Genetic Analysis of Moose Populations from Minnesota and Yellowstone National Park

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Abstract

By assessing the amount and geographic distribution of genetic variation in moose we can better understand how microevolutionary processes and landscape features have influenced that variation. How the distribution of moose changes in the future will be partially dictated by the amount and content of genetic variation moose populations possess. Therefore, it will be useful to acquire more moose population genetic data and to study declining populations. My thesis had two primary objectives: (1) to compare the efficacy of DNA extraction from different biological samples and (2) to genotype a subset of Minnesota moose at a locus known to be associated with chronic wasting disease in other cervid populations. DNA for genetic analyses was extracted from blood, tissue, and pellets. Extracted DNA from all source types was sufficient for genotyping using 15 microsatellites and Sanger sequencing. However, DNA extracted from pellets was of both lower quality and quantity than DNA extracted from blood and tissue. Minnesota moose contain polymorphisms that have been correlated with increased susceptibility to chronic wasting disease in cervids in other areas. These results provide valuable comparisons of efficiency and effectiveness of DNA extraction protocols for tissue, blood, and fecal pellets as well as baseline population genetic data that can be used to detect future genetic changes in these populations.

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Chapter 1 A Methodological Comparison between DNA Source Types in Moose

Introduction

An important advance in population and conservation genetics has been the ability to obtain DNA from multiple biological source types (Waits et al. 2005). The DNA source types used most often in population genetic research on ungulates are tissue and blood. Samples are obtained from dead animals from hunter harvest or vehicle collisions, or from live animals captured for research purposes (Coulon et al. 2004; Kangas et al. 2013; Wilson et al. 2015). Blood and tissue samples collected during GPS collar captures have been used as DNA sources in several cervid population genetic studies (Kangas et al. 2013; Wilson et al. 2015; Finnegan et al. 2011).

More recently, noninvasively collected fecal pellets have been used as an alternative source of DNA. Fecal pellets, referred to hereafter as pellets, can be collected from multiple individuals over a broad geographic region and are relatively easy to collect, particularly if fieldwork is conducted in winter months when fresh pellets are visible on snow. Pellets are an especially attractive DNA source in areas where noninvasive sampling may be desirable, such as parks.

It is also beneficial to use DNA collected noninvasively for rare, elusive, or otherwise difficult to study species. For example, DNA from pellets has been used to estimate population size in roe deer (*Capreolus capreolus*) (Ebert et al. 2012) and Sitka black-tailed deer (*Odocoileus hemionus sitkensis*) (Brinkman et al. 2011), two species that live in densely vegetated, concealing habitats that are otherwise hard to survey. Population size, survival rate, and rate of population size change have been estimated for

a protected subspecies of woodland caribou in Canada using DNA from noninvasively collected pellets (Hettinga et al. 2012).

Non-invasive genetic research based on DNA extracted from pellets has been done on several ungulate species, including mountain goat (*Oreamnos americanus*) (Poole et al. 2011), caribou (*Rangifer tarandus caribou*) (Hettinga et al. 2012), Sitka black-tailed deer (Brinkman et al. 2011; Brinkman et al. 2010a; Brinkman et al. 2010b), roe deer (Ebert et al. 2012), and red deer (*Cervus elaphus*) (Valière et al. 2007).

The quality of DNA extracted from fecal pellets is a potential issue. Higher quantity and quality DNA is obtained from tissue and blood than from feces (Ball et al. 2007; Waits & Paetkau, 2005). This is because DNA from feces is more degraded and more likely to be contaminated by other sources. The rate of DNA degradation is affected by the amount of time since deposition and environmental conditions (Brinkman et al. 2010b; Kreader, 1996; Piggott, 2004). High air temperature, rainfall, bacteria and fungi, and exposure to UV radiation increase DNA degradation rate (Brinkman et al. 2010b; Buś & Allen, 2014; Piggott, 2004).

DNA extracted from feces is further affected by the organism's diet. Herbivore pellets contain tannins and other substances from the vegetation they consume, which increases the number of PCR inhibitors in the extracted DNA (Kreader, 1996). Carnivore scat includes prey DNA, which may inflate DNA concentration measurements, but will not further affect results if genetic markers used are species-specific. In addition to inherent factors involving the sample itself, proper collection and lab techniques, including sample collection, storage methods, and specified extraction protocols, are

necessary to ensure quality results from all DNA source types (Beja-Pereira et al. 2009; Buś & Allen, 2014; Waits & Paetkau, 2005).

DNA quantity and quality are important because they directly affect PCR amplification, genotyping success, and error rates (Ball et al. 2007; Brinkman et al. 2010; Mckelvey & Schwartz, 2004; Taberlet et al. 1996; Waits & Paetkau, 2005). As DNA degrades, nucleic acid residues undergo chemical changes and strands become fragmented (Buś & Allen, 2014). DNA fragmentation results in lower PCR amplification success and increased genotyping errors (Taberlet et al. 1999). The most common PCR amplification errors using degraded DNA include false alleles, allelic dropout, and failure of DNA to amplify due to absence of usable DNA.

Genetic techniques have been tested using DNA from various source types for several species (Ball et al. 2007; Beja-Pereira et al. 2009; Brinkman et al. 2011; Ebert et al. 2012; Kreader, 1996; Paetkau, 2003; Piggott, 2004; Poole et al. 2011; Waits & Paetkau, 2005), but there are few direct comparisons between source types for ungulates (Valière et al. 2007; Wehausen et al. 2004), and none for moose. All published moose population genetic research to date has used DNA from blood, tissue, or a combination of the two sources. Therefore, the aim of this study is to provide a comparison of DNA extracted from moose tissue, blood, and fecal pellets. Source type affects the time and resources required for population genetic studies, therefore, it would be useful to know relative extraction and genotyping success from different DNA sources. Using moose DNA extracted from tissue, blood, and pellets, we measured average DNA yield and

compared PCR amplification and genotyping success rates, as well as identified ways in which certain steps can be improved.

Methods

We compared DNA from biological sources collected in two study areas: northern Minnesota (MN) and the northern range of Yellowstone National Park (YNP) (Fig 1.1).

Study areas

Minnesota

The study area includes northern Minnesota, transitioning from prairie in the west to mixed conifer-deciduous forests, bog, and swamp in the east. Northwestern (NW) MN is relatively flat and dominated by tallgrass prairie and farmland. Northeastern (NE) MN is characterized by conifer-deciduous forests, and conifer bogs and swamps. Vegetation in the NE consists of white pine (*Pinus strobus*), red pine (*P. resinosa*), quaking aspen (*Populus tremuloides*), paper birch (*Betula papyrifera*), white spruce (*Picea glauca*), balsam fir (*Abies balsamea*), and white cedar (*Thuja occidentalis*). Numerous small lakes, peatlands, and wet forest are also present throughout much of the NE portion of the state. The north-central region is a transition zone between the semi-arid prairie in the NW and relatively humid mixed conifer-deciduous forest in the NE.

Yellowstone National Park

The study area includes the portion of the Northern Yellowstone Elk Winter Range (Houston 1982) located within YNP as well as some creek drainages located

outside the park. Vegetation consists primarily of sage steppe and grassland at low elevation (<2,000 m), and coniferous forests at high elevation (3,000 m). The most common conifers are lodgepole pine (*Pinus contorta*), Engelmann spruce (*Picea engelmannii*), sub-alpine fir (*Abies lasiocarpa*), Douglas fir (*Pseudotsuga menziesii*), and whitebark pine (*Pinus albicaulis* Engelm.). Willow (*Salix* spp.) is present in drainages and other wet areas.

Samples

Minnesota

Blood samples were collected by the Minnesota Department of Natural Resources (MNDNR) and stored on Whatman FTA® Classic Cards (Whatman International Ltd., Maidstone, UK), hereafter termed blood (Fig. 1.2). The blood samples were from hunter harvested (n=116), GPS collared (n=132), and sick (n=6) moose from 2011-2013. Samples for hunter harvested moose were not available in 2013 after the cancellation of the moose hunt (DelGiudice, 2013). Frozen liver tissue (n=31) samples from sick moose were collected from 2009-2012 throughout northern MN (Fig. 1.2). Moose health was determined by MNDNR based on a range of observations, such as non-normal behavior associated with neurological impairment, emaciation, and inability to stand upright. Sick moose were either found dead or were euthanized. Sex for blood and tissue samples from Minnesota moose was determined via direct observation of the animal by MNDNR or other wildlife researchers. Samples are from males (n=148), females (n=108), and

individuals of unknown sex (n=29). FTA cards were stored at room temperature and frozen tissue was stored in -20°C freezers until analysis.

Yellowstone National Park

Pellet samples (n=489) were collected primarily along drainages in YNP (Fig. 1.3). Sampling was conducted during winter months when snow was present on the ground. A minimum of five pellets from each deposition pile were collected for each sampled moose. Pellets were stored in Whirl-Pak® or Ziploc freezer bags. Tissue samples (n=2) were opportunistically collected from dead animals, frozen, and stored in Whirl-Pak® bags. Pellets and tissue collected in YNP were kept frozen and sent to the University of Minnesota-Duluth for analysis. Date and time of sample collection, location, and estimated age of sample was provided for most samples. Sex was determined directly, by collecting pellet samples from an observed animal or indirectly, by using physiological and behavioral clues, such as size of snow bed, presence of a calf, or tree rubbing. The age of pellet samples at collection was estimated based on direct observation of the moose, and sometimes of the pellet deposition itself, or by using evidence from the pellets, moose tracks, or snow cover if the moose was not directly observed. Frozen tissue and pellets were stored in -20°C freezers until analysis.

Extraction

Blood

Whole genomic DNA was extracted from one drop of dried blood for each sample (n=251), using Fermentas/Thermo Scientific GeneJET Whole Blood Genomic DNA

Purification Mini Kit (Thermo Fisher Scientific Inc. Pittsburgh, PA) according to the manufacturer's protocol (Supplementary materials). Each blood drop was taken from individual FTA cards using a 4 mm hand punch. Blood drops were categorized as small, medium, and large with diameters averaging approximately 7.4 mm, 9.8 mm, and 11.8 mm, respectively. Extractions followed kit protocol, with one modification to improve final DNA yield, particularly for small blood drops. Small blood drops were eluted twice with 100 µl of Elution Buffer, the same 100 µl of Elution Buffer pipetted back into the spin column after being centrifuged, resulting in a final volume of 100 µl. Medium and large blood drops were eluted twice as well, but with new Elution Buffer for the second elution step, resulting in a final volume of 200 µl.

Tissue

DNA was extracted from 0.02 grams of frozen tissue (n=33) using Thermo Scientific GeneJET Genomic DNA Purification kit (Thermo Fisher Scientific Inc. Pittsburgh, PA) and the manufacturer's protocol with modifications to improve final DNA yield and purity, including: (1) overnight incubation with Digestion Solution and Proteinase K instead of the manufacturer's recommended 3-4 hours, (2) a minute added to each of the highest speed centrifugation times, (3) a minute added to the elution buffer incubation time, and (4) a second elution step, resulting in a final elution volume of 400 µl (Supplementary Material).

Pellets

Three DNA extraction kits, QIAamp DNA Stool Mini Kit (Qiagen Inc. Valencia, CA), Thermo Scientific GeneJET Genomic DNA Purification Kit (Thermo Fisher

Scientific Inc. Pittsburgh, PA), and PowerFecal DNA Isolation Kit (MO BIO Laboratories, Inc. Carlsbad, CA), were tested during a pilot study using 30 pellet samples. Qiagen's stool extraction kit produced sufficient DNA yield and PCR success, using our laboratory techniques (described below), and was chosen for large scale DNA extractions for pellet samples. DNA was extracted from pellets (n=489) using two Qiagen DNA extraction kits because the manufacturer discontinued the first kit we used mid-way through our study. The first method involved extracting DNA from one whole fecal pellet (n=301) using QIAamp DNA Stool Mini Kit (Qiagen Inc. Valencia, CA) and a modified protocol (Supplementary material). The protocol was designed to isolate DNA from intestinal cells sloughed off onto the surface of pellets (Estes-Zumpf et al. 2014). Inner pellet material can contain PCR inhibitors that lead to increased variability in PCR amplification and genotyping success rates (Flagstad et al. 1999; Wehausen et al. 2004). To exclude this material from the extraction process each pellet was submerged in stool lysis buffer, Buffer ASL, from the QIAamp DNA Stool Mini Kit, and agitated to rinse cells off the outer surface instead of vortexing, which can break up the pellet exposing the inner material.

For the second method of extracting DNA from pellets (n=188) we used QIAamp Fast DNA Stool Mini Kit (Qiagen Inc. Valencia, CA) and the manufacturer's protocol with several modifications to improve final DNA yield and purity, including: (1) centrifuging after step 2 to reduce bubbles caused by vortexing and (2) reducing centrifuge rates during step 14 to 6,000 x g instead of the manufacturer's recommended 20,000 x g (Supplementary material). This protocol required that a portion of the pellet,

instead of a whole pellet, be used. We used a razor to slice thin layers from the outer pellet material that contained the sloughed off cells. For both methods, pellets were kept frozen as long as possible, to prevent thaw and subsequent break up of pellets. We did not use pellets that were frozen together or pellets with ice or snow on them.

Sex determination

Sex was determined for each sample using SE47/SE48 primer pairs (Brinkman & Hundertmark, 2009). This primer pair produces a single band for females and a double band for males by PCR amplifying X- and Y-specific alleles of the amelogenin gene. This method has been previously used on moose and other cervid species (Brinkman & Hundertmark, 2009).

PCR amplification was attempted for each sample using DNA diluted 1:1 with 1X TE Buffer. DNA that successfully amplified required no further testing for sex. If PCR amplification was unsuccessful, PCR was attempted a second time, again using DNA diluted 1:1. If PCR failed after the second attempt, PCR was attempted a final time, using undiluted DNA. PCR products for sex identification were visualized using gel electrophoresis on 1.5% agarose gels stained with 10 mg/mL of ethidium bromide.

Genotyping

DNA extracted from blood (n=248) and pellets (n=269) was genotyped using one sex-linked and 15 autosomal microsatellites previously used for moose (Table 1.1). Each microsatellite primer pair was tested to determine optimal PCR conditions (Table 1.1).

All autosomal forward primers contain an M13 (-21) tail on the 5' end (Schuelke, 2000), and PCR products were labeled by incorporating a universal fluorescently-labeled M13 (-21) primer (FAM, PET, or VIC) during PCR. For DNA extracted from blood sources, all microsatellites were amplified in MultiGene Optimax Gradient Thermal Cycler TC9610 with a total volume of 13 µl containing sterile water, GoTaq DNA Polymerase and 1x GoTaq buffer (Promega Corporation, Madison, WI, USA), 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.08 µM forward primer, 0.8 µM reverse primer, 0.8 µM labeled primer, 1% bovine serum albumin (BSA), and 1 µl/reaction DNA. DNA used in PCR was either undiluted or diluted 1:1, based on what concentration was successful for sex determination. BSA was added to all PCR to bind potential inhibitors and improve amplification specificity (Kreader, 1996). The addition of BSA to PCR was initially implemented to increase amplification success using DNA extracted from pellets. It was added to PCR using DNA extracted from blood and tissue for consistency, and to potentially increase PCR amplification success for DNA from each source type.

