

aDNA LAB REPORT
(2013-07)

**Ancient DNA Analysis of Archaeological Elk Remains
from Historical Parks in the Western United States
(45-PC-106 & 45-SJ-24)**

**Ancient DNA Laboratory
Simon Fraser University**

Project Conducted by Antonia Rodrigues

Project Supervised by Dongya Yang

December 2013

- SUMMARY:** Ancient DNA analysis was conducted on four archaeological cervid remains recovered from two Historical National Parks in the Western United States (Lewis and Clark NHP (45-PC-106) and San Juan Island NHP (45-SJ-24)). All four ancient samples yielded at least 106 bp of mitochondrial DNA (mtDNA). Modern DNA analysis was carried out on six blood samples obtained from elk in Olympic National Park. One sample failed to generate clear DNA sequences for analysis, but additional lab work should be able to obtain usable sequences. Three of the archaeological remains and five of the modern samples generated at least 418 bp of mtDNA (D-loop region), enabling all eight samples to be confidently identified as *Cervus elaphus*. At least three different haplotypes were observed: each archaeological sample had a distinct haplotype, while the modern samples revealed two haplotypes (at least one ancient and modern haplotype overlapped). Due to a lack of strong phylogeographic patterning in modern elk subspecies, these mtDNA haplotypes cannot be confidently assigned to the subspecies level. Additional sampling of both archaeological and modern elk may be able to shed more light on the phylogenetic relationships of ancient and modern elk populations.
- ORIGIN:** Two archaeological elk bone samples were recovered from 45-PC-106, Middle Village-Station Camp, Lewis and Clark National Historical Park (OR, WA). Two additional archaeological elk bone remains were provided by the Burke Museum of National History and Culture and were recovered from 45-SJ-24, English Camp, San Juan Island National Historical Park (WA). Six modern elk blood samples were from elk captured within Olympic National Park (WA).
- CONTACT:** Dr. Ken Ames
Department of Anthropology
Portland State University
Portland, Oregon, 97207-0751
Tel: 503-725-3049, Fax: 503-725-3905
Email: amesk@pdx.edu
- MATERIAL:** Four archaeological elk bone samples were provided to Dongya Yang in March 2013; six modern elk blood samples were provided to Dongya Yang in October 2012 (see Table 1).

Table 1: Elk sample information.

SFU ID	Sample ID	Provenience/Capture Location	Sample Type	Sample Amount
KA1	FDCL4767	45-PC-106, Unit F40, Feature 62, Level 6, 75E/90N/36cmDB	archaeological, bone (caudal vertebrae)	0.761 g & 0.591 g
KA2	LEW18942	45-PC-106, Unit F52, Level 3, 100E/80N/36cmBD	archaeological, bone (tibia fragment)	1.588 g & 0.843 g
KA3	SAJH28667	45-SJ-24, 310300 IP 02 FS#12	archaeological, bone (proximal right humerus)	0.933 g
KA4	SAJH127905	45-SJ-24, OPDTAB 00 07	archaeological, bone (innominate)	0.879 g
KAM1	2010-49, 8503	Sol Duc-Heart Lake, Olympic NP	modern, blood	100 µL
KAM2	2010-34, 8482	Elwha-Buckinghorse, Olympic NP	modern, blood	100 µL
KAM3	2010-48, 6557	Queets-Skyline, Olympic NP	modern, blood	100 µL
KAM4	2010-36, 8479	East-LaCrosse, Olympic NP	modern, blood	100 µL
KAM5	2012-53, 899	Quinault-Graves Creek, Olympic NP	modern, blood	100 µL
KAM6	2010-46, 8477	Hoh-Hoh Lake, Olympic NP	modern, blood	100 µL

