

Optimization of multiplex PCR to genotype microsatellite alleles in bobcats (*Lynx rufus*)



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Background

- Bobcats (*Lynx rufus*) are ecologically pivotal mesocarnivores that inhabit diverse habitats in North America.
- Population sizes and densities of bobcats in Colorado are largely unknown.
- Genetic analysis of molecular markers in populations allows for determination of genetic diversity, migration rates, population structure and relatedness.
- Microsatellites are small DNA segments that have a high degree of variability and follow Mendelian inheritance patterns.
- Multiplex polymerase chain reaction (PCR) combines reagents from single target PCR to amplify two or more loci simultaneously.
- The goal of our research was to optimize a multiplex polymerase chain reaction (PCR) to amplify 16 microsatellite loci in a population of bobcats on the Western Slope of Colorado.
- This study is part of a larger study evaluating ecology of felids relative to landscape fragmentation. Other goals of the study include determining bobcat movement and home range by motion activated camera and GPS collar tracking.



Motion activated camera trap images of a bobcat on the Colorado Western Slope. Image courtesy of Jesse Lewis.

Methods

- DNA was extracted from blood or tissue samples from 26 bobcats from Western CO (Figure 1).
- Primers were paired for multiplex PCR based on ability to differentiate product size and minimization of primer interactions.
- Multiplex PCRs were designed to amplify 11 loci in four reactions.
- The remaining 5 loci were amplified with single locus PCR

