GENETICS, PHYLOGENY, AND BIOGEOGRAPHY OF THE MARTEN *Martes americana*

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GENETICS, PHYLOGENY, AND BIOGEOGRAPHY OF THE MARTEN *Martes americana*

by

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ABSTRACT

The purpose of this study was to examine the genetic variation within a small reintroduced population of American pine marten (*Martes americana*) in Terra Nova National Park, Newfoundland, as well as the variation and diversity within and among other North American pine marten and closely related species. DNA sequencing of several hundred base pairs of the 5' most end of the cytochrome *b* gene of the mitochonrdial DNA was completed and the sequences analysed.

It was determined that a reintroduced population and the source population shared identical DNA, based on 307 base pairs of data. A 401 base pair data base was compiled from samples of 12 subspecies of American pine marten (*Martes americana*) as well as European pine marten (*M. martes*), sable (*M. zibellina*), and American badger (*Taxidea taxus*). Genetic diversity was detected between certain subspecies of *Martes americana* as well as between all species of *Martes* studied. Two distinct lineages of *Martes americana* are apparent in North America ["*americana*" and "*caurina*" groups] whose pairwise sequence divergence is 1.5%. The two genetic groups correspond to the two former North American pine marten species *Martes caurina* and *Martes americana*. The average nucleon diversity (h) within the "*americana*" group is 0.22 and within the "*caurina*" group is 0.72.

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1.0 INTRODUCTION

The field of conservation biology has increasingly made use of genetic data in an attempt to answer elusive questions regarding wildlife species and population variability, diversity, and phylogeny (Allendorf *et al.* 1979, Avise *et al.* 1987, 1992, Bonnell and Selander 1974, Bowen *et al.* 1992, Brower 1996, Burger *et al.* 1996, Carr and Hughes 1993, Grakov 1994, Irwin *et al.* 1991, Kocher *et al.* 1989, Luikart and Allendorf 1996, McDermid *et al.* 1972, McKnight 1995, Mitton and Raphael 1990, O'Brien *et al.* 1986, Ohland *et al.* 1995, Perry *et al.* 1995, Seutin *et al.* 1995, Taylor *et al.* 1996, Vrana *et al.* 1994, Zink 1996). The concept of genetic variability and the negative effects of animal inbreeding (reproduction in closely related animals) is at least as old as the Bible. It is only in much more recent times that it has become possible to quantify genetic variation (O'Brien *et al.* 1983). Direct information of this type makes it possible to measure and manage such things as inbreeding effects in wildlife populations. Traditionally, morphological studies have been used to differentiate among, and classify species and intraspecific groups. Genetic techniques have become indispensable for answering historically difficult taxonomic questions such as the classification of the giant panda (*Ailuropoda melanoleuca*) (O'Brien *et al.* 1985). These same types of techniques are applicable to a wide array of organisms and problems.

The cytochrome *b* gene in mitochondrial DNA (mtDNA) has been used extensively to detect population level variation in several wildlife species (Carr and Marshall 1991, Collura and Stewart 1995, Graybeal 1993, Hardy *et al.* 1995, Honeycutt *et al.* 1995, Hosoda *et al.* in press, McKnight 1995, O'Reilly *et al.* 1993, Smith and Patton 1991). This molecule was therefore chosen to assess the level of variation of a small reintroduced population of American pine marten (*Martes americana*) in Terra Nova National Park on the island of Newfoundland, relative to its source population. It was hypothesized that a reduction in genetic variation had occurred in the new population as it was founded by a small number of individuals (eight) and the population has remained small in subsequent years (Hicks 1990, Simpson 1991). Several studies have cited "population bottlenecks" as a possible cause of low genetic variation, particularly in carnivores (Allendorf *et al.* 1979, Lehman *et al.* 1991, McDermid *et al.* 1972, Simonsen 1982). It was felt that if a reduction in genetic variation had occurred in the Terra Nova National Park marten population, it could be quantified with DNA sequencing techniques. If a loss of variation was detected, several wildlife management options could be employed to increase genetic variability in this population.

The aim of the research was to analyse differences in DNA sequences of pine marten native to regions throughout North America in the context of related Palaearctic species. There are two hypotheses to be tested: 1) the small

reintroduced Terra Nova National Park population of pine marten is less genetically variable than the source population from western Newfoundland, and 2) a genetic basis exists for the morphological diversity noted among American pine marten subspecies.

1.1 Analysis of genetic variation

Several genetic studies of Old World members of the genus *Martes* have been conducted. Simonsen (1982) used starch gel electrophoresis techniques and found no detectable genetic variation in two European pine marten (*Martes martes*) or 121 beech marten (*Martes foina*) studied. Hosoda *et al.* (1993) studied restriction site polymorphisms in ribosomal DNA of a single sample from eight species of canids and mustelids, including a single *Martes* species, the Japanese marten (*Martes melampus*). Results pertain primarily to carnivore phylogeny. A recent paper discussing the phylogeny of Japanese Mustelids has published cytochrome *b* sequence from the Japanese marten (*Martes melampus*) and the sable (*Martes zibellina*) (Masuda and Yoshida 1994). No genetic variation was found in the two sable sequences studied and *M. zibellina* and *M. melampus* showed 3.5% sequence differences between them. Hosada *et al.* (in press) have also detected cytochrome *b* sequence diversity among *M. zibellina*, and *M. melampus* as well as *M. flavigula* (yellow-throated marten). Several articles have

been written in Russian on DNA sequencing studies of European marten/sable hybrids known as a kidus (summarized in Grakov 1994). These genetic studies seem to indicate that *Martes* species in this area that lack a throat patch may be hybrids (*M. martes* X *M. zibellina*). This point is still debated.

Genetic research on the American marten includes a study by Mitton and Raphael (1990) who used starch gel electrophoresis techniques and reported a relatively high average heterozygosity in 10 Wyoming pine marten (*M. americana*). McGowan and Davidson (1994) used randomly amplified polymorphic DNA (RAPD) to assess the level of genetic variation in 23 Newfoundland pine marten and found that genetic variability was very low.

1.2 Mitochondrial DNA

Mitochondrial DNA (mtDNA) is a maternally-inherited circular molecule that is approximately 16-20 kilobases long in vertebrates. Mammalian mtDNA codes for 37 genes and also contains a noncoding region called the D-loop (Avise *et al.* 1987).

Mitochondrial DNA has been shown to evolve more rapidly than nuclear DNA (Vawter and Brown 1986) and typically shows a high level of intraspecific

polymorphisms (Avise *et al.* 1987). This allows mtDNA sequence variation to be detected in closely related species, subspecies, or populations. MtDNA is maternally inherited, therefore no recombination occurs. This simplifies the analysis of variation. These are the primary reasons mtDNA has been used extensively in population genetics and phylogenetic studies of wildlife (Avise *et al.* 1987, Carr and Marshall 1991, Kocher *et al.* 1989, Moritz *et al.* 1987, Wilson *et al.* 1985).

The cytochrome *b* gene codes for a protein component of complex III of the mitochondrial oxidative phosphorylation system (Hatefi 1985). This gene is approximately 1140 base pairs in length, and published sequence data from a large number of species are available for comparison e.g., Avise *et al.* (1987, 1992), Bermingham *et al.* (1986), Carr and Hughes (1993), Carr and Marshall (1991), Collura and Stewart (1995), Graybeal (1993), Hardy *et al.* (1995), Honeycutt *et al.* (1995), Hosada et al. (in press), Irwin *et al.* (1991), Kocher *et al.* (1989), Masuda and Yoshida (1994), McKnight (1995), Moore (1995), Moritz *et al.* (1987), O'Reilly *et al.* (1993), Smith and Patton (1991), Vawter and Brown (1986), Vrana *et al.* (1994), and Wilson *et al.* (1985).

1.3 Phylogeny, classification, and distribution of *Martes*

The genus *Martes* is comprised of three subgenera: *Pekania*, *Charronia*, and *Martes*. The subgenus *Pekania* contains a single species *M. pennanti*, the fisher of North America. The second subgenus is *Charronia* represented by two Asian species *M. flavigula*, the yellow-throated marten, and *M. gwatkinsi*, the Nilgiri marten. The third subgenus is *Martes*, the true martens. All extant species of true marten are thought to have descended from the extinct *M. vetus*. This Holarctic subgenus includes *M. americana* (American marten), *M. foina* (beech or stone marten), *M. martes* (European pine marten), *M. zibellina* (sable), and *M. melampus* (Japanese marten) (Nowak 1991), the last four of which are extremely similar in morphology and behavior. The ranges of these four Holarctic species are primarily allopatric. Because of their allopatric distribution, and similarity of morphological/behaviourial traits, Anderson (1970), and Hagmeier (1955, 1961), have suggested that they are closely related and possibly conspecific. Anderson (1970) suggests that these four species may be considered a superspecies. The American pine marten is thought to be a direct descendent of Eurasian *Martes* stock, which first reached North America from Asia initially 65,000 to 122,000 years ago (Anderson 1994).

The American pine marten was first described by Turton in 1806 as *Mustela americana*. The marten inhabiting the island of Newfoundland were originally referred to as a separate species *Mustela atrata* (Bangs 1897) and were described as being about the size of *Mustela americana*, but slightly larger, and considerably darker in colour. More recently, all marten in North America have been described as subspecies of a single species, *Martes americana* (Banfield 1974, Hall 1981). A number of historic classification schemes have been proposed for subspecies of this species (summarized in Hagmeier 1955). Presently fourteen subspecies of a single species *M. americana* are recognized (Hall 1981), while Hagmeier (1961) recognized six subspecies of the single species. In this study, Hall's (1981) subspecies names and ranges are used (see Table 1). Seven subspecies (*M. a. nesophila, M. a. caurina, M. a. vancouverensis, M. a. vulpina, M. a. origenes,* M. a. *humboldtensis*, and *M. a. sierrae),* inhabiting the British Columbia and Pacific Northwest coast, the Great Plains, and California, were once considered a distinct species, *Martes caurina*, from *Martes americana* (represented today by the subspecies *M. a. atrata, M. a. brumalis, M. a. americana, M. a. abieticola, M. a. abietinoides, M. a. actuosa, M. a. kenaiensis*), which inhabited eastern, central, northern and western North America (Merriam 1890, Rhoads 1902). Wright (1953) studied the morphology of marten from the zone of contact (British Columbia, Montana, and Idaho) between these two historically recognized species. Wright concluded that hybridization had occurred between *M. americana*

Table 1 The names and approximate geographic range of the fourteen subspecies of *Martes americana* (from Hall 1981).

Subspecies	**Geographic Range**
M. a. atrata	Newfoundland
M. a. brumalis	Labrador, Northern Quebec
M. a. americana	Eastern Canada excluding above
M. a. abieticola	Northern Manitoba, Saskatchewan
M. a. actuosa	Northwestern North America
M. a. kenaiensis	Kenai Peninsula, Alaska
M. a. abietinoides	Southeastern, Central British Columbia
M. a. caurina	Southwest British Columbia
M. a. vancouverensis	Vancouver Island, British Columbia
M. a. nesophila	Queen Charlotte Islands, British Columbia
M. a. origenes	Wyoming, Colorado
M. a. vulpina	Idaho, Montana
M. a. sierrae	Northeastern California
M. a. humboldtensis	Northwestern California

and *M. caurina* marten, based on intergradation of morphological characteristics noted in one region of Montana. Therefore, based on a strict interpretation of a "biological species" (Mayr 1969) he considered each of the "*caurina*" subspecies to be a subspecies of the single species *Martes americana* (Wright 1953). Considerable morphological differences exist between these two groups of subspecies. Anderson (1970) thought the "*caurina*" group of subspecies to be more closely related to Palearctic *Martes*, with which they show more dental and cranial similarities (Hagmeier 1961).

