OBSERVATIONS ON THE GENETIC HEALTH OF BOBCAT (*LYNX RUFUS*) POPULATIONS IN OKLAHOMA

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Abstract

The bobcat (*Lynx rufus*) is a significant meso-carnivore and an important game species within the state of Oklahoma. The bobcat is regularly harvested, with thousands of bobcats being legally hunted every season. To date, there have been limited studies on the genetic makeup and health of the species within the state. Collecting and analyzing genetic data from specimens collected across the state will provide information on the current health of the species, as well provide insight on the possible effect the removal of bobcats may have on the total population in the state. To assess the genetic health of bobcats in Oklahoma, 222 individuals will be genotyped for 10 microsatellite loci to establish a baseline of genetic diversity. To date, genotypes have been generated for 172 individuals. Future research will focus on comparing genetic diversity measures between hunting seasons.

Tissue Collection and Extraction

A total of 324 tissue samples were collected from bobcat specimens from 41 of 77 counties (Fig. 1) during the 2018 to 2019 season. Samples were obtained from all geographic regions of Oklahoma, including the panhandle. Tongues were removed from each bobcat by the Oklahoma Department of Wildlife Conservation and deposited at Oklahoma State University for parasite analyses. Tissue subsamples were taken from the tongues and DNA was extracted using the Qiagen DNeasy Tissue Extraction Kits. Samples were quantified using a Nanodrop-2000. Of these extractions, 222 samples representing 22 counties in Eastern, Central, and Western Oklahoma were used to identify baseline genetic diversity within the current bobcat population.

PCR and Genotyping

A pilot study was performed to test 19 microsatellite loci. A total of 10 loci were optimized and selected for the study (Table 1). Additional an SRY microsatellite marker is being used to identify sex in samples whose sex was not recorded during collection by ODWC. Samples are being genotyped on a 3500 Genetic Analyzer and allele calls are being made using GeneMapper. Preliminary diversity statistics were generated using Cervus 3.0.7 (Marshall et al., 1998; Slate et al., 2000; Kalinowski et al., 2007, 2010). An initial analysis of the number of distinct genetic groups in Oklahoma was performed using Structure 2.3.4 (Pritchard et al. 2000; Falush et al. 2003, 2007; Hubisz et al. 2009). Structure results were imported into Structure Harvester (Earl and Bridgett 2012) to determine the most likely value of K that best fit the data based on the Evanno et al. (2005) method.

Table 1.—Microsatellite primers to be used in this study to uniquely identify individual bobcats (*Lynx rufus*). The primer name, citation source, expected size range, and optimal annealing temperature are provided. BC = primers developed using bobcats, Fca = primers developed using domestic cats (*Felis catus*), and Lc = primers developed using Canada lynx (*L. canadensis*). All primers have been shown to work in bobcats.

Pimer Name	Source	Allele Size Range	Annealing Temp. (°C)
BC1AT	Faircloth et al. 2005	318	50-60
BCE5T	Faircloth et al. 2005	261	50
FCA77	Menotti-Raymond et al. 1999	143-155	40-62
FCA90	Menotti-Raymond et al. 1999	93-120	58-60
FCA96	Menotti-Raymond et al. 1999	184-224	53
FCA132	Menotti-Raymond et al. 1999	137-153	54-60
FCA126	Menotti-Raymond et al. 1999	139-145	58-60
FCA742	Menotti-Raymond et al. 1999	123-175	
FCA391	Menotti-Raymond et al. 1999	237-273	56-58
LC110	Carmichael et al. 2000	91-103	50-56

