ARE MOOSE (ALCES ALCES ANDERSONI) REPRODUCTIVELY ISOLATED IN THE ALEXANDER ARCHIPELAGO OF SOUTHEASTERN ALASKA?

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Introduction

The population genetics of moose in Southeast Alaska has not been studied in great detail. Moose have only been on the islands of the Alexander Archipelago since the 1920 - 1930s, and are thought to have originated from the Stikine River (*Alces alces gigas*) (“Moose Alces- alces”), although others hypothesize that the moose originated from western Canada (*Alces alces andersoni*) (“Alaska”). Where did moose in southeastern Alaska originate, and are they continuing to migrate or are they isolated by islands?

Moose are found only in a limited area of Southeast Alaska, and are essentially absent from the some of the islands (“Alaska”). In Alaska, moose live in a large area ranging from the Stikine River all the way to the Colville River on the Arctic Slope. The moose in Southeast Alaska are believed to be the sub-species *Alces alces andersoni* instead of the sub-species *Alces alces gigas* (Hundertmark et al, 2003; “Alaska”).

The home range size of moose varies seasonally between 3.6 to 9.2 km^2; largest in autumn and smallest in summer. Some studies show that moose are slow in redistributing themselves into vacant, low density, or heavily hunted areas (“Mooseworld”). Most of the moose in southern Southeast Alaska are localized in the Unuk River drainage (Timmermann, 1992).

Hundertmark et al. (2003) found that there were 12 separate haplotypes found in the Alexander Archipelago of Alaska. Mitochondrial diversity varied among the North American populations. Populations from within the range of *A. alces andersoni* of western Canada exhibited the greatest diversity, whereas populations from mainland Alaska, southeastern Alaska, and eastern North America showed less variation. He believes that the moose started out from one haplotype and then migrated out and then became isolated, resulting in a change in population structure (Hundertmark et al, 2003). Evidence from other data has indicated that small effective sizes of populations established in a similar manner have led to a rapid differentiation (Hundertmark et al, 2003). Is this same pattern observed in the colonization of the islands in southeastern Alaska by moose, and is 80-90 years enough time to establish variability in the mitochondrial DNA? This is a great project because we live in Southeast Alaska, and we would like to determine whether the moose on our island and neighboring islands have become reproductively isolated.

Purpose

Is this same pattern observed in the colonization of the islands in southeastern Alaska by moose, and is 80-90 years enough time to establish variability in the mitochondrial DNAo reproductive isolation due t? We think that the moose in the Southeastern Islands are their own haplotype, and not one of the ones from the Stikine or British Columbia.

Procedure

Moose samples were collect from the teeth and jaws of 2012 moose harvest records provided by ADF&G (Figures 1 and 2). Tissues were homogenized by using the Retsch MM 300. and stored at -20 degrees Celsius. DNA was extracted using the Wizard Genomic DNA purification kit protocols (Promega, 2010). Due to complications with tissue extraction, we used additional incubation time and increase temperatures (up to 70 degrees Celsius) according to Promega tech support recomindations.

Figure 1: Classmates working on cutting moose tissue out of the jaw.

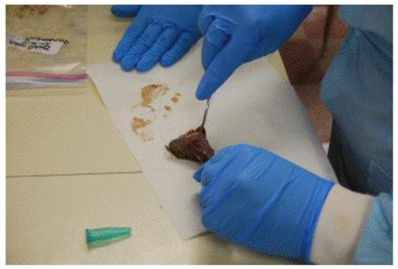

Figure 2: Classmates working with Alaska Department of Fish and Game.

PCR was completed using GE PowerBeads and the following primers: LGL283 (5’ TAC ACT GGT CTT GTA AAC -3’, Bickham et al, 1996) and a sequence of ISM015 (5’ ATG GCC CTG TAG AAA GAA -3’, r. Hundertmark) for a final concentration of . 5 uM. 5 ul of DNA template was used for each sample. The Bio-RAD thermocycler was used with a 2 minute soak at 94° C, followed by 30 cycles of 94° C, for 15 seconds, denaturation, 15 seconds of 50° C annealing, 45 seconds of extension at 72° C and 1 extension period of 10 minutes at 72° C. PCR products were sequenced in the University of Alaska CORE Lab.

All analysis was conducted using MEGA Software (Tamura et al, 2011). The current dataset was taken from the NCBI database using Hundertmark (2003) sequences while we wait for our dataset from the UAF CORE Lab. Sequences were aligned using Clustal W, and the number of base substitutions per site were determined using the Maximum Composite Likelihood Model (Tamura et al., 2004). The Minimum Evolution method was used to produce a possible phylogeny, with the tree produced using the neighbor-joining method (Tamura et al, 2004).

