Bighorn Sheep Research Activity 2016-17
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Wildlife Genomics & Disease Ecology Lab
Updated 04/27/2017 SMLS

Sample acquisition

Samples acquired to date from sheep captured or killed 2012-2017

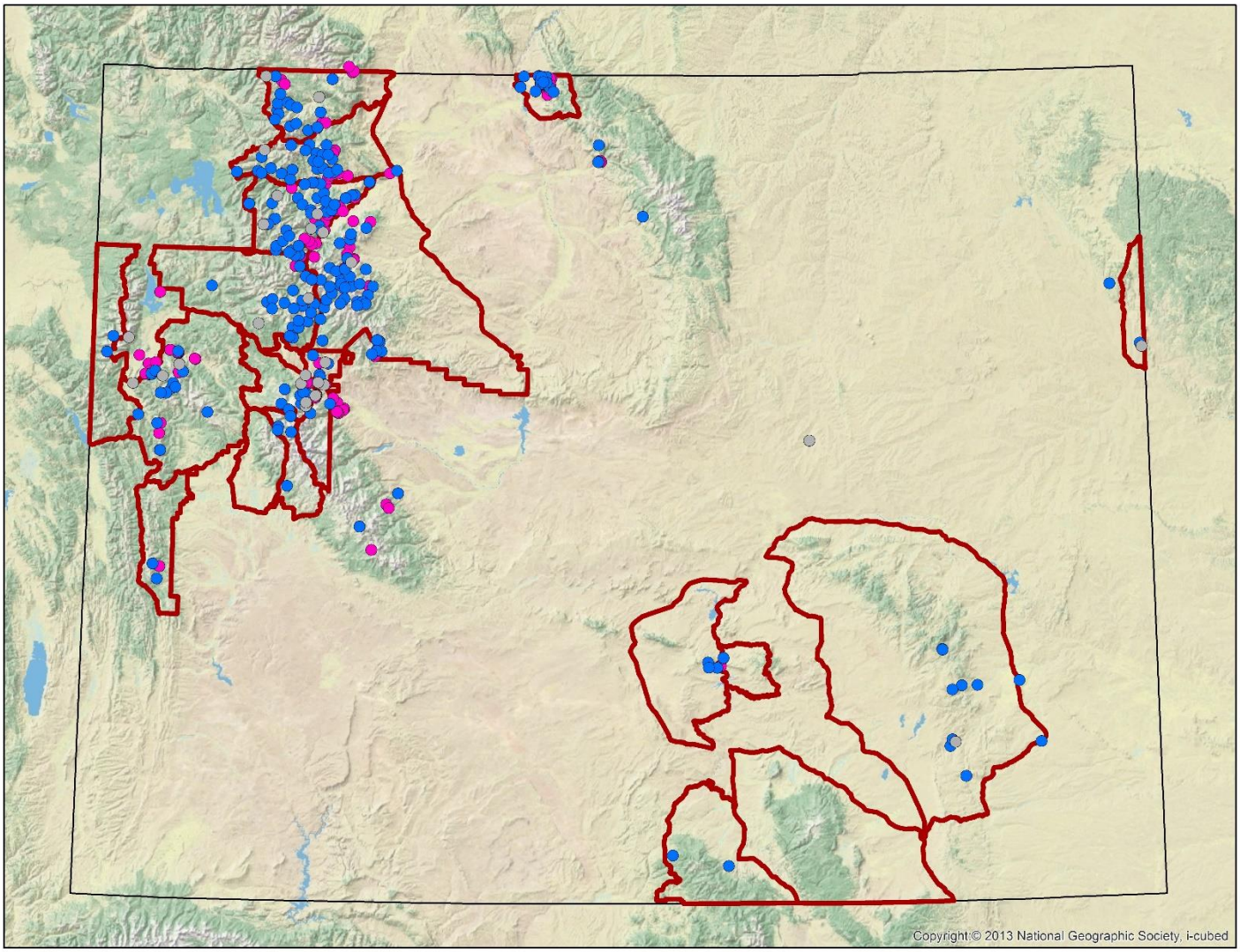

Figure 1- Distribution of bighorn sheep samples that were captured or killed 2014-2017 in Wyoming. Red outlines indicate the Wyoming Game & Fish Department Hunt Areas. Blue dots are samples from males (n = 263, mainly tissue samples from legally hunted animals), pink dots are females (n = 559, mix of whole blood and FTA card samples), gray dots are unknown sex (n = 138). Several locations such as Cody and Dubois are represented as a single point but multiple animals were sampled in those locations. Dots are representative of samples, not of unique individuals, which may be lower if animals were recaptured. Females are well sampled in the Jackson, Whiskey Mountain, and Absaroka herds but not in other herds which are only sampled by legally hunted rams and pickup heads. Outside of the northwest corner of the state, sampling is mainly limited to hunted rams. Dots outside hunt area boundaries are represent wandering rams, fecal collections, or planned captures. The number of samples from 2014-2016 available and analyzed from each hunt area are listed in Table 4.