DNA extracted from pellet sources was amplified using the same set of microsatellite loci; however, an additional step was taken for PCR using this DNA due to low PCR amplification success rates determined in a pilot study. For DNA extracted from pellets, microsatellite loci were amplified using either single step or pre-amplification PCR methods (Table 1.1). Pre-amplification is a two-step PCR method designed to increase the amount of DNA template for amplification and reduce genotyping error (Piggott et al. 2004). The success of the pre-amplification method has been questioned (Hedmark & Ellegren, 2006; De Barba & Waits, 2010); therefore, we conducted a pilot

study to test this method using DNA extracted from pellets amplified using our microsatellites. The pre-amplification method was used only on loci for which it increased PCR amplification success and subsequent genotyping success. PCR mixtures for both methods are shown in Table 1.2.

Analyses

PCR products were analyzed at the University of Minnesota Biomedical Genomics Center using an ABI 3730xl capillary genetic analyzer. Genotypes were assigned using GeneMarker (v.2.6.0, Softgenetics LLC, State College, PA) to score alleles for each locus. Failed or ambiguous allele scores were re-amplified and genotyped to reduce scoring errors and missing data.

<u>DNA quantification</u>

DNA extracted from blood, tissue, and pellets was quantified using a NanoDrop ND-1000 spectrophotometer to measure DNA concentration for each sample. Using DNA concentration and total extraction volume, we determined total DNA yield from each extraction for comparison. We also compared the two protocols for DNA extraction from pellets to determine which method resulted in higher DNA yield. DNA yield was compared between blood, tissue, and pellet samples using ANOVA, followed by pairwise comparisons between each sample type using two-sample t-tests.

<u>Sex determination</u>

Sex determination success rates were compared between DNA extracted from blood, tissue, and pellet samples using Fisher’s exact test. The accuracy of sex

determination using genetic methods was determined by comparing genetically determined sex with recorded sex from direct field observations using DNA from blood, tissue, and pellet samples. Additionally, we compared genetically determined sex with recorded sex from indirect field observations using DNA from pellet samples.

PCR and genotyping success rates

PCR amplification of autosomal microsatellites and genotyping success rates were calculated as a measure of DNA quality. Genotyping success rate is the proportion of microsatellites at which we were able to obtain genotypes for a given sample. To obtain overall genotyping success, PCR and allele scoring was attempted a second time for DNA samples if the first attempt failed. The genotyping success rate allows us to compare overall success using DNA from several DNA source types, however, it does not reflect the amount of effort and resources required to obtain that success. Therefore, we also calculated autosomal PCR success rate, the proportion of PCR amplification attempts that were successful, as a measure of effort required. PCR attempts were classified as successful if they produced viable product that was able to be used to genotype individuals. Autosomal PCR success rates using DNA extracted from blood and pellets were compared using chi-square test for independence. Genotyping success rates were compared using DNA extracted from blood and pellet samples using two-sample t-tests.

Genotyping error

A concern when working with lower quality and quantity DNA, primarily from fecal samples, is the increased risk of genotyping errors such as allelic dropout or false

alleles. To estimate genotyping accuracy, we repeated PCR and allele scoring for at least 12 and 24 individuals at each microsatellite locus using previously extracted DNA from blood and pellets, respectively. Because original repetitions using DNA extracted from pellet samples did not produce a usable amplicon, 95 additional DNA samples were repeated at 6 microsatellite loci. Genotyping error was calculated using these duplicated allele scores.

Predictors of downstream success

To identify ways to improve efficiency and success rates, we tested DNA yield and sex determination success on downstream success. We used linear regression and two-sample t-tests to determine whether higher DNA yield or PCR amplification success of SE47/SE48, respectively, resulted in greater genotyping success. If DNA yield or PCR amplification success leads to greater genotyping success, these methods can be used to identify poor quality samples for removal, reducing the amount of time and effort spent on those samples.

Effect of pellet age

Since numerous environmental factors degrade DNA, we investigated whether the time between deposition and collection affects DNA quality and quantity. Time since deposition was estimated for pellet samples based on observations made in the field during collection, including visual pellet characteristics, moose tracks, and snowfall. Using these data, DNA from pellets was separated into three age classes: <24 hours (n=84), 24-48 hours (n=65), and >48 hours (n=109) since deposition.

We compared DNA yield between age classes using ANOVA to determine if time since deposition affected the amount of DNA obtained from pellets. We compared sex-

linked and autosomal PCR amplification success between age classes using Fisher's exact and Chi-squared tests, respectively. Finally, we used two-sample t-tests to determine if age since deposition affected overall genotyping success. Because of potential difficulty in differentiating pellets deposited <24 hours and 24-48 hours since deposition, we compared PCR amplification and genotyping success rates between DNA from pellets collected <48 hours and >48 hours after deposition.

Results

DNA yield

We compared yield for DNA extracted from blood (n=251), tissue (n=33), and pellet (n=489) samples. Extracted DNA yield for blood, tissue, and pellet samples was sufficient for genotyping, however, tissue samples produced the highest average DNA yield, and pellets produced the lowest average DNA yield (Fig. 1.4). DNA extractions using sliced pellets had higher average DNA yield than extractions using whole pellets (Fig. 1.4).

Sex determination

We determined sex with primer pair SE47/SE48 using DNA extracted from all three sample sources. Sex determination was successful for 100% of blood samples (n=254), 91% of tissue samples (n=33), and 83% of pellet samples (n=460) (Table 1.3). We calculated the accuracy of sex determination using these methods by comparing results determined genetically to those in the field when the individual was observed

directly. Genetic and field sex determination was consistent for 219 of 225 blood samples (0.97), 24 of 25 tissue samples (0.96), and 64 of 67 pellet samples (0.96) when analyzed separately. When sex was determined in the field by indirect observation of moose in YNP, genetic and field determined sex were consistent for 74 of 100 pellet samples (0.74). Genetic sex determination was inconsistent with indirect field determined sex in similar proportions in samples identified as male (0.42) and female (0.58) in the field.

Genotyping and PCR success rates

We genotyped 517 total moose samples using DNA from blood (n=248) and pellets (n=269) at 15 autosomal microsatellite markers. Average PCR amplification success rate was higher for blood (0.81) than pellets (0.63) (Table 1.4). Similarly, average genotyping success rate, the percent of microsatellites that we were able to get data, was higher for DNA from blood (0.82) than pellets (0.76) (Table 1.4).

Genotyping error

Average genotyping error rate calculated by repeated PCR amplification and allele scoring using DNA extracted from blood (n=178) and pellet (n=541) samples was 0.16 and 0.10, respectively, for re-analyzed microsatellite loci. PCR did not produce usable amplicons for five microsatellite loci (IGF-1, RT1, RT5, NVHRT03, and CRFA) using DNA extracted from blood, and for three microsatellite loci (IGF-1, RT5, and CRFA) using DNA extracted from pellets.

Predictors of downstream genotyping success

DNA yield and PCR amplification success of the sex-linked primer pair SE47/SE48 were tested in order to determine whether they could be used as predictors of downstream success, particularly genotyping success. Linear regression detected almost no ability for DNA yield to predict genotyping success using DNA extracted from blood ($r^2 = 0.006$; $p = 0.229$) or pellets ($r^2 = 0.009$; $p = 0.127$) (Fig. 1.5). DNA extracted from pellets that successfully PCR amplified at the sex-linked loci had higher genotyping success at autosomal loci compared to those that failed to amplify at this locus (Table 1.5). The relationship between sex determination PCR and genotyping success could not be completed for DNA from blood or tissue samples because sex determination PCR success was high, resulting in insufficient sample size of failed sex determination PCR for comparison.

Effect of pellet age

Time since deposition was estimated for 447 samples as either <24 hours (n=178), 24-48 hours (n=114), or >48 hours (n=155). Average DNA yield for pellet samples from the three age classes was not significantly different (Table 1.6). Pellets collected <24 hours and 24-48 hours since deposition had the highest sex determination success rates and were not different from one another (0.85 and 0.92, respectively), whereas DNA from the oldest pellets had the lowest sex determination success rate (0.74) (Table 1.6).

In addition, moose pellet samples were compared to determine whether the pellet age at collection influenced PCR amplification or genotyping success rates. Similar to

sex determination success rates, pellets collected <24 and 24-48 hours since deposition had the highest PCR success rates and were not different from one another (0.67 and 0.69, respectively), whereas PCR success for pellets collected >48 hours after deposition was significantly lower (0.56) (Table 1.6). Genotyping success rate was the highest for DNA from pellets collected <24 and 24-48 hours after deposition (0.82 and 0.83, respectively) (Fig. 1.6). Pellets collected more than 48 hours after deposition had the lowest average genotyping success rate (0.69) and was significantly different from the two younger age classes. Similar results for PCR amplification and genotyping success rates were obtained when DNA extracted from pellets collected <48 hours since deposition was compared to DNA extracted from pellets collected >48 hours since deposition.

Discussion

DNA Yield

DNA was successfully extracted from blood, tissue, and both sliced and whole pellets. Tissue samples produced both the greatest amount of DNA on average and the largest total volume per extraction, while pellets produced the smallest average DNA yield and total volume per extraction. We used one drop of blood for DNA extractions from Whatman FTA® cards. DNA yield for blood samples may have been higher had we used more than one drop of blood.

DNA yield was not a reliable predictor of downstream genotyping success. While this measurement estimates the amount of DNA in a sample, it does not provide

information on DNA quality or the possible presence of PCR inhibitors. Samples with high DNA yield may actually have had some level of DNA degradation, and indeed, we found evidence supporting this.

DNA extracted from blood had the highest average PCR amplification success, for both sex-linked and autosomal microsatellites. This suggests higher quality for DNA extracted from blood than tissue, as tissue samples measured greater average DNA yield. DNA extracted from pellets had the lowest sex determination success, supporting evidence for lower average DNA yield.

Genotyping and PCR success

Overall we achieved similar genotyping success for samples originating from blood and pellets. However, the effort required to achieve those results was much greater for pellet samples compared to blood samples. This was because PCR amplification success was lower using DNA from pellets compared to blood, a consequence of using lower quantity and quality DNA. Genotyping error was higher for DNA extracted from blood compared to DNA from pellets; however, this was likely due to the smaller sample size using DNA from blood compared to pellets. For regions or populations where noninvasive genetic sampling is the only feasible option, moose pellet samples are an alternative source of DNA. For other areas with access to higher quality samples, such as blood or tissue, we found these samples to be more efficient and preferable.

Reported PCR amplification success using DNA from feces has been variable (Broquet et al. 2007; Wehausen, 2004), and is likely due to more factors than the DNA

source used. For example, methods of sample collection, storage, and extraction, can all affect DNA quality and downstream success (Wehausen, 2004; Roon et al. 2003; Waits & Paetkau, 2005). The effectiveness of these methods has been tested, but with no clear consensus (Beja-Pereira et al. 2009; Luikart et al. 2006). The lack of consensus is likely influenced by inherent variation among species and environmental variables (Waits & Paetkau, 2005), therefore it is essential to conduct a pilot study before beginning large-scale extractions (Taberlet, et al. 1999).

Improvements

We made many attempts to improve PCR success using DNA from pellets, including testing multiple DNA extraction kits and protocols, adding BSA to PCR to remove PCR inhibitors, using the pre-amplification method for PCR, and testing a variety of PCR conditions (optimizing PCR master mix ingredients and thermocycler programs) for each microsatellite locus.

The average amount of DNA extracted from sliced pellets was greater than that from whole pellets using the surface washing method. A similar comparison using whole pellets and sliced outer pellet material in bighorn sheep found no difference in extracted DNA yield (Wehausen 2004). However, the differences could be due to the sample size in our study being substantially larger, and differences in species, environmental conditions, and extraction protocols. We found slicing pellets to be a more time consuming process with increased probability of contamination through exposure to multiple laboratory surfaces (cutting surface, razor, and forceps), even though protocols

were in place to minimize this possibility. Additionally, accidental inclusion of inner pellet may increase PCR amplification failure and variation among samples (Wehausen 2004). Therefore, although the sliced pellet method resulted in higher average DNA yield, we prefer the whole pellet surface washing method.

Although not quantified, we saw noticeable improvement in PCR success using DNA extracted from pellets with the inclusion of BSA. BSA was not as beneficial when attempting to amplify DNA extracted from blood or tissue. BSA does not have a noticeable effect on PCR amplification success using DNA with low levels of PCR inhibitors (Kreader, 1996). Thus, the difference in the effect of including BSA provides evidence for increased levels of PCR inhibitors in DNA extracted from pellets compared to either blood or tissue.

The pre-amplification method for PCR was beneficial for six microsatellites; however, it required more resources and number of PCRs. In some cases, it caused non-specific amplification or amplification patterns that created difficulty for allele scoring. Therefore, this method should be used with caution.

Recommendations

For pellets, time since deposition affected downstream success. DNA is degraded by several environmental conditions (high temperature, precipitation, UV radiation, and microorganisms), thus the longer it is subjected to adverse conditions, the less likely the pellet will contain usable DNA. Therefore, selecting fresh pellets (<48 hours after deposition) will likely result in better quality and quantity DNA and improved PCR

amplification and genotyping success rates. However, samples collected in dry or protected areas may have a substantially larger window for collection (Brinkman et al. 2010b). In the future, decay functions could be created using more precise estimates of pellet age to determine the point or age in which pellets no longer contain usable viable DNA.

There is still variability in DNA quality and quantity from fresh pellets, and even between pellets from the same pellet pile (Taberlet et al. 1996). Poor quality samples can be removed from analysis based on PCR amplification success using primer pair SE47/SE48. Other microsatellites could also be used for this purpose; however, the benefit of using a sex-linked microsatellite is to confirm the sex of the individual, which is often missing from noninvasively collected samples.

Avenues of future work include testing the effectiveness of sample collection and storage methods. Blood and tissue samples were collected and stored before our study began, and we used reported methods from other noninvasive genetic studies for collection and storage (Ebert et al. 2012; Carr et al. 2010). However, there are several reasons why these factors may have influenced our results. First, the tissue samples used may have been inherently of lower starting quality. MN moose tissue samples came from sick moose that were either found dead or were euthanized due to their condition. Death and subsequent post-mortem decomposition result in DNA degradation, particularly in liver tissue (Alaeddini et al. 2010). Second, although attempts were made to preserve DNA by storing tissue samples in -20°C freezers, these methods have not proven entirely effective at eliminating DNA degradation (Dawson et al. 1998). This is especially true

after long storage periods. Storage length beyond six months decreases both DNA yield and PCR amplification success for multiple DNA source types (Roon et al. 2003). Not only were tissue samples from potentially compromised individuals, they were stored substantially longer than either blood or pellet samples. Extracted DNA quantity and quality from tissue may have been improved using samples collected more recently or if DNA had been extracted immediately following collection.

