Evaluation of the genetic status of Teton Range bighorn sheep in comparison to adjacent herds in Wyoming

Final Project Report
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Executive summary

We genotyped 185 bighorn sheep samples (29 tissue & 156 fecal samples) at 22 microsatellite loci, and 41 fecal samples at a sex identification marker. Genetic variation was lower in the Northern and Southern Teton bands of bighorn sheep than in the Jackson herd. Tests for population bottlenecks suggest the Northern Teton band has recently suffered a reduction in size and/or increased isolation (i.e. reduced gene flow). Our results show substantial genetic differentiation between bighorn in the Jackson herd and the Teton Range (F_{ST} = 0.18) and between the Northern and Southern bands within the Teton Range (F_{ST} = 0.12), and low to moderate genetic differentiation among the National Elk Refuge, Hoback, and Gros Ventre bands within the Jackson herd (F_{ST} = 0.05). Our loci have high power to correctly assign individuals to populations based on their multi-locus genotypes and allele frequency data from each subpopulation. This suggests high power to detect poaching and dispersal. For example, one individual (150) was sampled with the Southern Teton band, but assigned genetically to the Northern Teton band with high confidence, which suggests that this individual was born in the Northern band and moved into the southern part of the Teton Range (though this is not evidence that 150 attempted to breed in the Southern Teton band). None of the individuals we sampled in the Teton Range appeared to be the offspring of migrants between the Northern and Southern Teton bands. Lungworm parasite abundance ranged from zero up to almost 1200 larvae per gram of feces. We did not detect any associations between parasite abundance and individual inbreeding as measured by heterozygosity.

Considering the levels of genetic diversity and differentiation observed in this study, we have the following management recommendations:

1. The low levels of genetic diversity in the Northern and Southern Teton bands warrant considering management actions that would increase gene flow into (or within) the Teton Range, while carefully considering disease risks that may be associated with the movement of bighorn sheep. Such management actions could include the translocation of quarantined and vaccinated bighorn sheep (to ensure that they are disease free) from more genetically diverse bighorn herds (either nearby or from similar high-elevation habitats) or increasing connectivity with a nearby population(s).
2. We recommend considering the Northern and Southern Teton bands as distinct population units for management (e.g. harvest) and conservation purposes. This recommendation is based on the observed level of genetic differentiation and the lack of evidence for breeding between Northern and Southern band individuals.

3. We recommend managing the National Elk Refuge, Hoback, and Gros Ventre bands of sheep in the Jackson area as one population from a *genetic* standpoint. However, considering that there is some evidence for a low level of genetic differentiation among these three groups of sheep, it is possible that they could be somewhat demographically independent of one another (e.g. population sizes in the different bands *might* fluctuate independently).

DNA Extraction and Genotyping

We have extracted DNA from 29 tissue samples taken from helicopter-captured female bighorn sheep (*Ovis canadensis*) in the Teton Range, and from 156 fecal samples that were collected on foot from bands of bighorn in the Teton Range and the Jackson, Wyoming area. We also received historical blood samples from six individuals that were collected from bighorn sheep in the Teton Range in 1990-1995. Considering the limited information that could be gained from comparisons of genetic data on these samples with contemporary samples (due to small sample size), we did not genotype them.

Four multiplex polymerase chain reactions (PCRs) and one single-marker PCR containing a total of 24 microsatellite markers and one sex-linked insertion/deletion polymorphism were optimized for genotyping. Sixteen of these are neutral microsatellite markers, eight microsatellites are associated with candidate genes (genes thought to be important to fitness-related traits such as disease resistance), and the sex-linked insertion/deletion marker is used for sex identification. We confirmed the ability of the sex identification marker to be diagnostic on bighorn sheep of known sex from multiple study populations. After allele frequencies and amplification success for 27 tissue samples were examined, two of the microsatellite markers were dropped; IFN-λ was monomorphic, and FCB11 did not amplify reliably in a multiplex PCR. The remaining 22 polymorphic microsatellites were then genotyped on all tissue and fecal samples, and the sex identification marker was genotyped on 41 fecal samples. We independently repeat-genotyped each locus in each fecal sample three to eight times to maximize data quality and minimize genotyping error rates associated with fecal DNA. Samples with low quality DNA (those for which genotypes could not be reliably scored) were dropped from the study.

There were several fecal samples with identical genotypes (i.e. some individuals were sampled multiple times in the field: Table 1); hence the actual total number of genotyped unique individuals was 123: 29, 28, 22, 31, 13 individuals from the North Teton, South Teton, National Elk Refuge (NER), Gros Ventre, and Hoback bands, respectively.

Genetic Data Analysis

Consensus genotypes for fecal samples were based on data from multiple PCR reactions from individual samples. The following rules were used in consensus genotyping: for a sample to be heterozygous at a locus, both alleles had to be observed twice; for a sample to be homozygous, the same homozygous genotype had to be observed three times. We randomly chose 10% of samples for re-extraction and repeat genotyping to monitor for errors. One potential genotyping error was detected but further tests are necessary to determine its cause. Principal coordinates

analysis (PCoA) and multilocus genotype matching were done in GENALEX (Peakall and Smouse 2006) to identify outliers due to potential genotyping errors and fecal samples collected from the same individual. Amplification success rate, false allele rate, and allelic drop-out rate were computed as in Luikart et al. (2008b) with mean amplification success rate of 0.91, mean false allele rate of 0.002, and a mean allelic dropout rate of 0.03, across 22 microsatellites, reflecting the high quality of most samples (Figure 1).