1.4 Characteristics of the American marten

The pine marten or American marten (*Martes americana*) is a solitary mustelid carnivore. It inhabits mature coniferous and mixed forests throughout North America where it feeds mainly upon small mammals, although carrion, birds, insects, and fruits are consumed. Home range size has been reported to be from 1-20 km^2 with no intrasexual territorial overlap (Buskirk 1983, Clark *et al.* 1987, and Soutiere 1979).

A litter of one to four young is born in late March or April. Reproduction normally occurs at three years of age. Adult male marten are reported to weigh between 700 and 1600 grams with the males being approximately 15% longer and up to 65% heavier than females (Banfield 1974, Burt and Grossenheider 1976). Marten are inquisitive and often easily trapped. This fact, coupled with their relatively high pelt value, has made the marten a favourite of trappers (Clark *et al.*, 1987), and resulted in marten populations being reduced or eliminated in many parts of their range. The loss of mature forest habitat may also play a role in their decline (Banfield 1974, Clark *et al.* 1987, and Strickland *et al.* 1982). The problem of habitat loss, fragmentation, and low population numbers not only affect the Newfoundland pine marten (Thompson 1991), but many wildlife species throughout the world (Wilson 1992).

Pine marten population numbers on the island of Newfoundland have been low for many years. As early as 1934, a complete ban on trapping marten was instituted in Newfoundland due to a decrease in numbers. It is believed that the population has been in decline since that time (Bergerud 1969). Two recent population estimates, one in 1980-1983 and one in 1988, resulted in total population estimates of 630-875 and 150 respectively (Bissonette *et al.* 1988, and Snyder and Handcock 1985).

Continued accidental trapping/snaring, habitat loss, as well as a widespread disease outbreak has put severe pressure on this small population (Bissonette *et al.* 1988, WERAC 1991). In fact, Thompson (1991) has predicted the probable extinction of the Newfoundland pine marten within the next 50 years, based upon unfavourable forest demographics alone. The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) considers the population of pine marten of the island of Newfoundland to be threatened (COSEWIC 1986).

Pine marten were native to the area of Terra Nova National Park, Newfoundland, but were believed to have been extirpated from eastern Newfoundland (Bergerud 1969, Skinner 1979, Snyder 1985). A co-operative effort between the Newfoundland Wildlife Division, the Canadian Wildlife Service, and Parks Canada was initiated to re-introduce the pine marten to Terra Nova National Park. During 1982 and 1983 eight pine marten (three females, five males) were relocated from western Newfoundland to Terra Nova National Park in eastern Newfoundland during 1982 and 1983 (Bateman 1985). Because such a small founder population was utilized for the reintroduction of pine marten to Terra Nova National Park, the potential for inbreeding and genetic drift must be considered. Inbreeding can result in the loss of genetic variation and the expression of rare

recessive alleles. Ralls and Ballou (1983) present a convincing case as to the negative effects inbreeding can have on wildlife populations. They feel inbreeding (as one would expect in this small population) may cause "decreased fertility, increased juvenile mortality and general lack of vigour".

The taxonomic status of a species or subspecies can affect the priority assigned to the conservation of a given taxon (Cohn 1990). For example, the existence of a distinct Newfoundland marten subspecies is in question (Hagmeier 1961; and Hall 1981). Generally, if this population were shown to be genetically distinct from the marten found in the adjacent province of Labrador, a much greater emphasis might be placed on their preservation.

The purpose of this research was to assess intrasubspecific and intersubspecific differences in the DNA sequence of a portion of the cytochrome *b* gene in species of the subgenus *Martes*. Variation in the level of genetic variation within populations of *Martes americana atrata* would allow a determination of loss of variation within a small reintroduced population to be made. An assessment of intersubspecific genetic diversity within *Martes americana* would determine if a genetic basis for subspecies designations can be found in the cytochrome *b* and may provide insight into biogeographic influences and phylogenetic relationships. The study of diversity among other *Martes* species was also completed to allow

Nearctic results to be placed in a broader context. Mitochondrial DNA variation was examined in *Martes* from the population level to the species level in an effort to answer questions on loss of variability, phylogeny, classification, and biogeographic influences on marten.

2.0 MATERIALS AND METHODS

2.1 Tissue source and collection

Marten tissue samples (epithelial cheek cell scrapings and plucked hairs) were obtained for DNA analysis from live trapped animals handled by Terra Nova National Park Wardens. The Newfoundland and Labrador Wildlife Division provided liver tissue from accidentally killed marten from the western portion of the island as well as harvested animals from Labrador. Individuals from wildlife agencies, museums, universities, and furriers provided additional tissue samples (muscle tissue, liver, skin, and hair). Fresh tissue from suppliers was frozen and shipped via courier in an attempt to minimize tissue degradation. The identification number and geographic origin of each sample was logged in a catalogue.

Initial sampling was carried out in eastern Canada. Subsequently every effort was then made to obtain multiple samples from a broad range of North American populations/subspecies. This sampling strategy followed the geographic pattern of subspecies ranges as given by Hall (1981). In addition other species of mustelids were obtained from wildlife researchers, furriers, and personally collected from a road-killed animal (*M. martes*, *M. zibellina*, and *Taxidea taxus*).

2.2 Mitochondrial DNA isolation

Mitochondrial DNA was isolated from whole blood, liver, hair roots, skin, or muscle tissue using an acid guanidinium thiocyanate-phenol-chloroform DNA extraction technique adapted from Chomozynski and Sacchi (1987). Tissue samples (80-200mg) were placed in a 1.5 ml Eppendorf tubes and 400 uL of guanidinium buffer added (250 g guanidinium thiocyanate (Sigma) plus 293 mL water, 17.6 mL 0.75 M sodium citrate pH 7.0, and 26.4 mL 10% sarcosyl - stock, 0.36 mL 2-mercaptoethanol/50 mL stock added). The tissue was then physically disintegrated with a plastic homogenizer. Sixty (60) uL of 2 M sodium acetate was added and the solution vortexed, followed by 400 uL of phenol (saturated with 0.1 M Tris pH 8.0) and vortexed. Next, 160 uL of a 24:1 solution of chloroform:isoamyl alcohol was added and vortexed. This solution was incubated on ice for 15 minutes. The tubes were then shaken and centrifuged at 10,000 xg, 4° C, for 20 minutes in a Tomy MTX 150 tabletop centrifuge. The top aqueous phase was retained and 500 uL of isopropanol added, vortexed, and placed in the freezer for 1 hour to several days (normally overnight). Upon removal from the freezer the tubes were centrifuged at 18,000 xg, 4° C, for 15 minutes. The fluid was then poured off and 1 mL of ethanol added and centrifuged as above. A second extraction was carried out with 500 uL of chloroform:isoamyl alcohol. The

supernatant was removed and the precipitated DNA pellet dried in a speed vac. One hundred uL of 10 uM Tris pH 7.4 was used to resuspend the DNA.

Preserved skin tissues (1 cm^2) were lyophilized (freeze dried) and manually ground in liquid nitrogen before the extraction procedure to facilitate tissue break up and subsequent DNA extraction.

2.3 Cytochrome *b* amplification

The particular DNA fragment under study was amplified via the polymerase chain reaction (PCR). PCR is a means of enzymatically amplifying entire genes or gene sequences (Saiki *et al.* 1988).

Symmetric PCR amplification was carried out following the techniques described in Carr and Marshall (1991). Two pairs of amplification and sequencing oligonucleotide primers were utilized in this study. The first set of primers amplifies a 359 base pair (bp) region of the mitochondrial cytochrome *b* molecule (Kocher *et al.* 1989). Both primers are 26 bases in length, resulting in 307 bases being available for analysis. A second 455 base pair fragment which includes the 359 bp region is being used as well. Primers consist of one of the 26mers used in the 359 bp sequence (H15149) and the 28 base pair L14724 primer described in Irwin *et al.* (1991), yielding 401 bases of informative sequence data.

Primers for the 307 bp fragment are;

5'-ccatccaacatctcagcatgatgaaa-3' L14841

5'-gcccctcagaatgatatttgtcctca-3' H15149

Primers for the 401 bp fragment are;

5'-cgaagcttgatatgaaaaaccatcgttg-3' L14724

5'-gcccctcagaatgatatttgtcctca-3' H15149

Primers were obtained from the Milligen-Biosearch oligonucleotide synthesizer located at Memorial University as well as New England Biolabs Limited and Queen's University.

Symetric PCR amplifications were obtained in 25 uL volumes (100 uL reactions were also used with individual volumes increased proportionally). One or 2 uL of the mtDNA extract was combined with 67 mM Tris (pH 8-9), 2 mM $MgCl_2$, 20 uM dATP, dTTP, dGTP, dCTP (Pharmacia), 10 pmol of both the heavy and light strand primers, and 0.6 uL Amplitaq DNA polymerase (Perkin-Elmer Cetus). One drop of mineral oil was placed on top of this mixture to prevent

evaporation. The tubes were then placed in a Perkin Elmer Cetus Thermal Cycler on the following amplification cycle:

95°C 5 minutes - 93°C for 1 minute, 40°C for 1 minute, 55°C for 30 seconds, and 72°C for 2 minutes (35 cycles), followed by 72°C for 10 minutes and a 5°C soak.

PCR products were assayed by combining 4 uL of the amplified product with 1 uL of 5x stop dye and placed in wells along side of a DNA weight standard in a 2% ME agrose gel containing ethidium bromide. A 3% NuSieve agrose gel was also used. The gel was subjected to 50 mA of electric current for approximately 1 hour. DNA fragments were assessed for approximate concentration and purity under 302 nm ultraviolet light.

2.4 Double-stranded DNA desalting

The double stranded DNA product was desalted using Ultrafree-MC membrane filters (Millipore). The amplified product was placed in the tubes with 400 uL of sterile distilled water and centrifuged at 2000 xg for 10 minutes. The filtrate was then discarded and the procedure repeated two more times. After the final desalting procedure the DNA was resuspended in 100uL of sterile distilled water and stored in 0.5 uL microfuge tubes at -20°C.

Magic PCR Preps obtained from Promega Corporation were also used to desalt amplified DNA products. The technique followed the manufacturer's recommendations. The double-stranded product was combined with 100 uL of Direct Purification Buffer and 1 mL of Magic PCR Preps Resin. This mixture is drawn through the Magic Minicolumn and washed with 2 mL 80% isopropanol. The Minicolumn is dried and 50 uL of water added, the column is then centrifuged to elute the bound DNA.

