INTER- AND INTRASPECIFIC PHYLOGEOGRAPHY OF NORTH AMERICAN Odocoileus DEER BASED ON MITOCHONDRIAL DNA SEQUENCES

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INTER- AND INTRASPECIFIC PHYLOGEOGRAPHY OF NORTH AMERICAN *ODOCOILEUS* DEER BASED ON MITOCHONDRIAL DNA SEQUENCES

by

© Annette D. Greenslade, B.Sc. (Honours)

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ABSTRACT

Phylogenetic analysis of mitochondrial DNA (mtDNA) cytochrome *b* sequences identified four major mtDNA genotype assemblages among populations of *Odocoileus* in western North America. These assemblages correspond to northwestern black-tailed deer, southwestern mule deer, northwestern mule deer, and southwestern white-tailed deer. Approximate times of the divergence of these assemblages, calculated on the basis of pairwise nucleotide sequence divergence estimates, were used to construct a model of *Odocoileus* evolution in western North America. According to this model, black-tailed deer represent the ancestral *Odocoileus* mtDNA lineage. Mule deer and white-tailed deer diverged more recently in palaeontological history. The southwestern white-tailed deer mtDNA genotype assemblage is more closely related to the mule deer mtDNA assemblages than it is to the southeastern white-tailed deer lineage. This suggests that the southwestern white-tailed deer mtDNA lineage may have been effectively replaced by mule deer mtDNA through relatively recent hybridization between the two species.

Microgeographic analysis of deer from southern Alberta revealed a considerable degree of geographic structuring of mtDNA sequence genotypes, as did qualitative analysis of the geographic distribution of mule deer and white-tailed deer mtDNA genotypes in California and Alberta. The phylogeographic structure may be maintained by philopatry of the female (or family) social units of *Odocoileus* deer, despite their potentially high vagility.

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Gratitude is extended as appropriate to my supervisory committee (S. M. Carr, D. J. Innes, and E. H. Miller), my colleagues, and those researchers who provided deer samples: S. Lingle collected samples from the McIntyre Ranch near Magrath, Alberta, and the Waterton Lakes National Park, Alberta; M. Cronin (LGL Alaska) provided samples from southern California; C. Strobeck (University of Alberta, Edmonton) provided samples from British Columbia, Manitoba, Sasketchewan, and Ontario; and S. Carr (Memorial University of Newfoundland, St. John's) provided samples from western North America. DNA extractions of the samples from S. Lingle were carried out by J. Coffin *et al.* of C. Strobeck's research lab. S. Lingle also provided a UTM map showing the sampling location of most of the deer she collected; Figure 2 is based on this map.

Many thanks to M. Dennison and A. Lynch (both at The Royal Ontario Museum) for help with preparing the maps, to O. Haddrath (The Royal Ontario Museum) for advice with phylogenetic analyses, and to A. J. Baker (The Royal Ontario Museum) for reading an earlier draft of my thesis and offering invaluable advice (thanks for the encouragement).

Thank you to my supervisory committee for reading drafts of the thesis and for

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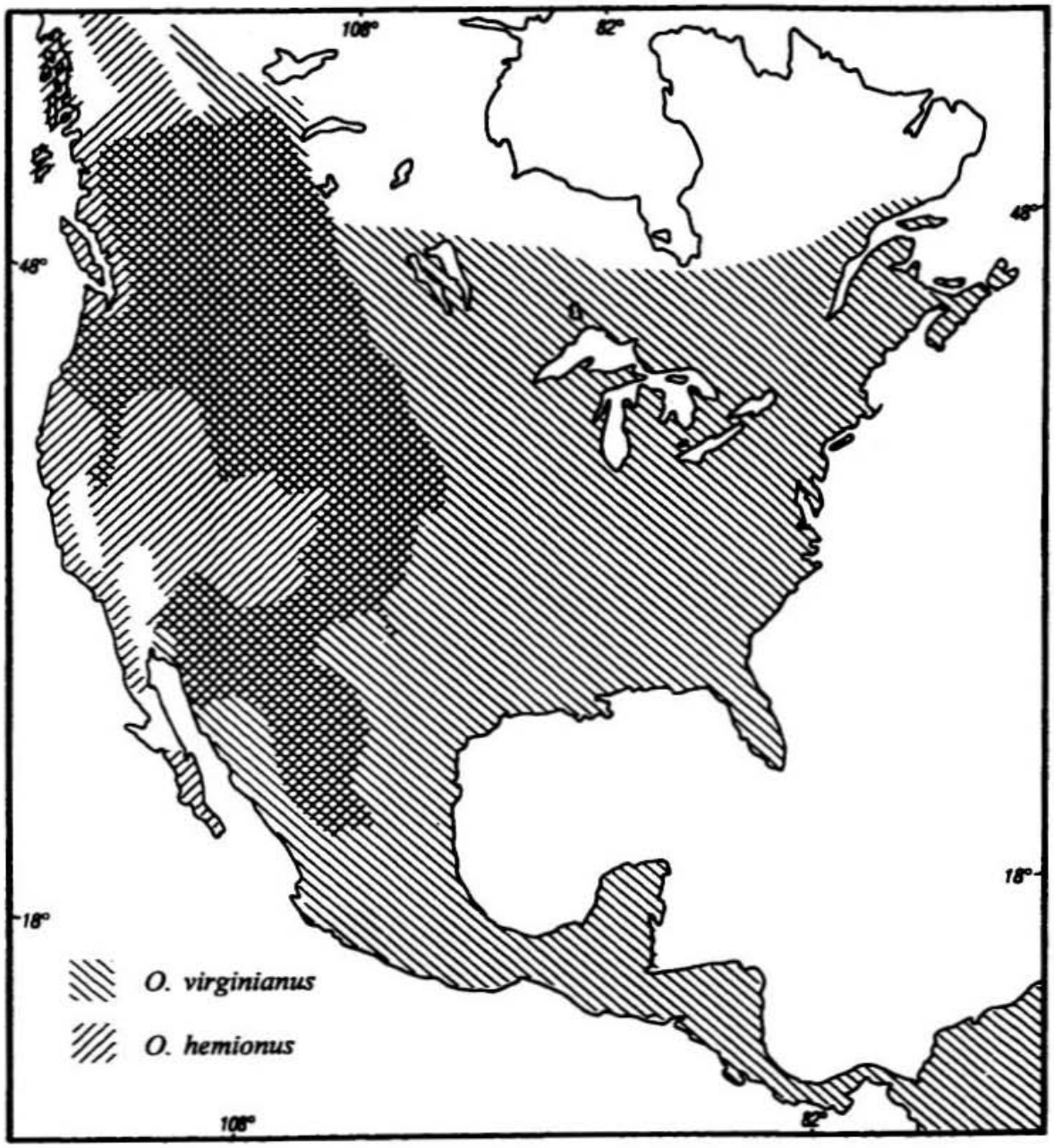
INTRODUCTION

The genus *Odocoileus* comprises two extant species, *O. virginianus* (white-tailed deer) and *O. hemionus* (mule deer and black-tailed deer). Grubb (1993) places them within the subfamily Capreolinae (family Cervidae, order Artiodactyla), whereas most authorities place them within their own subfamily, the Odocoileinae (Whitehead, 1972; Baker, 1984; Miyamoto *et al.*, 1993). The two species are sympatric over much of their range west of the Rocky Mountains (Figure 1); the 30 subspecies of *O. virginianus* currently recognized (Baker, 1984) are broadly distributed in mesic and tropical environments throughout North America and extend into Central and South America (McCullough, 1987), while the seven *O. hemionus* subspecies (Wallmo, 1981) occupy a more restricted range in the xeric environments of western North America, from southeastern Alaska to western Mexico. The distribution of *O. h. columbianus* (Columbian black-tailed deer) extends from central California to southern British Columbia, and is adjacent to the distribution of *O. h. sitkensis* (Sitka black-tailed deer), which extends northward along the coast of British Columbia to southeastern Alaska (Wallmo, 1981). All other subspecies of *O. hemionus* are referred to as mule deer, and occupy the remainder of the geographic area identified in Figure 1 as the *O. hemionus* species range. Subspecies of *O. hemionus* are characterized by

Figure 1: Geographic distribution of *Odocoileus virginianus* (white-tailed deer) and *O. hemionus* (mule deer and black-tailed deer) in North America. The map was assembled by S. Carr, based on Baker (1984). Cross-hatching indicates the area of sympatry between the species.


108°
82°
48°
48°
18°
18°
O. virginianus
O. hemionus
108°
82°

discontinuities in characters that result from barriers to gene flow, such as deserts and mountain ranges. In contrast, geographic variation in white-tailed deer is clinal, and differences among subspecies seem to be largely arbitrary (McCullough, 1987). The two species, however, can be readily distinguished on the basis of several characteristics, including those of morphology, ecology, and behaviour (Baker, 1984; Marchinton and Hirth, 1984). Some of the morphological features that differentiate the two species of *Odocoileus* are antler shape, length and position of the metatarsal gland, and appearance of tail, ear, and rump patch. For example, the antler tines bifurcate in mule deer, but not in white-tailed deer, and the beams tend to curve forward and turn inwards more in the latter species. White-tailed deer have longer tails of lighter colour. Metatarsal gland characteristics are considered to be the most reliable in distinguishing the two species (Derr *et al.*, 1991). The metatarsal ridge of white-tailed deer is considerably shorter (25 mm) relative to that of mule deer (125 mm), with no overlap in range; the metatarsal gland of white-tailed deer is located below the midpoint of the shank, whereas it is above the midpoint of the shank in most mule deer; and the metatarsal tuft is white in colour in white-tailed deer, and brown in mule deer (Wishart, 1980).

White-tailed deer and mule deer are regarded as reproductively isolated species (Wallmo, 1981), although evidence suggests that hybridization and introgression of

genetic material occurs between species or subspecies (Ballinger *et al.*, 1992; Carr and Hughes, 1993; Carr *et al.*, 1986; Hughes and Carr, 1993; McClymont *et al.*, 1982; Wishart, 1980). Deer with intermediate morphological characteristics have been produced in captivity, and reported in the wild in western North America. Wishart (1980) indicated that hybrids in Alberta are intermediate in some characters and more like one of the parental stocks in others. The tails of all hybrids are dark, shading into black towards the tip. The intermediate location and size of the metatarsal gland and the conspicuous white or mostly white hairs surrounding the gland are typical hybrid characters.

The occurrence of hybrid individuals has prompted a number of studies of *Odocoileus*, particularly at the genetic and behavioural levels. White-tailed and mule deer differ in habitat preference, with white-tailed deer preferring wetter, more wooded areas that offer much cover, whereas mule deer prefer more open and rugged areas (Kramer, 1973; Swenson *et al.*, 1983; Smith, 1987). This difference does not appear to be the result of competition for food resources, despite similar food preferences, nor does it seem to be caused by direct behavioural interactions. Geist (1981) suggested that the different antipredator responses of white-tailed and mule deer underlie their habitat segregation. White-tailed deer avoid predators at large distances and gallop when threatened, whereas mule deer approach disturbances and avoid predators by

means of an escape gait described as a stott. The escape gait of captive hybrid individuals, described as a bound, is intermediate between that of each of the parental species; it is slow and mechanically inefficient, which may result in ineffective responses to predators in the deer's natural habitat. This could in turn lower the chances of hybrid survival relative to white-tailed and mule deer, thus restricting contemporary genetic interchange between the two species (Lingle, 1992; Lingle 1993).

Many genetic studies of *Odocoileus* have investigated interspecific and intraspecific genetic variation. Several of these studies have involved analysis of the nuclear genome using protein electrophoresis. One of the earliest such studies was an analysis of blood serum protein variation between *O. virginianus* and *O. hemionus*, and among subspecies of *O. hemionus* (Cowan and Johnston, 1962). Other allozyme studies have focussed on *Odocoileus* populations in specific geographic locations, such as South Carolina (Cothran *et al.*, 1983; Ramsey *et al.*, 1979), Tennessee (Kennedy *et al.*, 1987), Maryland (Sheffield *et al.*, 1985), Texas (Stubblefield *et al.*, 1986), Alberta (McClymont *et al.*, 1982), Oregon and Washington (Gavin and May, 1988), and the southwestern United States (Derr, 1991).

A second approach has been the analysis of the mitochondrial genome, either as the primary source of genetic information or in conjunction with allozyme analysis.

Mitochondrial DNA (mtDNA) has been used in a large number of studies to investigate aspects of genetic diversity, phylogeography, population structure and dynamics, and population evolution. Animal mtDNA is a double-stranded, covalently closed circular molecule found within the mitochondria. It has a conserved gene content, consisting of two ribosomal RNA genes, 22 transfer RNA genes, and 13 protein genes which encode enzyme subunits that function in electron transport or ATP synthesis (Wilson *et al.*, 1985; Moritz *et al.*, 1987). Animal mtDNA lacks introns, repetitive DNA, and pseudogenes; intergenic sequences are small or absent (Avise *et al.*, 1987; Moritz *et al.*, 1987).

The popularity of mtDNA in evolutionary studies is due to a number of characteristics of the molecule, including its small size, high copy number, the relative ease with which it can be isolated from nuclear DNA, its fast rate of sequence evolution, conserved gene content, maternal inheritance, effective haploidy, and lack of recombination (Palumbi and Baker, 1994; Slade *et al.*, 1993; Zhang and Hewitt, 1996). The availability of universal primers for use in mtDNA amplification via the polymerase chain reaction (PCR), and the existence of a large data set for use in comparative studies have also made mtDNA the molecule of choice for many studies (Palumbi and Baker, 1994; Moritz *et al.*, 1987).

Although the uniparental inheritance of mtDNA makes it an effective marker in

studies of hybridization and matrilineal gene flow (Carr and Hughes, 1993; Hughes and Carr, 1993; Avise, 1994), it does not allow the reconstruction of paternal lineages (Wilson *et al.*, 1985; Avise *et al.*, 1987). This limitation may not affect studies of species that do not exhibit gender-biased dispersal, but will be important if the species under study have complex social behaviours and differences in the dispersal of males and females. In such cases, the population structure as indicated by maternally inherited mtDNA may be quite different from that indicated by biparentally-inherited nuclear DNA (Palumbi and Baker, 1994). However, comparisons of the spatial distribution of nuclear and mitochondrial markers can be used to study sexual bias in dispersal (Moritz *et al.*, 1987).

Because mtDNA does not undergo recombination, the evolution and inheritance of mtDNA are less complicated to decipher, relative to nuclear DNA. However, the entire mitochondrial genome must be regarded as a single genetic locus (Slade *et al.*, 1993; Palumbi and Baker, 1994); in the absence of recombination, the 37 genes effectively behave as a single genetic entity (Wilson *et al.*, 1985) that is passed on to progeny in its entirety (Attardi, 1985).

Other potential complications in studies based on mtDNA are the detection of nuclear copies (pseudogenes) of mitochondrial sequences, and paternal leakage of mtDNA. If mitochondrially-derived sequences are present in the nuclear genome, and a total

genomic DNA extract is used as the initial DNA source in PCR, the nuclear copies may co-amplify, thereby causing ambiguities in the data that appear as sequence heteroplasmy and genetic polymorphism (Zhang and Hewitt, 1996). Gyllensten *et al.* (1991) showed that a low proportion of paternal mtDNA could be produced in heteroplasmic experimental hybrids by repeated backcrossing between mouse species *Mus musculus* and *M. domesticus*. If the occurrence and degree of paternal leakage of mtDNA were great enough, mtDNA molecules could potentially recombine, thereby complicating the reconstruction and interpretation of mtDNA-based genealogies (Avise, 1991). Paternal leakage of mtDNA does not seem to be a factor in most studies, however.

Genetic studies of *Odocoileus* have reported low levels of hybridization in deer populations from Montana (Cronin *et al.*, 1988), Alberta (Hughes, 1990; Hughes and Carr, 1993; McClymont *et al.*, 1982), and the Pacific Northwest (Cronin, 1991; Gavin and May, 1988), and a high level of hybridization in Texas (Ballinger *et al.*, 1992; Carr *et al.*, 1986; Stubblefield *et al.*, 1986). With regards to mtDNA genotypes identified by direct sequence analysis, there are several cases of a genotype typical of one species being found in individuals that are phenotypically the other species. Hughes and Carr (1993) report one mule deer individual from Alberta that had a white-tailed deer mtDNA genotype (EDN), and one white-tailed deer, also from Alberta, that

had a mule deer mtDNA genotype (ELP). Such evidence suggests reciprocal hybridization between the two species.

The term "phylogeography" was first used by Avise *et al.* (1987) and is discussed in greater detail by Avise (1994). Avise (1994) has defined phylogeography as "the study of the principles and processes governing the geographic distributions of genealogical lineages, including those at the intraspecific level" (p. 233). The application of modern molecular techniques, especially those that use mtDNA, to investigations of population genetics has prompted the development of the discipline of intraspecific phylogeography. The advantageous characteristics of mtDNA (maternal, non-recombining mode of inheritance, and rapid rate of sequence evolution) often produce multiple alleles or genotypes that can be ordered phylogenetically within a species, thus producing an intraspecific phylogeny or gene genealogy. Studies have indicated that mtDNA clones and clades of some species are geographically structured, thus illuminating the link between phylogeny and geography, and therefore between evolution and the physical environment.

The study of mtDNA as a molecular marker allows maternal lineages to be traced through space and time, and thus permits reconstruction of the evolutionary history of a species. The present-day distribution of any given species represents the product of many factors, including those inherent in the environment and in the organism itself.

Physiogeographic factors such as mountain ranges and river systems may act as barriers to dispersal, while the vagility of the organism will also be of importance in establishing the geographic distribution of a species. It is expected that highly vagile organisms will not show a great deal of phylogeographic structure. Although this is the case for animals such as black bears (*Ursus americanus*) (Cronin *et al.*, 1991) and coyotes (*Canis latrans*) (Lehman and Wayne, 1991; Lehman *et al.*, 1991), populations of other species, such as humpback whales (*Megaptera novaeangliae*) (Baker *et al.*, 1990) are subdivided, as indicated by localized genealogical structure and/or major gaps in the mtDNA phylogeny across the species range (Avise, 1994).

Behavioural characteristics, such as philopatry and dispersal, will also affect the level of phylogeographic structure exhibited by a given species. While the social organization of a species may restrict dispersal, particularly if it involves gender-biased dispersal, its effects may not be obvious or detected in molecular studies of the nuclear genome. Such behaviour would appear to be of greater consequence when the molecular marker used is found within the mitochondrial genome; if the females of the species exhibit philopatric behaviour with respect to breeding, and the social organization involves the establishment of female-based family groups, as in *Odocoileus*, the mtDNA phylogeny is expected to indicate a high degree of spatial-genetic structure.

The current study analyzes the phylogenetic relationships among *Odocoileus* mtDNA genotypes in western North America, and interprets these relationships within a biogeographical context. Whereas previous genetic studies of *Odocoileus* have focused on specific populations or geographic regions within North America, the current study attempts to combine and extend existing mtDNA data sets and consider the large-scale inter- and intraspecific phylogenetic relationships within *Odocoileus*.

MATERIALS AND METHODS

SAMPLE COLLECTION

Samples used in this study came from a variety of sources. A set of white-tailed deer and mule deer tissue (ear notches or ear punches) and/or blood samples from fawns on the McIntyre Ranch near Magrath, Alberta, and Waterton Lakes National Park, Alberta, was collected (S. Lingle). Additional samples were from deer that were found dead, either as a result of predation or road accidents. Samples were collected between October 1993 and October 1994, with most from July 1994. The extraction of DNA from these samples was performed by C. Strobeck's lab, University of Alberta, Edmonton, Alberta, Canada. A sub-set of duplicate tissue samples were also sent directly to me.

Additional samples representing other geographic locations were also obtained. These included a small set of DNA extracts from deer collected in British Columbia, Manitoba, Saskatchewan, and Ontario (C. Strobeck); a set of mule deer DNA samples from southern California consisting of DNA from two representative individuals of each of eight mitochondrial DNA genotypes as identified by restriction-fragment-length-polymorphism (RFLP) analysis (Cronin and Bleich, 1995); and representative DNA samples for each of a series of previously identified cytochrome *b* mtDNA genotypes

within white-tailed, black-tailed and mule deer in western North America (Carr and Hughes, 1993; Hughes and Carr, 1993). A single moose (*Alces alces*) sample was also provided by S. Carr for use as an outgroup in phylogenetic analyses.

DNA EXTRACTION

The majority of samples were provided in the form of extracted DNA. Samples provided by C. Strobeck were extracted with a commercial kit, the QIAamp Tissue Kit (QIAGEN Inc., Chatsworth, CA), according to manufacturer's instructions. DNA samples from M. Cronin and S. Carr were extracted by standard techniques as described in Cronin and Bleich (1995) and Carr and Marshall (1991).