We estimated expected heterozygosity, tested for gametic (linkage) disequilibrium, and tested for departures from Hardy–Weinberg proportions (using exact tests and a Markov chain) using GENEPOP 3.4 (Raymond & Rousset 1995). None of the loci deviated significantly from Hardy-Weinberg proportions. Some evidence for gametic disequilibrium was evident between MHC2 and TCRG4 in the South Teton band, and KERA and KRT2 in the North Teton band, but neither of these associations was found in multiple subpopulations. If there would have been evidence for widespread gametic disequilibrium between these pairs of markers we would have dropped them from the study.

We compared levels of genetic diversity among populations with estimates of expected heterozygosity (H_e) and a bias-corrected estimate of allelic diversity ($N_{a\text{-}corr}$) which corrects for differences in sample sizes among populations (Kalinowski 2005). Data from the 22 microsatellites indicate that expected heterozygosity and allelic diversity are lowest in the Southern Teton band, and highest in the Jackson herd (Southern Teton: $N_{a(corr)} = 3.78$, $H_e = 0.50$; Northern Teton: $N_{a(corr)} = 4.02$, $H_e = 0.60$; Jackson: $N_{a(corr)} = 4.94$, $H_e = 0.61$). Heterozygosity was significantly different between the Jackson and South Teton subpopulations (paired t-test: $p = 0.02$) and between the North Teton and South Teton subpopulations (paired t-test: $p = 0.02$), but not between the Jackson and North Teton subpopulations. Allelic diversity was significantly different between the Jackson and Northern Teton subpopulations (paired t-test: $p = 0.005$) and between the Jackson and Southern Teton subpopulations (paired t-test: $p = 0.001$). There was no statistical support for a difference in allelic diversity between the Northern and Southern Teton bands. Allelic diversity and heterozygosity comparisons are summarized in Table 3.

We began assessing the population genetic structure of Jackson and Teton bighorn sheep by using principle coordinates analysis (PCoA). PCoA plots spatially cluster individuals based on pair-wise genetic distances among them (based on the sum of the squared differences in allele lengths between individuals). Groups of individuals who are genetically similar should cluster close to one another on the PCoA plots. This analysis suggests that the Jackson, Northern Teton, and Southern Teton groups of sheep are genetically differentiated from one another, and there is low genetic differentiation among the three bands of sheep within the Jackson herd (Figure 2).

We used the F_{ST} statistic to quantify the level of genetic differentiation among the three subpopulations (Weir & Cockerham 1984). F_{ST} is an estimate of the proportion of total genetic variation that is explained by differences among subpopulations. F_{ST} values of greater than zero are expected among subpopulations that are genetically differentiated from one another (i.e. when allele frequencies differ among subpopulations). Our analyses show substantial genetic differentiation between sheep in the Jackson and Teton areas ($F_{ST} = 0.18$) and between the Northern and Southern bands within the Teton Range ($F_{ST} = 0.12$), and low to moderate genetic differentiation among the NER, Hoback, and Gros Ventre bands within the Jackson area ($F_{ST} = 0.05$). Pair-wise F_{ST} values between bands of sheep are summarized in Table 2.

We also used an assignment-test-based approach to assess population genetic structure and to identify individuals that may be migrants or of mixed band ancestry (e.g. individuals with a father from another herd). Assignment tests were done in the program GENECLASS (Piry et

al. 2004) using the Bayesian algorithm of Rannala & Mountain (1997) to assign individuals to subpopulations based on individual genotypes and allele frequencies in different subpopulations. Approximately 92% of individuals (113/123) were assigned to the population from which they were sampled; this suggests that there is high power to correctly assign individuals to populations based on genotypes and information on allele frequencies in each subpopulation. Assignment test results are summarized in Figure 3.

All sheep in the North and South Teton bands assigned with >99% certainty to the putative band of origin except for one. Sample 150 (an adult female) was sampled within the putative range of the Southern Teton band, but assigned to the Northern Teton band with a very high assignment score (99.3%; Table 4). This suggests that this sheep was born in the Northern band and may have moved into the Southern band. However there is no evidence that this individual has reproduced in the Southern band. The sampling location of 150 in the Southern part of the Teton Range may be the result of seasonal movement during the summer, and is not necessarily evidence of a permanent migration with the potential for increasing gene flow between the Northern and Southern bands. No samples from the Teton bands show any signs of mixed band ancestry either between Teton bands, or with any of the bands in the Jackson herd.

Several individuals in the Jackson herd were assigned to bands other than the one they were sampled in; this suggests that there is frequent movement of individuals among bands within the Jackson herd. However, such movements do not necessarily result in gene flow (i.e. individuals may return to their bands of origin to breed), and may only be indicative of seasonal or temporary movements unrelated to reproduction. For example, sample 132 was collected within the range of the Gros Ventre band, but was assigned with 99.996% certainty to the NER band. Similarly, sample 109 was collected with the Hoback band, but assigned with 99.834% certainty to the NER band (Table 4).

Due to the low F_{ST} calculated between the bands in the Jackson herd, we would expect to see several individuals of mixed band ancestry. For instance, sample 112 was collected with the NER band, but was assigned with ~50% accuracy to both the Gros Ventre and NER bands. About half the genotypes for sample 112 have alleles more common in the Gros Ventre band, while the other half are alleles more common in the NER band, suggesting the sheep represents a first generation off-spring (F_1) of a Gros Ventre and NER mating.