Concluding Remarks

Population genetic studies of moose have traditionally used DNA extracted from tissue, blood, or a combination of those two DNA sources. Similar to what has been found for other ungulate species (Ebert et al. 2012; Brinkman et al. 2010a; Luikart et al. 2006) we have shown that moose fecal pellets are viable sources of DNA. However, the source type and methods used can significantly influence the amount of data able to be collected and the resources (time and money) required.

Table 1.1 Characteristics of 1 sex-linked and 15 autosomal microsatellites used in genetic analysis.

Locus	Primer sequences	Annealing Temp (°C)	PCR Method
SE47[¥]	5'-CAGCCAAACCTCCCTCTGC-3'	53	Single step
SE48[¥]	5'-CCCGCTTGGTCTTGTCTGTTGC-3'		
NVHRT01[#‡Π]	5'-GCAGTCTTCCCCTTTCTT-3'	50	Single step
	5'-GATTGCAGAGTTGGACACTA-3'		
RT1[‡Π φ]	5'-TGCCTTCTTTCATCCAACAA-3'	47	Pre-amp
	5'-CATCTTCCCATCCTCTTTAC-3'		
BM888[‡‼□]	5'-AGGCCATATAGGAGGCAAGCTT-3'	50	Single step
	5'-CTCGGTGAGCTCAAAACGAG-3'		
BM1225[† Ψ□π]	5'-TTTCTCAACAGAGGTGTCCAC-3'	50	Single step
	5'-ACCCCTATCACCATGCTCTG-3'		
KCSN[•]	5'-ATGCACCCTTAACCTAATCCC-3'	50	Pre-amp
	5'-GCACTTTATAAGCACCACAGC-3'		
BM2830[†Π]	5'-AATGGGCGTATAAACACAGATG-3'	50	Single step
	5'-GCATTTTTGTGTTAATTTCATGC-3'		
BM4513 [Ψ‡‼□ π]	5'-GCGCAAGTTTCCTCATGC-3'	50	Single step
	5'-TCAGCAATTCAGTACATCACCC-3'		
Cervid14 [π]	5'-TCTCTTGCGTCTCCTGCATTGAC-3'	54	Single step
	5'-AATGGCACCCACTCCAGTATTCTTC-3'		
BM848 [Ψ‼□]	5'-TGGTTGGAAGGAAAACTTGG-3'	54	Single step
	5'-CCCTCTGCTCCTCAAGACAC-3'		
RT9 [Ψ‡‼□φ]	5'-TGAAGTTTAATTTCCACTCT-3'	54	Single step
	5'-CAGTCACTTTCATCCCACAT-3'		
RT24 [Ψ‼□Π φ]	5'-TGTATCCATCTGGAAGATTTCAG-3'	54	Single step
	5'-CAGTTTAACCAGTCCTCTGTG-3'		
RT30[*‡‼□Π φ]	5'-CACTTGGCTTTTGGACTTA-3'	54	Single step
	5'-CTGGTGTATGTATGCACACT-3'		
Map2C [Ψ□]	5'-TTTACCAGACAGTTTAGTTTTGAGC-3'	54	Single step
	5'-AAGGATTCTGTCTGATACCACTTAG-3'		
NVHRT21[#‡Π]	5'-GCAGCGGAGAGGAACAAAAG-3'	50	Pre-amp
	5'-GGGGAGGAGCAGGGAAATC-3'		
BL42[Ψ‼□]	5'-CAAGGTCAAGTCCAAATGCC-3'	49	Pre-amp
	5'-GCATTTTTGTGTTAATTTCATGC-3'		
IGF-1[• π]	5'-GAGGGTATTGCTAGCCAGCTG-3'	54	Single step
	5'-CATATTTTTCTGCATAACTTGAACCT-3'		
RT5[‡‼Π φ]	5'-CAGCATAATTCTGACAAGTG-3'	54	Single step
	5'-AATTCCATGAACAGAGGAG-3'		

NVHRT03[#]	5'-TGGAGAGCTGAGTATGAAAG-3'	54	Pre-amp
	5'-AGAAATGCAGCTACCTAAAAG-3'		
CRFA•	5'-CTCGCTCACCTGCAGAAGCACC-3'	53	Pre-amp
	5'-GCTGAGCAGCCGTCTAAGTTGC-3'		

References: ‡Haanes et al. 2011; ∏Schmidt et al. 2009; □Murray et al. 2012; ‼Hundertmark 2009 †Broders et al. 1999; •Cronin et al. 2001; ΨFinnegan et al. 2011; φWilson et al. 1997; πWilson et al. 2003; ¥Brinkman and Hundertmark 2009; #Roed and Midthjell 1998.

Table 1.2 PCR mixtures for single step and pre-amplification methods

PCR Mixture	Single step	1st step pre-amp	2nd step pre-amp
GoTaq Flexi buffer	1x	1x	1x
$MgCl_2$	2 mM	3 mM	2 mM
dNTPs	0.2 mM	0.2 mM	0.2 mM
Forward primer	0.08 µM	0.1 µM each	0.08 µM
Reverse primer	0.8 µM	0.1 µM each	0.8 µM
Labeled primer	0.8 µM	-	0.8 µM
BSA	0.5 ng/µL	0.25 ng/µL	0.5 ng/µL
GoTaq DNA polymerase	0.05 µL/reaction	1 U	0.05 µL/reaction
DNA template	1 µL/reaction	2.4 µL/reaction	1.3 µL/reaction
Sterile water	to make 13 µL	to make 10 µL	to make 13 µL
Total reaction volume	13 µL	10 µL	13 µL

Table 1.3 Sex determination success rate using DNA extracted from blood, tissue, and pellet samples. Sex was determined genetically using sex-linked primer pair SE47/SE48. Sex determination success was significantly greater using DNA extracted from blood compared to DNA from tissue or pellets (Fisher's exact test, $p < 0.01$); however, sex determination success using extracted DNA from tissue and pellets were not significantly different (Fisher's exact test, $p > 0.05$).

DNA Source Type	n	Sex determination success rate
Blood	251	1.00
Tissue	33	0.91
Pellets	460	0.83

Table 1.4 PCR amplification and genotyping success rates using DNA extracted from blood and pellet samples. PCR amplification success rate, the proportion of PCRs that were successful, was greater using DNA extracted from blood than DNA extracted from pellets ($\chi^2 = 56.63$; d.f. = 1; $p < 0.001$). Genotyping success rate, the proportion of microsatellites that we were able to get information for, was also greater using DNA extracted from blood than DNA extracted from pellets ($t_{506} = 6.04$, $p < 0.001$). Both PCR amplification and genotyping success rates were calculated using 15 autosomal microsatellite markers.

		Success Rates	
DNA Source	n	PCR amplification	Genotyping
Blood	249	0.81	0.82
Pellets	269	0.63	0.76
Significance		***	***

NS $p > 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table 1.5 Average genotyping success rates for DNA extracted from moose pellets that successfully amplified and failed to amplify using sex-linked microsatellite (SE47/SE48). DNA samples that produced sex determination results had higher genotyping success rates than DNA that failed to amplify at this locus ($t_{36} = -4.61$, $p < 0.001$).

Sex Determination	n	Genotyping Success Rate
Successful	236	0.78
Failed	31	0.59
Significance	-	***

NS $p > 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table 1.6 Effect of moose pellet age on DNA yield, sex-linked microsatellite PCR amplification success (sex-linked PCR success), and autosomal microsatellite PCR amplification success (autosomal PCR success) using 15 microsatellite markers using pellets collected >24 (n=84), 24-48 (n=65), and >48 (n=109) hours after deposition. Pellets in >48 hours age class ranged from 48 hours to months since deposition. Average DNA yield was not significantly different between different age classes ($F_{2,255} = 1.73$, $p = 0.18$). Sex determination success for pellets in <24 and 24-48 age classes were not different ($p = 0.10$), but were different from pellets collected >48 hours after deposition ($p = 0.0003$). PCR success rate for pellets in <24 and 24-48 age classes were not different ($\chi^2 = 1.31$, d.f. = 2, $p = 0.25$), but were different from pellets collected >48 hours after deposition ($\chi^2 > 46$, d.f. = 2, $p < 0.001$).

Time Since Deposition (hours)	Average DNA Yield (μg)	Sex-linked PCR success	Autosomal PCR success
<24	2.54	0.85	0.67
24-48	2.45	0.92	0.69
>48	2.08	0.74	0.56

Figure 1.1 Location of two study areas, Yellowstone National Park and northeastern Minnesota, in North America. Area shaded in gray represents estimated moose distribution throughout North America.

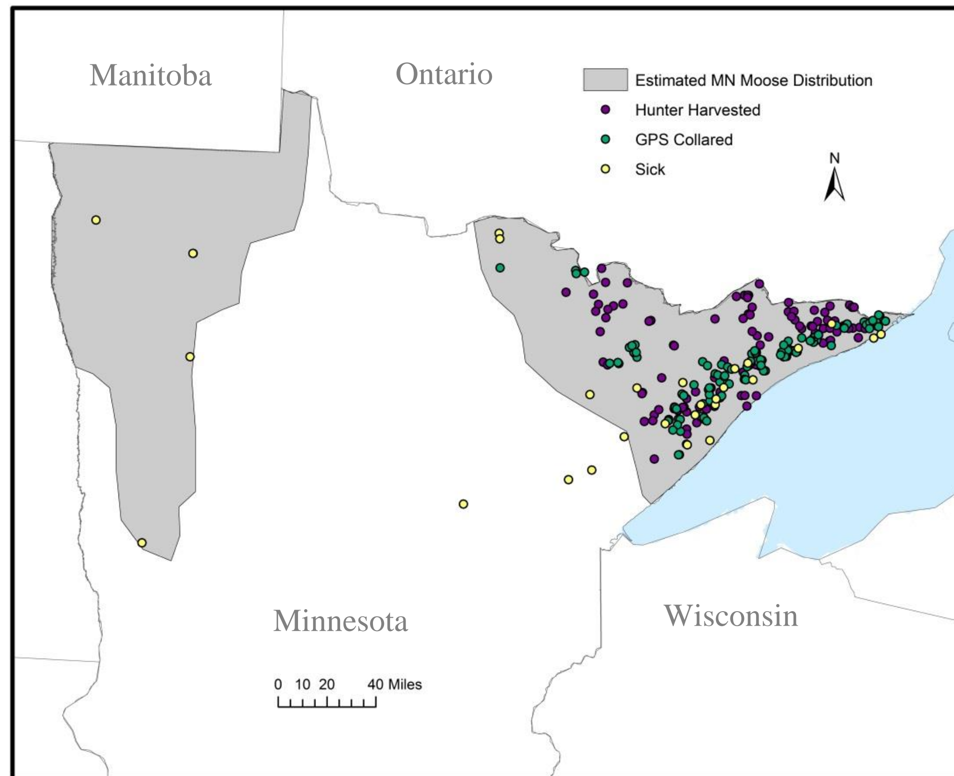


Figure 1.2 Location of hunter harvested (n=117), GPS collared (n=132), and sick (n=36) moose in Minnesota.

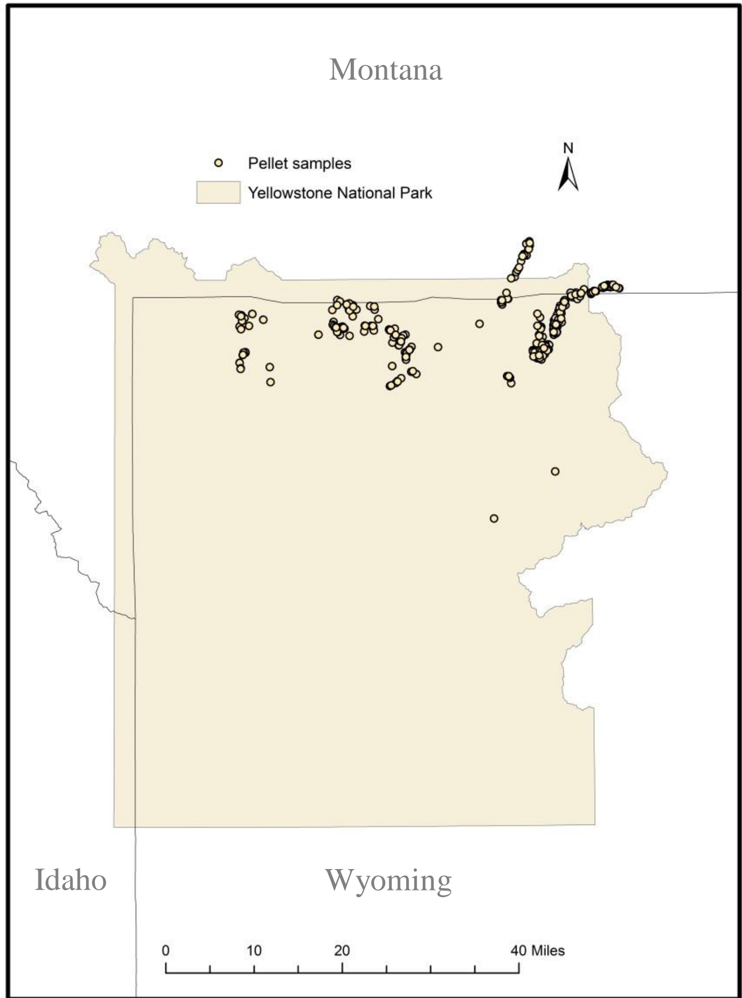


Figure 1.3 Location of noninvasively collected pellet samples (n=489) in the northern range of Yellowstone National Park.