2.5 DNA Sequencing

DNA sequences were determined with a *Taq* DNA polymerase/fluorescent dye-terminator sequencing chemistry on an Applied Biosystems 373A Automated DNA sequencer. A premix was used containing 50 ul of the four Applied Biosystems fluorescent "DyeDeoxy" terminators, 100 ul dNTP stock, composed of 150 uM dGTP, 150 uM dATP, 150 uM dCTP, and 150 uM dTTP from Pharmacia, 200 units of Perkin-Elmer Cetus Amplitaq enzyme in 40 ul storage buffer, and 400ul of Sigma 5X TACS buffer [400mM Tris-HCl (pH = 9), 10 mM $MgCl_2$, and 100 mM $(NH_4)_2SO_4$]. To 7 ul of the premix was added 3.2 pmole of DNA sample, 9 ul H_2O, and 3.2 ul of a 1 uM solution of one of the two primers. One drop of mineral oil was placed over the mixture before being placed in the Perkin-Elmer Cetus thermal cycler on the following cycle sequencing reaction; 98°C for 1

second, 50°C for 15 seconds, and 60°C for 4 minutes, repeated for 25 cycles and then a 4°C soak. The dye-labelled DNA was then precipitated with 2 volumes of isopropanol at 15,000 xg for 15 minutes. The resulting pellet was washed in the centrifuge two or three times with 70 % ethanol and dried in the speedvac. The DNA was resuspended in 5 ul of 5:1 Sigma deionized formamide:50 mM Na_2EDTA. Samples were then loaded into individual well of a 6% polyacrylamide (19:1 BIS), 7M urea gel on an Applied Biosystems 373A Automated DNA Sequencer. Electrophoresis was carried out at 30 W constant power (1200V, 30mA) for 7-10 hours.

Some adjustments were made to the procedure over time such as the use of Sephadex G-50F spin columns to clean up the reactions before sequencing. Prism kits were used for some of the final sequence data. Procedures followed manufacturers recommendations.

Automated sequencing was augmented with a double stranded manual sequencing technique. Specifically, *fmol* sequencing kits were obtained from Promega Corporation. The primers were end labelled with the radioisotope ^{32}P. Extension reactions and sequencing were carried out according to the manufacturers directions.

The labelled DNA was then subjected to 60 watts constant power for both 1.5 and 4 hours on 6% acrylamide gels to enable both ends of the sequence to be clearly read. Gels were affixed to paper, dried, and exposed with photographic film. Sequences were read manually into ESEE.

2.6 Data analysis

DNA sequences were analysed using the SeqEd (Applied Biosystems) and ESEE (Eyeball Sequence Editor) (Cabot 1988) programs. Phylogenetic analysis was carried out with the computer program PAUP (Phlyogenetic Analysis Using Parsimony) (v. 3.1) (Swofford 1993). The most parsimonious tree was identified using the heuristic search algorithm and delayed-character-transformation optimization. A bootstrap analysis (Felsenstein 1985) was used to estimate confidence limits on branches. Statistical analysis of morphological data was completed using the computer programs Excel, SPSS, and Minitab.

3.0 RESULTS

3.1 Cytochrome *b* variation

The research produced two data sets. The first is from the DNA sequence of a 307 bp region of the cytochrome *b* gene of mtDNA. The 307 bp data was obtained from sampling only *Martes americana* populations from eastern Canada. The second data set comprises 401 bases which completely overlaps the 307 bp region. The 401 bp sequences were obtained from *Martes americana* endemic to areas throughout North America and three other mustelids - European pine marten (*Martes martes*), sable (*Martes zibellina*), and American badger (*Taxidea* taxus). Some of the samples from the 307 bp database were used in the 401 bp study.

Variation within a 307 base pair region of the mitochondrial DNA sequence is shown in Figure 1. One variable site (genotype NNB) was found among 30 individual samples of pine marten from Atlantic Canada representing the subspecies *M. a. atrata*, *M. a. brumalis*, and *M. a. americana* (sample location, number, and genotype of subspecies sampled appear in Table 2). This single variable site occurred in two individuals out of twelve from northern New Brunswick. The average nucleon diversity (average proportion of pairwise nucleon substitutions per pair of individuals) (Nei and Tajima 1981) within the 307 base

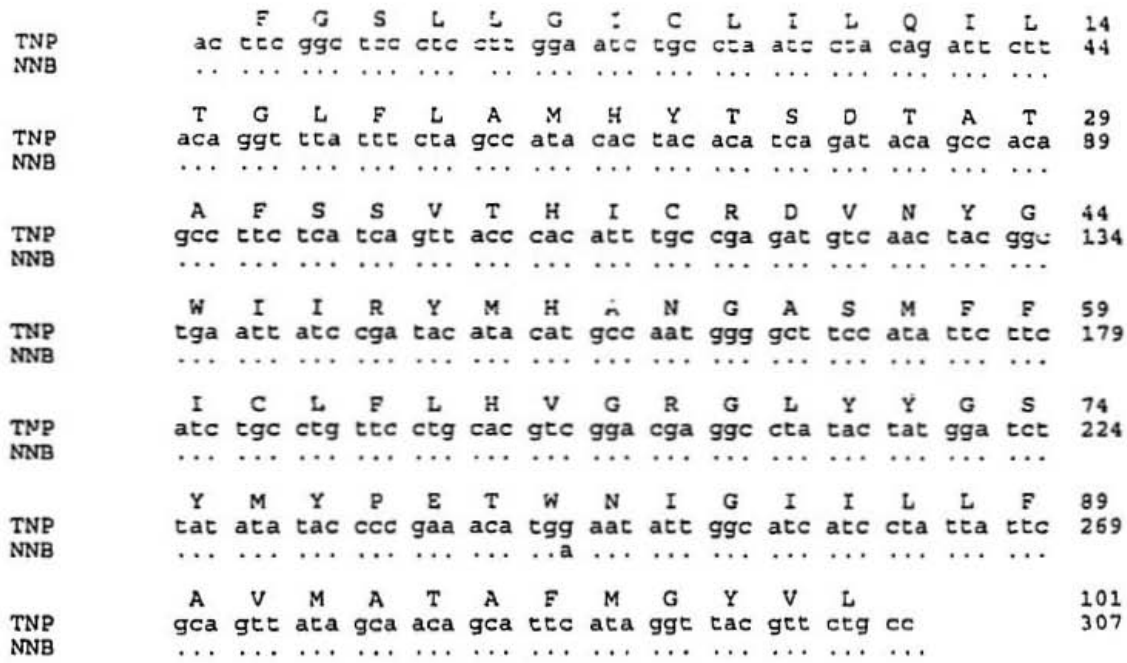


Figure 1 The 307 base pair cytochrome *b* mitochondrial DNA sequence from two genotypes of *Martes americana*. In the second sequence the nucleotides are identical except as indicated. The first line indicates the inferred amino acid sequence for the TNP genotype (International Union of Biochemists). The number adjacent to the first and second lines give the respective numbers of the protein and nucleotide sequences.

Table 2 *Martes americana* subspecies names, geographic origin, number of samples, and genotype in the 307 base pair database.

Subspecies	**Geographic Origin**	**#**	**Genotype**
M. a. atrata	Eastern Newfoundland	3	TNP (307)
M. a. atrata	Western Newfoundland	5	TNP (307)
M. a. brumalis	Eastern Labrador	10	TNP (307)
M. a. americana	Northern New Brunswick	10	TNP (307)
M. a. americana	Northern New Brunswick	2	NNB

pair database is 0.13. The single variant was a third position silent purine transition.

Within the 401 base pair portion of mitochondrial cytochrome *b* under study, 9 nucleotide substitutions were identified among eighteen individual specimens representing twelve subspecies of *M. americana* (Table 3 and Figure 2 and 3). Seven of the 9 observed substitutions are first or third position silent substitutions; the other two are first position substitutions that would result in amino acid changes. The sequence of a single specimen of *M. martes* differs from the *M. americana* TNP genotype by 17 nucleotide substitutions (4.2%), all of which are silent; one substitution is shared with one of the *M. americana* genotypes (SEL - position 180, Figure 2). The pairwise sequence divergences among the *M. americana* genotypes and *M. martes*, *M. zibellina*, and *M. melampus* are presented in Table 4. Interspecific differences range from 2.1% to 5.7%. Sequences were obtained for two European pine marten (*Martes martes*) from two areas in Sweden approximately 150 kilometers apart. Both of these DNA sequences were identical. A 375 bp fragment, homologous to the 5'-most end of the sequence, from a *M. melampus* was used in this analysis (Masuda and Yoshida 1994). The American badger (*Taxidea taxus*) sequence differs from the American pine marten by at least 43 base substitutions (10.7%).

Table 3 *Martes americana* subspecies names, geographic origin, number of samples, and genotype in the 401 base pair database.

Subspecies	Geographic Origin	#	Genotype
M. a. atrata	Eastern Newfoundland	1	TNP (401)
M. a. brumalis	Eastern Labrador	1	TNP (401)
M. a. americana	Northern New Brunswick	1	TNP (401)
M. a. abieticola	Northern Manitoba	1	TNP (401)
M. a. actuosa	Northwest Territory	2	TNP (401)
M. a. kenaiensis	Kenai Peninsula, Alaska	2	TNP (401)
M. a. abietinoides	Southeastern B.C.	1	SEL
M. a. caurina	Southwestern B.C.	2	VCI
M. a. vancouverensis	Vancouver Island, B.C.	2	VCI
M. a. nesophila	Queen Charlotte Island, B.C.	3	QCI
M. a. origenes	Southeastern Wyoming	1	WYO
M. a. vulpina	Northern Idaho	1	WYO

Table 4 The pairwise DNA sequence divergence among the *Martes americana* genotypes and *Martes martes* (*M.mar*), *Martes zibellina* (*M.zib*), and *Martes melampus* (*M.mel*) (*M. melampus* data are taken from Masuda and Yoshida (1994)).

	TNP	SEL	WYO	QCI	VCI	*M.mar*	*M.zib*	*M.mel*
TNP	-	0.3%	1.5%	1.7%	1.7%	4.2%	5.5%	3.5%
SEL		-	1.7%	2.0%	2.0%	4.0%	5.2%	3.2%
WYO			-	0.3%	0.3%	4.2%	5.5%	3.5%
QCI				-	0.5%	4.5%	5.7%	3.7%
VCI					-	4.5%	5.7%	3.7%
M.mar						-	2.2%	2.1%
M.zib							-	2.9%
M.mel								-