For those samples provided in tissue form, DNA was extracted using the acid quanidinium thiocyanate-phenol-chloroform procedure of Chomczynski and Sacchi (1987) as modified by Bartlett and Davidson (1991). Approximately 100-200 mg of tissue were homogenized using a sterile plastic pestle in 450 μL of a solution containing 4 M guanidinium thiocyanate, 25 mM sodium citrate (pH 7.0), 0.5% Sarkosyl^{R}, and 0.1 M 2-mercaptoethanol. A 50 μL volume of sodium acetate (2 M, pH 4.1) was added to the homogenate, followed by 300 μL of Tris-saturated phenol, and 150 μL of chloroform/isoamyl alcohol (24:1, v/v). The solution was mixed and left on ice for 15 minutes. Following this incubation, the sample was centrifuged at 10 000 x *g* for 15 minutes at 4°C, after which the upper (aqueous) phase was transferred to a new 1.5 mL

microfuge tube, 450 µL of chloroform/isoamyl alcohol (24:1, v/v) was added, and the solutions were mixed gently by inverting the tube. The sample was centrifuged at 10 000 x *g* for 15 minutes at 4°C, after which the upper (aqueous) phase was transferred to a new 1.5 mL microfuge tube and 400 µL of cold isopropanol was added. The solutions were mixed by inverting the tube and were left at -20°C for at least two hours (or overnight) to precipitate the nucleic acids. The sample was then centrifuged at 10 000 x *g* for 15 minutes at 4°C, and the isopropanol was removed and discarded, leaving the nucleic acid pellet in the tube. A 150 µL volume of fresh ice-cold (-20°C) 75% ethanol was added to the tube, and was followed by another centrifugation step (10 000 x *g*, 15 minutes, 4 °C). The ethanol was removed, the pellet was dried briefly (approximately 10 minutes) under vacuum and was resuspended in 100 µL of sterile distilled water.

AMPLIFICATION OF DNA

Amplification of a segment of the mitochondrial DNA genome was carried out using the polymerase chain reaction (PCR). The sequences of the primers used to amplify and sequence a 401-nucleotide fragment of the cytochrome *b* gene are as follows: 5'-GCCCCTCAGAATGATATTTGTCCTCA-3' (H15149) (modified from Kocher *et al.*, 1989) and 5'-CGAAGCTTGATATGAAAAACCATCGTTG-3' (L14724) (Irwin *et al.*,

1991). Primers were synthesized by the Oligonucleotide Synthesis Laboratory, Queen's University, Kingston, Ontario, Canada.

Amplifications were carried out in 100 µL final volume reactions containing 67 mM Tris-HCl (pH 9.0 at 25°C), 2 mM $MgCl_2$, 10 mM 2-mercaptoethanol (all Sigma), 0.2 mM each of dATP, dCTP, dGTP, and dTTP (Pharmacia), 10 pmol each of the heavy- and light-strand primers, and 1 unit of Amplitaq^R DNA polymerase (Perkin-Elmer Cetus). For each reaction, 2 µL of DNA extract were added. Most samples were amplified in a Perkin-Elmer Cetus GeneAmp 9600 Thermal Cycler with the following step-cycle profile: an initial 5 minute denaturation at 95°C, followed by 35 cycles of 93°C for 15 seconds, 40°C for 30 seconds, 55°C for 30 seconds, and 72°C for 45 seconds. This was followed by a final extension step of 72° for 2 minutes. The standard PCR cycle consists of a denaturation step, followed by a single annealing step, and then an elongation step. The cycle used here uses two annealing steps (40°C for 30 seconds and 55°C for 30 seconds) in order to prevent disassociation of the primer from the template DNA between the annealing and elongation phases. The intermediate step between the annealing and elongation steps makes the increase in temperature more gradual. An alternative approach is to use a temperature ramp. Some samples were amplified in a Perkin-Elmer Cetus TC-1 DNA Thermal Cycler using an equivalent cycle: an initial 5 minute denaturation at 95°C, followed by 35 cycles of 93°C for 1 minute, 40°C for 1 minute,

55°C for 30 seconds, and 72°C for 2 minutes. The final extension step was 72°C for 10 minutes. The differences in cycle profile reflect differences in the respective thermal cyclers; PCR products obtained using each machine were of comparable quality. The reaction mixtures were the same for both thermal cyclers, except that a drop of light mineral oil (Sigma) was added to each sample amplified in the TC-1 Thermal Cycler in order to prevent evaporation of the sample during amplification.

Following amplification, a 5 μL portion of the product was added to 1 μL of 5x tracking dye (25% glycerol [1.26 g/mL stock], 50 mM Na_2EDTA, 0.5% SDS, 0.1% bromophenol blue) and subjected to electrophoresis through a 2% NuSieve^R GTG^R agarose (FMC) gel in 1x TBE buffer (pH 7.4). The presence of amplified DNA was confirmed by visualization of the DNA using ethidium bromide (1 μg/mL) under 302 nm UV illumination. A molecular weight standard (*Hae*III digest of Φ X phage DNA; Pharmacia) was also run on the gel to estimate the size of the product and ensure amplification of the appropriate fragment. Direct purification of the product was carried out using the Magic^R PCR Preps DNA Purification System (Promega Corp.) according to manufacturer's instructions. The purified DNA product was quantified using a Hoefer model TKO 100 DNA Fluorometer (Hoefer Scientific Instruments) and a 250 μg/mL calf thymus DNA (Clontech) weight standard reference.

DNA SEQUENCING

The DNA sequence of each sample was determined using an Applied Biosystems model 373A automated DNA sequencer and the Applied Biosystems PRISM^{R} Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit. Reactions were set up according to manufacturer's instructions. A total of 400 ng of template DNA were used, which corresponds to 3.2 pmol of DNA. The amount of template DNA required in the sequencing reaction depends on the size of the fragment; equimolar amounts of primer and template DNA produce optimal results. The DNA was completely dried under vacuum prior to the addition of the reaction mixture (9.5 μL of the manufacturer's terminator premix, 3.2 μL (3.2 pmol) of primer, and 7.3 μL of sterile distilled water). The sequencing primers were the same primers used to amplify the target DNA (H15149 and L14724). Cycle sequencing of all samples was performed in a Perkin-Elmer Cetus TC-1 Thermal Cycler using the following step-cycle profile: 98°C for 1 second, 50°C for 15 seconds, and 60°C for 4 minutes, for a total of 25 cycles.

The samples were then purified by means of Sephadex^{R} G50 Fine (Pharmacia) spin column purification. Columns were prepared by filling empty columns (5 Prime -> 3 Prime) with Sephadex^{R}, placing the columns in 1.5 mL collecting tubes, and centrifuging for one minute at 1,100 x *g* in a clinical centrifuge. The excess water was removed from the collecting tubes, the columns were placed in the same set of tubes and centrifuged for

30 seconds at 1,100 x *g* to remove any remaining water. The water was removed from the collecting tubes and the entire cycle sequencing product was carefully loaded onto the center of the Sephadex^{R} resin bed (one sample per column). Each column was placed in a sterile labelled 1.5 mL collecting tube, and was then centrifuged for 3 minutes at 1,100 x *g* to collect the DNA. The DNA samples were completely dried under vacuum, resuspended in 4 μL of a mixture of 5μL of formamide and 1 μL of 50 mM Na_2EDTA, heated to 95°C for 2.5 minutes, and then chilled immediately on ice prior to being loaded on a 6% polyacrylamide denaturing gel. Gels were run for 8 hours at 32W on the 373A automated sequencer (Applied Biosystems).

DATA ANALYSIS

Data generated by the automated sequencer were collected and analyzed using the Data Collection (v.1.2), Analysis (v.1.2) and SeqEd™ (v.1.2) (Applied Biosystems, Inc.) programs. The DNA sequence data were exported to and aligned using the Eyeball Sequence Editor (ESEE) (version 2.00) program of Cabot and Beckenbach (1989). The Molecular Evolutionary Genetics Analysis (MEGA) program (v.1.01) (Kumar *et al.*, 1993) was used to compute pairwise distance estimates (transitions + transversions) under the Kimura 2-parameter substitution model (1980), and a neighbor-joining tree (Saitou

and Nei, 1987) was constructed using the distance matrix. The moose, *Alces alces*, was used as an outgroup. Neighbor-joining trees were also constructed in MEGA using only synonymous substitutions, and using only nonsynonymous substitutions, both with a Jukes and Cantor (1969) correction.

Puzzle, a maximum likelihood-based quartet puzzling program (Strimmer and von Haeseler, 1996), was also used in phylogenetic reconstruction. The type of analysis selected was tree reconstruction using the quartet puzzling tree search procedure over 1000 puzzling steps. *Alces alces* was used as an outgroup. The Tamura and Nei (1993) model of nucleotide substitution was selected. Puzzle assessed the robustness of the reconstructed tree via reliability percentages (*i.e.*, the number of times a group is reconstructed during the puzzling steps).

Cladistic analysis was performed on the sequence data using the Phylogenetic Analysis Using Parsimony (PAUP) (v.4.0d51 and subsequent versions) program (Swofford, 1996). The heuristic search algorithm was used to identify maximum parsimony trees, and analysis was carried out using ten random stepwise additions of taxa and the nearest-neighbour interchange branch-swapping option. Branches were collapsed, creating polytomies, in any cases where the maximum branch length equaled zero. All nucleotide substitutions were weighted equally, and *Alces alces* was used as the outgroup in the initial heuristic search that was performed on all taxa simultaneously. A 50% majority

rule consensus tree was generated from the 106 trees found by this search. Further heuristic searches were conducted based on this initial result, as detailed in the Results section.

Area cladograms that show the geographic distributions of the mtDNA lineages in Alberta and California were constructed by plotting the points at which each genotype occurred and enclosing the distribution of a given genotype in a minimum polygon. These figures were based on the collection sites of previous studies from which the samples sequenced in the current study were obtained.

A model of the evolutionary history of *Odocoileus* in western North America was constructed from estimates of divergence between pairs of nucleotide sequences within and among mtDNA lineages. Estimates of times since divergence were calculated as the number of pairwise differences observed between any two genotypes, divided by the total number of nucleotide bases analyzed per sequence. The approximate time of divergence of major genotype assemblages was then estimated using published rates for the mtDNA genome as outlined in the Discussion.

The data obtained from the deer samples from the Magrath, Alberta and Waterton Lakes National Park, Alberta area were analyzed statistically on a microgeographic scale. MEGA (Kumar *et al.*, 1993) was used to calculate pairwise haplotype divergences from the 401-nucleotide sequence data for each genotype identified in the region. These

divergence values and the frequency of occurrence of each genotype in mule deer and white-tailed deer were then used by the Restriction Enzyme Analysis Package (REAP) program (v.4.0) (McElroy *et al.*, 1991) to calculate the haplotypic or nucleon diversity (h) index for non-selfing populations and the nucleotide diversity (π) index of Nei and Tajima (1981) (equations 8.4 and 10.4 of Nei, 1987). The haplotypic diversity (h) index approximates the probability that any two individuals chosen at random from within a population will have different haplotypes. A value of 0.0 indicates complete fixation of a haplotype, whereas a value of 1.0 indicates that all individuals in the sample have different haplotypes (Nei, 1987). The nucleotide diversity (π) index measures the average pairwise nucleotide difference between individuals within a sample. This index corrects h for the size of the nucleon studied (Nei, 1987). These indices were used to estimate genetic heterogeneity within populations, with all mule deer being one population and all white-tailed deer being another population within the one geographic region. Estimates of nucleotide diversity and nucleotide divergence among populations were also calculated by REAP. The geographic distribution of mtDNA genotypes within this region was also plotted on a universal transect method (UTM) map.

RESULTS

Microgeographic analysis of deer from Magrath and Waterton Lakes National Park.

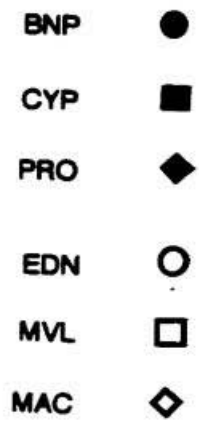
Of the 19 white-tailed deer and 42 mule deer from the Magrath and Waterton Lakes National Park area of Alberta for which mtDNA sequence data were obtained, three were identified as having mtDNA genotypes typically found in the other species. Two mule deer samples had the EDN genotype, and one white-tailed deer sample had the CYP genotype (Table 1). Based on previous studies (Carr and Hughes, 1993; Hughes and Carr, 1993), the EDN genotype is typically found only in white-tailed deer, whereas the CYP genotype has previously only been found in mule deer. One new genotype (MAC) was found in a single white-tailed deer; all other genotypes were previously reported, though not all genotypes identified by Hughes and Carr (1993) among *Odocoileus* in western Canada were found in this sampling location (Table 1).

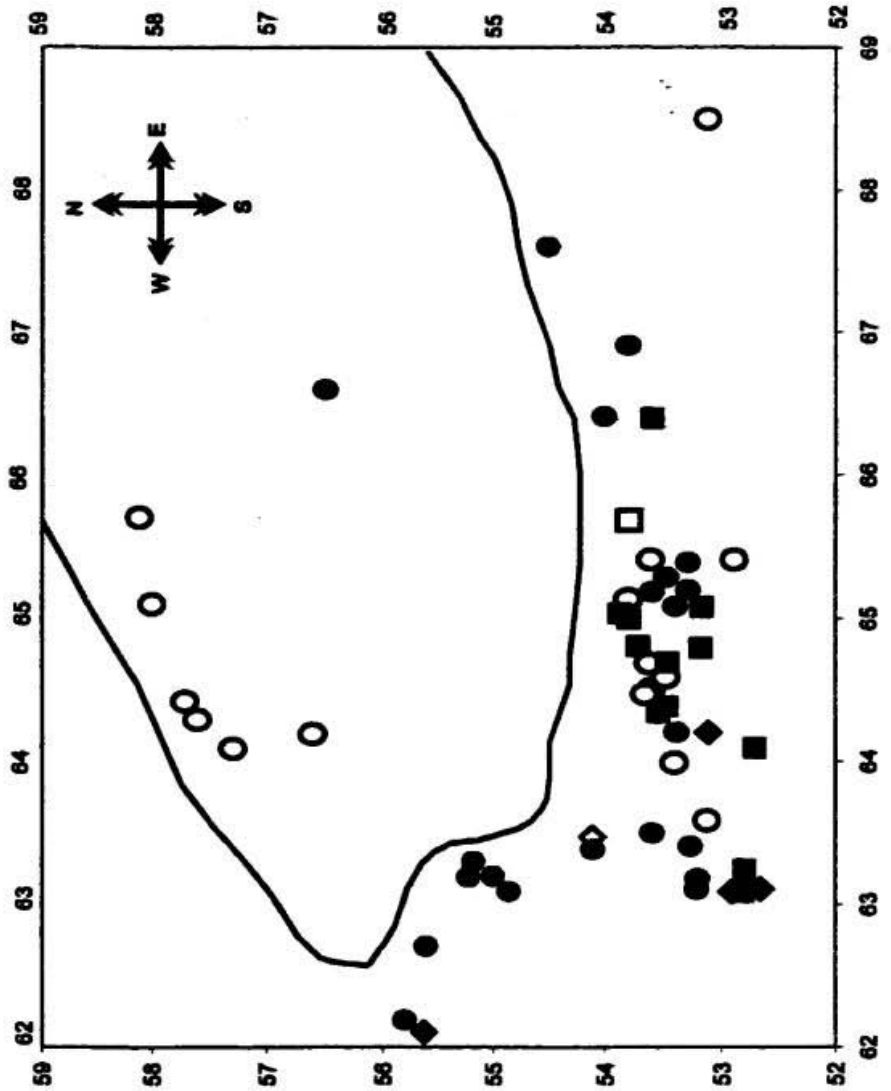
Figure 2 shows the origin of individual deer within southern Alberta at the time of sampling. Map co-ordinates were available for 55 of the 61 individuals for which sequence data were obtained and these appear on the figure. Most genotypes occur at higher altitudes (the area below the contour line on the figure). This pattern is more obvious in the mule deer genotypes, with only one representative individual occurring in

Table 1 : Mitochondrial DNA genotypes found in deer from the McIntyre Ranch near Magrath, Alberta, and Waterton Lakes National Park, Alberta. Genotype codes are those reported by Hughes and Carr (1993), with the exception of the single new genotype, MAC.

Genotype	Number of Deer	
	mule deer	white-tailed deer
BNP	24	0
PRO	4	0
CYP	12	1
EDN	2	16
MVL	0	1
MAC	0	1
Total	42	19

Figure 2: Sampling location of white-tailed and mule deer mtDNA genotypes within the southern Alberta collection site (McIntyre Ranch near Magrath, and Waterton Lakes National Park). Each genotype is represented by a distinct symbol, as indicated in the key. Mule deer genotypes are represented by filled circles and white-tailed deer genotypes are represented by open circles. The region below the contour line is more mountainous terrain, and the region below the line is predominantly lowland. Values on the abscissa and the ordinate correspond to UTM (universal transect method) A and B coordinates, respectively. A map of Canada indicates the geographic location of the collection site.




E
N
S
W
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58
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66
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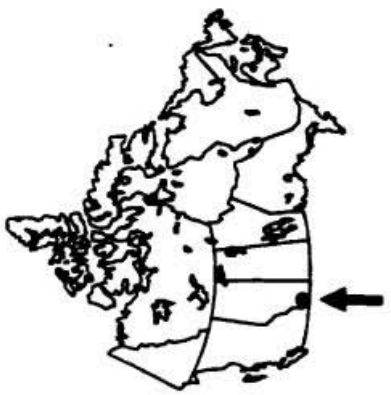
68
69



the lowlands (the area above the contour line). The most common mule deer genotype is BNP (Table 1). Individuals with the white-tailed deer genotype EDN, the most common white-tailed deer genotype in the region, occurred in both the highlands and the lowlands (Figure 2).

Mule deer and white-tailed deer from the Magrath and Waterton Lakes National Park region of Alberta were genetically characterized through the calculation of indices of haplotype and nucleotide diversity (Table 3). The pairwise haplotype divergence estimates upon which these calculations were based appear in Table 2. The haplotype diversity (h) estimates within mule deer and within white-tailed deer are 0.5875 and 0.2902, respectively, which indicate that the probability of two individual deer chosen at random having different genotypes is approximately 59% for mule deer and 29% for white-tailed deer. The nucleotide diversity (π) estimate for within-mule deer was 0.007079, which indicates that any two individual mule deer chosen at random differ by approximately three nucleotide substitutions in the 401-nucleotide region of the cytochrome *b* gene studied (*i.e.*, 401 x 0.007079 ≈ 2.8). The corresponding value for within-white-tailed deer is 0.002521, which indicates that any two individual deer of this species chosen at random differ by approximately one nucleotide substitution in the 401-nucleotide region of the cytochrome *b* gene studied. The within-species estimates of haplotype and nucleotide diversity for mule deer and white-tailed deer indicate that mule

Table 2: Pairwise haplotype divergence estimates for the mtDNA genotypes identified in mule deer and white-tailed deer from the McIntyre Ranch near Magrath, Alberta, and Waterton Lakes National Park, Alberta. The number of nucleotide substitutions between pairs of genotypes is shown in the upper matrix. The divergence estimates are shown in the lower matrix.

	BNP	PRO	CYP	EDN	MVL	MAC
BNP	-	7	3	5	5	3
PRO	0.01754	-	10	5	6	6
CYP	0.00752	0.02506	-	6	6	4
EDN	0.01253	0.01002	0.01504	-	2	2
MVL	0.01253	0.01504	0.01504	0.00501	-	2
MAC	0.00752	0.01504	0.01002	0.00501	0.00501	-

Table 3: Indices of haplotype (h) and nucleotide (π) diversity within populations of mule deer and white-tailed deer from the McIntyre Ranch near Magrath, Alberta, and Waterton Lakes National Park, Alberta.

Population	Haplotype Diversity	Nucleotide Diversity
Mule deer	0.5875	0.007079
White-tailed deer	0.2902	0.002521

deer are genetically more variable than white-tailed deer in the Magrath and Waterton Lakes National Park area of Alberta.

New mtDNA sequence genotypes identified.