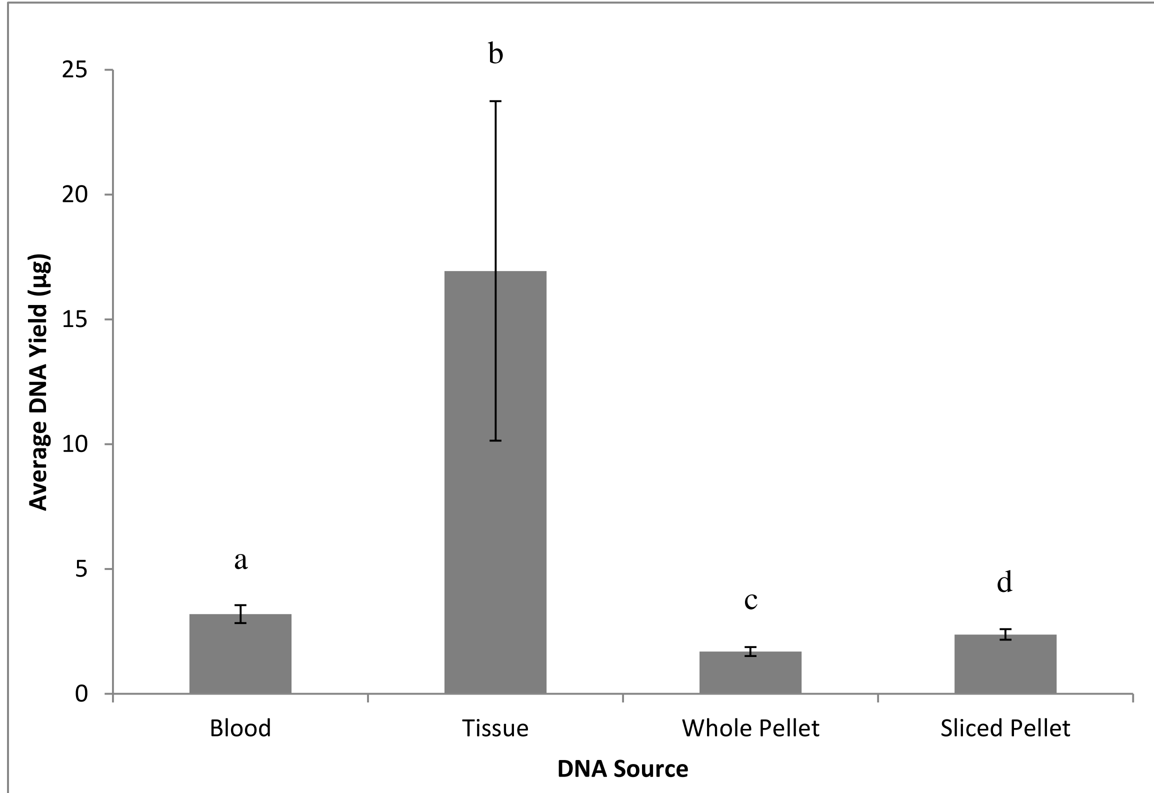


Figure 1.4 Average DNA yield extracted from multiple DNA source types. DNA sources were from moose in Minnesota (blood, n=251; tissue, n=31) and Yellowstone National Park (tissue, n=2; pellet, n=489). Pellets were extracted using two methods; using (1) whole pellets (n=188) and (2) outer slices of pellet (n=301). ANOVA detected differences in DNA yield between blood, tissue, and pellets ($F_{3,769} = 112.71$, $p < 0.001$). Values not sharing a common letter (a,b,c,d) differ significantly at $p < 0.05$.

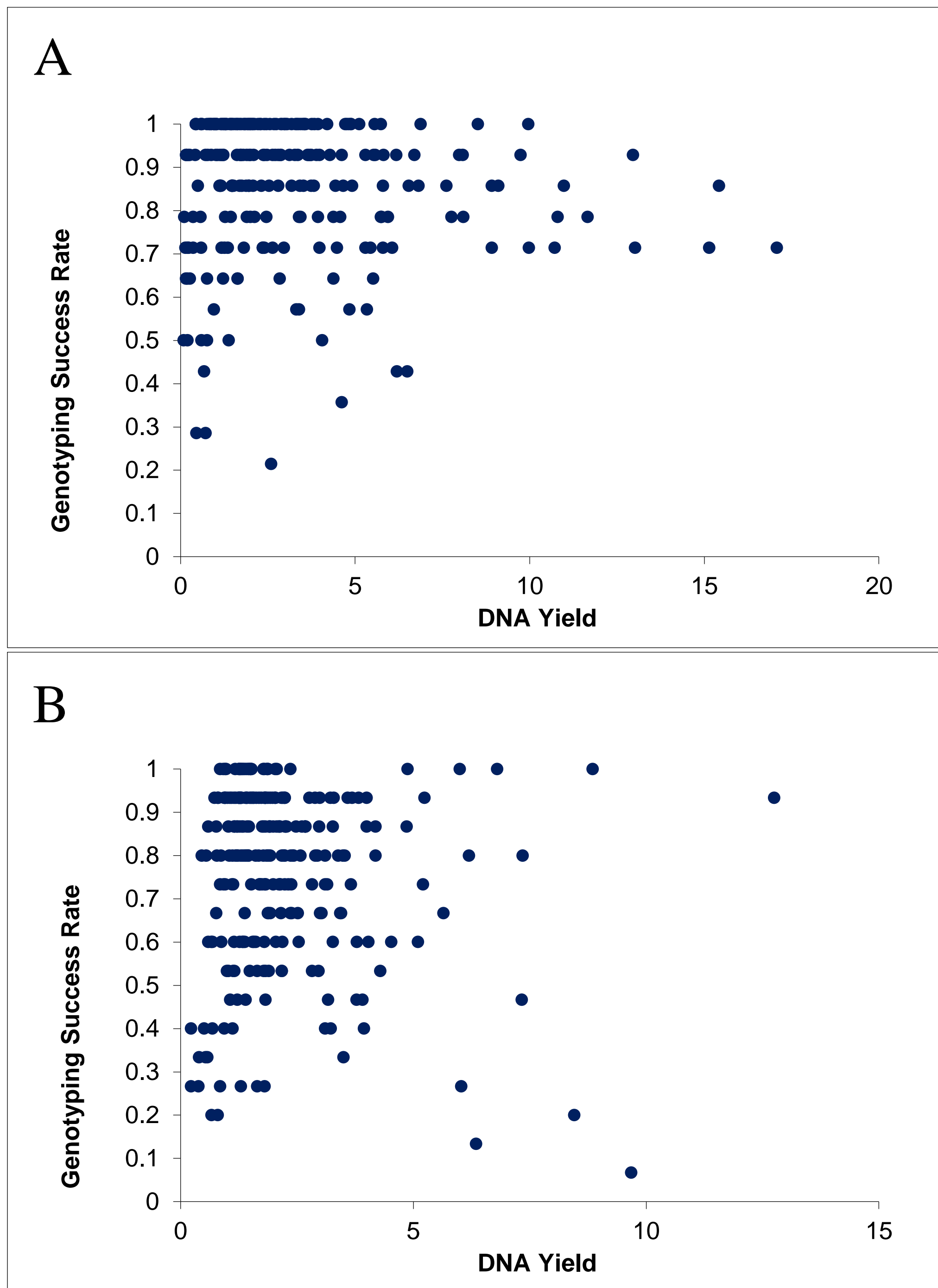


Figure 1.5 Genotyping success rate for (A) blood and (B) pellet samples of varying DNA yield (μg). Linear regression did not detect a relationship between DNA yield and genotyping success rate using DNA from blood ($\beta = -0.004$, $r^2 = 0.006$; $p = 0.229$) or pellets ($\beta = -0.012$, $r^2 = 0.008$; $p = 0.127$).

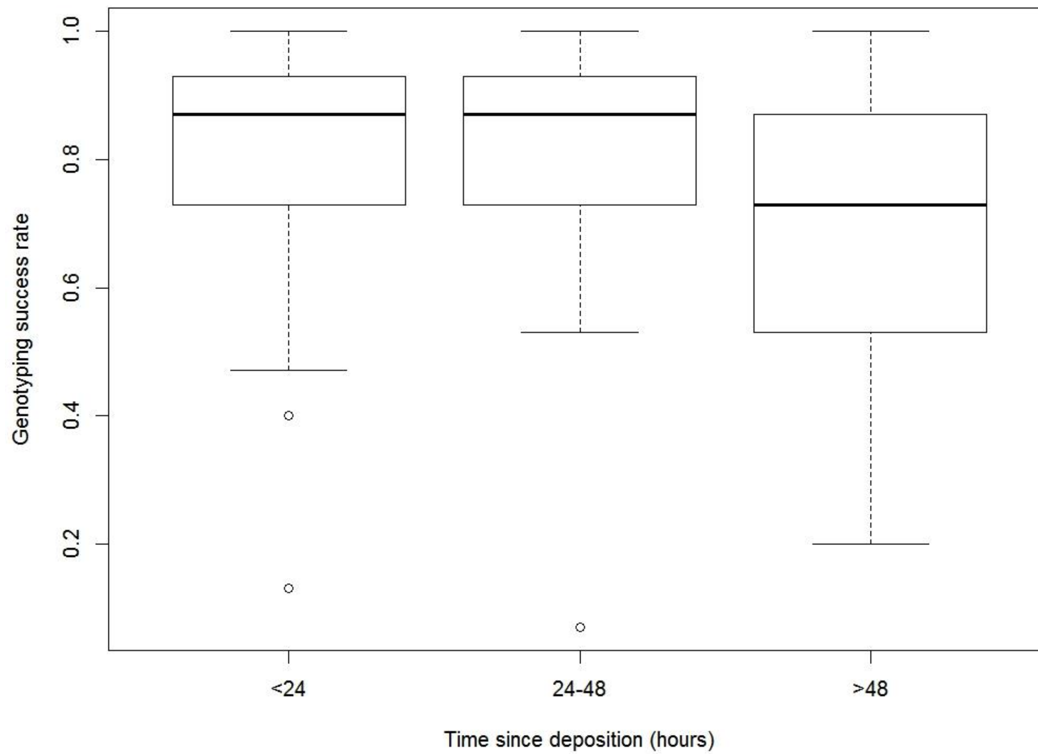


Figure 1.6 Effect of pellet age at collection (<24 hrs, n=84; 24-48 hrs, n=65; >48 hrs, n=109) on individual genotyping success rate. Pellets in >48 hrs age class ranged from 48 hours to months since deposition. Pellets in <24 hrs and 24-48 hrs age classes were not different ($t_{136} = -0.10$, $p = 0.92$), but were different from pellets collected >48 hrs after deposition ($t_{191} = -5.29$, $p < 0.001$).

Chapter 2 Prevalence of genetic polymorphisms in Minnesota moose associated with prion disease in other cervid populations

Introduction

Prion diseases, also called transmissible spongiform encephalopathies (TSEs), are fatal neurodegenerative diseases in mammals. The first documented case of TSE, scrapies, occurred in the mid-18th century in sheep (Aguzzi & Polymenidou, 2004). Since then, TSEs have been discovered in humans, cattle, mink, and cervids (Collinge, 2001; Gajdusek & Zigas, 1959; Hartsough & Burger, 1965; May, 1968; Prusiner, 1998; Williams & Young, 1980). Prion diseases are of concern because of their potential impact on human health (Belay & Schonberger, 2005), and possible economic and ecological consequences. Many captive animal facilities have been depopulated due to prion disease outbreaks (Belay et al. 2004), and prion disease outbreaks in free-ranging cervids may also decrease the number of individuals in the population (Gross & Miller, 2001).

Chronic wasting disease (CWD) is the form of prion disease associated with cervids. In 1967 CWD was identified in captive mule deer in Colorado (Williams & Young, 1980). It has since been discovered in captive and free-ranging cervid populations in 23 states in the U.S., and in Canada's Alberta and Saskatchewan provinces (NWHC, 2015). Detection of CWD may be related to surveillance effort, which has increased because the spread of CWD raises concerns about the possibility of transmission to humans (Belay et al. 2004). There is currently no cure or vaccine for CWD (Saunders et al. 2012) and it is highly transmissible within and among cervid species (Miller & Williams, 2003; Saunders et al. 2012; Sigurdson, 2008). CWD has been found in mule deer (*Odocoileus hemionus*), white-tailed deer (*O. virginianus*), elk (*Cervus elaphus*),

caribou (*Rangifer tarandus caribou*), red deer in Europe (*Cervus elaphus*) and moose (*Alces alces*) (Sigurdson, 2008; Williams, 2005; MNDNR, 2015a).

TSEs can be acquired spontaneously, genetically, or through horizontal transmission (Moreno-Gonzalez & Soto, 2011). Horizontal transmission appears to be the primary mechanism responsible for CWD infection (Saunders et al. 2012). The infectious agent responsible for prion disease is PrP^{Sc}, a misfolded isomer of the cellular protein (PrP^{C}). Once infected, PrP^{Sc} initiates the conversion of normal PrP^{C} to PrP^{Sc} (Collinge, 2001). There is a variable incubation period in the early stages of infection, but PrP^{Sc} is eventually found throughout the infected animal, with the highest concentrations found in the central nervous system (CNS). Amyloid prion plaques in the CNS are responsible for neuronal degeneration and encephalopathy, leading to eventual death.

No individuals appear to be immune to chronic wasting disease (Williams, 2005). However, there appears to be certain genetic polymorphisms in the *PrP* gene that result in increased susceptibility or decreased incubation period. White-tailed deer contain alleles coding for Glutamine (Q) or Histidine (H) at codon 95 and Glycine (G) or Serine (S) at codon 96 of the *PrP* gene. H and S were underrepresented in CWD+ individuals, suggesting that 95Q and 96G individuals are more susceptible to prion disease (Johnson et al. 2006; O'Rourke et al. 2004). There is a similar trend for susceptibility in elk at codon 132 (O'Rourke et al. 1999), where CWD+ individuals were more likely to be homozygous for the major allele than heterozygous or homozygous for the minor allele (Table 2.1).

Genetic variation in the *PrP* gene also affects prion disease incubation time (Table 2.1). Mule deer heterozygous at codon 225 for S and Phenylalanine (F) had longer incubation times compared to individuals homozygous for S (Jewell et al. 2005). Other *PrP* polymorphisms have been identified, but either have not yet been tested for association to prion disease or results have not been reported (Wik et al. 2012; Williams, 2005). For example, moose in Canada (*Alces alces andersonii*) contain a polymorphism at codon 36 coding for Threonine (T) or Asparagine (N), with individuals either being homozygous (T) or heterozygous (T/N) (Wik et al. 2012). These polymorphisms have not yet been tested for correlation with CWD because they have only been reported thus far in moose, and there have been few cases of CWD-infected moose.

Monitoring captive and free-ranging cervids genetically could provide useful information for management strategies in areas at risk for CWD. Several upper Midwest states near Minnesota (Iowa, Michigan, North Dakota, and Wisconsin) have recently reported cases of CWD in free-ranging deer (IADNR, 2015; MIDNR, 2015; NDGFD, 2015; WIDNR, 2015). Moose and other cervid species in Minnesota could be at risk of CWD infection if management strategies are unable to prevent its spread into the state. We investigated the *PrP* sequence in a subset of MN moose in order to (1) identify genetic polymorphisms in the *PrP* gene (2) determine if these genotypes have been associated with CWD in other ungulate populations, (3) identify what genotypes are present at specific locations known to be associated with CWD in other populations, and (4) determine how genetic polymorphisms are spatially distributed.

