GENETIC ANALYSES OF THE NORTH ATLANTIC RIGHT WHALE

POPULATION STRUCTURE AND GENETIC DIVERSITY OF THE NORTH ATLANTIC RIGHT WHALE (*EUBALAENA GLACIALIS*).

By

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ABSTRACT

The North Atlantic right whale (*Eubalaena glacialis*) is the most endangered species of large whale in the world (IUCN). Efforts for the recovery of this species were initiated almost 20 years ago, yet the North Atlantic right whale shows little signs of recovery. Reliance on particular habitat areas and the effect of factors such as ship collisions, net entanglement and habitat disturbances are hampering the recovery of this species. Therefore, it is important to assess the level of genetic diversity left in this population and to identify and assess all habitat areas for potentially lethal threats. This study has identified a subset of the reproductive females that do not use the Bay of Fundy as a nursery area, through the genetic structuring of mtDNA control region haplotypes. Genetic structuring of the control region haplotypes was established and maintained by site fidelity of reproductive females to specific nursery areas. These results have identified a list of reproductive females that will be the targets of satellite tagging to elucidate the location of the alternative nursery area(s) to the Bay of Fundy. Analysis of mtDNA control region haplotypes in North and South Atlantic right whale has identified five haplotypes in the 180 North Atlantic right whales analyzed compared to 10 haplotypes in the 16 South Atlantic right whales analyzed. The low level of haplotypic variability in the North Atlantic right whale is a direct consequence of the extensive whaling period endured by this species. The genetic divergence between the North and South Atlantic right whales was estimated to have occurred 3.0-9.0 mya. This is similar

to the genetic divergence of 2.0-5.3 mya found between the two clades identified in the South Atlantic samples.

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CHAPTER ONE

GENERAL INTRODUCTION

The right whale (*Eubalaena* spp.) is a large black baleen whale that is identified by its large head, callosity patterns, lack of dorsal fin and it's distinctive v-shaped blow (Leatherwood and Reeves 1983). These large solitary whales feed on copepods (Murison and Gaskin 1989) and can grow up to 17 meters in length.

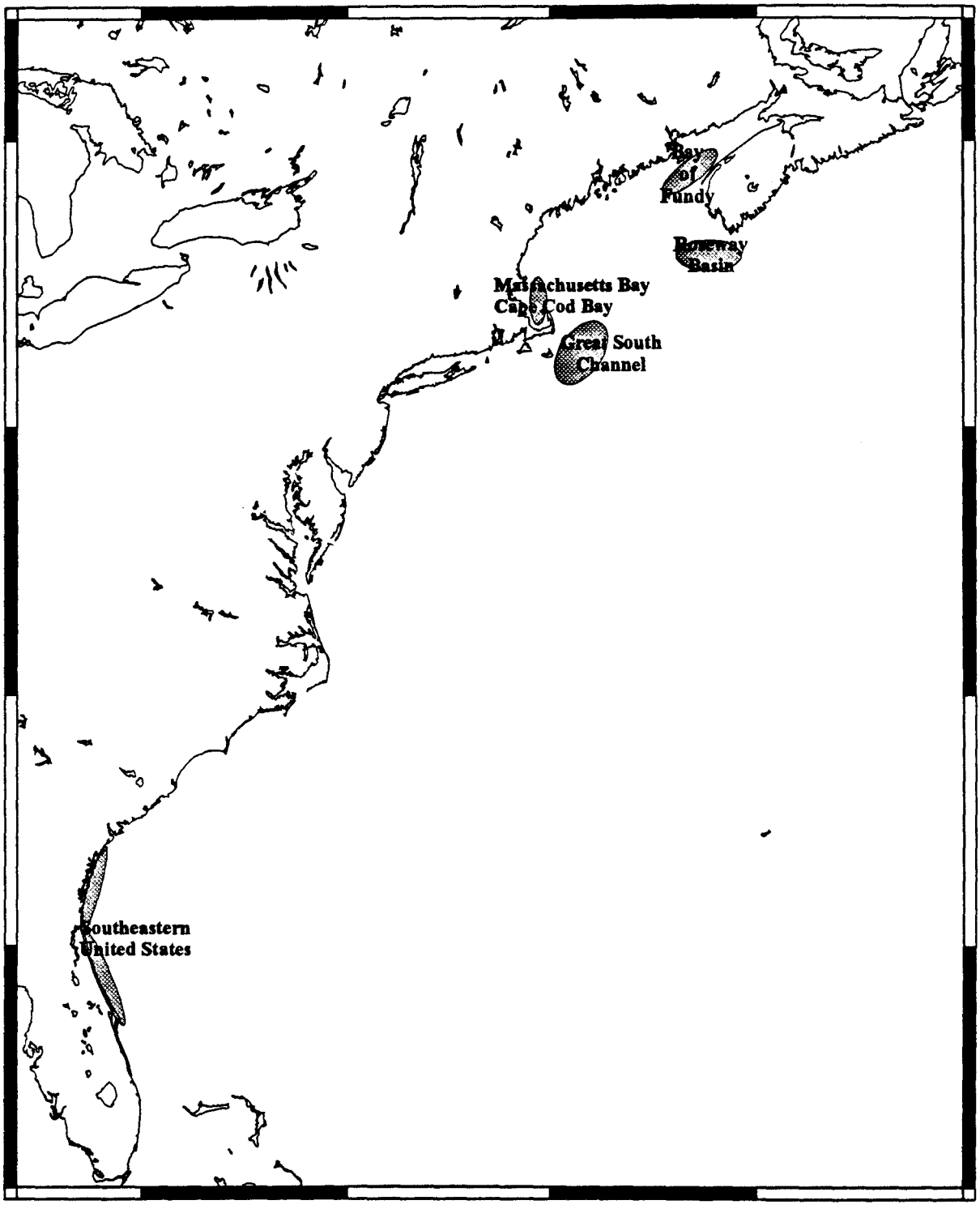
Distribution

The right whale (*Eubalaena*) is represented by two species, *Eubalaena glacialis* and *Eubalaena australis*. *Eubalaena glacialis*, the Northern right whale was represented by three populations, the Western and Eastern North Atlantic right whale and the North Pacific right whale. The Eastern North Atlantic right whale population is considered near extinction (IUCN status) as a result of extensive Basque whaling exploitation. Extensive exploitation has also listed the North Pacific right whale (*Eubalaena glacialsis japonica*) as near extinction (IUCN status). The southern hemisphere species is six months out of phase with the northern hemisphere species which presumably prevents interbreeding (Braham and Rice 1984). There are several possible southern hemisphere populations (*Eubalaena australis*) including the South American, the South African and South Pacific/Indian Ocean. These populations were also the targets of whaling but over a less prolonged period. Their IUCN status is vulnerable.

The focus of this thesis is the endangered (IUCN status) Western North Atlantic right whale (*Eubalaena glacialis*) from here on in referred to as the North Atlantic right whale since the second North Atlantic population is considered near extinction. The

North Atlantic right whale migrates along the east coast of North America. Five major habitat areas have been identified for this species (Figure 1.1): I. The coastal waters of southeastern United States is a winter calving ground where the majority of sightings are mother-calf pairs. Individuals are usually sighted between December and March. Low altitude aerial flights have surveyed the area in order to photograph mother-calf pairs for identification. II. Cape Cod Bay and Massachusetts Bay are late winter, early spring feeding grounds where the majority of individuals are juveniles and mother-calf pairs. III. The Great South Channel is a spring feeding ground. Most of the population is seen in this area from late March until July. IV. The Bay of Fundy is a summer-fall feeding and nursery area. Many individuals of the population are seen in this area, although not all mother-calf pairs that are sighted in southeastern United States use this area as a summer nursery (Schaeff *et al.* 1993, Brown 1994). The majority of biopsy darting has taken place in this area. V. Roseway Basin is a summer-fall feeding area. This area is predominantly used by juveniles and adult males. Courtship groups have also been observed in this area. It was also a location for biopsy darting until 1992. (Kraus *et al.* 1986, Schevill *et al.* 1986, Winn *et al.* 1986, Hamilton and Mayo 1990). Historically, this population had a range that extended from southern Greenland to Bermuda (Reeves and Mitchell 1986, Gaskin 1987) but was reduced by extensive whaling. Very few incidental sighting have occurred within the extended range in approximately the last decade (Lien *et al.* 1989, Knowlton *et al.* 1992).

Figure 1.1 Five known habitat areas of the North Atlantic right whale are: Southeastern United States between Cape Canaveral, Florida and Savannah, Georgia; Cape Cod Bay and Massachusetts Bay; Great South Channel; the Bay of Fundy between Maine and Nova Scotia and Roseway Basin between Browns and Baccaro Banks off the southern Scotian Shelf.


Bay of Fundy
Roseway Basin
Massachusetts Bay
Cape Cod Bay
Great South Channel
Southeastern United States
km
0
200
400

Whaling

As a result of the right whale's slow movement, tendency to float when dead, highly marketable baleen and high yield of oil (Reeves and Mitchell 1986) it was targeted as an easy and highly lucrative catch or as was named, the right whale to kill. Whaling on the Eastern North Atlantic right whale (*Eubalaena glacialis*) began as early as the 11th century by Basque whalers from France and Spain (Aguilar 1986). Stocks within the Bay of Biscay had depleted by the 16th century causing the Basques to move out in search for new stocks (Aguilar 1986). Basque whaling efforts in the Strait of Belle Isle (corridor between Labrador and Newfoundland) are documented to have begun by the late 16th century (Reeves and Mitchell 1986). By the late 17th century depletion of the Western North Atlantic right whale had resulted in a reduction of Basque whaling efforts (Reeves and Mitchell 1986). It has been estimated that the cumulative catch for this period was 25 000-40 000 individuals (Gaskin 1991 and Aguilar 1986). Despite reduced population size, New England whalers harvested the Western North Atlantic right whale along much of its migration route including Newfoundland, Labrador, Gulf of St. Lawrence, Long Island and Massachusetts through the 18th century till the mid 19th century (Reeves and Mitchell 1986a, 1986b and Mead 1986). The right whale received full protection in 1937 by signatory nations of the International Whaling Commission, although the genus was still vulnerable to non-signatory countries such as Brazil and Chile (Gaskin 1990). The current population estimation for the North Atlantic right whale is approximately 320-350 individuals (Crone and Kraus 1990). The historical population estimate for the Northern

right whale was 30 000 to 100 000 individuals (Braham and Rice 1984) and specifically the estimate for the Western North Atlantic right whale was 12 000 to 15 000 individuals (Gaskin 1991).

Right whale biology

Although the North Atlantic right whale is an endangered population with less than 350 individuals (Crone and Kraus 1990), it's small population size has allowed the North Atlantic right whale consortium to identify 369 individuals over a 17 year period between 1980-1996. Eighty-six of these individuals are known to be dead leaving 283 identified individuals.

Individuals of the North Atlantic right whale population are identified by the shape of callosity patterns on their head, rostrum and blowholes. The position and description of scars and the presence or absence of a ventral white side are also used for identification (Kraus *et al.* 1986). Once an individual is identified it is given an NEA (New England Aquarium) number that is used in all further surveillance data. It has been determined that the growth rate of the North Atlantic right whale population is 2.5% per year (Knowlton *et al.* 1994) which is approximately three times lower than the South Atlantic population (Best 1990 and Payne *et al.* 1990). The mean calving interval is 3.67 years (Knowlton *et al.* 1994) though longer intervals have been observed and the average age of recruitment of reproductive females is 7.57 years (Knowlton *et al.* 1994). The gestation period of the North Atlantic right whale is unknown. The gestation period for

the South Atlantic right whale has been estimated to be 12 months (Best 1994). A similar estimate for the North Atlantic right whale would predict mating to occur during the winter months when mother-calf pairs are observed in southeastern United States. The distribution area of adults and juveniles during these winter months has not yet been determined. Large courtship groups involving several males and one female have been observed in the Roseway Basin area in the summer months although it is unknown if they have resulted in any conceptions. Confirmed conceptions from the Roseway Basin area would infer a gestation period of 15 to 18 months for the North Atlantic right whale. This period seems extensive since the largest baleen whale, the blue whale (*Balaenoptera musculus*) only has a gestation period of 10 to 11 months (Leatherwood *et al.* 1988).

The South Atlantic right whale

The southern hemisphere right whale (*Eubalaena australis*) has a circumpolar distribution with many populations. The major populations are the South Atlantic, South African and the South Pacific/Indian Ocean. The South Atlantic right whale is often used as a comparison against the North Atlantic right whale due to the biological similarities between the two species and since the South Atlantic right whale experienced a less extensive whaling period of 200 years (Aguilar 1986). A major habitat area for the South Atlantic population is Peninsula Valdes, Argentina. This area has three separate aggregation areas with different age/sex distributions (Payne 1986). Individual identification and biopsy darting of the right whales in Peninsula Valdes is conducted in a

similar fashion to the methods used by the North Atlantic right whale consortium (Brown *et al.* 1994, Kraus *et al.* 1986).

Genetic studies

Mitochondrial DNA (MtDNA) is a powerful genetic marker, due to its lack of recombination, high rate of mutation and solely maternal inheritance. The mtDNA genome is a circular molecule approximately 16 500 bases in length in mammals. It is found in all tissues and organs and has 100 to 1000 times more copies than nuclear DNA (Brown *et al.* 1979). The mutation rate of this molecule is 5 to 10 times higher than nuclear DNA and specifically the control region has the highest mutation rate within the molecule (Hoelzel *et al.* 1991). In cetaceans this rate was found to be 0.5% to 1.0% per million years for odontocetes (Hoelzel *et al.* 1991) and 0.7% to 1.0% per million years in mysticetes (Arnason *et al.* 1993). The mtDNA genome is maternally inherited with little or no paternal leakage. During the union of egg and sperm all paternal mtDNA is located in the neck of the sperm which never enters the egg and is discarded after fertilization (Gyllensten *et al.* 1985). The above characteristics make mtDNA an excellent marker for examining maternal migration, population dynamics and matrilineal genetic structure (Moritz 1994).

Studies of maternal migration, population dynamics and matrilineal genetic structure are often based on techniques such as Restriction Fragment Length Polymorphism (RFLP) analysis and sequence analysis of mtDNA. RFLP analysis involves the entire molecule which is digested with restriction enzymes. The result is a

distinctive banding pattern for each RFLP haplotype. Differential patterns are formed when a new restriction site is found or lost due to a point mutation and sometimes when length changes occur due to insertions or deletions. Although this technique utilizes the entire molecule only a small amount of DNA is actually analyzed. For example, a 6 bp recognizing restriction enzyme will recognize a site every 6^4=1296 bp. If five of these 6 bp recognizing enzymes are used in a mtDNA study then 381 bp of the entire genome will be analyzed. RFLP analysis also requires 100 times more DNA than a technique that is Polymerase Chain Reaction (PCR) based. Control region sequence analysis is usually a PCR based technique in which the fragment containing the control region is amplified by using specific complementary primers (Saiki *et al.* 1986). Very little DNA is required as template since the PCR amplifies the specific area exponentially for analysis. A large area can be examined i.e. 300 to 500 bp and the exact type of mutation can be identified i.e. transition, transversion, insertion or deletion. Sequencing can also target areas with higher mutations rates in order to identify greater polymorphisms i.e. mtDNA control region. This technique offers greater resolution of haplotype identification than RFLP analysis (Walker *et al.* 1995).

Many cetacean studies have used these techniques to examine matrilineal genetic structure. Female philopatry to a specific summer/autumn habitat area has been the basis of genetic structuring of mtDNA haplotypes in many cetacean populations. Brennin *et al.* (1997) showed that a differential frequency of mtDNA haplotypes between the summering grounds was maintained by beluga whale philopatry (*Delphinapterus leucas*).

The genetic structuring of mtDNA haplotypes appears to have its origin in the Atlantic and Pacific beluga whale populations that were once separated by Arctic glaciation. Upon the retreat of glaciation, these separated populations with their divergent haplotypes came together in the Hudson Bay, and philopatry of the beluga to specific summer areas produced and maintained the genetic structuring that has been identified in this area.

Similar dynamics have been used to explain the genetic structuring exhibited by narwhals (*Monodon monoceros*) (Palsbøll *et al.* 1997). Maternally directed philopatry to summer/autumn feeding grounds has led to genetic structuring of control region haplotypes in narwhals. Relatively low levels of nucleotide diversity (0.0017) within this large population (27 600 to 42 500 individuals) (IWC 1992) has led to the conclusion that retreat of ice from glaciation created greater availability of suitable habitat and the subsequent expansion of this population from smaller numbers (Palsbøll *et al* 1997).

World-wide analysis of humpback whales (*Megatera novaeangliae*) has identified genetic structure of mitochondrial DNA haplotypes (Baker *et al.* 1994). Significant partitioning of world-wide variation has been observed between oceanic populations, stocks within oceanic populations and among seasonal habitats such as summer feeding areas within stocks (Baker *et al.* 1994). Much of the structuring is the result of limited mitochondrial gene flow and maternal philopatry (Larsen *et al.* 1996, Medrano-Gonzalez *et al.* 1995, Baker *et al.* 1994).

Within the North Atlantic right whale Schaeff *et al.* in 1993 observed nursery site philopatry among reproductive females. This philopatry resulted in RFLP haplotype

structuring between mothers that always used the Bay of Fundy nursery and mothers who always used an alternative nursery area that has not yet been located. Genetic structuring within this population was based on site fidelity but no statistical approaches were used to test this hypothesis.

Other genetic studies on the North Atlantic right whale have included gender identification using southern blot analysis of the pDP1007 Zfx/Zfy probe (Brown *et al.* 1994), a genetic variability study using DNA fingerprinting (Schaeff *et al.* 1997) and analysis of sequence variation in the North and South Atlantic right whales (Schaeff 1993). Brown *et al.* (1994) identified the gender of 95 North Atlantic right whales and determined the sex ratio to be 50:50. Brown *et al.* in 1994 also showed that only 38% of the females in the North Atlantic right whale population had been successful in reproduction in the 1980 to 1990 period compared to 54% in the closely related South Atlantic right whale population (South American) (Payne *et al.* 1990). This factor may be involved in the lack of measurable recovery of the North Atlantic right whale.

Schaeff et al. in 1997 compared the genetic variability in North and South Atlantic right whales using DNA fingerprinting. The results showed a higher bandsharing coefficient within North Atlantic right whales than within South Atlantic right whales. This result indicated that the North Atlantic right whales are more closely related to each other than the South Atlantic right whales. Within the North Atlantic right whale, mating is occurring between closely related individuals which may be a factor in the lower growth rate, increased calving period and reduced juvenile survivorship seen in

this population (Knowlton et al. 1994, Schaeff et al. 1997). In 1993 Schaeff also analyzed the mtDNA sequence variation within and between North and South Atlantic right whales using RFLP analysis. The results showed low levels of sequence diversity within the North and South Atlantic populations; 0.08 and 0.24 respectively. These levels are comparable to other cetacean species that have experienced intense exploitation such as humpbacks (Baker *et al.* 1993). Currently, genetic studies are aimed at paternity analysis and nuclear based genetic structure analysis using microsatellites (Waldick pers comm.).

Statement of Objectives

Only five habitat areas are known for the North Atlantic right whale. Not all of the population uses each of these areas. Specifically, in the summer months it is known that not all of the mother-calf pairs that were sighted in southeastern United States use the Bay of Fundy nursery area. Since this population shows reliance on particular habitat areas and anthropogenic factors such as whaling, ship collisions and net entanglement are hampering the recovery of this species; it is important to assess the level of genetic diversity left in the population and to identify and assess all habitat areas for potentially lethal threats. For this reason a program for the identification of the location of the alternative nursery area(s) was initiated. The most direct method of location would be through satellite tags on individuals that belong to the specific subpopulation that show significant site fidelity to this area. To facilitate this the objectives of my thesis are:

1. To increase the resolution of haplotypic diversity of mtDNA through analysis of control region sequences.
2. To determine the degree of genetic structuring present between mothers and calves that use the Bay of Fundy nursery area and mothers and calves that use an alternative site to the Bay of Fundy nursery area.
3. To determine the mechanism by which genetic structuring maybe maintained.
4. To identify a list of individuals that should be targets for satellite tagging, in order to elucidate the location of the alternative nursery area.
5. To examine and compare the genetic diversity present in the North and South Atlantic right whale populations.
6. To determine the phylogenetic relationship between the North and South Atlantic right whale populations.

CHAPTER TWO

ASSESSMENT OF GENETIC STRUCTURING AND HABITAT PHILOPATRY IN THE NORTH ATLANTIC RIGHT WHALE (*Eubalaena glacialis*).

Abstract

The North Atlantic right whale (*Eubalaena glacialis*) inhabits five seasonal areas along the east coast of North America. It has been found over 17 years of field observations that from late July until early October only a proportion of the females with a new born calf use the Bay of Fundy, Canada as a feeding and nursery area. The absence of some mother-calf pairs in this area has suggested that one or more additional nurseries exist. In a previous preliminary study mitochondrial DNA Restriction Fragment Length Polymorphisms (RFLPs) were used to establish genetic structuring based on nursery use. In this study 500 bp of the control region in mitochondrial DNA (mtDNA) have been analyzed in 269 individuals in order to confirm and refine the previous study. Seven polymorphic sites were found to be present within the 500 bp analyzed which defined five matrilines. Females were divided into three categories based on observed site fidelity to the Bay of Fundy nursery area. An Exact test for population differentiation with 2 000 Markov Chain permutations, identified significant genetic structuring of matrilines between mothers and calves that show site fidelity to the Bay of Fundy nursery area and mothers and calves that show fidelity to an alternative nursery area ($p=0.0004$). Significant site fidelity to a particular nursery area has been demonstrated by females who have had two and three calves ($p<0.05$). Site fidelity to a particular nursery and transmission of site fidelity appears to be the basis for maintaining genetic structuring. These results in combination with the categories established for genetic structuring analysis have also been used to identify females for the purpose of satellite tagging to elucidate the location of the alternative nursery area or areas.