Received in 2016-17

- 593 new samples acquired in 2016-17
- Variety of sample types, sample locations (see tables)

Table 1: 2016-17 samples acquired by sample type

Type	**Number**	**Notes**
FTA cards	147	66 cards have matching whole blood sample
Whole blood	59	60 samples have matching FTA cards or fecal
Bone	1	
Fecal	39	
Horn	8	
Tissue	122	
Total	**593**	Represents fewer than 593 unique individuals (includes duplicates and recaptures)

Table 2: 2016-17 samples acquired by collection method

Method	**Number**	**Notes**
Capture	406	For telemetry or other by WGFD or other researcher
Field collected	39	Fecal samples collected by WGFD
Legally hunted	124	Hunter kits distributed to WGFD regional offices
Necropsy	5	From general and capture mortalities
Pick up heads	11	Carcasses found in field and not necropsied
Wandering ram or unknown	9	4 individuals
Total	**593**	

Table 3: 2016-17 sample acquired by hunt area

Hunt Area	**Number**	**Notes**
1-Clark's Fork	13	
2-Trout Peak	16	
3-Wapiti Ridge	41	
4-Yount's Peak	26	
5-Franc's Peak	21	
6-Targhee	2	
7-Jackson	94	
8-Sheep Mountain	0	
9-Dinwoody	43	
10-Jakey's Fork	58	
12-Devil's Canyon	51	
17-Ferris-Seminoe	2	
18-Douglas Creek	0	
19-Laramie Peak	15	Includes one wandering ram outside Sybille facility
20-Kouba Canyon	71	Includes 68 FTA cards or DNA extractions of (<71) individuals captured 2012-2014 from Elk Mountain (HA 20) by WGFD and used for research by Brynn Parr, South Dakota State University
21-Encampment River	2	
22-Dubois Badlands	16	

23-Desolation Peak	21	
24-Big Piney	9	
26-Bennett Mountain	0	
2, 3, 5-Cody	70	
9,10,22-Dubois	15	
9,23-Temple Peak	6	
Total	**593**	

Requested

- Made and distributed >1000 hunter sample kits for bighorn and pronghorn
- Made and distributed > 100 fecal sample kits for bighorn sheep
- Standing requests at Wyoming State Veterinary Lab for tissue and fecal samples from necropsy specimens
- Agreement with Wyoming Cooperative Fish and Wildlife Research Unit and Wyoming Game & Fish Wildlife Health Laboratory to collect 3 mL of whole blood from captured animals

Table 4- Contemporary samples by hunt area and analysis method

Hunt Area	Available 2014-17**	msats*	mtDNA*	rfseq*	chip	Notes
1-Clark's Fork	44	20	21	15	0	MSU's region for SNP chip
2-Trout Peak	44	20	20	15	5	2 of Ours + shared data with E. Flesch
3-Wapiti Ridge	87	23	26	19	9	Shared data with E. Flesch
4-Yount's Peak	34	20	20	16	3	
5-Franc's Peak	66	21	35	14	15	Shared data with E. Flesch
6-Targhee	3	2	2	2	1	
7-Jackson	121	20	29	20	2	
8-Sheep Mountain	2	2	2	2	2	
9-Dinwoody	59	22	23	14	6	
10-Jakey's Fork	108	13	15	13	4	Includes fecal samples, possible duplicates
12-Devil's Canyon	120	21	23	19	14	
17-Ferris-Seminoe	13	12	12	1	0	
18-Douglas Creek	1	0	0	0	0	
19-Laramie Peak	18	14	15	7	2	
20-Kouba Canyon	71	20	20	0	0	
21-Encampment River	2	1	2	1	0	
22-Dubois Badlands	18	17	17	7	0	
23-Desolation Peak	21	9	10	3	0	
24-Big Piney	9	9	9	9	0	
26-Bennett Mountain	0	0	0	0	0	
1-5, 22-Absaroka	22	0	0	0	0	Samples received w/o detailed spatial data
2, 3, 5-Cody	62	4	4	0	0	Samples received w/o detailed spatial data
9, 10, 22-Dubois	21	0	0	0	0	Samples received w/o detailed spatial data
Sybille captive	13	0	0	0	0	
Total	**958**	**270**	**305**	**177**	**63**	

*includes pending data sent for genotyping and sequencing in April 2017; acronyms defined below
**reflects total number of samples, not unique individuals

DNA extraction

- 1,094 extraction attempts
- Validated extraction methods for different sample types (blood, tissue, bone, horn, FTA, fecal)
- Tested quality and quantity of interaction between sample type and extraction method (Qiagen, KingFisher, Phenol-Chloroform, Chelex)
- Yields of .05-300 ng/uL, 10 ng – 30,000 ng

Mitochondrial DNA sequencing ("mtDNA")