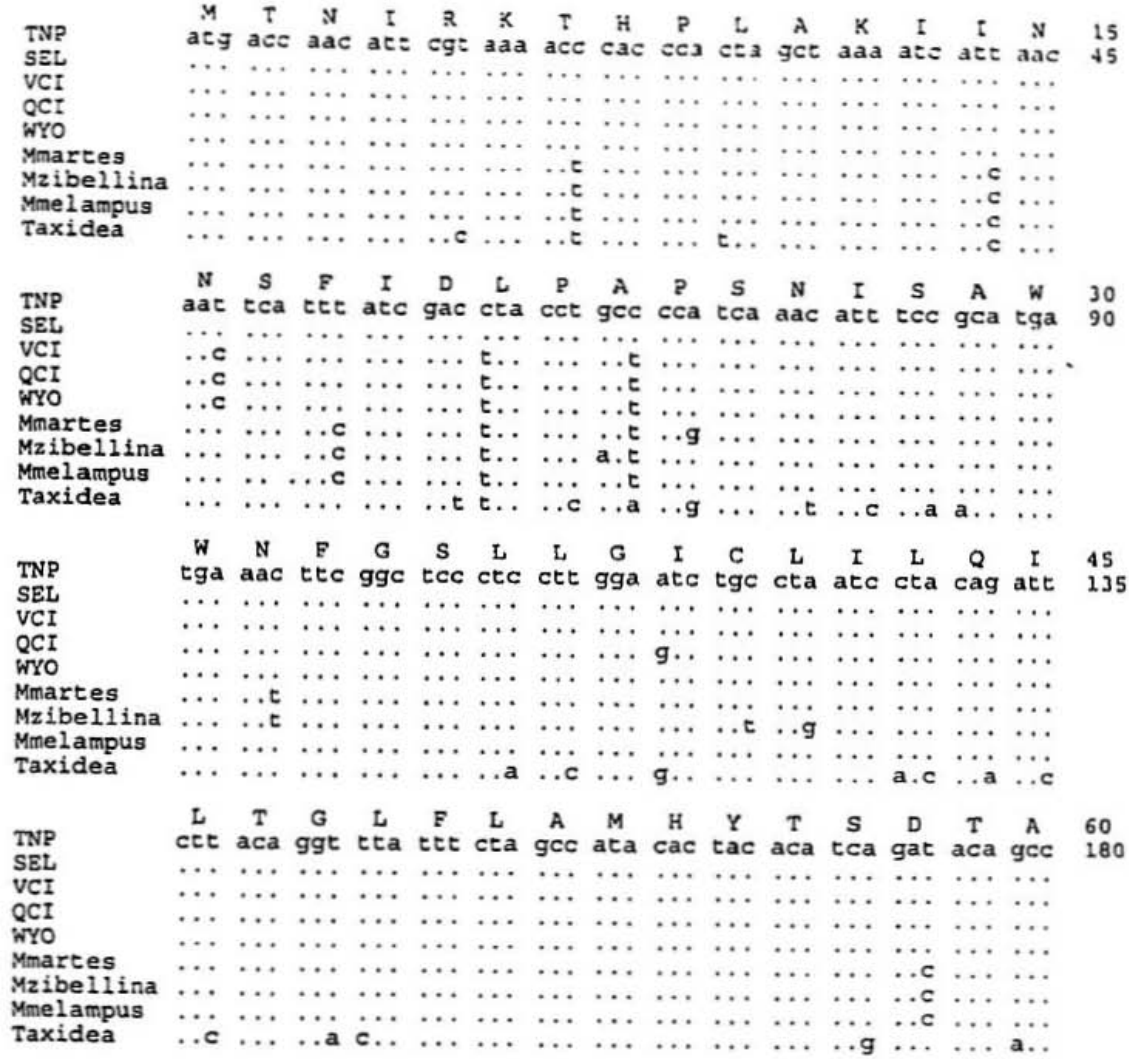
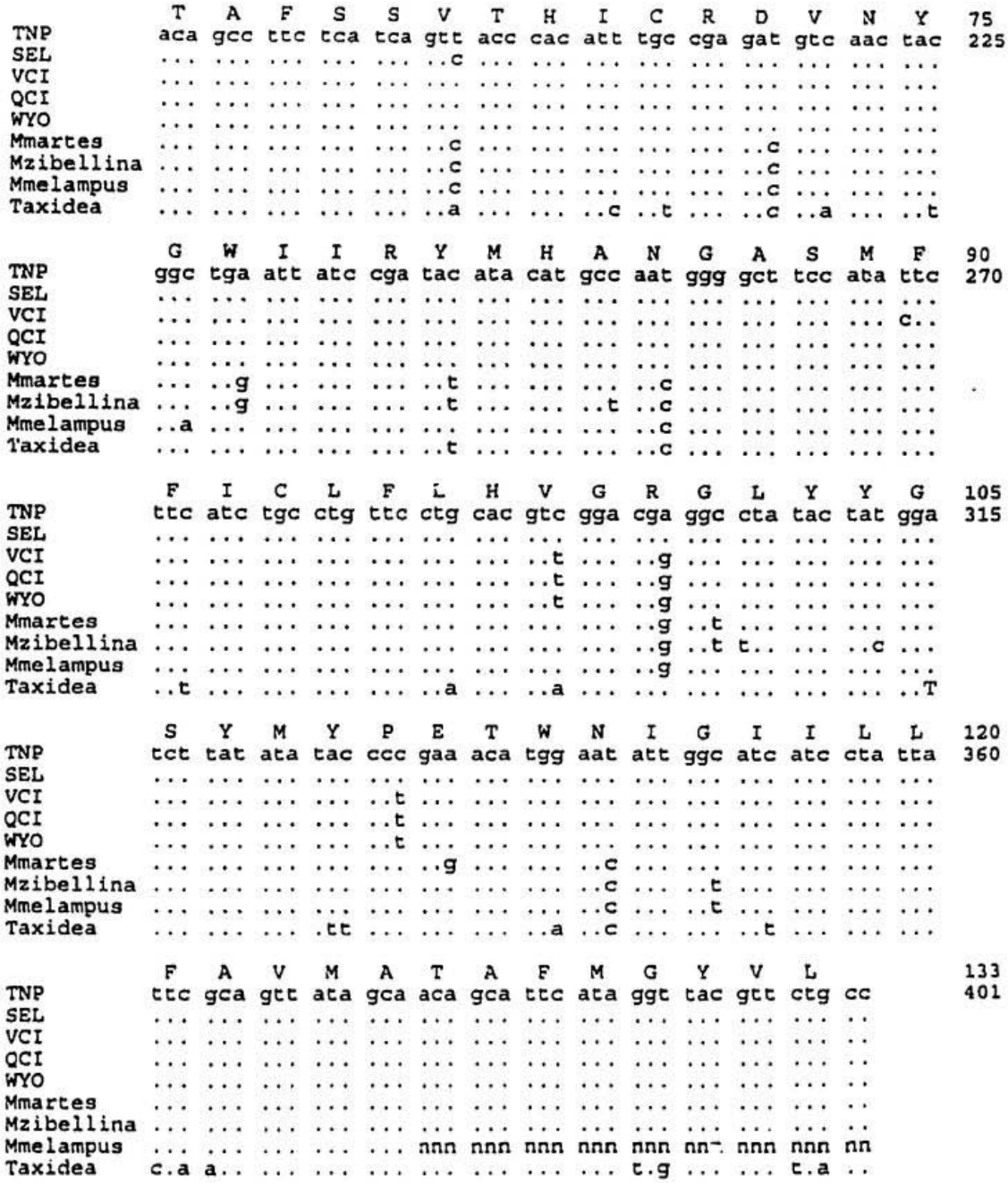


Figure 2 The 401 base pair cytochrome *b* mitochondrial DNA sequence five genotypes of *Martes americana*, two *Martes martes*, a *Martes zibellina*, a *Martes melampus*, and a *Taxidea taxus* (*M. melampus* data are taken from Masuda and Yoshida (1994)). In the last eight sequences the nucleotides are identical except as indicated. The first line indicates the inferred amino acid sequence of the TNP genotype (International Union of Biochemists). The number adjacent to the first and second lines give the respective numbers of the protein and nucleotide sequences.


T A F S S V T H I C R D V N Y 75
TNP aca gcc ttc tca tca gtt acc cac att tgc cga gat gtc aac tac 225
SEL ... ...c ... ...
VCI ... ...
QCI ... ...
WYO ... ...
Mmartes ... ...c ... ...c ... ...
Mzibellina ... ...c ... ...c ... ...
Mmelampus ... ...c ... ...c ... ...
Taxidea ... ...a ... .c ..t ... ..c ..a ... ..t
G W I I R Y M H A N G A S M F 90
TNP ggc tga att atc cga tac ata cat gcc aat ggg gct tcc ata ttc 270
SEL ... ...
VCI ... ... c..
QCI ... ...
WYO ... ...
Mmartes ... ..g ... ...t ... ...c ... ...
Mzibellina ... ..g ... ...t ... .t ..c ... ...
Mmelampus ..a ... ...c ... ...
Taxidea ... ...t ... ...c ... ...
F I C L F L H V G R G L Y Y G 105
TNP ttc atc tgc ctg ttc ctg cac gtc gga cga ggc cta tac tat gga 315
SEL ... ...
VCI ... ...t ... ..g ... ...
QCI ... ...t ... ..g ... ...
WYO ... ...t ... ..g ... ...
Mmartes ... ...g ..t ... ...
Mzibellina ... ...g ..t t.. ... ..c ...
Mmelampus ... ...g ... ...
Taxidea ..t ... ...a ... ..a ... ...T
S Y M Y P E T W N I G I I L L 120
TNP tct tat ata tac ccc gaa aca tgg aat att ggc atc atc cta tta 360
SEL ... ...
VCI ... ...t ... ...
QCI ... ...t ... ...
WYO ... ...t ... ...
Mmartes ... ...g ... .c ... ...
Mzibellina ... ...c ... ..t ... ...
Mmelampus ... ...c ... ..t ... ...
Taxidea ... ... .tt ... ...a ..c ... .t ... ...
F A V M A T A F M G Y V L 133
TNP ttc gca gtt ata gca aca gca ttc ata ggt tac gtt ctg cc 401
SEL ... ...
VCI ... ...
QCI ... ...
WYO ... ...
Mmartes ... ...
Mzibellina ... ...
Mmelampus ... ... nnn nnn nnn nnn nnn nn. nnn nnn nn
Taxidea c.a a.. ... ... t.g ... ... t.a .. ..

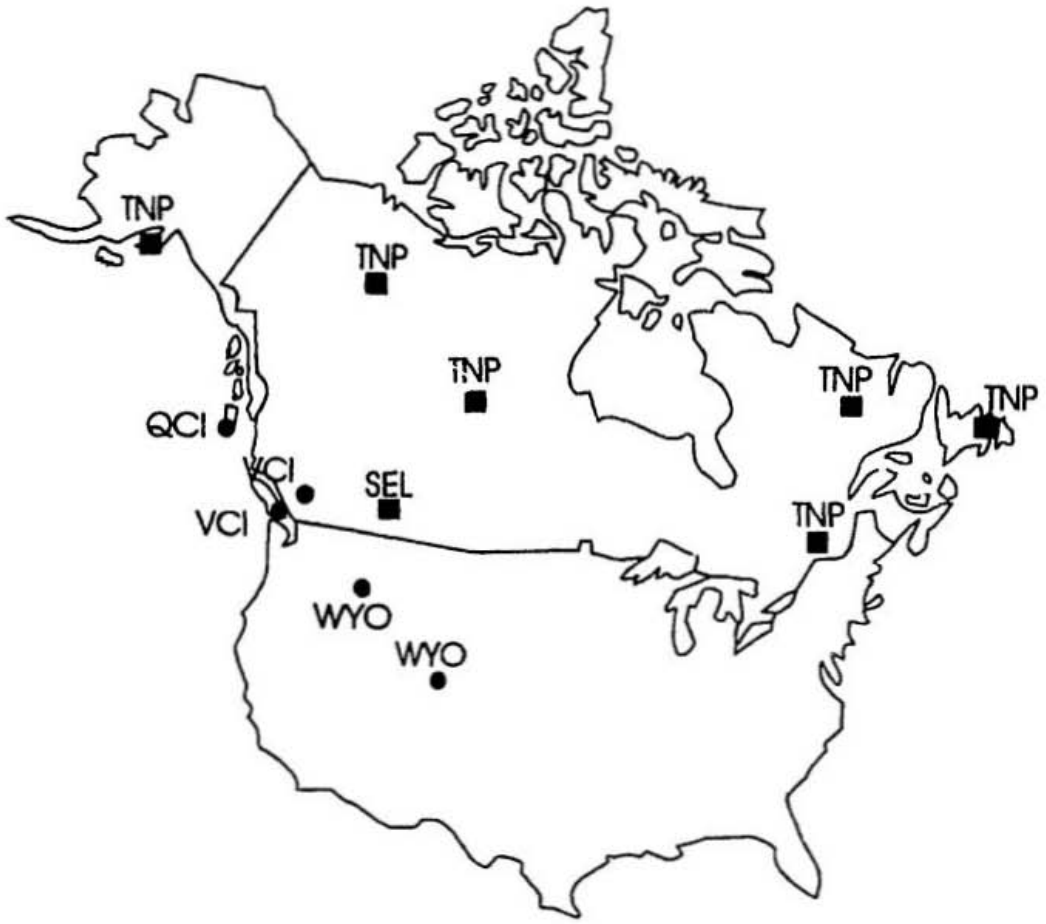


Figure 3 Geographic distribution of *Martes americana* sample sites shown in Table 3 (after Hicks and Carr 1995). The three letter code represents the genotypes from Table 3. Sample sites located in the range of the "*caurina*" group of marten are indicated with a circle. Samples from the "*americana*" group are identified with a square.