Introduction

The North Atlantic right whale (*Eubalaena glacialis*) is considered to be the most endangered species of large whale in the world (IUCN status). Since 1937 this whale has been protected internationally, but has yet to show any significant signs of recovery. Population studies indicate that the growth rate for this species to be 2.5% (Knowlton *et al.* 1994) whereas the closely related species, the South Atlantic right whale is growing between 6.8% to 7.1% (Best and Underhill 1990, Payne *et al.* 1990). Lack of species recovery has been attributed to inbreeding depression (Schaeff et al. 1997), human caused mortalities such as ship strikes, net entanglement (Kraus 1990), habitat degradation, chemical and noise-pollution (Reeves et al. 1978).

Five seasonal habitat areas along the east coast of North America have been identified: I. The coastal waters of southeastern United States, winter calving grounds; II. Cape Cod Bay and Massachusetts Bay, winter/spring feeding grounds; III. The Great South Channel, spring feeding grounds; IV. The Bay of Fundy, summer and fall feeding and nursery area and V. Roseway Basin, summer and fall feeding area (Kraus *et al.* 1986, Schevill *et al.* 1986, Winn *et al.* 1986 and Hamilton and Mayo 1990). Sighting data have shown that not all right whales are seen in all habitat areas, with the possible exception of Great South Channel (Winn *et al.* 1986). The population is segregated by age and sex (Brown 1994). The southeastern United States is predominantly used by mother-calf pairs, Cape Cod Bay and Roseway Basin are predominantly feeding areas where mother-calf pairs are not observed and the Bay of Fundy is not used by all the mother-calf pairs

(Schaeff *et al.* 1993, Brown 1994). Additional right whale sightings have been reported outside of the known habitat areas for the Western North Atlantic right whale. Including sightings off Newfoundland, Labrador, the Gulf of St. Lawrence and in the waters south of Greenland (Lien *et al.* 1989, Knowlton *et al.* 1992); all of which are within the historic migratory range of this species (Reeves *et al.* 1978). Of these, five whales have been photoidentified: the two animals seen in Newfoundland and Labrador waters were a reproductive female and a female of unknown age and the three seen south of Greenland were a reproductive female and her calf and an unsexed individual (Knowlton *et al.* 1992). Field sighting data showed that the Bay of Fundy nursery was not used by all mother-calf pairs and the few sightings documented outside the whale's current range suggest that an alternative nursery area or areas exist.

MtDNA analyses have revealed population subdivision among the nursery and feeding areas of many cetaceans, such as humpbacks (*Megatera novaeangliae*) (Baker *et al.* 1990), narwhals (*Monodon monoceros*) (Palsbøll *et al.* 1997) and belugas (*Delphinatperus leucas*) (Brennin *et al.* 1997). An RFLP (Restriction Fragment Length Polymorphism) study by Schaeff et al. (1993) showed genetic structuring of Western North Atlantic right whales in their summer nursery and feeding areas. Genetic structuring among habitat areas is often the result of site fidelity by females to a specific area. The presence of site fidelity or the consistent behaviour of a reproductive female to use a specific nursery area has also been studied in humpbacks (Larsen *et al.* 1996).

Transmission of site fidelity by a reproductive mother to her daughter may result in the establishment and maintenance of genetic structuring among nursery areas.

The purpose of this study was to increase the resolution of haplotypes through DNA sequence analysis of the control region over those identified by RFLP markers (Schaeff *et al.* 1993). Different frequencies of mtDNA haplotypes were used previously to identify genetic structuring. In this study control region haplotypes of previously analyzed individuals plus 96 newly biopsied individuals and six more years of field surveillance data will be used to assess mitochondrial structuring among mothers and calves that use different nursery areas. MtDNA is a powerful genetic marker as it is maternally inherited, has a rapid rate of mutation and lacks recombination (Brown *et al.* 1982, Avise *et al.* 1987). Specifically, the control region has often been targeted in many studies of cetacean populations (Baker *et al.* 1994, Rosel *et al.* 1995, Palsbøll *et al.* 1997) due to it higher rate of mutation than the remaining molecule (Hoelzel *et al.* 1991). This high rate of mutation also increases the resolution of matriline identification, than that available by RFLP analysis of the whole molecule. Sequence variation that distinguishes mtDNA control region haplotypes has been identified by polymerase chain reaction (PCR) amplification (Saiki *et al.* 1986) and sequencing. The population has been screened for sequenced haplotypes using single stranded conformation polymorphism (SSCP) analysis (Orita *et al.* 1989, Murray *et al.* 1995). Site fidelity and the transmission of site fidelity is often the basis or mechanism by which genetic structuring in a population is established and maintained. Assessment of site fidelity and the

transmission of site fidelity has been determined through analysis of field surveillance data using those females that have given birth to more than one calf between 1980 and 1996.

Despite complete protection from commercial whaling for the last 60 years this population of right whales is experiencing low growth and is under constant jeopardy by anthropogenic factors such as ship strikes and net entanglement. Critical habitat assessment has been the basis of many endangered population studies (Brown *et al.* 1995, Kraus and Brown 1992). Defining the use of an alternative nursery area or areas followed by location of these areas is critical to the conservation of this endangered species.

Materials and Methods

Samples

Samples used in this study were collected by skin biopsy sampling (Brown et. al. 1991) from North Atlantic right whales (*Eubalaena glacialis*) in the Grand Manan Basin, lower Bay of Fundy from August to October, 1988 to 1996 and in August and September from 1988 to 1992 in Roseway Basin, between Browns and Baccaro Banks off the southern Scotian shelf.

Sighting Data

Sighting data from 1980 to 1996 were used in this study. North Atlantic right whales have been individually identified using photographs of the callosity patterns on their heads and rostrums, photographs of scars on their bodies, the presence or absence of a white ventral side and the presence and location of lip crenulations (Kraus *et al.* 1986). The New England Aquarium (Boston, Massachusetts) curates a catalogue of photographs, that are maintained on individual whales seen in the western North Atlantic. All identified individuals are assigned an NEA (New England Aquarium) number. The NEA number is also used in sample assignment. For laboratory analyses all samples are also assigned a lab number such as, Egl 000 for Eubalaena glacialis.

Research efforts in the Bay of Fundy started in 1980 and continue to present. Research in southeastern United States (from Savannah, GA. to Cape Canaveral, Fl.) was

initiated in 1984. All other major habitat areas were the targets of research efforts by 1980.

DNA Extraction

Samples collected before 1995 were extracted according to protocols in Brown *et. al.* 1991 and Schaeff *et. al.* 1993. All other samples were extracted by grinding frozen tissue (0.3-0.5 g) and 4.0 mL of 1X lysis buffer (2X: 0.1 M Tris-HCl pH 8.0, 4 M urea, 0.2 M NaCl, 0.01 M 1,2 cyclohexanediamine and 0.5% n-laurylsarcosine) in liquid nitrogen. Samples were incubated at 37°C for one week. Proteinase K (83 units) (Boehringer Mannehiem) was added and the sample was allowed to incubate at 56°C for one hour. This was followed by a second addition of 83 units of proteinase K which was incubated at 37°C for 12 hours. Samples were then extracted with two equal volumes of phenol, chloroform and water (70:30) (Applied Biosystems Inc.); followed by an extraction with chloroform and water (24:1) (Applied Biosystems Inc.) (Sambrook *et. al.* 1989). DNA was precipated from the aqueous layer with 0.1X volume of 10 M ammonium acetate and one times the volume of isopropanol followed storage at -20°C for 12 hours. Samples were centrifuged at 3000rpm (Sorvall T 6000D rotor) for 30 minutes, washed in 70% ethanol and recentrifuged for 5 minutes. The pellet was dissolved in 200-500μL of TNE_2 (10 mM Tris-HCl pH 8.0, 10 mM NaCl and 2 mM disodium ethylene diamine tetraacetate•$2H_20$). The yield of DNA was measured using a DNA fluorometer (Model TKO-100, Hoefer Scientific Instruments) after staining with Hoechst dye 33258.

Agarose gel electrophoresis

The molecular weight of the extracted DNA was assessed by electrophoresis through a 1.25% agarose gel in 0.5X TBE (5X: 0.45 M Tris-borate pH 8.3, 0.01 M disodium ethylene diamine tetraacetate•$2H_2O$) and compared to a molecular weight ladder (GIBCOBRL). The gel was stained using ethidium bromide at a final concentration of 2ug/mL in 0.5X TBE for 30 minutes.

Polymerase Chain Reaction

DNA was amplified in a Perkin-Elmer Cetus Thermal Cycler model 480 under the following reaction conditions: 1X PCR buffer (GIBCOBRL) (20 mM Tris-HCl pH 8.4, 50 mM KCl), 1.5 mM $MgCl_2$, 0.2 mM dNTP's, 0.75 units Taq DNA polymerase (GIBCOBRL), 10 μmoles of each primer and 25 ng of template DNA in a 25 μL reaction. Control region amplification for SSCP analysis was completed in a 10 μL volume to prevent the production of excess radioactive material. Amplifications were performed under the following temperature regime: 95°C for 10 minutes, annealing temperature for specific locus 30 sec. (table 2.1) and 72°C for 30 sec. for one cycle followed by 30 cycles of 95°C for 15 sec., annealing temperature for specific locus 15 sec. and 72°C for 30 sec. Loci such as Zfx/Zfy and control region were amplified under these conditions with specific annealing temperatures (table 2.1). The size of the PCR products were assessed by agarose gel electrophoresis.

Table 2.1 Primers and annealing temperature for specific loci amplified using the polymerase chain reaction.

Locus	Primers	Annealing temperature
Zfx/Zfy	ZFY 1204 5' CAT TAT GTG CTG GTT CTT TTC TG 3'	60°C
	ZFY 0097 5' CAT CCT TTG ACT GTC TAT CCT TG 3'	
	(Schneider-Gädicke et al. 1989).	
Control region	AB6617 5' TAA TAT ACT GGT CTT GTA AAC C 3'	57°C
	AB6618 5' GGG TCG GAA GGC TGG GAC CAA ACC 3'	
	(Murray et al. 1995b)	
Control region	AB6617 5' TAA TAT ACT GGT CTT GTA AAC C 3'	59°C
reamplification for sequencing	H00034 5' TAC CAA ATG TAT GAA ACC TCA G 3'	
	(Rosel et. al. 1995)	
Control region	UP098 5' AAT CAC AGT ACT ATG TCA G 3'	55°C
for SSCP	LP585 5' GCT GAT TAG TAA TTA ACC C 3'	

Gender Identification

The gender of an individual was identified through PCR amplification of the Zfx/Zfy region followed by *Taq*I restriction enzyme digestion. *Taq*I digestion reaction (30 μL) contained: 10 μL of PCR product (Zfx/Zfy amplification), 2 μL of REACT® 2 buffer (GIBCOBRL) (50 mM Tris-HCl pH 8.0, 10 mM $MgCl_2$ and 50 mM NaCl) and 10 units of *Taq* I restriction enzyme (GIBCOBRL). The digestion was carried out at 65°C for 1 hour. The size of the fragments were assessed by agarose gel electrophoresis.

Sequencing of mtDNA control region

The control region was amplified and assessed by agarose gel electrophoresis as described above. The fragment was excised from the gel and the agarose was soaked in 30 μL of TE (10 mM Tris-HCl pH 8.0, 10 mM NaCl and 1 mM EDTA) to release DNA for reamplification. One μL of this solution was used in the second amplification using AB6617 and an internal primer, H00034 (table 2.1). Three of these reactions were amplified for each initial product. Successful amplification was tested by agarose gel electrophoresis. Triplicate samples were combined and extracted with an equal volume of phenol, chloroform and water (Applied Biosystems Inc.) (70:30) and once with chloroform and water (24:1) (Applied Biosystems Inc.). DNA was precipitated by 0.1X 10M ammonium acetate, 1X isopropanol and storage at -20°C overnight. DNA was collected by centrifugation at 12000 rpm for 30 min, washed in 70% ethanol and recentrifuged for 5 min. The ethanol was taken off the top and the pellet was allowed to

air-dry. The pellet was redissolved in 12μL of sterile H_2O and quantified using Hoechst dye 33258 and a TKO-100 fluorometer (Hoefer Scientific Instruments).

Sequencing of amplified products was performed by MOBIX Central Facility, Institute for Molecular Biology and Biotechnology, McMaster University, Hamilton, Ontario. Seventy-five to 150 ng of purified control region DNA was used for sequencing. The sequencing was done using the PRISM™ Ready Reaction Dye Deoxy Terminator Kit (Applied Biosystems Inc., Foster City, CA). Sequencing reactions were processed by a Perkin-Elmer 9600 Thermal Cycler and the Automated DNA Sequencing System 373A (Applied Biosystems Inc.).

Sequence Analysis

The sequences were aligned and analyzed for polymorphic sites using Genetic Data Environment 2.2-gde96 and Clustal W (1.4), Multiple sequence alignments. Within the Clustal W sequence alignment program the gap opening penalty was set at 10 in a range of 0.0-100.0 and the gap extension penalty was set at 0.05 in a range of 0.0-10.0.

Single stranded conformation polymorphism analysis (SSCP)

SSCP analysis was used on PCR amplified products of the mtDNA control region in order to screen the population for new mtDNA haplotypes and those that had been identified by sequence analysis. The reaction was carried out as described above except that the primers (table 2.1) were end-labeled with γ^{33}P-dATP (ICN) and only 0.16 μmoles

of each unlabeled primer and 0.15 μmoles of each labeled primer was used in each reaction.

The PCR products were diluted in a 1:4 ratio with water and heated to 95°C to allow double stranded DNA to denature and then placed on ice in order to form sequence dependent single stranded conformations (Orita *et al.* 1989, Murray *et al.* 1995a). These conformations were electrophoresed through a nondenaturing 5% acrylamide gel (59 acrylamide: 1 bis-acrylamide, 10% glycerol and 0.5X TBE) at 3-4 watts at room temperature for 20 hours. The SSCP gel was visualized by exposure to a PhosPhor Image screen and use of a PhosphoImager (Molecular Dynamics). Permanent audioradiographs were made by exposure to X-ray film.

Results

Sighting Data

For this study females were divided into three categories according to their site fidelity to the Bay of Fundy as a nursery area as described in Schaeff et al. (1993):

Category 1, *Bay of Fundy all* were mothers who had brought all of their calves to the Bay of Fundy and all of their offspring.

Category 2, *Bay of Fundy none* were mothers who had not brought any of their calves to the Bay of Fundy and all of their offspring.

Category 3, *Bay of Fundy some* were mothers who had brought some of their calves to the Bay of Fundy and all of their offspring.

These categories were used to assess mtDNA structuring, the presence of site fidelity and cultural transmission of site fidelity (Tables 2.2 to 2.4).

Zfx/*Zfy* amplification and analysis

The ZFY 1204 and ZFY 0097 primers (Palsbøll *et. al* 1992) were used to amplify an 1160 bp fragment of the X and Y chromosomes. *Taq*I restriction enzyme digestion shows a differential pattern for males compared to females. Three *Taq*I sites were present on the X chromosome sequence that produced two 439 bp fragments and one 182 bp fragment. The Y chromosome sequence has lost one of the *Taq*I sites found on the X chromosome and produced a restriction pattern of a 621 bp

Table 2.2 *Bay of Fundy* all females with their calves and their control region haplotypes.

[1]Mother	[2]Hap	Calf 1	Hap	Calf 2	Hap	Calf 3	Hap	Calf 4	Hap	Calf 5	Hap
1001	*D*	1301	D	1603	*D*	1911	D	2201	D		
1301	D	1931	D								
1118	A	1408	A	1702	A	2018	A				
1408	A	2608	A								
1142	A	1123	A	1411	A	2042	*A*	2642	A		
1123	A	2123	A								
1151	D	1707	D	2151	D						
1157	A	1134	*A*	1402	A	1703	*A*	2057	A	2557	*A*
1171	B	1170	B	1405	B	1971	B	2271	B		
1219	*D*	1701	D								
1701	D	2601	D								
1222	*B*	1250	B	1505	*B*						
1240	D	1241	D	1503	D	2140	D				
1241	D	1941	D	2541	D						
1503	D	2503	D								
1242	A	1243	A	1801	*A*	2142	A				
1243	A	2143	A								
1248	*A*	1249	A	1506	*A*	2048	A				
1281	A	1601	A	1981	*A*	2681	A				
1303	*A*	1403	*A*	1903	A	2303	*A*				
1135	D	1163	D	1406	D	1706	*D*	2135	D	2635	*D*
1163	D	1608	*D*	2163	D						
1406	D	2406	*D*								
1140	*D*	1245	D	1704	D	2040	D	2440	*D*		
1245	D	2645	*D*								
1025	*A*	1026	A								
1158	C	2158	C								
1425	*D*	2425	D								
1629	*D*	2029	D								
1713		1808									
1223	*A*	2223	A								
1179	X	calf, '94									
1815	D	2615	D								
1817		2617									
2610		2611									
1407	D	1426		1907	B	2307					
1705	C	2605	C								

[1]*Bay of Fundy all* females who had brought all of their calves to the Bay of Fundy and their offspring.
[2]Control region haplotypes (A,B,C,D and E) are included for each individual. For some individuals a DNA sample was not available, therefore the control region haplotype when possible was inferred through mother-calf relationships with individuals that were analyzed. These haplotypes are *italicized*. DNA of individuals that did not amplify and could not be inferred are marked with an X.

Table 2.3 *Bay of Fundy none* females with their calves and their control region haplotypes.

[1]Mother	[2]Hap	Calf 1	Hap	Calf 2	Hap	Calf 3	Hap	Calf 4	Hap	Calf 5	Hap
1007	*A*	1269	*A*	1609	A						
1034	D	1611	D	1934	D						
1204		1807		calf, '91		calf, '95					
1264		1265		1502							
1284	*D*	1708	D	calf, '90	*D*						
1334	*B*	calf, '83	*B*	calf, '86	*B*	1920	B	calf, '92	*B*	calf, '96	*B*
1509	*B*	1610	B	1909	B	2209	*B*				
1515	*D*	calf, '85	*D*	1806	D	calf, '92	*D*				
1612	C	1613	C	2212	C						
1619		1712		calf, '90							
1710	*D*	1711	*D*	2110	D	calf, '96	*D*				
1268		calf, '82		calf, '95							
1501		calf, '80		calf, '85							
1175	*B*	1239	B								
1321		calf, '92									
1412		1413									
1434		1435									
1620		calf, '96									
1810		calf, '94									
1970	C	calf, '96	*C*								
2420		calf, '94									
1316		1317									
1430	D	2230	D								
1604	A	2304	A								
1812		calf, '96									

[1]*Bay of Fundy none* females who had not brought any of their calves to the Bay of Fundy as well their offspring. In some cases calves had not been identified by NEA number, therefore calf and year of birth are in table.

[2]Control region haplotypes (A,B,C,D and E) are included for each individual. For some individuals a DNA sample was not available, therefore the control region haplotype when possible was inferred through the mother-calf relationship with individuals that were analyzed. These haplotypes are *italicized.*

Table 2.4 *Bay of Fundy some* mothers with their calves and their assigned haplotypes.

[1]Mother	[2]Hap	Calf 1	Hap	Calf 2	Hap	Calf 3	Hap	Calf 4	Hap	Calf 5	Hap
1004	D	1705^{F}	C	2004^{F}	*C*	2404^{O}	*C*				
1705	C	2605^{F}	C								
1012	*B*	1308^{F}	B	1605^{O}	*B*	calf, '91^{O}	*B*	calf, '96^{O}	*B*		
1014	A	1153^{F}	*A*	1302^{F}	*A*	1802^{F}	A	2130^{O}	*A*		
1153		calf, '93^{O}									
1114	D	2014^{O}	*D*	2614^{F}	D						
1127	D	1128^{F}	*D*	1404^{F}	*D*	1709^{O}	D	2027^{F}	D	2427^{O}	D
1145	D	1138^{F}	*D*	1410^{F}	*D*	2145^{F}	D	calf, '96^{O}	D		
1160	*A*	1161^{F}	*A*	1409^{F}	*A*	1804^{O}	A	calf, '91^{O}	*A*	calf, 96^{F}	*A*
1168	D	1427^{F}	D	1968^{F}	*D*	calf, '92^{O}	*D*				
1201	*C*	1429^{O}	C	1508^{O}	*C*	1901^{F}	C	2301^{F}	*C*		
1233	*A*	1933^{O}	A	2233^{F}	*A*						
1246	*C*	1247^{F}	C	calf, '86^{O}	*C*	1946^{F}	*C*				
1254	B	1607^{F}	B	1954^{F}	B	calf, '92^{O}	*B*	calf, '95^{O}	*B*		
1314	*D*	1315^{O}	*D*	1602^{F}	D	2114^{O}	D				
1315	*D*	2215^{O}	D								
1602	D	2602^{F}	D								
1318		1319^{O}		1504^{F}							
1013		calf, '80^{F}		calf, '88^{O}		calf, '94^{O}					
1310	*D*	1311^{F}	D	1606^{F}	*D*	2010^{F}	D	calf, '96^{O}	*D*		
1266	*D*	1267^{O}	D	1507^{F}	D	1803^{F}	D	2366^{F}	*D*		
1208	D	1510	A								

[1]*Bay of Fundy some* females who had brought some of their calves to the Bay of Fundy and not others as well as their offspring. Calves that were brought to the Bay of Fundy nursery are denoted with an ^{F}. Calves that were not brought to the Bay of Fundy nursery are denoted with and ^{O}. In some cases calves had not been identified by an NEA number, therefore calf and year of birth are in table.

[2]Control region haplotypes (A,B,C,D and E) are included for each individual. For some individuals a DNA sample was not available, therefore the control region haplotype when possible was inferred through the mother-calf relationship with individuals that were analyzed. These haplotypes are *italicized*. DNA of individuals that did not amplify and could not be inferred are marked with an X.