DNA EXTRACTION: For the archaeological samples (KA1-KA4), sample preparation and DNA extraction were conducted in the dedicated Ancient DNA Laboratory located in the Department of Archaeology at Simon Fraser University. Samples were chemically decontaminated through submersion in commercial bleach, 1N HCl and 1N NaOH, followed by UV irradiation for 60 min. Samples were manually crushed and incubated overnight in a lysis buffer (0.5 M EDTA pH 8.0; 0.5% SDS; 0.5 mg/mL proteinase K) in a rotating hybridization oven at 50 °C. Samples were then centrifuged and 2.0 mL of supernatant from each sample was concentrated to <100 µL using Amicon Ultra-4 Centrifugal Filter Devices (10 KD, 4mL, Millipore). Concentrated extracts were purified using QIAquick spin columns (QIAGEN, Hilden, Germany) based on the method developed by Yang et al. (1998). 100 µL of DNA from each sample was eluted from QIAquick column for PCR amplification.

For the modern samples (KAM1-KAM6), sample preparation and DNA extraction were conducted in the Modern DNA Laboratory located in the Department of Archaeology at Simon Fraser University. This facility is in a separate building from the Ancient DNA Laboratory. DNA was extracted from 100 µL of blood using the DNeasy Blood and Tissue kit (QIAGEN, Hilden, Germany) following the manufacturer's protocols.

PCR SETUP: For archaeological samples, PCR amplifications (60 cycles) were performed on an Eppendorf™ Mastercycler Gradient using a 30 µL reaction volume containing 1.5X Applied Biosystems™ Buffer, 0.3 µM of each primer, 2 mM MgCl₂, 0.2 mM dNTP, 1.0 mg/mL BSA, 3.0-4.0 µL DNA sample and 3.75 U AmpliTaq Gold LD (Applied Biosystems). PCR targeted a 640 bp fragment of the *Cervus* mtDNA D-loop region using five overlapping primer sets (EK-F23 & EK-R179; EK-F140 & EK-R306; EK-F202 & EK-R391; EK-F332 & EK-R572; EK-F483 & EK-R663).

For modern samples, PCR amplifications (35 cycles) were performed on an Eppendorf™ Mastercycler Gradient using a 25 µL reaction volume containing 1.5X Applied Biosystems™ Buffer, 0.3 µM of each primer, 2 mM MgCl₂, 0.2 mM dNTP, 2.5 µL DNA sample and 1.25 U AmpliTaq Gold LD (Applied Biosystems). PCR targeted the same 640 bp fragment of the *Cervus* mtDNA D-loop region but used a single set of primers (EK-F23 & EK-R663).

Five uL of PCR product from each sample was separated on a 2% agarose gel and visualized using SYBR Green™ on dark reader (Clare Chemical Research Co.). The results of the PCR amplifications and DNA sequencing can be found in Table 2.

SEQUENCING: All successfully amplified samples were sent to Eurofins MWG Operon (Huntsville, AL) for sequencing. All good quality sequences generated were compared to Genbank sequences through the BLAST application to determine their closest match, and to ensure that they did not match with any other unexpected species or sequences. Sample sequences were visually edited and base pair ambiguities were examined using ChromasPro software (www.technelysium.com.au). Primer sequences were truncated from DNA sequences and all fragments were assembled to generate a 418 bp sequence for phylogenetic analysis.

Multiple alignments of the ancient and modern sequences and published cervid reference sequences were achieved using ClustalW (Thompson, et al. 1994), through BioEdit (Hall 1998), and phylogenetic analysis was conducted using Mega 5.0 software (Tamura et al. 2011).

Appendix 1 displays multiple alignments of the obtained ancient and modern elk sequences and reference sequences obtained from Genbank. Appendix 2 displays the phylogenetic relationships between the ancient and modern D-loop haplotypes and reference sequences obtained from Genbank.

RESULTS: According to lab protocols, a species identification and haplotype is assigned to a sample only if it matches identically or very closely with published reference sequences, and if no other evidence, including reproducibility tests or additional sequencing of the same sample indicates the possibility of base pair ambiguities or an ambiguous species identity. All amplifications yielded sequences which matched identically or very closely with some Genbank *C. elaphus* references sequences (see Appendix 1 for accession numbers). However, confident haplotype designations cannot be assigned to the ancient elk samples due to some ambiguous bases within the obtained sequences (resulting from either DNA damage or unclear sequencing results).