We tested for recent population bottlenecks (i.e. reductions in effective population size [N_e]) with the software program BOTTLENECK (Piry et al. 1999). Reductions in N_e cause decreased allelic diversity and heterozygosity, but heterozygosity decreases at a slower rate than allelic diversity. Therefore, a recently bottlenecked population is expected to have an excess of heterozygosity relative to that expected based on observed allelic diversity and assuming mutation-drift equilibrium (Luikart et al. 1998). BOTTLENECK detects reductions in N_e by testing for an excess of heterozygosity. Wilcoxon sign rank tests in BOTTLENECK on the Northern Teton band are strongly indicative of a recent reduction in N_e. There was no evidence for recent bottlenecks in the Southern Teton or Jackson herds. The tests for bottlenecks are summarized in Figure 4.

Parasite Data and Analyses

To quantify the levels of parasitism, lungworm counts were conducted on 83 samples from the Jackson herd, and 62 samples from the Teton herd. The only lungworms detected were from the genus *Protostrongylus*, which are endemic in bighorn sheep almost everywhere they exist. The

parasite counts are summarized in Figure 5. Prevalence was high in both herds. Intensity ranged from zero up to almost 1200 larvae per gram of feces. The frequency distributions of *Protostrongylus* larvae among individuals are highly overdispersed (i.e. non-randomly distributed among individuals), which is typical of the distribution of macro-parasites within host populations. There was no evidence for correlations between parasite abundance and multi-locus heterozygosity (a measure of inbreeding) either in simple linear regression models, or multi regression analyses that included sex, age, and time of year as additional explanatory variables (Figure 6). Though this result is not indicative of inbreeding depression for resistance to lungworm parasites, it should not be taken as evidence for a lack of inbreeding depression for disease resistance. Power to detect a relationship between parasite abundance and heterozygosity can be low because due to the low precision and accuracy of fecal parasite counts and the potentially strong influence of other factors on parasite counts (e.g. exposure, parasite density, time of day, and food consumption; Michael 1989; Sithithaworn 1991; Seivwright et al. 2004). Additionally, the effects of inbreeding may vary when considering different types of parasites (e.g. micro-parasites such as *Pasturella* spp., or gastrointestinal nematodes). Further research is needed to better assess the effects of loss of genetic variation on disease resistance (e.g. resistance to gastrointestinal nematode parasites or micro-parasites). Fortunately, genome wide scans and disease-resistance gene sequencing is now feasible.

Discussion of preliminary results and description of ongoing work

Relative levels of genetic diversity: The estimates of allelic diversity and multi-locus heterozygosity among the three populations suggest that the Jackson herd is more genetically diverse (probably due to larger N_e and increased connectivity to adjacent populations) than the Northern and Southern Teton populations (Table 3). This is not surprising considering the larger estimated census size of the Jackson herd compared to either of the Teton populations, and relative isolation of the Teton Range from other bighorn sheep populations. Small population size, and limited immigration are expected to cause reduced genetic diversity relative to larger, more connected populations. Heterozygosity and allelic diversity can be very important to individual fitness (Hogg et al. 2006), and the ability to resist parasites and disease (Paterson et al. 1998; Coltman et al. 1999; Rijks et al. 2008).

Considering the relatively high level of genetic isolation, and low level of genetic diversity of bighorn sheep bands in the Tetons, we recommend considering management actions that would increase gene flow into (or within) the Teton Range, while taking potential disease risks into consideration. This could involve the translocation of bighorn sheep from more genetically diverse bighorn sheep herds (either nearby or from similar high-elevation habitats) or habitat management actions that would increase connectivity with a nearby population(s).

Population structure: Our results on population structure suggest that 1) bighorn sheep in the Jackson and Teton Range areas are genetically differentiated from one another; 2) the Northern and Southern bands of bighorn sheep within the Teton Range represent distinct breeding groups with relatively rare genetic exchange between them; and 3) there is relatively weak genetic differentiation among the bands of bighorn sheep within the Jackson herd. The relatively high level of genetic differentiation between sheep occupying the Jackson and Teton Range areas is not surprising considering their separation by Jackson Hole and the Snake River, which are likely to be areas of poor habitat quality and probable barriers to movement (though this 'barrier'

may not be 'absolute'). Our results warrant considering the Jackson, Northern Teton, and Southern Teton groups of bighorn sheep as separate populations.

The level of genetic differentiation between the Northern and Southern bands is rather surprising, considering that they are situated so close to one another in a relatively contiguous area of apparently adequate habitat within the same mountain range. Observed movements of individuals, along with knowledge of landscape features in the Teton Range should be considered to identify putative barriers to movement. Alternatively, one very important consideration is that genetic differentiation between the Northern and Southern bands within the Teton Range could be caused (or reinforced) by behavior (e.g. philopatry, or a tendency to breed where one is born).

Though our study is limited in scope, to our knowledge it is the first assessment of fine-spatial-scale population genetic structure of bighorn sheep (although G. Luikart found similar fine-scale structure over slightly larger spatial scales (30-40 kilometers) across Glacier National Park; unpublished data). The relatively high level of genetic differentiation between the Northern and Southern bands of sheep within the Teton Range suggests that bighorn sheep may have very strong philopatry even over very short distances connected by high quality habitat. This suggests that the strong population structure observed by other studies over larger spatial scales may be driven by very strong philopatry in addition to the fragmented nature of bighorn sheep habitat. Other studies of fine-scale population structure of bighorn sheep should be done to determine whether strong genetic differentiation over short distances in the absence of apparent barriers to movement may be commonly observed in this species.

