Amos B (1996) Levels of genetic variability in cetacean populations have changed little as a result of human activities. *Report of the International Whaling Commission*, **46**, 657–658.

Arnason U, Gullberg A (1994) Relationship of baleen whales based on cytochrome b gene sequence comparison. *Nature*, **367**, 726–728.

Arnason U, Gullberg A, Widegren B (1991) The complete nucleotide sequence of the mitochondrial DNA of the fin whale, *Balaenoptera physalus*. *Journal of Molecular Evolution*, **33**, 556–568.

Arnason U, Gullberg A, Widegren B (1993) Cetacean mitochondrial DNA control region: sequences of all extant baleen whales and two sperm whale species. *Molecular Biology and Evolution*, **10**, 960–970.

Baker CS, Medrano-Gonzalez L (2000) World-wide distribution and diversity of humpback whale mitochondrial DNA lineages. In: *Molecular Studies of Marine Mammals* (ed. Pfeiffer C). Krieger, Melbourne, FL.

Baker CS, Palumbi S (1994) Which whales are hunted? A molecular genetic approach to monitoring whaling. *Science*, **265**, 1538–1539.

Baker CS, Palumbi SR, Lambertsen RH *et al.* (1990) Influence of seasonal migration on geographic distribution of mitochondrial DNA haplotypes in humpback whales. *Nature*, **344**, 238–240.

Baker CS, Patenaude NJ, Bannister JL, Robins J, Kato H (1999) Distribution and diversity of mtDNA lineages among southern right whales (*Eubalaena australis*) from Australia and New Zealand. *Marine Biology*, **134**, 1–7.

Baker CS, Perry A, Bannister JL *et al.* (1993) Abundant mitochondrial DNA variation and world-wide population structure in humpback whales. *Proceedings of the National Academy of Sciences of the USA*, **90**, 8239–8243.

Bakke I, Johanse S, Bakke O, El-Gewely R (1996) Lack of population subdivision among minke whales (*Balaeanoptera acutorostrata*) from Icelandic and Norwegian waters based on mitochondrial DNA sequences. *Marine Biology*, **125**, 1–9.

Barnes LG (1984) Whales, dolphins and porpoises: origin and evolution of the Cetacea. *University of Tenessee, Studies in Geology*, **8**, 139–154.

Bérubé M, Aguilar A, Dendanto D *et al.* (1998) Population genetic structure of North Atlantic, Mediterranean Sea and Sea of Cortez fin whales, *Balaenoptera physalus* (Linnaeus 1758): analysis of mitochondrial and nuclear loci. *Molecular Ecology*, **7**, 585–600.

Best PB (1970) Exploitation and recovery of right whales *Eubalaena australis* off the Cape Province. *Investigative Reports of the Division of Sea Fisheries South Africa*, **80**, 1–20.

Best PB (1987) Estimates of the landed catch of right (and other whalebone) whales in the American Fishery. *Fishery Bulletin (USA)*, **85**, 403–418.

Bremer K (1994) Branch support and tree stability. *Cladistics*, **10**, 295–304.

Brownell RL, Clapham PJ, Miyashita T, Kasuya T (2000) Conservation status of North Pacific right whales. *Journal of Cetacean Research and Management, Special Issue*, in press.

Carretta JV, Lynn MS, LeDuc CA (1994) Right whale (*Eubalaena glacialis*) sighting off San Clemente Island, California. *Marine Mammal Science*, **10**, 101–105.

Clapham PJ, Young SB, Brownell RL Jr (1999) Baleen whales: conservation issues and the status of the most endangered populations. *Mammal Review*, **29**, 35–60.

Daugherty CH, Cree A, Hay JM, Thompson MB (1990) Neglected taxonomy and continuing extinctions of *Tuatara sphenodon*. *Nature*, **347**, 177–179.

Davies JL (1963) The antitropical factor in cetacean populations. *Evolution*, **17**, 107–116.

Davis JI, Nixon KC (1992) Populations, genetic variation, and the delimitation of phylogenetic species. *Systematic Biology*, **41**, 421–435.

