Phylogenetic status of North American wapiti (*Cervus elaphus*) subspecies

R.O. Polziehn, J. Hamr, F.F. Mallory, and C. Strobeck

Abstract: By the turn of the century, North American elk, or wapiti (*Cervus elaphus*), had been extirpated from all regions of the continent and two subspecies were extinct. The recovery of wapiti is largely a response to the large number of relocated Rocky Mountain (*C. e. nelsoni*) and Manitoban wapiti (*C. e. manitobensis*). A phylogenetic study was performed to determine the present genetic relationships among tule (*C. e. nannodes*), Roosevelt (*C. e. roosevelti*), Rocky Mountain, and Manitoban subspecies, using sequences from the D-loop region of the mitochondrial DNA of 28 individuals. All Roosevelt wapiti were grouped together, as were tule wapiti, which supports the classification of tule and Roosevelt subspecies. Yellowstone, Elk Island, and Riding Mountain National Parks have not introduced wapiti into their indigenous populations. When these populations were used, Manitoban wapiti were found to be monophyletic and Rocky Mountain wapiti to be paraphyletic. However, including animals from the Canadian Rocky Mountains places Rocky Mountain wapiti in clades by themselves or grouped with Manitoban wapiti. The clade containing a mixture of Manitoban and Rocky Mountain wapiti suggests that both types recently descended from a common ancestor. Hybridization or insufficient time for separation may explain the presence of the two types in the same clade.

Résumé : Déjà au tournant du siècle, le Grand Cerf nord-américain, ou Wapiti (*Cervus elaphus*), avait été exterminé de toutes les régions du continent et deux sous-espèces étaient déjà disparues. La remontée du wapiti est en grande partie le résultat de la relocalisation d'un grand nombre d'animaux des stocks des Montagnes rocheuses (*C. e. nelsoni*) et du Manitoba (*C. e. manitobensis*). Une étude phylogénétique a été entreprise pour déterminer les relations génétiques actuelles entre les sous-espèces de tule (*C. e. nannodes*), de Roosevelt (*C. e. roosevelti*), des Montagnes Rocheuses et du Manitoba, d'après les séquences de la boucle D de l'ADN mitochondrial de 28 individus. Tous les Wapitis de Roosevelt ont été regroupés et tous les Wapitis de tule ont formé un autre groupe, ce qui confirme la validité des deux sous-espèces de tule et de Roosevelt. Il n'y a pas eu d'introduction de wapitis dans les populations indigènes des parcs nationaux de Yellowstone, Elk Island et Riding Mountain. L'étude de ces populations a permis d'établir que les populations de wapitis du Manitoba sont monophylétiques et que celles des wapitis des Montagnes Rocheuses sont paraphylétiques. Cependant, l'intégration de wapitis des Rocheuses canadiennes dans les analyses place les wapitis des Montagnes Rocheuses dans des clades isolés, ou regroupés avec des wapitis du Manitoba. Le clade qui contient un mélange de wapitis du Manitoba et des Montagnes Rocheuses reflète probablement l'évolution récente des deux types à partir d'un ancêtre commun. La présence des deux types dans le même clade peut être attribuable à l'hybridation ou au fait que le laps de temps écoulé depuis la séparation des deux taxons est encore insuffisant. [Traduit par la Rédaction]

Introduction

Herds of North American elk (*Cervus elaphus*), also known as wapiti, recently inhabited nearly every region of North America. Wapiti populations were tenuously classified into six subspecies that corresponded to their biogeographical distribution and ecozones (Bryant and Maser 1982). The classification of wapiti has been examined using morphology, behavior, and, more recently, molecular characteristics (Bryant and Maser 1982; Cronin 1992). However, little consensus exists regarding subspecies distinctions. Determining evolutionary relationships among taxa can assist in the conservation and management of species. Populations that have been historically isolated and are likely to possess a unique evolutionary potential are called evolutionarily significant units (ESU; Moritz 1994). ESUs should be monophyletic for mitochondrial DNA (mtDNA) and show significant divergence of allele frequencies at nuclear loci (Moritz 1994). In this phylogenetic study, mtDNA was employed to determine the validity of North American wapiti subspecies.

Presently, six subspecies of wapiti are recognized in North America, including the extant Manitoban (*C. e. manitobensis* Millais, 1915), Rocky Mountain (*C. e. nelsoni* Bailey, 1935), Roosevelt (*C. e. roosevelti* Merriam, 1897), and tule wapiti (*C. e. nannodes* Merriam, 1905) and the extinct eastern (*C. e. canadensis* Erxleben, 1777) and Merriam (*C. e. merriami* Nelson, 1902) wapiti. Earlier classifications of wapiti, however, considered North American animals to be

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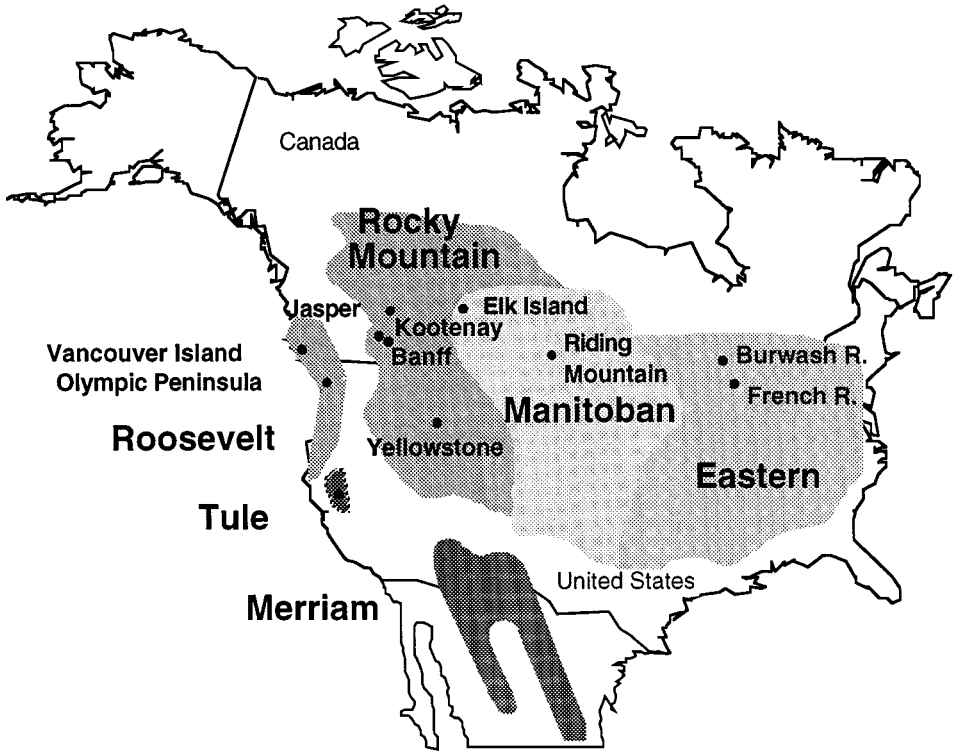
Fig. 1. Historical ranges of the Roosevelt, Rocky Mountain, Manitoban, eastern, Merriam, and tule wapiti, adapted from Bryant and Maser (1982). The locations of each of the four extant and one extinct subspecies in the sample used in this study are identified.



distinct from the European red deer and also had fewer divisions. This is demonstrated by Murie (1951), who accepted only two species of North American wapiti: *C. canadensis* and *C. nannodes*. The name *C. e. canadensis* was used to describe the eastern, Rocky Mountain, Manitoban, and even the Roosevelt wapiti (Bryant and Maser 1982). Currently, subspecies found in adjoining ranges are still considered by some to be one entity. Schonewald (1994) suggested that the extinct Merriam wapiti was an extension of the Rocky Mountain type.