Several new mtDNA sequence genotypes were identified in this study among samples collected in western Canada. One new genotype (MAC) was found in a single white-tailed deer from the Magrath and Waterton Lakes National Park area of Alberta, and a second new genotype (KNP) was obtained from a white-tailed deer from Kootenay National Park, British Columbia. A white-tailed deer from Saskatchewan had a mtDNA genotype (SSK) that was identical to a genotype (CRO03) identified in a mule deer collected in southern California. As a result, this genotype (SSK) was coded as a mule deer genotype in phylogenetic analyses. The samples from southern California represent the genetic variation as determined from RFLP analysis. The RFLP-genotypes listed in Table 4 have also been found at several other geographic locations within the United States, including Montana, Colorado, Utah, Oregon, Arizona and Washington (Cronin, 1991; Cronin and Bleich, 1995). Within the set of samples from southern California deer, only one individual had a sequence-genotype reported previously (SLO; Carr and Hughes, 1993); all other sequence-genotypes are new (Table 4). Two of the RFLP-genotypes (D and O) have identical sequence-genotypes (CRO04). Both samples

Table 4 : Correspondence of 401-nucleotide sequence genotype with 307-nucleotide sequence genotype (Carr and Hughes, 1993; Hughes, 1990; Hughes and Carr, 1993), and with RFLP-genotype (Cronin, 1991; Cronin and Bleich, 1995).

RFLP-genotype	307-nucleotide cyt *b* genotype	401-nucleotide cyt *b* genotype
A	CYP	CRO01
A	CYP	CRO02
B	SWN	CRO03
D	BNP	CRO04
O	------	CRO04
K	------	CRO05
L	------	CRO06
L	------	CRO07
M	------	CRO08
M	------	CRO09
N	SLO	CRO10
N	------	CRO11

representing RFLP-genotype K had the same sequence-genotype (CRO05), and both individuals of RFLP-genotype B had a sequence-genotype CRO03. For the remaining RFLP-genotypes (A, L, M, N), the two individuals within each pair could be distinguished from each other at the DNA sequence level; *e.g.*, the two individuals with RFLP-genotype A had distinct sequence-genotypes, CRO01 and CRO02.

Comparison of previously reported mtDNA sequence genotypes and new data.

Representative individuals of each mtDNA sequence-genotype reported by Carr and Hughes (1993) and Hughes and Carr (1993) based on 307 nucleotides of sequence data were reanalyzed to give 401 nucleotides of sequence data (ie, an additional 94 nucleotides of sequence at the 5-prime end of the gene fragment were obtained). Some of the mtDNA genotypes identified in deer collected in Canada and in the United States were assigned different three-letter codes when first reported on the basis of 307 nucleotides of sequence; *e.g.*, the genotype TEH reported by Carr and Hughes (1993) genotype ELP reported by Hughes and Carr (1993) have identical sequences. These two genotypes (TEH and ELP) were also identical when the additional 94 nucleotides of data were considered. In contrast, SAN differs from ETX by an RFLP, yet the two genotypes have identical 401-nucleotide sequences. BNP and REY are identical to each other on the basis of 401 nucleotides of sequence data, but were distinct on the basis of 307

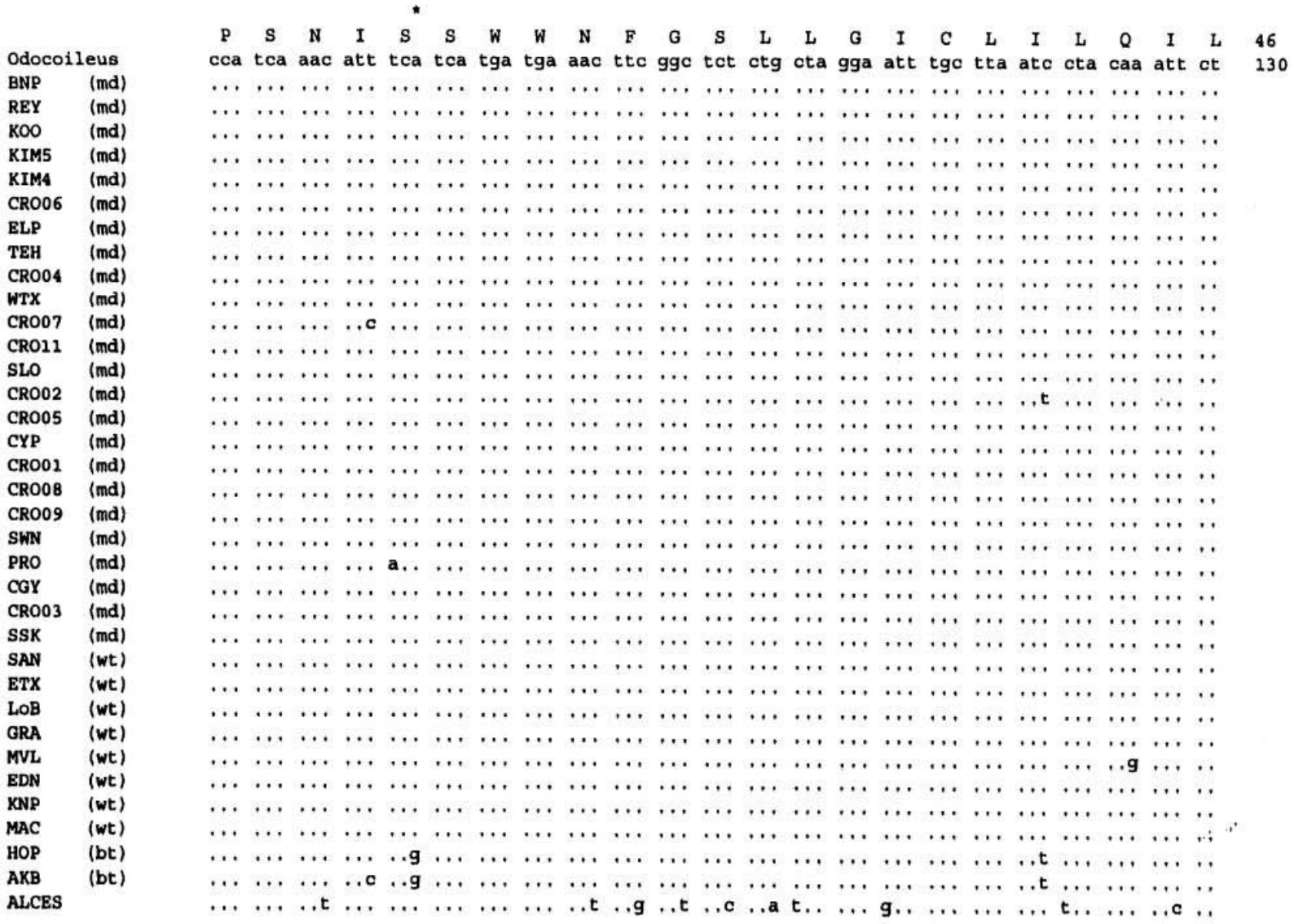
nucleotides of data. BNP and KIM can be distinguished from each other on the basis of nucleotide substitutions within the additional 94 nucleotides of information, as can SLO and CYP; the 307-nucleotide data set suggested that BNP and KIM, and SLO and CYP were identical in sequence composition. The two individuals representing the KIM genotype (Carr and Hughes, 1993) that were amplified and sequenced had different 401-nucleotide sequences; KIM5 had two nucleotide substitutions not present in KIM4 (Figures 3 and 4). In some cases, substitutions within the additional 94 nucleotides of the 401-nucleotide sequence identified variation within genotypes defined by the 307-nucleotide or RFLP genotypes. CRO01 and CRO02 are variant forms of the RFLP-genotype A / CYP sequence genotype. The 401-nucleotide sequence data shows three substitutions between CRO01 and CRO02, two substitutions between CRO02 and CYP, and one substitution between CRO01 and CYP. Similarly, SWN and CRO03 are variant forms of the RFLP-genotype B (Figures 3 and 4).

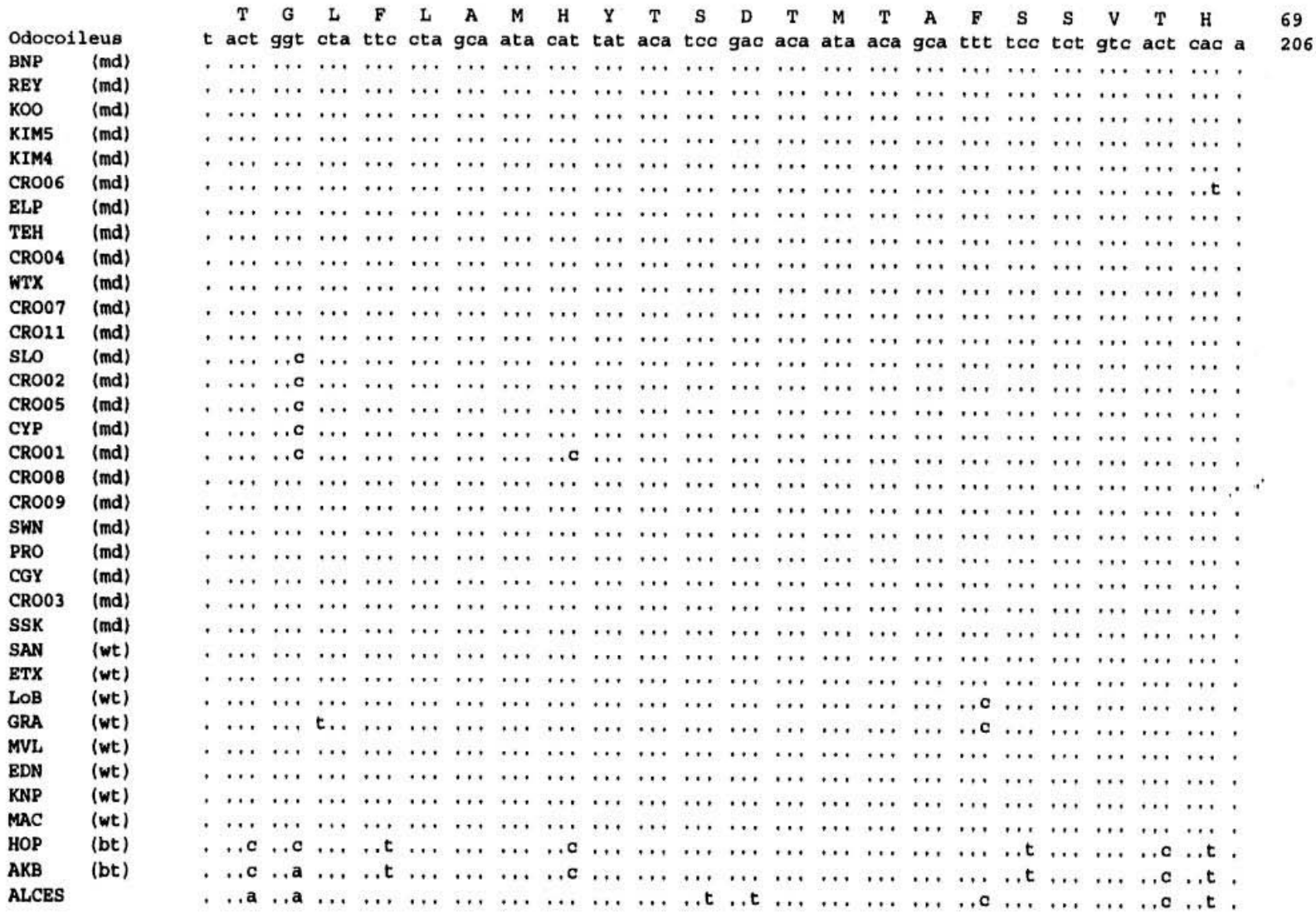
Pattern of nucleotide substitution among Odocoileus *mtDNA sequence genotypes.*

The distribution of nucleotide variance in the 401-nucleotide data is presented in Table 5. A total of 44 variable sites among *Odocoileus* genotypes was identified. Most of these sites were two-fold variable, and one was three-fold variable (T, C, A). Most of the substitutions were transitions (89%), with 83% at the third position. This pattern agrees

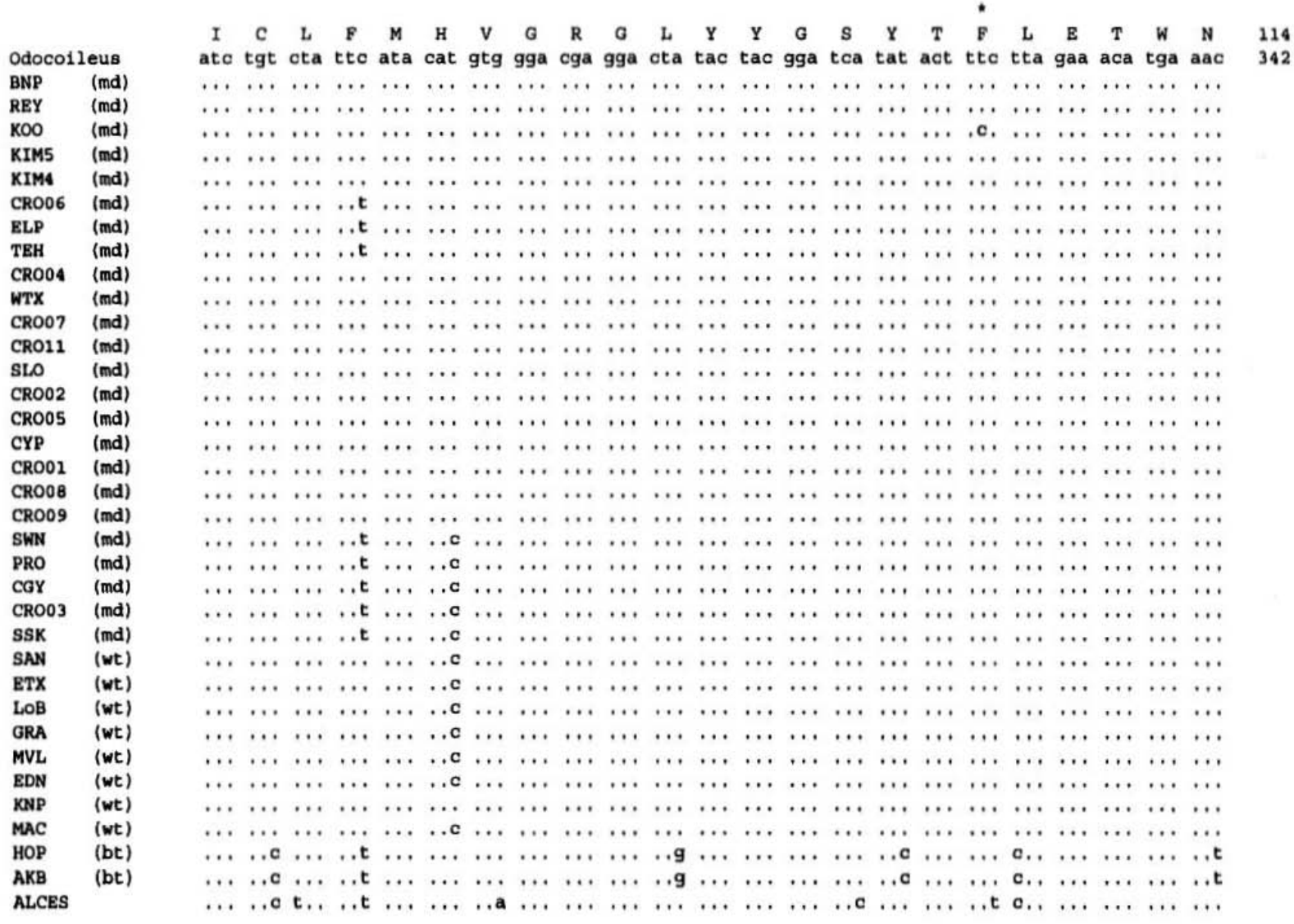
Figure 3 : Mitochondrial cytochrome *b* sequences of genotypes reported in *Odocoileus*. DNA sequences represent a 401-nucleotide fragment of the gene. The inferred amino acid sequence is presented in the first line, and the second line is the reference *Odocoileus* sequence. Numbers at the ends of the first and second lines indicate the positions in the amino acid and nucleotide sequences, respectively. Dots are used to indicate positions sharing the same nucleotide as the reference sequence. Those nucleotide substitutions resulting in amino acid replacements are indicated by asterisks. The moose (*Alcles alces)* sequence is presented as an outgroup.

```
                    *       *                       *       *
                    M   T   N   I   R   K   T   H   P   L   M   K   I   V   N   N   A   F   I   D   L   P   A   23
Odocoileus          atg acc aac atc cga aaa acc cac cca cta ata aaa att gta aac aac gca ttc att gat ctt cca gcc   39
BNP         (md)    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
REY         (md)    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
KOO         (md)    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
KIM5        (md)    ... g.. ... .c. ... ... ... ... ... ... ... ... ... ... ... ... a.g ... ... ... ... ... ...
KIM4        (md)    ... g.. ... .c. ... ... ... ... ... ... ... ... ... ... ... ... a.. ... ... ... ... ... ...
CRO06       (md)    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..c ... ... ...
ELP         (md)    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..g ... ... ..c ... ... ...
TEH         (md)    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..g ... ... ..c ... ... ...
CRO04       (md)    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
WTX         (md)    ... ... ... ... ... ... ... ... ... ... ... ... ... ... .c. ... ... ... ... ... ... ... ...
CRO07       (md)    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..c ..t
CRO11       (md)    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..t
SLO         (md)    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..t
CRO02       (md)    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..t
CRO05       (md)    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..t
CYP         (md)    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
CRO01       (md)    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
CRO08       (md)    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..c ... ...
CRO09       (md)    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..g ... ... ... ... ... ...
SWN         (md)    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..c ... ...
PRO         (md)    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..g ... ... ... ..c ... ...
CGY         (md)    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..g ... ... ... ..c ... ...
CRO03       (md)    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..g ... ... ... ..c ... ...
SSK         (md)    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..g ... ... ... ..c ... ...
SAN         (wt)    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..c ... ...
ETX         (wt)    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..c ... ...
LoB         (wt)    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..c ... ...
GRA         (wt)    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..c ... ...
MVL         (wt)    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..c ... ...
EDN         (wt)    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..g ... ... ... ..c ... ...
KNP         (wt)    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..g ... ... ... ..c ... ...
MAC         (wt)    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
HOP         (bt)    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..t ...
AKB         (bt)    ... ... ... ... ... ... ... ... ... ..c ... ... ... ... ... ... ... ... ... ... ... ..t ...
ALCES               ... ... ... ... ... ... ... ... ... t.. ... ... ... ... ..t ... ... ..t ... ... ... ... ...
```


P S N I S S W W N F G S L L G I C L I L Q I L 46
Odocoileus cca tca aac att tca tca tga tga aac ttc ggc tct ctg cta gga att tgc tta atc cta caa att ct 130
BNP (md)
REY (md)
KOO (md)
KIM5 (md)
KIM4 (md)
CRO06 (md)
ELP (md)
TEH (md)
CRO04 (md)
WTX (md)
CRO07 (md)
CRO11 (md)
SLO (md)
CRO02 (md)
CRO05 (md)
CYP (md)
CRO01 (md)
CRO08 (md)
CRO09 (md)
SWN (md)
PRO (md)
CGY (md)
CRO03 (md)
SSK (md)
SAN (wt)
ETX (wt)
LoB (wt)
GRA (wt)
MVL (wt)
EDN (wt)
KNP (wt)
MAC (wt)
HOP (bt)
AKB (bt)
ALCES


T G L F L A M H Y T S D T M T A F S S V T H 69
Odocoileus t act ggt cta ttc cta gca ata cat tat aca tcc gac aca ata aca gca ttt tcc tct gtc act cac a 206
BNP (md)
REY (md)
KOO (md)
KIM5 (md)
KIM4 (md)
CRO06 (md)
ELP (md)
TEH (md)
CRO04 (md)
WTX (md)
CRO07 (md)
CRO11 (md)
SLO (md)
CRO02 (md)
CRO05 (md)
CYP (md)
CRO01 (md)
CRO08 (md)
CRO09 (md)
SWN (md)
PRO (md)
CGY (md)
CRO03 (md)
SSK (md)
SAN (wt)
ETX (wt)
LoB (wt)
GRA (wt)
MVL (wt)
EDN (wt)
KNP (wt)
MAC (wt)
HOP (bt)
AKB (bt)
ALCES

