Predicting Disease Spread among Greater Yellowstone Elk using DNA Markers for Elk and their Parasites

Final Report, RM-CESU Task Agreement J1242077002

January 12, 2009

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Executive summary: There is a growing need to understand connectivity and movement patterns among elk herds and their parasites across the Greater Yellowstone Ecosystem (GYE). We developed microsatellite and mitochondrial (mt) deoxyribonucleic acid (DNA) makers to assess connectivity among elk from nine study areas. We sequenced 695 base pairs (bp) of mtDNA control region from 407 elk. Statistical analyses revealed moderately-high mtDNA differentiation (F_{ST} = 0.17), suggesting limited female-mediated gene flow ($Nm \cong 3$ migrants per generation) among study areas. We also genotyped 11 nuclear DNA microsatellites from 80 elk on four study areas. Statistical analyses revealed relatively low genetic differentiation (F_{ST} = 0.02), suggesting moderately high gene flow ($Nm \cong 50$ migrants per generation). The nearly 10-fold higher mtDNA differentiation suggests relatively limited female-mediated gene flow, possibly due to female philopatry. One microsatellite locus in a disease-related gene (IFNG) had relatively high F_{ST} (F_{ST} = 0.07), suggesting a possible adaptive function of IFNG. Five additional microsatellites were optimized, including three loci in genes with disease-related functions. All 16 microsatellite, mtDNA, and base line data represent important tools for long term, noninvasive monitoring of elk population connectivity across the GYE.

To study helminth parasites, we noninvasively sampled 554 fecal deposits during 2007 and 2008 from elk, bison, bighorn sheep, pronghorn, and cattle from across the GYE. We developed an internal transcribed spacer region 2 (ITS-2) nuclear gene sequencing assay for parasite species identification, and a cytochrome oxidase-1 (CO1) mitochondrial assay for assessing parasite gene flow. We produced ITS-2 polymerase chain reaction (PCR) products from 345 individual nematode parasites of ungulate hosts, and identified 223 (88%) to species. *Dictyocaulus viviparous* lungworms were at moderate prevalence (10-50%) in elk and bison. The most prevalent gastrointestinal (GI) parasite in elk was *Spiculopteragia spp.*, with ~5-10% prevalence. Bison in the GYE appear to commonly be infected with both *Cooperia oncophora* and *Ostertagia ostertagi*. Bighorn sheep are commonly infected with *Protostrongylus* lungworms and *Marshallagia* GI worms, which were not shared with other wild ungulates in the GYE. These DNA markers and preliminary data provide valuable tools for noninvasive monitoring of parasite transmission across the GYE.

To study *Brucella abortus*, the bacterium causing brucellosis, we genotyped 54 *B. abortus* isolates from bison, elk, and cattle using 10 microsatellite loci. Statistical analyses suggested that cattle isolates were nearly identical to elk isolates. This finding suggests elk were the source of two recent cattle outbreaks in Wyoming and Idaho. Finally, we cultured for *Yersinia enterocolitica* from over 150 bison fecal samples. We detected no strains that could cross-react with *Brucella* serology tests, suggesting that *Brucella*-seropositive bison were not "false positives" caused by *Yersinia*. The low *Yersinia* prevalence also suggests bison are not a likely source of *Yersinia* infections recently detected in GYE elk.

Introduction

The Greater Yellowstone Ecosystem (GYE) encompasses portions of Idaho, Montana, and Wyoming and supports world-renowned herds of elk that provide important benefits to the ecosystem, hunters, and local economies through guiding and tourism. However, these herds also harbor and transmit diseases such as brucellosis, sarcoptic mange (scabies), septicemic pasteurellosis, and perhaps yersiniosis (Thorne et al. 2002, Anderson et al. 2006). They also likely will soon be infected with chronic wasting disease, a fatal disease with no vaccine (Gross and Miller 2001). A critical need for minimizing the potential adverse conservation, economic, and social effects of these diseases is information on disease prevalence and transmission pathways, along with host population connectivity across the GYE.

We addressed this need by identifying deoxyribonucleic acid (DNA) markers from elk and their parasites to assess host and parasite population connectivity. The use of DNA markers from non-invasively collected feces facilitates sampling large numbers of elk and parasites from many locations. This sampling allowed us to estimate rates of gene flow and migration among elk populations, and to assess parasite prevalence and transmission across the GYE. This information can be used by natural resource managers to track the origin and spread of diseases, and predict the risks and geographic routes of transmission.

Herein, we describe mitochondrial (mt) DNA and microsatellite markers developed for elk and report on a pilot study to develop DNA markers to identify and track parasites of elk including several helminthes (macroparasites). Also, we describe results from the microsatellite genotyping of the bacteria *Brucella abortus,* and culture of the bacteria *Yersinia entercolitica*.

Elk Genetics

The specific objectives of this elk genetics project were to: 1) identify microsatellite DNA markers that are highly polymorphic in elk, including several markers in genes with important disease resistance functions; 2) identify a maternally-inherited mtDNA marker useful for assessing female-mediated elk gene flow and population connectivity; 3) optimize the analysis of microsatellite and mtDNA markers from fecal samples for noninvasive sampling; and 4) estimate sex-specific genetic population structure and gene flow among elk populations in the GYE.