Successful amplification and sequencing of all five overlapping mtDNA fragments was obtained for only one of the four ancient elk samples (KA3). KA2 and KA4 produced good quality sequences for four of the five fragments, while sample KA1 yielded only one good quality sequence (106 bp) and was excluded from further analysis (see Table 2).

Five out of six modern elk samples resulted in successful PCR amplification and sequencing of the targeted D-loop fragment (see Table 2). The single failed sample was successfully amplified; however, the amplicons generated poor quality DNA sequences. Additional lab work should yield clear, positive results.

At least three haplotypes were observed in the analyzed samples (see Table 2). The three ancient elk samples each show a distinct haplotype (arbitrarily designated them A, B, C and D). Haplotype A and B were observed in two and three of the modern samples, respectively. When compared to available modern elk haplotypes in GenBank (Polziehn et al. 1998; Polziehn and Strobeck 2002; Randi et al. 2001), haplotype A has been observed in modern populations of *C. e. roosevelti* and *C. e. canadensis*, while haplotype C has been observed in modern populations of *C. e. manitobensis* and *C. e. nelsoni*. Haplotype B has not been previously observed in any modern elk populations.

Table 2: PCR amplification and haplotype results.

SFU ID	Sample ID	----- PCR Amplification (Sequence Quality) -----						Haplotype
		EK-F23 & EK-R663	EK-F23 & EK-R179	EK-F140 & EK-R306	EK-F202 & EK-R391	EK-F332 & EK-R572	EK-F483 & EK-R663	
KA1	FDCL4767	n/a	✓ (messy)	✗	✗	✗	✓ (good)	n/a
KA2	LEWI8942	n/a	✓ (good)	✗	✓ (good)	✓ (good)	✓ (good)	C
KA3	SAJH28667	n/a	✓ (good)	✓ (good)	✓ (good)	✓ (good)	✓ (good)	Possible B or D
KA4	SAJH127905	n/a	✓ (good)	✗	✓ (good)	✓ (good)	✓ (good)	A
KAM1	2010-49, 8503	✓ (good)	n/a	n/a	n/a	n/a	n/a	A
KAM2	2010-34, 8482	✓ (good)	n/a	n/a	n/a	n/a	n/a	B
KAM3	2010-48, 6557	✓ (messy)	n/a	n/a	n/a	n/a	n/a	n/a
KAM4	2010-36, 8479	✓ (fair)	n/a	n/a	n/a	n/a	n/a	B
KAM5	2012-53, 899	✓ (good)	n/a	n/a	n/a	n/a	n/a	B
KAM6	2010-46, 8477	✓ (good)	n/a	n/a	n/a	n/a	n/a	A

The obtained mtDNA sequences cannot provide an unambiguous subspecies designation for the ancient elk in this study due to the lack of strong phylogeographic patterning in modern elk populations, most likely resulting from their relatively recent post-glacial divergence, as well as dramatic population declines during the historic period (Losey and Yang 2007; Polziehn et al. 1998). Additionally, 20th century conservation efforts often transplanted subspecies outside of their historic territories and subsequent hybridization events may have masked historic subspecies mtDNA patterning (Polziehn et al. 1998; Quayle and Brant 2003). Ancient DNA analysis of additional archaeological and modern elk samples, as well as amplification and sequencing of additional mtDNA loci, may be able to resolve the phylogenetic relationship of the ancient elk to modern populations.

AUTHENTICATION: The dedicated ancient DNA laboratory at SFU follows strict contamination control protocols such as the separation of modern DNA and ancient DNA work spaces; the separation of pre-PCR and post-PCR work spaces; the use of ancient DNA dedicated

equipment including clothing, equipment and reagents; and the analysis of negative controls alongside the ancient DNA samples. Ancient DNA amplicons and sequences are scrutinized to ensure that they follow expected amplification and phylogenetic patterns.

The results of the DNA amplification and sequences suggest that the recovered elk DNA is authentic. The contamination controls undertaken in this study were successful at eliminating any systematic contamination as no PCR amplification was observed in blank extracts and PCR negative controls. Additionally, multiple haplotypes were observed within the samples.

