The Use of Molecular Markers in Wild Sheep Research in North America: A Review

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***Abstract*:** Molecular markers increasingly have been used in ecological research as new technologies have improved automation and lowered costs. Researchers in applied disciplines such as wildlife management and conservation biology have begun to utilize genetic tools to address questions that are difficult or impossible to answer with more traditional approaches. For wild sheep in particular, molecular markers such as allozymes, mitochondrial fragments or sequence data, and microsatellites or gene sequences from the nuclear genome have been used to characterize genetic diversity, define population structure, and investigate natural history, behavior, and evolution of these species across North America. We review the literature on the use of molecular markers in North American wild sheep research, discuss the role molecular markers may play in wild sheep research and management in the future, and provide a detailed list of mitochondrial and microsatellite markers that have been used successfully to elucidate various aspects of wild sheep ecology and conservation.

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Population declines in the 1800's and early 1900's dramatically reduced North American wild sheep populations occurring south of Canada, extirpating numerous local populations throughout the range of the species in the United States. Fortunately, reintroduction and translocation efforts, beginning in the early 1920's, largely have reestablished wild sheep throughout much of their historical range (Toweill and Geist 1999, Krausman 2000). However, the process of restoring wild sheep herds has not been easy and the success rate for individual translocations over the past 80 years has been estimated to be only about 50% (Rowland and Schmidt 1981, Risenhoover et al. 1988). The most cited problems faced by wild sheep, especially newly established populations, include disease transmission from livestock (namely domestic sheep; Onderka and Wishart 1984, Jessup 1985), inbreeding (Berwick 1968, DeForge et al. 1979, DeForge et al. 1981, Hass 1989), and fragmentation of native habitats once connected by corridors (Risenhoover et al. 1988, Epps et al. 2006).

Over the past few decades, modern genetic tools involving a variety of molecular markers from the mitochondrial and nuclear genomes increasingly have been used to assist wildlife biologists in addressing many critical conservation and management issues facing wild sheep in North America (Ramey 1995, Gutiérrez-Espeleta et al. 2001, Coltman et al. 2003). "Molecular marker" is a generic term used to describe a variety of DNA attributes that can be used to infer differences in the

genetic code at the molecular level. Over evolutionary timescales, mutations arise in the genetic code creating variations in the DNA sequence (Hedrick 2005). Mutation rates vary widely for different regions of DNA, thus offering opportunities to examine evolution at varying time scales by selectively examining molecular markers from regions of DNA with mutation rates pertinent to the time scale of interest (Avise 2004). By selectively utilizing molecular markers associated with evolutionary processes occurring at different rates, researchers can discern signatures of past mutational events that reveal information at the species, population, and even individual levels. Thus, geneticists are constantly striving to understand the forces acting to create genetic variation and to develop new molecular markers that more accurately quantify changes that have occurred in the DNA code.

Genetic diversity is a metric used to describe the amount of genetic variation revealed by a particular molecular marker at a specific level of biological resolution (e.g., species, population, individual). There is abundant theoretical (Lacy 1987, Lacy 1997) and empirical evidence (see review by Frankham 2005) supporting the concept that increased levels of genetic diversity are important for individual fitness and population persistence. The relative abundance or paucity of genetic diversity is of particular importance in the context of wild sheep conservation efforts, where populations created through reintroduction or translocation efforts often are small and may have been established using only a few individuals (Fitzsimmons et al. 1997, Ramey et al. 2000). The concern for wild sheep populations is driven by the fact that small populations are highly susceptible to genetic problems including founder effects (starting from few individuals), bottlenecks (passing through few reproducers), and genetic drift (loss of genetic diversity due to stochastic differences in reproductive success or survival among individuals; Frankham 2005). The negative impacts of reduced genetic diversity, especially for small populations, have been well documented (reviewed by O'Grady 2006).

Although molecular tools have been used to study questions in evolutionary biology for decades, the formal recognition that molecular markers can be used to address questions of a purely ecological nature is a remarkably recent phenomenon. For instance, *Molecular Ecology*, a periodical dedicated to publishing ecological investigations that used molecular markers, only printed its first edition in 1992. The increased use of molecular markers in ecological research has been fueled in part by technological and methodological advances that have improved automation and reduced costs associated with molecular genetic analyses and the discovery of new markers that can provide information content relevant to studies conducted at population and individual levels. For example, many early molecular markers used by geneticists were capable of quantifying genetic variation only at very coarse levels of resolution; useful only for investigating broad taxonomic relationships such as those occurring at the species or subspecies level. Alternatively, many molecular markers available today can be used for individual identification, allowing researchers to examine population attributes such as fine scale genetic structure and reproductive variance among individuals contributing genes to subsequent generations.

Our overall goal is to review the literature in which molecular markers have been used to study North American wild sheep: including, desert bighorns (*O. c. nelsoni*), California bighorns (*O. c. californiana*), Rocky Mountain bighorn (*O.*

c. canadensis), Dall's sheep (*O. dalli dalli*), and Stone's sheep (*O. d. stonei*). When reviewing the progressive integration of genetic markers into management and conservation issues pertaining to wild sheep, a logical approach is to follow the sequence of development for the genetic markers used in such investigations. Thus, we have structured our review into a temporal sequence beginning with research utilizing the structural conformation of proteins to infer underlying patterns of diversity in nuclear DNA, moving to studies utilizing variation in the DNA sequence of the mitochondrial genome, and finishing with research employing a variety of molecular markers based on DNA variation in the nuclear genome. We also discuss several potential growth areas for the future use of molecular markers in wild sheep research and management. Finally, we provide an extensive list of microsatellite and mitochondrial markers (along with their primer sequences, annealing temperatures, and approximate sizes) that have been used successfully in wild sheep research in North America (Appendix).

Proteins

Protein electrophoresis was one of the earliest molecular methods adopted for genetic evaluation of wildlife populations. Through the cellular processes of transcription and translation, DNA from specific nuclear genes acts as a blueprint by which amino acids are assembled into proteins. In the laboratory, small amounts of soft tissue (such as liver) are homogenized to create a mix of cellular contents containing the proteins of interest: usually an enzyme (an assembly of several proteins) that acts to carry out cellular processes. Homogenized tissue from different individuals are placed side-by-side in a semi-solid medium such as a thin sheet of starch or agarose-based gel. Enzymes constructed from even slightly different sequences of DNA will vary in their shape and/or charge and will migrate through the medium at different rates during electrophoresis. After a specified length of time, the electrophoresis is stopped and the medium is soaked in a chemical solution designed to visibly dye the particular enzyme of interest (called an allozyme). Recipes for staining more than a hundred allozymes are available in the literature. The process results in a pattern of dark bands corresponding to the different conformations of the protein produced from the individual's DNA. Because proteins examined using electrophoresis of allozymes are products of nuclear genes, the underlying DNA sequences for the genes are biparentally inherited and are expressed as genotypes representing the contribution of one allele from each parent. A genotype is constructed for each individual based on the number and configuration of bands, and the relative distance they migrated through the medium. Thus, separating and visualizing the protein products from an individual is an effective method to document the underlying genetic diversity which created the different protein forms.

Allozymes reflect the functional product of one or more genes and are subject to selection when the genetic code changes enough to result in a different protein conformation. It is generally held that the vast majority of gene-code mutations that could result in a change in enzyme conformation are deleterious. Thus, selection is thought to reduce variation in allozyme conformation. Mutations that do not alter the conformation of the enzyme, called silent mutations, are retained in the genome, but this genetic variation is undetectable using allozymes. Therefore, allozymes have a lower resolution than other molecular markers. For this reason, studies

using allozymes are generally limited to identifying patterns of genetic variation at or above the population level. In wild sheep research, allozymes have been used to address questions related to 1) phylogeny and 2) the effect of reintroductions and harvest on genetic variation.

Phylogeny.--Sage and Wolff (1986) used allozymes to substantiate the hypothesis that glacial events reduced species wide genetic variability in Dall's sheep and other North American mammals. Jessup and Ramey (1995) also used allozymes to test the validity of sub-specific boundaries for bighorn sheep established from morphological characters. In a meta-analysis of studies using allozymes, Tiedman et al. (1996) found that the proportion of polymorphic loci and the ratio of heterozygosity to the proportion of polymorphic loci within species were predicted by body size, feeding type (carnivory vs. herbivory), mating system, and the geographic distribution of the mammalian species investigated. These papers illustrate the utility of allozymes for addressing questions at the scale of subspecies or higher.

