Phylogenetic Analysis of Cytochrome-b Mitochondrial DNA in Moose Along the Rocky Mountain Front

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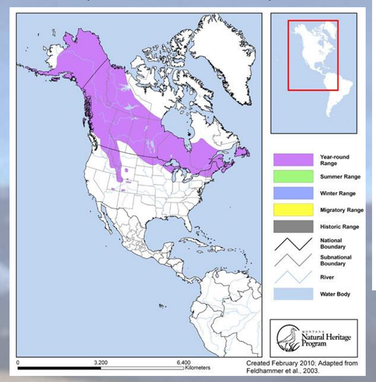
Introduction

The North American Moose, *Alces americanus*, can be found from Alaska down to the Northern part of Utah and throughout Canada spreading east to Maine (Fig.1. / Montana Field Guide[1]).

The purpose of our study was to isolate cytochrome-b loci on the mitochondrial DNA (Fig. 2) from fecal swab samples of moose along the Rocky Mountain Front in Montana and compare this gene to other moose in North America and throughout the rest of the world using evolutionary phylogenetic analysis.

Mitochondrial DNA is passed on through generations from mothers to their offspring and is an important indicator of population genetics because it evolves five to ten times faster than nuclear DNA (Castro et. al 1998[2]). Cytochrome-b was used in this study because it has been used to show differences in genetic relationships of North American mammals (Hundertmark et. al, 2002[3]) and has been successfully amplified from fecal samples of many large mammals (Zhang et. al, 2006[4]).

Fig. 1. Moose distribution throughout North America (Montana Field Guide[5]).



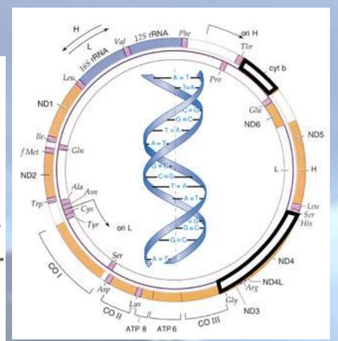


Fig. 2. Illustration of mitochondrial DNA with cyt-b loci outlined in black (upper right-hand side).

Methods

Fecal sample swabs were collected in collaboration with Jesse Newby , a Wildlife Biologist with Montana Fish, Wildlife & Parks (Fig. 3).

DNA was collected from epithelial cells on the fecal swabs of four different moose residing in the Rocky Mountain Front by using a DNA extraction kit from Invitrogen©. The four samples were designated moose A, B, C, and D. After the DNA was extracted, a forward primer H15149 and a reverse primer L14724 (Zhang et. al 2006[4]) were used to amplify the 5' end of the cytochrome-b gene through polymerase chain reaction (PCR) and the resulting products were ran through agarose gel electrophoresis (Fig. 4) to determine the size of the DNA fragment.

The PCR products of Moose A and Moose C were purified and sent to the University of Michigan Sequencing Core as those two samples displayed the best results out of the four samples. The sequences of the cytochrome-b gene were then analyzed and manually aligned through MEGA (Fig. 5) to generate a phylogenetic tree (Fig. 6). Sequences other than Moose A and C were acquired through GenBank for a total of 10 sequences.

Fig. 3. Fecal swabs provided by Jesse Newby from moose residing along the Rocky Mountain Front.

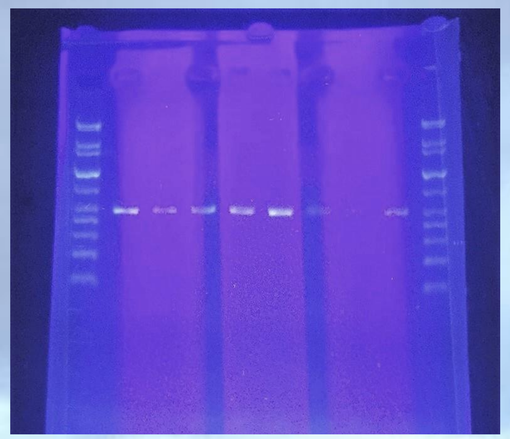


Fig. 4. Agarose gel electrophoresis of amplified mitochondrial cyt-b DNA at roughly 500 base pairs. Lanes 1 and 10 are a 1kb ladder containing 100bp DNA fragments. Lanes 2 and 3 contain sample from moose A, 4 and 5 from moose B, 6 and 7 from moose C, and 8 and 9 from moose D.