Additional:

COMPARISONS: A comparison was also made to 9 ancient elk DNA sequences recovered from Cathlapotle site (45CL1, AD 1450-1833, Clark County, WA) in the Ancient DNA Lab at SFU (Speller and Yang, 2009 Lab Report 2009-02) and 1 ancient elk DNA sequence (representing 4 individuals) from Par-Tee, a large shell midden in Seaside, Oregon, with primary occupation dating from 2300 cal B.P. to 800 cal B.P. (Losey and Yang 2007).

Since the sequences from the previous studies are shorter, approximately 160bp D-loop sequences were eventually compiled for all the ancient and modern DNA samples. Appendix 3 is the NJ phylogenetic tree created using Mega 5.0 software (Tamura et al. 2011). Although a longer DNA fragment is generally more desirable for phylogenetic analysis, the tree below (in appendix 3) has proven to be more informative when compared to the tree (in appendix 2) that was created using a longer fragment, as it is evident that all reference Roosevelt elk DNA sequences were clustered together in the tree in appendix 3 (diamonds in green) but not in the tree in appendix 2.

Some interesting patterns could be retrieved from the tree in appendix 3:

- One ancient DNA sequence from San Juan Island and 2 modern DNA sequences (blood samples collected from Olympic National Park) were clustered with the Roosevelt group (diamonds in green), indicating their identities to be of Roosevelt elk. This is an expected outcome since the three samples are all from the traditional territory of Roosevelt elk.
- Two modern blood samples from Olympic National Park were clustered with *C.e.nelsoni* (triangles in pink), indicating they may have some connection with Rocky elk. If there is no evidence showing that they could be translocated from a Rocky elk region or interbred with Rocky elk, it can be speculated that KAM2 and KAM5 might be indigenous Roosevelt elk who happened to share the DNA haplotype with *C.e.nelsoni*.
- It is interesting to notice that KA3 (upside down triangle in red), another ancient DNA sample from San Juan Island is quite different from any other ancient and modern DNA samples from the region in this study. Among others, one possible

explanation is that KA3 represents an extinct Roosevelt elk group. Antiquity data and archaeological contexts may prove to be useful to help elucidate the nature of this unique elk sample. KA3 also shows its uniqueness in the phylogenetic tree in Appendix 2 based on longer DNA fragments.

- It is striking to see that all archaeological elk samples from Oregon and Southern WA were clustered together (circles in dark blue) with all three other subspecies *C. c. manitobensis*, and *C. c. nelson* and potentially *C. c. nannodes*.
 - It is difficult to assign each individual sequence to a subspecies but it should be clear that none of the archaeological elk was from *C.c.roosevelt*.
 - The cluster also reveals a possible region-specific subgroup (circles in light blue) which includes five of the nice samples from the Cathlapotle site. The establishment of such unique regional subgroup can be made through the analysis of archaeological and biological contexts.
 - It is unexpected to see a modern elk DNA sample (KAM4) from Olympic National Park was also clustered with this big group, which may indicate a translocation or interbreeding event.

LAST WORDS: Although some interesting and insightful patterns have been observed from the phylogenetic trees in this study, however, cautions should be taken to interpret the data.

Natural distributions of elk subspecies have been significantly altered by anthropogenic factors such as interbreeding, as a result, subspecies A may contain a genetic signature of subspecies B. In reality, without any knowledge of such interbreeding, we may simply take a face value, believing that subspecies A has that genetic signature originally, which in turn may affect our ability to correctly reconstruct the genetic history of subspecies.

Therefore, historic and archaeological contexts should be brought in to help interpret DNA dataset.

REFERENCES:

Hall, T. A.

- 1999 BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids. Symp. Ser.* 41:95-98.

Losey, R. J. and D. Y. Yang

- 2007 Opportunistic whale hunting on the southern Northwest Coast: Ancient DNA, artifact, and ethnographic evidence. *American Antiquity* 72(4): 657-676.