Postglacial distributions of the various forms of wapiti (see Fig. 1) have been discussed by Geist (1971), Banfield (1974), Bryant and Maser (1982), and Peek (1982). Historically, the Rocky Mountain wapiti range followed the Rocky Mountains and extended across the northern Canadian boreal coniferous forest. The Manitoban wapiti range covered the region of the prairies known as the Great Plains. The eastern wapiti range corresponded to the eastern deciduous forests that lay parallel to the Manitoban wapiti range and the Atlantic coast, with a northern limit at the Great Lakes and a southern limit in northern Florida. The Merriam wapiti range was south of the Rocky Mountain wapiti range and covered the states of Arizona, Texas, New Mexico, and Mexico. The Roosevelt wapiti range extended along the west coast from southern British Columbia to northern California, while the tule wapiti range was enclosed by the Sierra Nevada – Cascade Mountains in southern and central California (Bryant and Maser 1982).

Hunting and ranching activities led to the extirpation of wapiti from most of their native ranges, and by 1900 only a few herds were found in North America. The tule animals (reports range from one pair to 100) were salvaged by Henry Miller during the mid-1870s and given refuge on his ranch in California (Bryant and Maser 1982). The Merriam wapiti is thought to have become extinct at the start of the 1900s, and the last eastern wapiti was seen in 1893 near North Bay, Ontario (Bryant and Maser 1982). Small herds of Roosevelt wapiti survived on Vancouver Island, British Columbia, on the Olympic Peninsula in Washington State, and in the Cascade Mountains of Oregon.

The difficult terrain in British Columbia provided refuge for several (10–20) isolated herds of Rocky Mountain wapiti (Spalding 1992). In Alberta, these wapiti were reduced to a few dozen in the Brazeau and Highwood river drainages and approximately 150–300 in the Oldman River drainage (Bryant and Maser 1982). Wapiti were never common in the valleys of Jasper and Banff National Parks (Kay et al. 1994). Legislated protection and inhospitable terrain also contributed to the survival of Rocky Mountain wapiti in Colorado, Montana, and Wyoming. The largest herd (>1000 animals) to survive the great extirpation was found in Yellowstone National Park (Houston 1974).

Manitoban wapiti, abundant throughout Alberta until 1810, were reduced to 24 animals in Elk Island National Park by 1906 (Blyth and Hudson),[2] and an unknown number of animals are thought to have existed in the Cypress Hills. Few

[2] C. Blyth and R. Hudson. Vegetation and ungulate management plan for Elk Island National Park. Unpublished status review, Department of Animal Science, University of Alberta, Edmonton. pp. 117–131.

animals survived on the open prairies and no Manitoban wapiti were found in the United States after 1900. The largest concentration of Manitoban wapiti was found in Riding Mountain National Park, Manitoba, which began with more than 500 animals (Banfield 1949).

The similarity in appearance of wapiti in the different ranges led to questions regarding their taxonomic status. However, morphological comparisons failed to reveal unique or indisputable characters that can discriminate between the different subspecies. Skull and antler characters both separated subspecies (McCullough 1969; Hutton 1972) and lumped them together (Green 1956; Blood and Lovaas 1966; Hutton 1972). Manitoban wapiti were described as both smaller (Soper 1946) and larger (Blood and Lovaas 1966) than Rocky Mountain wapiti. However, there is little dispute that the tule form is both smaller and lighter in coat color than other forms. As well, the Roosevelt form tends to be larger and heavier than the Rocky Mountain form, with more massive but shorter (crownlike) antlers, a shorter tail, longer hind feet, and a greater contrast between light and dark portions of the coat (Schwartz and Mitchell 1945; Quimby and Johnson 1951).

Morphological characters are encoded by the genetic components of DNA, but are influenced by the age, sex, and health of an animal, as well as by seasonal and habitat conditions (Berger and Peacock 1988; McHugh 1972; Geist 1991). Comparisons of strictly genetic components avoid these complex influences and still allow one to use characters that are under evolutionary constraints. Few studies have been directed at identifying the diversity of wapiti. Chromosome numbers vary within the genus *Cervus* (Fontana and Rubini 1990), but are constant among North American wapiti subspecies. Hemoglobin (Dratch 1986) and protein electrophoresis studies (Dratch and Gyllensten 1985) identified loci that were both unique and fixed in either red deer or wapiti, but they did not separate North American animals into subspecies. Glenn and Smith (1993) failed to differentiate among five of seven Rocky Mountain populations by means of protein variation. They did note that the number of polymorphic loci (P) was 0.087 in wapiti, with an average of 1.1 allele per locus, and that there was a slight difference between Roosevelt and Rocky Mountain populations. A lack of variation was also observed by Cameron and Vyse (1978), who found a P value of 0.0416 for wapiti in Yellowstone National Park, and Kucera (1991), who obtained a P value of 0.053 for tule wapiti. Random amplified polymorphic DNA analysis of wapiti suggested that similarity among individuals ranged from 0.976 to 0.947 (Comincini et al. 1996).

Clearly, genetic variation exists in wapiti populations, albeit reduced. DNA that has highly evolving sequences, such as the D-loop region of mtDNA, will usually produce more variable characters and is therefore best suited for distinguishing between closely related taxa. Total mtDNA analysis using restriction enzymes failed to uncover unique differences between 22 wapiti (Cronin 1991). While restriction analysis can assay at most a few hundred nucleotides, sequencing can assay thousands. In a study to determine genetic variation among subspecies, Cronin (1992) found one unique haplotype in the Rocky Mountain population. In addition to a common haplotype found among Rocky Mountain and Manitoban animals, restriction analysis of the D-loop region of mtDNA from 59 wapiti by Polziehn (1993) and Murray et al. (1995) confirmed a unique *Cfo*I restriction pattern for 15.8% (3/19) Rocky Mountain wapiti and a *Hin*fI restriction site for all 25 Roosevelt wapiti.

The relationship between genetic and geographic distribution has been used to augment classical taxonomy. However, employing genetic diversity to identify wapiti subspecies and their ranges has been complicated by numerous relocations of animals. Transplanting wapiti throughout North America gained popularity when populations started flourishing in Yellowstone, Olympic Peninsula, Elk Island, and Riding Mountain National Parks and on private land in California. Relocations of significance to this study are listed in Fig. 2. Many past introductions have involved moving wapiti from one subspecies into the range of another subspecies, and remarkably, similar events still occur. In 1984, for example, a group of Manitoban wapiti were released in the Kechikan River Valley, home of a native herd in coastal British Columbia.