The nine substitutions within *M. americana* define five genotypes that differ by between one and eight substitutions. Figure 4 shows the minimum-length mutational network connecting these genotypes with that of the European pine marten, the Eurasian sable and the Japanese marten. Two distinct groups of American marten genotypes are apparent. Samples from a broad geographic area across northern and eastern North America, from Alaska to Newfoundland, have identical DNA sequences (genotype TNP); a single individual from southeastern British Columbia has a unique genotype that differs from this common genotype by a single base transition (genotype SEL). This pyrimidine transition is shared with the three *Martes* species (parallel mutation) (position 198, Figure 2).

Pine marten inhabiting the same area as those with the TNP and SEL genotypes have been referred to as the "*americana*" group (Hagmeier 1961). In contrast, DNA sequences in pine marten from the southwestern portion of the species' range (Wyoming/Idaho) differ from the TNP genotype by six base substitutions (genotype WYO). Pine marten from three areas of coastal British Columbia comprise two other genotypes each differing from the more southern type by one nucleotide substitution (genotypes VCI and QCI). Marten from the area represented by the WYO, VCI, and QCI genotypes are referred to as the "*caurina*" group (Hagmeier 1961).

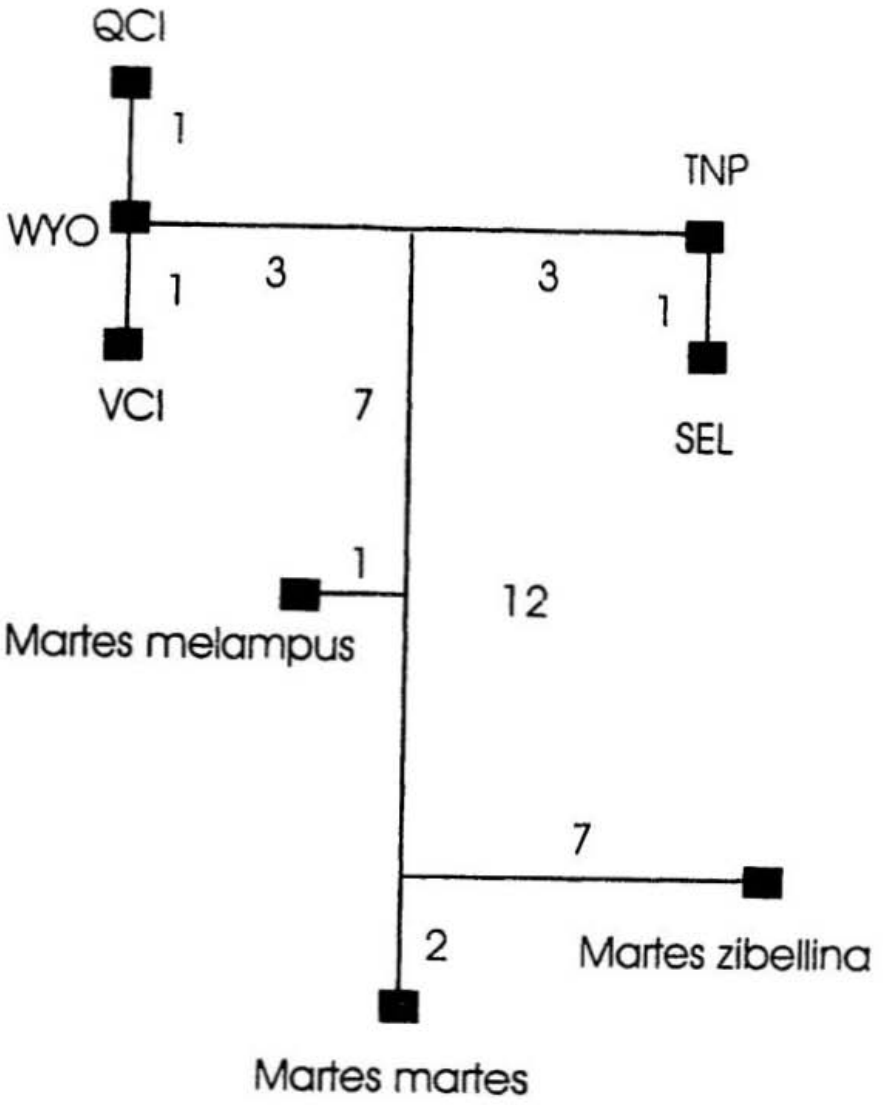


Figure 4 Network of mutational differences among five *Martes americana* cytochrome *b* mitochondrial DNA sequence genotypes, *Martes martes, Martes zibellina*, and *Martes melampus* (*M. melampus* data are taken from Masuda and Yoshida (1994)). Numbers on branches indicate the number of nucleotide substitutions. Parallel mutations are inferred in the branches leading to *M. martes* and genotype SEL (position 198, Figure 2).

The 307 bp variant, genotype (NNB), noted in two individual marten from New Brunswick is known to exist but was not sampled for the 401 bp database. The NNB genotype differs from the TNP genotype by a single base substitution as does the SEL genotype. The NNB variant shares a parallel mutation with the *Taxidea taxus* sequence, position 339, Figure 2.

Within subspecies represented by more than one individual, all DNA sequences (in the 401 base pair database) examined to date have been identical. The average nucleon diversity (h) (Nei and Tajima 1981) within the "*americana*" group of marten is 0.22. This is much lower than the h of 0.72 found within the "*caurina*" group.

3.2 Phylogenetic analysis of mtDNA genotypes

The phylogenetic analysis was based on the 401 base pair data. Figure 5 shows the inferred phylogenetic relationships of the various genotypes of the North American marten (*M. americana*), European marten (*M. martes*), Eurasian sable (*M. zibellina*), Japanese marten (*M. melampus*), and American badger (*Taxidea taxus*). The badger has been chosen as the outgroup comparison. The analysis was also carried out within a broader taxonomic context where the badger was shown to be

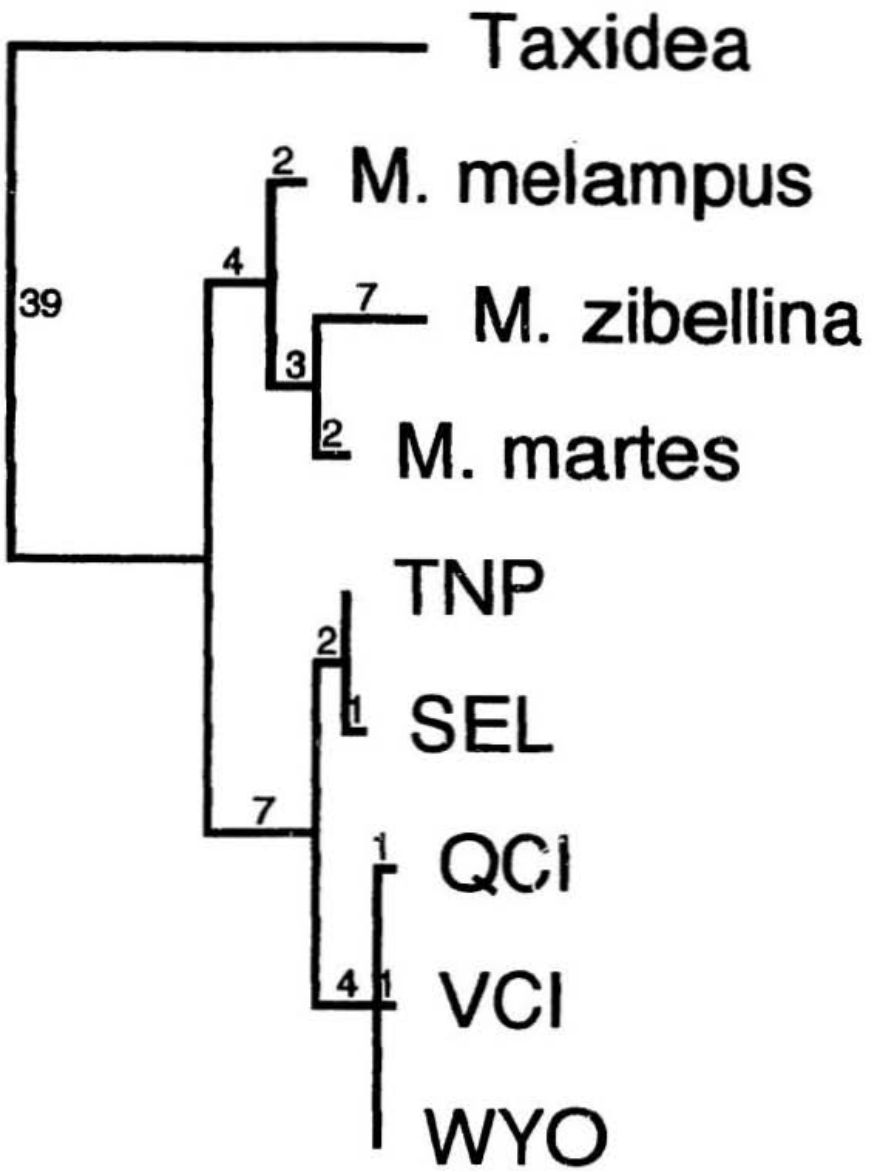


Figure 5 Phylogenetic tree of the mitochondrial cytochrome *b* sequences of five *Martes americana* genotypes, three other species of *Martes*, and *Taxidea taxus* (*M. melampus* data are taken from Masuda and Yoshida (1994)).

the outgroup to the *Martes*. Results were always similar with respect to placement of *Martes* species. The analysis used a heuristic search and delayed-character-transformation optimization for the shortest length tree using PAUP. There was only one tree of shortest length.

The heuristic search found a single tree having a length of 73 and the following characteristics; consistency index (CI) = 0.932 and homoplasy index (HI) = 0.068. The CI and HI excluding uninformative characters was 0.821 and 0.179 respectively. The retention index (RI) and the rescaled consistancy index (RC) were calculated as 0.872 and 0.812.

The bootstrap analysis of 100 trees provided the tree seen in Figure 6. The bootstrap analysis places confidence limits on phylogenies (Felsenstein 1985). In this analysis branches with less that 50% confidence were collapsed.

