Investigating Genetic Diversity of Oreamnos americanus by Microsatellite Markers

FORREST HOYT, Corvallis High School, Corvallis MT
TABITHA HOOTEN, Corvallis High School, Corvallis MT
JIM STREIBEL, Corvallis High School, Corvallis MT
ART RZASA, Corvallis High School, Corvallis MT

Abstract: For isolated species populations there is an inherent risk of small gene pools because of inbreeding. The genetic variability and configuration of Oreamnos americanus, populations were investigated using microsatellite markers. Both native and transplanted herds were examined for genetic variations at five microsatellite loci. Samples were collected by non-invasive gathering of scat and from donated tissue samples. The samples came from the Bitterroot, Beartooth, Absaroka, and Crazy Mountain ranges of Montana. The genetic variability based on number of alleles present in the sample population revealed low genetic variation for the loci used to date. Further examination of genetic diversity and statistical analysis using Popgene will help determine the existence of a genetic bottleneck. The finality of these results requires further assessment of sample population, and the studied loci. This study will make evident the need for management to maintain and diversify goat populations.

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Introduction

Oreamnos americanus, the Rocky Mountain goat, has inhabited the Rocky Mountains in Montana since the recession of the last ice age. As the result of environmental changes and human interaction, the natural goat habitats have been receding, restricting herds to smaller seasonal ranges. In a managerial effort, in the early 1940s, the Montana State Fish and Game Department began transplanting mature goat pairs from existing herds to stock ranges that had previously been uninhabited. In 1947, the estimated number of goats to inhabit two areas of study was 95 in the Crazy Mountains and 24 in the Beartooth Mountains. Populations in the Beartooth, Crazy, Gallatin, and Highwood have been isolated by geography from native goat herds because of both natural geology and by traditional goat ranges for almost fifty years. This separation of the transplanted herds makes genetic drift and low genetic diversity a possibility.

In 2005, a group of students began to study if it was plausible to determine the genetic diversity of Rocky Mountain goats by isolating DNA from scat. Taking the study a step further, the current aim is to examine the genetic diversity of a native goat herd as compared to goat herds that were transplanted in the 1940s. Using scat and donated tissue samples from the study areas, DNA has been isolated, amplified and studied using microsatellite markers. Gene lengths were examined at five loci utilizing electrophoresis.

Materials and Methods

Sample collection. The majority of sampling of Rocky Mountain goats was completed using scat samples obtained non invasively in the Bitterroot and Beartooth Mountain Ranges. Tissue samples were acquired from the Bitterroot, Beartooth, and Absorka Mountain Ranges through Fish Wildlife and Parks, and private donors. The location of where samples were collected