A phylogenetic analysis of the D-loop region of mtDNA was performed to investigate genetic variability among wapiti and to determine if the genetic relationships correspond to the distribution of subspecies. When all descendants of the most recent common ancestor were found to belong to one subspecies, the subspecies is called monophyletic. Monophyletic groups provide strong support for subspecific status. Paraphyly occurs when not all members of the most recent common ancestor are found in one subspecies. Paraphyly can occur among well-defined subspecies. Subspecies that arose from several recent common ancestors or lineages are called polyphyletic. Polyphyly is usually apparent when there has been insufficient time for populations to become distinct, or occurs as a consequence of hybridization or relocation. Polyphyly of subspecies provides evidence against the biological reality of such groups.

Materials and methods

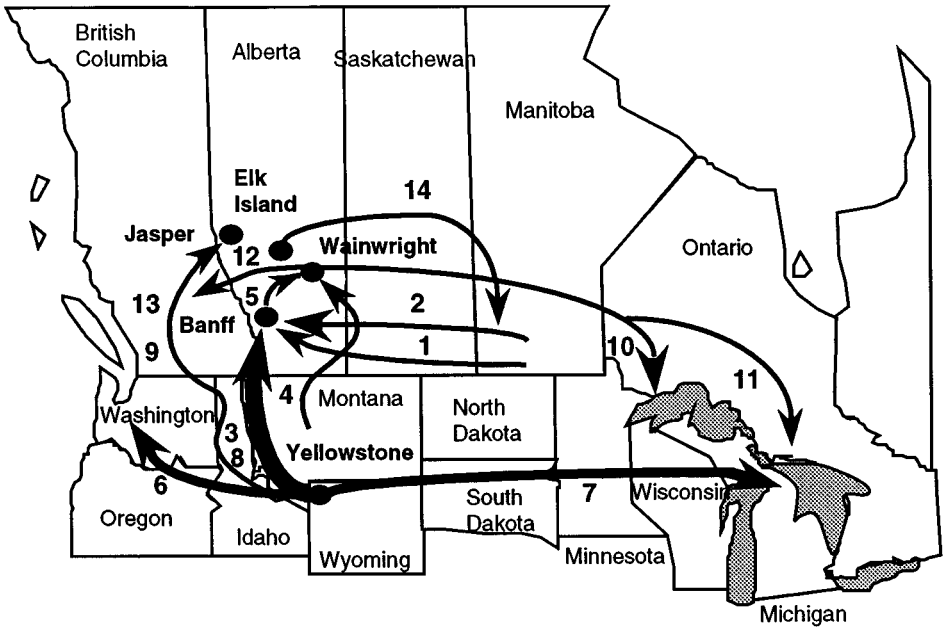
Collection

Samples representing Rocky Mountain wapiti were collected opportunistically from the following National Parks: Jasper, Alberta (91 and 92); Banff, Alberta (23, 37, and 14); Kootenay (KNP), B.C., and Yellowstone, Wyoming (1 and 2). Samples from Manitoban wapiti were collected from animals restrained for transport from Elk Island National Park, Alberta, and opportunistically from animals from Riding Mountain National Park, Manitoba. Samples potentially representing eastern wapiti were also collected from the French (B5A and B6) and Burwash (T1 and T5(55) river regions south of Sudbury, Ontario. Roosevelt samples (Roosevelt 33, 32, 29, 25, and 23) were supplied by the Fish and Wildlife Services in Alberta and British Columbia. The Forensics Laboratory of U.S. Fish and Wildlife supplied lyophilized samples from sika deer (215 and 226 samples), red deer (765 and 923 samples), tule wapiti (457 and 659), and Roosevelt wapiti (10 samples). The locations of samples from Canada and the United States are shown in Fig. 1.

Isolation and amplification (polymerase chain reaction)

DNA was isolated as in Bork et al. (1991) or using methods described in the Qiagen QIAamp tissue isolation kit (Chatsworth, Calif.). The D-loop region of mtDNA was enzymatically amplified in 100 μL of reaction mixture containing 0.06 mM each of dATP, dCTP, dGTP, and dTTP, 1× polymerase chain reaction (PCR) buffer (10 mM Tris buffer, pH 8.8, 0.1% Triton × 100, 50 mM KCl, and 0.16 mg/mL

Fig. 2. Relocations of wapiti in North America (Lloyd 1927; Bryant and Maser 1982; Stelfox and Stelfox 1993). Only a few of the hundreds of introductions that have taken place since 1900 are shown. These transfers illustrate the potential for hybridization to take place between subspecies.



Event No.	Date	Description
1	1900	Banff National Park received four bulls and one cow from Mrs. Ticknor of Morden, Manitoba.
2	1902	Banff National Park received one cow from Portage la Prairie, Manitoba (and one cow from Calgary, Alta.)
3	1910	Banff National Park purchased two cows and two bulls originating from Wyoming from Mr. J. Hill
4	1910	Wainwright Buffalo Park purchased two bulls and one cow from Michele Pablo of Montana
5	1910	Wainwright Buffalo Park received six wapiti from Banff National Park
6	1913	Yellowstone National Park shipped animals for 20 years into the Selkirk and Wenatchee Mountains
7	1915	Yellowstone National Park introduced 23 animals to Sturgeon County, Michigan
8	1916	Yellowstone National Park shipped 66 wapiti to Banff National Park and another 196 in 1920
9	1920	98 Yellowstone National Park wapiti were introduced into Jasper National Park
10	1930s	24 wapiti from Wainwright Buffalo Park were introduced to the Burwash Industrial Prison farm near Sudbury, Ont.
11	1920–1940	Wainwright Buffalo Park sent wapiti to the Nipigon–Onamon Game Preserve and an enclosure near Pemberton, Ont. Animals from the enclosure were relocated to the Bruce Peninsula, Abitibi, Peterborough, and Marten River
12	1927	Wainwright Buffalo Park shipped 25 wapiti to Cookson, B.C.; in 1933 another 25 animals went to Adams Lake, B.C.
13	1936	Yellowstone National Park wapiti shipped to Hinton, Alta., near Jasper National Park
14	1949	Elk Island National Park shipped an unknown number of animals to The Pas, Man.

bovine serum albumin), 1 unit of *Taq* polymerase, 2.0 mM magnesium chloride, and 20 pmol each of primers CST 2 and 39 (Table 1). Primer CST 2 anneals to the start of the transfer RNA (tRNA) proline gene upstream from the D-loop region and CST 39 anneals to the start of the 12S gene downstream from the tRNA phenylalanine gene and D-loop region. These primers were based on universal D-loop primers described by Kocher et al. (1989). Each 100-μL amplification reaction was performed on a 9600 Perkin Elmer Cetus thermocycler, using the following conditions: a 3-min denaturing step at 94°C; 30 cycles at 94°C for 15 s, 56°C for 30 s, and 72°C for 30 s; and a final 10-min extension at 72°C. The amplified products were separated from unincorporated primers by electrophoresis on a 1% agarose 0.5 × TBE gel. DNA fragments containing the D-loop region were excised from the gel with a scalpel and the DNA was isolated using a Qiagen Qiaquick Extraction kit. Samples were desiccated and resuspended in 36 μL of double-distilled water.