Reintroductions and harvest.--Persistence of bighorn sheep herds following reintroduction is a major concern in the ongoing effort to repopulate vacant sheep habitats (Berger 1990, Wehausen 1999). Small numbers of founding individuals (i.e., mean=15.2, SD=10.6 for 611 translocations of Rocky Mountain bighorn sheep calculated from Ramey 1993) involved in reintroductions coupled with low success rates for establishing new herds (Risenhoover 1988) has raised concerns about the role of founder effects and genetic bottlenecks in the persistence of these new populations. Fitzsimmons et al. (1997) used allozymes to document reduced heterozygosity relative to source populations in 3 of 4 reintroduced herds in Wyoming. Ramey et al. (2000) used allozymes (and other molecular markers) to investigate the possibility of a genetic bottleneck following reintroduction of bighorn sheep to the Badlands National Park. Luikart et al. (1998) used allozyme data from mountain sheep to test different methods for detecting genetic bottlenecks within populations. In a study documenting a positive correlation between allozyme heterozygosity and horn size in bighorn rams, Fitzsimmons et al. (1995) described the potential for loss of heterozygosity in small populations where large horned rams, the demographic group that was most heterozygous, are selectively harvested. In these studies, genetic data derived from allozymes were useful in describing genetic changes resulting from management activities, but also for informing potential management strategies for the remediation of those changes. Although analysis of allozymes using protein electrophoresis is a powerful tool for describing genetic variation, advances in technology and molecular methods have greatly reduced the use of this technique.

Mitochondrial DNA

Mitochondria are the source of energy for animal cells. They are located in the cell's cytoplasm separate from the nucleus and contain their own circular piece of DNA – almost all of which is functional (as opposed to non-coding). Because mitochondria are located in the cytoplasm and replicate via their own DNA, the only way for an offspring to acquire mitochondria is via the egg supplied by their mother. Sperm from the father usually contain little else besides nuclear DNA. Therefore, molecular markers based on mitochondrial DNA are maternally inherited, as opposed to the biparental inheritance that occurs with nuclear molecular markers.

Because of maternal inheritance and the highly conserved nature of many of the genes located in the mitochondrial genome, mitochondrial markers often can be used to resolve relationships spanning very long time periods and are relevant when considering questions of phylogenetic and taxonomic importance. In wild sheep research, mitochondrial markers have been used to 1) identify subspecies and other taxonomic relationships and 2) to describe genetically meaningful management units to facilitate conservation efforts.

Taxonomic relationships.--Describing taxonomic boundaries is important for mountain sheep conservation because conservation funding and management efforts usually are allocated relative to taxonomic designations. Ramey (1995), in the first published use of mitochondrial DNA markers in the wild sheep literature, questioned the validity of subspecific boundaries based on morphological data as established by Cowan (1940). Additionally, his results suggested strict philopatry among bighorn ewes as evidenced by haplotype differences between proximal habitats. Loehr et al. (2006), using DNA sequence data from the mitochondrial genome, found patterns of mitochondrial variation indicating a previously unsuspected glacial refuge for Dall's sheep in British Columbia, Canada, which they hypothesized may have been the source population from which Dall's sheep recolonized available habitats after the ice sheets retreated. In another recent paper, Latch et al. (2006) used mitochondrial DNA sequence data to assign a naturally recolonizing herd to one of 2 subspecies in Arizona and recommended caution in translocation efforts to preserve subspecific integrity. Finally, Groves and Shields (1996) sequenced mitochondrial DNA from 9 species of wild sheep to develop a molecular phylogeny for the subfamily of all North American wild sheep: Caprinae.

Describing genetically meaningful management units.--Mitochondrial markers are powerful tools for detecting relationships at the subspecies level, but also can be useful at the population level when enough sequence diversity exists. Bleich et al. (1996) combined Ramey's (1995) dataset with an analysis of historic and current sheep distributions in California to inform conservation efforts and make recommendations on how management efforts could improve connectivity of current wild sheep metapopulation in California. Boyce et al. (1999) used data from mitochondrial markers to demonstrate female philopatry in desert bighorn sheep and contended that conservation efforts in the southwestern United States should focus on retaining unique haplotypes and promoting connectivity among populations where evidence supports the existence of historical gene flow. Luikart and Allendorf (1996) analyzed mitochondrial DNA variation throughout the range of Rocky Mountain bighorn sheep and described the frequency and distribution of haplotypes within and among populations of this subspecies across their entire range. They suggested that observed patterns resulted either from fragmentation of a previously undivided (in evolutionary time) metapopulation, or from current rates of gene flow high enough to prevent fixation of haplotypes within populations but also low enough to allow haplotype frequencies to differ among populations.

Nuclear DNA

Nuclear DNA is biparentally inherited and molecular markers based on nuclear DNA from specific gene coding or non-coding regions are expected to segregate in a mendellion fashion. Although

several types of molecular markers from the nuclear genome are available, short, tandem, repetitive regions within the nuclear genome known as microsatellites currently are the most popular molecular marker for ecological studies (Avise 2004). Microsatellites are relatively small (<500 base pairs), non-coding portions of the nuclear genome composed of a series of short repeats in the base pair sequence. Microsatellites were first described in the late 1980's (Jarne and Lagoda 1996), but it wasn't until PCR methods became automated that their utility as a molecular marker was fully realized.

Because microsatellites reside in non-coding regions of the nuclear genome, are biparentally inherited, and segregate in a mendellion fashion, each individual receives 1 copy of the microsatellite repeat (called an allele) from each parent for a total of 2 alleles. These alleles are specific to a particular microsatellite and occupy a particular location (called a locus) in the genome. Therefore, an individual will have 2 alleles at every microsatellite locus. The genotype at each microsatellite locus may be composed of two alleles of the same length (a homozygous genotype) or two alleles of different lengths (a heterozygous genotype). It is the physical structure of microsatellites that makes them useful to molecular ecologists: microsatellites have a much higher rate of mutation (10^{-3}-10^{-4}; Dietrich et al. 1992, Weissenbach et al. 1992) than other portions of the genome. The high mutation rate is likely results from their repetitive sequence causing mistakes such as slippage and unequal crossing over during DNA replication and meiosis, respectively. The relatively high mutation rate of microsatellite alleles results in large amounts of polymorphism at most microsatellite loci (i.e., lots of alleles of different lengths). It isn't atypical for a given microsatellite locus to exhibit >20 alleles in a single population. When large numbers of microsatellite loci (plural) are used to evaluate genetic parameters of individuals within and among populations—a typical population genetics study uses between a few and 20 loci—microsatellites provide incredibly powerful resolution for quantifying genetic diversity.

Microsatellites are ideal molecular markers to identify patterns of genetic variation within and among populations, and have been applied to numerous questions pertaining to wild sheep management such as: 1) identification of subspecies boundaries; 2) conservation of established, reintroduced, and harvested populations; 3) investigation of natural history traits that are difficult to measure by traditional means; and 4) characterization of genetic variation associated with disease resistance genes. As a consequence of the number of markers available for wild sheep and the ease of access to genetic samples, several geneticists also have used data sets from wild sheep populations to address theoretical aspects of microsatellite evolution; these studies only will be cited as their results are beyond the scope of this review (Forbes et al. 1995, Forbes and Hogg 1999, Kalinowski and Hedrick 2001).

Subspecies boundaries.--Accurate classification of subspecies is an imperative for the conservation of wild sheep because conservation funding and efforts often are allocated along taxonomic lines. Worley et al. (2004) described concordance between genetic classification of Stone's and Dall's sheep using microsatellites and the supported current classification as separate subspecies derived from morphological characters. Conversely, Gutiérrez-Espeleta et al. (1998) and Gutiérrez-Espeleta et al. (2000) did not find evidence to substantiate current boundaries for 3 putative subspecies of desert sheep (Mexican (*O. c. mexicana*), desert, and Peninsular (*O. c. cremnobates*)

bighorn sheep), suggesting that subspecies assignments based on morphology in desert sheep were inadequate.