Estimates of genetic differentiation within the Jackson herd are indicative of weak substructure among the three bands of sheep in the area; however it is notable that even with the observed low level of genetic differentiation among the NER, Hoback and Gros Ventre bands, assignment tests appear to be useful for identifying the origin of individuals based on genotypes and allele frequency information. Though there is weak genetic differentiation between Jackson bands, they could conceivably be demographically independent from one another as relatively low levels of reproductive exchange among groups could genetically homogenize them.

Bottlenecks: There is strong evidence of a recent reduction in N_e in the Northern Teton population, and no evidence for recent bottlenecks in the Southern Teton or Jackson populations. Positive (significant) bottleneck tests suggest a population recently (e.g. < 2-15 generations ago) suffered a reduction in size and/or connectivity to other herds (e.g. Luikart and Cornuet 1998; Luikart et al. 1998). The lack of statistical evidence for bottlenecks in the Southern Teton and Jackson populations suggests that there have not been *recent, severe* bottlenecks in these populations. Severe bottlenecks in the more distant past or less severe recent bottlenecks are not expected to show the same genetic signature as a recent and severe reduction in N_e.

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Table 1. Samples with matching genotypes and their herd of origin.

Samples w/ Matching Genotypes	Herd
1, 127, 53	Jackson
2, 3	Jackson
15, 17	Jackson
16, 23	Jackson
48, 52	Jackson
49, 106	Jackson
56, 57, 633B	Jackson
60, 683	Teton
67, 87, 773	Teton
78, 893	Teton
86, 553	Teton
98, 99	Teton
107, 109	Jackson

Table 2. Summary of estimates of F_{ST} between each pair of bighorn sheep bands. Estimates within the box are those between bands in the Teton Range and in the Jackson area. Estimates outside the box are those within study areas. Notice that F_{ST} estimates that are for bands that are across Jackson Hole from one another are larger than those that are for pairs of bands that are within the same study area.

	South Teton	North Teton	Gros Ventre	NER
North Teton	0.12	-	-	-
Gros Ventre	0.19	0.15	-	-
NER	0.2	0.16	0.04	-
Hoback	0.22	0.17	0.05	0.08

Table 3. Summary of genetic diversity indices for the Jackson, Northern Teton, and Southern Teton bighorn sheep bands. N = number of individuals genotyped at the marker; N_a = the observed number of alleles; $N_{a(corr)}$ = the number of observed alleles corrected for differences in sample sizes among populations; H_o = observed heterozygosity; H_e = expected heterozygosity. $N_{a(corr)}$ values were estimated with the program HP-Rare (Kalinowski 2005) to be able to compare allelic diversity estimates among populations with different sample sizes.

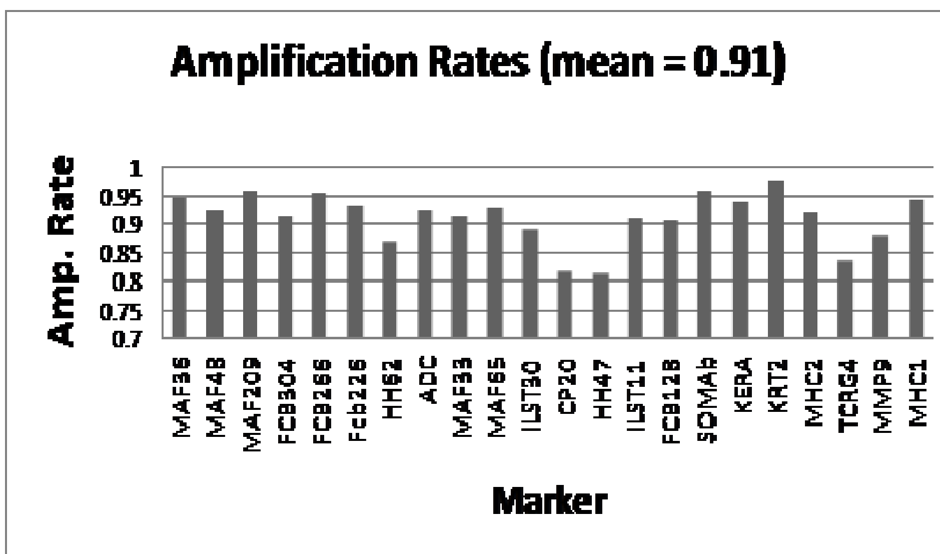
	Northern Teton					Southern Teton					Jackson				
Locus	**N**	**N_a**	**$N_{a(corr)}$**	**H_o**	**H_e**	**N**	**N_a**	**$N_{a(corr)}$**	**H_o**	**H_e**	**N**	**N_a**	**$N_{a(corr)}$**	**H_o**	**H_e**
MAF36	19	5	5	0.89	0.7	20	3	2.9	0.65	0.52	40	5	4.15	0.58	0.56
MAF48	19	4	4	0.79	0.69	20	5	4.9	0.45	0.48	40	4	3.96	0.78	0.6
MAF209	19	4	4	0.68	0.7	20	6	5.89	0.75	0.71	40	6	5.67	0.68	0.72
FCB304	19	4	4	0.68	0.67	20	4	4	0.85	0.66	37	3	2.47	0.49	0.5
FCB266	19	4	4	0.74	0.73	20	2	2	0.5	0.48	40	4	3.36	0.28	0.25
FCB226	19	3	2.95	0.16	0.15	20	2	2	0.3	0.32	40	2	2	0.38	0.33
HH62	19	4	4	0.68	0.62	20	6	5.88	0.75	0.73	40	7	6.51	0.85	0.77
ADC	19	3	2.95	0.37	0.37	19	2	1.95	0.05	0.05	39	2	2	0.36	0.48
MAF33	19	2	2	0.47	0.41	20	4	3.9	0.45	0.44	39	4	3.46	0.72	0.61
MAF65	19	3	3	0.63	0.59	20	3	3	0.45	0.6	39	6	5.54	0.59	0.66
ILST30	19	5	4.95	0.84	0.69	20	4	3.9	0.4	0.41	40	5	4.7	0.68	0.71
CP20	19	6	5.95	0.89	0.77	19	5	5	0.89	0.76	39	6	5.55	0.9	0.76
HH47	19	5	5	0.79	0.77	20	4	3.89	0.2	0.34	38	6	5.38	0.79	0.74
ILST11	18	3	3	0.61	0.63	19	6	5.89	0.74	0.71	40	9	7.29	0.8	0.8
FCB128	18	2	2	0.39	0.42	20	2	1.9	0.05	0.05	38	3	2.91	0.26	0.24
SOMAb	18	4	4	0.67	0.61	19	6	5.95	0.58	0.64	40	8	7.35	0.75	0.77
KERA	19	2	2	0.47	0.41	20	3	3	0.2	0.41	38	3	3	0.71	0.65
KRT2	19	3	2.95	0.26	0.31	19	2	2	0.68	0.5	40	3	2.44	0.2	0.18
MHC2	19	5	4.95	0.74	0.7	18	4	4	0.72	0.73	39	6	5.3	0.74	0.75
TCRG4	19	4	4	0.47	0.68	19	4	3.95	0.53	0.65	40	6	5.91	0.83	0.79
MMP9	18	5	4.95	0.5	0.51	18	3	3	0.5	0.5	40	7	6.86	0.78	0.81
MHC1	18	4	4	0.61	0.54	18	2	2	0.06	0.05	38	4	3.96	0.58	0.56
Mean:			**3.8**		**0.58**			**3.68**		**0.49**			**4.53**		**0.62**