Each sequencing reaction of the D-loop region was performed using 8 μL of purified PCR product, as described in the Perkin–Elmer Dye Terminator Cycle Sequencing Ready Reaction kit. Primers used for sequencing are given in Table 1. Cycle sequencing reaction parameters on the 9600 Perkin Elmer Cetus thermocycler were denaturation at 96°C for 15 s, annealing at 50°C for 1 s, and extension at 60°C for 4 min. Sequencing reactions were separated by electrophoresis on a ABI Prism 377 Perkin Elmer automated sequencer. Sequence data were processed and analyzed using ABI sequence software.

Table 1. Primers employed in the sequencing of the control region of mitochondrial DNA in cervids.

Primer	Location	Sequence (5′–3′)
2*	1–22	TAATATACTGGTCTTGTAAACC
25*	614–591	TCATGGGCCGGAGCGAGAAGAGG
39*	1216–1192	GGGTCGGAAGGCTGGGACCAAACC
139*	493–522	ATGTCAAATCTACCCTTGGCAACATGCGTA
149*	763–730	AGCACAGTTATGTGAGCATGGGCTGATTGG
463	714–733	CTCGATGGACTAATGACTAA
464	275–294	CTCGTAGTACATAAAATCAA
468	990–968	ATAAGGGGGAAAAATAAGAA

*Published in Polziehn (1993).

PCR products from the 10 Roosevelt samples from Olympic Peninsula National Park were restricted with the endonuclease *Hin*fI. It was thought that this enzyme distinguished Roosevelt wapiti from other wapiti subspecies in the D-loop region of mtDNA. The fragments were separated by electrophoresis on a vertical gel apparatus as described by Murray et al. (1995).

Phylogenetic analysis

Once sequences were aligned using the software Sequence Editor™ (Applied Biosystems), nucleotide substitutions, deletions, and insertions were identified. Sequences were analyzed for phylogenetic content using the heuristic branch-and-bound option of PAUP 3.1 (Swofford 1993). The PAUP program constructs phylogenetic trees based on parsimony criteria. Trees were constructed using both unweighted and weighted characters, where transversions were worth 2, 5, and 10 times more than transitions and gaps were equally weighted to transitions. Gaps generally occurred in tandom repeats of a single nucleotide. Trees were rooted using red deer and sika deer and examined for polyphyly. Bootstrapping was used to place confidence estimates on branches within the most parsimonious trees and was restricted to 100 replicates. Trees were constructed for pure populations as well as for populations known to have introductions.

Divergence

The following estimates of DNA divergence are taken from Nei (1987). The average number of nucleotide substitutions for haplotypes (d_x) in population X are estimated by

$$d_x = \frac{n_x}{n_x - 1} \sum_{ij} x_i x_j d_{ij}$$

where n_x is the number of sequences sampled and d_{ij} is the number of nucleotide substitutions per site between the ith and jth haplotypes. The average number (d_{xy}) of nucleotide substitutions between DNA haplotype from populations X and Y is estimated from

$$d_{XY} = \sum_{ij} x_i y_j d_{ij}$$

where d_{ij} is the number of substitutions between the ith haplotype from X and the jth haplotype from Y. The number of net nucleotide substitutions can be estimated by substracting the average intrapopulation distance from the intrapopulation distance, given as

$$\pi = d_{XY} - (d_X + d_Y)/2$$

Results

The D-loop region of mitochondrial DNA amplified from the four subspecies of North American wapiti was 1211 base pairs (bp) compared with 1135 bp for the red deer and 1215 bp for the sika deer. Compared with the North American wapiti, the red deer had four insertions and three deletions, with one deletion of 77 bp (Table 2). The sika deer had four insertions relative to the North American wapiti.

In addition to 2 red deer and 2 sika deer sequences, 25 unique sequences were recognized from the 28 wapiti analyzed. The sequences submitted to GenBank have Accession Numbers AF005196–5200, AF016953–16977, and AF016979–16980. In the phylogenetic analysis, there were 40 variable nucleotide sites among the wapiti sequences (Table 3), including 27 transitions, 9 tranversions, 2 insertions, and 2 deletions. There were 17 uninformative sites (113, 315, 476, 541, 681, 709, 798, 838, 852, 935, 942, 951, 986, 1025, 1054, 1117, and 1138) and 23 informative sites (181, 269, 440, 442, 444, 448, 450, 487, 488, 493, 627, 679, 694, 703, 717, 737, 808, 867, 960, 968, 981, 988, and 1154). Sites 440, 442, 448, 487, 488, 627, 679, 694, and 703 were homoplasic between wapiti and the out-groups, and characters 181, 450, 486 or 968, 960, and 988 were homoplasic within wapiti. Characters at sites 181, 960, 968, and 981 represent the absence/presence of a nucleotide in a long repeat of the identical nucleotide. Similarly, nucleotide substitutions found within a string of repeats include characters at sites 694, 709, 867, 951, and 1154. Replication errors are more likely to occur at runs of identical bases in the DNA (Ghosal and Saedler 1978), therefore mutations at these sites carry little phylogenetic weight.

The sequence of Yellowstone National Park wapiti (2) matched that of the KNP wapiti, Riding Mountain National Park sample 3 matched Riding Mountain National Park sample 4, and Burwash River sample T1 matched Burwash River sample T5(55). Because mtDNA is passed maternally, animals sharing female founders will have the same mtDNA sequences. The Riding Mountain National Park samples 3 and 4 were from the same herd, and similarly, Burwash River samples T1 and T5(55) were from the same herd. The KNP wapiti most likely descended from a Yellowstone animal relocated to Banff in the 1920s.

The number of nucleotides that varied between sequences ranged from 2 to 14 among Rocky Mountain and Manitoban animals, from 3 to 13 among Rocky Mountain and Roosevelt animals, and from 4 to 15 among Rocky Mountain and tule animals, with an average of 0.56% (6.53/1165) nucleotide

Table 2. Control region sequences from mtDNA of North American wapiti, Asian sika deer, and European red deer.