Conserving established, reintroduced, and harvested populations.-- Identifying factors that decrease the potential for interpopulation gene flow is of singular importance in the conservation of mountain sheep populations. Decreased gene flow is problematic especially for mountain sheep because the widely dispersed, insular habitats they now occupy strongly suggests they occur in a metapopulation-like structure (Levins 1970, Bleich et al. 1996) where movement between relatively small, isolated herds is imperative to avoid loss of genetic diversity due to genetic drift and inbreeding. Drift and inbreeding can combine in small populations to reduce fitness in the short term (Lacy 1987, Keller and Waller 2002) and theoretically reduce evolutionary potential in the long term (Lacy 1997). Epps et al. (2005) used a combination of microsatellites and mitochondrial markers to infer greatly reduced gene flow between desert bighorn populations bisected by human-constructed barriers (e.g., major highways, urban development, etc.). Epps et al. (2006) also used microsatellites to further describe the importance of habitat connectivity in maintaining genetic diversity among 25 desert bighorn populations in the face of climate change.

While genetic analyses using molecular markers can inform ongoing conservation efforts for established populations of wild sheep, such analyses also may contribute to efforts targeted at repopulating vacant mountain sheep habitat through reintroductions. Reintroduction of wild sheep throughout historic ranges is an ongoing effort, and to be most effective, source populations with adequate levels of genetic diversity must be identified to avoid genetic complications in newly established populations. Hedrick et al. (2001) investigated the suitability of the Tiburon Island population of desert bighorn sheep for continued use as a source population in reintroduction efforts. The population was founded using 20 individuals from the mainland in 1975 and apparently increased in size rapidly. However, results of genetic analysis suggest the Tiburon Island population suffers from low genetic diversity, likely due to a founder effect, and that it should be used as a source herd for reintroductions only in combination with another herd containing greater genetic diversity (Hedrick et al. 2001). In another study examining suitability of populations for use as sources for wild sheep reintroductions, Boyce and Ostermann (2002) described genetic variation in two populations of desert bighorn sheep and determined one was inadequate as a source due to low genetic diversity. These investigations are exemplary of conservation efforts informed using molecular markers. Establishing new populations from genetically depauperate stock can only exacerbate the potential for genetic problems (e.g., drift and inbreeding) in reintroduced herds.

Whittaker et al. (2004) examined genetic diversity in 5 California bighorn sheep herds established largely by within-state translocation in Oregon and compared them to 1 herd in Nevada, established from putatively more diverse stock. They reported extremely low levels of genetic diversity for the Oregon herds compared with levels exhibited within the Nevada herd and proposed the use of provisional, experimental efforts to increase genetic diversity in 2 of the Oregon herds through genetic management (defined as management action intended to increase genetic diversity; Frankham et al. 2002) via supplementation of more genetically diverse individuals into those populations. In a

comprehensive assessment of the potential for genetic management to benefit Rocky Mountain bighorn sheep in the National Bison Range in Montana, USA, Hogg et al. (2006) analyzed a 25-year, pedigree-based data set supplemented using data from a suite of microsatellites. They documented increased fitness for outbred individuals measured by increased adult reproductive success, survival, and many other life-history traits, suggesting that genetic management efforts for wild sheep may be a viable means to enhance population persistence (Hogg et al. 2006).

Finally, Coltman et al. (2003) described phenotypic effects of ram harvest on a population of bighorn sheep inhabiting Ram Mountain in Alberta, Canada. They demonstrated, using a quantitative genetics approach (discussed in the next section), that over time selective harvest of rams with the highest genetic quality for traits such as weight and horn growth resulted in a population level decline in those traits (Coltman et al. 2003). This study exemplifies the power of studies using molecular markers, although, in most circumstances, the population under investigation will not have the resolution of demographic data available from Ram Mountain.

Investigating natural history traits.-- Some aspects of natural history are difficult or impossible to investigate without using molecular markers. For example, in avian species extra-pair paternity was believed a rarity before parentage analysis (based on data from molecular markers) revealed it to be relatively common (Birkhead and Møller 1992). To investigate mating behavior in 2 populations of Rocky Mountain bighorn sheep, Hogg and Forbes (1997) used microsatellite data to determine paternity of 142 lambs. Examination of microsatellite paternity assignments in conjunction with extensive field observations revealed a surprisingly high success rate (range, 28% to 47%) for the alternative mating tactic called coursing, suggesting a high cost (as exhibited by defensive lapses) associated with the traditional mating tactic called defending (Hogg and Forbes 1997). In a study that similarly used microsatellites for paternity assignment coupled with extensive behavioral observations, Coltman et al. (2002) documented age-specific differences in mating success in the Ram Mountain herd of bighorn sheep. They suggested selective pressures might change with age, with younger rams increasing reproductive fitness by participating in alternative mating tactics while older rams acquire increased reproductive fitness by having larger horns (Coltman et al. 2002). These investigations of wild sheep natural history demonstrate the utility of microsatellites: useful not only in population analyses, but also in analyses focusing in scale down to the individual.

Disease resistance.—Disease epidemics resulting from contact with domestic sheep are cited as a major cause for declines in North American wild sheep populations and continue to be problematic when domestic sheep occur in areas of bighorn sheep reintroductions (Buechner 1960, Onderka and Wishart 1984, Jessup 1985). While much work has focused on the diseases affecting wild sheep from a disease pathology perspective (Bunch et al. 1999), molecular markers also have provided insights into the susceptibility of wild sheep to disease. For example, Luikart et al. (2008a) demonstrated a negative relationship between heterozygosity and parasite load in a population of bighorn sheep that had undergone a recent bottleneck.

The major histocompatability complex (MHC) is a linked set of genes important for immune response in mammals. It functions to identify pathogens and mobilizes the immune system to destroy

them. MHC regions of the genome are highly variable and thought to confer greater disease resistance (e.g., in a functional sense, different sequences can recognize different pathogens; Hedrick 1994). Variability of disease resistance genes may be particularly important in the context of wild sheep conservation where small founding population sizes and reduced connectivity limit overall genetic variation in many populations.

A variety of techniques have been used for analysis of nuclear DNA involved in immune function in wild sheep, not all involving microsatellites. Although microsatellites are non-coding portions of DNA, they can be useful if they are located within or in close proximity to the gene or genes of interest, in this case the MHC. Molecular markers located close to (i.e., linked to) areas under selection will "hitchhike"; acting as though they too are under selection (Maynard Smith and Haigh 1974, Slatkin 1995). Therefore, microsatellites, normally thought of as neutral markers, when located next to sections of DNA under selection (such as MHC) should behave as a proxy for the variation expected in those genes under selection.

Boyce et al. (1997) used a MHC linked microsatellite, 2 non-linked microsatellite loci, and a restriction fragment length polymorphism (RFLP) analysis (an older form of molecular marker with low levels of resolution) of a MHC gene to investigate patterns of variation within the MHC relative to that observed in the 2 neutral microsatellites. They found no evidence for variation at the MHC gene beyond that observed in the 2 neutral microsatellite loci and concluded that strong selection had not been acting on the MHC gene in bighorn sheep (Boyce et al. 1997). However, unlike the more modern technique of directly sequencing genes—a process that identifies the underlying base-by-base code of DNA—analysis of RFLPs revealed only a small portion of the genetic variation present at the MHC gene.

Gutiérrez-Espeleta et al. (2001) attempted to address the shortcomings of Boyce et al. (1997) by sequencing all alleles identified in a region of the MHC similar to that investigated by Boyce et al. (1997) using a technique known as single strand conformational polymorphism analysis (SSCP) to identify alleles. SSCP analysis is used to separate single strand sequences of DNA that differ in their molecular conformation much like protein electrophoresis separates different protein conformations. The method is useful because sequencing only unique alleles identified through SSCP confers a cost savings relative to sequencing every individual. They found high levels of variation within the MHC and discounted the hypothesis that population declines of bighorn sheep in the United States were related to low disease resistance resulting from low MHC variation (Gutiérrez-Espeleta et al. 2001).

Finally, Worley et al. (2006) analyzed DNA sequence data from three separate immune-functioning regions and a suite of neutral microsatellites in an attempt to detect balancing selection on immunity genes. After accounting for variation observed in their suite of neutral microsatellites, they could not detect effects of selection on the immune-functioning genes and cautioned against interpretations pertaining to the magnitude of selection in maintaining levels of MHC variation within populations without the context provided by simultaneously analyzing neutral markers from the same individuals (Worley et al. 2006).