Eriksson T (1998) *Autodecay*, Version 4.0 (program distributed by the author). Department of Botany, Stockholm University, Stockholm.

Excoffier L, Smouse PE (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.

Farris JS, Albert VA, Kallersjo M, Lipscomb D, Kluge AG (1996) Parsimony jackknifing outperforms neighbor-joining. *Cladistics*, **12**, 99–124.

Gendron D, Lanham S, Carwardine M (1999) North Pacific right whale (*Eubalaena glacialis*) sighting south of Baja California. *Aquatic Mammals*, **25**, 31–34.

Goddard P, Rugh D (1998) A group of right whales seen in the Bering Sea in July 1996. *Marine Mammal Science*, **14**, 136–141.

Goldstein PZ, Desalle R, Amato G, Vogler AP (2000) Conservation genetics at the species boundary: grand synthesis or methodological introgressions? *Conservation Biology*, **14**, 120–131.

Gray JE (1864) Notes on the whalebone-whales; with a synopsis of the species. *Annals and Magazine of Natural History*, **14**, 345–353.

International Whaling Commission (1998) *Chairman's Report of the 50th Annual Meeting*. International Whaling Commission, Cambridge.

Ivanova EI (1961a) K morfologii yaponskogo kita (*Eubalaena sieboldi* Gray) (The morphology of the Japanese right whale) in Russian. *(Eubalaena Sieboldi Gray)*, **34**, 216–251.

Ivanova EI (1961b) Proportsii tela I kharakter rosta kitov Dal'nego vostoka (Proportions of the body and the nature of growth in whales from the Far East) in Russian. *Trudy Soveshch. Ikhtiol. Korn.*, **12**, 72–78.

Jones DS, Hasson PF (1985) History and development of the marine invertebrate faunas separated by the Central American Isthmus. In: *The Great American Biotic Interchange* (eds Stehli FG, Webb D), pp. 325–355, Plenum Press, New York.

Kornev SI (1994) A note on the death of a right whale (*Eubalaena glacialis*) off Cape Lpatka (Kamchatka). *Report of the International Whaling Commission Special Issue*, **15**, 443–444.

Kraus SK, Moore KE, Price CA (1986) The use of photographs to identify individual North Atlantic right whales (*Eubalaena glacialis*). *Report of the International Whaling Commission Special Issue*, **10**, 145–151.

Lacépède BGE (1818) Note sur des Cétacées des mers voisines du Japon. *Mémoires Du Musée d'Histoire Naturelle*, **4**, 467–475.

Malik S, Brown MW, Kraus SD *et al.* (1999) Assessment of genetic structuring and habitat philopatry in the North Atlantic right whale. *Canadian Journal of Zoology*, **77**, 1–6.

Malik S, Brown MW, Kraus SD, White BN (2000) Analysis of mitochondrial DNA diversity within and between North and South Atlantic right whales. *Marine Mammal Science*, **16** (3), 545–558.

McLeod SA, Whitmore FC Jr, Barnes LG (1993) Evolutionary relationships and classification. In: *The Bowhead Whale* (eds Burns JJ, Montague JJ, Cowles CJ), pp. 45–70. The Society for Marine Mammalogy, Lawrence, Kansas.

Miyashita T, Kato H (2000) Recent data on the status of right whales in the Northwest Pacific Ocean. *Journal of Cetacean Research and Management, Special Issue*, in press.

Moritz C (1994) Defining 'Evolutionarily Significant Units' for conservation. *Trends in Ecology and Evolution*, **9**, 373–375.

Müller J (1954) Observations of the orbital region of the skull of the Mystacoceti. *Zoologische Mededelingen*, **32**, 239–290.

Nei M (1987) *Molecular Evolutionary Genetics*. Columbia University Press, New York.

Omura H (1958) North Pacific right whale. *Scientific Reports of the Whaling Research Institute*, **13**, 1–52.

Omura H, Ohsumi S, Nemoto T, Nasu K, Kasuya T (1969) Black right whales in the North Pacific whale. *Scientific Reports of the Whaling Research Institute*, **21**, 1–78.