Methods

Study area

Minnesota

The northern Minnesota study area transitions from prairie in the west to mixed conifer-deciduous forests, bog, and swamp in the east. Northwestern (NW) MN is relatively flat and dominated by tallgrass prairie and agriculture. Northeastern (NE) MN is characterized by conifer-deciduous forest, and conifer bogs and swamps. Vegetation in the NE consists of white pine (*Pinus strobus*), red pine (*P. resinosa*), quaking aspen (*Populus tremuloides*), paper birch (*Betula papyrifera*), white spruce (*Picea glauca*), balsam fir (*Abies balsamea*), and white cedar (*Thuja occidentalis*). Numerous small lakes, peatlands, and wet forest are also present throughout much of the NE portion of the state. The north-central region is a transition zone between the semi-arid prairie in the NW and relatively humid mixed conifer-deciduous forest in the NE.

Samples

Blood (n=61), liver tissue (n=31), and fecal pellet (n=10) samples were provided by the Minnesota Department of Natural Resources (MNDNR) (Fig. 2.1a). The blood samples are from hunter harvest (n=24), GPS collared (n=31), and sick (n=6) moose from 2011-2013, and were stored on Whatman FTA® Classic Cards (Whatman International Ltd., Maidstone, UK) at room temperature. Liver tissue and pellet samples were from sick moose collected between 2009-2012 and 2004-2012, respectively, throughout northern MN, and were stored in -20°C freezers prior to DNA extraction. Samples were

categorized as sick or healthy based on observations by the MNDNR (Fig. 2.1b). Moose categorized as sick exhibited abnormal behaviors associated with neurological impairment, emaciation, and/or an inability to stand upright, and were either found dead (n=22) or were euthanized as a result of their observed condition (n=17). Date of sample collection, location, and estimated age of sample was provided for all samples, if known. Samples are males (n=53), females (n=43), and individuals of unknown sex (n=6).

DNA extraction and sex determination

Whole genomic DNA was extracted from blood, tissue, and pellet samples and sex was determined genetically using methods described in Chapter 1. DNA extraction success was determined by measuring DNA yield for each extraction and testing DNA amplification success of the primer pair SE47/SE48 (Brinkman & Hundertmark, 2009) using PCR and gel electrophoresis.

Amplification and sequencing

DNA from tissue and pellets was sequenced using primers 5'-GCT GAC ACC CTC TTT ATT TTG C-3' and 5'-GCA AGA AAT GAG ACA CCA CCA C-3', which span the prion protein gene (*PrP*) open reading frame (ORF). A MultiGene Optimax Gradient Thermal Cycler TC9610 was used for polymerase chain reaction (PCR) amplification with a total volume of 13 µl containing sterile water, GoTaq DNA polymerase and 1x GoTaq Flexi buffer (Promega Corporation, Madison, WI, USA), 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.08 µM forward primer, 0.8 µM reverse primer, 1% bovine serum albumin (BSA), and 1 µl/reaction DNA. Amplification conditions included the

following: initial denaturation at 94°C for 2 min, followed by 10 cycles with 94°C for 30 sec (denaturation), 64°C for 30 sec (annealing), and 72°C for 1 min (extension), with annealing temperature decreasing by 1°C each cycle, followed by 30 cycles with 94°C for 30 sec (denaturation), 54°C for 30 sec (annealing), and 72°C for 1 min (extension), and a final extension at 72°C for 10 min. DNA was quantified by visualizing PCR product on a 1% agarose gel and comparing it to Bioline Hyperladder I (Bioline, London, UK) using Quantity One v.4.6.6 software (Bio-Rad, Hercules, CA). DNA samples that failed to PCR amplify after two attempts were removed from further analysis. PCR products that successfully amplified were purified with ExoSAP-IT (USB Corporation, Cleveland, OH) using the manufacturer's protocol, amplified a second time to incorporate BigDye Terminator v3.1 (Thermo Scientific, Waltham, MA), and sent to the University of Minnesota Biomedical Genomics Center for Sanger sequencing using an ABI 3730xl sequencer (Applied Biosystems, Carlsbad, CA). We used Geneious (Biomatters Ltd, Neward, NJ) software to analyze sequence data, and compare the quality of the sequence reads between DNA extracted from blood, tissue, and pellet samples. Our sequences were aligned to a cervid sequence from previous work imported from GenBank (*Alces alces alces* JQ290077). In MN moose, we identified observed genotypes at polymorphic regions in the *PrP* gene that have been correlated with CWD in other populations (Table 2.1), and identified polymorphisms at other locations.

Spatial distribution and statistical analysis

Spatial distribution of polymorphisms in Minnesota moose was plotted in ArcMAP 10.1. A Fisher's exact test for independence was used to determine whether any polymorphisms found in the *PrP* gene were significantly different between sick and healthy moose.

Results

Extraction and PCR success

DNA quantification was successful for 100%, 84%, and 40% of the total samples from blood, tissue, and pellets, respectively. DNA from five tissue and six pellet samples were removed from further analysis due to poor PCR amplification success. Of the DNA samples that were sequenced, we detected no differences in sequence read quality between the three sample sources. An average of 90.1% (±2.2) of the bases in each sequence read obtained using DNA from blood, tissue, and pellet samples were high quality, based on base call confidence in Geneious.

Polymorphisms in PrP gene

A sequence analysis of the *PrP* gene from 90 free-ranging moose in Minnesota identified polymorphisms at codon 209. Individuals were either homozygous for methionine (MM) or isoleucine (II) or heterozygous (MI) (Table 8). The allele encoding methionine (M) was present with a frequency of 0.35, and the allele encoding isoleucine (I) was present with a frequency of 0.65. Genotype frequencies for MM, MI, and II were 0.14, 0.41, and 0.44, respectively, and were not significantly different from Hardy-

Weinberg equilibrium (χ^2_2= 0.41; p = 0.52) (Table 2.2). Additionally, two silent substitutions, reported in other moose populations (Wik et al. 2012), were found at codons 63 and 246.

MN comparison with polymorphic regions in other ungulate populations

Although we did not find additional polymorphisms, we identified genotypes present at positions associated with CWD in other populations (Table 2.3). Minnesota moose were fixed for Q, G, K, and S at codons 95, 96, 132, and 225, respectively. Moose in MN were also fixed for T at codon 36, a polymorphism that has not yet been tested for association to CWD.

PrP polymorphisms and MN moose health

Both sick and healthy moose contained genotypes 209MM, 209MI, and 209II (Fig. 2.2). Both sick and healthy moose contained the genotype that has been experimentally infected with prion disease in moose (209MM); however, this genotype was found at the lowest frequency (Fig. 2.3). There was no relationship between health and genotype at codon 209 of the *PrP* gene (Fisher's exact test; p = 0.415).

Discussion

A sequence analysis of the *PrP* ORF in Minnesota moose identified a polymorphism at codon 209. This polymorphism has been reported in North American moose in other regions (Baeten et al. 2007; Huson & Happ, 2006; Kreeger et al. 2006; Wik et al. 2012). Minnesota moose homozygous at codon 209 (209MM) had identical genotypes at the *PrP* ORF as deer with the highest susceptibility to CWD in other

populations (Johnson et al. 2006; O'Rourke et al. 2004). However, polymorphisms at codon 209 have not been rigorously tested for a correlation with CWD, primarily because most cervid species are fixed at this site.

Shiras moose homozygous at codon 209 (209MM) have been infected with CWD both naturally (Baeten et al. 2007) and experimentally (Kreeger et al. 2006). Kreeger et al. (2006) experimentally inoculated three moose that had identical genotypes to MN moose, including the polymorphism at codon 209 (MM, MI, and II). All three moose died within 567 days post inoculation from causes other than CWD; however, one moose (209MM) showed signs of TSE in lymphoid and nervous tissues. For moose, more information is essential for a better understanding of the relationship between genetic polymorphisms and CWD susceptibility.

Moose containing the genotype associated with increased susceptibility to CWD appeared in NE MN, but not in NW MN. While this may be the result of small and biased sampling, it is worth further study for several reasons. First, this would indicate that some moose in northeastern Minnesota could be more susceptible to CWD. Second, certain polymorphisms in the *PrP* gene may be linked to other illnesses, as was found in Scandinavian moose afflicted with Moose Wasting Syndrome (MWS) (Wik et al. 2012). While we did not identify a relationship between health and genotype in our samples, the small sample size may have affected our ability to detect such a relationship. In addition, sick moose were categorized as such based on several physical or behavioral observations, which would create difficulties in identifying a relationship to a single cause or condition. None of the moose used in our study had CWD (Butler et al. 2013;

Carstensen et al. 2014). Further investigation using additional samples would produce a finer scale spatial distribution of polymorphisms for codon 209, and more statistical power to explore potential patterns.

Minnesota is bordered by several states with CWD in free-ranging deer. The MNDNR is actively managing to prevent CWD from entering the state. CWD has been detected in three captive elk herds (two in 2002 and one in 2009), one captive white-tailed deer herd (2006), one captive European red deer herd (2012), and a single free-ranging white-tailed deer in 2010 (MNDNR, 2015a). The MNDNR developed a comprehensive CWD monitoring program for free-ranging white-tailed deer in 2002 (MNDNR, 2015b). This program involves targeted monitoring of wild deer for the presence of CWD. A detailed contingency plan was also created for managing the disease if it is detected in captive or free-ranging cervids.

In several areas, controlling CWD after it has been detected has proven to be difficult because horizontal transmission is so efficient (Miller & Williams, 2003; Saunders et al. 2012) and CWD is hard to identify in free-ranging individuals (Williams et al. 2002). Infectious prions are released into the environment through shedding from infected individuals (e.g. blood, saliva, and feces) or decomposing carcasses (Belay et al. 2004; Mathiason et al. 2006; Miller et al. 2004; Miller & Williams, 2003; Saunders et al. 2012; Tamgüney et al. 2009; Williams et al. 2002). Infectious prions have been found in areas years after infected animals were last found there (Miller et al. 2004). Once released into the environment, infectious prions persist by binding to soil particles. Prions can become more infectious through conformational changes that occur as a result of binding

with soil particles (Johnson et al. 2007). For these reasons, detection and removal of CWD+ individuals before the disease becomes established is critical.

Genetic techniques such as those used here are a first step in incorporating genetic information into disease management strategies. We can identify individuals or groups of individuals with increased susceptibility to CWD or potentially other disease phenotypes, as a way to understand what would occur if a disease were to spread. Further, we can use genetic information to predict where CWD will spread, thereby providing wildlife managers with a more precise area to conduct disease surveillance. This approach has already been used to predict the spread of CWD in white-tailed deer from Wisconsin to Iowa (Lang & Blanchong, 2012). Microsatellite and mtDNA data provided evidence for efficient gene flow between the two areas, despite being separated geographically by the Mississippi River. CWD was detected in a hunter harvested deer in Iowa in 2013, and three additional cases were found the following year in 2014 (IADNR, 2015).

Additionally, population genetic and natural history information can be used in combination to better understand which individuals may be more likely to facilitate disease persistence and spread. For example, local CWD persistence in mule deer in Canada is thought to be driven by female philopatry (Cullingham et al. 2011). However, male mule deer behavior (greater dispersal, seasonal movements during the rut, and certain courtship displays) may facilitate the spatial spread of CWD.

The Centers for Disease Control and Prevention predicts CWD will continue to spread into new areas (Saunders et al. 2012), highlighting the need for more research and continued population monitoring. We sequenced the *PrP* gene in moose using tissue,

blood, and pellets, showing that this analysis can be completed using noninvasive sampling techniques with low quantity and quality DNA. Because the potential for spread is a major concern for many wildlife diseases, the incorporation of genetic analyses using multiple sample types could be applicable to a wide range of diseases and study systems.

Table 2.1 Polymorphisms found in the prion protein gene (*PrP*) of cervid species and their association with chronic wasting disease (CWD).

Species	**Codon**	**Amino acid (major allele)**	**Amino acid (minor allele)**	**Disease phenotype (associated genotype)**
Moose[€]	36	Asparagine (N)	Threonine (T)	Not tested
Moose[€]	109	Lysine (K)	Glutamine (Q)	Linked to MWS in Sweden (KQ)
Moose[#]	209	Methionine (M)	Isoleucine (I)	Experimentally inoculated with CWD (MM)
Moose*	209	Methionine (M)	Isoleucine (I)	Natural case of CWD (MM)
Elk[¥‡]	132	Leucine (L)	Methionine (M)	Increased susceptibility (MM)
White-tailed deer[∏!!]	95	Glutamine (Q)	Histidine (H)	Increased susceptibility (QQ)
White-tailed deer[∏!!]	96	Glycine (G)	Serine (S)	Increased susceptibility (GG)
Caribou[φ]	225	Phenylalanine (F)	Serine (S)	Shorter incubation period (SS)
Mule deer[π]	225	Phenylalanine (F)	Serine (S)	Shorter incubation period (SS)

References: €Wik et al. 2012; #Kreeger et al. 2006; *Baeton et al. 2007; ¥White et al. 2010; ‡O'Rourke et al. 1999; ∏Kelly et al. 2008; !!O'Rourke et al. 2004; φHapp et al. 2007; πJewell et al. 2005.

Table 2.2 Genotype and frequency of polymorphism found at *PrP* codon 209 in Minnesota moose. Genotype frequencies were not significantly different from Hardy-Weinberg equilibrium ($\chi^2 = 0.414$, d.f. = 1, $p = 0.52$).

Genotype	n	Genotype frequency
MM	13	0.14
MI	37	0.41
II	40	0.45

Table 2.3 Polymorphisms in the PRNP sequence of five cervid species. Highlighted amino acids have been correlated with CWD susceptibility or shorter incubation length in other populations.