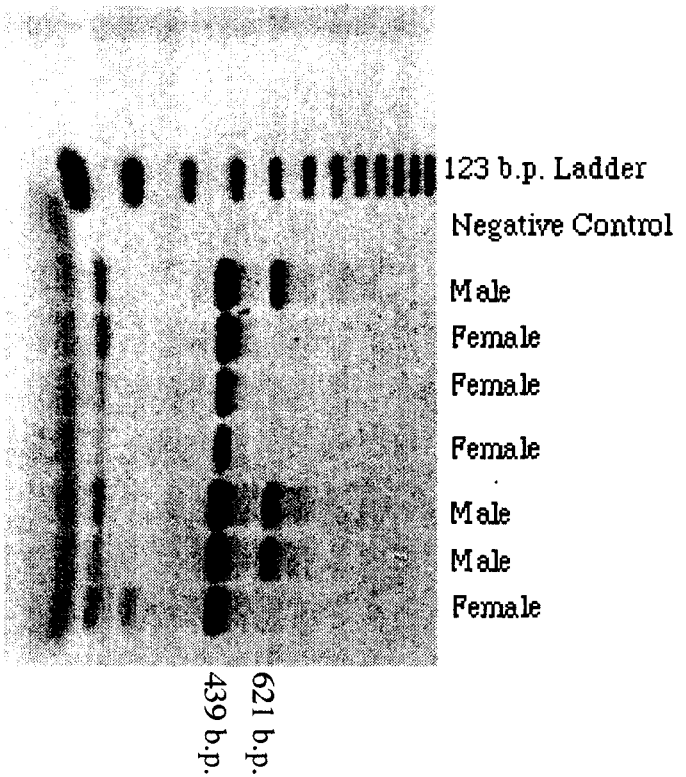
fragment and a 439 bp fragment. Therefore, males had an extra band at 621 bp when compared to a female on an agarose gel (Figure 2.1).

Gender identification was used in this study to confirm animals that were putatively identified as females by consistent association with a newborn calf as well as to identify unsexed calves. The New England Aquarium right whale catalogue, at the end of the 1980 to 1996 period included a cumulative total of 369 identified individuals. The sex of 286 of these individuals has been identified. One hundred and three individuals have been identified using Zfx/Zfy amplification and *TaqI* restriction enzyme digestion. One hundred and forty nine individuals have been identified by photographic observation of the genital area, 43 mothers have been putatively identified by consistent association with a newborn calf (Knowlton *et al.* 1994) and 41 individuals have been identified by hybridization with the Zfx/Zfy probe pDP1007 (Brown *et al.* 1994). Some overlap has occurred in order to confirm photographic analyses with genetic analyses. The gender of eighty three individuals remain unidentified due to lack of sample or photograph of the urogenital region. No mismatches were identified between the techniques, but the result of this confirmation identified one individual that had been misidentified at the time of sample collection.

Sequence analysis of mtDNA control region

The primers AB6617 and AB6618 (table 2.1) were used to amplify an approximately 1170 bp fragment for reamplification by a second set of primers;

Figure 2.1 Gender identification of Western North Atlantic right whale samples. The first lane to the left has a 123 bp molecular weight ladder to measure size fragments. The second lane is the PCR and restriction enzyme digestion negative control. The third lane is a male control and the fourth lane is a female control. Both positive control samples had previously been identified by hybridization of the Zfx/Zfy probe pDP1007 (Brown *et al.* 1994).


123 b.p. Ladder
Negative Control
Male
Female
Female
Female
Male
Male
Female
621 b.p.
439 b.p.

AB6617 and a nested primer H00034 (table 2.1). Reamplification produced a clean distinct 680 bp fragment that was used for sequencing. This product was sequenced in 14 unrelated individuals. Each haplotype was sequenced with both primers in three different individuals except for haplotype E which was only sequenced in two individuals. Multiple sequences as well as the use of both primers were used in order to confirm polymorphic sites. The sequences showed seven polymorphic sites within the 500 base pairs analyzed. This represented five haplotypes (Figure 2.2). All polymorphic sites were represented by transitional mutations. Sequence diversity ranged between 0.2% to 0.8%. Low sequence diversity combined with solely transitional mutations at all polymorphic sites suggests that these haplotypes are closely related.

SSCP analysis

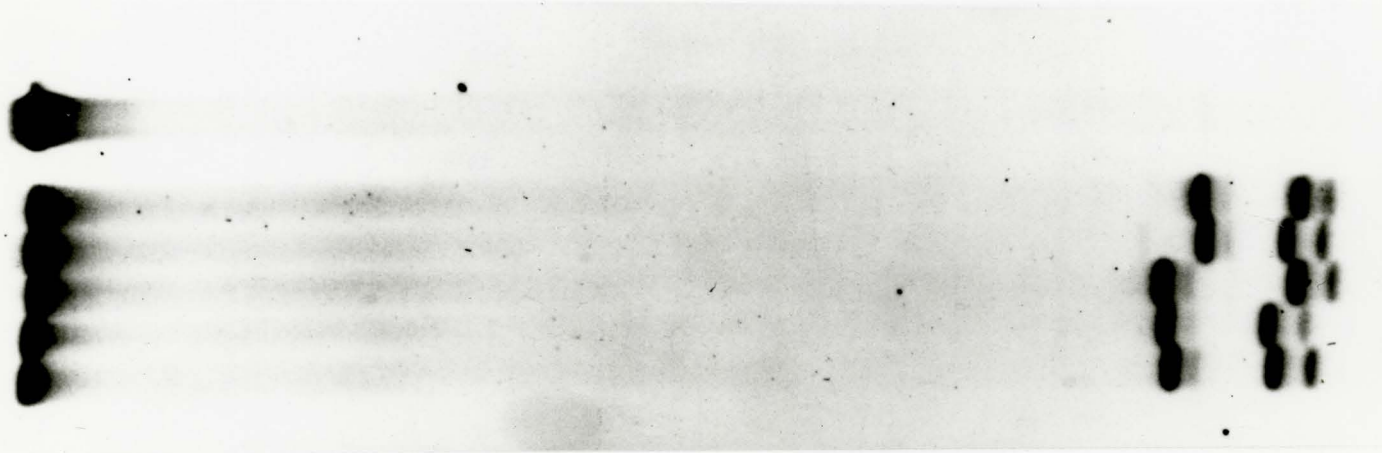
SSCP analysis was used to screen the mtDNA control region of 180 individuals in the population. Both UP098 and LP585 primers (table 2.1) were end-labeled with ^{33}P, producing two bands that represented each conformation. This procedure increased the accuracy of conformation polymorphism identification. Conformation polymorphism analyses were able to differentiate between sequences that differed by one base pair. For example haplotype C differs from B by one base pair, yet their conformation polymorphisms (Figure 2.3) are easily distinguishable. In Figure 2.3 at the bottom of each lane is a double-stranded product. The position of

Figure 2.2 Control region sequence alignment of five Western North Atlantic right whale haplotypes. Similarities between sequences are identified by '.'. Sequence differences are identified by the appropriate nucleotide change.

```
                1          11         21         31         41         51         61         70
                |          |          |          |          |          |          |          |
EglHapA     1   tactatactc cgccatcagc acccaaagct gaaattctat ttaaactatt ccctgaaaaa gtatattgta   70
EglHapB     1   .......... .......... .......... .......... .......... .......... ..........   70
EglHapC     1   .......... .......... .......... .......... .......... .......... ..........   70
EglHapD     1   .......... .......... .......... .......... .......... .......... ..........   70
EglHapE     1   .......... .......... .......... .......... .......... .......... ..........   70

                71         81         91         101        111        121        131        140
                |          |          |          |          |          |          |          |
EglHapA    71   gaacatcaca aaatcacagt actatgtcag tattaaaaat aaattatcct attacatatt actatgtaat  140
EglHapB    71   .......... .......... .......... .......... .......... .......... .........c  140
EglHapC    71   .......... .......... .......... .......... .......... .......... .........c  140
EglHapD    71   .......... .......... .......... .......... .......... .......... .........c  140
EglHapE    71   .......... .......... .......... .......... .......... .......... ........gc  140

                141        151        161        171        181        191        201        210
                |          |          |          |          |          |          |          |
EglHapA   141   ccgtgcatgt atgcactgcc acatggccaa tactagtcct gactcataaa ttgtacctat acatgctatg  210
EglHapB   141   t.........  .......a..  ..........  ..........  ..........  ..........  ..........  210
EglHapC   141   t.........  .......a..  ..........  ..........  ..........  ......t...  ..........  210
EglHapD   141   t.........  .......a..  ..........  ..........  ..........  ..........  ..........  210
EglHapE   141   t.........  ......c...  ..........  ..........  ..........  ..........  ..........  210

                211        221        231        241        251        261        271        280
                |          |          |          |          |          |          |          |
EglHapA   211   tataatcgtg cattcaatta ttttcactac gggaagttaa agctcgtatt aaattttatt tattttacat  280
EglHapB   211   ..........  ..........  ..........  ..........  ..........  ..........  ..........  280
EglHapC   211   ..........  ..........  ..........  ..........  ..........  ..........  ..........  280
EglHapD   211   ..........  ..........  ..........  ..........  ..........  ..........  ......g...  280
EglHapE   211   ..........  ..........  ..........  ..........  ..........  ..........  ..........  280

                281        291        301        311        321        331        341        350
                |          |          |          |          |          |          |          |
EglHapA   281   atgtacataa taatcattga tcgtgcatag tacatgtcct taaatcaatt caagtcaact gaatcttatg  350
EglHapB   281   ..........  ..........  ..........  ..........  ..........  ..........  ..........  350
EglHapC   281   ..........  ..........  ..........  ..........  ..........  ..........  ..........  350
EglHapD   281   ..........  ..........  ..........  ..........  ..........  ..........  ..........  350
EglHapE   281   ..........  ..........  ..........  ..........  ..........  ..........  ..........  350
```

```
                351         361         371         381         391         401         411         420
                |           |           |           |           |           |           |           |
EglHapA   351   gccgctccat tagatcacga gcttgatcag catgccgcgt gaaaccagca acccgctcgg cagggatccc  420
EglHapB   351   .......... .......... .......... .......... .......... .......... ..........  420
EglHapC   351   .......... .......... .......... .......... .......... .......... ..........  420
EglHapD   351   .......... .......... .......... .......... .......... .......... ..........  420
EglHapE   351   .......... .......... .......... .......... .......... .......... ..........  420

                421         431         441         451         461         471         481         490
                |           |           |           |           |           |           |           |
EglHapA   421   tcttctcgca ccgggcccat caattgtggg ggtagctatt taatggtctt tacaagacat ctggttctta  490
EglHapB   421   .......... .......... .......... .......... .......... .......... ..........  490
EglHapC   421   .......... .......... .......... .......... .......... .......... ..........  490
EglHapD   421   .......... .......... .......... .......... .......... .......... ..........  490
EglHapE   421   .......... .......... .......... .......... .......... .......... ..........  490

                491
                |
EglHapA   491   cttcagggcc                                                                    500
EglHapB   491   ..........                                                                    500
EglHapC   491   ..........                                                                    500
EglHapD   491   ..........                                                                    500
EglHapE   491   ..........                                                                    500
```

Figure 2.3 SSCP analysis of the five control region haplotypes found in the Western North Atlantic right whale with double-stranded and negative controls. The first two bands in each lane are the single-stranded conformation of each end-labeled primer. At the bottom of each lane is a double-stranded product. Location of the double-stranded product is used to ensure equal migration of all lanes.


DNA(ds)
Negative Control
Haplotype B
Haplotype E
Haplotype D
Haplotype C
Haplotype A

the double-stranded products allowed for assessment of any differential migration between lanes. The frequency of the five control region haplotypes found within the 180 individuals analyzed and 89 individuals whose haplotypes were inferred through mother-calf relationships were (Table 2.2-2.4): Haplotype A was found in 75 individuals, haplotype B was found in 39 individuals, haplotype C was found in 35 individuals, haplotype D was found in 117 individuals and haplotype E was found in 3 individuals (Table 2.5a). Another 9 individuals were not assigned a haplotype due to amplification failure. Their haplotypes could not be inferred as no mother-calf relationships were known. Control region haplotype E was found in only three individuals, all of which were male (NEA: 1041, 1126 and 1327) and were first sighted in the early eighties at an unknown age. No pedigree information for these individuals is known.

Analysis of mtDNA structuring

Assessment of mtDNA structuring was analyzed among *Bay of Fundy all* mothers and calves and *Bay of Fundy none* mothers and calves. An exact test for population differentiation (Raymond and Rousset 1995) was used with a Markov chain method in the computer package TFPGA (Miller 1997). An exact test is accurate and unbiased even for small sample sizes and low frequency alleles. It is constructed using the classical Fisher test for row by column contingency tables. While a Markov chain method allows for permutations of the data set. In this

Table 2.5a Control region haplotype frequencies for individuals that were analyzed and inferred in the North Atlantic right whale population and control region haplotype frequencies of mothers and their offspring from each category.

Control region haplotypes	Analyzed and inferred individuals of this population	Bay of Fundy all	Bay of Fundy none	Bay of Fundy some
A	75	41	5	15
B	39	9	12	10
C	35	4	5	14
D	117	48	16	38
E	3	0	0	0
Total	269	102	38	77

Table 2.5b Control region haplotypes of the analyzed population, distributed into their appropriate female, male and unknown sex categories.

Control region haplotypes	Females	Males	Unknown sex	Control region haplotypes of the analyzed population
A	30	29	16	75
B	16	11	12	39
C	10	21*	4	35
D	52	41	24	117
E	0	3	0	3
Totals	108	105	56	269

*There are two times more males with haplotype C than females with haplotype C.

analysis, 2000 dememorization steps were set up with 2000 permutations per batch and 20 batches. Table 2.5a shows the haplotype frequencies among the *Bay of Fundy all*, *none* and *some* categories. A pairwise comparison of frequency distributions among the *Bay of Fundy all* and *Bay of Fundy none* categories showed significant genetic structuring of haplotypes ($p=0.0004$). This was also seen in a pairwise comparison between the *Bay of Fundy all* category and the *Bay of Fundy some* category ($p=0.0010$). Genetic structuring was not observed in a comparison between the *Bay of Fundy some* and the *Bay of Fundy none* categories ($p=0.1360$). The absence of structuring between the *Bay of Fundy none* and the *Bay of Fundy some* category suggests that these categories have similar haplotypic diversity. Since the purpose of this analysis was to determine if there was genetic structuring of haplotypes among the individuals that always used the Bay of Fundy nursery compared to individuals that did not, a second analysis was also conducted. In this test the *Bay of Fundy all* category was compared to a combined category of *Bay of Fundy none* and some individuals. Use of an Exact test for population differentiation with the same parameters as described above; genetic structure analysis between the *Bay of Fundy all* category and a category of all others was found to be significant $p<<0.0001$.

Since, the majority of biopsy sampling has occurred in the Bay of Fundy, this sampling bias must be considered in the interpretation of all results. In Table 2.5b the control region haplotypes were analyzed by gender. The frequency of

identified females to identified males was 108 to 105, therefore there is no difference in the gender ratio. Brown *et al.* in 1994 also showed that the sex ratio in the North Atlantic right whale to be close to 50:50. Observations of the haplotype distribution showed that haplotype C is two times more prevalent in males than females. It is known that the biopsy darting from which these individuals were analyzed and inferred is a result of 8 years of biopsy darting in the Bay of Fundy and only 4 years of biopsy darting off the Scotian shelf. In the 4 years of darting off the Scotian shelf, the number of individuals sampled was less than that retrieved in the Bay of Fundy in the same years. This was due to difficult weather and sea conditions off the Scotian shelf in those years (Schaeff *et al.* 1993). It is most likely that the missing females of haplotype C do not frequent the Bay of Fundy waters. Therefore, these females probably belong to the *Bay of Fundy none* category. This exemplifies that there is a sampling bias against *Bay of Fundy none* individuals that is inherent to our data set.

Site Fidelity

Field surveillance data indicated site fidelity among females who used the Bay of Fundy nursery area or an alternative site. Site fidelity or the consistent use of a particular nursery site was analyzed using females within each of the three categories who had had two and three calves. The probability of these females bringing all of their calves to the Bay of Fundy, none of their calves to the Bay of Fundy or some of their calves to the Bay of Fundy was tested against the expected

probabilities based on the probability of a calf being brought to the Bay of Fundy or not. In order to test site fidelity among females with three calves, all females with three or more calves were used. To prevent a bias in the selection of three calves from females who had four or five calves, a table of random digits was used to select the individuals for the observed values. Figure 2.4 demonstrates the use of a random digit table in the

selection of individuals from mothers with more than three calves. The same method of analysis was used to test site fidelity in females with two calves. The expected probabilities were calculated using calf distribution data from 1980 to 1996 (Table 2.6). The probability of a calf being brought to the Bay of Fundy nursery was calculated by the summation of all calves that were brought to the Bay of Fundy during the 1980 to 1996 period, divided by the cumulative total of calves during the 1980 to 1996 period (Table 2.6). The probability of bringing a calf to the Bay of Fundy was calculated to be 0.59 whereas the probability on not bringing a calf to the Bay of Fundy was 0.41. The null hypothesis was that females who had had two or three calves would be distributed in the different nurseries according to expected frequencies based on these probabilities. A chi-square test was used to test the null hypothesis. Site fidelity analysis of females with three calves also used a Yates correction (Yates 1935), as each analysis contained a cell with a value lower than 5. Ten permutations were calculated for site fidelity analyses of females with three calves and two calves (Tables 2.7a and 2.7b). The chi-square results based on these

Figure 2.4 Illustration of the use of a random digit table for selecting three calves for analysis from families that had four or five calves.

In this figure one example of a mother with five calves is shown. Each calf is identified by a number and the nursery site to which it was taken by the mother. Bay of Fundy represents the use of the Bay of Fundy nursery and Other represents the use of an alternative nursery site. Below the diagram is a table which depicts the method of sampling using a table of random digits. In this analysis even numbers were used to include individuals and odd numbers were used to exclude individuals.

In the first permutation the first three calves are included and the last two are excluded. Therefore, this family would be included as one observation of a female that brought all three calves to the Bay of Fundy.

In the second permutation, two calves that were brought to the Bay of Fundy and one calf that was not brought to the Bay of Fundy were included. In this case this family was used as one observation of a female that brought two calves to the Bay of Fundy and not the third.

In the third permutation one calf that was brought to the Bay of Fundy nursery and one that was not were included. In this case three calves were not identified and this family was not counted for that permutation.

Mother	Calf 1 BoF	Calf 2 BoF	Calf 3 BoF	Calf 4 Other	Calf 5 Other
	Random Digit for Calf 1	Random Digit for Calf 2	Random Digit for Calf 3	Random Digit for Calf 4	Random Digit for Calf 5
Permutation one	2	4	6	1	1
Permutation two	6	1	4	8	3
Permutation three	1	3	4	6	7

Table 2.6 The frequency of either bringing a calf to the Bay of Fundy or not bringing a calf to the Bay of Fundy, calculated by distribution data of calves from 1980 to 1996.

Year	Calves brought to Bay of Fundy by *Bay of Fundy all* mothers	Calves not brought to Bay of Fundy by *Bay of Fundy none* mothers	Calves brought to Bay of Fundy by *Bay of Fundy some* mothers	Calves not brought to Bay of Fundy by *Bay of Fundy some* mothers	Percentage of calves brought to the Bay of Fundy	Percentage of calves not brought to the Bay of Fundy
1980	2	2	2	0	67	33
1981	4	1	3	0	88	12
1982	5	3	1	2	55	45
1983	1	2	3	2	50	50
1984	7	2	4	0	85	15
1985	3	3	2	2	50	50
1986	2	5	4	2	46	54
1987	6	3	1	1	64	36
1988	2	2	3	0	71	29
1989	7	3	4	2	69	31
1990	5	3	3	3	57	43
1991	7	3	2	5	53	47
1992	2	6	1	2	27	73
1993	2	2	2	0	67	33
1994	4	1	0	3	50	50
1995	3	2	0	1	50	50
1996	10	5	3	3	62	38
Total	72	48	38	28	59	41

Probability of bringing a calf to the Bay of Fundy: (72+38)/186= (0.59)
Probability of not bringing a calf to the Bay of Fundy: (48+28)/186=(0.41)

Table 2.7a The construction of permutation values from females with three or more calves using a random digit table. Expected probabilities were used to calculate the expected values from the total observations of each permutation. Expected values and the observed values from each of the ten permutations were used in a Chi-square test for analysis of site fidelity among females with three calves.

	Expected probabilities	Permutated observed values									
Females that brought all 3 calves to the Bay of Fundy	$(0.59)^3$	12	8	7	9	8	9	9	8	7	7
Females that brought 2 out of 3 calves to the Bay of Fundy	$3(0.59)^2(0.41)$	6	6	8	4	3	4	4	6	6	5
Females that brought 1 out of 3 calves to the Bay of Fundy	$3(0.59)(0.41)^2$	2	5	2	4	4	3	4	3	3	3
Females who brought zero out of 3 calves to the Bay of Fundy	$(0.41)^3$	5	5	5	5	4	5	4	5	5	4
Totals	1.0	25	24	22	22	19	21	21	22	21	19

A Chi-square test with 10 permutations and a Yates correction used for the assessment of site fidelity among females with three calves. Average Chi-square of ten permutations was 9.65, $p<0.025$ df = 3

Table 2.7b The construction of permutation values from females with two or more calves using a random digit table. Expected probabilities were used to calculate the expected values from the total observations of each permutation. Expected values and the observed values calculated in each of the ten permutations were used in a Chi-square test to analyze site fidelity among females with two calves.