Table 4. Summary of potential migrants and first generation off-spring of migrants identified by assignment test. Putative herd of origin is listed with assigned herd ranked from 1 to 3 based on the proportion of an individual's genotype assigned to each subpopulation.

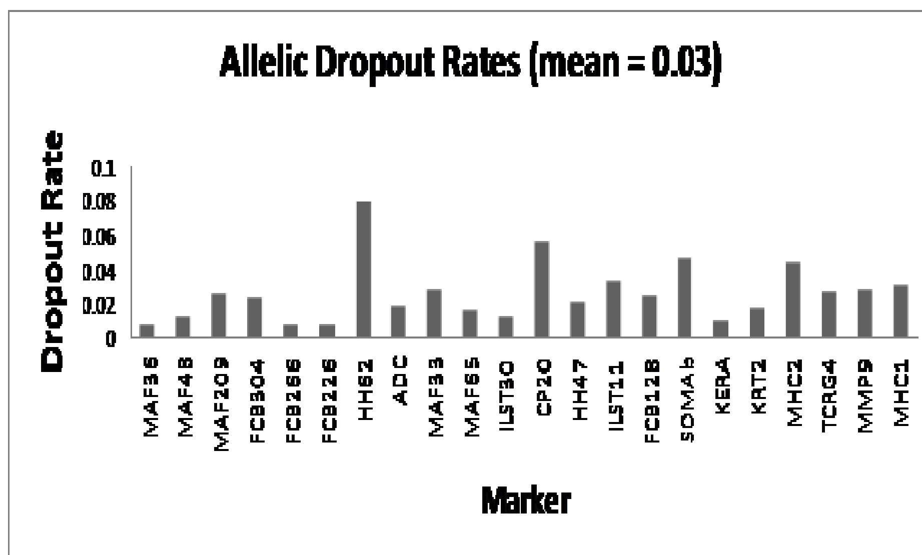
Sample #	Sex		Age Class	Herd of Sample Origin	Assigned Herd				
	Lab	Field			rank 1	score (%)	rank 2	score (%)	rank 3
43	M	M	Adult	Gros Ventre	NER	41.09	Gros Ventre	39.69	Hobac
132	M	M	Adult	Gros Ventre	NER	100	Hoback	0	Gros V
133		F	Adult	Gros Ventre	NER	85.47	Gros Ventre	14.52	Hobac
37		M	Adult	Gros Ventre	Gros Ventre	75.49	NER	24.51	Hobac
109		F	Adult	Hoback	NER	99.83	Hoback	0.12	Gros V
14	F	F	Adult	Hoback	Gros Ventre	82.85	NER	17.15	Hobac
25		F	Lamb	Hoback	Gros Ventre	99.48	NER	0.52	Hobac
126	F	F	Adult	NER	NER	73.61	Gros Ventre	26.39	Hobac
27	M	M	Adult	NER	Gros Ventre	98.98	Hoback	0.81	NER
112		F	Adult	NER	Gros Ventre	58.82	NER	41.01	Hobac
114	F	Unkn	Unkn	NER	Gros Ventre	90.34	NER	8.39	Hobac
13		F	Adult	NER	NER	80.44	Gros Ventre	19.56	Hobac
150	F	F	Adult	S Teton	N Teton	99.33	S Teton	0.67	Gros V

Figure 1. Summary of amplification success (a), allelic dropout (b), and false allele (c) rates in the Grand Teton bighorn sheep genotyping data set.

a)



b)



c)