Sampling Elk Tissue and Feces: During 2006-2008, we sampled an average number of 45 elk from each of nine study areas (i.e., populations) for DNA analysis (Table 1). We collected blood or tissue samples from radio-collared female elk in the Northern Range (NR) and Madison-Firehole (MF) areas of Yellowstone National Park, Paradise Valley (PV) of southern Montana, and Shoshone River (SR) area of Wyoming. We also obtained blood and tissue samples from hunter-killed elk in the Madison Valley (MV), PV, and Pioneer Mountains (PM) of southern Montana. In addition, we collected fecal pellets within a few hours of defecation from the Sand Creek (SC) area in Idaho and the Bench Corral (BC) and Muddy Creek (MC) areas of Wyoming. We used these samples in a mtDNA study of female genetic population structure. We also used a subset of the samples for a microsatellite DNA study (described below).

MtDNA Analyses: MtDNA is useful for assessing population structure and female gene flow because it is maternally inherited and has a high rate of mutation and random genetic drift. Understanding female movement and gene flow is important because females strongly influence

population processes such as colonization rates, demographic rescue, and the spread of diseases like brucellosis that are transmitted mainly or only by females (Thorne et al. 1979). MtDNA is also relatively easy to recover from fecal samples because of its high copy number in cells compared to nuclear DNA (e.g., microsatellite DNA).

Sequencing of mtDNA was completed for 695 base pairs (bp) from 407 elk in the nine study areas across the GYE (Table 1). Sequences were first analyzed for 16 animals at the Montana Conservation Genetics Laboratory at the University of Montana to confirm polymorphism was high, and then at the High Throughput Genomics Unit at the University of Washington (http://www.htseq.org/index.html). MtDNA sequences were corrected and aligned using DNAstar software. Diversity indices, genetic distances, and Mantel tests were computed using Arlequin (Schneider et al. 2000). Geographic distances between sampling areas were computed using ArcView software. Networks were computed using the median spanning algorithm (Bandelt et al. 1999), as implemented in Network 4.05 software (http://www.fluxus-engineering.com/contact.htm).

Data analyses of mtDNA revealed high diversity including 30 total haplotypes. There were an average of 12 haplotypes per sampling area, and mean haplotype diversity of 0.84 within study areas (Table 1). There was no phylogeographic structure as exemplified by the finding of phylogenetically divergent haplotypes in the same elk study area (Fig. 1). This was not surprising because development of phylogeographic structure requires substantial isolation of elk study areas for hundreds of generations to allow for divergent mtDNA lineages to evolve in separate geographic regions by accumulation of new mutations. This lack of phylogeographic structure suggests that gene flow has occurred across the GYE over the past hundreds of generations, either in a stepping-stone pattern of movement of females between adjacent study areas or perhaps by occasional long distance movements of females.

There was relatively high frequency differentiation among mtDNA haplotypes from the nine elk study areas as exemplified by the fairly high F_{ST}'s between study areas (0.10-0.25). The mtDNA F_{ST} is approximately 10 times higher than the microsatellite (0.02-0.03) across the GYE (see below; Hicks et al. 2006). This mtDNA F_{ST} was highly significant between all study areas ($P < 0.001$; Table 2). The relatively high F_{ST} suggests substantial genetic drift within study areas due to small female effective population size and low rates of female migration between study areas ($Nm_f \cong 3$ female migrants per generation assuming an island model of dispersal). However this migration rate is only a rough approximation because the island model used to infer Nm from F_{ST} requires numerous assumptions (e.g. mutation-migration equilibrium, symmetrical movement of females between populations) that are unlikely to hold in natural populations (Mills and Allendorf 1996).

It is unlikely that unrepresentative sampling could contribute to the high F_{ST} values because F_{ST}'s were consistent regardless of sampling strategy (e.g., hunter kill versus fecal pellet sampling) or year of sampling. No significant ($F_{ST} \sim 0$; $P > 0.05$) differences were detected between years in study areas with repeat samples between years such as Madison Valley and Muddy Creek.

There was no correlation between geographic distance and haplotype frequency differentiation between study areas (Mantel test, $P > 1.0$). The lack of correlation could result from female gene flow not corresponding to the linear distance between herds. For example, perhaps female elk tend to move more freely across continuous mountain or forest areas rather than across wide open valleys (e.g., between BC and MC, Fig. 2). These results are interesting from a disease perspective because if female migration rates are low, then it suggests

transmission rates of female-mediated diseases such as brucellosis are relatively low, at least over long distances. This finding is consistent with recent modeling results (Cross et al. 2009).

Microsatellite Analyses: We initially analyzed DNA from 20 individuals for each of four study areas (i.e., MC, NR, PV, PM) to identify 11 variable microsatellite DNA markers that co-polymerase chain reaction (PCR) amplify (multiplex) for analysis on an automated DNA sequencing machine. The 11 microsatellites PCR-amplified well on DNA from feces, blood, and tissue. Three of the microsatellite loci were in genes related to disease resistance (resistance to infectious bacteria such as *Brucella, Sallmonella, Mycobacterium*, and nematodes) in bovids and cervids, which could help detect fitness effects of disease on elk from geographic locations with high disease prevalence. Also, such loci often have a relatively high F_{ST}, which makes them useful for detecting immigrants using assignment tests (Cornuet et al. 1999).

All 11 microsatellites were moderately or highly variable (heterozygosity = 0.40-0.70; allelic richness = 3-10 alleles per locus) and, therefore, useful for estimates of gene flow and individual movement among populations across the GYE. The degree of genetic differentiation between populations (F_{ST} = 0.01-0.03; Table 4) is low, but sufficient to allow identification of inter-population migrants and estimation of migration rates (N_m) using direct estimates of current dispersal if approximately 20 highly polymorphic markers are analyzed (e.g., Cornuet et al. 1999, Wilson and Rannala 2003). One locus in a disease-related gene (IFNG) had relatively high F_{ST} between Muddy Creek feedgrounds in Wyoming and the Paradise Valley area in Montana (Figure 3; locus 9). The F_{ST} was nearly significantly higher than expected under neutrality using F_{ST}-outlier tests (Antao et al. 2008). This locus is known to have alleles associated with parasite susceptibility in ungulates (Coltman et al. 2001) and could be under selection. Thus, the high F_{ST} makes this locus especially useful for identifying migrants between study areas in Wyoming (e.g., Muddy Creek) and Montana.