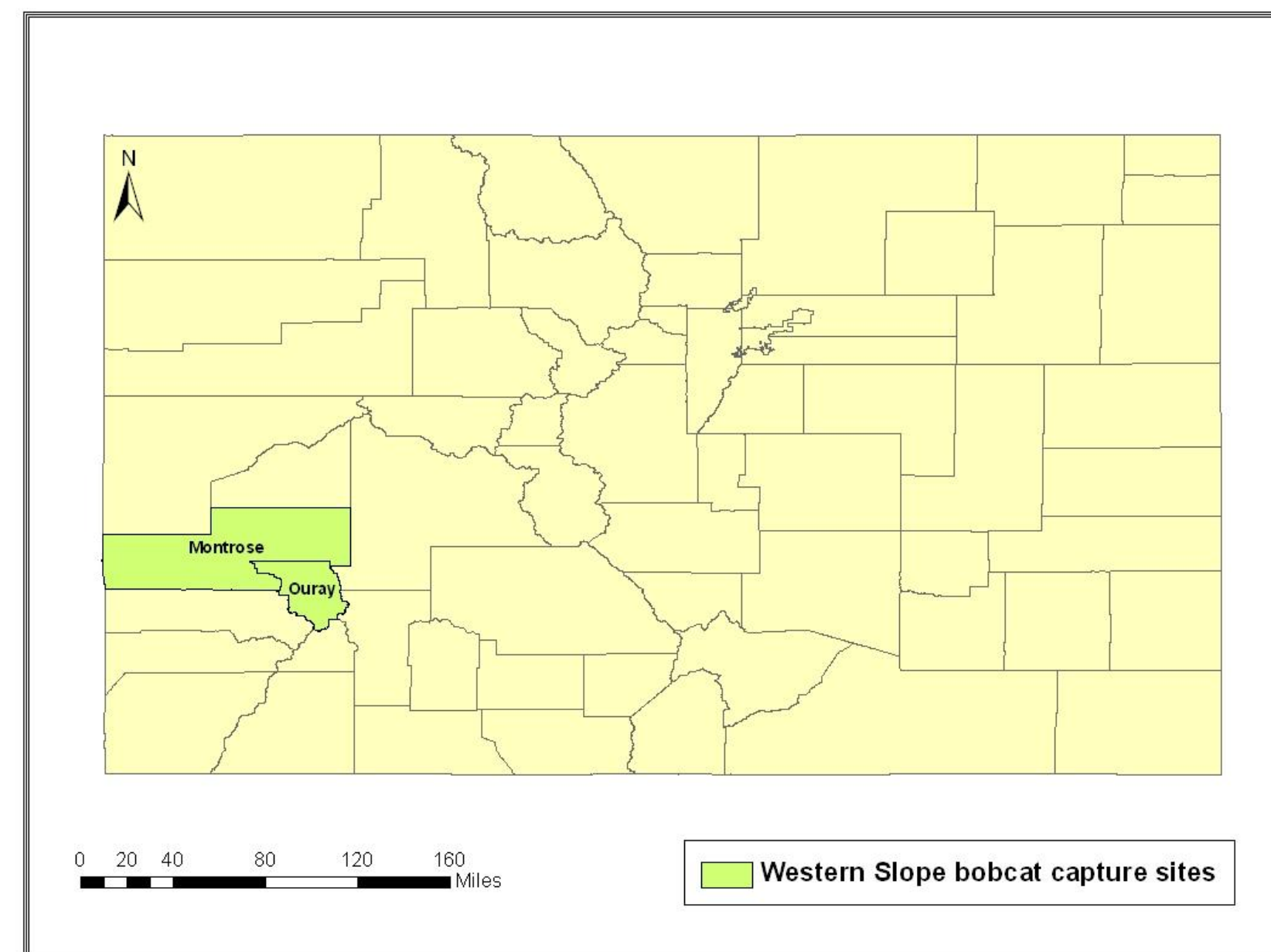