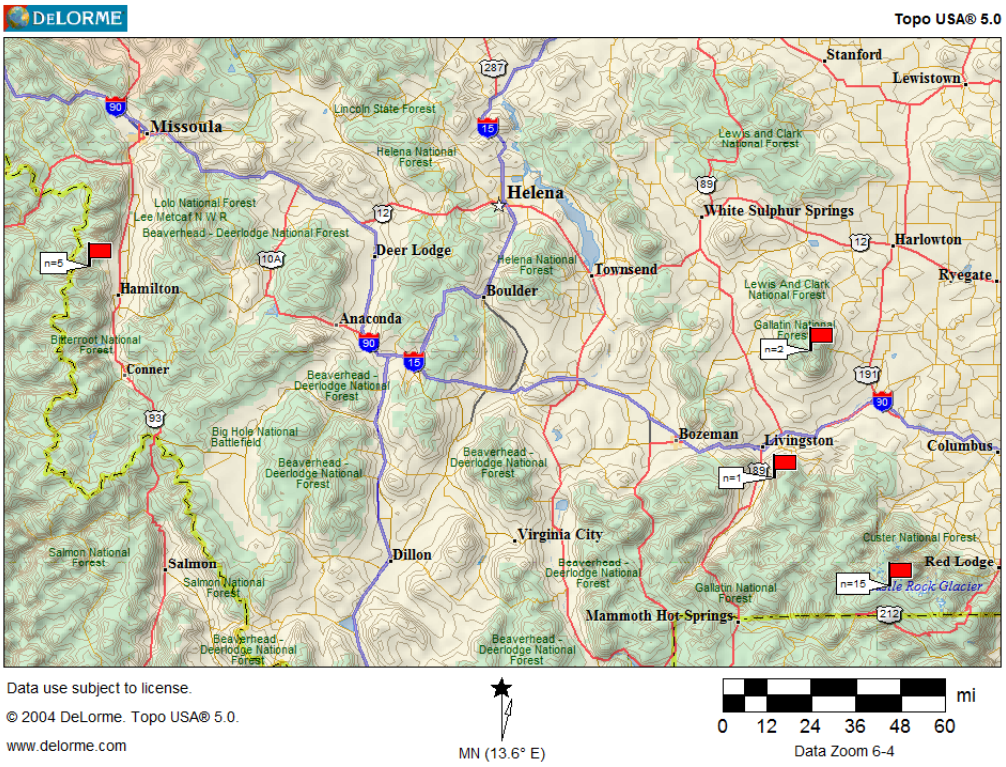

Figure 1: Location and number of samples per site used in the study.

are cataloged with GPS coordinates, date the sample was obtained, and the age of the goat if this information is known.

Molecular techniques. Genomic DNA was isolated using the procedure defined by the Qiagen Stool Sample and Qiagen DNeasy Tissue kits. To isolate DNA from scat required that a sample weighing 180-220 mg be prepared for the rest of the protocol. To obtain the most yield of DNA in each sample, four different methods were developed to isolate the epithelial cells from scat samples. (See figure 2). The selected method found that scraping the exterior of the goat pellet yielded the most positive samples of DNA. The weighed sample was then ready to have 1.6 ml Buffer ASL which is then homogenized with the sample. This buffer commences the refining of epithelial cells from plant and other inhibitors present in scat. The sample is centrifuged for one minute to pellet stool particles, 1.4 ml of the supernatant is pipetted into a 2 ml where one InhibitEX tablet is added. The solution is vortexed then incubated at room temperature to allow inhibitor to be absorbed by the Inhibit EX matrix, this solution is then centrifuged again for three minutes to collect inhibitors in a pellet form. The supernatant is then pipetted into a 1.5 ml microcentrifuge tube to be centrifuged again. 25 µl of Proteinase K is applied by pipette to a 2 ml microcentrifuge tube where 600 µl of supernatant is added. The tubes are then incubated at 70°C for ten minutes. When it is removed from the incubation