The Future of Molecular Markers in Wild Sheep Research

Research using molecular markers already has affected conservation and management of wild sheep in North America. The potential for future contributions of molecular genetics to wild sheep conservation and management will only increase as molecular methods become more accessible, cost effective, and practical. Several areas of molecular ecology that seem particularly ripe with applications for bighorn sheep management are noninvasive genetic sampling, quantitative genetics, and landscape genetics. Noninvasive genetic sampling (NGS) is the process of recovering DNA from animals without capturing, handling, or even necessarily observing them (Waits and Paetkau 2005). Typical sources of DNA used in wildlife studies involving NGS are plucked hair, feathers, and feces as opposed to the tissue or blood typically collected. The growing use of NGS to obtain DNA from rare or difficult to capture species has hastened development of effective sample storage (i.e., Frantzen et al. 1998, Piggott and Taylor 2003), extraction, and data screening (i.e., Roon et al. 2005) techniques to overcome two drawbacks associated with the use of NGS: the amount and quality of DNA generally is lower for samples collected noninvasively than for conventional samples. The promise of adapting NGS for collection of DNA for genetic analyses of wild sheep seems high. Wehausen et al. (2004) determined an effective method for extracting DNA from wild sheep fecal pellets and Luikart et al. (2008b) documented low error rates when using 18 microsatellites to genotype fecal samples from bighorn sheep. Effective extraction methods and lower error rates should allow NGS to become more prominent in wild sheep genetics research.

Quantitative genetics is a field in which researchers attempt to determine underlying contributions of specific genes or gene regions to morphological traits that vary quantitatively (i.e., height, weight, number of flowers, etc.). Previously this field was the dominion of animal breeders where complete pedigrees facilitated the determination of trait inheritance, or heritabilities. The primary application of quantitative genetics to animal breeding was for determining the potential for artificial selection (i.e., breeding programs) to produce a desired change in a specific trait. With the advent of molecular markers, complete pedigrees can be partially assembled via parentage analysis in wild populations. With data available from wild populations, researchers may be able to apply quantitative genetic methods to document the effects of natural selection (as opposed to artificial selection) on traits of interest. Pelletier et al. (2007) used data from the Ram Mountain bighorn sheep population where complete pedigrees were known from intense observation and paternity analysis was facilitated by microsatellites to address the evolutionary significance of body mass plasticity. Using this approach they found higher rates of recruitment among ewes with greater seasonal mass changes, suggesting selection favoring body mass plasticity (Pelletier et al. 2007). This is an example of classic quantitative genetics approach applied to a wild population of bighorn sheep with unusual levels of data. Where quantitative genetic methods likely exhibit the most potential to impact wild sheep management is in construction of marker-based estimates of heritability: estimates that do not require complete pedigrees. Coltman (2005) addressed this possibility with a large (n = 32) suite of microsatellites and was unable

to produce estimates consistent with those based on pedigrees. However, rigorous marker-based estimates of heritability may yet be possible as techniques such as genome mapping facilitate the construction of libraries of single nucleotide polymorphisms (SNP's; markers based on variants at a single nucleotide position). Some researchers feel SNP's have the potential to become the next molecular marker of choice for such applications in ecological studies (i.e., Seddon et al. 2005) because of their tractability for high-throughput analyses. This may be especially true for mountain sheep because of the relative ease with which molecular markers can be co-opted from those developed for domestic sheep. SNP's offer exciting possibilities for quantitative genetics and some ecological applications, but their utility may be limited in population genetics (Glaubitz et al. 2003, Schlötterer 2004), at least with current technologies and analytical methods.

Landscape genetics is the combination of landscape ecology and population genetics. Its application lies in the combination of spatially explicit biological and behavioral information with molecular data for the purpose of elucidating the relationship between biological and behavioral processes and genetic parameter estimates. Traditional approaches to population genetics require *a priori* identification of populations, whereas landscape genetics allows researchers to infer spatial genetic patterns using data from many individuals over large spatial scales without assigning preexisting population membership. Epps et al. (2007) applied landscape genetics to populations of desert bighorn sheep to document landscape features associated with gene flow between populations. Their analysis identified landscape features associated with corridors for and barriers to gene flow among desert bighorn populations and thus, can facilitate future efforts to maximize population connectivity. As new analytical methods are developed to facilitate landscape level analyses of molecular data, this field undoubtedly will increase its contributions to wild sheep conservation and management.

Literature Cited

Adamson, R., M. Logan, J. Kinnaird, G. Langsley, and R. Hall. 2000. Loss of Matrix Metalloproteinase 9 Activity in *Theileria annulata*-Attenuated Cells is at the Transcriptional Level and is associated with differentially Expressed AP-1 Species. Molecular and Biochemical Parasitology 106:51-61.

Avise, J. C. 2004. Molecular Markers, Natural History, and Evolution. Sinauer Associates, Sunderland, Massachusetts, USA.

Berger, J. 1990. Persistence of Different-sized Populations: An Empirical Assessment of Rapid Extinctions in Bighorn Sheep. Conservation Biology 4:91-98.

Berwick, S. H. 1968. Observations on the Decline of the Rock Creek, Montana, Population of Bighorn Sheep. M. S. thesis, University of Montana, Missoula.

Birkhead, T. R., and A. P. Møller. 1992. Sperm Competition in Birds: Evolutionary Causes and Consequences. Academic Press, London, United Kingdom.

Bishop, M. D., S. M. Kappes, J. W. Keele, R. T. Stone, S. L. F. Sunden, G. A. Hawkins, S. Solinas Toldo, R. Fries, M. D. Grosz, J. Yoo, and C. W. Beattie. 1994. A Genetic Linkage Map for Cattle. Genetics 136:619-639.

Blattman, A. N., and K. H. Beh. 1992. Dinucleotide Repeat Polymorphism within the Ovine Major Histocompatability Complex. Animal Genetics 23:392.

Bleich, V. C., J. D. Wehausen, R. R. Ramey II, and J. L. Rechel. 1996. Metapopulation Theory and Mountain Sheep: Implications for Conservation. Pages 353-373 *in* D. R. McCullough, editor. Metapopulations and Wildlife Conservation. Island Press, Washington, D.C., USA.

Boyce, W. and S. Ostermann. 2002. Genetic Consideration for Reintroducing Bighorn Sheep to the San Andres Mountains, New Mexico. Final Report to the United States Fish and Wildlife Service. Pages 1-17.

Boyce, W. M., P. W. Hedrick, N. E. Muggli-Crockett, S. Kalinowski, M. C. T. Penedo, and R. R. Ramey. 1997. Genetic Variation of Major Histocompatability Complex and Microsatellite Loci: A Comparison in Bighorn Sheep. Genetics 145:421-433.

Boyce, W. M., R. R. Ramey II, T. C. Rodwell, E. S. Rubin, and R. S. Singer. 1999. Population Subdivision among Desert Bighorn Sheep (*Ovis canadensis*) Ewes Revealed by Mitochondrial DNA Analysis. Molecular Ecology 8:99-106.

Buchanan, F. C., and A. M. Crawford. 1992a. Ovine Dinucleotide Repeat Polymorphism at the MAF209 Locus. Animal Genetics 23:183.

Buchanan, F. C., and A. M. Crawford. 1992b. Ovine Dinucleotide Repeat Polymorphism at the MAF33 Locus. Animal Genetics 23:186.

Buchanan, F. C., and A. M. Crawford. 1993. Ovine Microsatellites at the OarFCB11, OarFCB128, OarFCB193, OarFCB266 and OarFCB304 Loci. Animal Genetics 24:145.

Buchanan, F. C., L. J. Adams, R. P. Littlejohn, J. F. Maddox, and A. M. Crawford. 1994. Determination of Evolutionary Relationships among Sheep Breeds Using Microsatellites. Genomics 22:397-403.

Buchanan, F. C., P. A. Swarbrick, and A. M. Crawford. 1991. Ovine Dinucleotide Repeat Polymorphism at the MAF48 Locus. Animal Genetics 22:379-380.

Buchanan, F. C., P. A. Swarbrick, and A. M. Crawford. 1992. Ovine Dinucleotide Repeat Polymorphism at the MAF65 Locus. Animal Genetics 23:85.

Buechner, H. K. 1960. The Bighorn Sheep in the United States, Its Past, Present, and Future. Wildlife Monograph No. 4.

Bunch, T. D., W. M. Boyce, C. P. Hibler, W. R. Lance, T. R. Spraker, and E. S. Williams. 1999. Diseases of North American Wild Sheep. Pages 209-237 *in* R. Valdez and P. R. Krausman, editors. Mountain sheep of North America. University of Arizona Press, Tucson, USA.