Palsbøll PJ, Clapham PJ, Mattila DK *et al.* (1995) Distribution of mtDNA haplotypes in North Atlantic humpback whales: the influence of behavior on population structure. *Marine Ecology Progress Series*, **116**, 1–10.

Payne R (1986) Long term behavioral studies of the southern right whale (*Eubalaena australis*). *Report of the International Whaling Commission Special Issue*, **10**, 161–168.

Portway VA, Best PB, Clapham PJ *et al.* (2000) Mitochondrial DNA differentiation and genetic diversity in South Atlantic right whales (*Eubalaena australis*). *Marine Ecology Progress Series*, submitted.

Reeves RR, Leatherwood S (1994) *Dolphins, Porpoises and Whales 1994–98 Action Plan for the Conservation of Cetaceans*. International Union for Conservation of Nature and Natural Resource, Gland, Switzerland.

Rice DW (1998) *Marine Mammals of the World: Systematics and Distribution*. Allen Press-The Society for Marine Mammalogy, Lawrence, Kansas.

Rosenbaum HC, Egan MG, Clapham PJ, Brownell RL, Jr DeSalle R (1997) An effective method for isolating DNA from historic specimens of baleen. *Molecular Ecology*, **6**, 677–681.

Rosenbaum HC, Egan MC, Clapham PJ *et al.* (2000) Utility of North Atlantic right whale museum specimens in assessing changes in genetic diversity. *Conservation Biology*, in press.

Rowntree V, Darling JD, Silber G, Ferrari M (1980) Rare sighting of a right whale (*Eubalaena glacialis*) in Hawaii. *Canadian Journal of Zoology*, **58**, 309–312.

Scarff JE (1986) Historic and present distribution of the right whale (*Eubalaena glacialis*) in the eastern North Pacific south of 50 N and east of 180 W. *Report of the International Whaling Commission Special Issue*, **10**, 43–64.

Scarff JE (1991) Historic distribution and abundance of the right whale (*Eubalaena glacialis*) in the North Pacific, Bering Sea, Sea of Okhotsk and Sea of Japan from the Maury whale charts. *Report of the International Whaling Commission*, **41**, 467–489.

Schaeff CM, Hamilton PK (1999) Genetic basis and evolutionary significance of ventral skin coloration markings in North Atlantic right whales (*Eubalaena glacialis*). *Marine Mammal Science*, **15**, 701–711.

Schaeff CM, Best PB, Rowntree VJ *et al.* (1999) Dorsal skin color patterns among southern right whales (*Eubalaena australis*): genetic basis and evolutionary significance. *Journal of Heredity*, **90**, 464–471.

Schaeff C, Kraus S, Brown M (1991) Preliminary analysis of mitochondrial DNA variation within and between the right whale species *Eubalaena glacialis* and *Eubalaena australis*. In: *Genetic Ecology of Whales and Dolphins* (ed. Hoelzel AR), pp. 217–224, International Whaling Commission, Cambridge.

Schneider S, Kueffer J-M, Roessli D, Excoffier L (1997) *Arlequin,* Version 1.1: *A software for population genetic data analysis*. Genetics and Biometry Laboratory, University of Geneva, Geneva.

Swofford DA (1999) *PAUP*, *Version 4.0*. Sinauer, Sunderland, MA.

Takahata N, Palumbi SR (1985) Extranuclear differentiation and gene flow in the finite island model. *Genetics*, **109**, 441–457.

Tynan CT (1999) Redistribution of cetaceans in the Southeast Bering Sea relative to anomalous oceanographic conditions during the 1997 El Nino. *PICES Scientific Report*, **10**, 115–117.

Vogler A, DeSalle R (1994) Diagnosing units of conservation management. *Conservation Biology*, **8**, 354–363.

Wright S (1951) The genetical structure of populations. *Annals of Eugenics*, **15**, 323–354.

Yablokov A (1994) Validity of whaling data. *Nature*, **367**, 108.

This paper reflects a collaborative effort among an international team of researchers dedicated to the study and conservation of endangered right whales.