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

- 1998 Phylogenetic status of North American wapiti (*Cervus elaphus*) subspecies. *Canadian Journal of Zoology*. 76: 998–1010.

Polziehn, R. O. and C. Strobeck

- 1998 Phylogeny of Wapiti, Red Deer, Sika Deer, and Other North American Cervids as Determined from Mitochondrial DNA. *Molecular Phylogenetics and Evolution* 10(2):258.

Polziehn, R. O. and C. Strobeck

- 2002 A phylogenetic comparison of Red Deer and Wapiti using mitochondrial DNA. *Molecular Phylogenetics and Evolution* 22(3):356.

Quayle, J. F. and K. R. Brunt

- 2003 *The status of Roosevelt Elk (Cervus elaphus roosevelti) in British Columbia*. Ministry of Water, Land, and Air Protection, Biodiversity Branch, Victoria, BC.

Randi, E., N. Mucci, F. Claro-Hergueta, A. Bonnet and E. J. P. Douzery

- 2001 A mitochondrial DNA control region phylogeny of the Cervinae: speciation in *Cervus* and implications for conservation. *Animal Conservation* 4(1):1-11.

Tamura, K., N. Peterson, G. Stecher, M. Nei and S. Kumar.

- 2011 MEGA5: Molecular evolutionary genetics analysis using maximum likelihood evolutionary distance and maximum parsimony methods. *Molecular Biology and Evolution* (submitted).

Thompson, J. D., D. G. Higgins and T. J. Gibson

- 1994 CLUSTAL W: improving the sensitivity of progressive multiples sequence alignments through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research* 22:4673-4680.

1995

Speller, Camilla and Dongya Yang

- 2009 Ancient DNA analysis of cervid remains from Cathlapotle (45CL1), Lab Report 2009-02, Ancient DNA Laboratory, Simon Fraser University.

Yang, Dongya Y., Barry Eng, John S. Wayne, J. Christopher Duda, and Shelley R. Saunders

- 1998 Improved DNA extraction from ancient bones using silica-based spin columns. *American Journal of Physical Anthropology* 105:539-543.

APPENDIX 1: Multiple-alignment of ancient and modern elk D-loop sequences and reference sequences from Genbank (Accession numbers listed). The dots indicate identical base pairs, while the dashes represent insertion/deletion when compared to the *C. e. roosevelti* sequence at the top.