	Expected probabilities	Permutated observed values									
Females that brought both calves to the Bay of Fundy	$(0.59)^2$	14	18	20	17	11	19	15	13	11	18
Females that brought one out of two calves to the Bay of Fundy	2(0.59)(0.41)	8	10	9	10	10	11	10	12	8	8
Females that brought zero out of two calves to the Bay of Fundy	$(0.41)^2$	12	14	14	10	10	11	14	15	16	15
Totals	1.0	34	42	43	37	31	41	39	40	35	41

A Chi-square test with 10 permutations used for assessment of site fidelity among females with two calves. Average Chi-square of 10 permutations was 12.23, $p<0.005$, df=2.

permutations were averaged for the final chi-square value. The analysis showed that significant site fidelity was exhibited by females who had had three calves ($p<0.025$). Significant site fidelity was also exhibited by females with two calves ($p<0.005$). The null hypothesis was rejected and females with two and three calves show significant site fidelity to specific nursery areas.

Cultural transmission of site fidelity

Field surveillance data has also established several multigenerational families within the *Bay of Fundy all* category and the *Bay of Fundy some* category. Since females in the *Bay of Fundy all* category showed site fidelity, it was predicted that their offspring would show similar site fidelity. Cultural transmission of site fidelity is the use of a specific nursery site by different generations of the same family or pedigree. Sixty-nine pedigrees were identified using field data from 1980 to 1996 (Appendix 1). Twenty-six pedigrees out of the 69 represented the *Bay of Fundy all* category. Out of the 26 pedigrees, eight pedigrees were represented by three generations; grandmother, mother and daughter (i.e. pedigrees 001, 002, 004, 009, 012, 013, 053 and 054). These eight pedigrees include 12 generation II mothers. Ten out of 12 generation II mothers have used the Bay of Fundy nursery area. This trend indicates the cultural transmission of site fidelity, since the nursery site chosen by generation II mothers is the same as the nursery site used previously by the grandmothers or generation I. A Yates corrected chi-square analysis of the site

Table 2.8 Observed site fidelity by generation II mothers in a multigenerational family that has always used the Bay of Fundy nursery area. Expected probabilities are used to calculate expected numbers from total observations. Site fidelity is assessed by the use of a chi-square analysis with Yates correction for small cell values.

	Expected probabilities	Observed numbers	Expected numbers
Probability of bringing a calf to the Bay of Fundy	0.59	10	7
Probability of not bringing a calf to the Bay of Fundy	0.41	2	5

A Yate's corrected chi-square analysis of transmission of site fidelity among mothers or generation II in a multigenerational family (three generations). Yates correction chi-square 2.14, $p>0.05$, $df=1$

fidelity exhibited by generation II mothers indicate that the site fidelity shown by this generation is not significantly different ($p>0.05$) (Table 2.8) from the expected nursery distribution as calculated in Table 1.6. Therefore, there is no significant transmission of site fidelity between generations. The *Bay of Fundy none* category was represented by 25 pedigrees. Out of the 25 pedigrees, no daughters or generation II have produced an offspring. Four daughters are due to produce an offspring within the next four years (i.e. pedigrees 022, 030, 031 and 035). The *Bay of Fundy some* category was represented by 18 pedigrees. None of these pedigrees exhibited site fidelity to a particular nursery area and therefore cannot be analyzed for transmission of site fidelity. The transmission of site fidelity between generations has been observed in *Bay of Fundy all* pedigrees but shows no statistical significance. Lack of significance is most likely a result of small sample sizes.

Discussion

MtDNA structuring or geographic heterogeneity has often been observed in many marine mammal species across their geographic range (Baker *et al.* 1994, Rosel *et al.* 1995, Brennin *et al.* 1997, Palsbøll *et al.* 1997). Assessment of mtDNA structuring in the Western North Atlantic right whale showed that matrilineal diversity is significantly different between females that always use the Bay of Fundy nursery area and females that do not always use the Bay of Fundy nursery area. This result is concordant with the Schaeff *et al.* (1993) RFLP study which defined genetic structuring between *Bay of Fundy all* and *Bay of Fundy none* as well as between *Bay of Fundy all* and *Bay of Fundy some* females. Schaeff *et al.* (1993) identified three haplotypes by RFLP analysis and 3 categories of nursery use. Females were divided into *Bay of Fundy all*, *none* or *some* categories based on similar traits used to categorize reproductive females in our study.

RFLP haplotypes in the Schaeff *et al.* (1993) study could be combined with control region haplotypes to provide an even greater degree of haplotype resolution than can be provided by either study alone. Table 2.9 shows the relationships between RFLP haplotypes and control region haplotypes of individuals that were analyzed by both studies. There are ten combined haplotypes but we feel three are the result of some error as there would have to be two independent mutations to create either the same RFLP or the same control region sequence. Therefore, as A2, A3 and C2 are represented by only one animal each they are improbable outcomes since the probability of a point mutation causing the same restriction enzyme change or the same control region sequence change

Table 2.9 The relationship between control region haplotypes and RFLP haplotypes* in the North Atlantic right whale. Ten mtDNA haplotypes are identified. Haplotypes A2, A3 and C2 are most likely the result of sample error. Refer to text for more information.

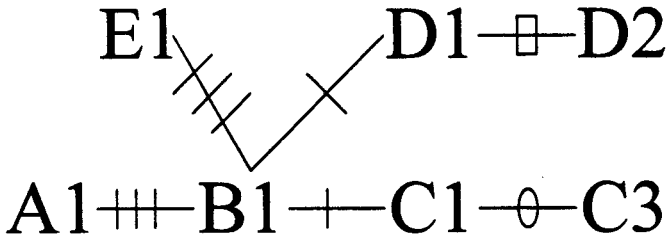
Control region haplotypes	RFLP haplotype 1	RFLP haplotype 2	RFLP haplotype 3
A	24	1	1
B	13	0	0
C	2	1	7
D	4	29	0
E	1	0	0

* Schaeff *et al.* (1993)

in two different lineages is highly unlikely. It is likely that these are from the same form of assignment error. Figure 2.5 illustrates a possible haplotype network of the mtDNA haplotypes established from the combination of control region sequence analysis and RFLP analysis. The haplotype network indicates the number of mutational events between each haplotype. Although, the combination of control region analysis and RFLP analysis have increased the resolution of haplotypic diversity, future use is impractical. This combination would require all samples that have only been analyzed by control region sequence analysis and all new samples to be analyzed for RFLPs. RFLP analysis requires a 100 fold more DNA than PCR based techniques. It therefore restricts the number of molecular techniques and studies that one sample can be involved in. As an alternative, other mitochondrial markers may be examined by PCR analysis to increase or maintain this haplotypic resolution.

Our study has shown, the presence of site fidelity and the suggestion of the transmission of site fidelity. These results have identified a group of females that do not use the Bay of Fundy (*Bay of Fundy none*) as a nursery area and will be targeted for use in satellite tagging studies to elucidate the location of the alternative nursery area. The use of sequence analysis has increased the resolution of haplotype or matrilineal diversity from 3 to 7 haplotypes. The distribution of these haplotypes between females and males has reinforced the known sampling bias against *Bay of Fundy none* females since they do not frequent areas where biopsy darting has occurred. Genetic structuring of control

Figure 2.5 Control region and RFLP haplotype relationships of the North Atlantic right whale. The haplotype network shows the number of nucleotide changes between 7 North Atlantic right whale mtDNA haplotypes. Haplotypes 1-3 are based on the RFLP study by Schaeff *et al.* (1993) and A-E are based on control region haplotype sequences. A cross hatch indicates a transitional change within the control region sequence, an □ indicates a change at a *Bam*HI restriction site and a ○ indicates a change at a *BstE*II site within the mitochondrial genome.


E1
D1
D2
A1
B1
C1
C3

region haplotypes has been observed between the *Bay of Fundy all* and the *Bay of Fundy none* categories as well as between the *Bay of Fundy all* and the *Bay of Fundy some* categories. The *Bay of Fundy none* and the *Bay of Fundy some* categories showed no significant genetic structuring. Although, upon observation of haplotypic frequencies in Table 2.5a; haplotype A is more common in the *Bay of Fundy some* category than in the *Bay of Fundy none* category indicating a similarity between the *Bay of Fundy some* and *all* categories. This suggests that the *Bay of Fundy some* category may be a mixture of the *Bay of Fundy all* and *Bay of Fundy none* categories. The composition of the *Bay of Fundy some* category is consistent with the following hypothesis.

The *Bay of Fundy some* individuals are a subset of the *Bay of Fundy none* and *Bay of Fundy all* individuals and have expanded their summer nursery range as a result of habitat disturbances and extensive whaling. Extensive whaling and habitat destruction caused a reduction in experienced reproductive females. It has been well documented that reproductive females and their calves were often the focus of many whaling expeditions as they inhabited coastal waters and the killing of one often resulted in an easy catch of the other (Reeves and Mitchell 1986a, Oldfield 1988). It has been proposed that experienced reproductive females were important to proper population dynamics and growth as they provided experience to newborn calves, juveniles or inexperienced females through learned behaviour or social facilitation.

The loss of experienced reproductive females and the disturbance of habitat areas would have greatly affected the mechanisms of site fidelity. A possible outcome of such

disturbances may have been the increase of female movement between habitat areas and the subsequent development of *Bay of Fundy some* females that use both the Bay of Fundy nursery and an alternative nursery area(s).

It is also possible that the loss of experienced females may have caused some females to rely on physical oceanographic factors such as temperature or food availability to govern their movements to a particular nursery area. Right whales require dense and mature copepod patches for effective foraging (Wishner *et al.* 1995, Woodley and Gaskin 1996). Fluctuations in physical oceanography such as temperature and onset of seasons may have a large effect on zooplankton concentrations and in turn on right whale distributions (Murison and Gaskin 1989, Kenney *et al.* 1995). An example of such an effect was recently shown by Kenney (1997) where low calving years are found to correspond with El Niño years.

This study has established that significant site fidelity is seen in females with two or three calves. The presence of site fidelity is often the mechanism by which genetic structuring is established and maintained (Bowen *et al.* 1992, Baker *et al* 1994). The mechanisms that may maintain site fidelity within a population are social facilitation and learned behaviours.

Social facilitation may have been a mechanism used by first time mothers who followed experienced mothers on their migratory route to a nursery area (Bowen *et al.* 1992). The mechanism of social facilitation may have allowed a first time mother who had a favourable experience to fix this site for future migrations. Learned behaviour or

cultural transmission may have been another mechanism by which site fidelity was established. Since a calf completed a migratory cycle with it's mother in the first year (Best 1994, Hamilton *et al.* 1995), subsequent site fidelity may have been a result of the calf's early maternal experience (Brodie 1969, Clapham and Mayo 1987, Baker *et al.* 1994). Differentiation between these mechanisms depends on several factors: Continued field surveillance data is needed in order to increase sample size in cultural transmission analyses. Statistically significant transmission of site fidelity would mean that learned behaviours was the mechanism used to establish site fidelity. In order to discern whether social facilitation is the mechanism used, satellite tagging of inexperienced females with their calf and aerial surveys are needed. Satellite tags are needed to trace the migration route and aerial surveys will determine if inexperienced mother-calf pairs are alone in their migration.

Conservation Management Recommendations

It is currently not possible to determine if an alternative nursery to the Bay of Fundy is a specific location where many individuals congregate in similar fashion to the Bay of Fundy or if mother-calf pairs remain secluded from other mother-calf pairs in obscure coastal waters or out at sea. These questions can be answered by satellite tagging followed by aerial surveys. As a result of site fidelity and cultural philopatry analyses a specific list of individuals that would utilize a nursery other than the Bay of Fundy was established. Any mother from the *Bay of Fundy none* category would be a good target for

satellite tagging since they have all exhibited site fidelity to a nursery other than the Bay of Fundy. Specifically, individuals 1204, 1334, 1509, 1515, 1612 and 1268 would be key targets since they are due to produce an offspring in the next four years and have exhibited a reliable calving interval. The most opportune times for satellite tagging is during the migration period in March to July as most of the individuals in the population are sighted in Great South Channel (Kenney *et al.* 1995). *Bay of Fundy none* mothers with newborn calves of that year should be the focus of satellite tagging in the area. Preliminary aerial surveys could potentially increase the effectiveness of one tag if the tagged mother-calf pair were not alone in their migration.

Identification of the location and assessment of the alternative nursery area or areas is important in order to determine the reason behind the movement between nurseries by *Bay of Fundy some* females. Assessment of potentially lethal threats and the protection of this critical habitat area is necessary to prevent further disturbance to this endangered species.

Continued field surveillance for at least the next four years is required in order to determine if cultural transmission of site fidelity occurs in *Bay of Fundy none* category. This information would also be valuable in determining the mechanism of site fidelity such as learned behaviours or social facilitation. It is also valuable in analyses with physical oceanographic factors in order to determine if correlations exist that affect population distribution and reproduction.

Expanded efforts are required in the habitat areas in which biopsy darting occurs. Since 1992, darting has been restricted to the Bay of Fundy area. Efforts are required in the Great South Channel area since most individuals in the population are seen in this area (Winn *et al.* 1986) and the southeastern United States since all mother-calf pairs are sighted here. These efforts may also increase the amount of haplotype diversity that has currently been identified.

It is highly likely that, haplotypic diversity has been affected by extensive whaling. It has been documented that Basque whaling efforts were concentrated to the waters of Newfoundland, Labrador, Gulf of St. Lawrence and Eastern Nova Scotia (Aguilar 1986, Reeves and Mitchell 1986a). This area represents the species' historical geographic range (Gaskin 1991) which may have housed different haplotypes or haplotype frequencies maintaining the basis of genetic structuring. Since this area was exploited for a period of over 100 years (Aguilar 1986) it is possible that many haplotypes were greatly reduced. Remnant examples of these highly reduced haplotypes may still exist. Haplotype E is a possible example of a rare remnant haplotype and has only been found in three males. The lack of haplotype E females suggests that this haplotype is confined to the alternative nursery to the Bay of Fundy and that it may soon be lost. Since only 48% of all reproductive females have been darted and the majority of these dartings have occurred in the Bay of Fundy and in Roseway Basin it is highly probable that Haplotype E mothers belong to *Bay of Fundy none* category. It is also probable that *Bay of Fundy none* females utilize areas that were within the historic

geographic range. Other rare haplotypes may still exist in very low frequencies, but a different darting strategy is needed in order to sample these individuals and identify their haplotypes. Specifically, biopsy darting in spring feeding areas where most of the population is present and in the calving area in southeastern United States is necessary to increase our population sample size and screen for rare haplotypes.

The immediate conservation recommendations for this species are:

1. Identification of the location of an alternative nursery area or areas by satellite tagging and aerial surveillance.
2. Expanded biopsy darting efforts in Great South Channel and southeastern United States to collect missing individuals, especially the *Bay of Fundy none* mothers and calves to analyze full haplotype diversity and provide samples for parentage analysis.
3. Continued field surveillance in all major habitat areas to continually analyze the population for site fidelity and transmission of site fidelity.

Future recommendations are:

1. Critical habitat areas such as calving, nursing and breeding areas should be identified and protected by legislation.

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CHAPTER THREE

GENETIC DIVERSITY IN THE CONTROL REGION OF MITOCHONDRIAL DNA WITHIN AND BETWEEN NORTH AND SOUTH ATLANTIC RIGHT WHALES.

Abstract

Eight hundred years of whaling has left the North Pacific and Eastern Atlantic populations of *Eubalaena glacialis* near extinction while the Western North Atlantic population is listed as endangered (IUCN status). In the southern hemisphere *Eubalaena australis* populations have experienced less extensive whaling over a period of approximately 200 years, yet these populations are still considered vulnerable (IUCN status). Little is known about the evolutionary history of the right whale, *Eubalaena*. Through the use of RFLP (restriction fragment length polymorphism) analysis a previous study has shown sequence diversity of North Atlantic right whale haplotypes in the low range compared to within population estimates in other species and similar levels of haplotypic diversity between North and South Atlantic right whale populations.

The purpose of this study was to provide a finer resolution of the estimation of genetic diversity within and between North and South Atlantic right whale populations. Control region DNA sequences were used to identify the genetic variation and estimate the genetic divergence within and between these populations. One hundred and eighty Western North Atlantic and 16 South Atlantic right whale samples have been analyzed by DNA sequence and single stranded conformation polymorphism (SSCP) analysis. Five haplotypes have been found in the 180 Western North Atlantic individuals compared to ten haplotypes in the 16 South Atlantic individuals. Sequence divergence for the five North Atlantic right whale haplotypes was between 0.2%-0.8% and for the ten South Atlantic right whale haplotypes it was between 0.6%-3.2%. Genetic divergence between

the North and South Atlantic populations was estimated to have occurred 3.0-9.0 mya. This is similar to the genetic divergence estimate of 2.0-5.3 mya found between the two clades identified in the South Atlantic samples.

Introduction

Many marine mammal species have been exploited for their oil, hide, baleen and meat (Reeves and Mitchell 1986a). The right whale (*Eubalaena*) was an extremely lucrative catch due to it's high yield of oil and marketable baleen (Reeves and Mitchell 1986a). The right whales preferential use of inshore coastal areas as well as the tendency to float when killed made these whales an easy catch well before the initiation of modern commercial whaling.

Basque whalers from France and Spain were the first to initiate commercial whaling as early as 1059 (Aguilar 1986). Initial Basque whaling in the Bay of Biscay had focused on the Eastern North Atlantic right whale population (*E. glacialis*). Catch results indicate the depletion of this stock by the 16th century (Aguilar 1986). With the declining harvests in the local coastal waters, Basque whalers moved out in search for new stocks. Archival and archeological material show the presence of Basque whaling in the Strait of Belle Isle region (corridor between Labrador and Newfoundland) by 1567 (Reeves and Mitchell 1986a). By the late 17th century depletion of the Western North Atlantic right whale had resulted in the decline of Basque whaling efforts (Reeves and Mitchell 1986a). The approximate cumulative catch for the period between the 11th century and 17th century was 25 000-40 000 whales. It is unknown what proportion of these were bowheads, since the southern range of the bowhead overlapped with this area for the first couple of months of each season (Aguilar 1986). Despite, the decline in Basque whaling; New England pelagic whalers utilized Newfoundland, Labrador and the Gulf of St.

Lawrence during the 18th century till the mid 19th century (Reeves and Mitchell 1986a). Concurrently the southern hemisphere species in coastal areas and off of oceanic islands were being exploited over a period of 200 years starting in the mid 16th century (Best 1988). Between 1785 and 1939 a minimum of 38 609 animals were taken by French, US and South African whalers in the South Atlantic (IUCN).

The southern hemisphere right whales have a circumpolar distribution with many identified stocks and habitats. Samples for this study were taken from the South American population. Two other major populations are the South African and the South Pacific/Indian Ocean. Samples were obtained from the Peninsula Valdes, Argentina habitat area, where the population estimate is 1200 individuals. This is a winter/spring habitat area with three areas of aggregation (Payne 1986). There is a differential age/sex composition within these areas. One area is predominantly mother-calf pairs, a second is mainly males and adult females without calves and the third is a mixture of juveniles and mother-calf pairs (Payne 1986).

There is no information about the pre-exploited population structure, dynamics or genetic composition of the right whale. Recent studies have shown the growth rate of the North Atlantic right whale to be 2.5% (Knowlton *et al.* 1994) which is 2-3 times lower than the population growth rate for the South Atlantic right whale (Best and Underhill 1990, Payne 1990). Studies have also examined the genetic diversity of the North and South Atlantic populations through RFLP analysis (Schaeff 1993) and the genetic variability between North and South Atlantic samples using DNA fingerprinting (Schaeff

et al. 1997). These studies have shown sequence divergence of mtDNA in the North Atlantic right whale to be 0.08% compared to 0.24% in the South Atlantic right whales and both with similar haplotypic variability; three haplotypes were identified in ten North Atlantic individuals and four haplotypes were identified in ten South Atlantic individuals (Schaeff 1993). DNA fingerprinting results have shown higher bandsharing coefficients in the North Atlantic right whale from the South Atlantic right whale, indicating a higher degree of relatedness within the North Atlantic population.

The purpose of this study was to provide finer resolution to the estimation of genetic diversity within and between North and South Atlantic right whale populations by analysis of mtDNA control region sequence variation. The control region is an area within the mtDNA genome with a high rate of mutation (Hoelzel *et al.* 1991). Sequence analysis of the control region, provides greater resolution in identifying mtDNA haplotypes compared to RFLP analysis (Walker *et al.* 1995). MtDNA is also solely maternally inherited and lacks recombination (Brown *et al.* 1982) and is therefore an excellent marker for the analysis of matrilineal structure and population dynamics. The results of this study will also provide a context in which historical DNA sequences from samples from around the world may be analyzed in a comprehensive study on the genus *Eubalaena* to elucidate the world-wide genetic distribution, structure and the degree of migration.

Materials and Methods

Samples

The North Atlantic right whale (*E. glacialis*) samples used in this study were collected by skin biopsy sampling (Brown et. al. 1991). Sampling was done in the Grand Manan Basin, lower Bay of Fundy from August to October, 1988 to 1996 and in August and September from 1988 to 1992 in Roseway Basin, between Browns and Baccaro Banks off the southern Scotian shelf. South Atlantic right whale (*E. australis*) samples used in this study were also collected by biopsy darting off the shores of Peninsula Valdes, Argentina by J. Perkins. A bowhead (*Balaena mysticetus*) (accession number X72195) (Arnason et al. 1993) sequence was used as an outgroup in our sequence analysis.