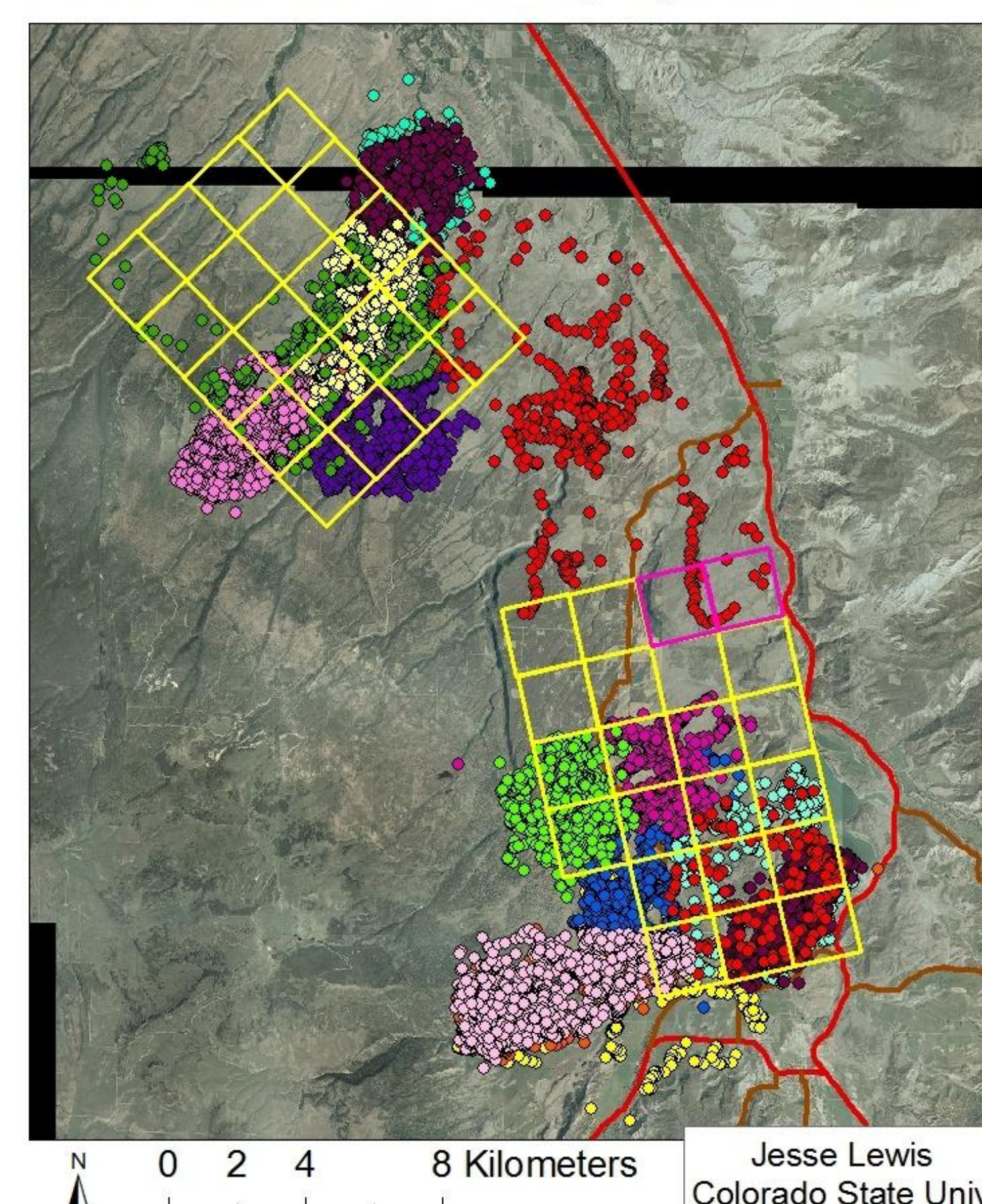