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                                                                                                  60
Wapiti      TAATATACTG  GTCTTGTAAA  CCAGAAAAGG  AGAGCAACCA  ACCTCCCTAA  GACTCAAGGA
Sika deer   ..........  ..........  ..........  ..........  ..........  ..........
Red deer    ..........  ..........  ..........  ........T.  ..........  ..........
                                                                    *             120
Wapiti      AGAAGCCATA  GCCCCACTAT  CAACACCCAA  AGCTGAAGTT  CTATTTAAAC  TATTCCCTGA
Sika deer   ..........  ..........  ..........  ..........  ..........  ..........
Red deer    T.........  ..........  ..........  ..........  ..........  ..........
                                                                                      180
Wapiti      CGCTTATTAA  TATAGTTCCA  TAAAAATCAA  GAACTTTATC  AGTATTAAAT  TTCCAAAAAA
Sika deer   ..........  ..........  ..........  ..........  ..........  ..........
Red deer    ..........  ..........  ..........  ..........  ..........  .........G
            *                                                                         240
Wapiti      -TTTAATATT  TTAATACAGC  TTTCTACTCA  ACATCCAATT  TACATTTTAT  GTCCTACTAA
Sika deer   T.........  ..........  ..........  ...C......  ......C...  .C....T...
Red deer    T.........  .C........  ....C.....  ...C...T..  .........C  A...-..C..
                                                      *                               300
Wapiti      TTACACAGCA  AAACACGTGA  TATAACCTTA  TGCGCTCGTA  GTACATAAAA  TCAATGTGCT
Sika deer   CC.....A..  ..G.......  ..........  ..T...T...  ..........  .T....CATC
Red deer    .......A..  ...T.T..A.  ......TA..  ......TA..  .......G..  .T.....A..
                        *                                                             360
Wapiti      AGGACATGC-  ATGTATAACA  GTACATGAGT  TAGCG-TATA  GGACATATTA  TGTATAATAG
Sika deer   .A.....A.T  .....CGGT.  ........AA  CC.GTA....  .......C..  ..........
Red deer    .......A.T  ..........  ....------  ----------  ----------  ----------
                                                                                      420
Wapiti      TACATAAATT  AATGTATTAA  GACATATTAT  GTATAATAGT  ACATTATATT  ATATGCCCCA
Sika deer   ..........  .........G  A.........  ..........  ..........  ..........
Red deer    ----------  ----------  ----------  ----------  ----......  ..........
                                    *   * *   * *                           *         480
Wapiti      TGCTTATAAG  CATGTACTTC  TCACTATCTG  AAGTACATAG  TACATAATGT  TGTTCATCGT
Sika deer   ..........  ......T..T  CT.T.....A  T.........  .....G....  CA..T.....
Red deer    ...A......  .........T  CT.T...T.A  T.........  .....G....  ..........
                  **        *                                                         540
Wapiti      ACATAGTACA  TTAAGTCAAA  TCAGTCCTTG  TCAACATGC   GTATCCCGTCC  CCTAGATCAC
Sika deer   ......C...  ..........  ..........  ..........  ..........  ..........
Red deer    ......CG..  ..........  ..........  ..........  ..........  ..........
            *                                                                         600
Wapiti      GAGCTTAATT  ACCATGCCGC  GTGAAACCAG  CAACCCGCTG  GGCAGGGATC  CCTCTTCTCG
Sika deer   ......G...  ..........  ..........  .........A  ..........  ..........
Red deer    ......G...  ..........  ..........  ..........  ..........  ..........
                                          *                                           660
Wapiti      CTCCGGGCCC  ATGAACCGTG  GGGGTAGCTA  TTTAATGAAT  TTTATCAGAC  ATCTGGTTCT
Sika deer   ..........  ..........  ......A...  .........C  ..........  ..........
Red deer    ..........  ......T...  ..........  .........C  ..........  ..........
                                 *   *           *           *           *            720
Wapiti      TTTTTCAGGG  CCATCTCATC  TAAAATCGCC  CACTCCTTGT  AAC - ATAAGA  CATCTCGATG
Sika deer   ..........  ........C.  ..........  .........C  ..TT.C....  ..........
Red deer    ..........  ........C.  ..........  .........C  ..T.......  ..........
                        *                                                             780
Wapiti      GACTAATGAC  TAATCAGCCC  ATGCTCACAC  ATAACTGTGG  TGTCATACAT  TTGGTATTTT
Sika deer   ..........  ..........  ..........  ..........  ..........  ..........
Red deer    ..........  ..........  ..........  ..........  ..........  ..........
                           *           *                                      *      840
Wapiti      TAATTTTTGG  GGGGATGCTT  GGACTCAGCA  ATGGCCGTCT  GA-GGCCCCG  TCCCGGAGCA
Sika deer   ..........  ..........  ..........  ..........  ..........  ..........
Red deer    ..........  ..........  ..........  ...A......  .GC..T....  ..........
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Table 2. (*concluded*)

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                          *           *                                                  900
Wapiti      TGAATTGTAG  CTGGACTTAA  CTGCATCTTG  AGCATCCCCA  TAATGGTAGG  CGCAGGGCAT
Sika deer   ..........  ..........  ..........  ..........  ..........  ATG.......
Red deer    ..........  ..........  ..........  ..........  ..........  AT-.......
                                                    *           *           *          960
Wapiti      TACAGTCAAT  GGTCACAGGA  CATAGTTATT  ATTTCATGAG  TCAACCCTAA  GATCTATTTT
Sika deer   .G........  ..........  ..........  ..........  ..........  ..........
Red deer    GG........  .........   ....A.C...  ..........  ..........  ..........
                    *                 *   *  *                                        1020
Wapiti      CCCCCCCCTT  CTTATTTTTT  -CCCCCTTAT  ATAGTTATCA  CCATTTTTAA  CACACTTTCC
Sika deer   ..........  ..........  ..........  .......C..  T.........  ........T.
Red deer    .........G  ..A.......  ..........  ..........  ..........  ........T.
                  *                                   *                              1080
Wapiti      CCTAGATATA  ATTTTAAATT  TATCACATTT  CCAATACTCA  AAATAGCACT  CCAGAGGGAG
Sika deer   .........T  ..........  ..........  ..........  ..T.......  ..........
Red deer    .........T  ..........  ..........  ..........  ..TC......  ....G...T.
                                                        *           *                  1140
Wapiti      GTAAGTATAT  AAACGCCAAT  TTTTCCCTAA  TTATGCATAG  TTAATGTAGC  TTAAACAGCA
Sika deer   ..........  ..........  ..........  ..........  ..........  ..........
Red deer    ....C.....  ..........  ..........  .....T.C..  ..G.......  .....TG...
                        *                                                            1200
Wapiti      AAGCAAGGCA  CTGAAAATGC  CTAGATGAGT  ATATTAACTC  CATAAAACAC  ATAGGTTTGG
Sika deer   ..........  ..........  ..........  ..........  ..........  ..........
Red deer    ..........  ..........  ..........  ..........  ..........  ..........