This pilot study demonstrated we can reliably analyze DNA from elk feces, and that 11 microsatellite DNA markers are moderate-to-highly polymorphic and useful for quantifying rates of gene flow and movement between areas. However, we must analyze more individuals (approximately 40 per study area) and more loci to achieve precise estimates of elk gene flow. Therefore, we developed more microsatellites and started genotyping more individuals.

We developed and optimized five additional microsatellites (MMP9, IGF1, BL42, BM4107, and BM203) to be co-PCR amplified and genotyped reliably on fecal or tissue DNA. Three of the loci are in genes with a disease-related function (i.e., matrix metalloproteinase 9 gene, insulin-like growth factor, and interferon gamma). We genotyped the five loci on ~200 elk samples from seven study areas. This brought the total number of elk genotyped to approximately 40 elk in several populations (e.g., MV, PV, and MC). However, the total number of elk genotyped is still less than 30 in other populations (e.g., SC, SR, BC, and NR). Thus, we are still working toward finishing analyses and will publish the microsatellite data after obtaining additional genotypes.

Macroparasite Detectability and DNA Markers

Numerous parasite species can be isolated from fecal samples (e.g., Ezenwa 2003), which facilitates noninvasive sampling and studies of parasite transmission. The objectives of this pilot study were to: 1) identify parasites that can be sampled noninvasively from elk feces and that have high enough prevalence (>10%-20% in elk) that they can be used to assess elk movement

and parasite transmission across the GYE; 2) identify DNA markers that are highly polymorphic in parasites; and 3) infer patterns of gene flow and transmission of the parasites among elk populations and between elk and other ungulate species (e.g., bison, mule deer) in the GYE.

Herein, we report results from work on several helminth species (i.e., macroparasites) and two bacteria (i.e., microparasites; *Yesinia enterocolitica* and *Brucella abortus*). We included host species other than elk to better understand transmission among host species. Parasite species or strains that are specific to elk could be useful for understanding elk movement. Also, parasites specific to elk should help us map and predict geographic areas of disease transmission between elk populations. Parasites with transmission between elk and other host species will be useful eventually for predicting and mapping locations of inter-specific disease transmission. For example, as described in detail below, we detected a lungworm (*Dictyocaulus viviparous*) prevalence of approximately 10-55% in elk and bison feces collected in and near Grand Teton and Yellowstone National Parks.

Helminth Parasite Sampling: Samples from 554 fecal deposits were collected noninvasively during 2007 and 2008 from elk, bison, bighorn sheep, pronghorn, and cattle from several locations across the GYE (Tables 5 and 6). All samples were collected within 30-60 minutes after observing defecation from approximately 50 to 300 meters away. This sampling protocol prevented any disturbance of the animals but, in many cases, allowed for the identification of the sex and age class of the individuals voiding the feces.

We isolated individual gastrointestinal and lungworm parasites using standard veterinary techniques (e.g., a modified Baermann beaker technique; Foreyt 2001). To recover gastrointestinal helminths, we cultured fecal samples for 10 days before using the Baermann technique to allow eggs to hatch into first stage larvae that can readily be isolated. To date, we have isolated approximately 2500 individual nematode parasites and prepared them for DNA extraction. The host species-specific proportions of fecal samples infected with particular classes of parasites for each sampling location are shown in Tables 5 and 6. Note that *Dictyocaulus viviparous* is the only parasite we encountered that is easily identified to species by examination of morphological characteristics with the aid of microscopy and without DNA sequence analysis.

Dictyocaulus Lungworm Infection in Elk and Bison: *Dictyocaulus viviparous* is a common, environmentally-transmitted lungworm parasite of ungulates that has a simple (single host) lifecycle and is common in cervids and especially bovids. First-stage larvae are passed out in the feces of the host and then mature to the third, infective stage in the environment, at which point they infect new hosts when they are consumed during grazing.

First stage *D. viviparous* lungworms are readily identified to species by observation of distinctive morphological characteristics. We have identified and isolated *D. viviparous* in elk and bison feces from Grand Teton and Yellowstone National Parks. *D. viviparous* was isolated from 10 of 46 (22%) fecal samples collected from elk, and 7 of 25 (28%) fecal samples collected from bison, in Grand Teton National Park during summer 2007. *D. viviparous* was isolated from 22 of 39 (56%) fecal samples collected from elk, and 3 of 29 (10%) fecal samples collected from bison, in Yellowstone National Park during summer 2007.

We successfully amplified cytochrome oxidase-1 (CO1), a mitochondrial genetic marker that is commonly used in parasite population genetics studies, from *D. viviparous*. Sequence data from CO1 and other mitochondrial markers such as subunit 4 of NADH dehydrogenase (NAD4)

and the control region can be used to infer information about intra- and inter-species patterns of transmission via estimates of genetic differentiation (F_{ST}) and gene flow (N_m) among host species and geographic areas across the GYE.