Figure 1. Study location in Colorado includes rural counties in the Western Slope

GIS Movement Data

Bobcat Locations -- Uncompahgre Plateau 2009



Bobcat locations for 17 Individuals fitted with GPS collars on the UP in 2009. Image courtesy of Jesse Lewis

Results

- Eight of nine loci in multiplex reactions were visualized by gel electrophoresis (Figure 2).
- However, only 4 of 9 loci initially yielded reliable genotypes by capillary electrophoresis (Figure 3). Results for the remaining loci are pending.
- Only the largest products in each multiplex reaction produced reliable signals even though smaller bands were visualized on gels (Figures 3 and 4).

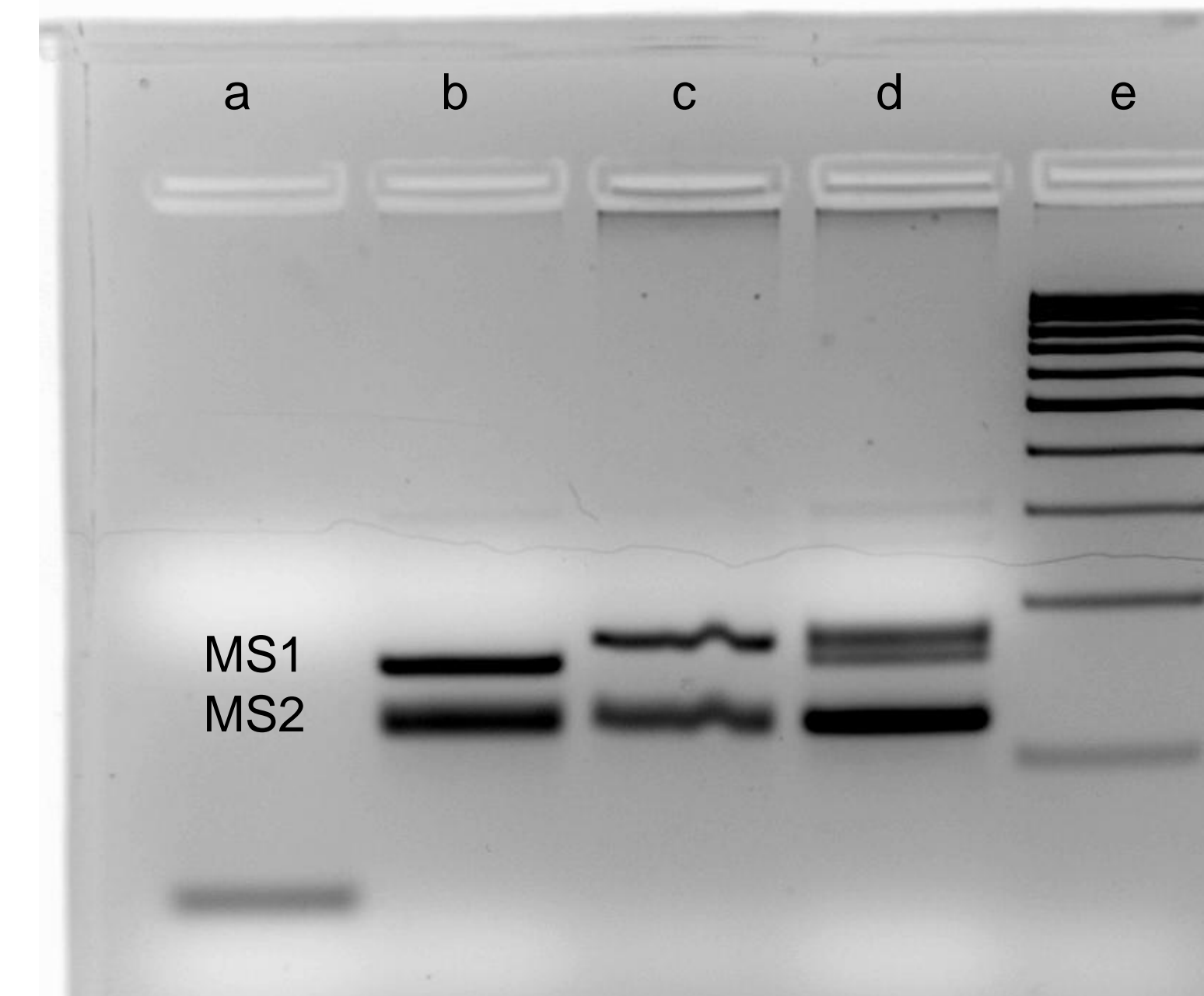


Figure 2. Successful amplification of bobcat microsatellite alleles in a multiplex PCR in 3 bobcats. (a) negative control (b & c) homozygous for MS1 and MS2. (d) heterozygous for MS1 and homozygous for MS2 (e) 100 bp DNA ladder. MS1 = BCD8T, MS2 = FCA742