		I	C	R	D	V	N	Y	G	W	I	I	R	Y	M	H	A	N	G	A	S	M	F	F	91
Odocoileus		tc	tgc	cga	gac	gtt	aac	tat	ggc	tga	atc	att	cga	tat	ata	cat	gcc	aat	gga	gca	tcc	ata	ttc	ttt	274
BNP	(md)	..	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
REY	(md)	..	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
KOO	(md)	..	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
KIM5	(md)	..	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
KIM4	(md)	..	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
CRO06	(md)	..	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
ELP	(md)	..	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
TEH	(md)	..	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
CRO04	(md)	..	...	...	...	...	...	...	...	...	..t	...	...	...	...	...	...	...	...	...	...	...	...	...	
WTX	(md)	..	...	...	...	...	...	...	...	...	..t	...	...	...	...	...	...	...	...	...	...	...	...	...	
CRO07	(md)	..	...	...	...	...	...	...	...	...	..t	...	...	...	...	...	...	...	...	...	...	...	...	...	
CRO11	(md)	..	...	...	...	...	...	...	...	...	..t	...	...	..c	...	...	...	...	...	...	...	...	...	...	
SLO	(md)	..	...	...	...	...	...	...	...	...	..t	...	...	..c	...	...	...	...	...	...	...	...	...	...	
CRO02	(md)	..	...	...	...	...	...	...	...	...	..t	...	...	..c	...	...	...	...	...	...	...	...	...	...	
CRO05	(md)	..	...	...	...	...	...	...	...	...	..t	...	...	..c	...	...	...	...	...	...	...	...	...	...	
CYP	(md)	..	...	...	...	...	...	...	...	...	..t	...	...	..c	...	...	...	...	...	...	...	...	...	...	
CRO01	(md)	..	...	...	...	...	...	...	...	...	..t	...	...	..c	...	...	...	...	...	...	...	...	...	...	
CRO08	(md)	..	...	...	...	...	...	...	...	...	...	...	...	..c	...	...	...	...	...	...	...	...	...	...	
CRO09	(md)	..	...	...	...	...	...	...	...	...	...	...	...	..c	...	...	...	...	...	...	...	...	...	...	
SWN	(md)	..	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
PRO	(md)	..	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..c	
CGY	(md)	..	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
CRO03	(md)	..	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
SSK	(md)	..	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	
SAN	(wt)	..	...	...	...	...	...	...	...	...	...	...	...	..c	...	...	...	...	...	...	...	...	...	...	
ETX	(wt)	..	...	...	...	...	...	...	...	...	...	...	...	..c	...	...	...	...	...	...	...	...	...	...	
LoB	(wt)	..	...	...	...	...	...	...	...	...	...	...	...	..c	...	...	...	...	...	...	...	...	...	...	
GRA	(wt)	..	...	...	...	...	...	...	...	...	...	...	...	..c	...	...	...	...	...	...	...	...	...	...	
MVL	(wt)	..	...	...	...	...	...	...	...	...	...	...	...	..c	...	...	...	...	...	...	...	...	...	...	
EDN	(wt)	..	...	...	...	...	...	...	...	...	...	...	...	..c	...	...	...	...	...	...	...	...	...	...	
KNP	(wt)	..	...	...	...	...	...	...	...	...	...	...	...	..c	...	...	...	...	...	...	...	...	...	...	
MAC	(wt)	..	...	...	...	...	...	...	...	...	...	...	...	..c	...	...	...	...	...	...	...	...	...	...	
HOP	(bt)	..	...	...	...	..c	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..c	
AKB	(bt)	..	...	...	...	..c	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..c	
ALCES		..	...	...	..t	..a	..t	..c	...	...	...	...	...	...	..g	...	..a	..c	...	..c	..a	...	..t	..c	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Odocoileus atc tgt cta ttc ata cat gtg gga cga gga cta tac tac gga tca tat act ttc tta gaa aca tga aac 342
BNP (md)
REY (md)
KOO (md)
KIM5 (md)
KIM4 (md)
CRO06 (md)
ELP (md)
TEH (md)
CRO04 (md)
WTX (md)
CRO07 (md)
CRO11 (md)
SLO (md)
CRO02 (md)
CRO05 (md)
CYP (md)
CRO01 (md)
CRO08 (md)
CRO09 (md)
SWN (md)
PRO (md)
CGY (md)
CRO03 (md)
SSK (md)
SAN (wt)
ETX (wt)
LoB (wt)
GRA (wt)
MVL (wt)
EDN (wt)
KNP (wt)
MAC (wt)
HOP (bt)
AKB (bt)
ALCES

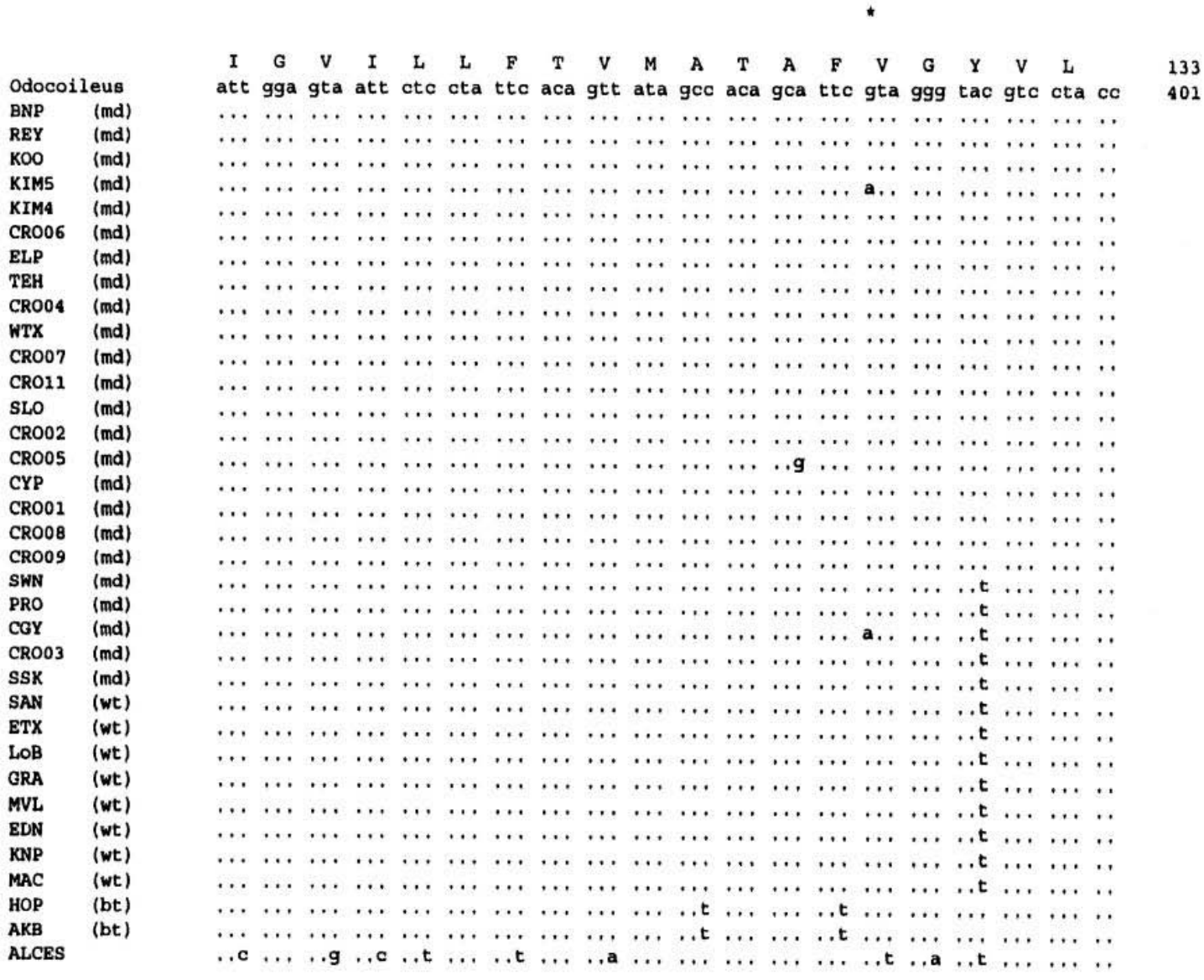

I G V I L L F T V M A T A F V G Y V L 133
Odocoileus att gga gta att ctc cta ttc aca gtt ata gcc aca gca ttc gta ggg tac gtc cta cc 401
BNP (md)
REY (md)
KOO (md)
KIM5 (md)
KIM4 (md)
CRO06 (md)
ELP (md)
TEH (md)
CRO04 (md)
WTX (md)
CRO07 (md)
CRO11 (md)
SLO (md)
CRO02 (md)
CRO05 (md)
CYP (md)
CRO01 (md)
CRO08 (md)
CRO09 (md)
SWN (md)
PRO (md)
CGY (md)
CRO03 (md)
SSK (md)
SAN (wt)
ETX (wt)
LoB (wt)
GRA (wt)
MVL (wt)
EDN (wt)
KNP (wt)
MAC (wt)
HOP (bt)
AKB (bt)
ALCES

Figure 4 : **Character matrix of the variable nucleotide sites used in the phylogenetic analysis of *Odocoileus* relationships. The position of each nucleotide in the original 401-nucleotide sequence data (Figure 3) is given in the first three lines, followed by the *Odocoileus* reference sequence. Dots indicate positions where the genotype sequence matches the reference. The moose (*Alces alces*) is presented as an outgroup sequence.**

```
                                                  11 111 111 111 111 111 122 222 222 222 222 222 222 333 333 333 333 333 333 33
                                  12 344 455 666 678 889 900 001 223 344 456 778 900 112 234 455 667 778 899 012 222 445 556 678 888 99
                                 418 045 914 036 981 246 925 895 672 514 502 149 214 692 543 625 140 390 514 681 678 251 473 951 457 03
BNP       (md)                   ATC AAC GAC TTA CCT TAC CCT GCA CCA TTT CCT CCT CTC CTC TCT ACT ACC TTC CTG AAT TCT CTA TCC TCA CGA GC
REY       (md)                   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
KOO       (md)                   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... C.. ... ... ... ..
KIM5      (md)                   GC. ... AG. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .A. ..
KIM4      (md)                   GC. ... A.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..
CRO06     (md)                   ... ... ... C.. ... ... ... ... ... ... ... ... ..T ... ... ... ... ... T.. ... ... ... ... ... ... ..
ELP       (md)                   ... ... .G. C.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... T.. ... ... ... ... ... ... ..
TEH       (md)                   ... ... .G. C.. ... ... ... ... ... ... ... ... ... ... ... ... ... ... T.. ... ... ... ... ... ... ..
CRO04     (md)                   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... .T. ... ... ... ... ... ... ... ... ... ..
WTX       (md)                   ... .C. ... ... ... ... ... ... ... ... ... ... ... ... ... .T. ... ... ... ... ... ... ... ... ... ..
CRO07     (md)                   ... ... ... .C. T.C ... ... ... ... ... ... ... ... ... ... .T. ... ... ... ... ... ... ... ... ... ..
CRO11     (md)                   ... ... ... ... T.. ... ... ... ... ... ... ... ... ... ... .TC ... ... ... ... ... ... ... ... ... ..
SLO       (md)                   ... ... ... ... T.. ... ... ... ... ..C ... ... ... ... ... .TC ... ... ... ... ... ... ... ... ... ..
CRO02     (md)                   ... ... ... ... T.. ... ... ... T.. ..C ... ... ... ... ... .TC ... ... ... ... ... ... ... ... ... ..
CRO05     (md)                   ... ... ... ... T.. ... ... ... ... ..C ... ... ... ... ... .TC ... ... ... ... ... ... ... ... ..G ..
CYP       (md)                   ... ... ... ... ... ... ... ... ... ..C ... ... ... ... ... .TC ... ... ... ... ... ... ... ... ... ..
CRO01     (md)                   ... ... ... ... ... ... ... ... ... ..C ..C ... ... ... ... .TC ... ... ... ... ... ... ... ... ... ..
CRO08     (md)                   ... ... ... .C. ... ... ... ... ... ... ... ... ... ... ... ..C ... ... ... ... ... ... ... ... ... ..
CRO09     (md)                   ... ... .G. ... ... ... ... ... ... ... ... ... ... ... ... ..C ... ... ... ... ... ... ... ... ... ..
SWN       (md)                   ... ... ... .C. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... TC. ... ... ... ... ... ... .T
PRO       (md)                   ... ... .G. .C. ... A.. ... ... ... ... ... ... ... ... ... ... ... ... C.. TC. ... ... ... ... ... ... .T
CGY       (md)                   ... ... .G. .C. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... TC. ... ... ... ... ... .A. .T
CRO03     (md)                   ... ... .G. .C. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... TC. ... ... ... ... ... ... .T
SSK       (md)                   ... ... .G. .C. ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... TC. ... ... ... ... ... ... .T
SAN       (wt)                   ... ... ... .C. ... ... ... ... ... ... ... ... ... ... ... ..C ... ... ... .C. ... ... ... ... ... ... .T
ETX       (wt)                   ... ... ... .C. ... ... ... ... ... ... ... ... ... ... ... ..C ... ... ... .C. ... ... ... ... ... ... .T
LoB       (wt)                   ... ... ... .C. ... ... ... ... ... ... ... ..C ... ... ..C ... ... ... .C. ... ... ... ... ... ... .T
GRA       (wt)                   ... ... ... .C. ... ... ... ... ... ... T.. ..C ... ... ..C ... ... ... .C. ... ... ... ... ... ... .T
MVL       (wt)                   ... ... ... .C. ... ... ... ... ..G ... ... ... ... ... ..C ... ... ... .C. ... ... ... ... ... ... .T
EDN       (wt)                   ... ... .G. .C. ... ... ... ... ... ... ... ... ... ... ..C ... ... ... .C. ... ... ... ... ... ... .T
KNP       (wt)                   ... ... .G. .C. ... ... ... ... ... ... ... ... ... ... ..C ... ... ... ... ... ... ... ... ... ... .T
MAC       (wt)                   ... ... ... ... ... ... ... ... ... ... ... ... ... ... ..C ... ... ... .C. ... ... ... ... ... ... .T
HOP       (bt)                   ... ... ... ..T ... .G. ... ... T.. .CC .TC ... TCT .C. ... ... ... CC. T.. G.C ..C T.. ... .T. T.. ..
AKB       (bt)                   ... C.. ... ..T ..C .G. ... ... T.. .CA .TC ... TCT .C. ... ... ... CC. T.. G.C ..C T.. ... .T. T.. ..
ALCES                            ..T ..T ..T ... .T. ..T GTC ATG .T. CAA ... TTC .CT TAT C.. GAC CAT CCT T.A .C. .TC .CG CTT A.. ..T AT
```

Table 5: Frequencies and distributions of variant nucleotides in *Odocoileus* within the 401-nucleotide cytochrome *b* sequence data. A total of 44 variable sites was identified, one of which is three-fold variable.

	Codon Position			
	First	Second	Third	Total
Transitions	4	3	33	40
Transversions	1	1	3	5
	5	4	36	45

with that reported in a previous study of *Odocoileus* in western Canada (Hughes, 1990). Transversions accounted for 11% of the variance. Seven of the nucleotide substitutions result in amino acid substitutions (Figure 3).

Phylogenetic analyses of mtDNA sequence genotypes.

The neighbor-joining tree constructed from the Kimura 2-parameter distance matrix (Figure 5) shows the presence of several groups of sequence genotypes. The black-tailed deer genotypes (HOP and AKB) clustered together and are the most divergent of the *Odocoileus* genotypes. Most of the mule deer genotypes are distributed across the middle portion of the tree, with the exception of a group of five genotypes (SWN, CGY, PRO, CRO03, and SSK) that is located within the white-tailed deer genotype cluster (LoB, GRA, ETX, MVL, SAN, MAC, EDN, and KNP). The mule deer genotypes CRO08 and CRO09 are also part of the white-tailed deer cluster. The neighbor-joining tree constructed using synonymous sites only (Figure 6) is almost identical to the tree that results from analysis of all sites simultaneously; the same major clusters of genotypes are present. In contrast, the neighbor-joining tree constructed using nonsynonymous sites only (Figure 7) shows essentially no resolution of the phylogenetic relationships among *Odocoileus* genotypes.

While the results of the maximum-likelihood quartet puzzling analysis (Figure 8) shows

Figure 5: Neighbor-joining tree constructed from Kimura-2 parameter pairwise distance estimates (transitions + transversions). The moose (*Alces alces*) was used as an outgroup.

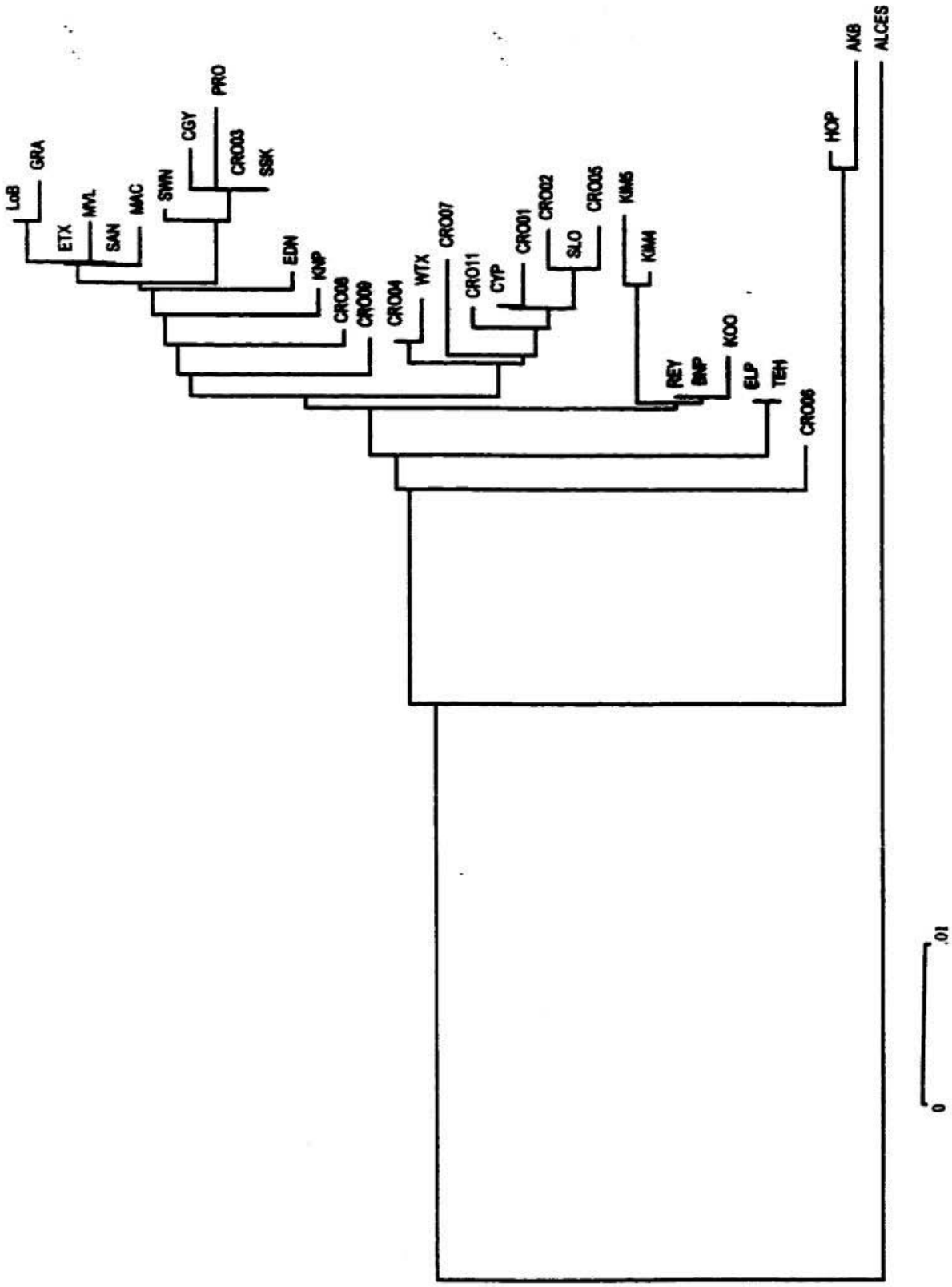

LoB
GRA
ETX
MVL
SAN
MAC
SWN
CGY
PRO
CRO03
SSK
EDN
KNP
CRO08
CRO09
CRO04
WTX
CRO07
CRO11
CYP
CRO01
CRO02
SLO
CRO05
KIM5
KIM4
REY
BNP
KOO
ELP
TEH
CRO06
HOP
AKB
ALCES
.01
0

Figure 6: **Neighbor-joining tree constructed using synonymous substitutions with a Jukes and Cantor correction. The moose *(Alces alces)* was used as an outgroup.**