The branch leading to the *M. martes, M. zibellina, M. melampus* group was supported in 68% of replications, while the *M. martes, M. zibellina* was found in 92%. The branch containing all the *M. americana* genotypes was supported 99% of the time, with the "*americana*" group (TNP and SEL), and the "*caurina*" group (WYO, QCI, and VCI) being observed in 74% and 96% of replicates respectively.

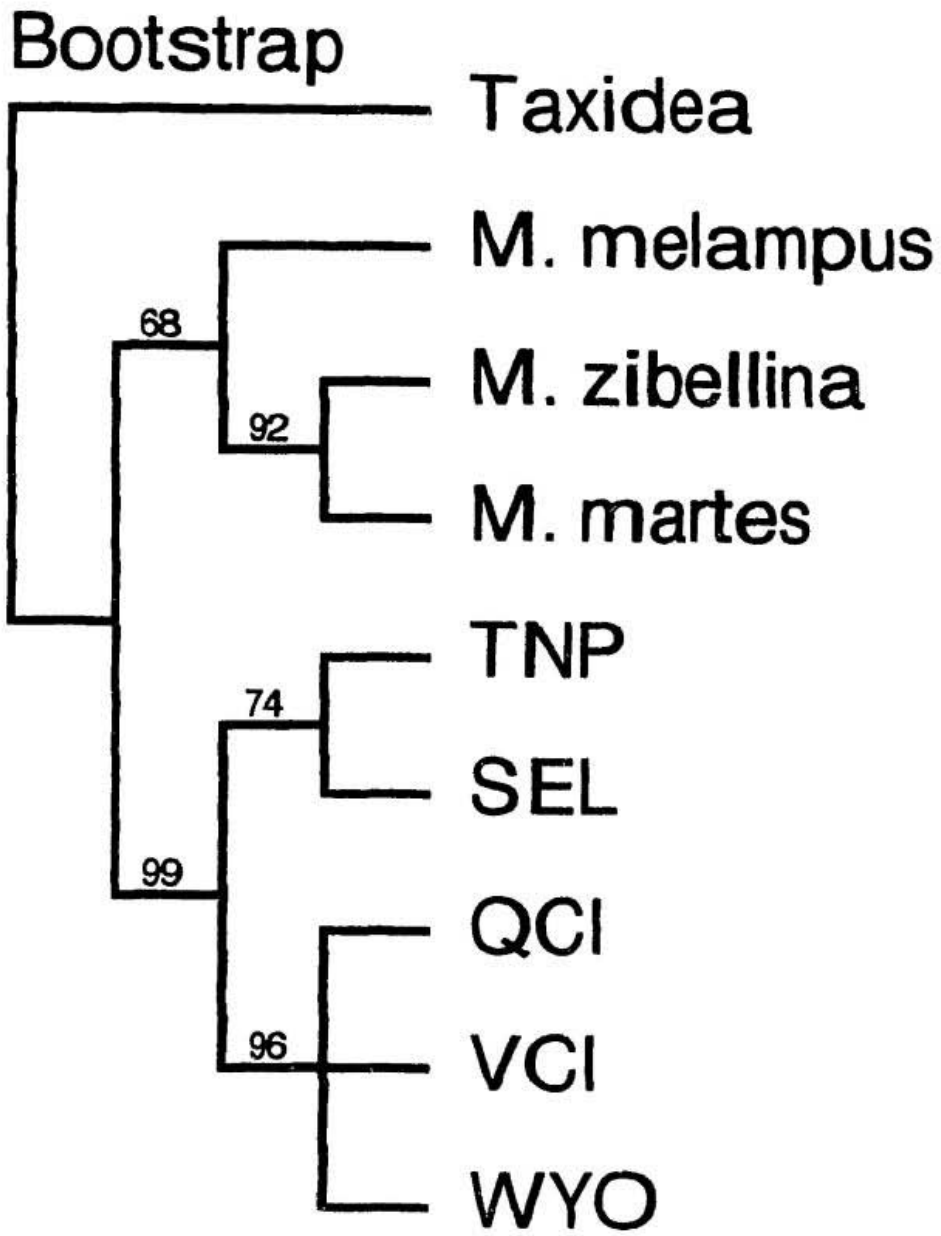


Figure 6 Bootstrap analysis of phylogenetic relationships within five *Martes americana* genotypes, *Martes martes*, *Martes zibellina*, and *Martes melampus* (*M. melampus* data are taken from Masuda and Yoshida (1994)). Branch numbers represent the number of times this placement was supported in 100 bootstrap replications.

4.0 DISCUSSION

4.1 Genetic variation

The 307 base pair portion of the cytochrome *b* DNA sequence from Newfoundland, Labrador, and New Brunswick marten showed one variable site but it is not phylogenetically informative. No genetic diversity was found within any of these three subspecies. These results have previously been reported in Hicks and Carr (1992). No variants were found in the 307 bp DNA sequences of pine marten from Terra Nova National Park (3) or western Newfoundland (5). Small sample sizes are, in large part, a function of the small estimated marten population on the island (Bissonnette *et al.* 1988).

Intraspecific and population-level variation has been noted in a wide range of species such as deer (Carr *et al.* 1986) and Atlantic cod (*Gadus morhua*) (Carr and Marshall 1991) for the same 307 bp region of the cytochrome *b* gene used in this study. The same marker showed very low genetic variation in *Martes*. These findings are in contrast to those of Mitton and Raphael (1990) who found high genetic variation in 10 pine marten from Wyoming, although their techniques were very different (DNA sequencing versus starch gel electrophoresis). In the current study the sequence under study was expanded to include an additional 94 base

pairs, bringing the total to 401 base pairs, but no additional variants were noted in the *Martes americana* from eastern Canada.

Based on the 401 bp data, two unique genetic groups have been identified within the American marten. The "*americana*" group includes seven subspecies and is broadly distributed across most of northern and eastern North America, and the "*caurina*" type including pine marten assigned to five (of seven in total) subspecies occurring over a much smaller geographic area in the southwestern portion of the species' range.

Subspecies of the "*americana*" group are characterized by very low levels of genetic diversity across a large geographic range. The genetic variation within the group of subspecies is also very low. A higher level of genetic diversity exists among subspecies of the "*caurina*" group. The two groups differ from one another by at least 1.5% of the 401 bp of cytochrome *b* sequence studied and this difference may be caused by biogeographical factors. Hicks and Carr (1995 and In Press) discuss some of the 401 base pair data representing marten throughout North America from biogeographic and species perspectives.

4.1.1 *"americana"* Group

This research shows that the marten of Terra Nova National Park have an identical 307 bp DNA sequence to those of their source population in western Newfoundland. The 401 bp data from the "*americana*" group of marten supports the 307 bp data in that genetic variation was found to be very low within the entire range of the "*americana*" subspecies group (Table 3 and Figure 2). The inclusion of marten samples from a broad geographic range provided an indication of intersubspecific genetic diversity within the "*americana*" group but only in a single sample from one subspecies (genotype SEL compared to the typical TNP) from the southwestern portion of their range (h = 0.22). For comparison, h = 0.13 for the 307 base pair data which is comprised of "*americana*" group animals from eastern Canada. The phylogenetic tree (Figure 5) provides good support for genotypes TNP and SEL forming a distinct clade. The branch placement of the phylogenetic tree itself is well supported based on the CI and HI figures (Swofford 1993).

The conserved nature of the cytochrome *b* in *M. americana,* originally noted in the 307 base pair data from marten native to Terra Nova National Park and eastern Canada, appears to be the typical condition for the "*americana*" group, as evidenced by the 401 base pair data from a broader geographic area. Higher resolution genetic techniques may resolve questions regarding the possible loss of

genetic variation in marten from Terra Nova National Park. These techniques may also detect a level of genetic diversity between marten of the island of Newfoundland and adjacent mainland areas as well as between other subspecies. Caution must be exercised in interpreting these current data with respect to the validity of the Newfoundland pine marten as a subspecies or a genetically unique entity. It cannot be extrapolated from these data that the entire genome of the currently recognized subspecies *M. a. atrata* and *M. a. brumalis* are identical, simply because 401 base pairs of mitochondrial DNA from 2 samples (in addition to 18 from the 307 database) showed no differences.

Another available procedure is randomly amplified polymorphic DNA (RAPD) analysis which is considered to be a high resolution genetic technique (Gibbs *et al.* 1994). McGowan and Davidson (1994) found very little RAPD variation among Newfoundland pine marten. Their study included samples from three areas of western Newfoundland, but not from the Terra Nova National Park area. Inclusion of samples from the park may provide some insight into the variability of this population. However McGowan and Davidson (1994) concluded that another technique, microsatellite analysis, may be preferable for intensive wildlife management of these marten.

It has been hypothesised that American marten were restricted to southern Pleistocene glacial refugia. Low population numbers may have been experienced at that time. This population "bottleneck" could have resulted in a loss of genetic variation. This restricted population is believed to have repopulated North America following the withdrawal of the Wisconsin ice sheet (Anderson 1970, Clark *et al.* 1987). The ensuing years (approximately 18,000-10,000)(Pielou 1992) is a relatively short time in which to increase genetic variation (Wilson *et al.* 1985). The genetic evidence provides some support for the theory that marten endemic to eastern Canada, and indeed throughout the entire range of the "*americana*" group, may have descended from a small, genetically depauperate refugial population. Nevertheless, marten on the island of Newfoundland have been isolated from others of their kind and have evolved in a unique insular environment (Snyder 1985).

It has been suggested that a second glacial refugium for "*americana*" pine marten may have been present in the Rocky Mountains (Dillon 1961). The only variant 401 base pair genotype (SEL) found within the "*americana*" group was from that area. This may support the second refugium theory but as only one specimen was sampled from the area it is unclear if this single variant is characteristic of the population / subspecies. If the SEL genotype were present in the area in a high frequency or if the genotype is exclusive to this area, it might suggest that the

population has been physically isolated from the other marten of their subspecies group for some time possibly in a second glacial "*americana*" refugium.

Low levels of genetic variation have previously been reported in mammals, and large carnivores in particular (Allendorf *et al.* 1979, Bonnell and Selander 1974, O'Brien *et al.* 1983, 1985, 1986, Sage and Wolff 1986, Sage *et al.* 1982, Simonsen 1982, and Wooten and Smith 1985). These data suggest that low genetic variability among carnivores is common. Simonson (1982) found a complete lack of genetic variation in the European and stone martens using electrophoretic techniques. In contrast, Mitton and Raphael (1990) found pine marten from Wyoming to have an average heterozygosity of 17%.

4.1.2 "*caurina*" Group

The "*caurina*" group of American marten has historically been found to be morphologically different from the "*americana*" group (Hagmeier 1955, 1958, and 1961, Merriam 1890, Wright 1953). The genetic evidence, based on the 401 bp data, suggests the two groups are distinct (Figure 2 and 4). The phylogenetic tree (Figure 5) supports placing the QCI, WYO, and VCI genotypes in a second clade, separate from the TNP and SEL genotypes. This pattern of genetic diversity, superimposed on the North American landscape, closely resembles the ranges accorded the morphologically divergent "*americana*" and "*caurina*" subspecies

groups (Hagmeier 1955), therefore, this terminology will be used here. The current range of marten from the "*caurina*" group includes western and southern British Columbia extending into the states of Washington, Oregon, Idaho, Wyoming, and Montana, Colorado, New Mexico, Nevada, Utah, and California.