DNA Extraction

Samples that were processed before 1995 were extracted according to protocols in Brown *et. al.* 1991 and Schaeff *et. al.* 1993. All other samples were extracted by grinding frozen tissue (0.3-0.5 g) and 4.0 mL of 1X lysis buffer (2X: 0.1 M Tris-HCl pH 8.0, 4 M urea, 0.2 M NaCl, 0.01 M 1,2 cyclohexanediamine (CDTA) and 0.5% n-laurylsarcosine) in liquid nitrogen. Samples were incubated at 37°C for one week. Proteinase K (83 units) (Boehringer Mannehiem) was added and allowed to incubate at 56°C for one hour. This was followed by a second addition of 83 units of proteinase K which was incubated at 37°C for 12 hours. Samples were then extracted with two equal volumes of phenol, chloroform and water (70:30) (Applied Biosystems Inc.); followed by an extraction with

chloroform and water (24:1) (Applied Biosystems Inc.) (Sambrook *et. al.* 1989). DNA was precipitated from the aqueous layer with 0.1X volume of 10 M ammonium acetate and one times the volume of isopropanol followed by storage at -20°C for 12 hours. Samples were centrifuged at 3000rpm (Sorvall T 6000D rotor) for 30 minutes, washed in 70% ethanol and recentrifuged for 5 minutes. The pellet obtained was dissolved in 200-500μL of TNE_2 (10 mM Tris-HCl pH 8.0, 10 mM NaCl and 2 mM disodium ethylene diamine tetraacetate•$2H_20$). The yield of DNA was measured using a DNA fluorometer (Model TKO-100, Hoefer Scientific Instruments) and Hoechst dye 33258.

Agarose gel electrophoresis

The molecular weight of extracted and amplified DNA was assessed by electrophoresis through a 1.25% agarose gel in 0.5X TBE (5X: 0.45 M Tris-borate pH 8.3, 0.01 M disodium ethylene diamine tetraacetate•$2H_2O$) and comparison to a molecular weight ladder (GIBCOBRL). The gel was stained using ethidium bromide which was added in a final concentration of 2ug/mL to the storage buffer (0.5X TBE) for 30 minutes followed by destaining in water for 10 minutes.

Polymerase Chain Reaction

DNA was amplified in a Perkin-Elmer Cetus Thermal Cycler model 480 under the following reaction conditions: 1X PCR buffer (GIBCOBRL) (20 mM Tris-HCl pH 8.4, 50 mM KCl), 1.5 mM $MgCl_2$, 0.2 mM dNTP's, 0.75 units Taq DNA polymerase

(GIBCOBRL), 10 μmoles of AB6617 5' TAA TAT ACT GGT CTT GTA AAC C 3' and AB6618 5' GGG TCG GAA GGC TGG GAC CAA AAC 3' (Murray *et al.* 1995b) and 25 ng of template DNA in a 25 μL reaction. Amplification was performed under the following temperature regime: 95°C for 10 minutes, 57°C for 30 sec. and 72°C for 30 sec. for one cycle followed by 30 cycles of 95°C for 15 sec., 57°C for 15 sec. and 72°C for 30 sec. The size of the PCR products were assessed by agarose gel electrophoresis.

Sequencing of the mtDNA control region

The mtDNA control region was amplified and assessed by agarose gel electrophoresis as described above. The control region fragment within the agarose gel was excised and soaked in 30 μL of TE (10 mM Tris-HCl pH 8.0, 10 mM NaCl and 1 mM EDTA) to elute the fragment for reamplification. One μL of this solution was used in the second amplification using AB6617 and an internal primer, H00034 5' TAC CAA ATG TAT GAA ACC TCA G 3' (Rosel *et al.* 1995). Three of these reactions were amplified for each initial product. Successful amplification was tested by agarose gel electrophoresis. Triplicate samples were combined and extracted with an equal volume of phenol, chloroform and water (Applied Biosystems Inc.) (70:30) and once with chloroform and water (24:1) (Applied Biosystems Inc.). DNA was precipitated by 0.1X 10M ammonium acetate, 1X and storage at -20°C overnight. DNA was pelleted by centrifugation at 12000 rpm for 30min, washed in 70% ethanol and recentrifuged for 5min. The ethanol was taken off the top and the pellet was allowed to air-dry. The pellet

was redissolved in 12μL of sterile H_2O and quantified using Hoechst dye 33258 and aTKO-100 fluorometer (Hoefer Scientific Instruments).

Amplified products were sequenced by MOBIX Central Facility, Institute for Molecular Biology and Biotechnology, McMaster University, Hamilton, Ontario. Seventy-five to 150 ng of purified control region DNA was used for sequencing. Sequencing was done using the PRISM™ Ready Reaction Dye Deoxy Terminator Kit (Applied Biosystems Inc., Foster City, CA). Sequencing reactions were processed by a Perkin-Elmer 9600 Thermal Cycler and the Automated DNA Sequencing System 373A (Applied Biosystems Inc.).

Sequence Analysis

Sequences were aligned and analyzed for polymorphic sites using Genetic Data Environment 2.2-gde96 and ClustalW (1.4), Multiple sequence alignments. Within the ClustalW sequence alignment program gap opening penalties was set at 10 within a range of 0.0-100.0 and the gap extension penalty was set at 0.05 where the range was 0.0-10.0. Sequence diversity estimates were calculated by the ratio of substitutions to the number of bp analyzed. DNA distances of pairwise sequence comparisons were determined using the Kimura "2-parameter" model (1980) within the DNADIST program (Phylip 3.55c, Felsenstein 1993). This model assumes a 2:1 transition to transversion ratio at all mutation sites.

Phylogenetic relationships among haplotypes identified in each species as well as the outgroup species (*Balaena mysticetus*) were determined using neighbor-joining, DNA parsimony and maximum likelihood algorithms within the programs NEIGHBOR, DNAPARS and DNAML respectively, in the Phylogenetic Inference Package (PHYLIP) version 3.55c (Felsenstein 1993).

A bootstrap analysis was performed on the phylogenies produced by the neighbor-joining and DNA parsimony algorithms. The program SEQBOOT (PHYLIP 3.55c) resampled the input data set 1000 times and estimated the variation among the replicates using the DNADIST program. These estimates were used in constructing 1000 trees in the NEIGHBOR program. A majority consensus tree was then generated from the 1000 bootstraps using CONSENSE (PHYLIP 3.55c). A bootstrap analysis of the DNA parsimony estimate was constructed in similar fashion with the exception of the use of the DNADIST program.

A maximum likelihood analysis was conducted using the default substitution rate of 2:1, transitions: transversions. Global rearrangement was also conducted which allowed each possible group to be removed and re-added to the tree once the last species was added. This strengthens the position of each species as its position is reconsidered.

Results

Sequence Analysis

Twelve individuals of the North Atlantic right whale population were sequenced. Four mtDNA control region haplotypes were found. From a previous study (refer to Ch.2) an additional 166 individuals were screened for these and new haplotypes using single stranded conformation polymorphism (SSCP) analysis. Haplotype E was identified through SSCP analysis and subsequently sequenced in two individuals. Therefore, a total of 180 individuals were screened and five haplotypes were identified in the North Atlantic right whale. The mtDNA of sixteen South Atlantic right whales was sequenced. Ten control region haplotypes were identified and no samples were screened by SSCP analysis. Of the 15 haplotypes identified none, appeared in both populations. A sequence alignment of the five North Atlantic right whale sequences, ten South Atlantic right whale sequences and the one bowhead haplotype is shown in figure 3.1. The alignment represents 500 bp sequence, with the bowhead sequence starting 55 bp from the 5' end of the analyzed sequence. When comparing North Atlantic sequences to South Atlantic sequences, 44 polymorphic sites were found, all of which showed transitional mutations except for one at position 381. Twelve of these sites showed fixed differences between North and South Atlantic sequences. Five sites (positions 140, 141, 157, 158 and 197) were synapomorphic or had common polymorphic sites between the two species. Sequence comparisons between the five North Atlantic control region haplotypes (EglHapA to EglHapE) showed sequence divergence between 0.2%-0.8% and among the ten South Atlantic haplotypes (EauHapA to EauHapJ) sequence divergence was 0.6%-3.2%.

FIGURE 3.1 Comparison of 500 bp of the North and South Atlantic right whales. Similarity to EglHapA is noted by '.'. Sequence differences are noted by the appropriate nucleotide changes. North Atlantic haplotypes are labeled with Egl. South Atlantic haplotypes are labeled with Eau. The bowhead sequence is labeled as Bmy.

```
                1          11         21         31         41        50
                |          |          |          |          |         |
EglHapA     1   tactatactc cgccatcagc acccaaagct gaaattctat ttaaactatt   50
EglHapB     1   .......... .......... .......... .......... ..........   50
EglHapC     1   .......... .......... .......... .......... ..........   50
EglHapD     1   .......... .......... .......... .......... ..........   50
EglHapE     1   .......... .......... .......... .......... ..........   50
EauHapA     1   .....c.... .a........ .......... .........c ..........   50
EauHapB     1   .....c.... .a........ .......... .........c ..........   50
EauHapC     1   .....c.... .a........ .......... .........c ..........   50
EauHapD     1   .....c.... .a........ .......... .........c ..........   50
EauHapE     1   .....c.... .a........ .......... .........c ..........   50
EauHapF     1   .......... .a........ .......... .........c ..........   50
EauHapG     1   .......... .a........ .......... .........c ..........   50
EauHapH     1   .......... .a........ .......... .........c ..........   50
EauHapI     1   .......... .a........ .......... .........c ..........   50
EauHapJ     1   .......... .a........ .......... .........c ..........   50
Bmy         1   ---------- ---------- ---------- ---------- ----------    0

                51         61         71         81         91       100
                |          |          |          |          |         |
EglHapA    51   ccctgaaaaa gtatattgta gaacatcaca aaatcacagt actatgtcag  100
EglHapB    51   .......... .......... .......... .......... ..........  100
EglHapC    51   .......... .......... .......... .......... ..........  100
EglHapD    51   .......... .......... .......... .......... ..........  100
EglHapE    51   .......... .......... .......... .......... ..........  100
EauHapA    51   .......... .......... ..g....... ...c...... .t........  100
EauHapB    51   .......... .......... ..g....... ...c...... .t........  100
EauHapC    51   .......... .......... ..g....... ...c...... .t........  100
EauHapD    51   .......... .......... ..g....... ...c...... .t........  100
EauHapE    51   .......... .......... ..g....... ...c...... .t........  100
EauHapF    51   .......... .......... ..g....... ...c...... .t........  100
EauHapG    51   .......... .......... ..g....... ...c...... .t........  100
EauHapH    51   .......... .......... ..g....... ...c...... .t........  100
EauHapI    51   .......... .......... ..g....... ...c...... .t........  100
EauHapJ    51   .......... .......... ..g....... ...c...... .t........  100
Bmy         1   -----g.... a......... ..g....... ...c...... ..........   45

                101        111        121        131        141      150
                |          |          |          |          |         |
EglHapA   101   tattaaaaat aaattatcct attacatatt actatgtaat ccgtgcatgt  150
EglHapB   101   .......... .......... .......... .........c t........  150
EglHapC   101   .......... .......... .......... .........c t........  150
EglHapD   101   .......... .......... .......... .........c t........  150
EglHapE   101   .......... .......... .......... ........gc t........  150
EauHapA   101   .......... .......... .......... .tc......c ..........  150
EauHapB   101   .......... .......... .......... .tc....... ..........  150
EauHapC   101   .......... .......... .......... .tc....... ..........  150
EauHapD   101   .......... .......... .......... .tc....... ..........  150
EauHapE   101   .......... .......... .......... .t........ ..........  150
EauHapF   101   .......... .......... .......... .tc....... ..........  150
EauHapG   101   .......... .......... .......... .t........ t........c  150
EauHapH   101   .......... .......... .......... .tc....... t........c  150
EauHapI   101   .......... .......... .......... gt....cg.c t........  150
EauHapJ   101   .......... .......... .......... .tc......c t.......ac  150
Bmy        46   ....g..... ..c....tt. ...g...... .........c t........   95
```

```
                151         161         171         181         191         200
                |           |           |           |           |           |
EglHapA  151    atgcactgcc  acatggccaa  tactagtcct  gactcataaa  ttgtacctat  200
EglHapB  151    .......a..  ..........  ..........  ..........  ..........  200
EglHapC  151    .......a..  ..........  ..........  ..........  ......t...  200
EglHapD  151    .......a..  ..........  ..........  ..........  ..........  200
EglHapE  151    ......c...  ..........  ..........  ..........  ..........  200
EauHapA  151    .......a..  ..........  ..t.g.....  ..........  ..........  200
EauHapB  151    ..........  ..........  ..t.g.....  ..........  ..........  200
EauHapC  151    ..........  ..........  ..t.g.....  ..........  ..........  200
EauHapD  151    ..........  ..........  ..t.g.....  ..........  ..........  200
EauHapE  151    ..........  ..........  ..t.g.....  ..........  ..........  200
EauHapF  151    .......a..  ..........  ..t.g.....  ......c...  ......t...  200
EauHapG  151    ......ca..  ..........  ..t.g.....  ..........  ......t...  200
EauHapH  151    ......ca..  ..........  ..t.g.....  ..........  ......t...  200
EauHapI  151    ......ca..  .....a....  ..t.g.....  ..........  ......t...  200
EauHapJ  151    ......c...  ..........  ..t.g.....  ..........  ......t...  200
Bmy       96    ......ca..  ........g.  .........c  a.........  .....tg...  145

                201         211         221         231         241         250
                |           |           |           |           |           |
EglHapA  201    acatgctat-  gtataatcgt  gcattcaatt  attttcacta  cgggaagtta  249
EglHapB  201    ..........  ..........  ..........  ..........  ..........  250
EglHapC  201    ..........  ..........  ..........  ..........  ..........  250
EglHapD  201    ..........  ..........  ..........  ..........  ..........  250
EglHapE  201    ..........  ..........  ..........  ..........  ..........  250
EauHapA  201    ..........  .......t..  ..........  ..........  ..........  250
EauHapB  201    ..........  ..........  ..........  ..........  ..........  250
EauHapC  201    ..........  ..........  ..........  ..........  ..........  250
EauHapD  201    ..........  ..........  ..........  ..........  ..........  250
EauHapE  201    ..........  ..........  ..........  ..........  ..........  250
EauHapF  201    ..........  ..........  ..........  ..........  ..........  250
EauHapG  201    ..........  ..........  ..........  ..........  ..........  250
EauHapH  201    ..........  ..........  ..........  ..........  ..........  250
EauHapI  201    ..........  ..........  ..........  ..........  ..........  250
EauHapJ  201    ..........  ..........  ..........  ..........  ..........  250
Bmy      146    .........t  ..........  ..........  ..........  ..........  195

                251         261         271         281         291         300
                |           |           |           |           |           |
EglHapA  250    aagctcgtat  taaatttttat  ttattttaca  tatgtacata  ataatcattg  299
EglHapB  251    ..........  ..........  ..........  ..........  ..........  300
EglHapC  251    ..........  ..........  ..........  ..........  ..........  300
EglHapD  251    ..........  ..........  .......g..  ..........  ..........  300
EglHapE  251    ..........  ..........  ..........  ..........  ..........  300
EauHapA  251    ..........  ..........  ..........  ..c.......  ..........  300
EauHapB  251    ..........  ..........  ..........  ..........  ..........  300
EauHapC  251    ..........  ..........  ..........  ..c.......  ..........  300
EauHapD  251    ..........  ..........  ..........  ..c.......  ..........  300
EauHapE  251    ..........  ..........  ..........  ..c.......  ..........  300
EauHapF  251    ..........  c.........  ..........  ..c.......  ..........  300
EauHapG  251    ..........  c.........  ..........  ..c.......  ..........  300
EauHapH  251    ..........  c.........  ..........  ..c.......  ..........  300
EauHapI  251    ..........  c.........  ..........  ..c.......  ..........  300
EauHapJ  251    ..........  c.........  ..........  ..c.......  ..........  300
Bmy      196    .........a  .g........  ..........  ..c.......  ..........  245
```

```
                301         311         321         331         341         350
                |           |           |           |           |           |
EglHapA   300   atcgtgcata  gtacatgtcc  ttaaatcaat  tcaagtcaac  tgaatcttat   349
EglHapB   301   ..........  ..........  ..........  ..........  ..........   350
EglHapC   301   ..........  ..........  ..........  ..........  ..........   350
EglHapD   301   ..........  ..........  ..........  ..........  ..........   350
EglHapE   301   ..........  ..........  ..........  ..........  ..........   350
EauHapA   301   ..........  .......ct.  ..........  ..........  c.........   350
EauHapB   301   ..........  .......ct.  ..........  ..........  c.........   350
EauHapC   301   ..........  .......ct.  ..........  ..........  c.........   350
EauHapD   301   ..........  .......ct.  ..........  ..........  c.........   350
EauHapE   301   ..........  .......ct.  ..........  ..........  c.........   350
EauHapF   301   g....a....  ..g....ct.  ..........  .........t  ......c...   350
EauHapG   301   g....a....  ..g....ct.  ..........  ..........  c.........   350
EauHapH   301   g....a....  ..g....ctt  ..........  ..........  ..........   350
EauHapI   301   g....a....  ..g....ct.  ..........  ..........  c.........   350
EauHapJ   301   .....a....  ..g....ct.  ..........  ..........  ..........   350
Bmy       246   .........g  ...t......  .c.....g..  ..........  ......c...   295

                351         361         371         381         391         400
                |           |           |           |           |           |
EglHapA   350   ggccgctcca  ttagatcacg  agcttgatca  gcatgccgcg  tgaaaccagc   399
EglHapB   351   ..........  ..........  ..........  ..........  ..........   400
EglHapC   351   ..........  ..........  ..........  ..........  ..........   400
EglHapD   351   ..........  ..........  ..........  ..........  ..........   400
EglHapE   351   ..........  ..........  ..........  ..........  ..........   400
EauHapA   351   ..........  ..........  ......g...  ..........  ..........   400
EauHapB   351   ..........  ..........  ......g...  ..........  ..........   400
EauHapC   351   ..........  ..........  ......g...  ..........  ..........   400
EauHapD   351   ..........  ..........  ......g...  ..........  ..........   400
EauHapE   351   ..........  ..........  ......g...  ..........  ..........   400
EauHapF   351   ..........  ..........  ......g...  ..........  ..........   400
EauHapG   351   ..........  ..........  ......g...  ..........  ..........   400
EauHapH   351   ..........  ..........  ......g...  ..........  ..........   400
EauHapI   351   ..........  ..........  ......g...  ..........  ..........   400
EauHapJ   351   ..........  ..........  ......g...  c.........  ..........   400
Bmy       296   ..........  ..........  ......g...  ..........  ..........   345

                401         411         421         431         441         450
                |           |           |           |           |           |
EglHapA   400   aacccgctcg  gcagggatcc  ctcttctcgc  accgggccca  tcaattgtgg   449
EglHapB   401   ..........  ..........  ..........  ..........  ..........   450
EglHapC   401   ..........  ..........  ..........  ..........  ..........   450
EglHapD   401   ..........  ..........  ..........  ..........  ..........   450
EglHapE   401   ..........  ..........  ..........  ..........  ..........   450
EauHapA   401   ..........  ..........  ..........  ..........  ..g..c....   450
EauHapB   401   ..........  ..........  ..........  ..........  .....c....   450
EauHapC   401   ..........  ..........  ..........  ..........  .....c....   450
EauHapD   401   ..........  ..........  ..........  ..........  .....c....   450
EauHapE   401   ..........  ..........  ..........  ..........  .....c....   450
EauHapF   401   ..........  ..........  ..........  ..........  .....c....   450
EauHapG   401   ..........  ..........  ..........  ..........  .....c....   450
EauHapH   401   ..........  ..........  ..........  ..........  .....c....   450
EauHapI   401   ........t.  ..........  ..........  ..........  .....c....   450
EauHapJ   401   ..........  ..........  ..........  ..........  .t...c....   450
Bmy       346   ..........  ..........  ..........  ..........  .t...ca...   395
```

```
                451         461         471         481         491         500
                |           |           |           |           |           |
EglHapA    450  gggtagctat ttaatggtct ttacaagaca tctggttctt acttcagggc  499
EglHapB    451  .......... .......... .......... .......... ..........  500
EglHapC    451  .......... .......... .......... .......... ..........  500
EglHapD    451  .......... .......... .......... .......... ..........  500
EglHapE    451  .......... .......... .......... .......... ..........  500
EauHapA    451  .......... ......a... .......... .......... ..........  500
EauHapB    451  .......... ......a... .......... .......... ..........  500
EauHapC    451  .......... ......a... .......... .......... ..........  500
EauHapD    451  .......... ......a... c......... .......... ..........  500
EauHapE    451  .......... ......a... .......... .......... ..........  500
EauHapF    451  .......... ......a... .......... .......... ..........  500
EauHapG    451  .......... ......a... .......... .......... ..........  500
EauHapH    451  .......... ......a... .......... .......... ..........  500
EauHapI    451  .......... ......a... .......... .......... ..........  500
EauHapJ    451  .......... .......... .......... .......... ..........  500
Bmy        396  .......... ........t. .......... .......... ..........  445
```

Distance measurements

DNA distance measurements were calculated using the Kimura "2-parameter" model. This model assumes a 2:1 transition to transversion ratio. The transition bias has been observed among three cetacean species: minke, orca and Commerson's dolphin (Hoelzel *et al.* 1991) and is consistent with the expectations of mtDNA (Avise *et al.* 1987, Moritz *et al.* 1987). The genetic distance between each pairwise comparison of haplotypes estimates the total branch length and time of divergence between those two haplotypes (DNADIST, PHYLIP 3.55c Felsenstein 1993). Distance measurements were calculated for all North and South Atlantic haplotypes as well as a bowhead haplotype (Table 3.1). Intraspecific distance variation among North Atlantic haplotypes ranged from 0.002-0.009. Intraspecific distance variation among South Atlantic haplotypes ranged from 0.002-0.037. Interspecific variation between the two species ranged from 0.030-0.063. These distance measurements indicate that most of the haplotypes within each species are more closely related than between the species. Some intraspecific distance measurements among the South Atlantic samples are similar to the interspecific distance measurements between the North and South Atlantic samples.