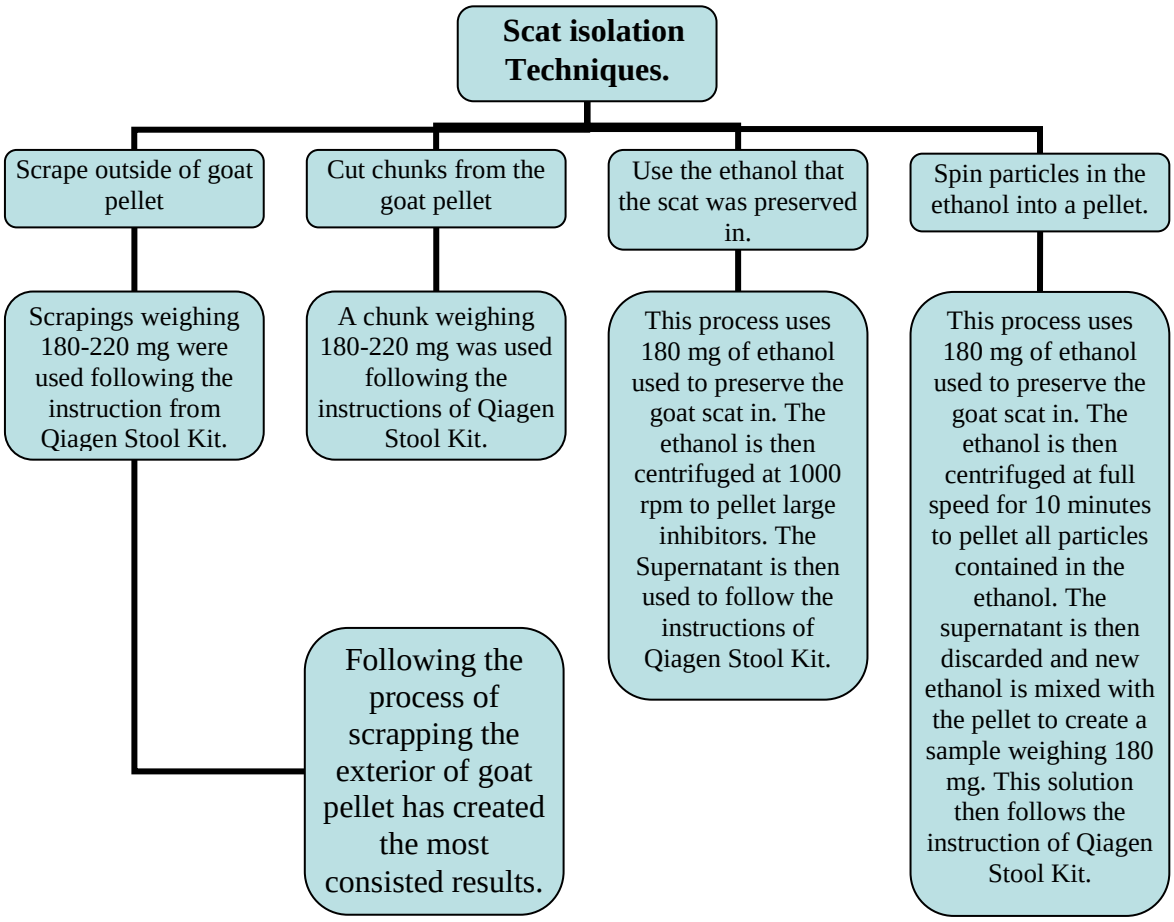

Figure 2: This chart depicts the different processes that were studied to determine the most effective procedure for isolating DNA from scat.

device, 600 µl of ethanol (96%-100%) is added and mixed with the lysate. 600 µl of this mixture is then pipetted into a QIAamp spin column with 2 ml collection tube. This collection is then centrifuged at full speed for one minute. The spin column is placed in a new 2 ml collection tube where 500 µl of Buffer AW1 is added, then centrifuged at full speed for a minute again. This step is repeated with Buffer AW2, centrifuging for 3 minutes. The final step to isolate the DNA is to transfer the QIAamp spin column to a 1.5 ml microcentrifuge tube where 300 µl of Buffer AE is applied directly to the spin column membrane. To elute DNA the column and tube are centrifuged at full speed for one minute. Isolating DNA using Qiagen DNeasy Tissue Kit follows similar steps of purifying DNA from the make up of cells. Using a 25 mg. portion of a goat sample the process begins by adding180 ml Buffer ATL to the sample. Before being place in a water bath at 55°C, 20 µl of Protenaise K is added. This mixture remains in the water bath for 2-3 three hours to allow for cell structures to begin breaking down. After the bath, the sample is mixed before 200 µl Buffer AL is added and then mixed again. The sample is again placed in the water bath, which is 70°C, and allowed to sit for ten minutes. When removed, 200 µl of ethanol (96%-