Species	Codon						
	36	95	96	109	132	209	225
Moose (this study)	T	Q	G	K	M	M/I	S
Moose[#€χ*]	N/T**	Q	G	**K/Q*** K**	M	M* **M**/I**	S
Elk[¥‡]	-	Q	G	-	L/**M**	M	S
White-tailed deer[∏!!]	**-**	**Q**/H	**G**/S	-	M	M	S
Caribou[φ]	-	Q	G	-	M	M	F/**S**
Mule deer[π]	-	Q	G	-	M	M	F/**S**

*European moose (*Alces alces alces*), **N. American moose (*Alces alces gigas, A.a.shirasii*)
References: €Wik et al. 2012; #Kreeger et al. 2006; χHuson and Happ 2006; *Baeton et al. 2007; ¥White et al. 2010; ‡O'Rourke et al. 1999; ∏Kelly et al. 2008; !!O'Rourke et al. 2004; φHapp et al. 2007; πJewell et al. 2005

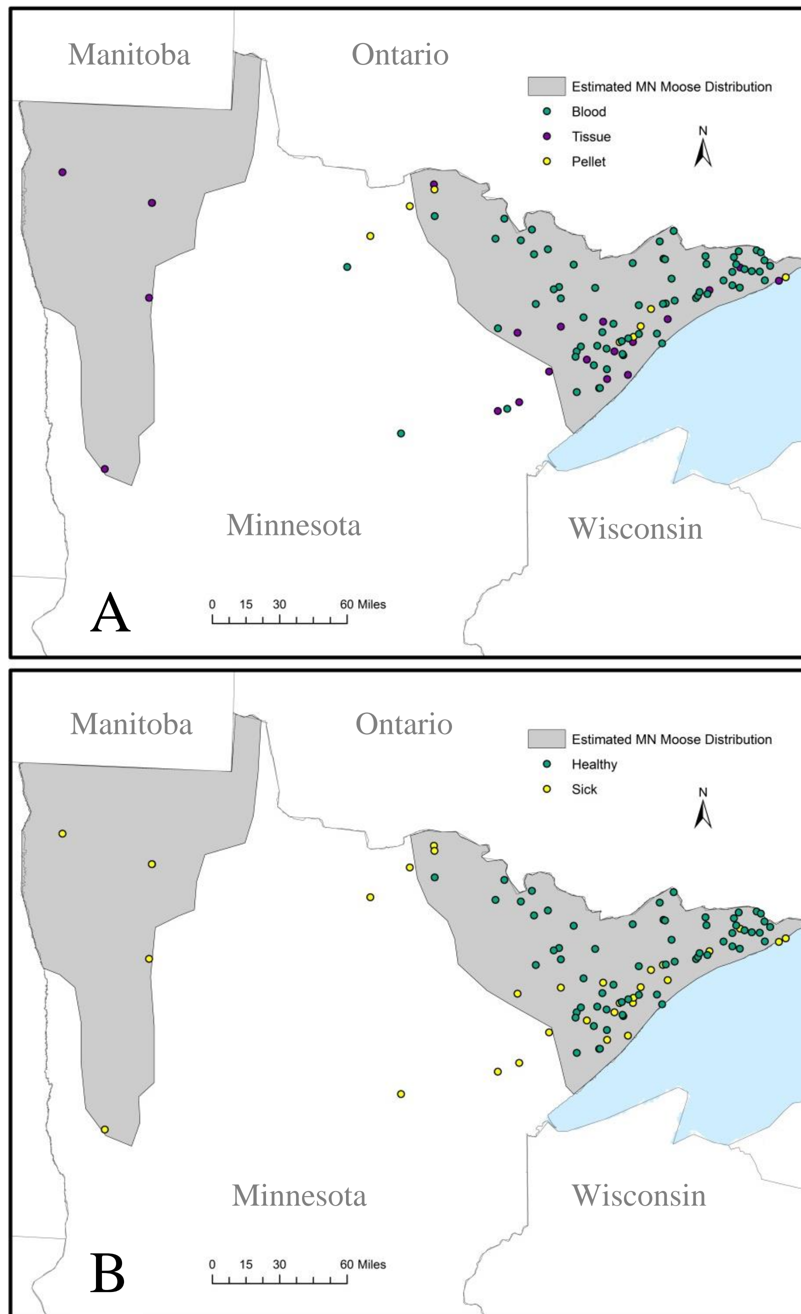


Figure 2.1 Location of moose samples in Minnesota sequenced at the prion protein gene (*PrP*). A) Location of samples by DNA source type: blood (n=61), tissue (n=31), and pellet (n=10). B) Location of samples further categorized as healthy or sick based on observations by the MNDNR. All moose samples have been tested for prion disease, and produced negative results.

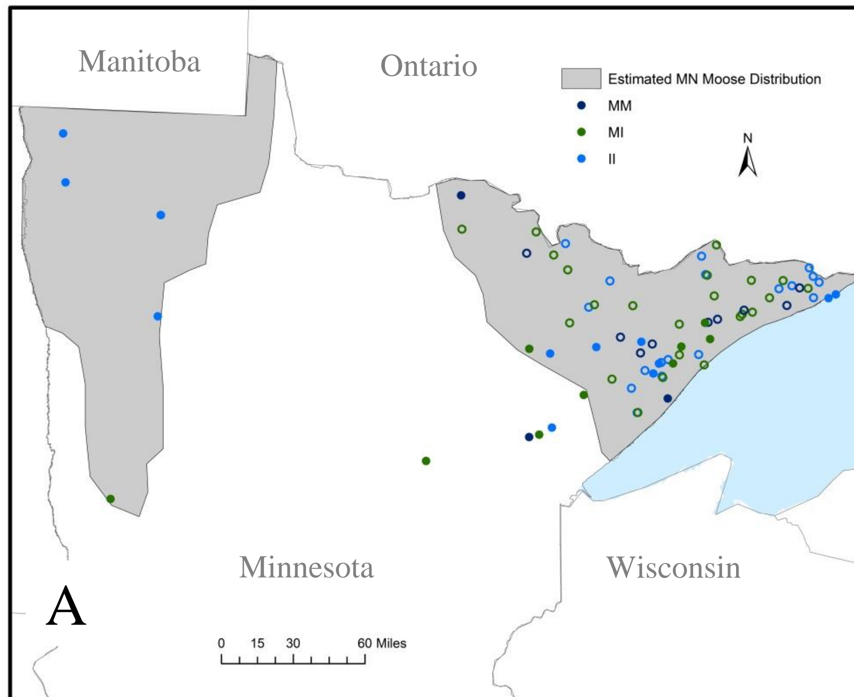


Figure 2.2 Distribution of polymorphic genotypes for sick (filled in circles) and healthy (hollow circles) moose in northern Minnesota. Moose were either homozygous for methionine (MM) or isoleucine (II) or heterozygous (MI). Sick moose were categorized as such for several observed conditions, but all tested negative for chronic wasting disease.

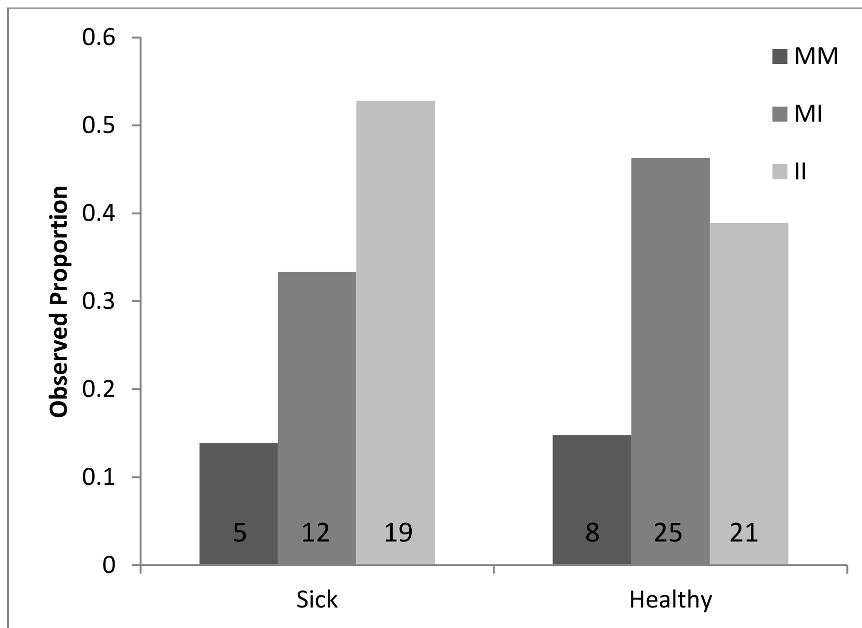


Figure 2.3 Observed genotypes for sick (n=36) and healthy (n=54) moose at codon 209 of the prion protein gene (*PrP*). Moose were either homozygous for methionine (MM) or isoleucine (II) or heterozygous (MI). The number of individuals with each genotype is represented within each corresponding bar. There was no association between health and genotype at this location (Fisher's exact test, $p = 0.415$).

Literature Cited

Aguzzi, A., & Polymenidou, M. 2004. Mammalian prion biology: one century of evolving concepts. *Cell*, *116*(2), 313–327.

Alaeddini, R., Walsh, S. J., & Abbas, A. 2010. Forensic implications of genetic analyses from degraded DNA—A review. *Forensic Science International: Genetics*, *4*(3), 148–157.

Baeten, L. a, Powers, B. E., Jewell, J. E., Spraker, T. R., & Miller, M. W. 2007. A natural case of chronic wasting disease in a free-ranging moose (*Alces alces shirasi*). *Journal of Wildlife Diseases*, *43*(2), 309–314.

Ball, M. C., Pither, R., Manseau, M., Clark, J., Petersen, S. D., Kingston, S., Morrill, N., & Wilson, P. 2007. Characterization of target nuclear DNA from faeces reduces technical issues associated with the assumptions of low-quality and quantity template. *Conservation Genetics*, *8*(3), 577–586.

Beja-Pereira, A., Oliveira, R., Alves, P. C., Schwartz, M. K., & Luikart, G. 2009. Advancing ecological understandings through technological transformations in noninvasive genetics. *Molecular Ecology Resources*, *9*(5), 1279–1301.

Belay, E. D., & Schonberger, L. B. 2005. The public health impact of prion diseases. *Annual Review of Public Health*, *26*, 191–212.

Belay, E. D., Maddox, R. A., Williams, E. S., Miller, M. W., Gambetti, P., & Schonberger, L. B. 2004. Chronic wasting disease and potential transmission to humans. *Emerging Infectious Diseases*, *10*(6), 977–984.

Brinkman, T. J., & Hundertmark, K. J. 2009. Sex identification of northern ungulates using low quality and quantity DNA. *Conservation Genetics*, *10*(4), 1189–1193.

Brinkman, T. J., Person, D. K., Chapin, F. S., Smith, W., & Hundertmark, K. J. 2011. Estimating abundance of Sitka black-tailed deer using DNA from fecal pellets. *Journal of Wildlife Management*, *75*(1), 232–242.

Brinkman, T. J., Person, D. K., Schwartz, M. K., Pilgrim, K. L., Colson, K. E., & Hundertmark, K. J. 2010a. Individual identification of sitka black-tailed deer (*Odocoileus hemionus sitkensis*) using DNA from fecal pellets. *Conservation Genetics Resources*, *2*(1), 115–118.

Brinkman, T. J., Schwartz, M. K., Person, D. K., Pilgrim, K. L., & Hundertmark, K. J.

2010b. Effects of time and rainfall on PCR success using DNA extracted from deer fecal pellets. *Conservation Genetics*, *11*(4), 1547–1552.

Broders, H. G., Mahoney, S. P., Montevecchi, W. A., & Davidson, W. S. 1999. Population genetic structure and the effect of founder events on the genetic variability of moose, *Alces alces*, in Canada. *Molecular Ecology*, *8*, 1309-1315.

Broquet, T., Menard, N., & Petit, E. 2007. Noninvasive population genetics: a review of sample source, diet, fragment length and microsatellite motif effects on amplification success and genotyping error rates. *Conservation Genetics*, *8*, 249-260.

Buś, M. M., & Allen, M. 2014. Collecting and preserving biological samples from challenging environments for DNA analysis. *Biopreservation and Biobanking*, *12*(1), 17–22.

Butler, E. A., Carstensen, M., Hildebrand, E., & Giudice, J. 2013. *Northeast Minnesota moose herd health assessment 2007-2012*. Minnesota Department of Natural Resources, St. Paul, MN, USA. <http://www.dnr.state.mn.us/publications/wildlife/research2012.html> Accessed 01 January 2015.

Carr, N. L., Rodgers, A. R., Kingston, S. R., Hettinga, P. N., Thompson, L. M., Renton, J. L., & Wilson, P. J. 2010. Comparative woodland caribou population surveys in Slate Islands Provincial Park, Ontario. *Rangifer*, *20*, 205-217.

Carstensen, M., Hildebrand, E. C., Pauly, D. C., Wright, R. G., & Dexter, M. H. 2014. Determining cause-specific mortality in Minnesota's northeast moose populations. Pages 133-143 in Cornicelli, L., Carstensen, M., Grund, M., Larsen, M., & Lawrence, J. Summaries of wildlife research findings, 2013. Minnesota Department of Natural Resources, Wildlife Populations and Research Unit, St. Paul, MN.

Collinge, J. 2001. Prion diseases of humans and animals: their causes and molecular basis. *Annual Review of Neuroscience*, *24*, 519–550.

Cronin, M. A., Patton, J. C., Courtois, R., & Crete, M. 2001. Genetic variation of microsatellite DNA in moose in Quebec. *Alces*, *37*(1), 175-187.

Dawson, M. N., Raskoff, K. A., & Jacobs, D. K. 1998. Field preservation of marine invertebrate tissue for DNA analyses. *Molecular Marine Biology and Biotechnology*, *7*(2), 145–152.

De Barba, M., & Waits, L. P. 2010. Multiplex pre-amplification for noninvasive genetic sampling: Is the extra effort worth it? *Molecular Ecology Resources*, *10*(4), 659–665.

DelGiudice, G. D., 2014. *2013 Aerial Moose Survey*. Minnesota Department of Natural Resources, St. Paul, MN, USA. <http://files.dnr.state.mn.us/recreation/hunting/moose /moose_survey_2013.pdf> Accessed 21 January 2014.

Ebert, C., Sandrini, J., Spielberger, B., Thiele, B., & Hohmann, U. 2012. Non-invasive genetic approaches for estimation of ungulate population size: a study on roe deer (*Capreolus capreolus*) based on faeces. *Animal Biodiversity and Conservation*, *35*, 267–275.

Estes-Zumpf, W. A., Zumpf, S. E., Rachlow, J. L., Adams, J. R., & Waits, L. P. 2014. Genetic Evidence Confirms the Presence of Pygmy Rabbits in Colorado. *Journal of Fish and Wildlife Management*, *5*(1), 118–123.

Finnegan, L. A., Wilson, P. J., Price, G. N., Lowe, S. J., Patterson, B. R., Fortin, M. J., & Murray, D. L. 2012. The complimentary role of genetic and ecological data in understanding population structure: a case study using moose (*Alces alces*). *European Journal of Wildlife Research*, *58*, 415–423.

Flagstad, O., RØed, K., Stagy, J. E., & Jakobsen, K. S. 1999. Reliable noninvasive genotyping based on excremental PCR of nuclear DNA purified with a magnetic bead protocol. *Molecular Ecology*, *8*(5), 879–883.

Gajdusek, D. C., & Zigas, V. 1959. Kuru; clinical, pathological and epidemiological study of an acute progressive degenerative disease of the central nervous system among natives of the Eastern Highlands of New Guinea. *The American Journal of Medicine*, *26*(3), 442–469.

Gross, J. E., & Miller, M. W. 2001. Chronic wasting disease in mule deer: disease dynamics and control. *The Journal of Wildlife Management*, *65*(2), 205–215.

Haanes, H., Roed, K. H., Solberg, E. J., Herfindal, I., & Saether, B. E. 2011. Genetic discontinuities in a continuously distributed mobile ungulate, the Norwegian moose. *Conservation Genetics*, *12*, 1131-1143.