Coltman, D. W. 2005. Testing Marker-based Estimates of Heritability in the Wild. Molecular Ecology 14:2593-2599.

Coltman, D. W., M. Festa-Bianchet, J. T. Jorgenson, and C. Strobeck. 2002. Age-dependent Sexual Selection in Bighorn Rams. Proceedings of the Royal Society B 269: 165-172.

Coltman, D. W., P. O'Donoghue, J. T. Jorgenson, J. T. Hogg, C. Strobeck, and M. Festa-Bianchet. 2003. Undesirable Evolutionary

Consequences of Trophy Hunting. Nature 246:655-658.

Cowan, I. M. 1940. The Distribution and Variation in the Native Sheep of North America. American Midland Naturalist 24:505-580.

Crawford, A. M., F. C. Buchanan, and P. A. Swarbrick. 1991. Ovine Dinucleotide Repeat Polymorphism at the MAF92 Locus. Animal Genetics 22:371-372.

DeForge, J. R., C. W. Jenner, A. J. Piechner, and G. W. Sudmeier. 1979. Decline of Bighorn Sheep (*Ovis canadensis*): The Genetic Implications. Desert Bighorn Council Transactions 23:63-66.

DeForge, J. R., C. W. Jenner, G. W. Sudmeier, R. L. Graham, and S. V. Segreto. 1981. The Loss of Two Populations of Desert Bighorn Sheep in California. Desert Bighorn Council Transactions 25:36-38.

de Gortari, M. J., B. A. Freking, S. M. Kappes, K. A. Leymaster, A. M. Crawford, R. T. Stone, and C. W. Beattie. 1997. Extensive Genomic Conservation of Cattle Microsatellite Heterozygosity in Sheep. Animal Genetics 28:274-290.

Dietrich, W., H. Katz, S. E. Lincoln, H. S. Shin, J. Friedman, N. C. Dracopoli, and E. S. Lander. 1992. A Genetic Map of the Mouse Suitable for Typing Intraspecific Crosses. Genetics 131:423-427.

Diez-Tascón, C., J. F. Maddox, K. G. Dodds, and A. M. Crawford. 2002. Ovine T-cell Receptor γ Genes Contain Polymorphic Microsatellites. Animal Genetics 33:468-470.

Ede, A. J., C. A. Pierson, and A. M. Crawford. 1995. Ovine Microsatellites at the OarCP9, OarCP16, OarCP20, OarCP21, OarCP23 and OarCP26 Loci. Animal Genetics 26:129-130.

Ede, A. J., C. A. Pierson, H. Henry, and A. M. Crawford. 1994. Ovine Microsatellites at the OarAE64, OarHH22, OarHH56, OarHH62 and OarVH4 Loci. Animal Genetics 25:51.

Ellegren, H., C. J. Davies, and L. Andersson. 1993. Strong Association between Polymorphisms in an Intronic Microsatellite and in the Coding Sequence of the *BoLA-DRB3* Gene: Implications for Microsatellite Stability and PCR-based *DRB3* Typing. Animal Genetics 24:269-275.

Epps, C. W., J. D. Wehausen, V. C. Bleich, S. G. Torres, and J. S. Brashares. 2007. Optimizing Dispersal and Corridor Models Using Landscape Genetics. Journal of Applied Ecology 44:714-724.

Epps, C. W., P. J. Palsbøll, J. D. Wehausen, G. K. Roderick, and D. R. McCullough. 2006. Elevation and Connectivity Define Genetic Refugia for Mountain Sheep as Climate Warms. Molecular Ecology 15:4295-4302.

Epps, C. W., P. J. Palsbøll, J. D. Weyhausen, G. K. Roderick, R. R. Ramey II, and D. R. McCullough. 2005. Highways Block Gene Flow and Cause a Rapid Decline in Genetic Diversity of Desert Bighorn Sheep. Ecology Letters 8:1029-1038.

Fitzsimmons, N. N., S. W. Buskirk, and M. H. Smith. 1995. Population History, Genetic Variability, and Horn Growth in Bighorn Sheep. Conservation Biology 9:314-323.

Fitzsimmons, N. N., S. W. Buskirk, and M. H. Smith. 1997. Genetic Changes in Reintroduced Rocky Mountain

Bighorn Sheep Populations. Journal of Wildlife Management 61:863-872.

Forbes, S. H. and J. T. Hogg. 1999. Assessing Population Structure at High Levels of Differentiation: Microsatellite Comparisons of Bighorn Sheep and Large Carnivores. Animal Conservation 2:223-233.

Forbes, S. H., J. T. Hogg, F. C. Buchanan, A. M. Crawford, and F. W. Allendorf. 1995. Microsatellite Evolution in Congeneric Mammals: Domestic and Bighorn Sheep. Molecular Biology and Evolution 12:1106-1113.

Frankham, R. 2005. Genetics and Extinction. Biological Conservation 126:131-140.

Frankham, R., J. D. Ballou, and D. A. Briscoe. 2002. Introduction to Conservation Genetics. Cambridge University, Cambridge, United Kingdom. 617 pp.

Frantzen, M. A., J. B. Silk, J. W. H. Ferguson, R. K. Wayne, and M. H. Kohn. 1998. Empirical Evaluation of Preservation Methods for Faecal DNA. Molecular Ecology 7:1423-1428.

Georges, M., and J. M. Massey. 1992. Polymorphic DNA Markers in Bovidae. Patent WO/1992/013102.

Glaubitz, J. C., O. E. Rhodes Jr., and J. A. DeWoody. 2003. Prospects for inferring pairwise relationships with single nucleotide polymorphisms. Molecular Ecology 12:1039-1047.

Groves, P. and G. F. Shields. 1996. Phylogenetics of the Caprinae Based on Cytochrome b Sequence. Molecular Phylogenetics and Evolution 5:467-476.

Gutiérrez-Espeleta, G. A., P. H. Hedrick, S. T. Kalinowski, D. Garrigan, and W. M. Boyce. 2001. Is the Decline of Desert Bighorn Sheep from Infectious Disease the Result of Low MHC Variation? Heredity 86:439-450.

Gutiérrez-Espeleta, G. A., S. T. Kalinowski, W. M. Boyce, and P. H. Hedrick. 2000. Genetic Variation and Population Structure in Desert Bighorn Sheep: Implications for Conservation. Conservation Genetics 1:3-15.

Gutiérrez-Espeleta, G. A., S. T. Kalinowski, W. M. Boyce, and P. H. Hedrick. 1998. Genetic Variation in Desert Bighorn Sheep. Transactions of the Desert Bighorn Council 42:1-10.

Hass, C. C. 1989. Bighorn Lamb Mortality: Predation, Inbreeding, and Population Effects. Canadian Journal of Zoology 67:699-705.

Hedrick, P. W. 1994. Evolutionary Genetics of the Major Histocompatability Complex. The American Naturalist 143:945-964

Hedrick, P. W. 2005. Genetics of Populations. Jones and Bartlett, Sudbury, Massachusetts, USA.

Hedrick, P. W., G. A. Gutiérrez-Espeleta, and R. N. Lee. 2001. Founder Effect in an Island Population of Bighorn Sheep. Molecular Ecology 10:851-857.

Henry, H. M., J. M. Penty, C. A. Pierson, and A. M. Crawford. 1993. Ovine Microsatellites at the OarHH35, OarHH41, OarHH44, OarHH47 and OarHH64 Loci. Animal Genetics 24:222.

Hogg, J. T. and S. H. Forbes. 1997. Mating in Bighorn Sheep: Frequent Male Reproduction via a High-risk "Unconventional" Tactic. Behavior, Ecology, and Sociobiology 41:33-48.

Hogg, J. T., S. H. Forbes, B. M. Steele, and G. Luikart. 2006. Genetic Rescue of an Insular Population of Large

Mammals. Proceedings of the Royal Society B 273:1491-1499.

Hulme, D. J., A. J. Smith, J. P. Silk, J. M. Redwin, and K. J. Beh. 1995. Polymorphic Sheep Microsatellites at the McM2, McM131, McM135, McM136, McM140, McM200, McM214, McM373, McM505, McM507, and McM512 Loci. Animal Genetics 26:365-374.

Jarne, P., and P. J. L. Lagoda. 1996. Microsatellites, from Molecules to Populations and Back. Trends in Ecology and Evolution 11:424-429.