Although the phylogenetic tree cannot resolve the relationships among the "*caurina*" marten, the mutational network (Figure 4) suggests that both of the "*caurina*" genotypes presently found in western Canadian island populations are derived independently from the more southerly genotype. Further sampling or the addition of sequences from other portions of the genome may help to resolve phylogenetic relationships within the "*caurina*" group.

Not only does the "*caurina*" group of marten differ markedly from the "*americana*" group (1.5%) but intersubspecific differences within the "*caurina*" group ($h = 0.72$) are much greater than that found in the "*americana*" group marten ($h = 0.22$) and may result from biogeographic factors.

As sample sizes from the "*caurina*" subspecies are low it is possible that the observed variants are not distributed throughout the entire local population / subspecies being sampled. Other base substitutions may also be present which have not been detected. The "*caurina*" marten as a group may simply have a higher level of genetic variation as opposed to their being a number of genetically diverse

populations/subspecies. Another possible explanation for this increased number of substitutions is that isolated subspecies may have retained or developed genetic diversity over time.

Refugia for marten of the *"caurina"* group may have been present on coastal islands (Foster 1965) or the extreme southern portion of their present range (Graham and Graham 1994). As noted above, the mutational network in Figure 4 suggests that the VCI and QCI genotypes are derived from the WYO genotype. This pattern of genetic diversity could be explained by either hypothesis (i.e. southern or coastal refugia).

Pine marten inhabiting the Queen Charlotte Islands (*M. a. nesophila*) have been described as morphologically distinct from pine marten from other areas of North America (Giannico 1986, and Hagmeier 1955). The possibility exists that pine marten survived in glacial refugia in the area. The genetic data for pine marten show that a unique genotype (QCI) exists on the Queen Charlotte Islands which differs by two base substitutions from that found in marten of the subspecies inhabiting the adjacent mainland. This finding provides additional support for the theory that these marten may have been isolated from other *"caurina"* marten populations. Sample sizes are small, therefore additional sampling is warranted before strong conclusions can be drawn from the genetic data.

In addition to the effects of glaciation, geological, and geographical features such as water bodies, mountain ranges, and any large areas devoid of forest cover may have had a major impact on range expansion and isolation of marten populations. During the hypsithermal (the most recent period of highest average air temperature) 4,000-10,000 years ago, the extent of forested areas is thought to have decreased and been replaced by drier plains (Hoffmann and Jones 1970). These plains may have reduced the amount of habitat available to forest species and served as barriers to the movement of marten. This may have been the case in present day northwestern United States and may account for the disjointed nature of the *"caurina"* group of marten's range (Graham and Graham 1994). At that time the *vulpina* and *origenes* subspecies may have been completely disjunct from any other marten population and may have only more recently come into contact with marten from the "*americana*" group, as temperatures have cooled and forest environments have descended to lower elevations.

It has been proposed that pine marten, possibly of the "*americana*" type, could have existed in Beringia during the last glaciation, as occurred with many other North American wildlife species (Hagmeier 1955). In addition to a refugium, Beringia may have provided access to North America for new *Martes* immigrants from Asia (the postulated second incursion of sable-like *"caurina"* group ancestors)(Anderson 1994). However, the habitat is thought to have resembled

tundra while present day marten require mature forests (Dillon 1961). There have been suggestions, based on the fossil record, that habitat requirements for *Martes* may have been broader in the past (Graham and Graham 1994).

Another issue is the exact identity of fossil remains which some researchers feel belong to a large, extinct North American marten described as *Martes nobilis* (Anderson 1970). Others believe this fossil is simply a large specimen of *Martes americana* possibly belonging to the *"caurina"* group (Hall 1926, Youngman and Schueler 1991). If tissue could be located from this fossil "species", DNA sequence comparisons with the current data set should solve this question and possibly provide insight into the evolution of North American *Martes*.

The present data from mitochondrial DNA show the "*caurina*" marten as a group are genetically distinct from the "*americana*" group as all sequences studied differed by 1.5% between the two groups. Within the "*caurina*" group subspecies show low levels of genetic differences. However the sample sizes are small, therefore, additional sampling would be required to confirm that the genetic diversity noted between several subspecies is fixed.

4.1.3 Two Species?

The pattern of genetic diversity shown in this study (Figure 2, 3, and 4) is consistent with patterns of morphological diversity within the species, as reflected in historical species/subspecies accounts (reviewed in Hagmeier 1955). Researchers have long recognized the morphological differences between the two groups of true marten in North America. These two groups were once considered separate species (Merriam 1890) and are now thought to represent two different subspecies groups in *M. americana* (Anderson 1970, Hagmeier 1961, Hall 1981, and Wright 1953).

One explanation for the differences between the two subspecies groups is the possibility that the "*caurina*" group is more closely related to Palearctic *Martes* stock with which it displays greater morphological similarities (Hagmeier 1955, 1961, and Anderson 1970). If this were the case phylogenetic analysis of DNA sequences would be expected to show a closer relationship between *"caurina"* marten and Old World forms. The phylogenetic tree (Figure 5) does not support this theory. The *"caurina"* and *"americana"* groups are more closely related to each other than to other *Martes* species. Additionally the phylogeny of the *Martes* subgenus is characterized by divergent Nearctic and Palearctic lineages as can be seen in Figure 5. If the current diversity of Nearctic true marten is not a result of

multiple founder events, the possibility that marten have existed on this continent for greater periods of time than previously thought should be reconsidered (Dillon 1961).

The pairwise sequence divergence between the North American subspecies groups (1.5%) is only slightly less than that between European and Japanese pine marten (2.1%), or between European pine marten and sable (2.2%). This further suggests that there may be cause to reconsider *M. americana* (Turton 1806) and *M. caurina* (Merriam 1890) as distinct species.

Wright (1953) studied the morphology of what was then considered two allopatric species of true martens *Martes caurina* and *Martes americana*, in their zone of contact in Montana. He reported intergradation of morphological characteristics between the two species in one area of Montana. He concluded that they were interbreeding and therefore were not "good" species as defined by the biological species concept (Mayr 1969). He suggested that all subspecies of the former *M. caurina* species be relegated to separate subspecies of *Martes americana* and this is the currently accepted taxonomic practice, though some debate remains over the exact number of subspecies and their ranges (Hagmeier 1955, and Hall 1981).

There are several cases in which two closely related species of mammals are known to hybridize and produce fertile offspring, such as mule deer (*Odocoileus hemionus*) and whitetailed deer (*Odocoileus virginianus*) (Carr *et al.* 1986, Hughes 1990). These animals have retained their specific designation as they are distinct evolutionary lineages. Another classic case is that of the coyote (*Canis latrans*) and the North American Grey Wolf (*Canis lupus*) where genetic evidence has proven the two hybridize in the wild but they are still considered separate species (Lehman *et al.* 1991). Among mustelids, sympatric populations of European marten (*Martes martes*) and sable (*Martes zibellina*) are said to hybridize in the wild and are known to interbreed in captivity, yet they have not been considered as a single species (Grakov 1994). In fact Grakov (1994) states that although in the wild the "kidus" may be numerous, captive studies show the hybrid F_1 generation is only partially fecund and are a "biological dead lane".

Wright (1953) identified eight populations, numbered 1 through 8 in his Figure 2. Populations 1 - 4 (from Barkerville BC, Suswap BC, Whitefish Range MT, and Northern Idaho, respectively) are from the range of the "*americana*" group, and populations 6 - 8 (from Clearwater Region ID, Sapphire Range MT, and Red Lodge Region MT) belong to the "*caurina*" group. Wright's (1953) population 5 inhabited the Swan, South Fork and Sun rivers region of Montana and was considered by him

to be the intergrade group based on the intermediate nature of cranial measurements and pelage characteristics.

A re-analysis of Wright's original data was carried out to determine if significant differences in crainial measurements existed between these eight populations and if so were these differences in a pattern consistent with the ranges of the two former species *M. americana and M. caurina*.

Wright's (1953) original data for auditory bulla, inner moiety of the inner molar, and the width of rostum in males (Appendix 1) were analysed using the one-way analysis of variance (ANOVA). Significant differences in the means existed among the eight populations regarding the auditory bulla ($F = 29.72$, $P << 0.01$), the inner moiety of the inner molar ($F = 41.04$, $P << 0.01$), and the width of rostum ($F = 11.33$, $P << 0.01$).

Pairwise ANOVA testing was carried out on the auditory bulla of all eight populations (Table 5). If population 5 (the hypothesised intermediate group) is removed from consideration, populations 1, 2, and 3 (the "*americana*" marten) differ significantly (alpha = 0.01) from populations 6, 7, and 8 (the "*caurina*" marten) (Table 5). All of the "*americana*" group populations of *M. americana* are significantly different from the "*caurina*" group populations. It can be noted from Figure 2 of Wright's (1953) data that differences are not simply a matter of size,

Table 5 One-way analysis of variance results from auditory bulla measurements of the eight populations of American pine marten studied in Wright (1953). A significant difference at the 0.05 level is indicated between populations by a single asterisk (*) in the appropriate box, two (**) indicate significance at the 0.01 level. Population identification numbers used here follow those of Wright's (1953) Figure 1. Populations 1 through 4 represent "*americana*" group *Martes americana*, while populations 6, 7, and 8 belong to the "*caurina*" group, and population 5 is the proposed intergrade population.

	"americana"				intergrade	*"caurina"*		
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	-			*	**	**	**	**
2		-		*	**	**	**	**
3			-	**	**	**	**	**
4				-		**	**	*
5					-	**	**	
6						-		
7							-	*
8								-

although the "*caurina*" marten tend to have a small auditory bulla, the trend is towards larger inner moiety of the inner molar and width of rostum. This is suggestive of a different skull shape, not simply a difference in size.

A 3 dimensional plot of these 3 measurements (normalized using the greatest length of skull measurement) indicates that if group 5 is removed the *"americana"* and the *"caurina"* marten can easily be differentiated (Figure 7). A principal components analysis revealed similar results (not shown). The genetic and morphological differences between the two subspecies groups forces the reconsideration of the former species *Martes americana* and *Martes caurina* .

Genetic and ecological studies of the marten in the Montana zone of contact should be conducted to ascertain whether or not these animals are hybridizing and if so the outcome of these matings determined. If the hypothesised F_1 and subsequent hybrid generations are not completely viable, as occurs in the sable/European marten hybrid (Grakov 1994), the case for the existence of two separate species must be rethought. Such a research program, in conjunction with this and past studies, should allow a conclusive answer to the question of the taxonomic status of these two species/subspecies groups. In the interim it may be wise to manage the two subspecies groups as distinct genetic entities.