Happ, G. M., Huson, H. J., Beckmen, K. B., & Kennedy, L. J. 2007. Prion protein genes in caribou from Alaska. *Journal of Wildlife Diseases*, *43*(2), 224–228.

Hartsough, G. R., & Burger, D. 1965. Encephalopathy of mink: 1. Epizootiologic and climical observations. *The Journal of Infectious Diseases*, *115*(4), 387–392.

Hedmark, E., & Ellegren, H. 2006. A test of the multiplex pre-amplification approach in microsatellite genotyping of wolverine faecal DNA. *Conservation Genetics*, *7*(2), 289–293.

Hettinga, P. N., Arnason, A. N., Manseau, M., Cross, D., Whaley, K., & Wilson, P. J. 2012. Estimating size and trend of the north interlake woodland caribou population using fecal-DNA and capturerecapture models. *Journal of Wildlife Management*, *76*(6), 1153–1164.

Hundertmark, K. J. 2009. Reduced genetic diversity in two introduced and isolated moose populations in Alaska. *Alces*, *45*, 137-142.

Huson, H. J., & Happ, C. M. 2006. Polymorphisms of the prion protein gene (PRNP) in Alaskan moose (*Alces alces gigas*). *Animal Genetics*, *37*(4), 425–426.

Iowa Department of Natural Resources (IADNR). 2015. *Chronic Wasting Disease*. <http://www.iowadnr.gov/Hunting/Deer-Hunting/CWD-EHD-Information> Accessed 21 January 2014.

Jewell, J. E., Conner, M. M., Wolfe, L. L., Miller, M. W., & Williams, E. S. 2005. Low frequency of *PrP* genotype 225SF among free-ranging mule deer (*Odocoileus hemionus*) with chronic wasting disease. *Journal of General Virology*, *86*(8), 2127–2134.

Johnson, C. J., Pedersen, J. A., Chappell, R. J., McKenzie, D., & Aiken, J. M. 2007. Oral transmissibility of prion disease is enhanced by binding to soil particles. *PLoS Pathogens*, *3*(7), 874–881.

Johnson, C., Johnson, J., Vanderloo, J. P., Keane, D., Aiken, J. M., & McKenzie, D. 2006. Prion protein polymorphisms in white-tailed deer influence susceptibility to chronic wasting disease. *Journal of General Virology*, *87*(7), 2109–2114.

Kelly, A. C., Mateus-Pinilla, N. E., Diffendorfer, J., Jewell, E., Ruiz, M. O., Killefer, J., Beissel, T., & Novakofski, J. 2008. Prion sequence polymorphisms and chronic wasting disease resistance in Illinois white-tailed deer (*Odocoileus virginianus*). *Prion*, *2*(1), 28–36.

Kreader, C. A. 1996. Relief of amplification inhibition in PCR with bovine serum albumin or T4 gene 32 protein. *Applied and Environmental Microbiology*, *62*(3), 1102–1106.

Kreeger, T. J., Montgomery, D. L., Jewell, J. E., Schultz, W., & Williams, E. S. 2006. Oral transmission of chronic wasting disease in captive Shira's moose. *Journal of Wildlife Diseases*, *42*(3), 640–645.

Lang, K. R., & Blanchong, J. A. 2012. Population genetic structure of white-tailed deer: Understanding risk of chronic wasting disease spread. *Journal of Wildlife Management*, *76*(4), 832–840.

Luikart, G., Zundel, S., Rioux, D., Miquel, C., Keating, K. A., Hogg, J. T., Steele, B., Foresman, K., & Taberlet, P. 2006. Low genotyping error rates and noninvasive samling in bighorn sheep. *Journal of Wildlidfe Mangement*, *72*(1), 299-304.

Mathiason, C. K., Powers, J. G., Dahmes, S. J., Osborn, D. A., Miller, K. V, Warren, R. J., Mason, G. L., Hays, S. A., Hayes-Klug, J. Seelig, D. M., Wild, M. A., Wolfe, L. L., Spraker, T. R., Miller, M. W., Sigurdson, C. J., Telling, G. C., & Hoover, E. A. 2006. Infectious prions in the saliva and blood of deer with chronic wasting disease. *Science*, *314*(5796), 133–136.

May, W. 1968. Creutzfeldt-Jakob Disease. *Acta Neurology Scandinavica*, *44*, 1–32.

Mckelvey, K. S., & Schwartz, M. K. 2004. Genetic errors associated with population estimation using non-invasive molecular tagging: problems and new solutions. *Journal of Wildlife Management*, *68*(3), 439–448.

Michigan Department of Natural Resources (MIDNR). 2015. *Chronic wasting disease (CWD)*. < http://www.michigan.gov/emergingdiseases/0,4579,7-186-25806---,00.html> Accessed 14 January 2014.

Miller, M. W., & Williams, E. S. 2003. Prion disease: horizontal prion transmission in mule deer. *Nature*, *425*(6953), 35–36.

Miller, M. W., Williams, E. S., Hobbs, N. T., & Wolfe, L. L. 2004. Environmental sources of prion transmission in mule deer. *Emerging Infectious Diseases*, *10*(6), 1003–1006.

Minnesota Department of Natural Resources (MNDNR). 2015a. *Managing chronic wasting disease*. <http://www.dnr.state.mn.us/hunting/deer/cwd/index.html> Accessed 21 January 2014.

Minnesota Department of Natural Resources (MNDNR). 2015b. *Chronic wasting disease response plan*. <http://files.dnr.state.mn.us/fish_wildlife/wildlife/disease/cwd/cwdresponseplan.pdf> Accessed 14 January 2014.

Moreno-Gonzalez, I., & Soto, C. 2011. Misfolded protein aggregates: mechanisms, structures and potential for disease transmission. *Seminars in Cell & Developmental Biology*, *22*(5), 482–487.

Murray, D. L., Hussey, K. F., Finnegan, L. A., Lowe, S. J., Price, G. N., Benson, J., Loveless, K. M., Middel, K. R., Mills, K., Potter, D., Silver, A., Fortin, M. J., Patterson, B. R., & Wilson, P. J. 2012. Assessment of the status and viability of a population of moose (*Alces alces*) at its southern range limit in Ontario. *Canadian Journal of Zoology*,

90, 422-434.

North Dakota Game and Fish Department (NDGFD). 2015. *Chronic wasting disease*. <http://gf.nd.gov/wildlife/fish-wildlife/wildlife-diseases/cwd> Accessed 14 January 2014.

O'Rourke, K. I., Besser, T. E., Miller, M. W., Cline, T. F., Spraker, T. R., Jenny, A. L., Wild, M. A., Zebarth, G. L, & Williams, E. S. 1999. PrP genotypes of captive and free-ranging Rocky Mountain elk (*Cervus elaphus nelsoni*) with chronic wasting disease. *Journal of General Virology*, *80*(10), 2765–2769.

O'Rourke, K. I., Spraker, T. R., Hamburg, L. K., Besser, T. E., Brayton, K. A., & Knowles, D. P. 2004. Polymorphisms in the prion precursor functional gene but not the pseudogene are associated with susceptibility to chronic wasting disease in white-tailed deer. *Journal of General Virology*, *85*(5), 1339–1346.

Paetkau, D. 2003. An empirical exploration of data quality in DNA-based population inventories. *Molecular Ecology*, *12*(6), 1375–1387.

Piggott, M. P. 2004. Effect of sample age and season of collection on the reliability of microsatellite genotyping of faecal DNA. *Wildlife Research*, *31*(5), 485–493.

Piggott, M. P., Bellemain, E., Taberlet, P., & Taylor, A. C. 2004. A multiplex pre-amplification method that significantly improves microsatellite amplification and error rates for faecal DNA in limiting conditions. *Conservation Genetics*, *5*, 417–420.

Poole, K. G., Reynolds, D. M., Mowat, G., & Paetkau, D. 2011. Estimating mountain goat abundance using DNA from fecal pellets. *Journal of Wildlife Management*, *75*(6), 1527–1534.

Prusiner, S. B. 1998. Prions. *Proceedings of the National Academy of Sciences*, *95*(23), 13363–13383.

Roed, K. H. and Midthjell L. 1998. Microsatellites in reindeer, *Rangifer tarandus*, and their use in other cervids. *Molecular Ecology*, *7*, 1771-1788.

Roon, D.A., Waits, L.P., Kendall, K. C. 2003. A quantitative evaluationi of two methods for preserving hair samples. *Molecular Ecology Notes*, *3*, 163–166.

Saunders, S. E., Bartelt-Hunt, S. L., & Bartz, J. C. 2012. Zoonotic potential of chronic wasting disease. *Emerging Infectious Diseases*, *18*(3).

Schmidt, J. I., Hundertmark, K. J., Bowyer, R. T., and McCracken, K. G. 2009. Population Structure and Genetic Diversity of Moose in Alaska. *Journal of Heredity*,

100(2), 170-180.

Schuelke, M. 2000. An economic method for the fluorescent labeling of PCR fragments. *Nature Biotechnology*, *18*(2), 233–234.

Sigurdson, C. J. 2008. A prion disease of cervids: chronic wasting disease. *Veterinary Research*, *39*(4), 1–12.

Taberlet, P., Griffin, S., Goossens, B., Questiau, S., Manceau, V., Escaravage, N., Waits, L., & Bouvet, J. 1996. Reliable genotyping of samples with very low DNA quantities using PCR. *Nucleic Acids Research*, *24*(16), 3189–3194.

Taberlet, P., Waits, L. P., & Luikart, G. 1999. Noninvasive genetic sampling: look before you leap. *Trends in Ecology and Evolution*, *14*(8), 323–327.

Tamgüney, G., Miller, M. W., Wolfe, L. L., Sirochman, T. M., Glidden, D. V, Palmer, C., Lemus, A., DeArmond, S. J., & Prusiner, S. B. 2009. Asymptomatic deer excrete infectious prions in faeces. *Nature*, *461*(7263), 529–532.

Valière, N., Bonenfant, C., Toïgo, C., Luikart, G., Gaillard, J. M., & Klein, F. 2007. Importance of a pilot study for non-invasive genetic sampling: Genotyping errors and population size estimation in red deer. *Conservation Genetics*, *8*(1), 69–78.

Waits, L., & Paetkau, D. 2005. Noninvasive genetic sampling tools for wildlife biologists: a review of applications and recommendations for accurate data collection. *Journal of Wildlife Management*, *69*(4), 1419–1433.

Wehausen, J. D., Ramey, R. R., & Epps, C. W. 2004. Experiments in DNA extraction and PCR amplification from bighorn sheep feces: The importance of DNA extraction method. *Journal of Heredity*, *95*(6), 503–509.

White, S. N., Spraker, T. R., Reynolds, J. O., & O'Rourke, K. I. 2010. Association analysis of PRNP gene region with chronic wasting disease in Rocky Mountain elk. *BMC Research Notes*, *3*(1), 314.

Wik, L., Mikko, S., Klingeborn, M., Simonsson, M., & Linne, T. 2012. Polymorphisms and variants in the prion protein sequence of European moose (*Alces alces*), reindeer (*Rangifer tarandus*), roe deer (*Capreolus capreolus*) and fallow deer (*Dama dama*) in Scandinavia. *Prion*, *6*(3), 1–5.

Williams, E. S. 2005. Chronic Wasting Disease. *Veterinary Pathology*, *42*, 530–549.

Williams, E. S., & Young, S. 1980. Chronic wasting disease of captive mule deer: a spongiform encephalopathy. *Journal of Wildlife Diseases*, *16*(1), 89–98.

Williams, E. S., Miller, M. W., Kreeger, T. J., Kahn, R. H., & Thorne, E. T. 2002. Chronic wasting disease of deer and elk: a review with recommendations for management. *Journal of Wildlife Management*, *66*(3), 551–563.

Wilson, P. J., Grewal, S., Rodgers, A., Rempel, R., Saquet, J., Hristienko, H., Burrows, F., Peterson, R., & White, B. N. 2003. Genetic variation and population structure of moose (*Alces alces*) at neutral and functional DNA loci. *Canadian Journal of Zoology*, *81*, 670-683.

Wilson, G. A., Strobeck, C., Wu, L., & Coffin, J. W. 1997. Characterization of microsatellite loci in caribou *Rangifer tarandus*, and their use in other artiodactyls. *Molecular Ecology*, *6*, 697-699.

Wisconsin Department of Natural Resources (WIDNR). 2015. *Chronic wasting disease*. <http://dnr.wi.gov/topic/wildlifehabitat/regulations.html > Accessed 14 January 2014.

Supplementary Materials

Moose DNA extraction protocol for FTA cards
Fermentas/Thermo Scientific GeneJET Whole Blood Genomic DNA Purification Kit (#K0782)

Before Starting:

1. Check to make sure all stocks of chemicals and supplies are available.
2. Turn on water bath (56°C)
3. Prepare extraction supplies
   Ethanol and kim-wipes
   Sterilized 4 mm hand punch
   Pipette and pipette tips
   1.5 mL microcentrifuge tubes
   Fermentas/Thermo Scientific GeneJET Whole Blood Genomic DNA Purification Kit

4. Label 2-1.5 mL microcentrifuge tubes (not included in kit) and 1 spin column (included in kit) for each sample with sample lab ID#.
5. Use sterile 4 mm hand punch to punch out one full dried blood drop and place into labeled microcentrifuge tube. Replace FTA card with remaining drop back into sample envelope and put in 'Done' sample bag. Sterilize hand punch with ethanol between samples.
6. Add 200 µl of 1x PBS and incubate 10 minutes at room temperature.
7. Add 20 µl of Proteinase K Solution to 200 µl of whole blood, mix by vortexing.
8. Add 400 µl of Lysis Solution, mix thoroughly by vortexing to obtain a uniform suspension.
9. Incubate sample at 56°C for 60 minutes, vortexing samples every 10 minutes.
10. Add 200 µl of (96-100%) ethanol and mix by pipetting.
11. Transfer the prepared mixture to labeled spin column. Centrifuge for 1 minute at 6,000 x g.
12. Discard the collection tube containing the flow-through solution. Place the column into a new 2 mL collection tube (included in kit).
13. Add 500 µl of Wash Buffer WB I (with ethanol added). Centrifuge for 1 minute at 8,000 x g. Discard the flow-through and place the column back into the collection tube.
14. Add 500 µl of Wash Buffer II (with ethanol added) to the column. Centrifuge for 3 minutes at 21,000 x g.
15. Empty the collection tube. Place the purification column back into the tube and re-spin the column for 1 minute at 21,000 x g.