DNA Sequencing to Identify Gastrointestinal Parasite Species: Gastrointestinal parasite species were identified via comparison of gene sequences of the ribosomal second internal transcribed spacer (ITS-2, a nuclear gene) from individual parasites with those catalogued in GENBANK (http://www.ncbi.nlm.nih.gov/sites/entrez?db=nucleotide; National Center for Biotechnology Information). ITS-2 sequences have been widely used to reliably identify nematode parasites to species (Gasser 2001). DNA sequencing was done by the High Throughput Genomics Unit at the University of Washington (http://www.htseq.org/index.html).

We currently have ITS-2 PCR products from 345 individual nematodes representing parasites of ungulate hosts across the GYE. We identified 223 (88%) of these parasites to species with ITS-2 sequence analysis. The other 12% of sequences were not of high enough quality to provide reliable species identification.

The highest prevalences of gastrointestinal parasites among GYE ungulates were *Cooperia oncophora* and *Ostergtagia ostertagia* in bison, *Spiculopteragia* spp. in elk, and *Marshallagia* spp. in bighorn sheep. For each host species, we provided details on helminth parasites identified in and near Grand Teton National Park, Yellowstone National Park, and northern Wyoming (Figs. 4-6). The number of individuals of each host species from each sampling location with successfully identified parasites is shown in Table 7.

It should be noted that species identification of parasites in the genera *Marshallagia* and *Spiculopteragia* was ambiguous. The two candidate *Marshallagia* species are *M. occidentalis* and *M. marshalli*. The two candidate *Spiculopteragia* species are *S. asymmetrica* and *S. quadrispiculata*. The ambiguity in species identification appears to be the result of highly similar ITS-2 sequences in multiple species within these two genera. Alternatively, this ambiguity could result from spurious species designations in the literature that could result from un-described plasticity in morphological characteristics within parasite species. We will analyze sequences of other genes that are likely to be species-diagnostic (e.g. cytochrome oxidase-1) to clarify species identification within the *Marshallagia* and *Spiculopteragia* genera.

Bighorn Sheep Gastrointestinal Helminth Parasites: Bighorn sheep in and near Grand Teton National Park are commonly infected with *Protostrongylus* lungworms and *Marshallagia* gastrointestinal worms. These parasites do not appear to be shared with other wild ungulates in the GYE, though they may be shared with domestic sheep in the ecosystem. Genetic analyses of these parasites could potentially be used to infer relative rates of parasite transmission and bighorn movement among populations, but comprehensive sampling for these parasites among domestic sheep will be necessary to determine whether domestics may be a strong determinant of parasite gene flow and movement across the ecosystem. Alternatively, if these parasites are commonly found in domestic sheep, it would provide an opportunity to study the rates and routes of parasite transmission between domestic and bighorn sheep.

We have not yet optimized methods to identify parasites in the *Marshallagia* and *Protostongylus* genera to species. As mentioned above, *M. occidentalis* and *M. marshalli* appear to have identical ITS-2 sequences. We will explore the use of alternative species-diagnostic genetic markers (e.g., CO1) to identify parasites in this genus to species. The rate of success in

ITS-2 PCR amplification in *Protostrongylus* is low (~10%), which may be due to low complimentarity of the universal ITS-2 primers in *Protostrongylus* or inherently low quantities of DNA in *Protostrongylus* larvae, which are quite small compared to other nematode parasites we have amplified ITS-2 from. Additionally, there are only ITS-2 sequences in GENBANK for two out of as many as eight *Protostrongylus* species that could potentially be found in bighorn sheep. To improve our ability to identify *Protostrongylus* parasites to species, we will test primers for other genetic markers that are likely to be species-diagnostic (e.g., CO1), and modify our DNA extraction technique (e.g., longer incubation times and larger amounts of protein digesting enzymes) to improve DNA recovery from individual larvae.

Cattle and Pronghorn Gastrointestinal Helminth Parasites: Because of difficulties we had early in this study with parasite storage and DNA extraction, we were not able to obtain high quality DNA from some of the parasites of cattle and pronghorn that were collected. Therefore, we have not been able to identify many of them to species. Since experiencing these problems, we have improved our parasite storage and DNA extraction techniques to increase our PCR amplification success rate and obtain high quality sequence data from parasites of pronghorn and cattle.

Microparasites

We initiated studies on *Brucella* and *Yersinia* bacteria from Yellowstone ungulates. Herein, we report on microsatellite DNA genotyping and PCR diagnostics results from *Brucella,* and on culture results from *Yesinia*. For culture of *Brucella*, we obtained cultures and DNA from 7 of 27 bison consigned to slaughter facilities during the winter of 2007-08.

Brucella DNA Markers: In collaboration with the National Animal Disease Center (Dr. B. Bricker), we genotyped 10 microsatellites loci on over 50 *Brucella* isolates from elk and bison from Idaho, Montana, and Wyoming. Elk and cattle *Brucella* were highly similar, but both were quite divergent from bison *Brucella* (Fig. 7). This finding suggests that recent cattle outbreaks in Wyoming and Idaho originated from elk, not bison.

We have subsequently sequenced the genomes of two *Brucella* isolates from the GYE (one from cattle and one from bison) and are developing SNP (single nucleotide polymorphism) markers to study *Brucella* transmission among elk, bison, and cattle. We are collaborating with the National Animal Disease Center and National Veterinary Laboratory Service in Ames, Iowa to genotype more isolates with approximately 20 microsatellites and 20 SNPs. The National Veterinary Laboratory Service is culturing new *Brucella* isolates from bison and elk tissues recently obtained from hunter-killed animals.

Yersinia culture and QPCR: Wade See, an undergraduate student at the University of Montana, tested if *Yersinia enterocolitica* exists in bison sampled from Yellowstone and Grand Teton National Parks. He used cultures provided by Hank Edwards from the Wildlife Disease Laboratory operated by the Wyoming Game and Fish Department, and PCR at University of Montana to test for *Yersinia*.