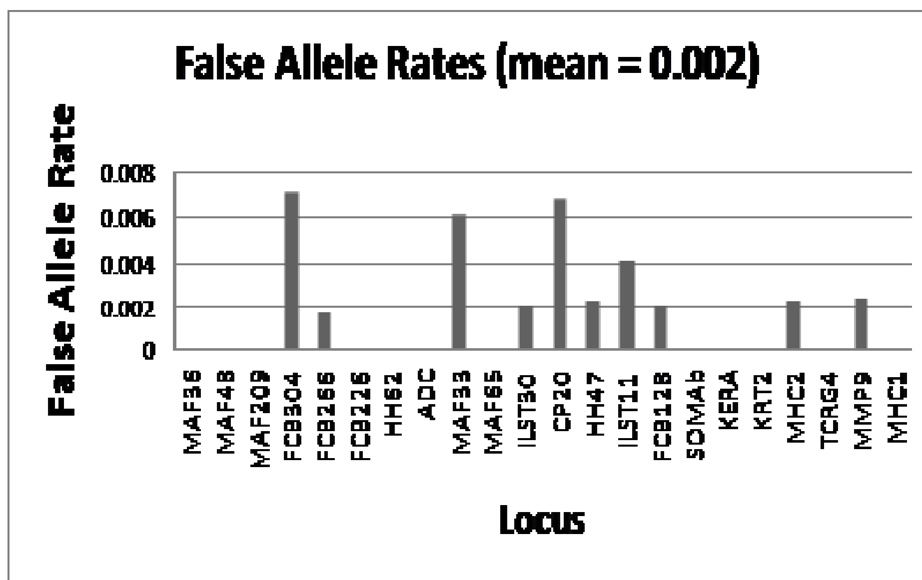


Figure 2. Principal coordinates analysis of Jackson and Teton bighorn sheep bands. Principal coordinate one separated out the Jackson and Teton groups, and principal coordinate two separated the Northern and Southern bands of the Teton Range.

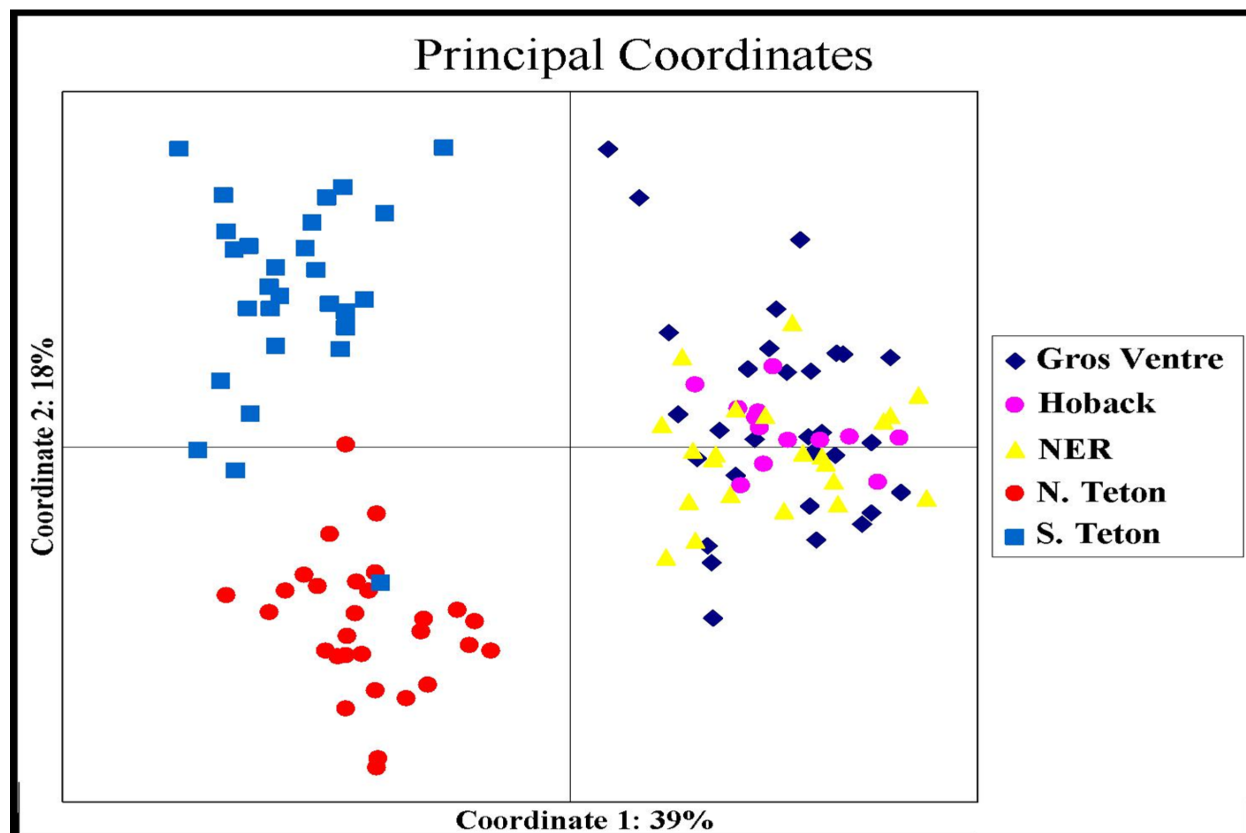


Figure 3. Results of GENECLASS assignment tests. Each vertical bar represents an individual. The subpopulation in which each individual was sampled is indicated at the bottom. The proportion of an individual's 'assigned population' bar is made up of color(s) indicating the proportion of the individual's genotype associated with each population. The population with the highest assignment score is always lowest on an individual's 'assigned population' bar.