Neighbor-joining and parsimony analysis (figure 3.2 and 3.3) showed the clustering of South Atlantic haplotypes into two clades. The definition of these clades was also supported by intraspecific and interspecific genetic distance measurements within and between the clades. Intraspecific variation among clade A haplotypes ranged between 0.002-0.011 and among clade B ranged between 0.007-0.032. Interspecific

Table 3.1 Genetic distance measurements among North and South Atlantic control region sequences and a bowhead sequence. Measurements are based on Kimura's two parameter distance measurements. North Atlantic right whales are labeled as Egl, South Atlantic right whales are labeled as Eau and the bowhead is labeled as Bmy.

EglHapA															
EglHapB	0.007														
EglHapC	0.009	0.002													
EglHapD	0.009	0.002	0.005												
EglHapE	0.009	0.007	0.009	0.009											
EauHapA	0.041	0.039	0.041	0.041	0.046										
EauHapB	0.030	0.037	0.039	0.039	0.039	0.011									
EauHapC	0.032	0.039	0.041	0.041	0.041	0.009	0.002								
EauHapD	0.034	0.041	0.044	0.044	0.044	0.011	0.005	0.002							
EauHapE	0.030	0.037	0.039	0.039	0.039	0.011	0.005	0.002	0.005						
EauHapF	0.051	0.053	0.051	0.055	0.060	0.027	0.025	0.023	0.025	0.025					
EauHapG	0.051	0.048	0.046	0.051	0.051	0.027	0.025	0.023	0.025	0.020	0.018				
EauHapH	0.053	0.051	0.048	0.053	0.053	0.030	0.027	0.025	0.027	0.027	0.016	0.0067			
EauHapI	0.063	0.055	0.053	0.058	0.058	0.034	0.037	0.034	0.037	0.032	0.030	0.0158	0.030		
EauHapJ	0.053	0.051	0.048	0.053	0.048	0.034	0.032	0.030	0.032	0.032	0.030	0.0204	0.018	0.032	
Bmy	0.073	0.065	0.065	0.068	0.068	0.083	0.085	0.083	0.085	0.080	0.090	0.0850	0.088	0.092	0.083

variation between the two clades ranged from 0.020-0.037. As stated above the interspecific distance measurement between clade A and clade B in the South Atlantic population is similar to the distance measurement between the North and South Atlantic species. Genetic distance measurements indicate that the haplotypes among clades are less divergent then between clades. Genetic distance measurements between the bowhead haplotype and the right whale haplotypes ranged between 0.065-0.090.

Genetic distance measurements along with the divergence rate in mysticetes (Arnason *et al.* 1993) were used to estimate the times of divergence. Arnason *et al.* 1993 calculated the divergence rate to be 0.7-1.0% / Myrs. This estimate was based on sequences from humpback *Megaptera novaeangliae*, fin *Balaenoptera physalus*, blue *Balaenoptera musculus*, sei *Balaenoptera borealis*, gray *Eschrichtius robustus* and bowhead *Balaena mysticetus*. Hoelzel *et al.* 1991 had previously determined the divergence rate to be 0.5-1.0% / Myrs, but this estimate was based mainly on odontocete sequences. Using the mysticete divergence rate with the distance measurements we estimate the division between right whales and bowheads to have occurred 6.5 to 13 million years ago (mya) which would have been in the late Miocene. The estimate for divergence between the North and South Atlantic right whales is 3-9 mya in the late Miocene to early Pliocene. This is consistent with the fossil record since, the family Balaenidae are found by the early Miocene, 20-25 mya (Barnes *et al.* 1985).

Within the South Atlantic haplotypes there were two clades; A and B. The divergence time between clade A and clade B is 2.0-5.3 mya. This estimate overlaps with the estimate of species divergence between the North and South Atlantic right whales.

Phylogenetic tree analysis

The phylogenetic relationship of the variable mtDNA control region sequences among North and South Atlantic right whales were constructed using Neighbor-Joining (Saitou and Nei 1987), parsimony (Felsenstein 1993) and Maximum Likelihood (PHYLIP 3.55c) analysis.

The Neighbor-joining method produces a tree under the principle of minimum evolution; it minimizes the sum of branch lengths at each stage of clustering of the operational taxonomic units (Saitou and Nei 1987). The Neighbor-joining tree was rooted with a homologous bowhead whale sequence (Figure 3.2). The branching order of the two right whale species and the two major lineages among the South Atlantic samples was supported by a majority rule consensus of the Neighbor-joining trees produced by 1000 bootstrap simulations (PHYLIP 3.55c). In figure 3.2 the percentages show the agreement in consensus of 1000 permutations. The South Atlantic haplotypes (EAU) are divided into two major lineages with a 87% confidence interval for clade A and 72% confidence interval for clade B. The North Atlantic samples are a distinct group from the South Atlantic samples with no overlap of haplotypes.

FIGURE 3.2 A phylogenetic tree based on Neighbor-Joining distance measurements of control region sequences from North and South Atlantic right whales. Results of a majority rule consensus tree are provided at the nodes of the Neighbor-Joining tree. North Atlantic samples are labeled as Egl and South Atlantic samples are labeled as Eau. Bmy is a bowhead sequence which was used as an outgroup. The number located at the nodes indicates the percentage of bootstrap trees that contain this pattern. The scale can be used to estimate the genetic distance between any two individuals. It is based on the Kimura's two pararmeter model (Table 3.1).

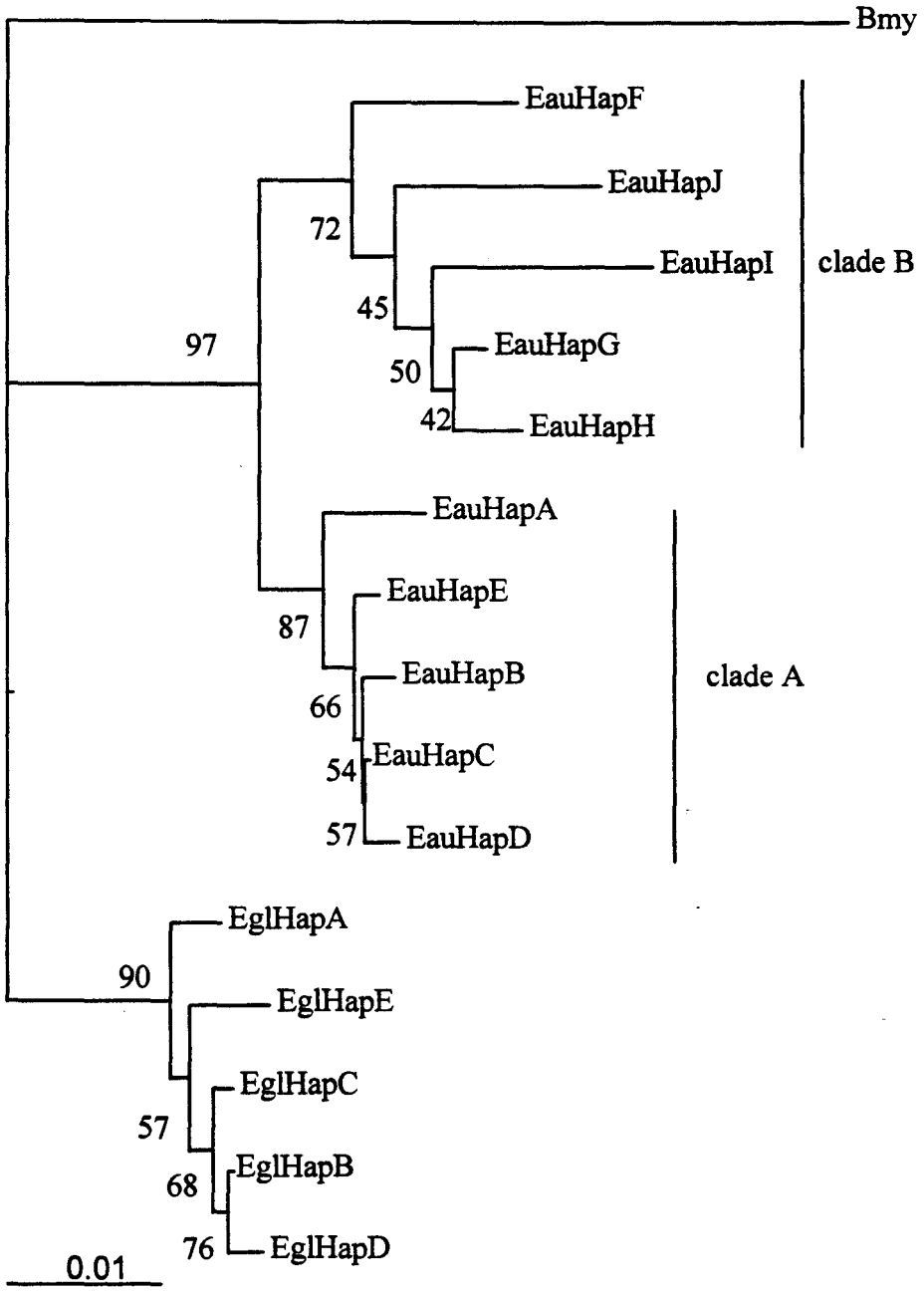
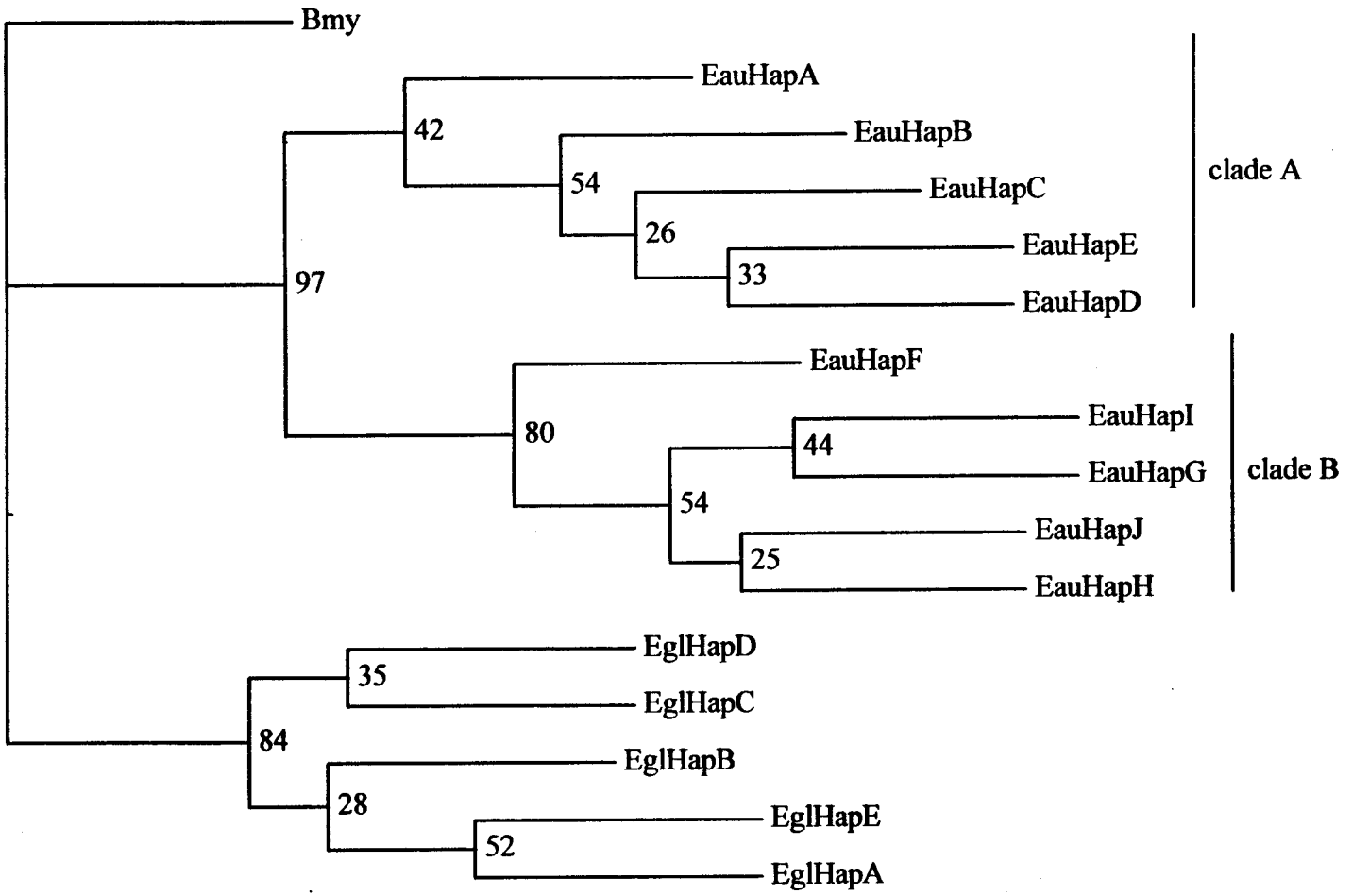

Bmy
EauHapF
EauHapJ
72
EauHapI
clade B
45
97
EauHapG
50
42
EauHapH
EauHapA
EauHapE
87
EauHapB
clade A
66
EauHapC
54
57
EauHapD
EglHapA
90
EglHapE
EglHapC
57
EglHapB
68
76
EglHapD
0.01

FIGURE 3.3 Consensus tree of 1000 bootstrap parsimony distance trees based on North and South Atlantic control region sequences. Bmy is a bowhead control region sequence used as an outgroup. North Atlantic samples are labeled with Egl and South Atlantic samples are labeled with Eau. The number located at the nodes indicates the percentage of bootstrap trees that contain this pattern.


Bmy
EauHapA
42
EauHapB
54
EauHapC
26
EauHapE
33
EauHapD
clade A
97
EauHapF
80
EauHapI
44
EauHapG
54
EauHapJ
25
EauHapH
clade B
EglHapD
35
EglHapC
84
EglHapB
28
EglHapE
52
EglHapA

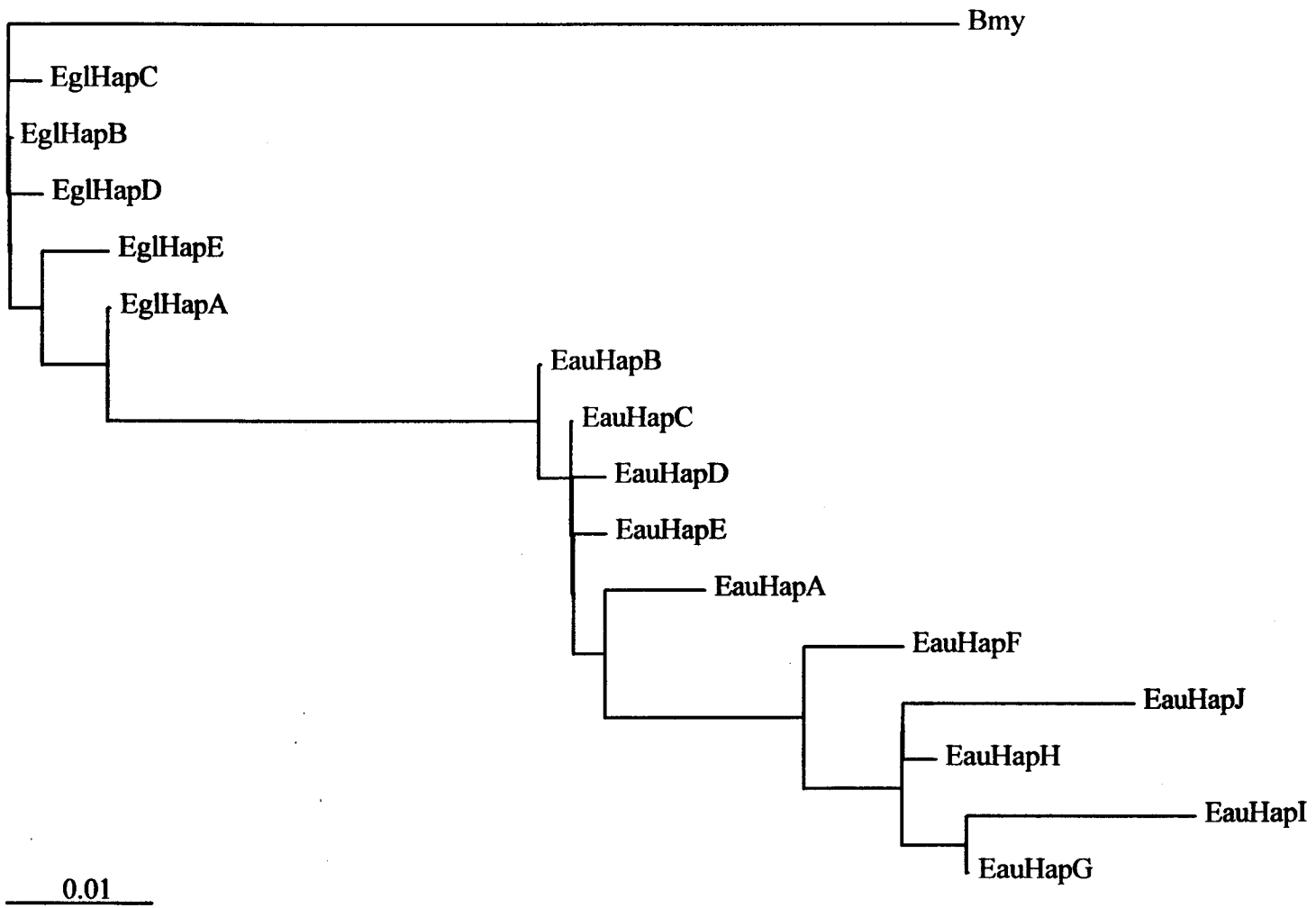
Parsimony analysis is also consistent with the division of South Atlantic haplotypes into two major lineages (clade A and B) and the division between North and South Atlantic haplotypes. The most parsimonious tree with the fewest mutational steps is estimated based on similarities among sequences ie. synapomorphies (Felsenstein 1993). One thousand trees were generated from distance measurements using parsimony analysis. Figure 3.3 (DNA parsimony tree) shows a similar topology to figure 3.2 (Neighbor-joining tree). Confidence limits within internal branches of the major lineages differ in the percentage agreement between the Neighbor-joining and parsimony trees, yet branching order of major lineages remains similar.

The maximum likelihood method is based on the analysis of all sites within a sequence and not just those that have changed or are phylogenetically important. This method minimizes the bias towards long branch lengths (Felsenstein 1993). Figure 3.4 shows the tree topology based on the maximum likelihood method with global rearrangement and the bowhead outgroup rooting the tree. Maximum likelihood method shows similarities to Neighbor-joining (figure 3.2) and parsimony analysis (figure 3.3). North and South Atlantic samples have grouped separately with a similar division of lineages among South Atlantic samples.

Discussion

Analysis of the control region sequences of the 180 North Atlantic right whales identified five haplotypes and analysis of the 16 South Atlantic right whales identified 10

FIGURE 3.4 Phylogenetic tree of North and South Atlantic right whale control region sequences based on Maximum likelihood method with global rearrangements. North Atlantic samples are labeled by Egl and South Atlantic samples are labeled with Eau. This tree is rooted with a bowhead control region sequence, labeled as Bmy. The scale can be used to measure the genetic distance between any two individuals. It is based on a 2:1 substitution ratio of transitions to transversions.


Bmy
EglHapC
EglHapB
EglHapD
EglHapE
EglHapA
EauHapB
EauHapC
EauHapD
EauHapE
EauHapA
EauHapF
EauHapJ
EauHapH
EauHapI
EauHapG
0.01

haplotypes. The limited amount of haplotype diversity identified in the North Atlantic right whale compared to the South Atlantic right whale is hypothesized to have been reduced by the prolonged whaling period endured by the North Atlantic right whale. The five haplotypes or matrilines identified in the North Atlantic right whale suggests that only a minimum of five reproducing females survived the whaling bottleneck. Many haplotypes may have been completely obliterated by the extensive whaling experienced by this population. The low genetic distance measurements estimated for the North Atlantic right whale population may also be explained by the loss of older haplotypes that would have increased these measurements. The detrimental effects of extensive whaling have also been observed in the high bandsharing coefficients of the North Atlantic right whale compared to the South Atlantic right whale (Schaeff *et al.* 1997). The high levels of bandsharing in the North Atlantic population suggests that individuals within this population are highly related to each other. The result of high relatedness is increased inbreeding (Schaeff *et al.* 1997).

RFLP analysis of mtDNA (Schaeff 1993) had identified 3 RFLP haplotypes in the North Atlantic right whale and 4 in the South Atlantic right whale. Although RFLP haplotypes could be combined with control region haplotypes to further increase haplotypic resolution, it is impractical because samples that have only been analyzed through control region sequence would need to be analyzed for RFLPs. RFLP analysis requires 100 fold more DNA than any PCR based technique and therefore would restrict the number of future studies on any given sample.

Among the five North Atlantic right whale haplotypes, sequence divergence was calculated to be 0.2%-0.8%. In the 16 South Atlantic right whales the ten haplotypes were between 0.6%-3.2% sequence divergence. Both of these estimates are higher than the sequence divergence measurements calculated by Schaeff *et al.* (1993). Schaeff *et al.* in 1993 estimated sequence diversity in the North Atlantic right whale to be 0.08% and in the South Atlantic right whale to be 0.24%. Sequence diversity estimates of the North Atlantic right whale in our study was similar to the level of diversity seen in harbor porpoises of the North Atlantic 0.90% (Rosel *et al.* 1995) and slightly higher than the sequence diversity of humpbacks of the Pacific 0.38% (Baker *et al.* 1990). The range of sequence diversity estimated in the South Atlantic right whale is large. At the high end of this range estimates are similar to between population sequence divergence measurements of harbor porpoises in the North Atlantic compared to the Black Sea 2.4% (Rosel *et al.* 1995). These similarities suggest that two historically separate populations may be present in the South Atlantic samples taken from Peninsula Valdes.