Yersinia enterocolitica serotype O:9 has identical O-antigens to those of *Brucella abortus* and has caused false positive brucellosis tests, particularly in elk in Montana. We investigated whether a similar phenomenon was occurring in Yellowstone bison that were tested for

brucellosis and consigned to slaughter after they tested seropositive for *Brucella* antibodies. Of the approximately 50 seropositive bison consigned to slaughter, no fecal cultures grew *Yersinia enterocolitica* serotype O:9. Furthermore, only two of the 100 additional bison fecal samples from Yellowstone and Grand Teton National Parks yielded cultures of *Yersinia enterocolitica* and the cultured strain was not serotype O:9 and did not cross react with the brucellosis tests. Controlled experiments by Hank Edwards of the Wyoming Game and Fish Department have demonstrated that *Yersinia* can be reliably cultured from frozen feces. Thus, we conclude that *Yersinia enterocolitica* O:9 cross reactivity with *Brucella* antigen tests are unlikely to have caused false positive tests in Yellowstone bison.

Future Research

Our longer-term research goals are to map elk and parasite population connectivity patterns across the GYE by combining genetic data from elk and their parasites, with movement data (e.g., telemetry locations) from elk. This will facilitate testing of hypotheses about how environmental variables and landscape features influence elk movements and parasite transmission. This research will help biologists manage infectious diseases, advance our understanding of parasite and disease ecology, and predict the affects of climate change and land use change on elk populations and their diseases.

One objective of this research was to obtain preliminary data for use in obtaining larger grants. We have submitted a grant to the National Science Foundation that would allow completion of genotyping of microsatellites on elk, as well as genotyping of many *Brucella* isolates to help understand elk movement and brucellosis transmission across the GYE. We are actively seeking additional funding and collaborations to continue and extend this research.

Acknowledgments

This study was funded by the National Park Service, University of Wyoming-National Park Service Research Center, Montana Integrated Learning Experience for Students fellowship program of the Howard Hughes Foundation through the University of Montana, and a Christine Stevens Award through the Animal Welfare Institute. We thank Kathy Tonnessen NPS and Rocky Mountains Cooperative Ecosystem Studies Unit (RM-CESU) in Missoula, Montana, Celeste Havener from the University of Wyoming, Laramie, and Lisa Gerloff, RM-CESU and University of Montana, Missoula for facilitating cooperative funding agreements. DNA analyses were facilitated by Jay Chen, Raquel Godihno, Albano Beja-Pereira, and especially Kristy Pilgrim and Mike Schwartz. Claudia Almendra assisted with bison tissue sampling for *Brucella* genotyping. Dan Bingham assisted with helminth analyses and figure production. We also thank the following individuals for assisting with data collection, analyses, and/or administration of the project: Neil Anderson, Steve Cain, Julie Cunningham, Troy Davis, Sara Dewey, Hank Edwards, Robert Garrott, Sara Gregory, Ken Hamlin, Matthew Kauffman, Doug McWhirter, Jarod Rodgerson, Karen Rudolf, Josh Rydalch, Brandon Scurlock, John Stevenson, Rick Wallen, and Pete Zager.

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Table 1. Study areas, sample sizes, and mtDNA diversity for nine elk populations in the Greater Yellowstone Ecosystem.

Study area (letter code)	Individuals sampled	Number of Haplotypes	Haplotpe diversity (SE)	Nucleotide diversity* (SE)
1. Paradise Valley, Montana (PV)	61	16	0.89 (0.02)	0.0058 (0.003)
2. Madison Valley, Montana (MV)	80	17	0.85 (0.03)	0.0055 (0.003)
3. Pioneer Mountains Montana (PM)	27	6	0.76 (0.05)	0.0049 (0.003)
4. Northern Range, Yellowstone (NR)	44	15	0.85 (0.03)	0.0049 (0.003)
5. Madison-Firehole, Yellowstone (MF)	42	12	0.85 (0.04)	0.0049 (0.003)
6. Shoshone River, Wyoming (SR)	59	15	0.84 (0.03)	0.0056 (0.003)
7. Muddy Creek, Wyoming (MC)	62	12	0.75 (0.05)	0.0035 (0.002)
8. Bench Corral, Wyoming (BC)	13	7	0.87 (0.07)	0.0045 (0.003)
9. Sand Creek, Idaho (SC)	19	10	0.91 (0.04)	0.0059 (0.003)
Mean	**~45**	**12**	**0.84**	**0.0051**
Total	**407**	**30**		

*Proportion of nucleotide sites expected to be different between two randomly sampled elk

Table 2. Pairwise F_{ST} values between study areas computed from haplotype frequencies. See Table 1 for study area acronyms, and Figure 2 for geographic locations of study areas. The highest F_{ST} values are depicted in bold font.

	PV	MV	PM	NR	MF	SR	MC	BC
MV	0.131							
PM	0.167	0.187						
NR	0.139	0.159	0.199					
MF	0.125	0.146	0.185	0.155				
SR	0.137	0.157	0.196	0.167	0.153			
MC	0.182	0.201	**0.245**	**0.213**	0.200	0.209		
BC	0.118	0.141	0.186	0.151	0.135	0.149	0.202	
SC	0.103	0.125	0.167	0.134	0.119	0.133	0.184	0.110

Table 3. Microsatellite expected heterozygosity, mean allelic richness, and probability of identity using 11 loci in four study areas. Probability of identity is the probability of randomly sampling two individuals with identical multi-locus genotypes.