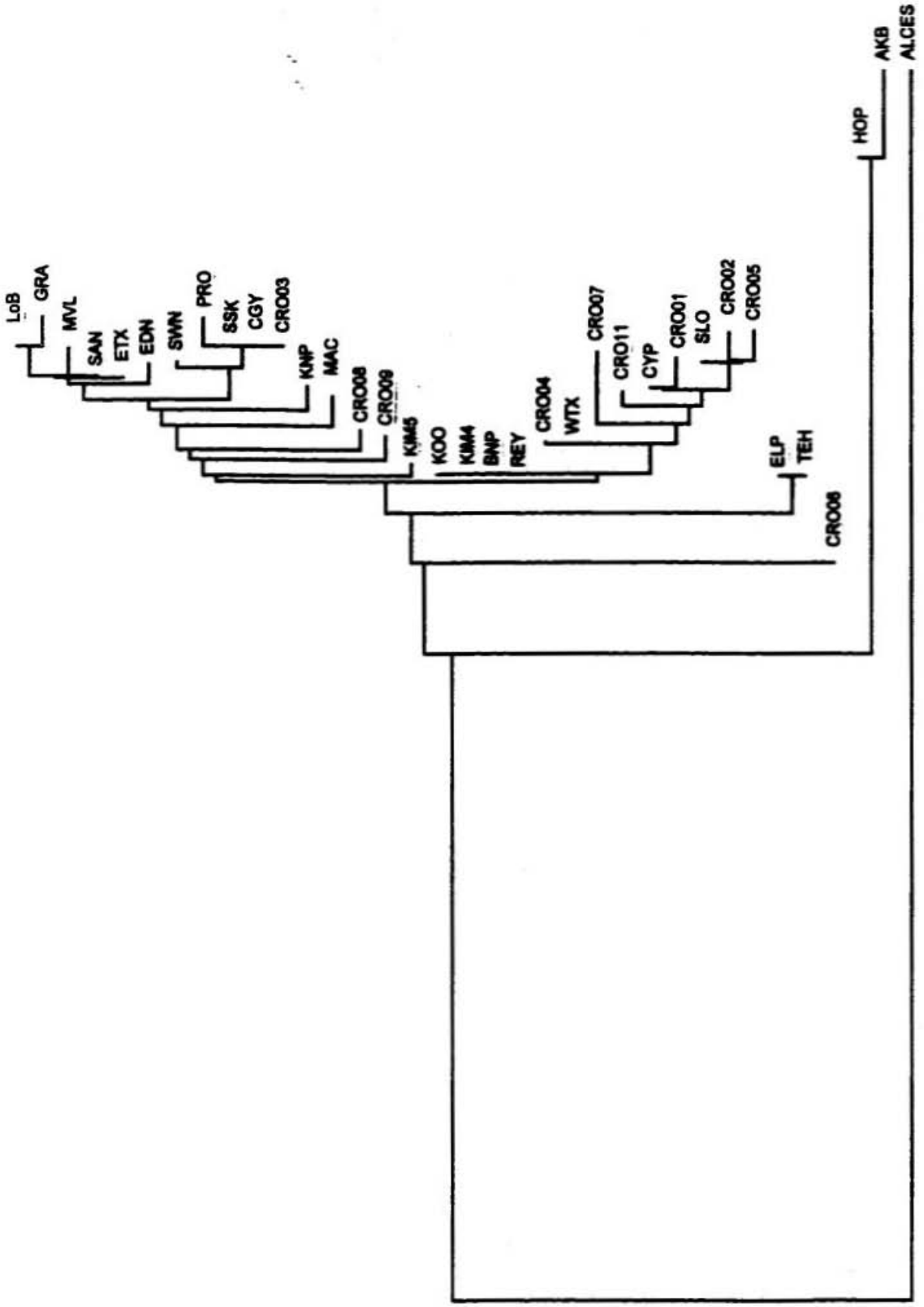

LoB
GRA
MVL
SAN
ETX
EDN
SWN
PRO
SSK
CGY
CRO03
KNP
MAC
CRO08
CRO09
KIM5
KOO
KIM4
BNP
REY
CRO04
WTX
CRO07
CRO11
CYP
CRO01
SLO
CRO02
CRO05
ELP
TEH
CRO06
HOP
AKB
ALCES
.1
0

Figure 7: **Neighbor-joining tree constructed using nonsynonymous substitutions with a Jukes and Cantor correction. The moose (*Alces alces*) was used as an outgroup.**

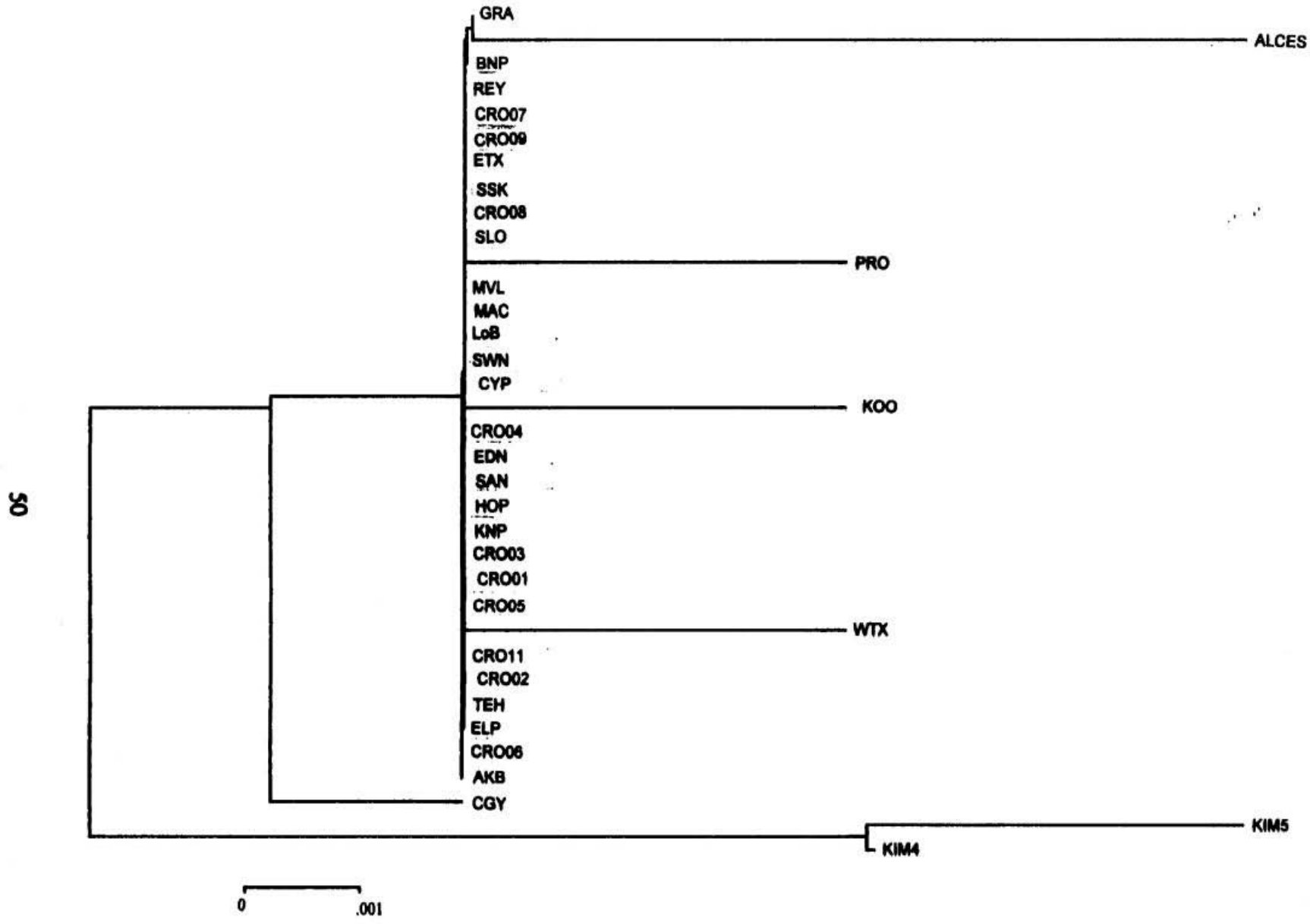
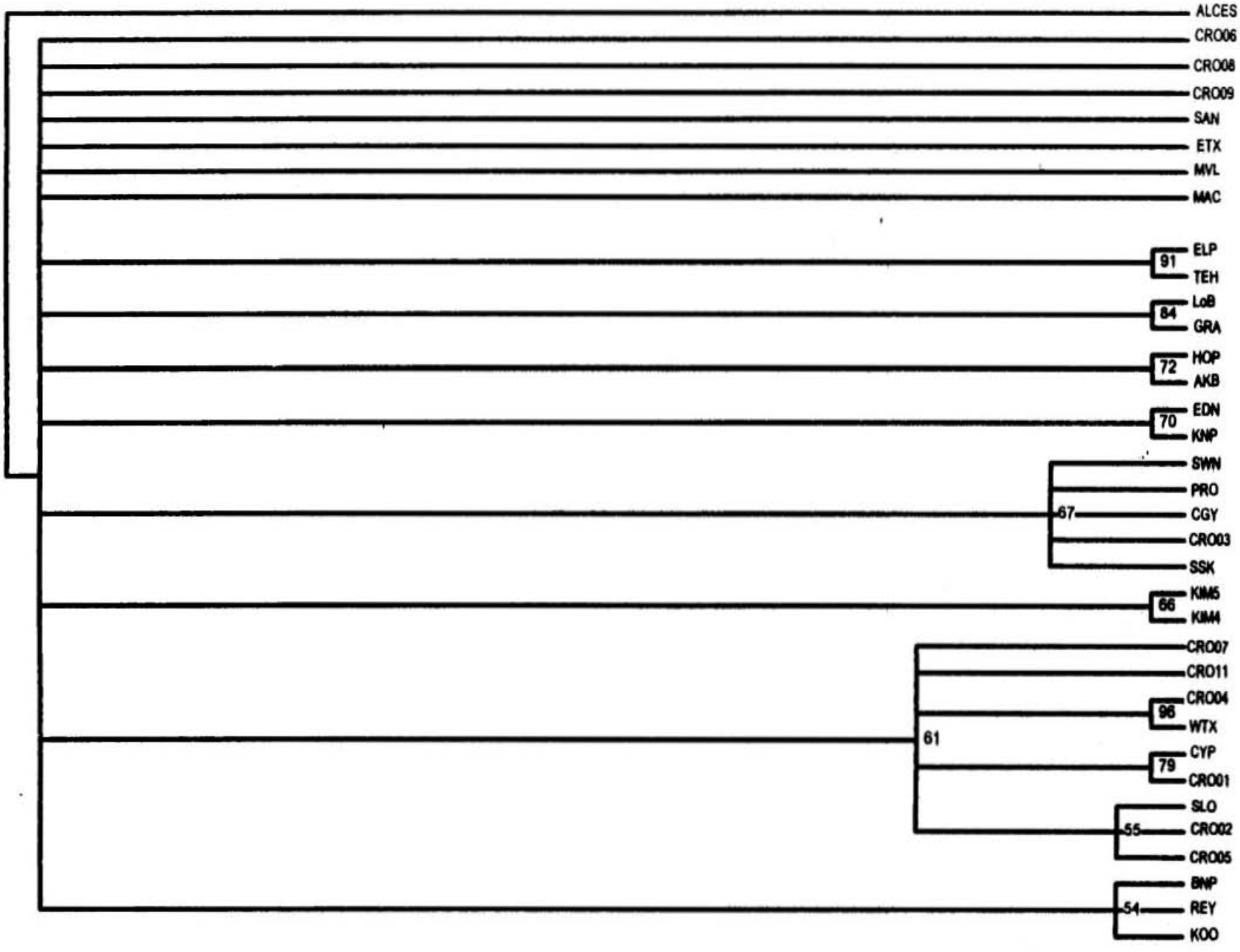

GRA
ALCES
BNP
REY
CRO07
CRO09
ETX
SSK
CRO08
SLO
PRO
MVL
MAC
LoB
SWN
CYP
KOO
CRO04
EDN
SAN
HOP
KNP
CRO03
CRO01
CRO05
WTX
CRO11
CRO02
TEH
ELP
CRO06
AKB
CGY
KIM5
KIM4
0
.001
50

Figure 8: **Quartet puzzling tree of the phylogenetic relationships among *Odocoileus* mtDNA sequence genotypes. The moose (*Alces alces)* was used as an outgroup. Reliability percentages are given for the internal nodes.**

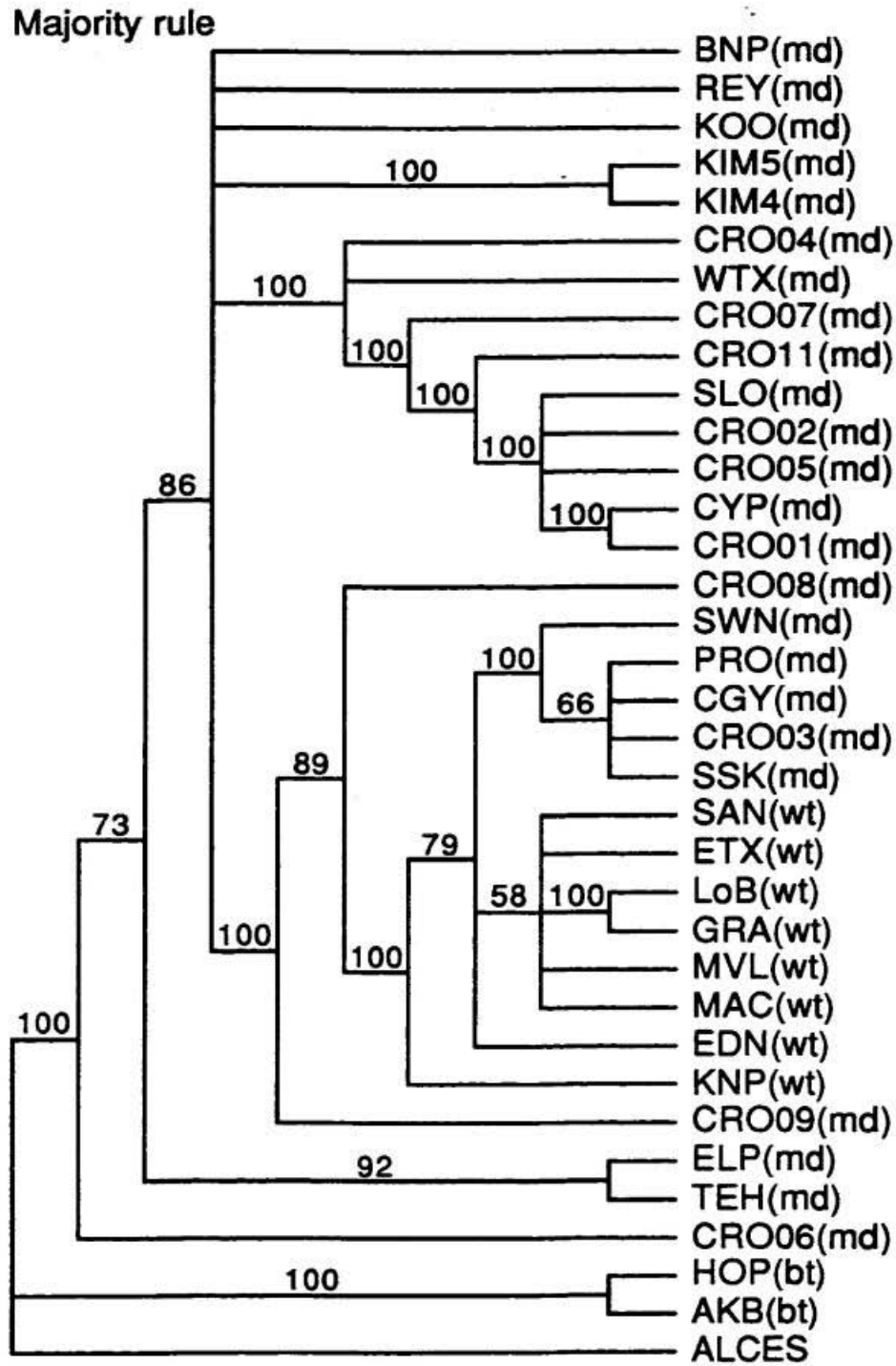

ALCES
CRO06
CRO08
CRO09
SAN
ETX
MVL
MAC
91
ELP
TEH
84
LoB
GRA
72
HOP
AKB
70
EDN
KNP
SWN
PRO
67
CGY
CRO03
SSK
66
KIM5
KIM4
CRO07
CRO11
96
CRO04
WTX
61
79
CYP
CRO01
SLO
55
CRO02
CRO05
BNP
54
REY
KOO

less resolution than the neighbor-joining analysis of all sites, some of the same clusters of genotypes are present in both trees. The set of five mule deer genotypes (SWN, PRO, CGY, CRO03, SSK) is present as a group in the quartet puzzling tree as well as the neighbor-joining tree, for example. This group was reconstructed during 67% of the 1000 puzzling steps.

The complete 401-nucleotide sequence data set included variants at 44 positions among the *Odocoileus* genotypes; cladistic analysis was performed on this subset of the data (Figure 4). Initial analysis of the original character matrix identified 106 identical minimum-length trees of length 100 and consistency index 0.8000. The 50% majority rule consensus tree of the 106 minimum-length trees (Figure 9) shows a comparable degree of resolution to the neighbor-joining tree (Figure 5). The two black-tailed deer genotypes (HOP and AKB) form a distinct clade relative to the other *Odocoileus* sequence genotypes. The same set of mule deer genotypes (SWN, PRO, CGY, CRO03, and SSK) appear within the white-tailed deer genotype clade (SAN, ETX, LoB, GRA, MVL, MAC, EDN, and KNP), as do the mule deer genotypes CRO08 and CRO09. The relationships among all genotypes were not fully resolved by the initial heuristic search. As a result, two exploratory searches were conducted.

The distribution of the variation among *Odocoileus* genotypes (Figure 4) suggests the presence of four main groups. Black-tailed deer (HOP and AKB) are clearly a separate

Figure 9: **Maximum parsimony 50% majority rule consensus tree of the 106 minimum length trees recovered by a heuristic search of the unmodified mtDNA sequence genotype data. The percentage of the trees in which groupings occurred appear on the tree. The moose (*Alces alces*) was used as an outgroup.**


Majority rule
BNP(md)
REY(md)
KOO(md)
KIM5(md)
KIM4(md)
CRO04(md)
WTX(md)
CRO07(md)
CRO11(md)
SLO(md)
CRO02(md)
CRO05(md)
CYP(md)
CRO01(md)
CRO08(md)
SWN(md)
PRO(md)
CGY(md)
CRO03(md)
SSK(md)
SAN(wt)
ETX(wt)
LoB(wt)
GRA(wt)
MVL(wt)
MAC(wt)
EDN(wt)
KNP(wt)
CRO09(md)
ELP(md)
TEH(md)
CRO06(md)
HOP(bt)
AKB(bt)
ALCES
100
100
100
100
100
86
66
100
89
73
79
58
100
100
100
100
92
100

group since the two genotypes share a number of substitutions unique to that group. White-tailed deer genotypes (SAN, ETX, LoB, GRA, MVL, EDN, KNP, and MAC) also share many of the same substitutions within their group; most genotypes share the same substitutions at sites 63, 243, 291, and 393. In terms of the mule deer genotypes, there appear to be at least two groups, one of which shares three substitutions with the white-tailed deer genotypes (SWN, PRO, CGY, CRO03, SSK; sites 63, 291, 393), and the other (all remaining mule deer genotypes) of which shares only one substitution with the white-tailed deer genotypes (site 243). The presence of a T <-> C transition at site 243 in all white-tailed deer genotypes and some mule deer genotypes appears to be the result of convergent evolution (homoplasy), since it is the only piece of evidence grouping these mule deer genotypes with the white-tailed deer genotypes. The noisiness of this site obscures the phylogenetic signal of the data, as it causes these mule deer genotypes to sometimes group with the white-tailed deer genotypes even though there are more pieces of evidence grouping these mule deer genotypes with the remaining mule deer genotypes.

In order to overcome the problems associated with homoplasy, this character state ('C' at site 243) was recoded as 'A' in all mule deer genotypes that had a 'C'. An initial heuristic search carried out on white-tailed deer genotypes produced a single minimum-length tree of length 6, while the equivalent analysis of mule deer genotypes identified a single minimum-length tree of length 31. The mule deer tree was used as a

backbone constraint tree in a second round of analysis that included all white-tailed deer genotypes. It is possible to search under topological constraints to restrict the set of trees retained by a heuristic search. A backbone constraint tree contains a subset of the study taxa. When searching under topological constraints, only those trees that share the topology of the constraint tree will be retained when the constraint tree is used as a filter. A trial tree is compatible with the constraint tree only if pruning those taxa on the trial tree that are not present on the backbone tree results in a topology identical to the backbone (Swofford and Begle, 1993). This type of constraint tree forces a relative topology, since other taxa may be added at any point on the backbone tree, providing the backbone is not violated (Swofford and Begle, 1993). The second round of analysis identified 77 minimum-length trees of length 39. When the mule deer constraint tree was used as a filter, five trees were retained that were compatible with the constraint tree, and were saved for later use as constraint trees. The two black-tailed deer genotypes were then added to the data set for analysis. The heuristic search identified 162 minimum-length trees of length 61. When the mule deer genotype constraint filter was applied, 14 trees were retained; independent application of the [mule deer + white-tailed deer] constraint filters retained between 3 and 11 trees per constraint tree. The fact that a single tree was not recovered suggested that there may be other sites within the data set that are phylogenetically noisy.