Phylogenetic analysis of the sequences indicated the divergence between right whales and bowheads to have occurred 6.5-13 mya. The divergence estimate between North and South Atlantic right whales was 3-9 mya. These estimates are consistent with the fosssil record that indicates the family Balaenidae to be present by early Miocene, 20-25 mya (Barnes *et al.* 1985).

The North Atlantic right whale population showed intraspecific genetic distance measurements of haplotypes between 0.22%-0.90%. These low genetic distance

measurements may underestimate the time of divergence for this species. Reeves and Mitchell in 1986 suggested that at one time Northwestern and Northeastern Atlantic right whales may have been a part of one breeding population. Information about the pre-exploited matrilineal structure of this population will be enlightened by the analysis of historical samples from this population.

Within the South Atlantic samples the results have shown two clades which diverged 2.0-5.3 mya. This estimate of divergence is similar to the estimate of population divergence between the North and South Atlantic right whales. This raises the question about whether one population with genetic structuring or two populations inhabit these waters. Peninsula Valdes, Argentina from where these samples were taken is a winter and spring habitat area from June-December for many individuals in the population (Payne 1986). At Peninsula Valdes, right whales concentrate in three separate areas, which have a mixed composition of mother-calf pairs, juveniles and adults (Payne 1986).

It is possible that the genetic distance measurements calculated for the South Atlantic samples represents a population where two distinct historical populations have come together resulting in genetic structuring. During the peak of the last glacier event, 18 000 years ago, the Antarctic ice sheet by conservative measures was in close proximity to the southern tip of South America (CLIMAP 1976). At times the corridor between the southern tip of South America and the Antarctic ice sheet may have been blocked by glacial expansion. This barrier may have separated a distinct right whale population in the South Eastern Pacific from the South Atlantic population. Upon glacier

retreat these populations may have come together in the South Atlantic resulting in the genetic structuring. Similar genetic structuring has been observed in Arctic beluga populations, (Brennin *et al.* 1997) upon the retreat of Arctic glaciation.

It is also possible that two separate populations come together on Peninsula Valdes from June through December. These populations would be represented by clade A and clade B of our phylogenetic analyses. Yet, it is unknown whether frequent migration between populations occurs. The southern right whale has a circumpolar distribution with many identified stocks and habitats. Three main right whale populations of southern hemisphere are recognized: 1. South Atlantic, 2. South African and 3. South Pacific/Indian Ocean. Photoidentification and field surveillance of the South Atlantic right whale has resulted in six instances of long-range movements of right whales in the Southern Atlantic (Best *et al.* 1993). These documented cases include individuals sighted at Peninsula Valdes and resighted in south central Atlantic islands, such as Tristan da Cunha, Gough Island and South Georgia. They also include an individual sighted in the south central Atlantic islands and resighted off the coast of South Africa. These examples demonstrate the ability for long-range movement and thereby population migration, but only by a few possibly transient individuals. In order to understand the presence of two clades with the South Atlantic samples more information about the individuals from whom the samples came from, the population dynamics and population distribution within the Peninsula Valdes area is needed.

In order to ascertain the effects of extensive historic whaling on the North Atlantic right whale population, an understanding of pre-exploitation genetic structure and variation is needed. Control region sequence analysis of historical samples from the Western North Atlantic right whale and the previous northern hemisphere populations will provide this information. The Molecular Systematics Laboratory of the American Museum of Natural History is currently in the process of obtaining historic samples of baleen and bone from Northern hemisphere right whales (Rosenbaum *et al.* 1997). Currently, samples have been obtained from early 16th and 20th century Western North Atlantic samples and 19th century Western Pacific samples. Future acquistion of samples includes late 19th century Eastern North Atlantic samples. Particular interest lies in Eastern North Atlantic samples and their relationship with Western North Atlantic samples in the context of population genetic structure and migration. In order to clarify the genetic structure and variation seen in the South Atlantic; samples from South Georgia, England that have recently been acquired will be added to this data set. South Georgia is located in the south-central Atlantic and is the area where exchanges have been observed between the South American and South African populations. Future collaborations with the groups studying the South Atlantic, South African and South Pacific/Indian Ocean populations are also possible. Such collaborations would in combination with Northern hemisphere studies of the right whale result in the world-wide analysis of genetic structure and evolution of the genus *Eubalaena*.

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CHAPTER FOUR

GENERAL DISCUSSION

The recovery program for the North Atlantic right whale has provided a unique opportunity to all who study it's behaviour, biology, population dynamics and genetics. This population is highly endangered with as few as 320 to 350 individuals left (Crone and Kraus 1990) yet, through collaborative efforts of the right whale consortium at the end of 1996, 283 extant individuals had been identified. This is approximately 81%-88% of the population. Individual identification coupled with field surveillance data on the majority of these individuals in their habitat areas as far back as 1980 and biopsy samples of 208 individuals has lent this population to thorough scientific studies.

Analysis of female philopatry to nursery areas has demonstrated mtDNA control region haplotype frequency differences between mothers and calves that use the Bay of Fundy nursery area and those that have not used the Bay of Fundy nursery area. The presence of significant site fidelity has provided a mechanism for the maintenance of genetic structuring and probability of the transmission of site fidelity from mothers to daughters. This study has also identified a group of reproductive females that do not use a specific nursery area but instead alternate between nursery areas with different calves. These females may demonstrate the ability of this species to change its patterns in response to habitat disturbances. A combination of these results has also helped identify for a group of females as prime targets for satellite tagging in order to elucidate the location of the alternative nursery area or areas to the Bay of Fundy.

Analysis of genetic diversity between and within North and South Atlantic right whales has shown that haplotypic variability is far greater among South Atlantic right

whales than among North Atlantic right whales. Low haplotypic variability in the North Atlantic right whale is undoubtedly a function of the extensive exploitation endured by this species. Genetic diversity analysis of the South Atlantic right whale identified two clades within the samples analyzed. Genetic divergence between the two clades was estimated to be 2.0-5.3 mya, which is similar to the estimate of genetic divergence between North and South Atlantic right whale species (3.0-9.0 mya). The origin of these clades may be the combination of historical populations or Peninsula Valdes, Argentina may be a habitat area used by two discrete populations. Further analysis of South Atlantic samples will help resolve this.

The North Atlantic right whale has shown genetic structuring of control region haplotypes between the *Bay of Fundy all* and *Bay of Fundy none* categories but the presence of a sampling bias against the *Bay of Fundy none* females has made it difficult to infer the haplotype frequencies in this group. The same bias exists in estimating haplotype frequencies for the whole population. In order to estimate these frequencies to the total population individuals can be divided into two subpopulations. One derived from animals brought to the Bay of Fundy and the other from animals that were not. The total population is estimated to be between 320-350 individuals (Crone and Kraus 1990). In Table 2.6 the probability of a calf being brought to the Bay of Fundy nursery was calculated to be 0.59 and therefore the probability of a calf not being brought to the Bay of Fundy was 0.41. The use of these probabilities in combination with the total population size of 320 can be used to estimate that 189 individuals could belong to the

Table 4.1 Control region haplotypes of reproductive females, their offspring and males with no matrilineal information of the North Atlantic right whale population were extrapolated to the total population size to examine haplotype frequency differences.

	Haplotypes of Bay of Fundy all females and their offspring	Extrapolated haplotypes of the proportion of the total population that went to the Bay of Fundy nursery area	The percentage of each haplotype in the proportion of the population that went to the Bay of Fundy nursery area	Haplotypes of all other reproductive females and their offspring + males	Extrapolated haplotypes of the proportion of the total population that did not go to the Bay of Fundy nursery area.	The percentage of each haplotype in the proportion of the population that did not go to the Bay of Fundy nursery area
A	41	76	**40**	34	26	23
B	9	17	9	25	19	**23**
C	4	7	4	34	25	**23**
D	48	89	**47**	79	59	**60**
E	0	0	0	3	2	2
Totals	102	189	100	175	131	100

Haplotype A was more common in the Bay of Fundy all category. Haplotypes B and C were more common in the all other category. Haplotype D was a predominant haplotype in both categories

Bay of Fundy nursery area subpopulation and 131 to the non Bay of Fundy subpopulation. In Table 4.1 these estimates for population distribution are used to extrapolate the frequency of control region haplotypes within the *Bay of Fundy all* category and the combined category of *Bay of Fundy some* and *none* or in other words the all other category. In order to compensate for the sampling bias against non Bay of Fundy females, the haplotypes of males that have no known mother-calf relationship to any analyzed individual and were first sighted in 1980 or earlier as a juvenile were included into the all other category. Since the mother-calf relationship for these males is not known it can be assumed that the majority of them belonged to mothers that were never identified and therefore compensate for the sampling bias. Males sighted after 1980 were not included into the all other category because in their first year they were probably reported as a calf with their mother and were never identified in that year. The haplotypes of these unidentified calves have already been included into the analysis (Tables 2.3 and 2.4). There is still a biased measure since some of the males included in the analysis are probably the offspring of the *Bay of Fundy all* category but a mother-calf relationship was never identified. The extrapolated population frequencies show that haplotype A is more common in the *Bay of Fundy all* category while haplotypes B and C are more common in the all other category. Haplotype D represents a large percentage of both categories. In Table 4.2 a similar extrapolation of predicted control region haplotype frequencies was estimated. In this case the extrapolation was based solely on the offspring of each category, therefore the control region haplotypes of females missing

Table 4.2 Control region haplotypes analyzed in the calves of the North Atlantic right whale population, extrapolated to the total population size of 320 in order to identify haplotype frequency differences

	Bay of Fundy all calves	Bay of Fundy all Predicted population	Haplotype percentages of predicted population	All other offspring	All other Predicted population	Haplotype percentages of predicted population
A	29	79	**42**	15	23	18
B	7	19	10	17	27	**20**
C	2	6	3	14	22	**17**
D	31	85	**45**	38	59	**45**
E	0	0	0	0	0	0
Totals	69	189	100	84	131	100

Haplotype A was more common in the Bay of Fundy all predicted population while haplotypes B and C were more common in the all other predicted population and haplotype D was predominant in both categories.

due to the sampling bias was not corrected for. Table 4.2 demonstrates that haplotype A is more common in the *Bay of Fundy all* category and haplotypes B and C are more common in the all other category. Haplotype D one again represents a large percentage of both distributions and haplotype E was not included in this extrapolation since it was only identified in adult males that had no known mother-calf relationships.

Chapter one also identified a group of reproductive females that did not use the Bay of Fundy nursery for all of their offspring. This group, the *Bay of Fundy some* category in subsequent analyses was included into the all other category since it did not show strict site fidelity to the Bay of Fundy. The movement between nursery areas by the *Bay of Fundy some* category was explained to be a result of habitat disturbances and extensive whaling that disrupted the mechanism by which site fidelity was established such as social facilitation or learned behaviours. Therefore, originally these individuals may have shown site fidelity to one nursery area. As table 2.5a has shown, the haplotype frequencies exhibited by the *Bay of Fundy some* category show similarities to the *Bay of Fundy all* and *Bay of Fundy none* categories. The *Bay of Fundy some* category also shows no significant structuring when compared to the *Bay of Fundy none* category. Therefore, these individuals may be a subset of both the *Bay of Fundy all* and *Bay of Fundy none* categories.

The last seventeen years of field surveillance data (1980-1996) has provided researchers with an immense amount of detailed information about individual North Atlantic right whales. The lengthy period over which this data was obtained allows

researchers to examine trends and fluctuations in the distribution of this species. One such trend is the increase of individuals sightings in the Bay of Fundy over the last four years 1992-1996. The average number of individuals identified per year in the last four years has been 168 individuals per year. This rate is 2.5 times higher than the previous four years 1989-1992 when the rate was 66 individuals per year. Based on personnel communications with M. Brown, it was confirmed that sighting effort has been consistent over the 17 year period and due to the large influx of individuals in the last four years, efficiency may have even slightly decreased. M. Brown also confirmed that the number of individuals sighted in Roseway Basin off the southern Scotian shelf has decreased in the last four years. It is highly likely that these individuals have moved into the Bay of Fundy for summer/fall feeding. The basis of this move is unknown but may be correlated with food availability of disturbances in the previously used habitat area. This movement can be confirmed by the higher ratio of males to females (ranging from 1.4-1.7) in the Bay of Fundy over the last four years since Roseway Basin was predominantly used by adult and juvenile males. Analysis of the individuals sighted in the Bay of Fundy over the last five years shows only two *Bay of Fundy none* females; NEA 1430 and 1812 in this area. These females were sighted in three consecutive non-calving years, therefore they still belong to the *Bay of Fundy none* category. Despite the sighting of two *Bay of Fundy none* mothers another 14 extant mothers of the *Bay of Fundy none* category have not been sighted in the Bay of Fundy. This suggests that these females still show strong

site fidelity to an alternative nursery area even in non-calving years despite the recent influx of individuals to the Bay of Fundy.

Currently, genetic research continues on the North Atlantic right whale to identify the sex and control region haplotype of all new samples to the right whale database. All samples are being used in a genealogy study to establish paternity through microsatellite or STR (short tandem repeat) analysis (Waldick pers. comm.). This study will also determine the effective population size and genetic structuring based on nuclear analyses.

Recommendations for future studies

1. Identification of the location of the additional nursery area or areas is essential to the understanding of the population distribution and habitat assessment for the North Atlantic right whale. A list of females that will lead to this area have been identified for satellite tagging. The combination of aerial surveys with the satellite tags will increase the effectiveness of one tag if the tagged mother-calf pair are not alone in their migration.
2. Expanded biopsy darting efforts in the Great South Channel or southeastern United States are necessary to collect missing individuals from the North Atlantic right whale database. These individuals will be used to assess full haplotypic diversity and provide samples for parentage analysis.
3. Seventeen years of field surveillance data has provided researchers a unique opportunity to trace the fluctuations and trends in the distributions and movements of

the North Atlantic right whale. Continued field surveillance in all major habitat areas will allow researchers to study the correlations between habitat and distribution as well as to analyze the population for the transmission of site fidelity.

4. Collaborative studies using historical samples will provide information about the degree of haplotypic diversity that has been lost through the whaling bottleneck experienced by the North Atlantic right whale.
5. Further analyses of the South Atlantic right whale will also provide a context in which the genetic structure exhibited by Peninsula Valdes samples will be revealed.
6. Future research to identify all critical habitat areas such as breeding, calving and nursery areas is essential in order to assess the individual habitats for potentially lethal threats.

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APPENDIX ONE

Pedigrees have been drawn for each matriline identified and analyzed. It is very important to note that due to the limitations of the computer package (Cyrillic) used to draw these pedigrees, each reproductive female is mated to the same male for all offspring which in reality is unlikely. No information is known about the parental component of any of the offspring. Currently, a pedigree analysis study is being conducted which will identify the reproductive males and the mechanism of mating i.e. monogamy, polygamy etc. (Waldick pers. comm.)

Under each individual is the NEA (New England Aquarium) number that is used for field identification, the Egl number given to the lab sample and the haplotype which was determined through control region sequence analysis and SSCP analysis. No inferred haplotypes are included in this appendix. It should be noted that within the *Bay of Fundy some* category, before the haplotype identification there is either 'other' or 'BoF' printed. This refers to the nursery area that this individuals was taken to. 'BoF' corresponds to the calf being brought to the Bay of Fundy while 'other' corresponds to the calf not being brought to the Bay of Fundy.

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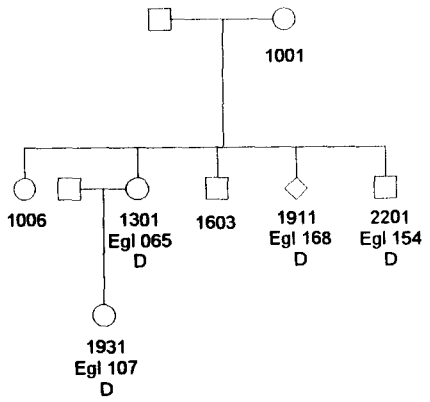

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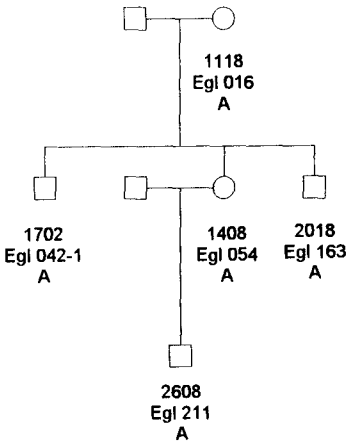

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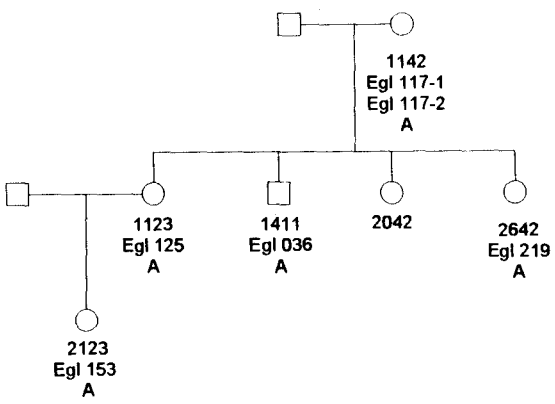

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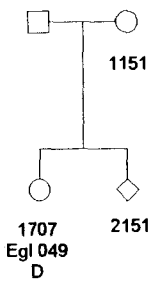

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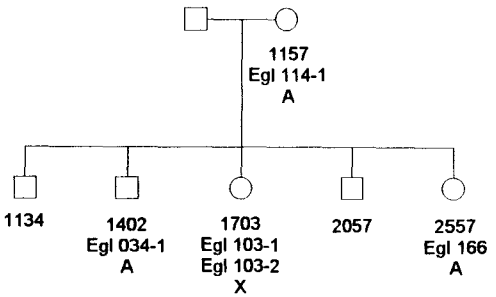

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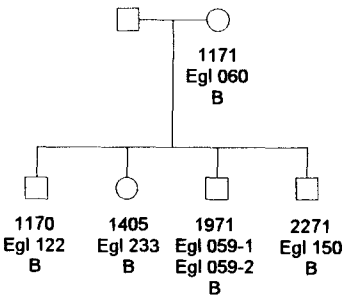

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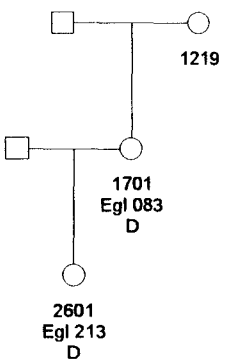

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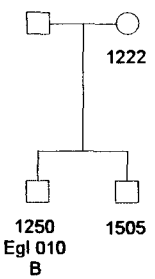

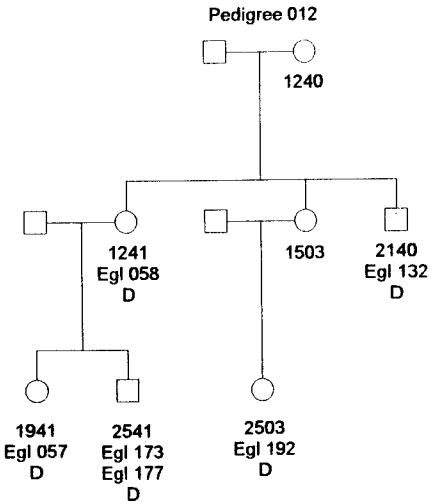
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Egl 058
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D
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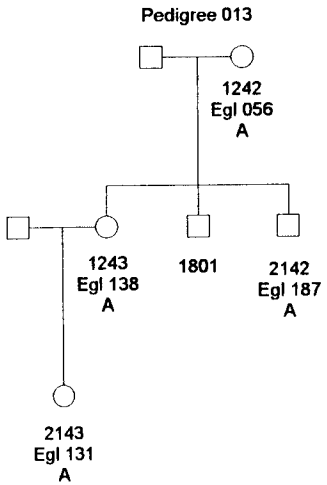
D
2503
Egl 192
D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2142
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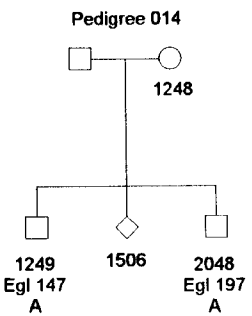
2143
Egl 131
A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1506
2048
Egl 197
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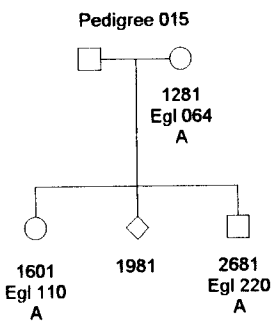
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1981
2681
Egl 220
A

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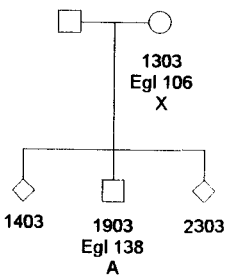

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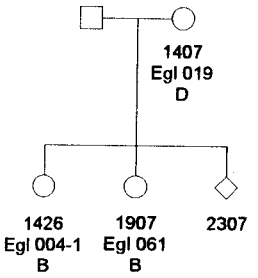

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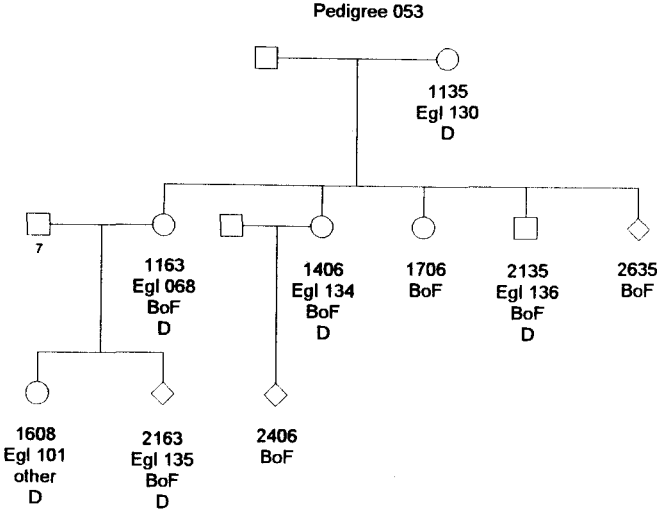