Study area	*H*e	Alleles/locus	Probability of identity
Muddy Creek	0.62	3.6	< 1/100,000
Northern Range	0.59	3.6	< 1/100,000
Paradise Valley	0.60	3.8	< 1/100,000
Pioneer Mountains	0.56	3.6	< 1/100,000

Table 4. F_{ST} between pairs of sampling areas.

Area A	Area B	F_{ST}
Pioneer	NR Yellowstone	0.021
Muddy Creek	NR Yellowstone	0.017
Muddy Creek	Pioneer	0.019
Paradise Val.	NR Yellowstone	0.014
Paradise Val.	Pioneer	0.029
Paradise Val.	Muddy Creek	0.020

Table 5. Summary of 2007 host species-specific sample sizes, sampling locations, and prevalence (proportion of individuals infected) with each class of parasite.

Location	Host species	Number of fecal samples collected	*Dictyocaulus viviparous* lungworm	Other lungworm species**	Gastrointestinal nematodes
Grand Teton National Park, WY	elk	46	0.22	0	0.1*
	bison	32	0.28	0.08	0.44
	bighorn sheep (Teton Ridge)	19	0.05	1 (*Protostrongylus*)	1
	pronghorn	2	0	0	0
	cattle (Pinto Ranch)	35	0	0.03	0.15
Yellowstone National Park	elk	39	0.56	0	0.75
	pronghorn	15	0	0.87***	0.8
	bison	41	0.1*	0.13	0.76
Northern Wyoming (outside national parks)	cattle	22	0	0.09	0.53
	pronghorn	13	0	0.92	0.91
Bench/Jewett Feedgrounds in Wyoming	elk	20	0.58	NA	0.08

* These samples were rather old when analyzed, so the prevalence estimates are likely biased low.
** Significant differences in morphology were used to distinguish between *D. viviparous* and other lungworm species. The morphological characteristics of *Protostrongylus* lungworms are distinctive enough to identify them reliably to the genus level, but not to species.
*** Species could not be identified by morphological characters and therefore will require ITS-2 sequencing.
Note: "NA" means that analyses were not done and data are not available.

Table 6. Summary of 2008 host species-specific sample sizes, sampling locations, and parasite infection prevalence.

Location	Host species	Number of fecal samples collected	Lungworms*	Gastrointestinal nematodes
Grand Teton National Park	elk	60	NA	0.13
	bison	35	NA	0.37
	bighorn sheep (Jackson Herd – near Grand Teton NP)	52	1 (*Protostrongylus*)	0.83
	bighorn sheep (Teton Herd)	30	0.83 (*Protostrongylus*)	NA
	pronghorn	6	NA	0
Yellowstone National Park	cattle (Paradise Valley)	40	NA	0.05
	bison	50	NA	0.62
Sand Creek Wildlife Management Area, ID	elk	24	NA	0

Table 7. Number of host species by location with successfully sequenced (ITS-2) gastrointestinal parasites.

Location	Host species	# of hosts with successfully sequenced worms
Grand Teton National Park	bison	11
	elk	9
	moose	1
	bighorn sheep	7
Yellowstone National Park	elk	9
	pronghorn	1
	bison	35
Wyoming (outside national parks)	pronghorn	2
	cattle	4

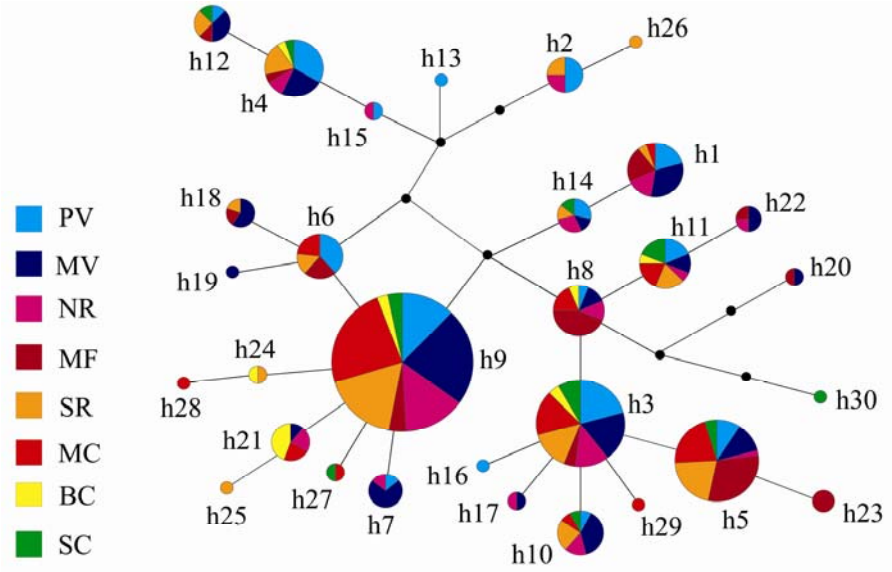

Figure 1. MtDNA haplotype network. The size of each circle is proportional to the frequency of the haplotype. Colors in circles represent one of eight locations from which the haplotype was sampled. The Pioneer Mountains study area was not included because it is a reintroduced population outside of the Greater Yellowstone Ecosystem in western Montana. Note that divergent haplotypes (e.g., h7, h18, h22, h12) occur within the same study area (e.g., Madison Valley (MV) shown in dark blue).