Further inspection of the data set suggested that the distribution of character state 'G' at character eight (nucleotide 51 of the original data set) was also the result of convergent mutations in mule deer and white-tailed deer genotypes. Character eight was therefore excluded from a second exploratory analysis. The complete (or partial, as in the case of recoding a character) exclusion of clearly unreliable data from an analysis is an acceptable solution to the problem of homoplasy (Swofford *et al.*, 1996). The same search criteria were used in phylogenetic analysis of the modified character data matrix. The exclusion of character eight caused some genotypes to collapse onto each other; for example, SWN and CRO03/SSK become identical (Figure 4). The second modification (*i.e.*, exclusion of character eight) was used in all subsequent analysis.

Analysis of only the mule deer genotypes identified a single minimum-length tree of length 27 (Figure 10), and independent analysis of only the white-tailed deer genotypes identified a single minimum-length tree of length 5 (Figure 11). When the mule deer genotypes and the white-tailed deer genotypes were analysed together in the same heuristic search, a single minimum-length tree of length 32 (Figure 12) was identified. The black-tailed deer genotypes were then added to the analysis; this identified only two minimum-length trees, each requiring 54 steps (Figures 13 and 14). These trees differ with respect to the position of the black-tailed deer genotypes relative to the mule deer and the white-tailed deer genotypes. Constraint trees were not used as filters in this

Figure 10 : **Unrooted minimum-length network of phylogenetic relationships among mule deer mitochondrial DNA genotypes in North America. Filled circles represent genotypes; bars indicate the number of nucleotide substitutions inferred to occur along each branch of the network.**

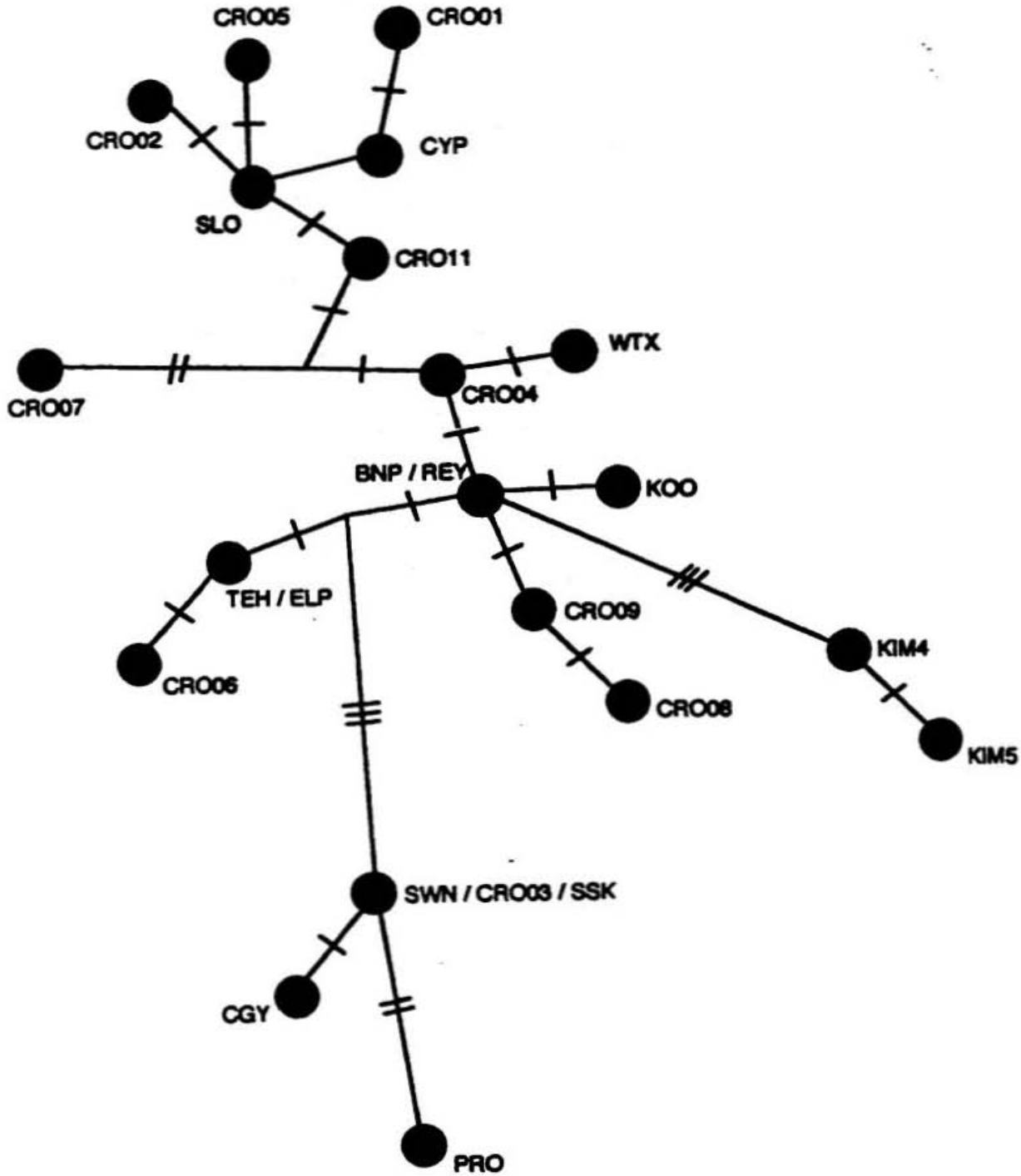

CRO05
CRO01
CRO02
CYP
SLO
CRO11
WTX
CRO07
CRO04
BNP / REY
KOO
TEH / ELP
CRO09
KIM4
CRO06
CRO08
KIM5
SWN / CRO03 / SSK
CGY
PRO

Figure 11 : **Unrooted minimum-length network of phylogenetic relationships among white-tailed deer mitochondrial DNA genotypes in North America. Open circles represent genotypes; bars indicate the number of nucleotide substitutions inferred to occur along each branch of the network.**

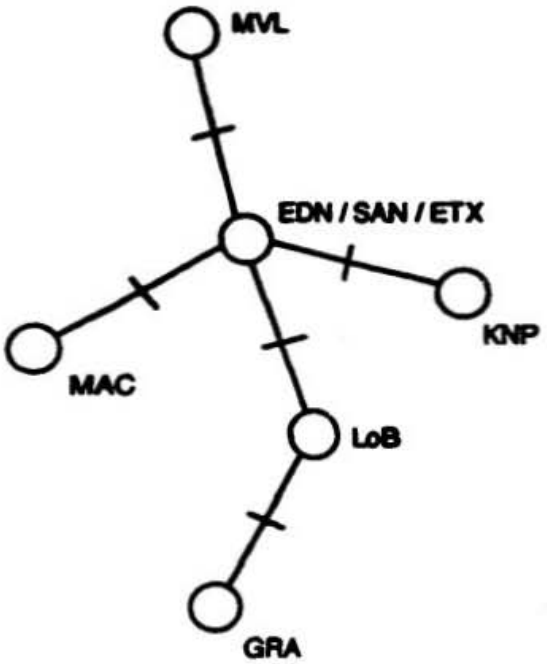

MVL
EDN / SAN / ETX
KNP
MAC
LoB
GRA

Figure 12 : Unrooted minimum-length network of phylogenetic relationships among mule deer and white-tailed deer mitochondrial DNA genotypes in North America. Mule deer genotypes are represented by filled circles and white-tailed deer genotypes are represented by open circles. Bars indicate the number of nucleotide substitutions inferred to occur along each branch of the network.

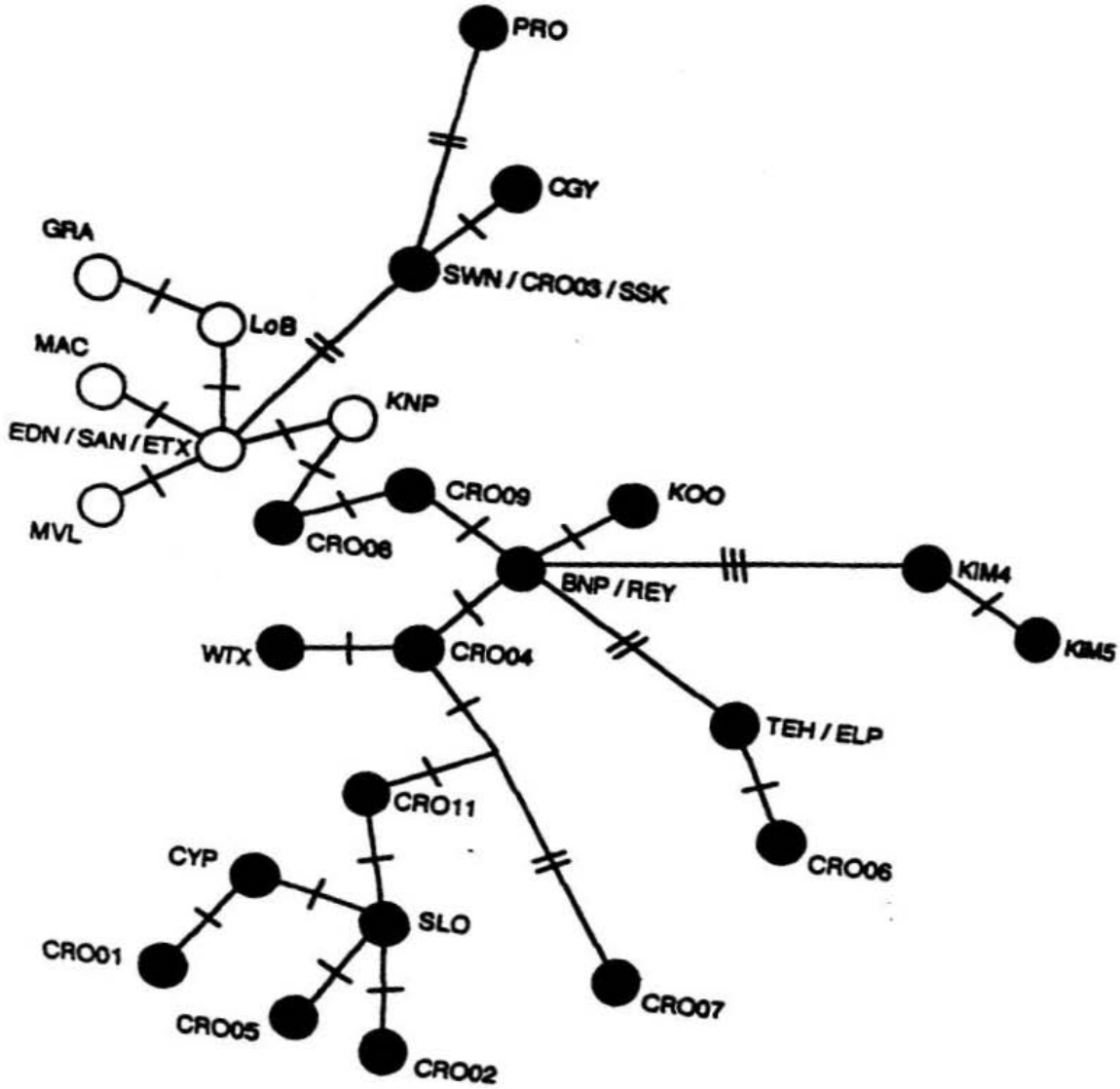

PRO
CGY
GRA
SWN / CRO03 / SSK
LoB
MAC
KNP
EDN / SAN / ETX
CRO09
KOO
MVL
CRO08
KIM4
BNP / REY
KIM5
WTX
CRO04
TEH / ELP
CRO11
CRO06
CYP
SLO
CRO01
CRO07
CRO05
CRO02

Figure 13: One of two unrooted minimum-length networks of phylogenetic relationships among mule deer, white-tailed deer, and black-tailed deer mitochondrial DNA genotypes in North America. Mule deer genotypes are represented by filled circles, white-tailed deer genotypes are represented by open circles, and black-tailed deer genotypes are represented by circles with diagonal lines. Bars indicate the number of nucleotide substitutions inferred to occur along each branch of the network. The number of nucleotide substitutions differentiating the black-tailed deer genotypes from the mule deer and white-tailed deer genotypes is indicated by numerals.

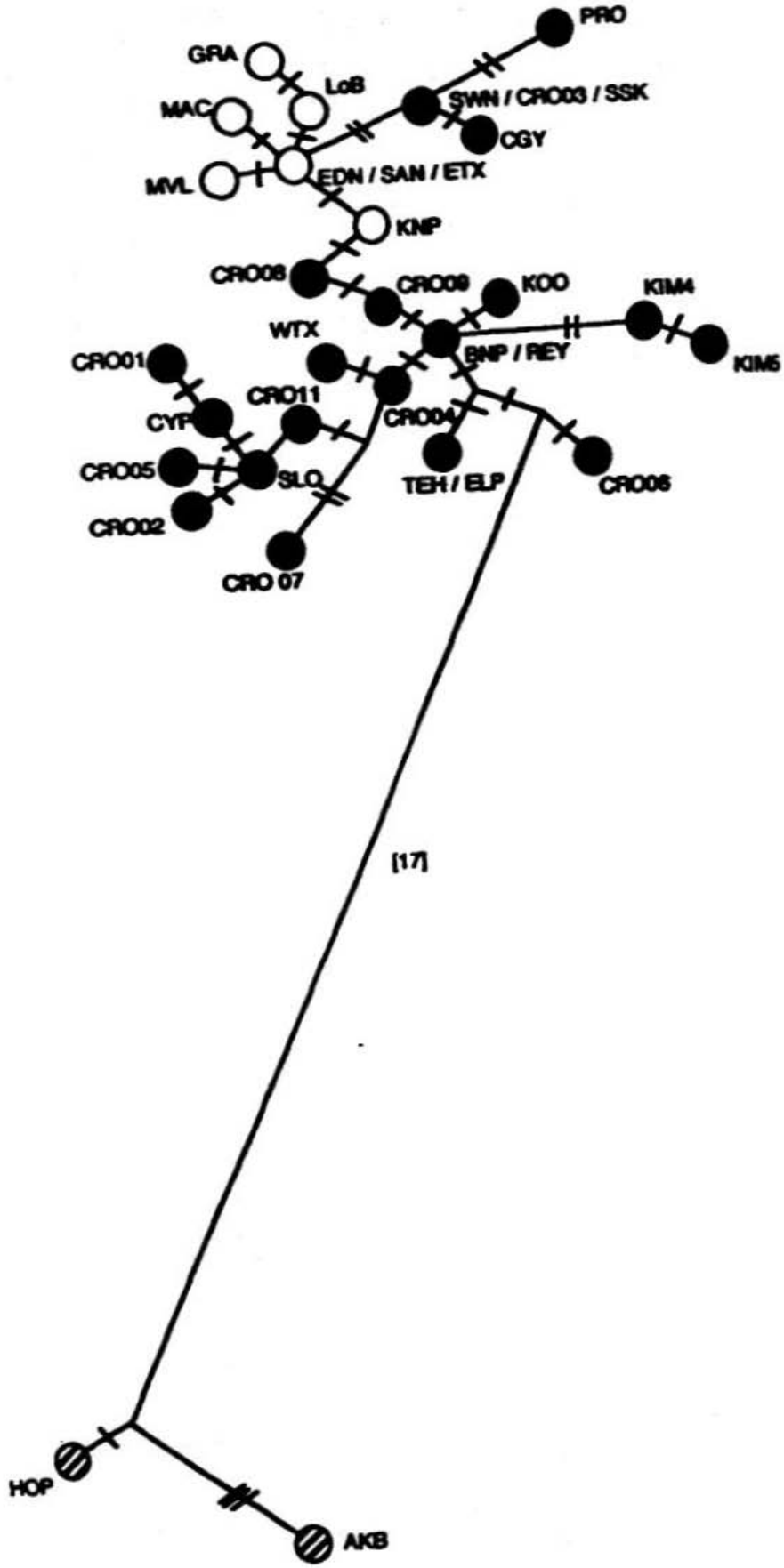
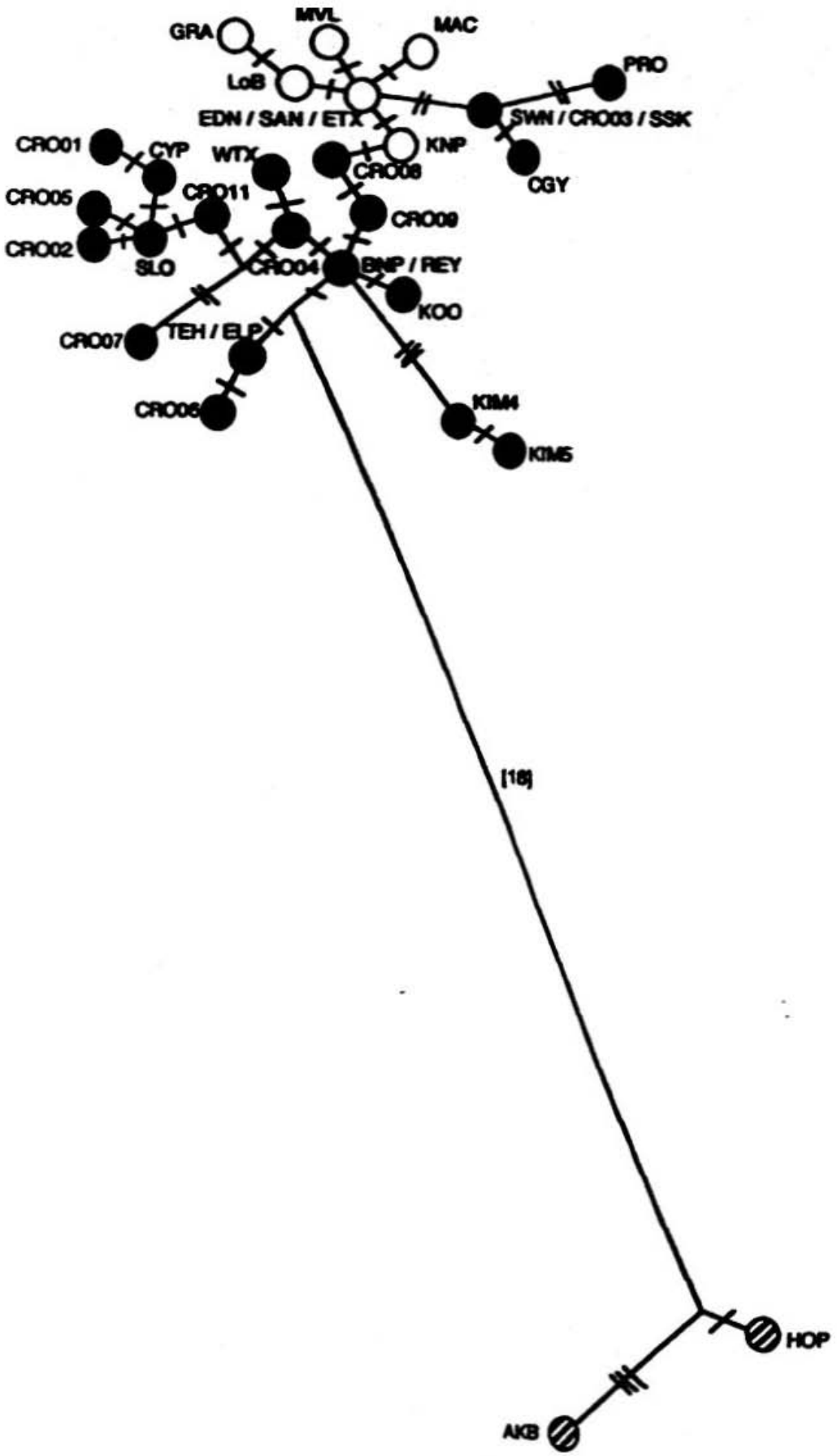

PRO
GRA
LoB
MAC
SWN / CRO03 / SSK
CGY
MVL
EDN / SAN / ETX
KNP
CRO08
CRO09
KOO
KIM4
WTX
BNP / REY
KIM5
CRO01
CRO11
CYP
CRO04
CRO05
SLO
TEH / ELP
CRO06
CRO02
CRO 07
[17]
HOP
AKB

Figure 14 : The second of two unrooted minimum-length networks of phylogenetic relationships among mule deer, white-tailed deer, and black-tailed deer mitochondrial DNA genotypes in North America. Mule deer genotypes are represented by filled circles, white-tailed deer genotypes are represented by open circles, and black-tailed deer genotypes are represented by circles with diagonal lines. Bars indicate the number of nucleotide substitutions inferred to occur along each branch of the network. The number of nucleotide substitutions differentiating the black-tailed deer genotypes from the mule deer and white-tailed deer genotypes is indicated by numerals. This network differs from that presented as Figure 13 in terms of the position of the black-tailed deer genotypes relative to the mule deer and the white-tailed deer genotypes.