C e roosevelti AF016970 A T A A A A A T C A A G A C T T T A T C A G T A T T A A A T T C C A A A A A - T T T A T A T T T T A T A C A G C T T T C T A C T C A C A T C C A A T T T A C A T T T T A T G T C C T A C T A
 C e roosevelti AF016971
 C e roosevelti AF016969
 C e roosevelti AF016968
 C e roosevelti AF016967
 C e canadensis AY970666
 C e canadensis AF129410
 C e manitobensis AF01695 A
 C e manitobensis AF01696 A
 C e manitobensis AF01695 A
 C e manitobensis AF01695 A
 C e manitobensis AF01695 A
 C e manitobensis AF01695
 C e manitobensis AF01695
 C e manitobensis AF01695 A
 C e manitobensis AF00520
 C e manitobensis AF00519
 C e manitobensis AF00519
 C e manitobensis AF00519 A
 C e manitobensis AF00519
 C e nannodes AF016976
 C e nannodes AF016977
 C e nelsoni AF291882
 C e nelsoni AF016979
 C e nelsoni AF016964
 C e nelsoni AF016980
 C e nelsoni AF016966
 C e nelsoni AF016965
 C e nelsoni AF016963 A
 C e nelsoni AF016962
 C e nelsoni AF016961
 C e nelsoni AF291882
 KA2 (Middle Village-Stat
 KA3 (English Camp)
 KA4 (English Camp)
 KAM1 (Sol Duc-Heart Lake
 KAM2 (Elwha-Buckinghamhor
 KAM4 (East-LaCrosse)
 KAM5 (Quinault-Graves Cr
 KAM6 (Hoh-Hoh Lake)
 C e roosevelti AF016970 A T T A C A C A G C A A A C A C G T G A T A T A A C C T T A T G C G G T C G T A G T A C A T A A A A T C A A T G T G C T A G G A C A T G C A T G T A T A A C A G T A C A T G A G T T A G C G T A T A G
 C e roosevelti AF016971
 C e roosevelti AF016969
 C e roosevelti AF016968
 C e roosevelti AF016967
 C e canadensis AY970666
 C e canadensis AF129410
 C e manitobensis AF01695
 C e manitobensis AF01696
 C e manitobensis AF01695
 C e manitobensis AF01695
 C e manitobensis AF01695
 C e manitobensis AF01695
 C e manitobensis AF01695
 C e manitobensis AF01695 G
 C e manitobensis AF01695
 C e manitobensis AF01695
 C e manitobensis AF00520
 C e manitobensis AF00519
 C e manitobensis AF00519
 C e manitobensis AF00519
 C e manitobensis AF00519
 C e nannodes AF016976 C
 C e nannodes AF016977 C
 C e nelsoni AF291882
 C e nelsoni AF016979
 C e nelsoni AF016964
 C e nelsoni AF016980
 C e nelsoni AF016966
 C e nelsoni AF016965
 C e nelsoni AF016963
 C e nelsoni AF016962
 C e nelsoni AF016961
 C e nelsoni AF291882
 KA2 (Middle Village-Stat
 KA3 (English Camp)
 KA4 (English Camp)
 KAM1 (Sol Duc-Heart Lake
 KAM2 (Elwha-Buckinghamhor
 KAM4 (East-LaCrosse)
 KAM5 (Quinault-Graves Cr
 KAM6 (Hoh-Hoh Lake)

```

      210      220      230      240      250      260      270      280      290      300
C e roosevelti AF016970 GACATATTATGTATAAATAGTACATAAATTAAATGTTAAGACATATTATGTATAAATAGTACATTATATATGCCCCATGCTTATAAGCATGTACTTCT
C e roosevelti AF016971 .....
C e roosevelti AF016969 .....
C e roosevelti AF016968 .....
C e roosevelti AF016967 .....
C e canadensis AY970666 .....T.
C e canadensis AF129410 .....
C e manitobensis AF01695 .....
C e manitobensis AF01696 .....
C e manitobensis AF01695 .....
C e manitobensis AF01695 .....
C e manitobensis AF01695 .....
C e manitobensis AF01695 .....
C e manitobensis AF01695 .....
C e manitobensis AF00520 .....
C e manitobensis AF00519 .....
C e manitobensis AF00519 .....
C e manitobensis AF00519 .....
C e manitobensis AF00519 .....
C e nannodes AF016976 .....
C e nannodes AF016977 .....
C e nelsoni AF291882 .....
C e nelsoni AF016979 .....T.
C e nelsoni AF016964 .....
C e nelsoni AF016980 .....
C e nelsoni AF016966 .....
C e nelsoni AF016965 .....
C e nelsoni AF016963 .....
C e nelsoni AF016962 .....
C e nelsoni AF016961 .....
C e nelsoni AF291882 .....
KA2 (Middle Village-Stat .....
KA3 (English Camp) .....Y.
KA4 (English Camp) .....
KAM1 (Sol Duc-Heart Lake .....
KAM2 (Elwha-Buckinghamhorse .....
KAM4 (East-LaCrosse) .....
KAM5 (Quinault-Graves Cr .....
KAM6 (Hoh-Hoh Lake) .....