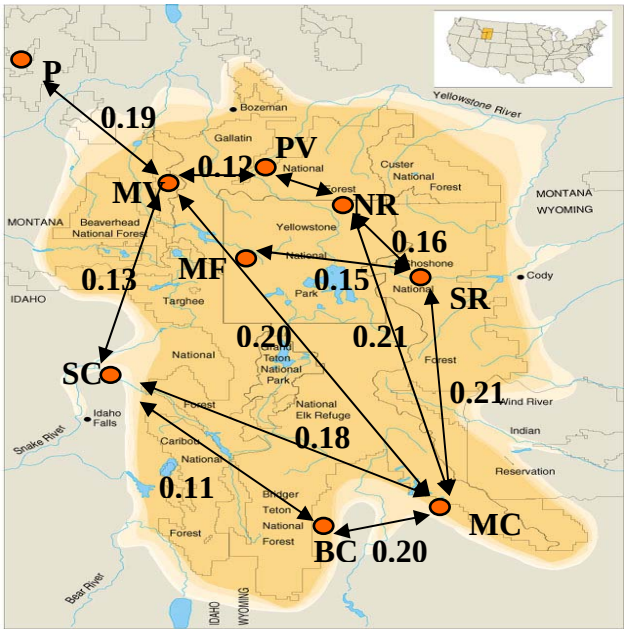

Figure 2. Sampling locations (dots) of nine elk study areas, and F_{ST} between some areas. The two southern most populations are elk feed grounds in Wyoming where prevalence of some diseases is high due to high densities of elk during the winter feeding period (approximately December to March). Brucellosis prevalence is 10-30% among elk on many of the 23 feed grounds, but only 2-5% away from the feed grounds (Thorne et al. 2002) except in several areas (e.g., SR) where prevalence recent rose above 10% (Cross et al. 2009).

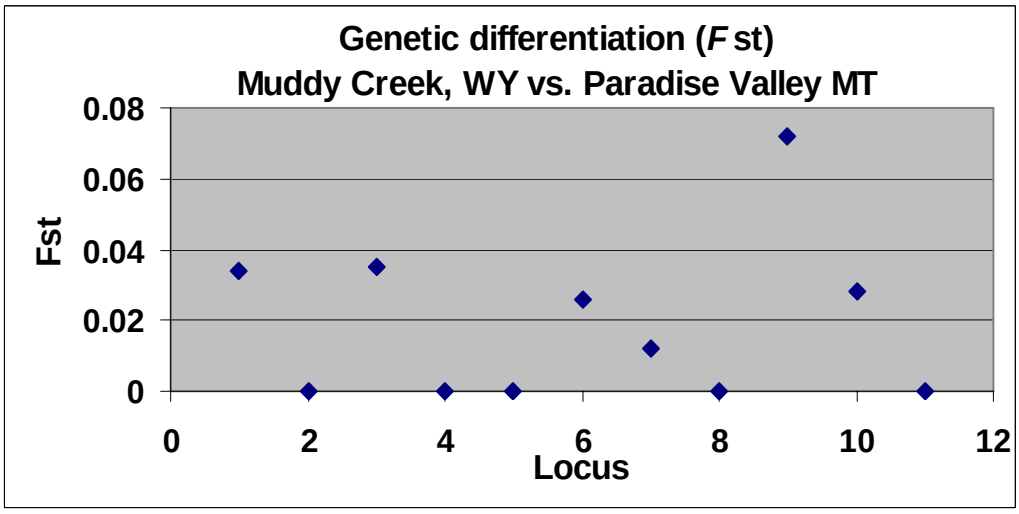

Figure 3. F_{ST} between the Muddy Creek feed ground in Wyoming and the Paradise Valley in Montana for each of 11 microsatellite loci (dots). Mean F_{ST} is 0.02, but locus 9 (IFNG) has a relatively high F_{ST} of approximately 0.07.

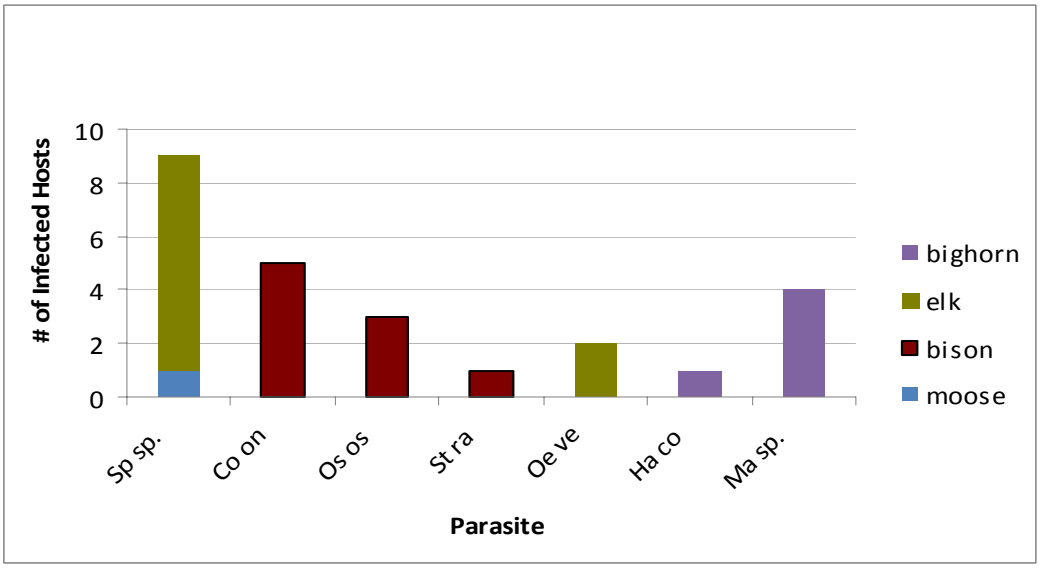

Figure 4. Distribution of the number of hosts infected with each gastrointestinal parasite species in Grand Teton National Park. Note: Co on = *Cooperia oncophora*; Ha co = *Haemonchus contortus*; Ma sp. = *Marshallagia* species; Oe ve = *Oesophagostomum venulosum*; Os os = *Ostertagia ostertagi*; Sp sp. = *Spiculopteragia* species; St ra = *Steinernema rarum*