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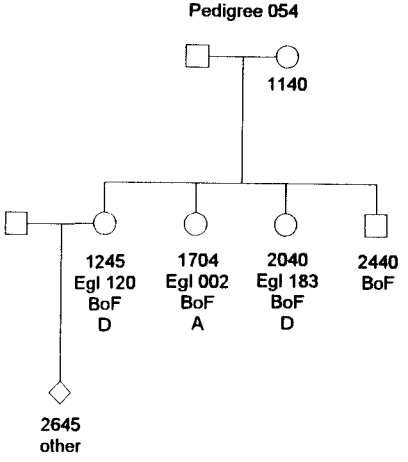

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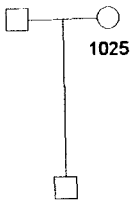

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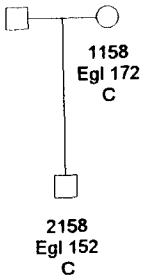

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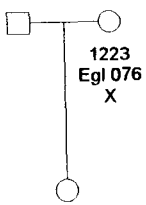

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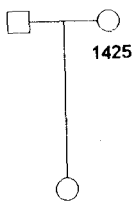

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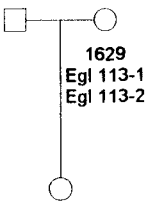

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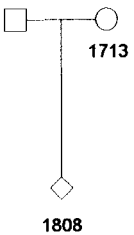

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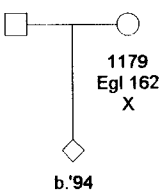

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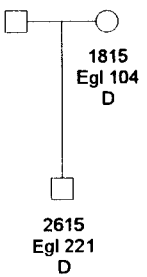

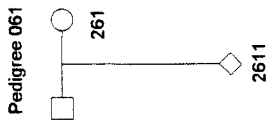
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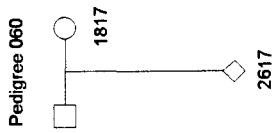
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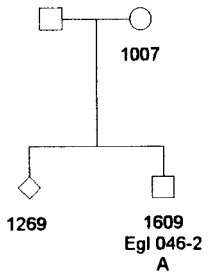
Bay of Fundy none

Pedigree 021



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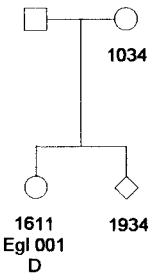

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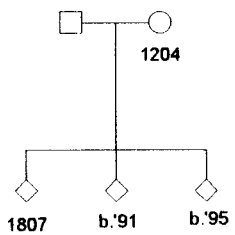

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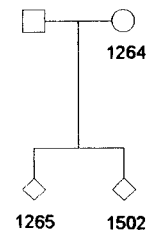

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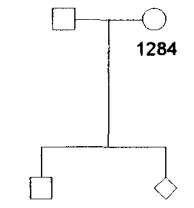

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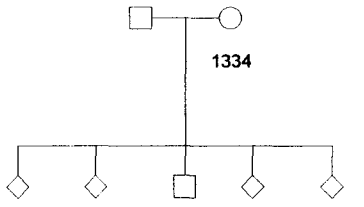

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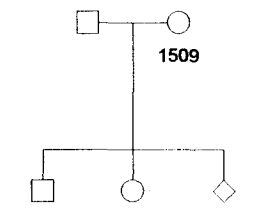

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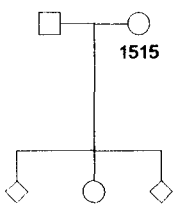

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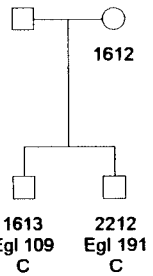

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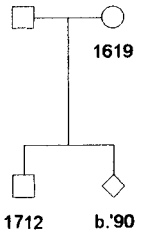

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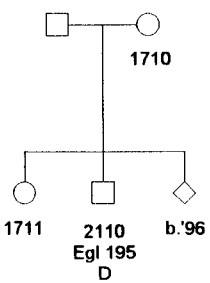

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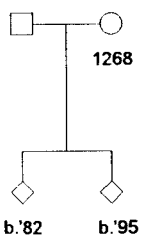

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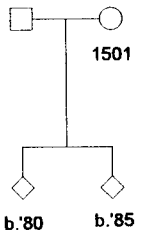

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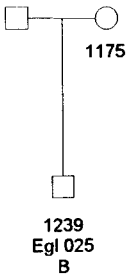

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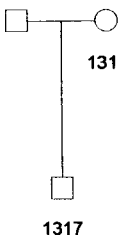
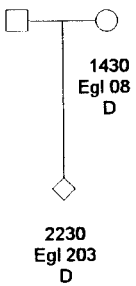
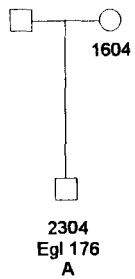


Pedigee 029



Pedigree 032



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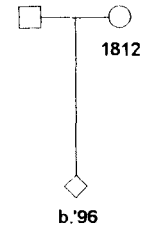
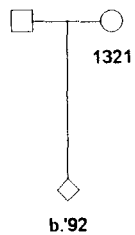


Pedigree 063



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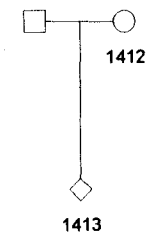

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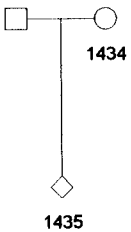

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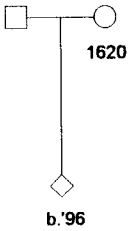

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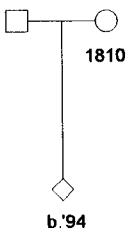

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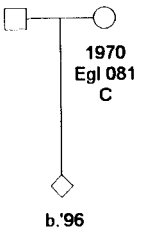
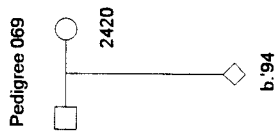
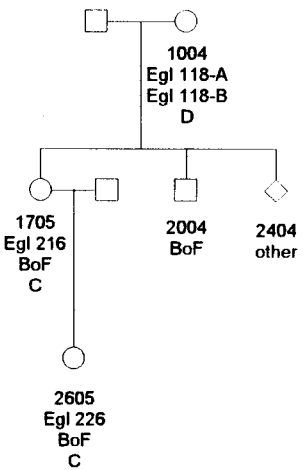



Pedigree 069
2420
b.'94

Pedigree 036



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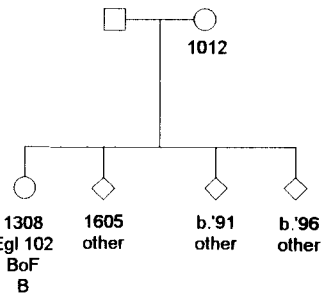
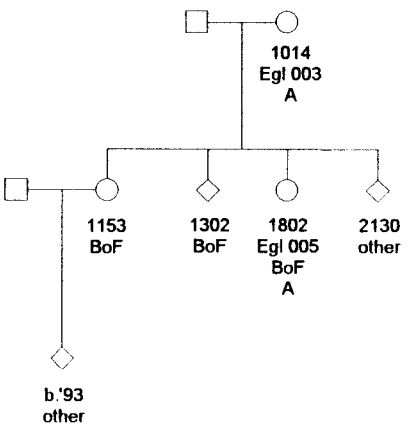
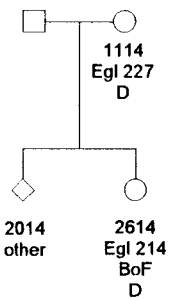


Pedigree 038



Pedigree 039



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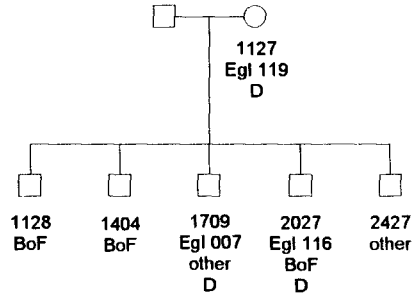
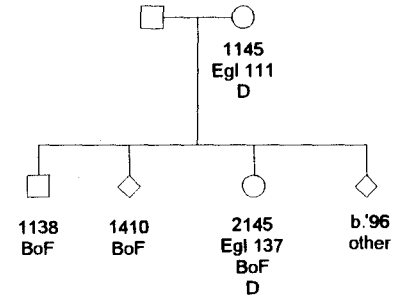
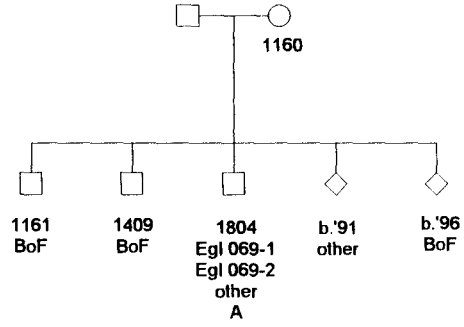


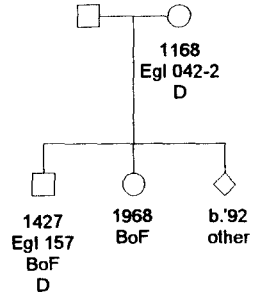
Pedigree 041



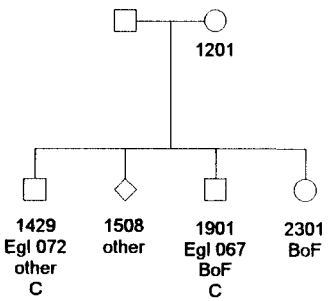
Pedigree 042



Pedigree 043



Pedigree 044



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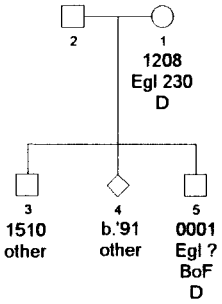
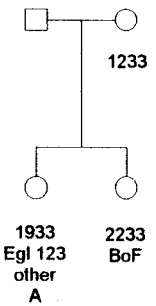


Pedigree 046



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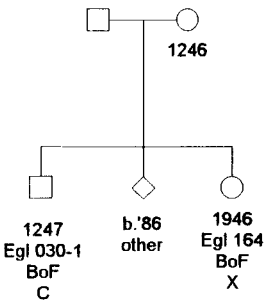
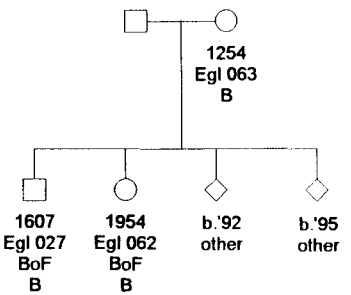


Pedigree 048



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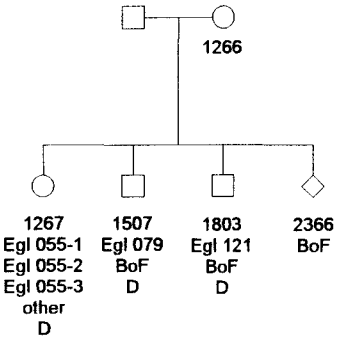
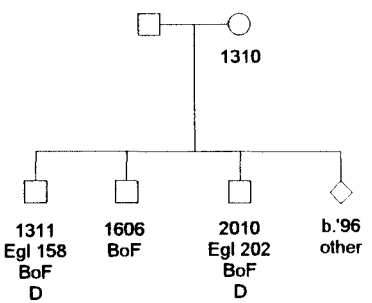


Pedigree 050



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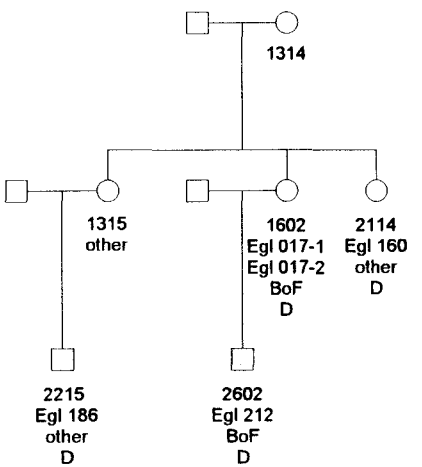
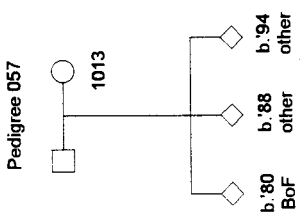
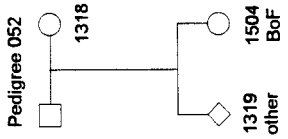



Pedigree 057
1013
b.'80
BoF
b.'88
other
b.'94
other


Pedigree 052
1318
1319
other
1504
BoF

APPENDIX TWO

This spreadsheet includes the sex, NEA (New England Aquarium) number, Egl code from the laboratory, control region haplotype and RFLP haplotype (Schaeff 1993) of each individual analyzed in this thesis. Control region haplotypes of individuals for which there was no sample were inferred through matrilineal relationships. The control region haplotypes for these individuals are *italicized*. Some control region haplotypes have been identified by an X. These are samples where the DNA did not amplify and no matrilineal relationships were known.

	A	B	C	D	E
1	Sex	NEA	Egl	Haplotype	RFLP
2	F	1611	001	D	2
3	F	1704	002	A	2
4	F	1014	003	A	1
5	F	1426	004	B	1
6	F	1802	005-2	A	1
7	F	1168	006	A	1
8	M	1709	007	D	2
9	M	1270	008	B	1
10	M	1250	010	B	
11	M	1331	011	A	1
12	M	1625	012	A	1
13	F	1027	013-1	D	2
14	M	1238	014	D	2
15	M	1130	015	A	1
16	F	1118	016	A	1
17	F	1602	017-2	D	2
18	M	1803	018-2	D	2
19	F	1407	019-2	D	1
20	M	1510	020	A	1
21	M	1708	021	D	2
22	M	1283	022-2	D	2
23	M	1333	024	D	2
24	M	1239	025	B	
25	M	1607	026	B	1
26	M	1607	027	B	
27	M	1424	028-2	C	3
28	M	1247	030-2	C	3
29	M	1610	031-2	B	1
30	M	1276	032	D	2
31	M	1402	034-1	A	1
32	M	1709	035	D	
33	M	1411	036	A	1
34	M	1155	037	A	1
35	M	1048	039	C	1
36	M	1702	041-1	A	
37	F	1168	042-2	D	
38	M	1609	046-2	A	1
39	M	1032	047-2	D	2

	A	B	C	D	E
40	M	1306	048	A	1
41	F	1707	049	D	2
42	M	1170	052	B	1
43			053-1	D	
44	F	1408	054	A	1
45	F	1267	055-1	D	2
46	F	1242	056	A	1
47	F	1941	057	D	
48	F	1241	058	D	2
49	M	1971	059-1	B	1
50	F	1171	060	B	1
51	F	1907	061	B	1
52	F	1954	062	B	1
53	F	1254	063	B	1
54	F	1281	064	A	1
55	F	1301	065	D	2
56	M	1401	066	C	3
57	M	1901	067	C	
58	F	1163	068	D	2
59	M	1804	069-1	A	1
60	M	1613	070	C	3
61	M	1323	071	A	1
62	M	1429	072	C	3
63	M	1166	073-1	X	2
64	M	1422	074	C	3
65	M	1149	075	A	1
66	F	1223	076	*A*	1
67	M	1150	077	C	3
68	M	1428	078-2	D	1
69	M	1507	079	D	2
70	F	1950	080	A	
71	F	1970	081	C	
72	M	1174	082	X	2
73	F	1701	083	D	2
74	M	1155	085	B	
75	M	1627	086	X	1
76	F	1430	087	D	2
77	M	1226	088	D	2
78	M	1207	089	A	1

	A	B	C	D	E
79	M	1238	090	D	
80	M	1126	091	E	1
81	M	0	092	D	
82	M	1036	093	X	2
83	M	1113	094	D	2
84	M	1514	095	X	3
85	M	1052	096	X	
86	M	1203	097	A	1
87	M	1510	098	A	
88	M	1813	099	C	1
89	M	1112	100	A	3
90	F	1608	101	*D*	2
91	F	1308	102	B	1
92	F	1703	103-1	*A*	1
93	F	1815	104	D	2
94	F	1123	105	A	
95	F	1303	106	*A*	1
96	F	1931	107	D	
97	M	1147	108	C	2
98	M	1613	109	C	
99	F	1601	110	A	1
100	F	1145	111	D	2
101	F	2029	112	D	
102	F	1157	114-1	A	
103	M	1152	115-2	D	2
104	M	2027	116	D	
105	F	1142	117-2	A	
106	F	1004	118	D	
107	F	1127	119	D	2
108	F	1245	120	D	2
109	M	1803	121	D	
110	M	1170	122	B	
111	F	1933	123	A	
112	M	1429	124	C	
113	F	1123	125	A	1
114		0	126	X	
115	F	0	127	B	
116	F	1135	130	D	2
117	F	2143	131	A	

	A	B	C	D	E
118	M	2140	132	D	
119	F	1243	133	A	1
120	F	1406	134	D	2
121	U	2163	135	D	
122	M	2135	136	D	
123	F	2145	137	D	
124	M	1903	138	A	
125	M	1930	139	C	
126	M	1271	141	A	
127	M	1327	142	E	
128	M	1017	143	C	
129	M	1320	144	D	
130	M	1176	145	C	
131	M	1805	146	D	
132	M	1249	147	A	
133	F	1806	148	D	
134	F	2223	149	A	
135	M	2271	150	B	
136	M	1121	151	D	
137	M	2158	152	X	
138	F	2123	153	A	
139	M	2201	154	D	
140	M	1516	155	D	
141	M	1818	156	A	
142	M	1427	157	D	1
143	M	1311	158	D	
144	M	1920	159	B	
145	F	2114	160	D	
146	M	1307	161	D	
147	F	1179	162	X	
148	M	2018	163	A	
149	F	1946	164	C	3
150	F	2120	165	C	
151	F	2557	166	A	
152	M	1102	167	D	
153	U	1911	168	D	
154	F	2450	169	D	
155	M	1041	170	E	
156	M	1623	171	C	

	A	B	C	D	E
157	F	1158	172	C	
158	M	2541	173	D	
159	M	1021	174	D	
160	M	1019	175	D	
161	M	2304	176	A	
162	M	2541	177	D	
163	F	2030	178	A	
164	F	2050	179	C	
165	M	1102	180	D	
166	M	1156	181	D	
167	F	2120	182	C	
168	F	2040	183	D	2
169	M	1203	184	A	
170	M	1819	185	C	
171	M	2215	186	D	
172	M	2142	187	A	
173	M	1170	188	B	
174	F	2450	189	D	
175	F	1909	190	B	1
176	M	2212	191	C	
177	F	2503	192	D	
178	M	1050	193	B	
179	M	1624	194	D	
180	M	2110	195	D	
181	M	1960	196	A	
182	M	2048	197	A	
183	F	2320	198	B	
184	M	2310	199	B	
185	F	2460	200	A	
186	F	2430	201	B	
187	M	2010	202	D	
188	F	2230	203	D	
189	M	1167	204	C	
190	M	2410	205	A	
191	F	2425	206	D	
192	M	2340	207	A	
193	M	1026	208	A	
194	F	1045	209	D	
195	M	1033	210	D	

	A	B	C	D	E
196	M	2608	211	A	
197	M	2602	212	D	
198	F	2601	213	D	
199	F	2614	214	D	
200	F	2642	215	A	
201	F	1705	216	C	
202	F	2029	217	D	
203	M	1716	218	C	
204	F	2642	219	A	
205	M	2681	220	A	
206	M	2615	221	D	
207	F	1611	222	D	
208	M	2158	223	C	
209	M	2304	224	A	
210	F	2460	225	A	
211	F	2605	226	C	
212	F	1114	227	D	
213	M	1616	228	D	
214	M	1122	229	C	
215	F	1208	230	D	1
216	M	0	231	D	
217	M	2427	232	D	
218	F	1405	233	B	1
219	F	2430	234	B	
220	M	1424	235	C	
221	M	1272	236	D	
222		1603		*D*	
223	F	1001		*D*	
224		2042		*A*	
225	F	1151		*D*	
226		2151		*D*	
227		1134		*A*	
228	F	1219		*D*	
229	F	1222		*B*	
230		1505		*B*	
231	F	1240		*D*	
232	F	1503		*D*	
233		1801		*A*	
234	F	1248		*A*	

	A	B	C	D	E
235		1506		*A*	
236		1981		*A*	
237		1403		*A*	
238		2303		*A*	
239		2307		*B*	
240		1706		*D*	
241		2406		*D*	
242	F	1140		*D*	
243		2645		*D*	
244		2440		*D*	
245	F	1007		*A*	
246		1269		*A*	
247	F	1034		*D*	
248		1934		*D*	
249	F	1284		*D*	
250	F	1334		*B*	
251	F	1509		*B*	
252		2209		*B*	
253	F	1515		*D*	
254	F	1612		*C*	
255	F	1710		*D*	
256		1711		*D*	
257	F	1012		*B*	
258		1605		*B*	
259		1153		*A*	
260		1302		*A*	
261		2130		*A*	
262		2014		*D*	
263		1128		*D*	
264		1404		*D*	
265		1138		*D*	
266		1410		*D*	
267	F	1160		*A*	
268		1161		*A*	
269		1409		*A*	
270		1968		*D*	
271	F	1201		*C*	
272		1508		*C*	
273		2301		*C*	

	A	B	C	D	E
274	F	1233		*A*	
275		2233		*A*	
276	F	1246		*C*	
277	F	1314		*D*	
278	F	1315		*D*	
279	F	1310		*D*	
280	F	1266		*D*	
281		2366		*D*	
282	F	1025		*A*	
283	F	1425		*D*	
284	F	1629		*D*	
285	F	1175		*B*	
286	F	1604		*A*	