Wapiti      TCCCAGCCTT  CCGACCC
Sika deer   ..........  .......
Red deer    ..........  .......
```

Note: Nucleotide substitutions are given and gaps are indicated by a dash. Nucleotide substitutions in wapiti are marked above the consensus by an asterisk and can be found in Table 4.

substitutions between wapiti. As estimated from sequence divergence, Manitoban and Rocky Mountain wapiti had the closest genetic distance (π), 0.00767, followed by Rocky Mountain and tule wapiti, where π = 0.00826. The greatest distances were found between tule and Manitoban wapiti, where π = 0.01256, and tule and Roosevelt wapiti, where π = 0.01288. The comparison of the numbers of nucleotide differences among subspecies places Rocky Mountain wapiti central to all other subspecies (Table 4).

Comparisons of nucleotide substitutions (Table 3) in tule wapiti revealed that site 269 was unique to tule wapiti, site 627 was shared with sika deer, and site 703 was shared with both red deer and sika deer. Comparisons of nucleotide substitutions in Roosevelt wapiti showed that site 493 was unique to all members of this group, site 1154 was unique to most of the Roosevelt wapiti, and site 450 was shared among several Roosevelt and Rocky Mountain wapiti. No informative nucleotide sites were shared by all Rocky Mountain or Manitoban wapiti, although the insertion at site 981 and deletion at site 960 were exclusive to several Manitoban wapiti. Nucleotide substitutions at sites 487 and 968 were found among both Rocky Mountain and Manitoban wapiti. The nucleotide change at site 488 created a unique recognition site for endonuclease *Cfo*I among several Rocky Mountain wapiti. The nucleotide substitution at site 493 identified the unique recognition site for *Hin*fI found in Roosevelt wapiti.

Trees were constructed using weighted and nonweighted characters and both including and excluding gaps. Roosevelt and tule wapiti were found in monophyletic clades regardless of constraints or weights. If gaps were not considered informative and transversions were not given extra weight, then both Manitoban and Rocky Mountain animals were found to be paraphyletic. By including gaps as a new state character but no extra weighting on transversions, the heuristic search placed Rocky Mountain animals into clades that (*i*) branch before all other animals (Yellowstone 1, Banff 23, Banff 37, and Jasper 91), (*ii*) include Manitoban animals (Banff 14 and Jasper 92), and (*iii*) form a sister-clade to tule and Roosevelt animals. This would make the Rocky Mountain group polyphyletic, while the Manitoban group would remain paraphyletic. Weighting transversions twice as heavily as transitions, and including gaps as characters, also resulted in paraphyly of Manitoban and polyphyly of Rocky Mountain types (see Fig. 3*a*). Weighting transversions to transitions more strongly (5:1 or 10:1) and counting gaps caused some interesting changes: Elk Island animals 20 and 72 grouped closely with the Yellowstone 1 animal in a clade that also included Riding Mountain National Park individuals; Yellowstone 2 and KNP animals moved to a clade containing both tule and Roosevelt animals. Banff 14 and Jasper 92 animals again grouped with the remaining Riding Mountain and Burwash River animals, and Banff 23, Banff 37, and Jasper 91 animals

branched early in the tree. Both Manitoban and Rocky Mountain groups became polyphyletic with increasing weights on transversions.

The consensus of 26 equally parsimonious trees, using transversions weighted twice as much as transitions, and gaps equal to new state characters (shown in Fig. 3), illustrates the relationships observed among wapiti common to most trees of weighted and nonweighted characters. The consistency index was 0.928 and branch lengths were equal to 262 steps. It is important to note that the Yellowstone 2 / KNP animal can be moved to the clade containing the Manitoban wapiti without additional steps by changing the order in which characters 968 and 487 appear in the tree. Branch lengths varied between 230 and 562 for bootstraps on unweighted trees that saved only one tree per replication. Bootstraps for 100 replications were performed using the ratio 2:1 for transversions to transitions found in wapiti where branch lengths varied from 230 to 562 steps. The bootstrap (not shown) used 280 steps, and CI = 0.821. Roosevelt wapiti were grouped together with 56% frequency in the weighted bootstrap consensus tree. Rocky Mountain wapiti from Jasper, Kootenay, Yellowstone, and Banff 14 and the Manitoban sample Riding Mountain 1 also did not sort into any one clade. The number of homoplasies and the absence of unique informative characters do not lead to a consistent division of Rocky Mountain or Manitoban wapiti into subspecies.

Analysis of populations that have had no introductions, including those from Elk Island, Riding Mountain, and Yellowstone National Parks, was also performed using the same restraints as noted above. Rocky Mountain wapiti were paraphyletic and Manitoban, tule, and Roosevelt wapiti were monophyletic (Fig. 3*b*) if Elk Island animals are of the Manitoban type.

Digests of the 10 Olympic Peninsula National Park Roosevelt samples using the restriction enzyme *Hin*fI revealed six individuals with fragment sizes of approximately 450, 340, 300, and 135 bp and four individuals with fragment sizes of 750, 340, and 135 bp. The first restriction fragment length pattern was formerly found among only Roosevelt individuals, while the second was common to Rocky Mountain and Manitoban forms. *Hin*fI sites can be found at sequence sites 493, 809, 906, and 1148.

Discussion

Historically, North American wapiti populations were assigned to subspecies largely on the basis of their geographic distribution, which has made the taxonomic classification particularly suspect for Rocky Mountain, Manitoban, eastern, and Merriam wapiti. The phylogenetic relationships of wapiti in this study are discussed with regard to the invasion and distribution of wapiti in North America and the large number of reintroductions of animals into both historical and nonhistorical ranges.