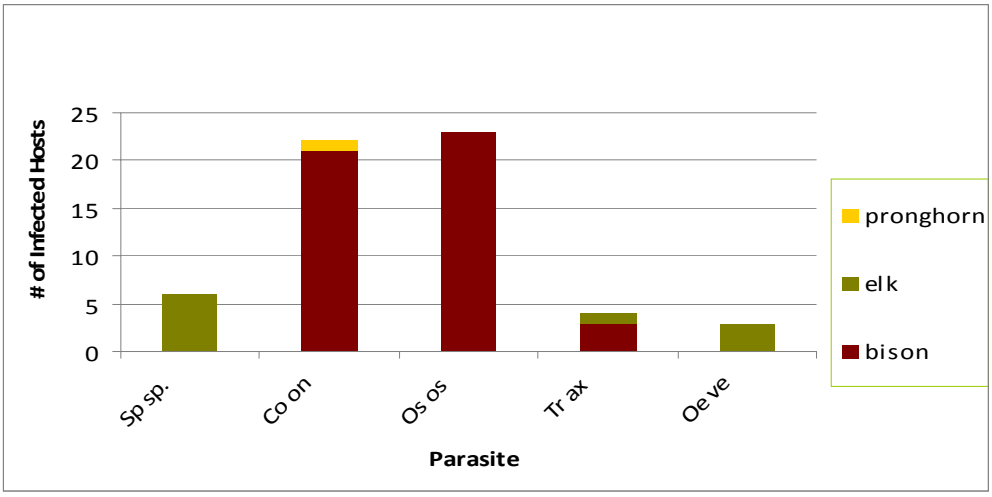

Figure 5. Distribution of the number of hosts infected with each gastrointestinal parasite in Yellowstone National Park. Parasite species codes are listed in the caption of Figure 4.

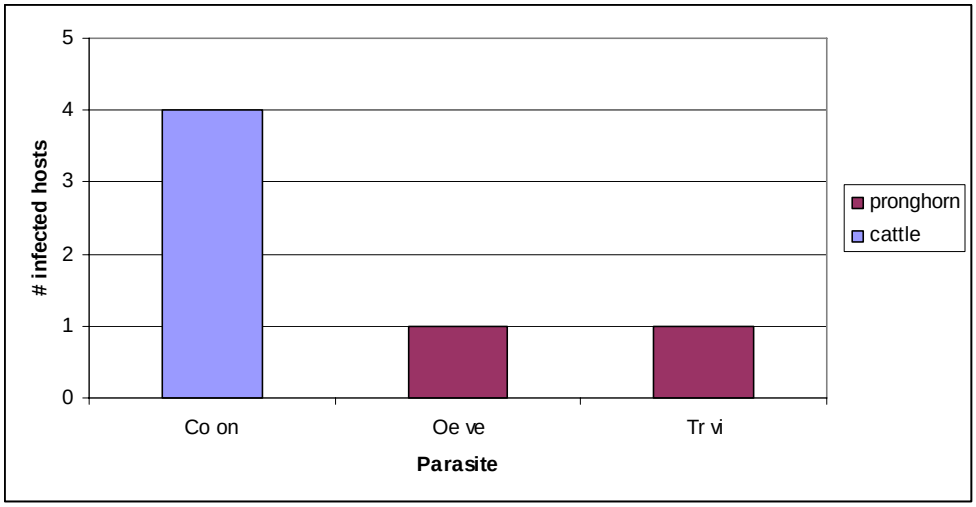

Figure 6. Distribution of the number of hosts infected with each parasite in northern Wyoming.

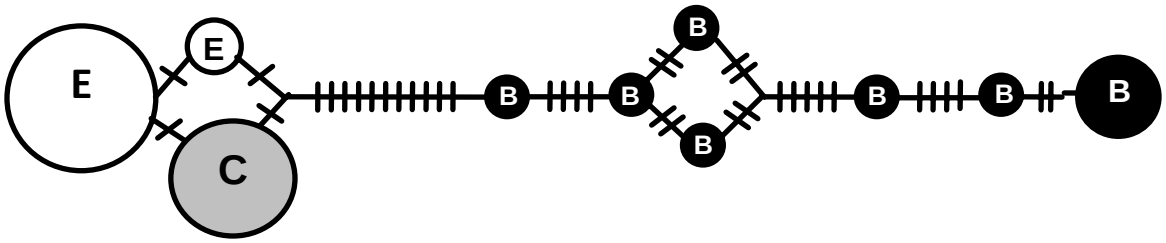

Figure 7. Haplotype network for the major *Brucella* haplogroups showing that cattle and elk *Brucella* are nearly identical, but highly divergent from all bison *Brucella* isolates. Haplotypes consist of unique multi-locus alleles from the 10 microsatellite loci (also called Variable Number of Tandem Repeat (VNTR) loci). Haplotypes from each host species are shown by a different color and letter: white are elk (E), gray are cattle (C); and black are bison (B). The size of each circle is proportional to the frequency of that haplotype. Each cross-hatch line represents one mutational step. Thus, *Brucella* haplotypes of bison all differ by at least 12 mutational steps from elk and cattle.