GRA
MVL
MAC
LoB
PRO
EDN / SAN / ETX
SWN / CRO03 / SSK
CRO01
CYP
WTX
KNP
CRO08
CGY
CRO05
CRO11
CRO09
CRO02
SLO
CRO04
BNP / REY
KOO
CRO07
TEH / ELP
CRO06
KIM4
KIM5
[18]
HOP
AKB

analysis.

Phylogenetic analyses identified four major assemblages of *Odocoileus* mtDNA genotype: (1) black-tailed deer genotypes (AKB and HOP), (2) white-tailed deer genotypes (KNP, EDN, MVL, MAC, LoB, and GRA), (3) a group of mule deer genotypes predominantly found in western Canada (PRO, SWN, and CGY), and (4) a group of mule deer genotypes found predominantly in the western United States (the remaining 17 mule deer genotypes) (Figures 13 and 14).

The geographic organization of the genotype assemblages can be summarized as follows: assemblage (1) western North American black-tailed deer, (2) western North American mule deer, (3) western Canadian mule deer, and (4) western North American white-tailed deer. The minimum and maximum pairwise nucleotide sequence divergence estimates within and among genotype assemblages are presented in Table 6. The maximum interspecific sequence divergence is 6.98% between western North American white-tailed deer and black-tailed deer, while the maximum intraspecific sequence divergence is 6.73% between western North American mule deer and black-tailed deer.

The approximate geographic distributions of mtDNA genotypes in California and Alberta (Figures 15, 16 and 17) indicate that there is a considerable degree of geographic structure to the sequence genotypes. Within both *O. hemionus* and *O. virginianus*, some genotypes are widespread while others are more localized. For example, among white-

Table 6: Minimum and maximum pairwise nucleotide sequence divergences within and among *Odocoileus* mtDNA genotype assemblages. The estimated sequence divergences were calculated as (number of pairwise differences) / 401.

	Genotype assemblage			
Genotype assemblage	1	2	3	4
1. western North American black-tailed deer	0.75%			
2. western North American mule deer	4.75% - 6.73%	0.25% - 2.50%		
3. western Canadian mule deer	5.49% - 6.48%	1.00% - 2.99%	0.25% - 0.75%	
4. western North American white-tailed deer	5.74% - 6.98%	0.50% - 2.74%	0.50% - 1.75%	0.25% -1.00%

Figure 15: Approximate distribution of mule deer (*Odocoileus hemionus)* mtDNA genotypes in California. The extent of occurrence of a given genotype is bounded by a minimum polygon. Numbers are used to represent genotypes as follows:

1	HOP
2	TEH
3	BNP
4	SLO
5	CRO01
6	CRO03
7	CRO05
8	CRO06
9	CRO11
10	CRO07
11	CRO02
12	CRO09
13	CRO04
14	CRO08

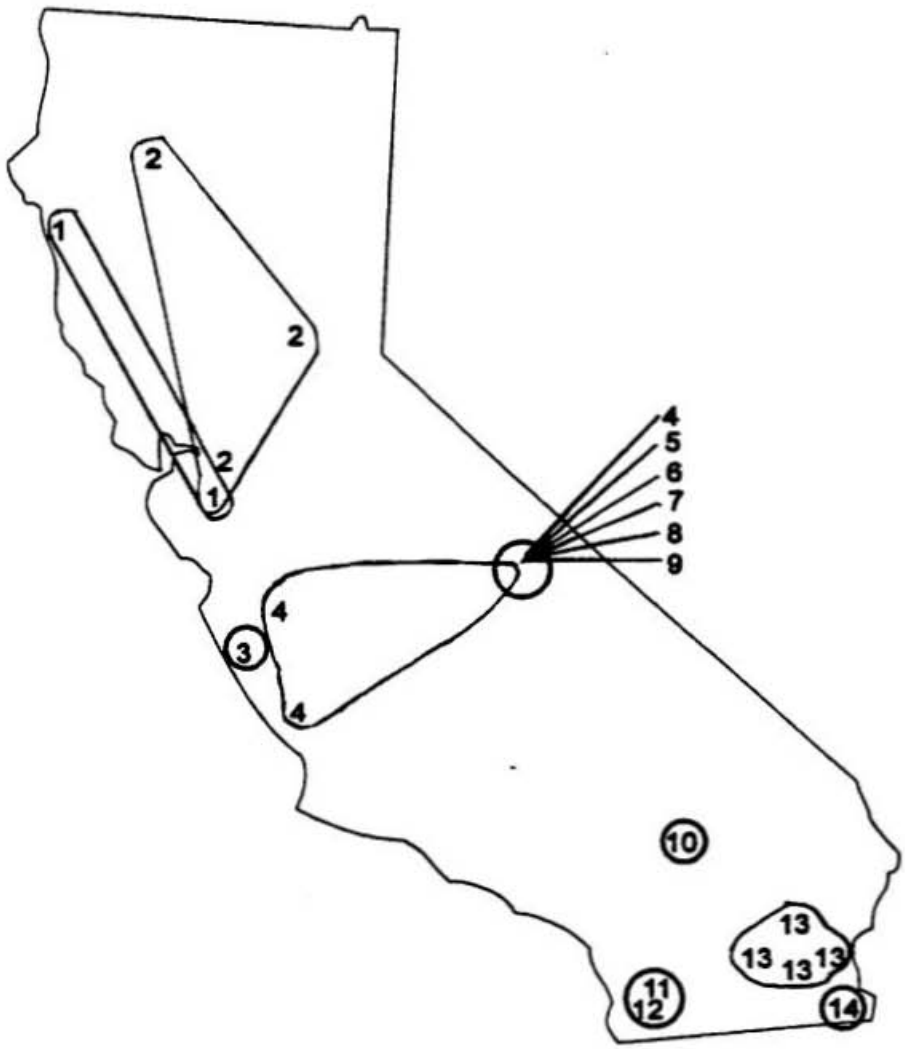

1
2
2
2
1
2
4
5
6
7
8
9
3
4
4
10
13
13
13
13
11
12
14

Figure 16: Approximate distribution of mule deer (*Odocoileus hemionus)* mtDNA genotypes in Alberta. The extent of occurrence of a given genotype is bounded by a minimum polygon. Numbers are used to represent genotypes as follows:

1	SWN
2	PRO
3	BNP
4	TEH
5	KOO
6	CGY
7	CYP

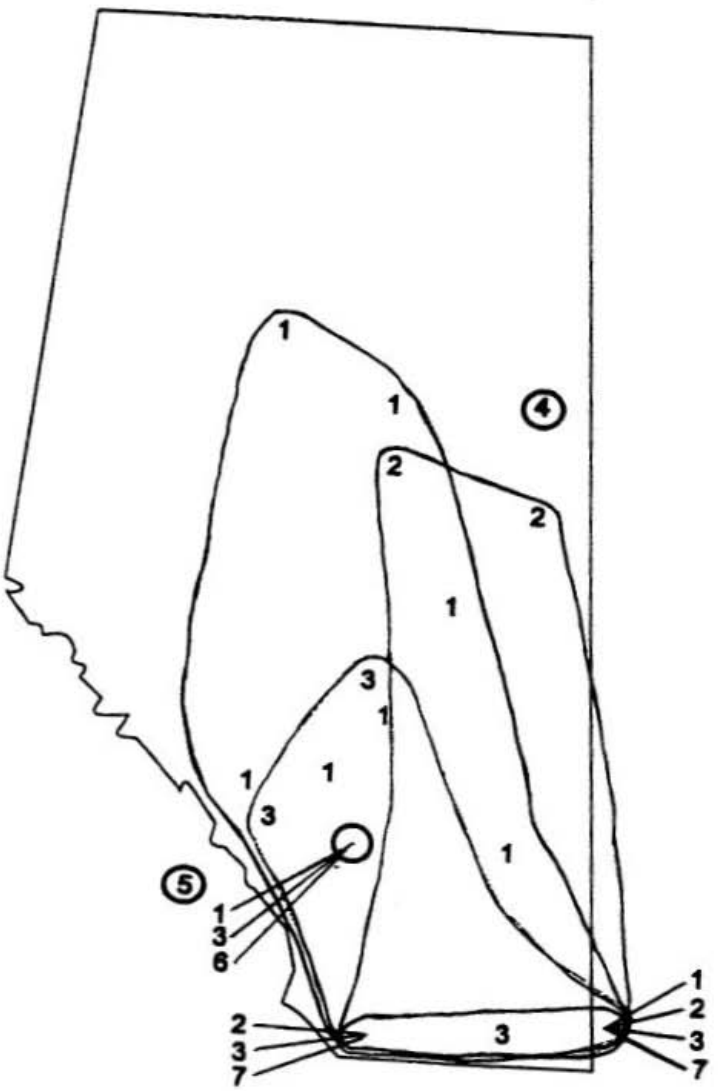
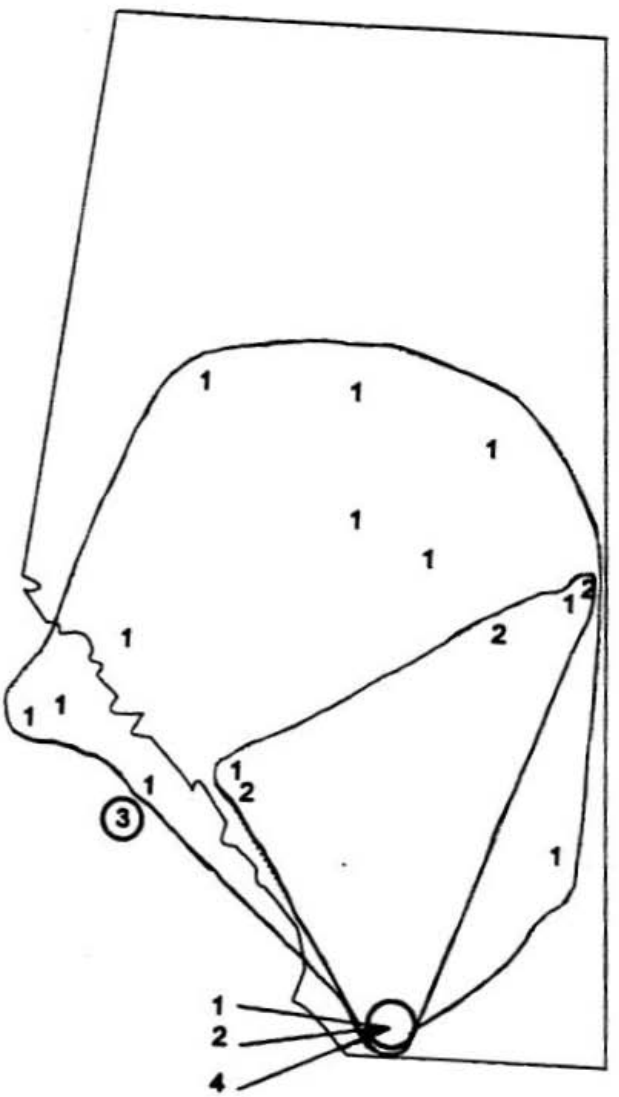

1
1
4
2
2
1
3
1
1
1
3
1
5
1
3
6
2
3
7
3
1
2
3
7

Figure 17: Approximate distribution of white-tailed deer (*Odocoileus virginianus)* mtDNA genotypes in Alberta. The extent of occurrence of a given genotype is bounded by a minimum polygon. Numbers are used to represent genotypes as follows:

1 EDN
2 MVL
3 KNP
4 MAC


1
1
1
1
1
1
2
1
2
1
1
1
1
2
1
3
1
1
2
4

tailed deer genotypes in Alberta, EDN is the most widespread, while MAC and KNP have been found at one location each (Figure 12).

DISCUSSION

Palaeontological record of Odocoileus.

Fossil material attributed to *Odocoileus* first appeared 3.5×10^6 years ago. Material classified as *O. virginianus* dates to 3.2×10^5 years ago, and *O. hemionus* material first appears in the Irvingtonian ($0.7 - 1.9 \times 10^6$) (Kurtén and Anderson, 1980). Fossils attributed to *O. virginianus* are found in Blancan Florida and Texas deposits. The North American Land Mammal Ages are defined by faunal assemblages, and have been independently dated using radiometric and palaeomagnetic techniques. There is some variation in the start and end dates of palaeontological ages, depending on which authority is consulted; for example, Martin and Barnosky (1993) state that the Blancan extended from $4.8 - 4.0 \times 10^6$ years ago to 1.9×10^6 years ago, while Kurtén and Anderson (1980) state that the Blancan began 3.5×10^6 years ago. By the Irvingtonian, fossils attributed to white-tailed deer were present in several of the central and eastern states, and by the Rancholabrean were very common and widespread. Most of these records are from central and eastern North America, though there are also several western fossils of *Odocoileus* that may be *O. virginianus*. Fossils recognizable as *O. hemionus* are limited to about 15 sites in western North America; several other western fossils of *Odocoileus*, most of which date to the Rancholabrean, may also be *O. hemionus* (Kurtén

and Anderson, 1980). The palaeontological record as conventionally interpreted would thus suggest that white-tailed deer are ancestral to both mule deer and black-tailed deer.

Evolutionary history of Odocoileus *based on morphological and mtDNA sequence data.*

Phylogenetic analysis of mtDNA sequence genotypes suggests a contrasting evolutionary history of *Odocoileus*. The sequence data suggest that black-tailed deer are ancestral to both mule deer and white-tailed deer (Figures 5, 13 and 14); interpretation of morphological variation within *O. hemionus* also suggests that black-tailed deer are ancestral to mule deer, and possibly all *Odocoileus* species (Carr and Hughes, 1993; Geist, 1981). Among the criteria that segregate ancestral from derived forms of ungulates, Geist states that more highly evolved forms are typically larger and have a more distinctly patterned pelage, a larger rump patch, and a shorter tail. The adaptive trend involves a shift from forest to open habitat, from low latitudes and altitudes to higher ones, and from old glacial refugia into more recently glaciated zones. On the basis of these criteria, the ancestral subspecies of *O. hemionus* is the black-tailed deer that inhabits the Alaskan coast, the Sitka deer (*O. h. sitkensis*), and the most derived subspecies is the Rocky Mountain mule deer (*O. h. hemionus*) (Geist, 1981).

The maximum sequence divergence between a black-tailed deer genotype (AKB) and any other *Odocoileus* genotype (the white-tailed deer genotype GRA) is 6.98% (Table 7).

On the basis of RFLP data, Brown *et al.* (1979) estimated the rate of sequence divergence for the entire mtDNA molecule as 2% per million years per pair of lineages, or 20×10^{-9} substitutions per nucleotide site per year per pair of lineages. Nei (1985, cited in Hartl and Clark, 1989) estimated the rate as 0.71% per million years, or 7.1×10^{-9} substitutions per nucleotide site per year per pair of lineages, a three-fold difference. Previous work on *Odocoileus* (Carr and Hughes, 1993) indicates that the portion of the cytochrome *b* gene under study evolves slightly faster than the entire molecule: they calculated the sequence divergence between black-tailed deer and white-tailed deer as 7.5% for a 307bp fragment of the cytochrome *b* gene, whereas the divergence between the same two *Odocoileus* genotypes calculated from RFLP data for the whole mtDNA molecule was 6.0%. Using the corrected cytochrome *b* divergence rate of Carr *et al.* (1995), which is based on Nei's (1985) divergence rate and corrects for the faster rate of evolution of the cytochrome *b* gene, the 6.98% sequence divergence between the two most divergent *hemionus* and *virginianus* genotypes (AKB and GRA, respectively) suggests a divergence time of *ca.* 7.9×10^6 years ago. The calculation from Brown *et al.*'s (1979) rate, corrected for cytochrome *b*, used by Carr and Hughes (1993) suggests a divergence time of *ca.* 2.8×10^6 years ago. The first estimate is incompatible with the palaeontological record, since it antedates the appearance of the genus, or even New World deer, in the fossil record. The more recent estimate obtained on the basis of Brown *et al.*'s (1979) rate is compatible

with the fossil record; all subsequent estimates of time since divergence are based on Brown *et al.*'s (1979) rate.

The earliest estimated time since divergence between a mule deer genotype and a white-tailed deer genotype is considerably more recent; on the basis of Brown *et al.*'s figure, the 2.74% sequence divergence between KIM5 and GRA (Table 6) corresponds to a divergence time of *ca.* 1.1×10^6 years ago. Mule deer and black-tailed deer genotypes diverged from each other a maximum of *ca.* 2.7×10^6 years ago (maximum sequence divergence of 6.73% between AKB and CRO05; Table 6). Based on these estimates, the black-tailed deer genotypes represent the oldest *Odocoileus* mtDNA lineage in North America. The mule deer mtDNA lineage diverged from the black-tailed deer, and the white-tailed deer mtDNA lineage subsequently diverged from the mule deer lineage. This general order is indicated by the branching pattern shown in the phylogenetic networks (Figures 13 and 14).

The lack of concordance between the evolutionary patterns among *Odocoileus* lineages as determined from mtDNA sequence analysis and from the fossil record may be due in part to the fact that many of the *Odocoileus* fossils have not been classified to a particular species, let alone subspecies. Species designation assigned to a given fossil may also be influenced by the collection site: *Odocoileus* specimens from eastern or central North America may be assumed to be *O. virginianus* simply for geographic reasons (C. Dailey,

pers. comm. to S. Carr). Kurtén and Anderson (1980) suggest that Pleistocene *Odocoileus* may require revision.

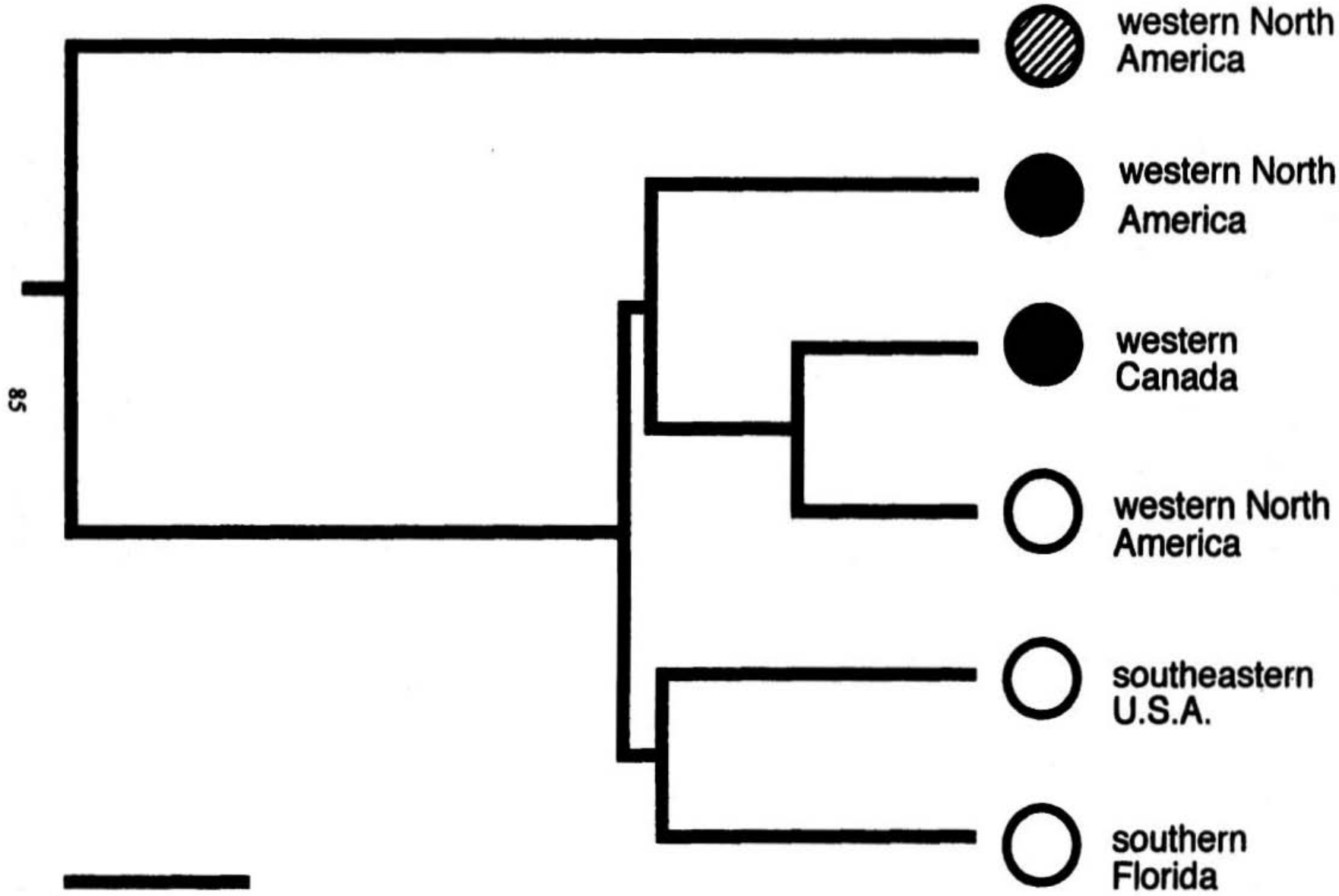
The evolutionary history of *Odocoileus* as indicated by molecular data is complex. Previous studies have demonstrated a discordance of nuclear and mitochondrial genetic differentiation: mtDNA sequence divergence was found to be high between conspecific mule and black-tailed deer and low between the two species, *O. hemionus* and *O. virginianus*, whereas the relationships indicated by allozyme data (McClymont *et al.*, 1982; Stubblefield *et al.*, 1986) were in agreement with the classical species and subspecies designations (Carr and Hughes, 1993; Cronin, 1991; Cronin *et al.*, 1988). The current study indicates the same pattern of high intraspecific sequence divergence and low interspecific sequence divergence among mtDNA genotypes: the maximum intraspecific divergence for *O. hemionus* genotypes (6.73% between AKB and CRO05) exceeds the maximum intraspecific divergence for *O. virginianus* genotypes (1.00% between GRA and KNP) in western North America (Table 6). RFLP analysis of mtDNA among white-tailed deer in the southeastern United States indicates that the level of nucleotide sequence divergence among southeastern white-tailed deer RFLP-genotypes (maximum of 2.81%) is greater than that found in white-tailed deer populations from other regions of North America (Ellsworth *et al.*, 1994). Direct DNA sequence analysis of deer from the southeastern United States would be expected to show a similar pattern

as the RFLP analysis of DNA.