Data

Eight samples produced PCR product. The analysis that follows currently uses data that we have gotten from the University of Alaska Fairbanks through NCBI (Hundertmark, 2003), and is not based on our PCR sequences yet. When we do get our data, we will analyze the sequences similarly and use the Hundertmark sequences to anchor the tree.

There is fairly low diversity in the mitochondrial DNA segment sequenced within groups, and large diversity between groups (Table 1). British Columbia sequence #3 was the most different from all samples within the mainland/BC group. Mitkof Island #1 was the most different within its group (Table 1). The interior Alaska sequences had the lowest base substitution rate amongst all pairwise comparisons.

Table 1: Matrix showing nucleotide subtitution rates between sequences. Analysis included eleven nucleotide sequences.

	1	2	3	4	5	6	7	8	9	10	11
1. SEAK2											
2. SEAK1	0.002										
3. BC3	0.004	0.002									
4. INTAK1	0.008	0.010	0.012								
5. BC2	0.010	0.012	0.014	0.006							
6. INTAK2	0.010	0.012	0.014	0.002	0.008						
7. BC1	0.014	0.012	0.014	0.006	0.008	0.008					
8. Mitkof Island 1	8.718	8.717	8.718	8.719	8.720	8.719	8.719				
9. Mitkof Island 2	8.720	8.719	8.720	8.721	8.722	8.721	8.721	0.012			
10. Kupreanof Island	8.720	8.719	8.720	8.721	8.722	8.721	8.721	0.020	0.008		
11. Stikine Area	8.719	8.719	8.719	8.720	8.721	8.721	8.721	0.014	0.002	0.010	

The phylogenety produced by this analysis shows interior Alaska moose as a clade. The British Columbia moose samples are on separate branches of the tree. Two samples represent their own group while a third is more similar to the southeastern Alaska moose sequences. The moose samples from the islands are also their own separate branches of the tree. None of these samples are connected to the interior Alaska, British Columbia, or southeastern Alaska.

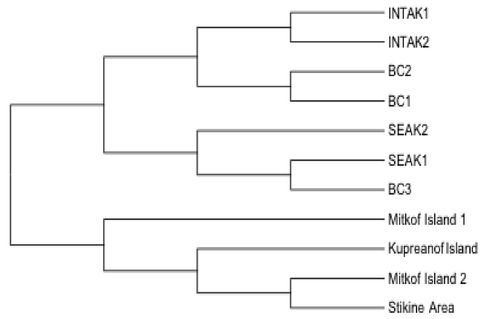

Figure 3: Phylogenetic tree showing possible relationship using the neighbor joining method. INTAK = interior Alaska, BC = British Columbia and SEAK = southeastern Alaska. Sequences were determined by Hundertmark (2003) and accessed via NCBI Blast (2013).

Discussion

We hypothesized was that the moose in the Alexander Archipelago would have become isolated reproductively from up the Stikine River and thus showing diversity between the islands. We thought we might find evidence showing that the moose are reproductively isolated, i.e., not swimming back and forth between islands. The moose found in the Alexander Archipelago appear to be genetically different based on the phylogenetic tree (Figure 3). What we did not think we would find is that they are actually different from the moose samples that were collected by Hundertmark for southeastern Alaska. This could be because we do not know where the Hundertmark moose came from in southeastern Alaska, or because the moose sample came from a different area than we are testing in southeast Alaska.

Some possible sources of error revolve around tissue digestion as Promega technical support recommended lengthy incubation windows at separate times during the protocol, yet consultation with different technical support personnel suggested that this process would have degraded the DNA. The low effectiveness of the PCR may have been impacted by this.

Our four samples suggest the moose are genetically different and therefore reproductively isolated to some degree in southeastern Alaska. It would have been nice if we would have had all our own samples sequenced.

We can conclude that the moose have differentiated genetically, and thus have become genetically isolated. Our hypothesis was correct in that the moose from the islands are not the same haplotype as the moose in interior Alaska or British Columbia. The moose that were from the islands were on their own separate branches, different from British Columbia or interior Alaska. From reading on this topic we could conclude that there has not been a mitochondrial DNA test on the moose in Southeast Alaska to address this research question. Kevin White with ADF&G, indicated that Kevin Colson has defended his dissertation with preliminary results using genomic microsatellites suggesting that eight years has been enough time to find distinct populations within the Alexander Archipelago (White, pers. comm, 2013).

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