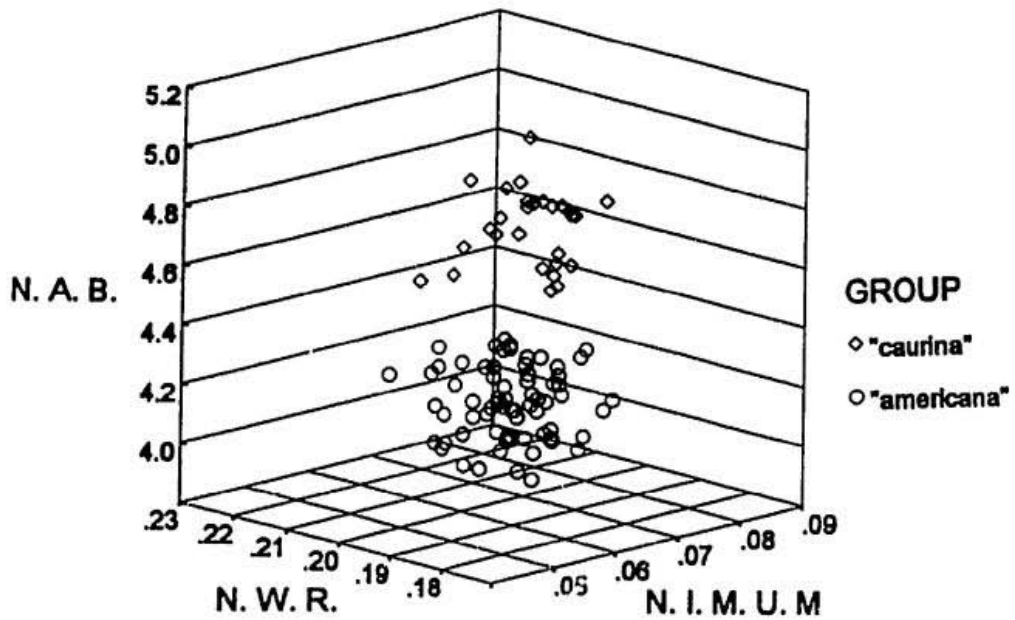


Figure 7 A three dimensional graph of the three morphological measurements (normalized) used by Wright 's (1953) Figure 2 (normalized auditory bulla - N.A.B., normalized inner moiety of the upper molar - N.I.M.U.M. and normalized width of rostum - N.W.R.). Wright's populations 1 through 4 have been combined and called *"americana"*, populations 6 through 8 were combined and referred to as *"caurina"*. Wright's intergrate population number 5 has been excluded for clarity.

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Appendix 1 Four morphological measurements (greatest length of skull, auditory bulla, inner moiety of the upper molar, and width of rostum) taken from eight populations of American pine marten (from Wright 1953).

G. Length of Skull	Auditory Bulla	Inner Moiety Upper Molar	Width of Rostum	Location
86.1	21.1	4.4	15.7	Barkerville, B.C.
78.9	18.5	4.4	15.5	Barkerville, B.C.
80	18.2	4.3	15.8	Barkerville, B.C.
78.2	19.2	4.2	15.5	Barkerville, B.C.
82.8	20.1	4.8	15.8	Barkerville, B.C.
79.8	18.7	4.7	15.2	Barkerville, B.C.
83.9	19.8	4.7	15.8	Barkerville, B.C.
79.5	19.1	4.5	15.4	Barkerville, B.C.
80	19.5	4.3	14.8	Barkerville, B.C.
84.7	19.7	4.6	15	Suswap, B.C.
80.2	18.4	4.4	15.2	Suswap, B.C.
80.8	18.7	4.2	15.9	Suswap, B.C.
81	19.6	4.6	14.9	Suswap, B.C.
80	19.4	4.5	14.9	Suswap, B.C.
77.3	18.8	4.5	14.6	Suswap, B.C.
78.2	18.4	4.9	14.4	Suswap, B.C.
80.3	18.3	4	14.6	Suswap, B.C.
78.6	19	4.5	13.7	Suswap, B.C.
79.9	19.4	4	15.3	Suswap, B.C.
77.3	18.6	4.6	14.1	Suswap, B.C.
79.9	20	4.7	15	Suswap, B.C.
79.3	19.6	4.1	14.8	Suswap, B.C.
83.8	19.2	5.1	15.8	Whitefish Range, MT
81.6	20.5	4.8	15.1	Whitefish Range, MT
82.2	19.3	4.9	15.3	Whitefish Range, MT
	20.2	5.3	15.6	Whitefish Range, MT
82.9	18.7	4.6	15.6	Whitefish Range, MT

81.2	18.6	5.3	14.9	Whitefish Range, MT
78.7	18.5	5.1	13.9	Whitefish Range, MT
80.6	19.8	5.3	15.4	Whitefish Range, MT
79.5	18.7	4.3	14.7	Whitefish Range, MT
81.7	19.3	4.6		Whitefish Range, MT
78.2	19.7	4.6	15.5	Whitefish Range, MT
80.9	19.9	4.8	15	Whitefish Range, MT
79.2	18.7	4.5	14.4	Whitefish Range, MT
82.3	19.4	4.7	15.6	Whitefish Range, MT
82.4	19.9	4.7	16.5	Whitefish Range, MT
81.8	19.7	4.2	15.5	Whitefish Range, MT
83.6	20.1	4.5	15.3	Whitefish Range, MT
84.4	20.3	5	16.4	Whitefish Range, MT
82.6	19.5	4.5	15.8	Whitefish Range, MT
81.8	18.6	4.8	15	Whitefish Range, MT
84.8	19.1	4.6	15.7	Whitefish Range, MT
81.2	18.7	5.7	15.3	Whitefish Range, MT
77.9	18.2	4.9	14.4	Whitefish Range, MT
81.6	18.6	5.1	15.9	Whitefish Range, MT
81.5	18.7	4.8	15.2	Whitefish Range, MT
78.4	18.7	4.5	14.6	Whitefish Range, MT
77.4	18.1	4.2	14.3	Whitefish Range, MT
81.8	19.1	4.6	14.8	Whitefish Range, MT
76.1	18.7	5.2	14.2	Whitefish Range, MT
85.4	20.5	4.7	15.1	Whitefish Range, MT
83.1	19.9	5.3	15.9	Whitefish Range, MT
79.1	18.2	4.7	14.3	Whitefish Range, MT
83.1	18.7	4.4	14.4	Whitefish Range, MT
82	19.4	5.2	14.5	Whitefish Range, MT

81.7	20.2	4.5	16.2	Whitefish Range, MT
85.8	20.5	4.7	17.1	Whitefish Range, MT
82	18.9	5.3	16.1	Whitefish Range, MT
82.7	19.5	4.6	17.3	Whitefish Range, MT
78.8	18.7	5.1	16.6	Whitefish Range, MT
81.8	18.8	4.6	15.5	Whitefish Range, MT
83.7	20	5	15.9	Whitefish Range, MT
77.8	18.7	4.4	13.9	Northern ID
80.5	19.1	4.6	15	Northern ID
79.5	19.4	4.6	15	Northern ID
79	18.2	4.4	14.4	Northern ID
78.1	17.7	4.6	14.8	Northern ID
76.5	18.4	4.7	15	Northern ID
81.2	18.7	4.7	15	Northern ID
74	17.3	4.6	15	Northern ID
78.9	18.4	4.9	14.6	Northern ID
81.4	18.2	4.9	16	Northern ID
83.3	17.9	5.5	16.6	S.F., S., S., Rivers, MT
82.7	19.1	5.1	16.1	S.F., S., S., Rivers, MT
81.8	18.7	4.8	15.3	S.F., S., S., Rivers, MT
79.4	19	4.9	15.5	S.F., S., S., Rivers, MT
82.2	17.4	5.9	16.7	S.F., S., S., Rivers, MT
81.4	18.2	5.4	16	S.F., S., S., Rivers, MT
80	17.8	5.7	17.2	S.F., S., S., Rivers, MT
83.2	18.8	5.6	16.5	S.F., S., S., Rivers, MT
79.5	17.8	5.5	16.2	S.F., S., S., Rivers, MT
79.6	17	6	16.9	S.F., S., S., Rivers, MT
77.9	17.3	5.4	15.4	S.F., S., S., Rivers, MT
79	17.6	5.3	15.8	S.F., S., S., Rivers, MT

80.6	17.7	5.7	16.3	S.F., S., S., Rivers, MT
83.1	19.6	5.7	16.3	S.F., S., S., Rivers, MT
84.9	18.7	5.6	16.4	S.F., S., S., Rivers, MT
		5.5	14.6	Clearwater, ID
81.1	17.1	5.1	16.1	Clearwater, ID
80.6	17.2	5.4	16.3	Clearwater, ID
80.5	17.8	5.7	15.7	Clearwater, ID
81.3	16.9	5.4	15.9	Clearwater, ID
77.3	16.3	5.2	15.6	Clearwater, ID
78.2	16.5	5.8	15.3	Clearwater, ID
82.1	17.1	5.7	15.7	Clearwater, ID
80.6	17.4	5.7	15.7	Clearwater, ID
81.5	17.7	5.5	15.8	Clearwater, ID
81.5	16.8	5.7	16.4	Clearwater, ID
77.3	16.8	5.5	14.9	Clearwater, ID
80.8	17.9	5.4	17	Clearwater, ID
80	16.7	5.6	15.9	Sapphire Range, MT
80.3	16.7	5.6	15.5	Sapphire Range, MT
81.3	17.3	6.4	17.1	Sapphire Range, MT
78.9	16.4	5.9	15	Sapphire Range, MT
79.7	16.6	5.7	16.4	Sapphire Range, MT
80.9	16.8	5.5	15.6	Sapphire Range, MT
78.8	17.3	5.2	15	Sapphire Range, MT
79.3	17.6	6.3	16.3	Sapphire Range, MT
83.7	16.6	5.6	16.4	Sapphire Range, MT
79.8	17.6	5.8	15.8	Sapphire Range, MT
79.6	16.7	5.7	15.4	Sapphire Range, MT
80.2	18.1	5.6	17.7	Red Lodge, MT
81.9	17.6	5.9	16.7	Red Lodge, MT

83.3	17.2	5.6	17.3	Red Lodge, MT
79.5	16.8	6.1	16.3	Red Lodge, MT
81	17.9	6.1	17.7	Red Lodge, MT

Appendix 2 One-way analysis of variance results for the auditory bulla of eight pine marten populations (data are taken from Wright (1953)).

	Group 2			Group 3			Group 4			
	F	P	F Critical	F	P	F Critical	F	P	F Critical	
Group 1	0.84088	0.37007	4.35125	0.166678	0.684934	4.047109	7.308391	0.015067	4.451322	
Group 2	4			0.675461	0.41498	4.030397	6.955037	0.015403	4.324789	
Group 3							11.96953	0.001145	4.042647	
Group 4										
Group 5										
Group 6										
Group 7										
Group 8										

	Group 5			Group 6			Group 7			Group 8		
	F	P	F Critical	F	P	F Critical	F	P	F Critical	F	P	F Critical
	11.86963	0.002307	4.300943	52.81696	6.76718E-	4.380751	63.22469	2.67263E-	4.413862	17.30405	0.001323	4.747221
	11.97441	0.001875	4.225199	80.13219	5.91732E-	4.279343	102.5802	9.56954E-	4.300943	27.32107	8.30493E	4.493998
	24.51328	7.88652E	4.023007	95.49701	3.47010E-	4.034319	108.5098	5.09969E-	4.038383	28.39541	3.42734E	4.067047
	0.665288	0.423066	4.279343	28.81446	2.96787E-	4.351250	40.82126	3.96900E-	4.380751	7.532221	0.016712	4.667185
				16.6943	0.000397	4.241699	23.27009	6.49865E-	4.259675	3.105633	0.094994	4.413863
							0.922687	0.347701	4.324789	2.112299	0.166720	4.543068
										5.550411	0.033582	4.600111