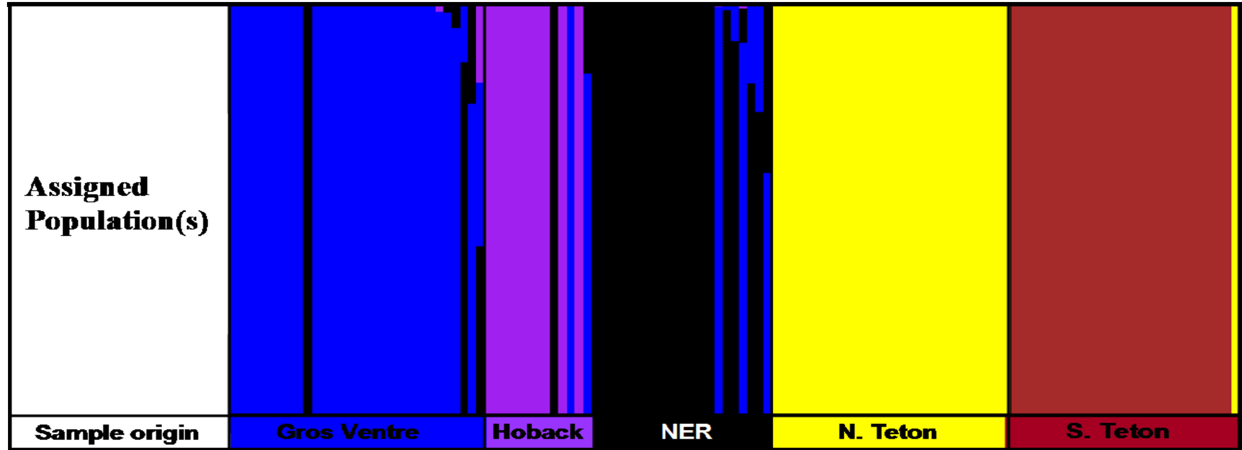


Figure 4. Summary of BOTTLENECK tests for recent reductions in effective population size. A range of plausible mutation models were considered (80-95% stepwise mutation model).

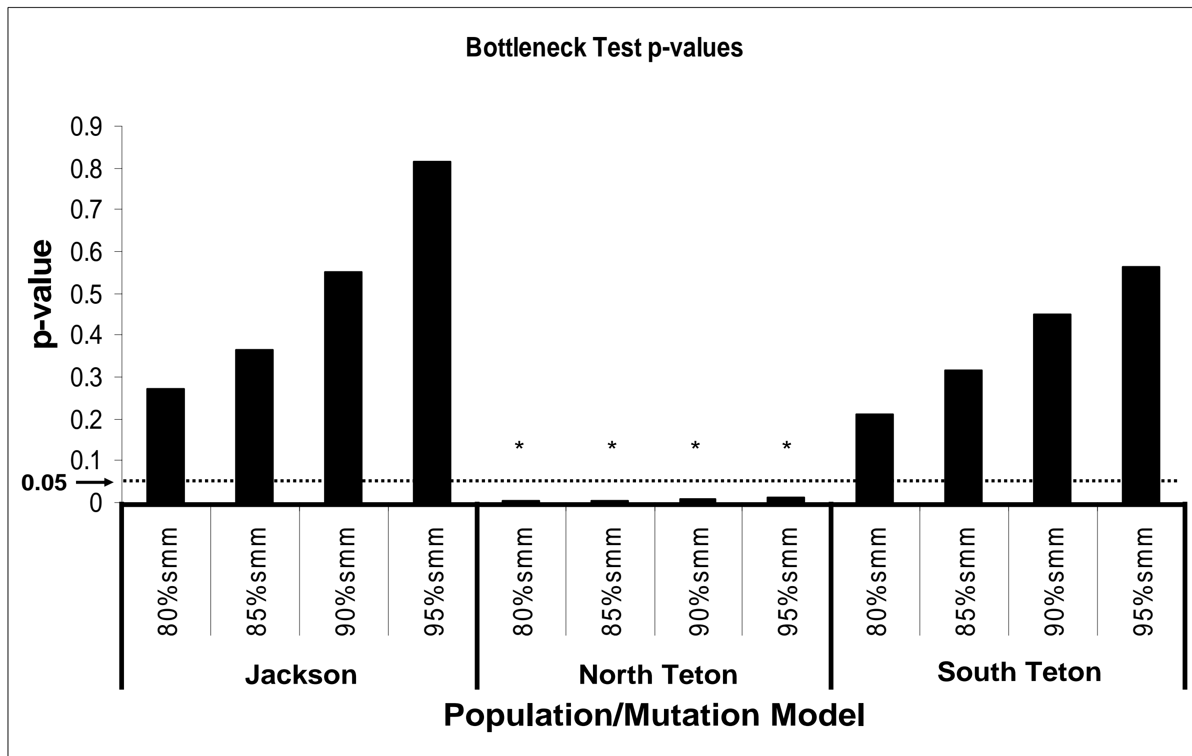


Figure 5. Histograms showing the frequency distributions of *Protostrongylus* lungworms from individual sheep from the Jackson, North Teton, and South Teton bighorn sheep populations.