Wapiti originated in Asia and entered North America by crossing the Bering land bridge (Guthrie 1966). The land bridge between the two continents is thought to have disappeared 10 000 – 15 000 years ago when the sea level rose (Pielou 1991). The post-Wisconsin stage (10 000 – present) was marked by gradual climate and habitat changes that may have led to the extinction of the Alaskan population, the division of the large central population into montane/boreal, prairie, and deciduous forest ecotypes, and further isolation of the Californian and west coast populations by the Cascade and Rocky mountains (Guthrie 1966). Prior to the arrival of Europeans, Seton estimated that there were 10 000 000 wapiti in North America, with numbers dwindling to less than 100 000 by 1907 (Bryant and Maser 1982). Both numbers are likely overestimates, but they illustrate that wapiti were once widely distributed across North America, with the exception of the tule and Roosevelt wapiti residing along the west coast. According to the phylogenetic tree, all wapiti subspecies appear to have descended from one common ancestor, which clearly suggests a close relationship among North American animals.

As the wapiti population expanded and herds dispersed into new habitats, a few founders would eventually have moved into the remote coastal regions and given rise to the tule and Roosevelt populations. Murie (1951) suggested that the Rocky Mountain wapiti possibly gave rise to the tule and Roosevelt wapiti, although Bailey (1936, p. 78) found no fossil records to indicate that the range of Rocky Mountain wapiti was ever connected with that of the Roosevelt animals. Movement across the mountain ranges was not impossible, but likely not extensive. Both the Roosevelt and tule populations are monophyletic, which suggests that each is derived from a single lineage. Populations isolated for long periods of time generally accumulate nucleotide differences not found in other populations. These differences translate into greater genetic distances between populations. The largest number of nucleotide differences was found in comparisons between tule and Roosevelt animals. Tule and Roosevelt wapiti (Vancouver Island) have maintained their monophyletic status as a result of isolation brought about by habitat changes, reduction of their populations caused by human intervention, and the fortuitous lack of relocations of wapiti into or out of these populations.

The Roosevelt population from Olympic Peninsula National Park is comprised of a mixture of individuals with haplotypes unique to the Roosevelt form and individuals with haplotypes common to the Rocky Mountain and Manitoban forms. Presently, Washington State is home to large populations of both Roosevelt and Rocky Mountain animals (Bryant and Maser 1982), and movement between the two populations may account for the presence of Rocky Mountain / Manitoban haplotypes in wapiti in Olympic Peninsula National Park. The introduction of Yellowstone wapiti into the Wenatchee Mountains between 1913 and 1933 would have placed Rocky Mountain wapiti within reasonable travelling distance of the Olympic Peninsula population. When restriction data from Polziehn (1993), where eight Olympic Peninsula National Park animals also had the unique *Hin*fI restriction site, are included, the frequency of Rocky Mountain / Manitoban haplotypes in the Olympic Peninsula population is 22%.

A panmictic or clinal distribution was suggested for eastern, Manitoban, and Rocky Mountain wapiti (Bryant and Maser 1982; Schonewald 1994). With a few exceptions, Schonewald (1994) found a decrease in cranial size from north to south and from western Europe to North America. Blyth and Hudson (see footnote 2) suggested that the park-

Table 3. Nucleotide substitutions in the control region of mitochondrial DNA among North American wapiti subspecies.

	Position of nucleotide substitution																			
Wapiti sample	113	181	269	315	440	442	444	448	450	476	487	488	493	541	627	679	681	694	703	709
Consensus	T	–	T	A	C	C	C	C	G	A	T	A	A	G	G	T	T	T	C	G
Riding Mountain 1	.	.	.	G	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Riding Mountain 2	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Riding Mountain 3/4	.	A	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.
Riding Mountain 5	A	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Riding Mountain 7	.	A	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.
French River 5A	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	C	.	.	.	A
French River B6	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	C	.	.	.	.
Burwash River T1/T5(55)	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Elk Island 20	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.
Elk Island 63	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.
Elk Island 72	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.
Banff 14	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	C	.	.
Banff 23	.	.	.	.	.	.	T	T	A	.	C	G	.	.	.	.	.	.	.	.
Banff 37	.	.	.	.	.	.	T	T	A	.	C	G	.	.	.	.	.	.	.	.
KNP/Yellowstone 2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Jasper 91	.	.	.	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.	.	.
Jasper 92	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.
Yellowstone 1	.	.	.	.	T	.	.	.	A	.	.	.	.	.	.	.	.	C	.	.
Roosevelt 23	.	.	.	.	.	.	.	.	.	G	.	.	G	.	.	.	.	.	.	.
Roosevelt 25	.	.	.	.	.	.	.	.	A	.	.	.	G	.	.	.	.	.	.	.
Roosevelt 29	.	.	.	.	.	.	.	.	A	.	.	.	G	.	.	.	.	.	.	.
Roosevelt 32	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.
Roosevelt 33	.	.	.	.	.	.	.	.	A	.	.	.	G	A	.	.	.	.	.	.
Tule 457	.	.	C	.	.	.	.	.	.	.	.	.	.	.	A	.	A	.	T	.
Tule 659	.	.	C	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	T	.
Sika deer 226	.	T	.	.	T	T	T	.	A	.	C	.	.	.	A	C	.	.	T	.
Sika deer 215	.	T	.	.	T	T	T	.	A	.	C	.	.	.	A	C	.	.	T	.
Red deer 765	.	T	.	.	T	T	T	.	A	.	C	G	.	.	.	C	.	C	T	.
Red deer 923	.	T	.	.	T	T	T	.	A	.	.	G	.	.	.	C	.	.	T	.

Note: Nucleotide substitutions that vary from the wapiti consensus sequence are given, and deletions are indicated by a dash.

Table 4. Divergence of mtDNA D-loop sequences from wapiti subspecies calculated from the number of nucleotide differences between individuals from each type. Values in boldface type indicate sequence variation within the subspecies (d_X), values above the diagonal represent uncorrected sequence variation within the species (d_{XY}), and values below the diagonal represent sequence divergence between subspecies corrected for intraspecific variation (π).

	Wapiti subspecies			
	Manitoban	Rocky Mountain	Roosevelt	Tule
Manitoban	**0.00326**	0.01110	0.01344	0.01505
Rocky Mountain	0.00767	**0.00343**	0.01047	0.01084
Roosevelt	0.01044	0.01257	**0.00275**	0.01511
Tule	0.01257	0.00826	0.01288	**0.00172**

land area of central Alberta serves as a transition zone between boreal and prairie habitats, and that Rocky Mountain and Manitoban wapiti in Alberta may have genetic affinities because of overlapping ranges. A similar relationship between the historical ranges of the Manitoban and eastern subspecies can be suggested.