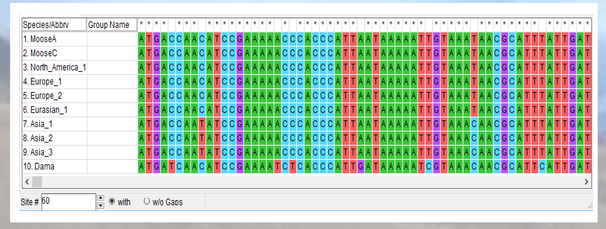


Fig. 5. Sequence alignment of a cytochrome-b DNA fragment that was prepared and analyzed with MEGA in order to generate a phylogeny. Number 10 on the alignment table, *Dama dama* or fallow deer was the root ancestor that DNA of the moose were compared to for genetic differences.

Results

Pairwise sequence comparisons show the differences in the DNA base pairs found in the moose from the Rocky Mountain Front compared to other individuals from around the world (Table 1). There were no nucleotide differences between moose A and C, or between them and the North American 1 and Eurasian moose, but there were 2 European haplotypes, and 3 Asian haplotypes with Asia 2 being the most different from moose A, moose C, and North America 1.

The topology of the phylogenetic tree (Fig. 6) show three distinct clades: a North American sub-group that includes our moose A and C, an Asian sub-group, and a European sub-group. The North American subgroup has no sequence variation between each other, but clear variation between the European and Asian subgroups.

	Moose A	Moose C	NA1	Eurasian	Eur1	Eur2	Asia1	Asia2
Moose A								
Moose C	0							
NA1	0	0						
Eurasian	0	0	0					
Eur1	2	2	2	2				
Eur2	2	2	2	2	2			
Asia1	2	2	2	2	4	4		
Asia2	4	4	4	4	6	6	2	
Asia3	3	3	3	3	5	5	1	1

Table 1. Pairwise comparisons of sequences used and the number of nucleotide differences between them.

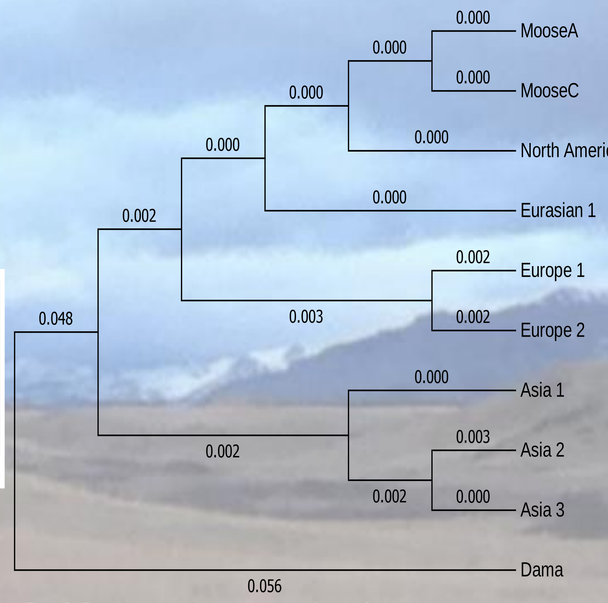


Fig. 6. Resulting phylogenetic tree of the 10 cyt-b sequences. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model[5]. The tree with the highest log likelihood (-753.9944) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The analysis involved 10 nucleotide sequences. There were a total of 435 positions in the final dataset. Evolutionary analyses were conducted in MEGA7[6].

Fig. 7 North American Moose, *Alces americanus* on the Jefferson River near Whithall, MT.

Conclusions

The results of our study show that there is little genetic diversity in cytochrome-b between populations of moose around the world. Moose A and Moose C had identical sequences to each other and to the North American and Eurasian samples, indicating that Montanan moose could possibly be direct descendants to that lineage. The fact that there is more genetic diversity between moose in Asia indicates that populations there have been longer established with more time to evolve. In fact, evidence shows that moose originated in Asia and migrated to North America ~14,000 years ago when the two continents were connected by a land bridge (Hundertmark et. al, 2003[7]).

The lack of genetic variation between the moose of the Rocky Mountain Front and in North America supports the fact that moose on this continent are a relatively new species without enough time to significantly evolve. Fourteen thousand years is not very long on the evolutionary time scale. However, more extensive studies on different genes in moose mitochondrial DNA could potentially show more diversity among individual populations as single gene analysis is a very small representation.

Future Research

To better understand the genetic diversity between Montanan moose and moose populations throughout the world, sequencing and analyzing different genes in the mitochondria could be more insightful. The hypervariable domain of the $tRNA^{thr}$ or $tRNA^{pro}$ genes have been shown to be useful in phylogenies of *cervids* and for intraspecific population studies (Hundertmark et. al, 2003[7], Swislocka et. al, 2008[8]) and could be used for analysis of Montana populations. In addition, analyses of other allele frequencies not associated with mitochondrial DNA could also be done.

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Acknowledgements

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