Jessup, D. A. 1985. Diseases of Domestic Livestock which Threaten Bighorn Sheep Populations. Desert Bighorn Council Transactions 29:29-33.

Jessup, D. A., and R. R. Ramey II. 1995. Genetic Variation of Bighorn Sheep as Measured by Blood Protein Electrophoresis. Desert Bighorn Council Transactions 39:17-25.

Kalinowski, S. T., and P. W. Hedrick. 2001. Estimation of Linkage Disequilibrium for Loci with Multiple Alleles: Basic Approach and an Application using Data from Bighorn Sheep. Heredity 87:698-708.

Kato, Y., T. Kudo, and S. Sato. 1996. Cloning and Sequence Analysis of the Bovine Leukemia Inhibitory Factor (LIF) Gene. Animal Science and Technology 67:165-141.

Keller, L. F., and D. M. Waller. 2002. Inbreeding Effects in Wild Populations. Trends in Ecology and Evolution 17:230-241.

Krausman, P. R. 2000. An Introduction to the Restoration of Bighorn Sheep. Restoration Ecology 8:3-5.

Lacy, R. C. 1987. Loss of Genetic Diversity from Managed Populations: Interacting Effects of Drift, Mutation, Immigration, Selection, and Population Subdivision. Conservation Biology 1:143-158

Lacy, R. C. 1997. Importance of Genetic Variation to the Viability of Mammalian Populations. Journal of Mammalogy 78:320-335.

Latch, E. K., J. R. Heffelfinger, B. F. Wakeling, J. Hanna, D. Conrad, and O. E. Rhodes, Jr. 2006. Genetic Subspecies Identification of a recently Colonized Bighorn Sheep Population in Central Arizona. Managing Wildlife in the Southwest (in press)

Levins, R. 1970. Extinction. Pages 75-107 *in* M. Gestenhaber, editor. Some Mathematical Problems in Biology. American Mathematical Society, Providence, Rhode Island, USA.

Loehr, J., K. Worley, A. Grapputo, J. Carey, A. Veitch, and D. W. Coltman. 2006. Evidence for Cryptic Glacial Refugia from North American Mountain Sheep Mitochondrial DNA. Journal of Evolutionary Biology 19:419-430.

Lucy, M. C., G. S. Johnson, H. Shibuya, C. K. Boyd, and W. O. Herring. 1998. Rapid Communication: Polymorphic $(GT)_n$ Microsatellite in the Bovine Somatotropin Receptor Gene Promoter. Journal of Animal Science 76:2209-2210.

Luikart, G., and F. W. Allendorf. 1996. Mitochondrial-DNA Variation and Genetic-population Structure in Rocky Mountain Bighorn Sheep (*Ovis canadensis canadensis*). Journal of Mammology 77:109-123.

Luikart, G., K. Pilgrim, J. Visty, V. O. Ezenwa, and M. K. Schwartz. 2008a. Candidate Gene Microsatellite Variation is Associated with Parasitism in Wild

Bighorn Sheep. Biology Letters 4:228-231.

Luikart, G., S. Zundel, D. Rioux, C. Miquel, K. A. Keating, J. T. Hogg, B. Steele, K. Foresman, and P. Taberlet. 2008b. Low Genotyping Error Rates and Noninvasive Sampling in Bighorn Sheep. Journal of Wildlife Management 72:299-304.

Luikart, G., W. B. Sherwin, M. M. Steele, and F. W. Allendorf. 1998. Usefulness of Molecular Markers for Detecting Populations Bottlenecks via Monitoring Genetic Change. Molecular Ecology 7:963-974.

Maddox, J. F. 2002. Linkage Mapping of Microsatellites Associated with the Glycosylation Dependent Cell Adhesion Molecule (GLYCAM1) and Thyroid Hormone Receptor Alpha Chain (THRA) Genes to Ovine Chromosomes 3 and 11. Animal Genetics 33:240-242.

Maynard Smith, J., and J. Haigh. 1974. The Hitch-hiking Effect of a Favourable Gene. Genetical Research 23:23–35.

McLaren, R. J., G. R. Rogers, K. P. Davies, J. F. Maddox, and G. W. Montgomery. 1997. Linkage Mapping of Wool Keratin and Keratin-associated Protein Genes in Sheep. Mammalian Genome 8:938-940.

Moore, S. S., W. Barendse, K. T. Berger, S. M. Armitage, and D. J. S. Hetzel. 1992. Bovine and Ovine DNA Microsatellites from the EMBL and GENBANK Databases. Animal Genetics 23:463-467.

O'Grady, J. J., B. W. Brook, D. H. Reed, J. D. Ballou, D. W. Tonkyn, R. Frankham. 2006. Realistic Levels of Inbreeding Depression Strongly Affect Extinction Risk in Wild Populations. Biological Conservation 133:42-51.

Onderka, D. K., and W. D. Wishart. 1988. Experimental Contact Transmission of *Pasteurella haemolytica* from Clinically Normal Domestic Sheep causing Pneumonia in Rocky Mountain Bighorn Sheep. Journal of Wildlife Disease 24:663-667.

Pelletier, F., D. Réale, D. Garant, D. W. Coltman, and M. Festa-Bianchet. 2007. Selection on Heritable Seasonal Phenotypic Plasticity of Body Mass. Evolution 61:1969-1979.

Penty, J. M., H. M. Henry, A. J. Ede, and A. M. Crawford. 1993. Ovine Microsatellites at the OarAE16, OarAE54, Oar AE57, OarAE119 and OarAE129 Loci. Animal Genetics 24:219.

Piggott, M. P., and A. C. Taylor. 2003. Extensive Evaluation of Faecal Preservation and DNA Extraction Methods in Australian Native and Introduced Species. Australian Journal of Zoology 51:341-355.

Ramey, R. R., II. 1993. Evolutionary Genetics and Systematics of North American Mountain Sheep. PhD thesis, Cornell University, Ithaca, New York, USA.

Ramey, R. R., II, G. Luikart, and F. J. Singer. 2000. Genetic Bottlenecks Resulting from Restoration Efforts: The Case of Bighorn Sheep in Badlands National Park. Restoration Ecology 8:85-90.

Ramey, R. R. II. 1995. Mitochondrial DNA Variation, Population Structure, and Evolution of Mountain Sheep in the South-western United States and Mexico. Molecular Ecology 4:429-439.

Risenhoover, K. L., J. A. Bailey, and L. A. Wakelyn. 1988. Assessing the Rocky Mountain Bighorn Sheep

Management Problem. Wildlife Society Bulletin 16:346-352.

Roon, D. A., L. P. Waits, and K. C. Kendall. 2005. A Simulation Test of the Effectiveness of Several Methods for Error-checking Non-invasive Genetic Data. Animal Conservation 8:203-215.

Rowland, M. M., and J. L. Schmidt. 1981. Transplanting Desert Bighorn Sheep – A Review. Desert Bighorn Council Transactions 25:25-28.

Sage, R. D. and J. O. Wolff. 1986. Pleistocene glaciations, fluctuating ranges and low genetic variability in a large mammal (*Ovis dalli*). Evolution 40:1092-1095.

Schlötterer, C. 2004. The Evolution of Molecular Markers – Just a Matter of Fashion? Nature Reviews Genetics 5:63-69.

Seddon, J. M., H. G. Parker, E. A. Ostrander, and H. Ellegren. 2005. SNPs in Ecological and Conservation Studies: A Test in the Scandinavian Wolf Population. Molecular Ecology 14:503-511.

Slatkin, M. 1995. A Measure of Population Subdivision Based on Microsatellite Allele Frequencies. Genetics 139:457-462.

Steffen, P., A. Eggen, A. B. Dietz, J. E. Womack, G. Stranzinger, and R. Fries. 1993. Isolation and Mapping of Polymorphic Microsatellites in Cattle. Animal Genetics 24:121-124.

Swarbrick, P. A., F. C. Buchanan, and A. M. Crawford. 1991a. Ovine Dinucleotide Repeat Polymorphism at the MAF36 locus. Animal Genetics 22:377-378.

Swarbrick, P. A., F. C. Buchanan, and A. M. Crawford. 1991b. Ovine Dinucleotide Repeat Polymorphism at the MAF64 Locus. Animal Genetics 22:375-376.

Tate, M. L. 1997. Evolution of ruminant chromosomes. Dissertation, University of Otago, Dunedin, New Zealand.

