

Development of a technique to extract DNA from Dall's sheep feces: Preliminary results

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Introduction

Little is known of the genetic structure of Dall's sheep (*Ovis dalli dalli*) populations within contiguous mountain ranges, such as the Wrangell, Chugach, and St. Elias Mountains of Southcentral Alaska. Dall's sheep in Alaska occur at ancestral population levels and are well-distributed compared to fragmented bighorn sheep (*O. canadensis*) populations, and therefore serve as a useful baseline to assess natural patterns of genetic diversity in wild sheep. However, broad scale evaluations of sheep genetics are limited by the expense of tissue acquisition from live sheep or the availability of archived samples.

Feces can be a good source of DNA for population level studies because it is relatively easy to collect from a large sample of individuals over a wide geographical area, and is less expensive than collecting tissues from captured individuals. Methods of DNA extraction from feces have been developed for other species of wild sheep and have proven to be as effective and accurate as blood and tissue samples (Maudet et al. 2004, Wehausen et al. 2004).



Genetics: We used DNA extracted from the tissue samples to test a set of 15 microsatellite loci previously used in bighorn sheep (*O. canadensis*; Luikart et al. 2008), and DNA extracted from both tissues and feces to screen and develop markers to access sequence data from three mitochondrial DNA genes, including the cytochrome oxidase I (COXI) and cytochrome b (cytb) genes, and the control region (CR). We used these markers to test the feasibility of using DNA extracted from the fecal samples. We estimated the power of the microsatellite markers to assign individuals to a population of origin (Manel et al. 2002). We used analyses of variance (*F_{st}*) to ascertain the level of population structuring among sampled sheep.

Study Area

The sheep population of Wrangell St. Elias National Park and Preserve has been estimated at 15-25,000 constituting 25-30% of Alaska's sheep. We collected the majority of samples in the Chitina River drainage containing an estimated 2,500 – 3,600 sheep (Strickland et al. 1993). This area covers approximately 20,000 km² of mountainous terrain subdivided by large glaciers (>20 km long, >1 km wide) and major river drainages (fig. 1.). Sheep groups are typically located between 900 – 1,500m elevations in the alpine zone.

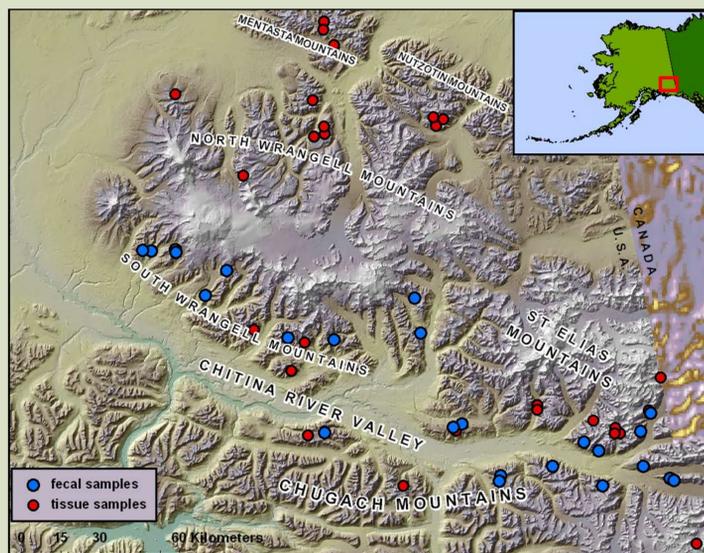


Figure 1. Study area and locations of fecal and tissue samples collected in 2007.



Objectives

1. Develop and validate noninvasive fecal DNA analysis techniques for large-scale evaluations of the genetic structure of free-ranging Dall's sheep populations.
2. Assess levels of genetic diversity and gene flow within the sheep population of the Chitina River drainage using maternally and biparentally inherited genetic markers.
3. Evaluate the genetic structure of the Chitina River drainage sheep population to determine the level and scale of population differentiation (*F_{st}* > 0.00)

Methods

Field collection: We collected feces from 47 adult sheep in late summer 2007 using a fixed-wing aircraft to locate sheep groups and a helicopter to approach them. We collected 4-8 fecal pellets from each individual and stored them in 95% ethyl alcohol (ETOH) at room temperature until they were analyzed.

We also obtained 30 tissue samples from rams taken during the 2007 annual hunting season (August 10 – September 20) over a larger geographical area than the fecal samples (fig. 1) to provide a basis for comparing the genetic structure of sheep populations with limited geneflow.

Results

Biparentally-inherited microsatellite loci: Using tissue samples we determined that all 15 microsatellite loci were variable within Dall's sheep in our study. Number of alleles ranged from 2 to 10. Expected heterozygosity ranged from 0.064 to 0.755; average heterozygosity was 0.544. Probability of identity values for the 15-locus genotype is 1.818 x 10⁻¹¹. This suggests there is adequate power to discern individuals from the non-invasive samples. Analyses of variance uncovered substantial structuring within the Southcentral Alaska populations (Table 1).

Table 1. Pairwise comparison of *F_{st}* values for microsatellite DNA data. All values below the diagonal, neutral loci only above the diagonal.

CHUGACH MOUNTAINS	NORTH WRANGELL MOUNTAINS	SOUTH WRANGELL MOUNTAINS	ST. ELIAS MOUNTAINS	
0	0.125	0.139	0.109	CHUGACH MOUNTAINS
0.104	0	0.084	0.056	NORTH WRANGELL MOUNTAINS
0.15	0.066	0	0.099	SOUTH WRANGELL MOUNTAINS
0.12	0.051	0.1	0	ST. ELIAS MOUNTAINS

Table 2. Pairwise comparison of *F_{st}* values for mitochondrial DNA control region data. Values in bold are significant at *P* < 0.05

CHUGACH MOUNTAINS	NORTH WRANGELL MOUNTAINS	SOUTH WRANGELL MOUNTAINS	ST. ELIAS MOUNTAINS	
0	0.411	0.068	0.001	CHUGACH MOUNTAINS
	0	0.402	0.421	NORTH WRANGELL MOUNTAINS
		0	0.059	SOUTH WRANGELL MOUNTAINS
			0	ST. ELIAS MOUNTAINS

Table 3. Comparison of genetic diversity at microsatellite loci and mtDNA control region among populations of Dall's sheep in WRST.

Population	MITOCHONDRIAL DNA ¹				MICROSATELLITE DNA ²			
	N	k	h	π	N	Loci	H _o	H _e
CHUGACH MTS	13	1	0	0	2	15	0.6	0.375
NORTH WRANGELLS	11	7	0.873	0.151	12	15	0.592	0.599
SOUTH WRANGELLS	21	6	0.729	0.025	2	15	0.6	0.492
ST. ELIAS MTS	23	8	0.676	0.02	9	15	0.473	0.539

¹Includes fecal and tissue samples.

²Tissue samples only

N = sample size, for each analysis; k = number of haplotypes; h = nucleotide diversity (Nei 1987); π = nucleotide diversity (Nei 1987); Loci = number of microsatellite loci used for each analysis; H_o and H_e = observed and expected heterozygosity

Future Research

Microsatellite analyses for the fecal samples collected in 2007 will soon be completed. We plan to collect an additional 150 fecal samples and 100 tissue samples during 2008 and 2009. We will use genetic markers developed in this study to assess levels of genetic diversity and gene flow within the sheep population and to determine the level and spatial scale of population differentiation. Increased understanding of the extent of genetic partitioning of the sheep population over a large montane landscape will provide useful assessments of natural patterns of genetic variability in Dall's sheep and the appropriate geographic scales for population monitoring and harvest management.