The phylogenetic networks show four main assemblages of mtDNA genotypes: (1) black-tailed deer genotypes (AKB and HOP), (2) white-tailed deer genotypes (KNP, EDN, MVL, MAC, LoB, and GRA), (3) a group of mule deer genotypes predominantly found in western Canada (PRO, SWN, and CGY), and (4) a group of mule deer genotypes found predominantly in the western United States (the remaining 17 mule deer genotypes) (Figure 13). The placement of the white-tailed deer assemblage within the network is of particular interest: the white-tailed genotypes phylogenetically separate the two mule deer assemblages. Inspection of the sequence data indicates that the white-tailed deer genotypes are quite similar to the mule deer genotypes from western Canada (Figure 4). In order to estimate approximate times since divergence among the major assemblages, the most widespread genotype within each assemblage was used as a representative sequence, and times of divergences were estimated using Brown *et al.*'s (1979) 2% per million years per pair of lineages rate of sequence divergence, corrected for the cytochrome *b* gene.

A model for *Odocoileus* mtDNA evolution suggested by the current study is presented as Figure 18. According to the relationships shown in the model, the black-tailed deer diverged from the ancestral stock somewhat more than 2 MYBP. The next divergence event did not occur until about 1 MYBP; this event separates the southeastern white-

Figure 18: Model of evolutionary patterns among *Odocoileus* mtDNA assemblages in North America. Mule deer genotype assemblages are represented by filled circles, white-tailed deer genotype assemblages by open circles, and the black-tailed deer assemblage by a circle with diagonal lines. Branch points indicate approximate sequence divergence date estimates as calculated from mtDNA sequence data. No sequence data were available for the southeastern U.S.A. and southern Florida assemblages; estimated divergence times for these assemblages are based on RFLP analysis of the mitochondrial genome as reported by Ellsworth *et al.* (1994).


western North America
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southern Florida
85
c. 0.5 m.y.a

tailed deer from all *Odocoileus* genotypes currently represented in western North America. On the basis of a recent RFLP analysis of mtDNA variation among white-tailed deer in the southeastern United States, there is a southern Florida assemblage of white-tailed deer RFLP-genotypes that can be distinguished cladistically from other southeastern RFLP-genotypes (Ellsworth *et al.*, 1994). Divergence of this southern Florida assemblage is estimated to have occurred somewhat less than 1 MYBP. The divergence at about the same time or slightly before separates the western United States mule deer assemblage from the mule deer assemblage found in western Canada and the white-tailed deer found in western North America. The most recent divergence suggested by mtDNA sequence data occurred within the last 0.50×10^6 MYBP and separates Canadian mule deer genotypes from western North American white-tailed deer genotypes.

Glacial refugia, stochastic lineage sorting and introgressive hybridization of mtDNA.

Two main hypotheses that have been suggested as explanations for the discordance between nuclear and mitochondrial genetic differentiation in *Odocoileus* are stochastic lineage sorting of mtDNA, and extensive introgressive hybridization of mtDNA between the two species (Carr and Hughes, 1993; Cronin, 1991). These processes can also explain the evolutionary patterns observed over the geographic range studied here. Given the

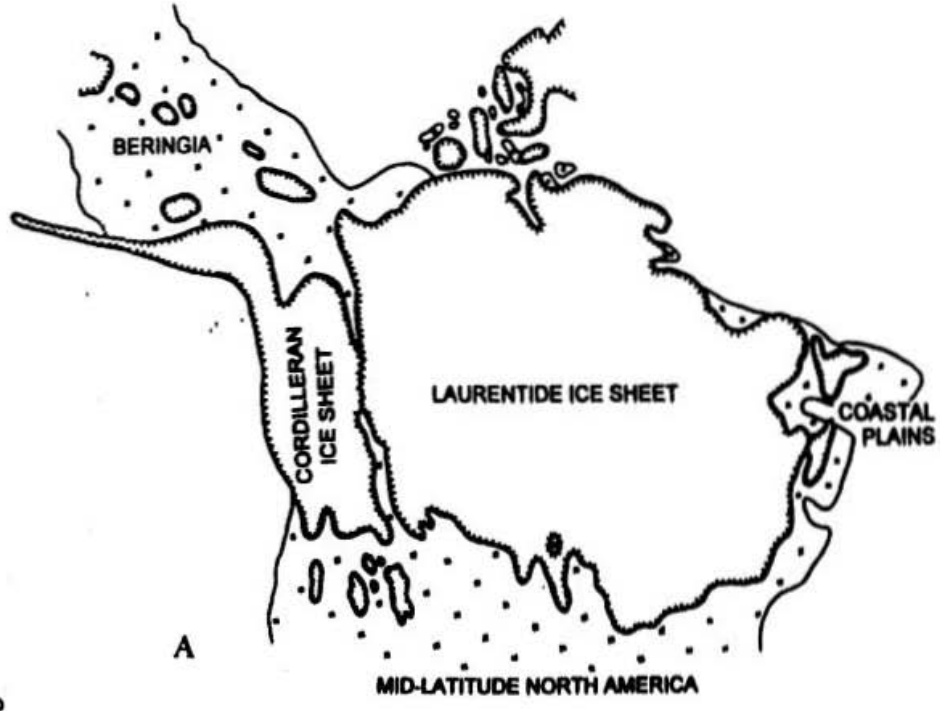
extreme divergence of the black-tailed deer assemblage relative to the other *Odocoileus* assemblages, it can be hypothesized that the black-tailed deer genotypes represent an ancient mtDNA lineage that has persisted over evolutionary time. The estimated time of separation of this lineage from other lineages antedates the separation of the species (Carr and Hughes, 1993). Through the process of lineage sorting, all other black-tailed deer mtDNA lineages would have been eliminated, leaving only the current lineage represented by AKB and HOP. The persistence of this lineage may be attributable to its isolation from other *Odocoileus* lineages during the Wisconsin Glaciation. Black-tailed deer probably occupied a coastal refugium in the Pacific Northwest, while mule deer may have occupied a refugium in the southwest (Cronin, 1991). Contemporary populations of black-tailed deer are largely coastal; the distribution of Columbian black-tailed deer (*O. h. columbianus*) extends from central California to southern British Columbia, and is contiguous with that of Sitka black-tailed deer (*O. h. sitkensis*), which extends northward along the coast of British Columbia to southeastern Alaska (Carr and Hughes, 1993).

The high degree of genetic variation in terms of both numbers of distinct mtDNA genotypes and sequence divergences among genotypes within all observed mule deer genotypes suggests that the mule deer also represent an old lineage that survived the Ice Age in one or more refugia. The three large regions that served as the primary terrestrial refugia during the Wisconsin Glaciation were mid-latitude North America (*i.e.*, the land

south of the Laurentide and Cordilleran Ice Sheets), Beringia (present-day Yukon and Alaska, plus the beds of the Bering and Chuckchi Seas), and the coastal plains east of the ice sheet (the currently submerged continental shelf off New England and Atlantic Canada) (Pielou, 1991; Figure 19). Mule deer may have occupied a southwestern refugium (Cronin, 1991) or Beringia. The proliferation of mule deer genotypes would have occurred while the species occupied its refugium and as it extended its range to the east and south as the ice sheets receeded.

White-tailed deer would also have occupied refugia during the Ice Age. While a Florida refugium has been suggested for southeastern populations of *O. virginianus* (Ellsworth *et al.*, 1994), the central and western populations may have occupied a southwestern refugium or may have moved northwards to Beringia as the ice sheets advanced. If white-tailed deer and mule deer occupied the same refugia, or at least the same general area, at the same time in palaeontological history, a comparable level of genetic polymorphism would be expected in both species. The results of mtDNA sequence analysis suggest the contrary: 20 distinct mule deer mtDNA genotypes have been identified in western North America, while only 7 white-tailed deer mtDNA genotypes have been identified in the same broad geographical region. The lack of extensive polymorphism in western white-tailed deer may indicate that this mtDNA genotype assemblage is of more recent origin than either the western mule deer or the

Figure 19: Extent of ice coverage over Northern North America during the Wisconsin Glaciation. The maps show the increase in the relative amount of ice-free land available for colonization by organisms at 18,000 years ago (A), 13,000 years ago (B), 10,000 years ago (C), and 7,000 years ago (D). These maps are modified from Figures 1.2 - 1.5 in Pielou (1991).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A
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COAST PLAI
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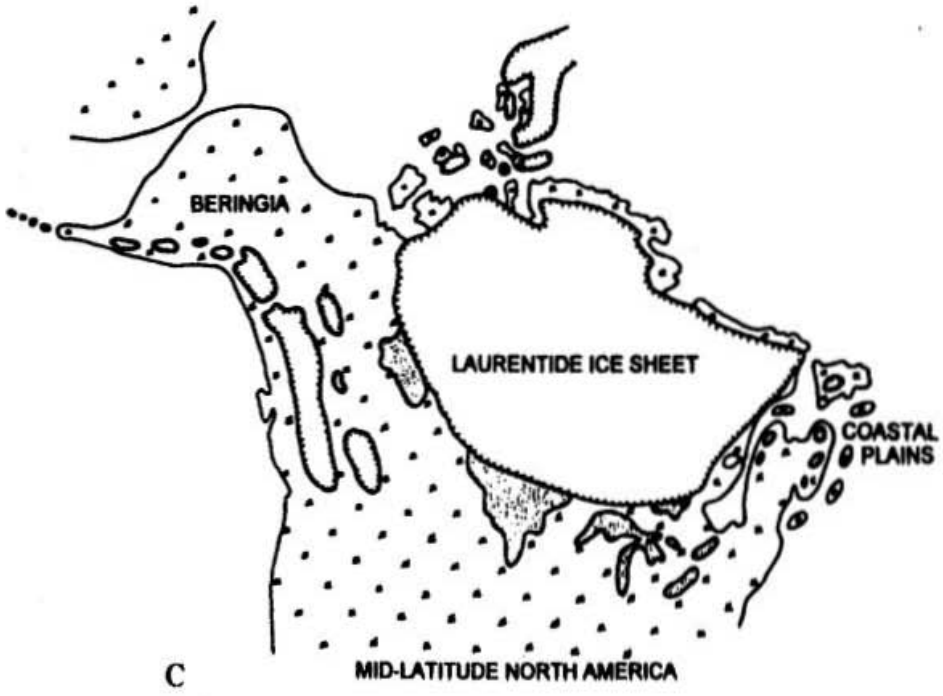
MID-LATITUDE NORTH AMERICA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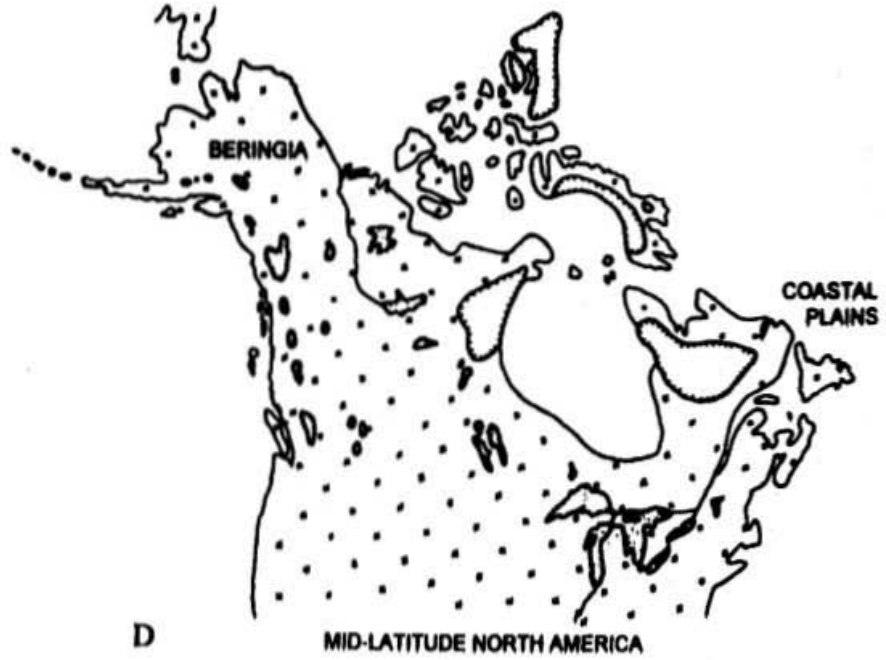
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southeastern white-tailed deer. Given that both species were well established before the onset of the Wisconsin Glaciation, and that there are several western fossils of *Odocoileus* that may be *O. virginianus* (Kurtén and Anderson, 1980), it is reasonable to assume that there was a western white-tailed deer mtDNA lineage. The fate of this lineage may be accounted for by an episode of ancient hybridization between mule deer and white-tailed deer, with introgression of mtDNA from mule deer into white-tailed deer. Such a scenario would effectively replace the white-tailed deer mtDNA lineage in western North America with mule deer mtDNA. The divergence date estimate suggests that such an event occurred fairly recently in palaeontological time (Figure 18).

Gene trees and species trees.

While the hypotheses of stochastic lineage sorting of mtDNA and of extensive introgressive hybridization of mtDNA between the two species of *Odocoileus* can be used to explain the observed discordance between nuclear and mitochondrial genetic differentiation in these deer, it is important to realize that the phylogenetic tree generated from mtDNA sequence data represents a gene tree, and may differ from the species tree. A species tree represents the actual evolutionary pathway of a group of species, whereas a gene tree is a phylogenetic tree constructed from DNA sequences for a single gene in each of the species involved (Li, 1997; Pamilo and Nei, 1988). Gene trees and species

trees can differ as a result of such factors as retention of ancestral polymorphisms and hybridization events. If the time of divergence between the genes is roughly equal to the time of divergence between populations, the gene tree and the species tree will show the same topology.

In the case of *Odocoileus* mtDNA, the observation that mule deer are more divergent from the conspecific black-tailed deer than from the congeneric white-tailed deer may reflect the difference between a gene tree and a species tree. Classical species and subspecies designations are based on morphological and ecological characteristics of the biological entities called black-tailed deer, mule deer, and white-tailed deer. Analysis of allozyme data suggests an evolutionary history consistent with the classical taxonomy (Cronin, 1991; McClymont *et al.*, 1982; Stubblefield *et al.*, 1986). In contrast to allozyme analysis in which several loci are examined in the same study, mtDNA studies are effectively based on a single locus, a consequence of the nonrecombining mode of inheritence of the mtDNA genome (Moritz *et al.*, 1987). As a result of this mode of inheritance of mtDNA, the effects of hybridization events and lineage sorting of ancestral polymorphism are potentially retained through subsequent generations, and may result in the gene tree recovering a different evolutionary history than the species tree. The phylogenetic trees presented in the current study represent the evolutionary history of the mtDNA sequence genotypes only; the fact remains that black-tailed deer,

mule deer, and white-tailed deer are distinct biological entities, regardless of the observed discordance between nuclear and mitochondrial DNA evidence.

Phylogeography of Odocoileus.

The data obtained from *Odocoileus* samples from southern Alberta can be used to illustrate some of the principles of phylogeography on a microgeographic scale. Figure 2 indicates the relative distributions of mtDNA sequence genotypes identified among individuals from the McIntyre Ranch near Magrath, and Waterton Lakes National Park. Since most individuals were sampled as fawns, the geographic co-ordinates corresponded to localities within the respective maternal ranges. In some cases, fawns suspected of being siblings were sampled. As expected from the mode of maternal inheritance, all putative siblings shared the same mtDNA sequence genotype. There are two basic social units among *Odocoileus* species: doe (or family) groups, and buck groups (Marchinton and Hirth, 1984). A doe group consists of an adult doe plus her fawn and offspring from the previous year or years. While males typically disperse far from the maternal range as yearlings (12 - 24 months) to establish solitary residence on new ranges, most female offspring remain in or near the ranges of their mothers (Bunnell and Harestad, 1983; Hawkins and Klimstra, 1970; Nelson and Mech, 1984; Tierson *et al.*, 1985). Studies have indicated that female dispersal is very limited in comparison to that

of males (Nelson and Mech, 1992).

Since dispersal is male-biased, with females exhibiting philopatric behaviour, distributional patterns of mtDNA variation would be expected to show geographic structure. Figure 2 indicates clustering of genotypes within the microgeographic study site. Similarly, qualitative analysis of the geographic distributions of mtDNA genotypes on a broader scale reveals a level of geographic structure, with some genotypes being found at only one location and other genotypes being quite widespread (Figures 15, 16 and 17).

Site fidelity will have its most profound effect on phylogeographic structure if it involves fidelity to reproductive site, since it is there that any exchange of genetic material will occur. For example, a phylogeographic analysis of mtDNA control-region sequence variation in dunlins (*Calidris alpina*) indicated that this species of migratory shorebird exhibits a strongly subdivided genetic population structure that is being maintained by philopatric behaviour (Wenink *et al.*, 1996). In contrast, mtDNA variation in song sparrows (*Melospizia melodia*) was not geographically structured, despite geographic variation in size and plumage colour across its North American range (Zink and Dittmann, 1993). RFLP analysis of mtDNA in humpback whales (*Megaptera novaeangliae*) revealed a strong segregation of mtDNA haplotypes among subpopulations as well as populations from the North Pacific and western North Atlantic

Ocean populations. This pattern of geographic structure was interpreted to be the result of maternally directed fidelity to migratory sites (Baker *et al.*, 1990). A study of another marine mammal, the grey seal (*Halichoerus grypus*), revealed a pronounced segregation between western North Atlantic and eastern North Atlantic groups, as indicated by the absence of shared haplotypes (Boskovic *et al.*, 1996).

Some of the recent studies of *Odocoileus* examine the genetic aspects of its evolution. Ellsworth *et al.* (1994) specifically studied the historical biogeography and contemporary patterns of mtDNA variation in white-tailed deer. The study site was restricted to the southeastern United States, and revealed three main groups of RFLP-genotypes that were geographically oriented and spatially concordant with patterns observed in unrelated species. Most of the other *Odocoileus* studies focus on the genetic interactions between the two species and/or their subspecies in terms of hybridization and genetic introgression. The current work examines the phylogenetic relationships among *Odocoileus* genotypes over a broader geographic range. While independent studies have been carried out on deer populations throughout their species ranges, these studies have tended to be geographically localized. Most used RFLP or allozyme analysis of genetic variation as a means of studying population dynamics. Given the difference in resolution between RFLP analysis and mtDNA sequence analysis, as indicated by the current analysis of a 401 base pair fragment of the cytochrome *b* gene, the data from other

analyses cannot be directly incorporated. This effectively reduces the extent of geographic sampling of *Odocoileus* populations to those regions for which tissue and/or DNA samples were available for DNA sequence analysis. Conclusions made on the basis of sequence data may not reflect the phylogeographic patterns that would be observed if the entire *Odocoileus* range had been sampled. Samples from southeastern U.S.A. for DNA sequence analysis would be particularly valuable, given the phylogenetic patterns among white-tailed deer reported here and by Ellsworth *et al.* (1994); if all western North America white-tailed deer mtDNA genotypes have effectively been replaced by mule deer mtDNA genotypes via introgressive hybridization, then attention should be turned eastward in order to gain insight into the white-tailed deer evolutionary history as indicated by mtDNA.

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