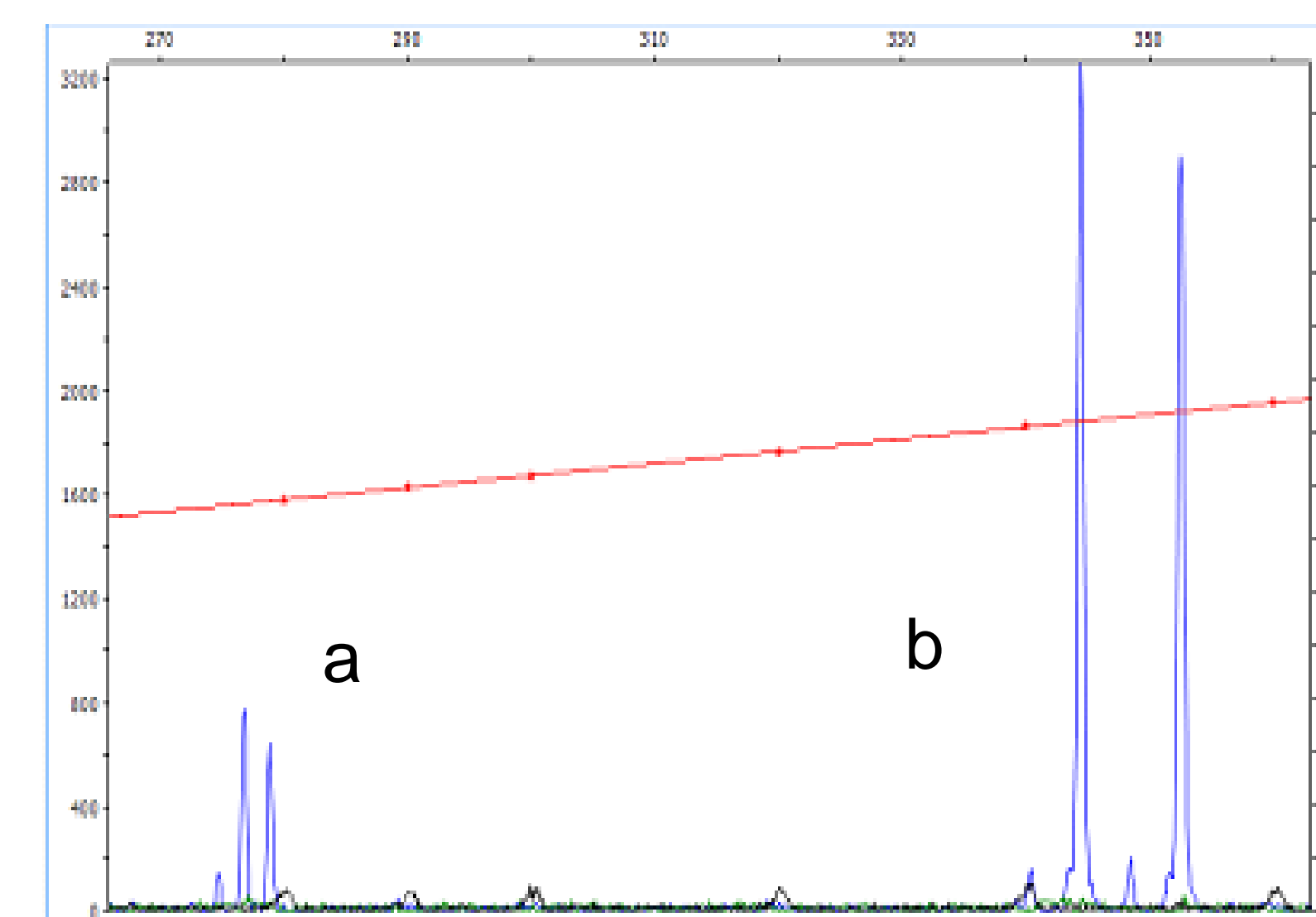


Figure 3. Electropherogram of multiplex PCR group 3 (Table 1). (a) BCG8T (b) FCA740. No signal was detected for FCA026.

Microsatellites

Primer Name	Size	# alleles	Multiplex PCR grouping
FCA077	130-140	6	1
FCA132	182-194	7	1
BCE5T	256-280	7	1
FCA023	144-158	6	2
FCA096	189-209	8	2
FCA031	237-255	8	2
FCA026	138-166	13	3
BCG8T	275-299	11	3
FCA 740	333-353	6	3
BCD8T	156-180	5	4
FCA742	104-134	7	4
FCA008	140-156	8	
FCA045	147-173	7	
FCA090	108-126	7	
FCA149	133-149	9	
FCA559	115-135	6	

Table 1. Approximate product sizes and number of alleles for microsatellite loci.

Future Steps

- Completion of microsatellite determination will allow comparison of individual movement data with kinship analysis to estimate relatedness within the population of bobcats.
- Compare genetic diversity between the two sample sites
Hypothesis: The two sample sites are not genetically different because the sites are separated by only 6 km.
- Relatedness of male/female pairs sharing home ranges
Hypothesis: The individuals sharing home ranges will not be related.
- Relatedness of all females and relatedness of males in the study sites
Hypothesis: The females are more related to each other than the males because of sex biased dispersal by males.
- Pathogen characteristics between male/female pairs. Do they share pathogen profiles?

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