Tiedemann, R., S. Hammer, F. Suchentrunk, and G. B. Hartl. 1996. Allozyme Variability in Medium-sized and Large Mammals: Determinants, Estimators, and Significance for Conservation. Biodiversity Letters 3:81-91.

Toweill, D. E., and V. Geist. 1999. Return of Royalty: Wild Sheep of North America. Boone and Crockett Club and Foundation for North American Wild Sheep, Missoula, Montana, USA.

Waits, L. P., and D. Paetkau. 2005. Noninvasive Genetic Sampling Tools for Wildlife Biologists: A Review of Applications and Recommendations for Accurate Data Collection. Journal of Wildlife Management 69:1419-1433.

Wehausen, J. D. 1999. Rapid Extinction of Mountain Sheep Populations Revisited. Conservation Biology 13:378-384.

Wehausen, J. D., R. R. Ramey II, and C. W. Epps. 2004. Experiments in DNA Extraction and PCR Amplification from Bighorn Sheep Feces: The Importance of DNA Extraction Method. Journal of Heredity 95:503-509.

Weissenbach, J., G. Gyapay, C. Dib, A. Vignal, J. Morissette, P. Millasseau, G. Vaysseix, and M. Lathrop. 1992. A Second-generation Linkage Map of the Human Genome. Nature 359:794-801.

Wilson, G. A., C. Strobeck, L. Wu, and J. W. Coffin. 1997. Characterization of Microsatellite Loci in Caribou *Rangifer tarandus*, and Their Use in Other Artiodactyls. Molecular

Ecology 6:697-699.

Whittaker, D. G., S. D. Ostermann, and W. M. Boyce. 2004. Genetic Variability of Reintroduced California Bighorn Sheep in Oregon. Journal of Wildlife Management 68:850-859.

Wood, N. J., and S. H. Phua. 1993. A Dinucleotide Repeat Polymorphism at the Adenylate Cyclase Activating Polypeptide Locus in Sheep. Animal Genetics 24:329.

Worley, K., C. Strobeck, S. Arthur, J. Carey, H. Schwantje, A. Veitch, and D. W. Coltman. 2004. Population Genetic Structure in North American Thinhorn Sheep (*Ovis dalli*). Molecular Ecology 13:2545-2556.

Worley, K., J. Carey, A. Veitch, and D. W. Coltman. 2006. Detecting the Signature of Selection on Immune Genes in Highly Structured Populations of Wild Sheep (*Ovis dalli*). Molecular Ecology 15:623-637.

Appendix. Microsatellite (nDNA) and mitochondrial (mtDNA) markers used in wild sheep research in North America. Primer sequences, annealing temperature, sizes, reference in which they were first used for wild sheep and the citation for their original description (if applicable) are listed. Microsatellite information is from the original description unless specified. Information for microsatellite TCRBV624 (Luikart et al. 2008b) was unavailable.

Locus	Forward Primer	Reverse Primer	T_A	Allele (bp) Min	Allele (bp) Max	Reference	Original Description
nDNA							
ADCYAP1	CCAGACGCCGACT TCGCCCGAGG	GCCTGAAGTCCACT GAGAAGAAAGGAG	60	85	115	Hogg et al. 2006	Wood and Phua 1993
BM1225[1]	TTTCTCAACAGAG GTGTCCAC	ACCCCTATCACCAT GCTCTG	54	245	259	Coltman et al. 2002	Bishop et al. 1994
BM1818[1]	AGCTGGGAATATA ACCAAAGG	AGTGCTTTCAAGGT CCATGC	54	257	273	Coltman et al. 2002	Bishop et al. 1994
BM203	GGGTGTGACATTTT GTTCCC	CTGCTCGCCACTAG TCCTTC	58	217	247	Boyce and Ostermann 2002	Bishop et al. 1994
BM4025	TCGAATGAACTTTT TTGGCC	CACTGACTATCTGA CTTTGGGC	50	140	230	Coltman et al. 2003	Bishop et al. 1994
BM4107	AGCCCCTGCTATTG TGTGAG	ATAGGCTTTGCATT GTTCAGG	55	144	178	Boyce and Ostermann 2002	Bishop et al. 1994
BM4505[1]	TTATCTTGGCTTCTG GGTGC	ATCTTCACTTGGGA TGCAGG	54	265	277	Coltman et al. 2002	Bishop et al. 1994
BM4513[1]	GCGCAAGTTTCCTC ATGC	GCGCAAGTTTCCTC ATGC	54	139	153	Coltman et al. 2002	Bishop et al. 1994
BM6506	GCACGTGGTAAAG AGATGGC	AGCAACTTGAGCA TGGCAC	58	199	217	Boyce and Ostermann 2002	Bishop et al. 1994
BM848[1]	TGGTTGGAAGGAA AACTTGG	CCTCTGCTCCTCAA GACAC	54	219	237	Coltman et al. 2002	Bishop et al. 1994
Locus	Forward Primer	Reverse Primer	T_A	Allele (bp) Min	Allele (bp) Max	Reference	Original Description
BMC1009	GCACCAGCAGAGA GGACATT	ACCGGCTATTGTCC ATCTTG	58	274	282	Boyce and Ostermann 2002	Bishop et al. 1994

Locus	Forward Primer	Reverse Primer	T_A	Allele (bp) Min	Allele (bp) Max	Reference	Original Description
BMC1222[1]	CCAATTTTGCAGATAAGAAAACA	CCTGAGTGTTCCTCCTGAGT	54	286	292	Coltman et al. 2002	de Gortari et al. 1997
CELB9[2]	TCACCTTAATATGGAGGCAGAAATA	GATGCATTTCAGATTATGGCTTATC	63	235	237	Boyce and Ostermann 2002	Tate 1997
CELJP15[2]	GGAAATACCTTATCTTTCATTCTTGACTGTGG	CCTTCTTCTCATTGCTAACTTATATTAAATATCC	63	151	157	Boyce and Ostermann 2002	J. Permberton, unpub. data
CELJP23	GAAAATCCAAGCGACAAAGG	CCGCAGAACAACTAAGCCCAAG	-	-	-	Boyce and Ostermann 2002	J. Permberton, unpub. data
DRB3	GAGAGTTTCACTGTGCAG	CGCGAATTCCCAGAGTGAGTGAAGTATCT	50	159	219	Boyce and Ostermann 2002	Ellegren et al. 1993
DS52 (ETH152)	TACTCGTAGGGCAGGCTGCCTG	GAGACCTCAGGGTTGGTGATCAG	55	190	210	Gutierrez-Espeleta et al. 1998	Steffen et al. 1993
GLYCAM1	CCTCGGTCCCAAGCTCCCTAT	GCTTGAGTCTGCCTTCTCTGGCT	58	165	215	Luikart et al. 2008	Maddox 2002
IRBP	GTATGATCACCTTCTATGCTTCC	CCCTAAATACTACCATCTAGAAG	55 - 65	286	290	Boyce and Ostermann 2002	Moore et al. 1992
KERA	GTACTGAACCAAATAGTACAGCAGCCAAT	GCATGGCAACCCACTCCAGTAT	63	172	196	Luikart et al. 2008a	J. F. Maddox, unpub. data
KRT2	GCCTGTAGGCGTGAGGGTTTT	AAGGGCCAAGAGTCATTCACAT	55	135	137	Luikart et al. 2008a	McLaren et al. 1997
LIF	CTGCAGGGCAAGTGATTGGATT	TCAGCCCTTGGGCGTCAGT	58	108	122	Luikart et al. 2008a	Kato et al. 1996
MAF209	TCATGCACTTAAGTATGTAGGATGCTG	GATCACAAAAAGTTGGATACAACCGTGG	63	109	135	Hedrick et al. 2001	Buchanan & Crawford 1992a
MAF33	GATCTTTGTTTCAATCTATTCCAATTTC	GATCATCTGAGTGTGAGTATATACAG	60	121	141	Hedrick et al. 2001	Buchanan & Crawford 1992b
MAF36	CATATACCTGGGAGGAATGCATTACG	TTGCAAAAGTTGGACACAATTGAGC	63	99	125	Hedrick et al. 2001	Swarbrick et al. 1991a
MAF48	GGAAACCAAAGCCACTTTTCAGATGC	AGACGTGACTGAGCAACTAAGTACG	50	122	138	Hedrick et al. 2001	Buchanan et al. 1991
MAF64	CTCATGGAATCAGACAAAAGGTAGG	AATAGACCATTCAGAGAAACGTTGAC	63	109	141	Coltman et al. 2003	Swarbrick et al. 1991b
MAF65	AAAGGCCAGAGTATGCAATTAGGAG	CCACTCCTCCTGAGAATATAACATG	60	123	135	Hedrick et al. 2001	Buchanan et al. 1992
MAF92	TAGAATGTCATGTTCTCAGCATTCCC	AACCCATGAATCATCTCTAACTAACTC	52	122	134	Coltman et al. 2003	Crawford et al. 1991
MCM527	GTCCATTGCCTCAAATCAATTC	AAACCACTTGACTACTCCCCAA	50	165	175	Coltman et al. 2003	Hulme et al. 1995
MMP9	CTTGCCTTCTCATGCTGGGACT	GTGAGGATAGCACTTGGTCTGGCT	58	189	205	Luikart et al. 2008a	Adamson et al. 2000
OarAE16[1]	CTTTTTAATGGCTCGGTAATATTCCTC	CATCAGAGGAATGGGTGAAGACGTGG	54	82	104	Coltman et al. 2002	Penty et al. 1993