Elk Island National Park wapiti were assumed to be of the Manitoban form because their mtDNA grouped with that of other Manitoban wapiti, and the Manitoban wapiti range was thought to extend into this region. However, animals used to describe the Rocky Mountain form by Bailey (1935) included wapiti from Fort Saskatchewan, which is approximately 20 km west of Elk Island National Park. Perhaps the Manitoban wapiti ranged farther west than was previously believed, and animals from Fort Saskatchewan should not have been included in the Rocky Mountain group. However, the Elk Island National Park wapiti most likely represent animals in the transition zone, which have morphological and genetic affinities with both types. The existence of this population is likely the greatest proof that Rocky Mountain and Manitoban subspecies are the least differentiated wapiti subspecies.

Yellowstone, Elk Island, and Riding Mountain National Parks have not introduced animals from outside sources into their resident populations. Using only these three populations, one would conclude that Manitoban wapiti have a monophyl-

Table 3. (*concluded*)

Position of nucleotide substitution																			
717	737	798	808	838	852	867	935	942	951	960	968	981	986	988	1025	1054	1117	1138	1154
G	G	C	G	G	T	C	C	C	G	T	C	–	C	T	G	A	A	G	A
.	.	.	.	.	.	.	.	.	.	.	–	.	.	.	.	.	.	.	.
.	C	.	.	.	.	.	.	.	.	–	–	.	.	.	.	.	.	.	.
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.	C	.	.	.	.	.	.	.	.	–	–	.	.	.	.	.	.	.	.
.	.	.	.	A	.	.	.	G	.	.	–	T	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.	.	–	T	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.	.	–	T	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.	–	–	.	.	.	.	.	.	.	.
C	.	A	T	.	.	T	.	.	.	.	–	T	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.	.	–	T	.	.	.	.	.	.	.
C	.	.	T	.	.	T	.	.	.	.	–	T	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	G	G	A	.
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
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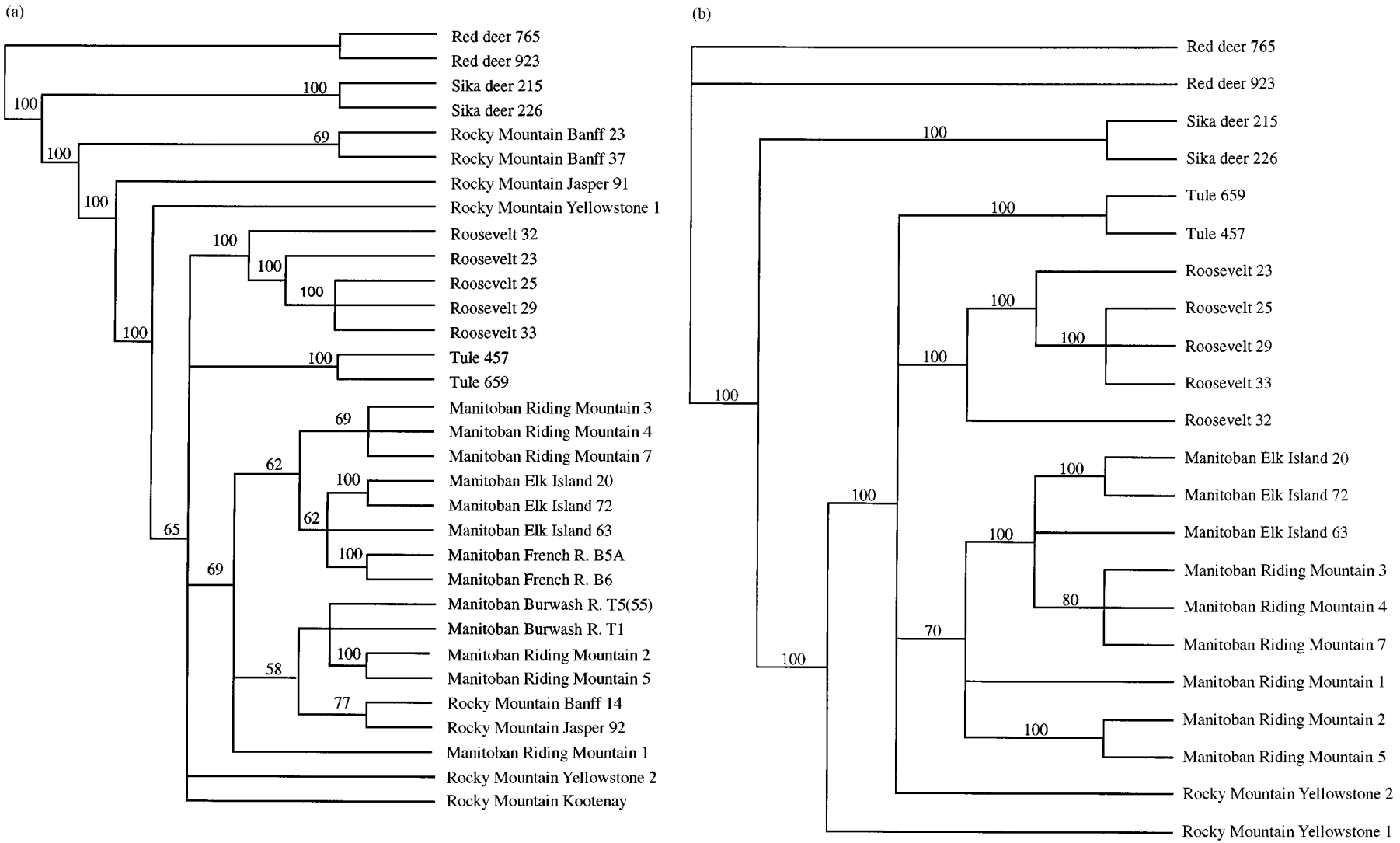
etic origin, assuming that both Elk Island and Riding Mountain National Park populations are of the Manitoban type. The Rocky Mountain population would be paraphyletic, as one lineage branches early in the tree and another branch shares a node with all other forms. This study provides support for the Manitoban subspecies status of wapiti in Elk Island and Riding Mountain National Parks. The sample size from Yellowstone, however, is too small to allow any strong conclusions to be drawn regarding the relationship between Rocky Mountain and Manitoban wapiti.

Using the complete data set, which assumes that wapiti in the Rocky Mountains are most likely of this type, results in a phylogeny that places a few animals of the Rocky Mountain and Manitoban forms in the same clade. This suggests that separation between these two groups is only in the early stages of development. The longer populations are isolated, the more likely it is that shared lineages will be lost and a transition from polyphyly to paraphyly to monophyly will occur. The Rocky Mountain animals (Banff 14 and Jasper 92) found within the clade containing Manitoban wapiti are likely descendants of animals in Yellowstone National Park, as any Elk Island National Park animals were transplanted outside these parks. The shortest genetic distances were found between these two subspecies, which suggests that separation between them is recent.

In the comparison of sequences from the mtDNA D-loop region, Burwash River and French River wapiti presently living in the range of the extinct eastern wapiti were placed in the same clades as the Manitoban or Manitoban / Rocky Mountain group. The absence of unique differences among these sequences suggests that the Burwash River and French River populations are likely not the same as those formerly belonging to the eastern wapiti. The founders of these recent populations originate from the Wainwright herd, which contained descendants from Montana, Wyoming, and (or) Ontario. If the Burwash River and French River animals are descended from lineages that could be directly linked to either Montana or Wyoming, both the Rocky Mountain and Manitoban forms would be polyphyletic.

Outside of park boundaries, one would expect to find even less evidence of distinction between Rocky Mountain and Manitoban subspecies. The Rocky Mountain wapiti in Canada are surrounded by transplanted Elk Island National Park animals, and most likely exhibit hybridization. However, isolated populations in Yellowstone National Park in the United States should represent the true Rocky Mountain

Fig. 3. (*a*) The phylogenetic relationships among North American wapiti, based on the D-loop region of mtDNA. The majority rule consensus of 26 most parsimonious trees, using a 2:1 weighting of transversions to transitions, requires 262 steps and has a CI value of 0.928. (*b*) The phylogenetic relationships among pure populations of North American wapiti, based on the D-loop region of mtDNA. The majority rule consensus of 10 most parisomonious trees, using a 2:1 weighting of transversions to transitions, requires 225 steps and has a CI value of 0.951.



type. Neither Elk Island nor Riding Mountain National Park have had Rocky Mountain animals released within their borders, but both populations have the potential to hybridize with free-ranging and game-ranched animals of Rocky Mountain origin.

Overall, there is a clear lack of mtDNA variation within North American wapiti that corresponds well to the results of previous genetic studies and the lack of morphological differences. The average 0.560% genetic difference in mtDNA among North American wapiti is comparable to the 0.364% (2/549 nucleotides) observed in North American moose (*Alces alces*; Mikko and Andersson 1995) but substantially less than the 2.5% found in white-tailed deer (*Odocoileus virginianus*; Ellsworth et al. 1994).

This phylogenetic study has shown that there is a slight difference between pure wapiti populations, most likely because of the limited number of founders and the absence of wapiti introductions into these populations. Both Roosevelt wapiti from Vancouver Island and tule wapiti are monophyletic, which, by definition, supports their subspecific status. In the absence of geographic barriers, hybridization likely took place at some time between neighboring Rocky Mountain and Manitoban animals, and both forms are found within one clade. The lack of distinction between some Rocky Mountain and Manitoban animals suggests that these two groups are at the early stages of subspeciation.

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