```

```

      310      320      330      340      350      360      370      380      390      400
C e roosevelti AF016970 CACTATCTGAAGTACATAGTACATAATGTTGTCATCGTACATAGTACATTGAGTCAAAATCA-GTCCTTGTCAACATGCGTATCCCGTCCCCTAGATCAC
C e roosevelti AF016971 .....A.
C e roosevelti AF016969 .....A.
C e roosevelti AF016968 .....G.
C e roosevelti AF016967 .....A.
C e canadensis AY970666 .....A.
C e canadensis AF129410 .....
C e manitobensis AF01695 .....C. A.
C e manitobensis AF01696 .....C. A.
C e manitobensis AF01695 .....A.
C e manitobensis AF01695 .....C. A.
C e manitobensis AF01695 .....A.
C e manitobensis AF01695 .....T. A.
C e manitobensis AF01695 .....C. A.
C e manitobensis AF01695 .....A.
C e manitobensis AF00520 .....C. A.
C e manitobensis AF00519 .....C. A.
C e manitobensis AF00519 .....C. A.
C e manitobensis AF00519 .....C. A.
C e nannodes AF016976 .....A.
C e nannodes AF016977 .....A.
C e nelsoni AF291882 .....A.
C e nelsoni AF016979 .....A.
C e nelsoni AF016964 .....T. T. A. CG. A.
C e nelsoni AF016980 .....A.
C e nelsoni AF016966 .....C. A.
C e nelsoni AF016965 .....T. T. A. CG. A.
C e nelsoni AF016963 .....A.
C e nelsoni AF016962 .....A.
C e nelsoni AF016961 .....C. C. A.
C e nelsoni AF291882 .....A.
KA2 (Middle Village-Stat .....T. A.
KA3 (English Camp) .....T. A.
KA4 (English Camp) .....
KAM1 (Sol Duc-Heart Lake .....
KAM2 (Elwha-Buckinghamhorse .....C. A.
KAM4 (East-LaCrosse) .....C. A.
KAM5 (Quinault-Graves Cr .....C. A.
KAM6 (Hoh-Hoh Lake) .....

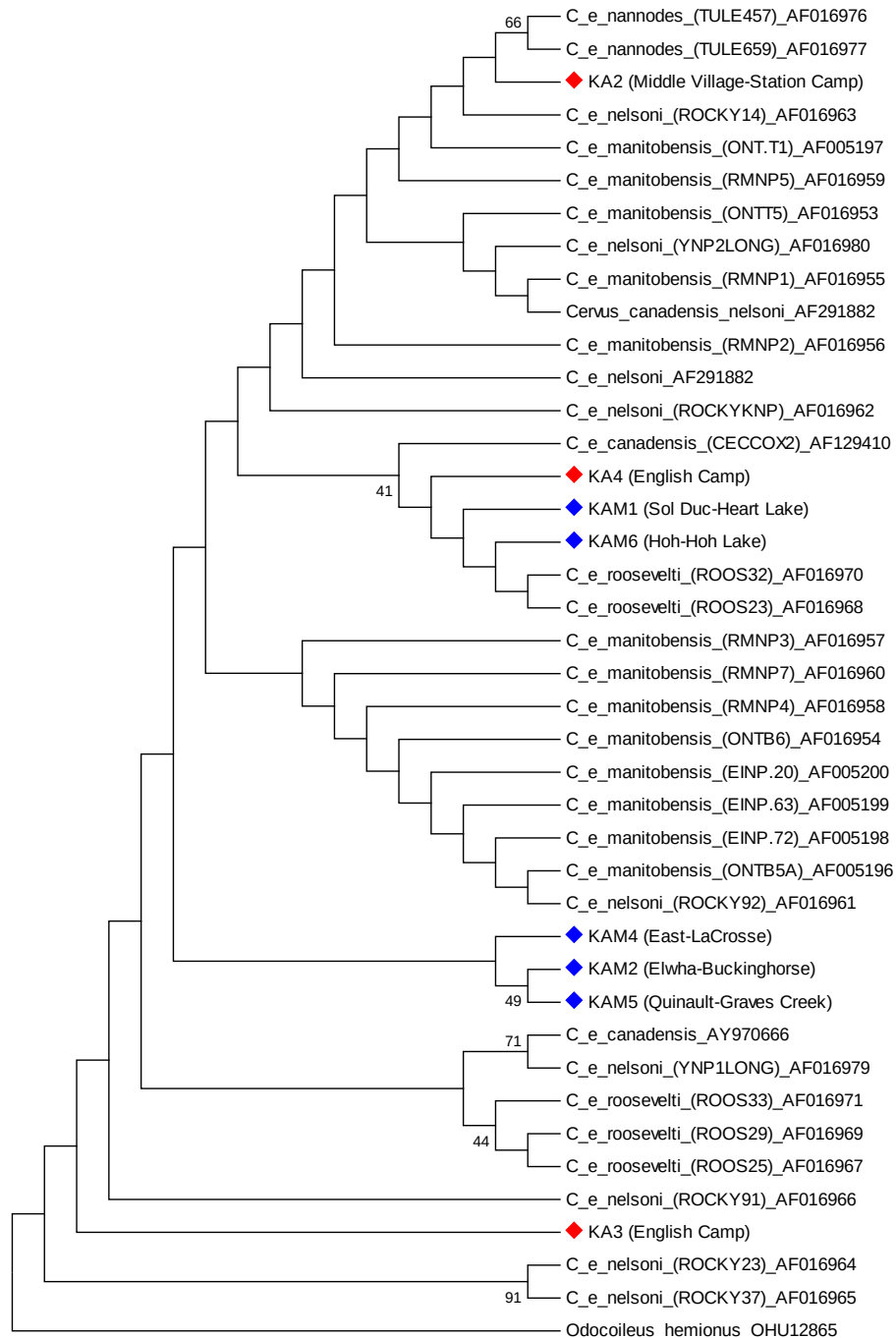
```

```

                                410
                                .....|.....|.....|.....
C e roosevelti AF016970  AAGCTTAATTACCATGCCG
C e roosevelti AF016971  G.....
C e roosevelti AF016969  G.....
C e roosevelti AF016968  G.....
C e roosevelti AF016967  G.....
C e canadensis AY970666  G.....
C e canadensis AF129410  G.....
C e manitobensis AF01695  G.....
C e manitobensis AF01696  G.....