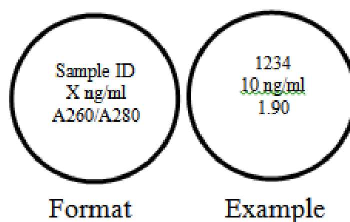
16. Discard the collection tube containing the flow-through solution and transfer the column to second 1.5 mL labeled, sterile microcentrifuge tube (not included in kit).
17. Add 100 µl of Elution Buffer to the center of the column membrane to elute genomic DNA. Incubate for 2 minutes at room temperature, then centrifuge for 1 minute at 8,000 x g.
18. Repeat previous step (add 100 µl Elution Buffer, incubate for 2 minutes, and centrifuge).
19. Discard the purification column.
20. Nanodrop the DNA sample. Record concentration and A260/A280 directly on sample label and in lab notebook.

Before you go

Enter lab IDs concentration, purity (A260/280 ratio), and other sample information into the extraction database.

Sterilize equipment used in extraction and put away all equipment and supplies.

Turn off hot bath.



Final 1.5 mL
microcentrifuge
tube label

Moose DNA extraction protocol for tissue
Thermo Scientific GeneJET Genomic DNA Purification Kit (#K0721)

Before Starting:

1. Check to make sure all stocks of chemicals and supplies are available.
2. Turn on water bath (56°C)
3. Prepare extraction supplies
    a. Forceps
    b. Razor blade
    c. Ethanol (EtOH)
    d. Cutting board
    e. 12 1.5 mL microcentirifuge tubes, labeled with sample IDs
    f. Digestion solution (from extraction kit)
    g. Proteinase K (from freezer)
4. Using forceps and a razor blade, cut and weigh 0.02 grams of muscle tissue and place into corresponding 1.5 mL microcentrifuge tube. Repeat for remaining samples, making sure to wipe down forceps, razor blade, and cutting board with ethanol between samples.
5. Write sample IDs on top of muscle tissue tubes, to indicate that they have been extracted.
6. Add 180 µl Digestion solution and 20 µl Proteinase K to each sample. Vortex thoroughly.
7. Incubate samples in 56°C water bath until the tissue is completely lysed and no particles remain (overnight).
8. Add 20 µl RNase A (from freezer) to each tube; mix by vortexing. Incubate for 10 minutes at room temperature.
9. While samples are incubating, gather the remaining supplies:
    a. 12 DNA purification columns (labeled with sample ID)
    b. 12 extra collection tubes
    c. Lysis solution
    d. Wash buffer I
    e. Wash buffer II
    f. Elution buffer
    g. 12 1.5 mL microcentrifuge tubes (labeled with sample ID)
    h. 50% EtOH
    i. 50 mL waste tube
10. Add 200 µl Lysis solution to each tube. Mix by vortexing for 15 seconds, until mixture is homogenous.

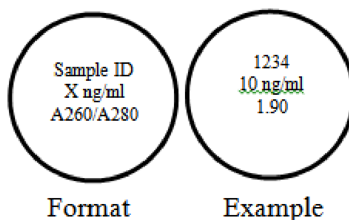
11. Add 400 µl 50% EtOH to each tube; mix by vortexing.
12. Transfer each lysate to the corresponding DNA purification column.
13. Centrifuge columns for 1 minute at 6,000 x g.
14. Discard the collection tubes containing the flow-through; keep the purification columns. Place each DNA purification column into a new 2 mL collection tube. Add 500 µl Wash buffer I.
15. Centrifuge columns for 1 minute at 8,000 x g.
16. Discard the flow-through into the waste tube using a pipette, and place DNA purification column back into the collection tube. Add 500 µl Wash buffer II.
17. Centrifuge columns for 3 minutes at 20,000 x g.
18. Transfer the DNA purification columns to sterile, labeled 1.5 mL microcentrifuge tubes. Discard collection tubes with flow-through.
19. Add 200 µl Elution buffer to each DNA purification column membrane. Incubate for 3 minutes at room temperature.
20. Centrifuge for 2 minutes at 8,000 x g.
21. Repeat elution and centrifuge steps (steps 19 and 20 above).
22. Discard the purification columns.
23. Use NanoDrop to determine concentration and purity of extractions. Record concentration and purity of each sample directly on sample label and in lab notebook.

Before you go

Enter lab IDs concentration, purity (A260/280 ratio), and other sample information into the extraction database.

Sterilize equipment used in extraction and put away all equipment and supplies.

Turn off hot bath.



Final 1.5 mL
microcentrifuge
tube label

Moose pellet DNA extraction protocol for whole pellets
Qiagen QIAamp DNA Stool Mini Kit (Cat. No. 51504)

Before Starting:

1. Check to make sure all stocks of chemicals and supplies are available
2. Check Buffer ASL for precipitate. If necessary, incubate in hot water bath until precipitate is dissolved
3. Turn on water bath (70°C)
4. Prepare extraction supplies
   Ethanol and kim-wipes
   Sterilized forceps
   10 mL pipette and bulb
   Pipette and pipette tips
   50 mL, 1.5 mL, and 2.0 mL microcentrifuge tubes
   Large and small racks inside hood
   Qiagen QIAamp DNA Stool Mini Kit
5. Determine how many extractions you are doing (up to 12). Select samples to extract and assign each sample a Lab ID (see YNP_Complete_Data.xls for correct Lab ID to assign. Lab IDs are unique and continuous). Record field and lab IDs in lab notebook.
6. Label 1-50 ml (P00_), 1-2 ml (P00_-X), 1-1.5 ml (P00_), 1-2 ml (P00_-K), 1-spin column (P00_), and 1-1.5 ml tube with Lab ID, date, and initials on top/Lab ID and Field ID on side.
7. Place InhibitEx tablet into tube labeled P00_-X. Do not touch tablet or set it on counter surface.
8. Pipette 25 µl Proteinase K into each tube labeled P00_-K. Qiagen Proteinase K stored in kit.

Pellet Extraction Procedure

9. Remove selected sample from freezer and bring to hood.
10. Record Field ID in lab book. Record initials, date, and assigned Lab ID on Field ID paper inside sample bag.
11. Open pellet sample and use sterile forceps to remove one pellet and place in corresponding 50 mL tube.
    Note: Select "loose" pellet, not one frozen to others. If all are frozen together, thaw enough so pellets will come apart, but do not thaw more than is absolutely necessary.
12. Re-close sample tightly and put remaining pellets back into freezer in 'Done' container.

13. Repeat steps 1-4 for all your pellet samples. Be sure to use sterilize instruments between samples with ethanol and kim-wipes.
14. Using 10 mL pipette, add enough Buffer ASL to cover the pellet (~4-5 mL).
15. Agitate samples for 1-2 minutes.
16. Incubate at 70°C for 5 minutes.
17. Pipette 1.4 mL (700 µl twice) of supernatant (liquid, not feces) to labeled 2 mL tube, containing InhibitEx tablet. Vortex immediately and continuously for at least 1 minute until the tablet is completely suspended.
18. Centrifuge samples at 13,500 rpm (17,500 x g) for 3 minutes to pellet stool particles and inhibitors bound to the tablet. The layer of liquid on top of the pellet particles will be used in further steps.

    Note: If pellet begins mixing with supernatant between steps (i.e. this step and the next, re-centrifuge).
19. Immediately after the centrifuge stops, pipette all (~650 µl) of the supernatant into a new labeled 1.5 mL microcentrifuge tube and discard the 2 mL tube with the pellet into the biohazard bag.
20. Centrifuge the samples at 13,500 rpm (17,500 x g) for 3 minutes.
21. Immediately after the centrifuge stops, pipet 600 µl supernatant from previous step into 2 mL tube containing 25 µl Proteinase K. Discard the 1.5 mL centrifuge tube into the biohazard bag.
22. Add 600 µl Buffer AL and vortex for 15 seconds.
23. Incubate samples at 70°C for 10 minutes.
24. Centrifuge (quick spin) to pull liquid down off the top of the lids.
25. Add 600 µl 100% ethanol and vortex for 10 seconds. The solution in the tube is called "lysate."
26. Add 600 µl lysate to the spin column (there will be some remaining lysate in tube – save for further steps). Centrifuge at 6,000 rpm (3,500 x g) for 1 minute.
27. Place the spin column in a new collection tube. Pour filtrate into initial 50 ml tube for later disposal. Discard the used collection tube into the biohazard bag.
28. Repeat two previous steps until all lysate is gone.
29. Add 500 µl Buffer AW1 to the spin column. Centrifuge at 6,000 rpm (3,500 x g) for 1 minute. Discard the filtrate and the old collection tube as before. If there is liquid remaining in spin column, spin the filter again.
30. Add 500 µl Buffer AW2 to the spin column. Centrifuge at 6,000 rpm for 1 minute, then 13,300 rpm (17,000 x g) for 2 minutes.
31. Transfer the spin column to a clean 1.5 mL centrifuge tube, with top cut off. (Cut top off directly before use, do not prepare beforehand).
32. Add 100 µl Buffer AE directly onto the filter. Incubate at 70°C for 5 minutes. Centrifuge at 13,500 rpm (17,500 x g) for 1 minute to elute the DNA.

Note: DO NOT DISCARD THE FILTRATE (liquid in the collection tube – this is the DNA).

33. Pipette the filtrate (from the collection tube – 100 μl) back onto the spin column. The second pass through the filter should increase yield. Incubate at 70°C for 5 minutes. Centrifuge at 13,500 rpm (17,500 x g) for one minute to elute the DNA. Discard the spin column in the biohazard bag.

    Note: DO NOT DISCARD THE FILTRATE (liquid in the collection tube – this is the DNA).

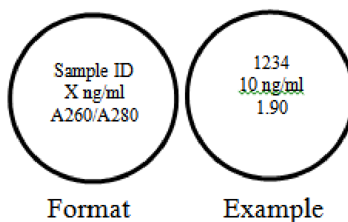
34. The filtrate in the 1.5 mL microcentrifuge tube is the DNA. Be sure the tube is correctly and completely labeled. Use NanoDrop to determine concentration and purity of extractions. Record concentration and purity of each sample directly on sample label and in lab notebook.
35. Store extracted DNA samples in appropriate box in freezer - labeled 'Yellowstone Moose DNA'.

Before you go

Enter field and lab IDs, concentration, A260/280 ratio, and other sample information into the extraction database before leaving.

Sterilize equipment used in extraction (forceps, inside hood) and put away all equipment and supplies.

Turn off hot bath and hood light.



Final 1.5 mL microcentrifuge tube label

Moose pellet DNA extraction protocol for sliced pellets
QIAamp Fast DNA Stool Kit (Cat. No. 51604)

Before Starting:

1. Ensure that Buffers AW1 and AW2 have been prepared according to the label instructions
2. Check InhibitEx Buffer and Buffer AL for precipitate. If necessary, incubate in hot water bath until precipitate is dissolved
3. Turn on water bath (70°C)
4. Prepare extraction supplies
   - Ethanol and kim-wipes
   - Sterilized forceps, razor, and cutting surface
   - Analytical scale and weigh paper
   - Pipette and pipette tips
   - 50 mL, 1.5 mL, and 2.0 mL microcentrifuge tubes
   - Cooler with ice
   - Large and small racks inside hood
   - QIAamp Fast DNA Stool Kit

Pellet Extraction Procedure

5. Remove sample from freezer. Remove single pellet from sample bag using forceps, and place on cutting surface.
6. Slice ~0.28 g of outer material from pellet using razor. Weigh using analytical balance, and place slices in 50 mL centrifuge tube using forceps once desired sample size is acquired. Discard remaining pellet in biohazard. Place centrifuge tube on ice. Replace remaining, unused pellets back in freezer.
7. Sterilize razor, forceps, and cutting surface using ethanol and kim-wipes. Repeat for remaining samples to be extracted.
8. Pipet 2.8 mL InhibitEx Buffer into each 50 mL centrifuge tube. Vortex for 1 minute until the stool sample is thoroughly homogenized. Centrifuge 50 mL tubes until bubbles/foam reduced.
9. Pipet 2 mL of lysate into a labeled 2 mL microcentrifuge tube. Centrifuge sample at 20,000x g for 1 minute to pellet any stool debris.
10. Pipet 20 µl proteinase K into a new, labeled 2 mL microcentrifuge tube. Pipet 200 µl supernatant from step 9 into the 2 mL microcentrifuge tube containing proteinase K.
11. Add 600 µl Buffer AL and vortex for 15 seconds.

12. Incubate at 70ºC for 10 minutes. Vortex for 5 seconds. Centrifuge tube briefly using minicentrifuge to remove drops from the inside of the tube lid.
13. Add 600 µl ethanol (96-100%) to the lysate, and mix by vortexing. Centrifuge tube briefly using minicentrifuge to remove drops from the inside of the tube lid.
14. Carefully apply 600 µl lysate from step 9 to the QIAamp spin column. Close the cap and centrifuge at 6,000 x g for 1 minute. Place the QIAamp spin column in a new 2 mL collection tube, and discard the tube containing the filtrate.
15. Repeat step 14 until all of the lysate has been loaded on the column.
16. Carefully open the QIAamp spin column and add 500 µl Buffer AW1. Centrifuge at full speed for 1 min. Place the QIAamp spin column in a new 2 mL collection tube, and discard the collection tube containing the filtrate.
17. Carefully open the QIAamp spin column and add 500 µl Buffer AW2. Centrifuge at >20,000 x g for 3 minutes. Discard the collection tube
containing the filtrate.
18. Place the QIAamp spin column in a new 2 mL collection tube (not provided) and discard the old collection tube with the filtrate. Centrifuge at 20,000 x g for 3 minutes.

This step helps to eliminate the chance of possible Buffer AW2 carryover.

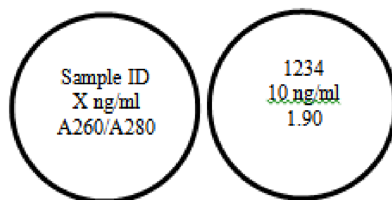
19. Transfer the QIAamp spin column into a new, labeled 1.5 mL microcentrifuge tube (not provided) and pipet 100 µl Buffer ATE directly onto the QIAamp membrane. Incubate for 2 minutes at room temperature, then centrifuge at full speed for 1 minute to elute DNA.
20. Pipet 100 µl of Buffer ATE back onto spin column membrane (the Buffer ATE that you just spun through), incubate for 2 minutes at room temperature, then centrifuge at full speed for 1 minute to elute DNA.
21. The filtrate in the 1.5 mL microcentrifuge tube is the DNA. Be sure the tube is correctly and completely labeled. Immediately NanoDrop samples using Buffer ATE to blank NanoDrop. Record results in lab notebook and in extraction database.
22. Store extracted DNA samples in appropriate box in freezer - labeled ‘Yellowstone Moose DNA’.

Before you go

Enter field and lab IDs, concentration, A260/280 ratio, and other sample information into the extraction database before leaving.

Sterilize equipment used in extraction (forceps, inside hood) and put away all equipment and supplies.

Turn off hot bath and hood light.



Final 1.5 mL
microcentrifuge
tube label