Locus	Primer Sequence	Ta°C	Allele Length Range	# of Alleles	
				Oreamnos americanus	Cervus canadensis
BM1818	F: 5'-AGCTGGGAATATAACCAAAGG-3'	50°C	250-280 bp	2	3
	R:5'-AGTGCTTCAAGGCCATGC-3'				
ILSTS005	F: 5'-GTTTCTTTGTTCTGTGAGTTTGTAAGC-3'	50°C	185-220 bp	1	5
	R: 5'-GGAAGCAATTGAAATCTATAGCC-3'				
TGLA122	F: 5'-CCCTCCTCCAGGTAAATCAGC-3'	50°C	130-256 bp	1	3
	R: 5'-AATCACATGGCAAATAAGTACATAC-3'				
BM4513	F: 5'-GCGCAAGTTTCCTCATGC-3'	50°C	140-160 bp	1	
	R: 5'-TCAGCAATTCAGTACATCACC-3'				
BM4208	F: 5'-TCAGTACACTGGCCACCATG-3'	50°C	150-175 bp	1	
	R: 5'-CACTGCATGCTTTTCCAAAC-3'				

Figure 3: This table expresses the number of alleles that have been observed in the study.

100%) is added and mixed together. This solution is then pipetted into a QIAamp spin column contained in a 2 ml collection tube to be centrifuged at 8000 rpm for one minute. When the spin column is placed in a new collection tube, 500 µl of Buffer AW1 is added. Again the spin column is centrifuged at 8000 rpm for one minute. After the spin column is secured in a new collection tube, 500 µl of Buffer AW2 is added before being centrifuged at full speed for three minutes. The final step to isolate DNA is to place the spin column into a 1.5 ml microcetrifuge tube and pipette 200 µl of Buffer AE onto the QIAamp spin column membrane. The tube and column should incubate at room temperature for 1 minute before being centrifuged at 8000 rpm for one minute to elute.

Polymerase Chain Reaction was then carried out for gene amplification and duplication. Five primers were utilized for comparison. These primers were BM1818, BM4513, BM4208, ISTLS005, and TGlA122. For all five primers the annealing temperature was 50°C. The PCR program had an initial denature cycle of 92°C for 1 minute followed by 40 cycles of 94°C for 30 seconds, 50°C for 20 seconds, and 72°C for 10 seconds. These cycles were followed by one cycle at 72°C for 10 minutes. 25 µl reactions volumes were used which contained 18 µl of purified water, 2 µl of a primer mix composed of forward and reverse primers, and 5 µl of the DNA template. The reaction tubes that were used were then run on a BioRad Gene Cycler.

Using the PCR reactions, allele length can be observed through the process of electrophoresis. Using an Invitrogen E-Gel and E-Gel 2% with SYBR Safe Starter Pak gels, the results was seen after 30 second pre-run and a 15 minute run time. Using a BioRad ultra violet light and a photographic filter, the results were recorded by observation and with camera.

Statistical analysis. Data that was attained was observed using five microsatellite loci to observe the heterozygosity and allele frequency of the representative goats. Bottleneck hypothesis will be used once 20-30 individual samples per population are available. With the bottleneck, the mutation-drift will be observed based on heterozygosity excess of

Figure 4: Two gel runs to reveal the genetic diversity of Montana mountain goats.

deficiency.

Results

The results to date using five microsatellite makers have revealed low allelic frequencies. Four of the five primers: BM4513, BM4508, ILSTS005, and TGLA122, rendered only one allele per samples tested. For BM1818 two alleles were present, one at 200-220 bp and another at 220-240 bp. To view all allele range data refer to Figure 3 for more information. The amount of samples observed with electrophoresis was fourteen, five from the Bitterroot Mountains, eight from the Beartooth Mountains, and one from the Absaroka Mountains.

Analysis

The results showed that current number of samples tested have low genetic diversity, but do not specifically indicate a low genetic diversity for the goat population as a whole. The genes present using the current microsatellite markers all run to similar lengths for the entirety of animals that have been tested. To make the results of this study more conclusive, the number of goats tested needs to be increased to twenty to thirty goats per sample range. When all samples have been tested a Genetic Bottleneck Analysis can be performed on the sample populations. Another element that needs to be evaluated to make the study more conclusive is the current microsatellite markers that are being used. It may be possible that the sequences coded for by the markers are genes that are unanimously similar for each goat. Once these quandaries have been assessed, a better picture will appear of the genetic diversity of mountain goat populations in the Montana Rockies.

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