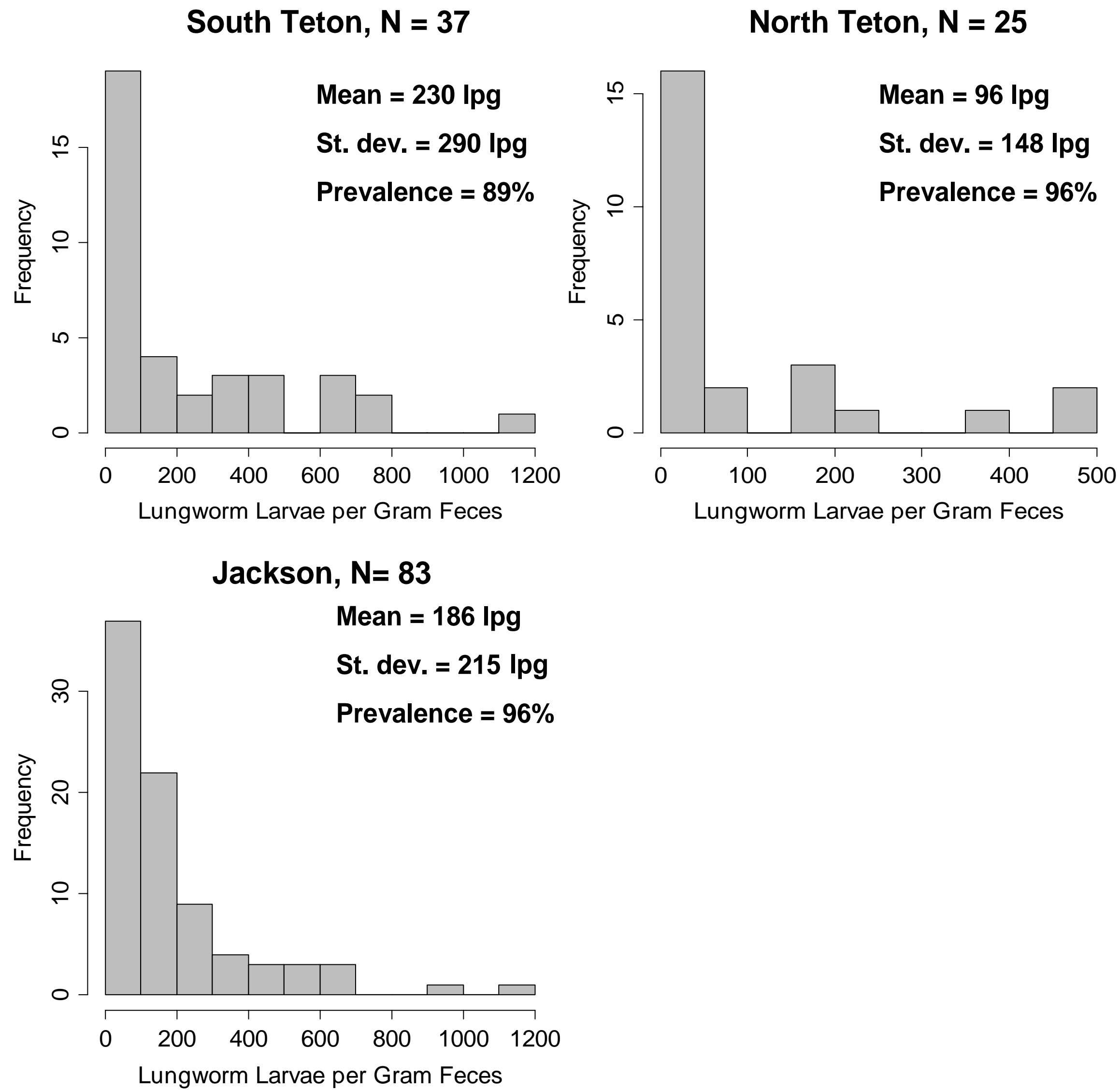
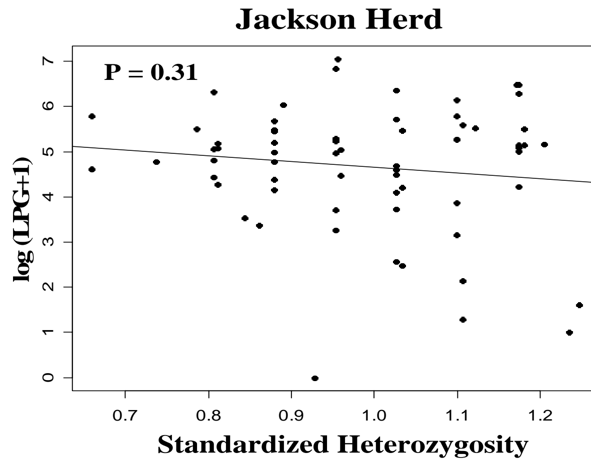
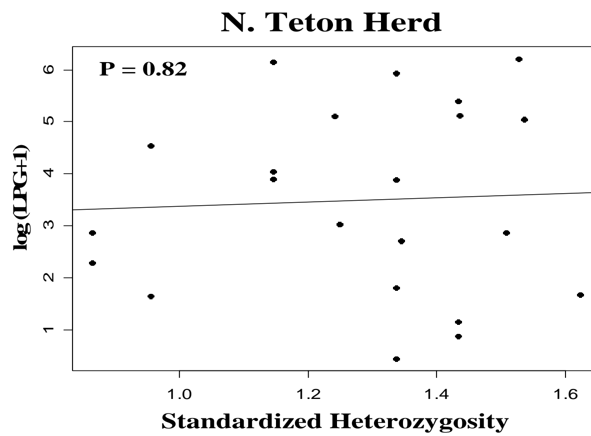


Figure 6. Summary of simple linear regression tests for correlations between fecal lungworm counts and heterozygosity.

a.



b.



c.