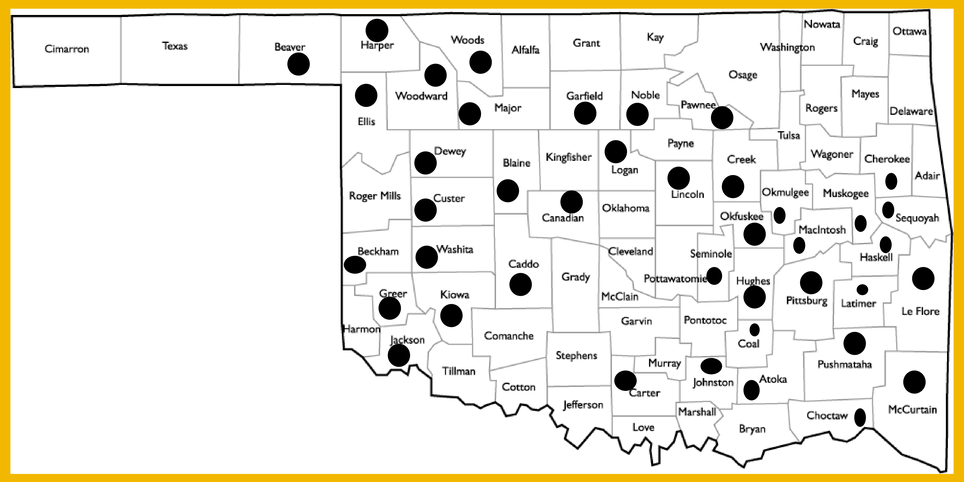

Fig. 1.— Map of Oklahoma showing counties from which bobcat samples were obtained and genotyped for this study.

Table 2.—Preliminary diversity statistics for 10 microsatellite loci. The locus name, number of alleles (k), number of individuals genotyped (N), observed heterozygosity (H_O), expected heterozygosity (H_E), and polymorphic information content (PIC) is provided for each marker.

Locus	k	N	H_O	H_E	PIC
BC1AT	27	124	0.903	0.910	0.899
BCE5T	22	142	0.803	0.871	0.855
FCA77	22	135	0.770	0.894	0.881
FCA90	25	143	0.615	0.921	0.912
FCA96	32	114	0.746	0.944	0.937
FCA132	24	120	0.583	0.917	0.907
FCA126	33	128	0.813	0.926	0.917
FCA742	24	119	0.538	0.861	0.845
FCA391	29	118	0.568	0.803	0.787
LC110	22	158	0.816	0.888	0.877

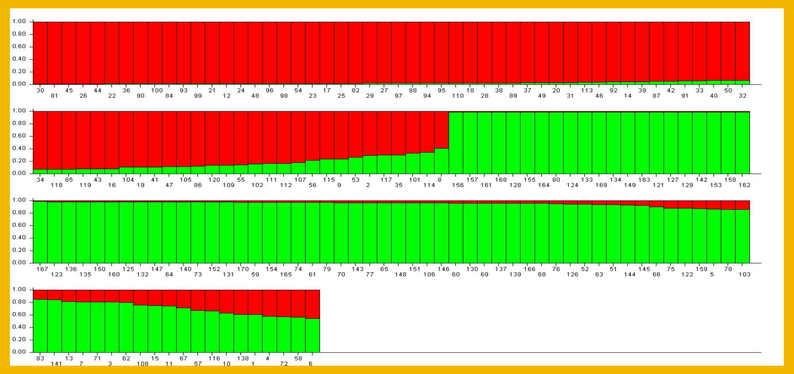

Fig. 2.— Outcome of Structure run showing k = 2.

Results and Future Work

Initial allele calls have been made for 172 of the 222 samples. Preliminary results from Cervus analyses (Table 2) show a high level of heterozygosity and high polymorphic information content, and minimal levels of homozygosity, indicating a healthy level of genetic diversity across the state. The results from Structure and Structure Harvester suggest there are 2 genetic units represented by these samples (Fig. 2). However, there was evidence of additional structure that needs to be further assessed. We currently are in the process of genotyping the remaining samples, finalizing allele calls for all samples, and analyzing the SRY.

The data from this study will be used as a baseline of genetic diversity and structure for future comparisons to understand the effects of removing bobcats from the greater population. Future research will focus on genotyping the remaining 102 samples from the 2018-2019 season, and extracting and genotyping samples from the 2019-2020 season. The 2019-2020 trapping season includes samples from additional counties.

Photograph 1. — Bobcat recorded in Oklahoma, taken by Nathan Proudman.

Acknowledgements

Funding for this research was provided by the University of Central Oklahoma Office of Research and Sponsored Programs RCSA grant; Oklahoma Department of Wildlife, Wildlife Restoration Grant; and the UCO College of Mathematics and Science Dean's Molecular Fund. We would like to thank J. Davis (ODWC) for obtaining the tongue samples, Dr. W. S. Fairbanks (OSU) for providing tissue subsamples, and C. Smith, T. Gray, C. Jennings, K. Patatanian, and R. Frank for assistance in the lab. Thanks to Nathan Proudman for the bobcat photograph used in the poster.

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