C e manitobensis AF01695  G.....
C e manitobensis AF01695  G.....
C e manitobensis AF01695  G.....
C e manitobensis AF01695  G.....
C e manitobensis AF01695  G.....
C e manitobensis AF01695  G.....
C e manitobensis AF00520  G.....
C e manitobensis AF00519  G.....
C e manitobensis AF00519  G.....
C e manitobensis AF00519  G.....
C e manitobensis AF00519  G.....
C e manitobensis AF00519  G.....
C e nannodes AF016976    G.....
C e nannodes AF016977    G.....
C e nelsoni AF291882     G.....
C e nelsoni AF016979     G.....
C e nelsoni AF016964     G.....
C e nelsoni AF016980     G.....
C e nelsoni AF016966     G.....
C e nelsoni AF016965     G.....
C e nelsoni AF016963     G.....
C e nelsoni AF016962     G.....
C e nelsoni AF016961     G.....
C e nelsoni AF291882     G.....
KA2 (Middle Village-Stat G.....
KA3 (English Camp)      G.....
KA4 (English Camp)      G.....
KAM1 (Sol Duc-Heart Lake G.....
KAM2 (Elwha-Buckinghorse G.....
KAM4 (East-LaCrosse)    G.....
KAM5 (Quinault-Graves Cr G.....
KAM6 (Hoh-Hoh Lake)     G.....

```

APPENDIX 2: Phylogenetic tree displaying the relationships between obtained D-loop haplotypes and cervid reference sequences (Genbank accession numbers and haplotypes are listed). Red diamonds represent analyzed ancient elk samples; blue diamonds represent analyzed modern elk samples. The tree (NJ with Kimura 2-parameter, 2000 replications) was composed using Mega5 software (Tamura et al. 2011). Bootstrap values below 40% are not shown.



APPENDIX 3: Phylogenetic tree displaying the relationships between all ancient and modern DNA samples from this study and previous studies (Losey and Yang 2007; Speller and Yang, aDNA Lab Report 2009-02), and reference sequences (Genbank accession numbers and haplotypes are listed). The tree (NJ with Kimura 2-parameter, 2000 replications) was composed using Mega5 software (Tamura et al. 2011). All bootstrap values are shown in the tree.