Locus	Forward Primer	Reverse Primer	T_A	Allele (bp) Min	Max	Reference	Original Description
OarCP20	GATCCCCTGGAGG AGGAAACGG	GGCATTTCATGGCT TTAGCAGG	55	71	87	Hogg et al. 2006	Ede et al. 1995
OarCP26[1]	GGCCTAACAGAAT TCAGATGATGTTGC	GTCACCATACTGA CGGCTGGTTCC	54	131	163	Coltman et al. 2002	Ede et al. 1995
OarFCB11	GGCCTGAACTCAC AAGTTGATATATCT ATCAC	GCAAGCAGGTTCT TTACCACTAGCACC	63	121	143	Hedrick et al. 2001	Buchanan & Crawford 1993
OarFCB128	CAGCTGAGCAACTA AGACATACATGCG	ATTAAAGCATCTTC TCTTTATTTCCTCGC	60	99	131	Hedrick et al. 2001	Buchanan & Crawford 1993
OarFCB193	TTCATCTCAGACTG GGATTCAGAAAGG C	GCTTGGAAATAAC CCTCCTGCATCCC	65	104	118	Boyce and Ostermann 2002	Buchanan & Crawford 1993
OarFCB20	AAATGTGTTTAAGA TTCCATACAGTG	GGAAAACCCCCAT ATATACCTATAC	55	92	112	Hogg et al. 2006	Buchanan et al. 1994
OarFCB226	CTATATGTTGCCTTT CCCTTCCTGC	GTGAGTCCCATAG AGCATAAGCTC	63	119	153	Hogg et al. 2006	Buchanan et al. 1994
OarFCB266[1]	GGCTTTTCCACTAC GAAATGTATCCTC AC	GCTTGGAAATAACCC TCCTGCATCCC	54	88	100	Coltman et al. 2002	Buchanan & Crawford 1993
OarFCB304	CCCTAGGAGCTTTC AATAAAGAATCGG	CGCTGCTGTCAACTG GGTCAGGG	63	150	188	Hedrick et al. 2001	Buchanan & Crawford 1993
OarHH47	TTTATTGACAAACT CTCTTCCTAACTCC ACC	GTAGTTATTTAAAAA AATATCATACCTCTT AAGG	60	124	148	Hogg et al. 2006	Henry et al. 1993
OarHH62	TAATGAGTCAAACA CTACTGAGAGAC	AATATATAAAGAGAA AAGCTGGGGTGCC	62	114	138	Hogg et al. 2006	Ede et al. 1994
Locus	Forward Primer	Reverse Primer	T_A	Allele (bp) Min	Max	Reference	Original Description
OLADRBps	CTGCCAATGCAGAG ACACAAGA	GTCTGTCTCCTGTCTT GTCATC	62	273	295	Luikart et al. 2008a	Blattman & Beh 1992
RT9[1]	TGAAGTTTAATTTC CACTCT	CAGTCACTTTCATCC CACAT	54	118	140	Coltman et al. 2002	Wilson et al. 1997
SOMAb	GTGCTCTAATCTTTT CTGGTACCAGG	CCTCCCCAAATCAAT TACATTTTCTC	62	96	120	Luikart et al. 2008a	Lucy et al. 1998
TCRG4	AGAACAAATATCTG GAATGGTGATGCT	TGCTATAGGATGACA TGAAGGCAAAT	58	170	176	Luikart et al. 2008a	Diez-Tascón et al. 2002
TGLA116	GCACAGTAATAAGA GTGATGGCAGA	TGGAGAAGATTTGGC TGTGTACCCA	52	80	109	Ramey et al. 2000	Georges and Massey 1992
TGLA122[1]	CCCTCCTCCAGGTA AATCAGC	AATCACATGGCAAAT AAGTACATAC	54	134	150	Coltman et al. 2002	Georges and Massey 1992
TGLA126[1]	CTAATTTAGAATGA GAGAGGCTTCT	TTGGTCTCTATTCTCT GAATATTCC	54	116	124	Coltman et al. 2002	Georges and Massey 1992
TGLA137[2]	GTTGACTTGTTAAT CACTGACAGCC	CCTTAGACACACGTG AAGTCCAC	55	124	136	Ramey et al. 2000	Georges and Massey 1992
TGLA188	TCATCTGCCCTATTT	GATCTTTGCAAATGG	-	-	-	Ramey et	Georges

	TTTAATTCCAAACCTA	TATTTCTGATAAGGGGTTAAT				al. 2000	and Massey 1992
TGLA387[1]	CAAAGTCTTAGAATAAACTGGATGG	GTCCCTTTGTTTACTTTGATAAAAC	54	134	154	Coltman et al. 2002	Georges and Massey 1992
TGLA427	GCCACCTTCTCATCAACAAATCCATGCAAGCGTTCTCTGCATTATGCCGCTTTTCACTCACAAGTTTTATTTTTCACTAGAGAAGCACTTAGCCCAAATAAGACAATTTGCTGTGGAAC	CCTCACTGCAGTGCTCCTATTATGATAATGGGAATTTATGCACATGAGTATTT	-	-	-	Ramey et al 2000	Georges and Massey 1992
TGLA94	CATCAAAACAGTGAAGGATGATTGCCAG	CGAATCTCTTCTAGGGATTGAGACTGTG	52	125	135	Boyce and Ostermann 2002	Georges and Massey 1992

Locus	Forward Primer	Reverse Primer	Region	Size (bp)	Reference
mtDNA					
L14724/ H15149	CGAAGCTTGATATGAAAAACCATCGTTG	AAACTGCAGCCCCTCAGAATGATATTTGTCCTA	Cyt. B	969-983	Groves and Shields 1996
L14841/ H15149	AAAAAGCTTCCATCCAACATCTCAGCATGATGAAA	AAACTGCAGCCCCTCAGAATGATATTTGTCCTA	Cyt. B		Groves and Shields 1996
L15513/ H15915	CTAGGAGACCCTGACAACTA	AACTGCAGTCATCTCCGGTTTACAAGAC	Cyt. B		Groves and Shields 1996
L15069/ H15338	GCCTATACTACGGATCATACAC	CTGTTTCGTCCACCAAGAG	Cyt. B		Groves and Shields 1996
L15275/ H15608	GACAAAGCATCCCTCACCCG	TAGGCTAGAACTCCGCCTAG	Cyt. B		Groves and Shields 1996

Locus	Forward Primer	Reverse Primer	Region	Size (bp)	Reference
mtDNA					
-	AACCTCCCTAAGACTCAAGG	GTGTGAATTTGAGTATTGAGG	Control	515	Boyce et al. 1999
-	ACTTCCAAACATATAACAC	AGGATACGCATGTTGACTAG	Control		Boyce et al. 1999
-	TGGACATACGTAATTAATGG	GTAGACTCATCTAGGCAT	Control		Boyce et al. 1999
L15712/ BETH	AACCTCCCTAAGACTCAAGG	ATGGCCCTGAAGAAAGAACC	Control	515	Epps et al. 2005
L15999/ H16498	ACCATCAACACCCAAAGCTGA	CCTGAAGTAGGAACCAGATG	Control	604	Loehr et al. 2006

[1]Annealing temperature and allele sizes from wild sheep reference
[2]Annealing temperature and allele sizes as determined in the Rhodes Lab for a test set of 30 California bighorn sheep