- Optimized PCR methods for sequencing ~500 bp of control region
- Haplotypes identified for 238 individuals
- 66 additional individuals in preparation
- Tested methods for identifying unique haplotypes and cluster related haplotypes

Microsatellite genotyping ("Msat")

- 255 individuals genotyped at 40 autosomal loci + 3 sex markers
- 14 individuals in progress at the University of California Davis Veterinary Genetics Lab
- Compared genotyping calls between machines and facilities, rerun 10% of individuals
- Tested methods for identifying individuals, removing null alleles, and clustering

Genomic SNP genotyping

SNP Chip ("chip")

- Genotyped 36 individuals on the Illumina Ovine HD BeadChip (600K loci)
- Shared data on additional 24 individuals from Greater Yellowstone Ecosystem
- Developed pipeline for filtering genotypes based on "missingness", allele frequency, Hardy-Weinberg, etc.
- Tested methods for clustering

Restriction fragment sequencing throughout whole genome ("Rfseq")

- Validated library preparation methods for enzyme combination and Illumina sequencing
- Sequenced 92 individuals using library preparation method of Parchman et al. (2012)
- Additional 92 individuals in progress at University of Texas Genomic Sequencing & Analysis Facility
- Developing pipeline for cleaning and filtering data, aligning to reference genome, and calling variable sites
- Developing workflow for identifying individuals and clustering
- Found that FTA cards and low template samples do not sequence well

Funding requested

- Applied for $24,725 from the Wyoming Wild Sheep Foundation to conduct a detailed genetic study on the Whiskey Mountain herd (denied)
- Applied for $40,000 over 2 years from the Wild Sheep Foundation (national organization) to study the genetic effects of translocation on bighorn sheep herds (received $10,000 for year one)
- Applied for $99,755 from the Safari Club International Foundation to study the genomics of disease history in bighorn sheep (denied but reviewed favorably, and invited for 2018 application)

- Requested additional funding from the Eastern Wild Sheep Foundation and Iowa Chapter of the Foundation of North American Wild Sheep to support the state-wide genetic assessment of bighorn sheep that is currently funded by the Wyoming Governor's Big Game License auction and the Wyoming Wild Sheep Foundation (no answer back)

Collaboration

- Research agreement with Bob Garrott, Jennifer Thomson, and Elizabeth Flesch at Montana State University RE: Greater Yellowstone Ecosystem, including sample and data sharing, geographic partitioning, and publishing
- Methods and conceptual discussion with Oliver Ryder (San Diego Zoo), Lisette Waits (University of Idaho), Kim Andrews (UI), and Paul Hohenlohe (UI)
- Sample sharing with Brynn Parr, South Dakota State University
- Sample sharing and discussion with Wyoming Game & Fish Wildlife Forensics and Fish Health Laboratory
- Sample sharing and discussion with Wyoming Cooperative Fish and Wildlife Research Unit
- Sample sharing and discussion with Wyoming Game & Fish Wildlife Health Laboratory
- Sample sharing and discussion with Wyoming State Veterinary Lab
- Attended Northern Wild Sheep & Goat Council meeting in Moscow, ID in May 2016
- Presented at the Wyoming Wild Sheep Foundation meeting in Casper, WY in June 2016

Plans for 2017

- Submit manuscript on DNA extraction methods for mammalian blood preserved on FTA cards
- Sequence additional individuals using rfseq, mtDNA sequencing
- Prepare manuscript on genetic clustering and diversity of bighorn sheep in Wyoming
- Present results at Wyoming Wild Sheep Foundation meeting (June 2017), The Wildlife Society meeting (September 2017)
- Outreach presentation at the National Bighorn Sheep Center in Dubois (July 2017)
- Apply for additional funding from sources such as the Wyoming Governor's Big Game License Grant Coalition, Wyoming Wild Sheep Foundation, Midwest Wildlife Sheep Foundation, Iowa Chapter of the Foundation of North American Wild Sheep, Pope & Young Club, Camp Fire Conservation Fund, and Dallas Safari Club

Narrative of preliminary results

Without specifying herd or hunt area identity *a priori*, analyses of microsatellite genotypes recover 5 main clusters that correspond to the core native herds: Jackson, Whiskey Mountain, Absaroka, Devil's Canyon, and Kouba Canyon/Elk Mountain. Individuals from Ferris-Seminoe and Laramie Peak tend to cluster with Devil's Canyon or Whiskey Mountain herds, likely reflecting translocation history. Analyses of mitochondrial haplotypes suggest high diversity, with less obvious population structuring than the microsatellite data. Most haplotypes from Wyoming form unique clades but others cluster with published bighorn sheep DNA sequences from North America. Genomic SNP recovery is high but depends greatly on filtering decisions. rfseq data align to the published draft bighorn sheep genome. SNP chip data recovers most domestic sheep loci but the percentage of polymorphic loci is low.