JM
Journal of Mammalogy, 101(1):10–23, 2020
DOI:10.1093/jmammal/gyz163
Published online November 30, 2019

Phylogeography of moose in western North America

Nicholas J. DeCesare,* Byron V. Weckworth, Kristine L. Pilgrim, Andrew B. D. Walker, Eric J. Bergman, Kassidy E. Colson, Rob Corrigan, Richard B. Harris, Mark Hebblewhite, Brett R. Jesmer, Jesse R. Newby, Jason R. Smith, Rob B. Tether, Timothy P. Thomas, and Michael K. Schwartz

Montana Fish, Wildlife and Parks, Missoula, MT 59804, USA (NJD)
Panthera, New York, NY 10018, USA (BVW)
Rocky Mountain Research Station, United States Forest Service, Missoula, MT 59801, USA (KLP, MKS)
British Columbia Ministry of Forests, Lands, Natural Resource Operations and Rural Development, Penticton, British Columbia V2A 7C8, Canada (ABDW)
Colorado Parks and Wildlife, Fort Collins, CO 80526, USA (EJB)
Alaska Department of Fish and Game, Palmer, AK 99645, USA (KEC)
Alberta Environment and Parks, Edmonton, Alberta T5K 2M4, Canada (RC)
Washington Department of Fish and Wildlife, Olympia, WA 98501, USA (RBH)
University of Montana, Missoula, MT 59812, USA (MH)
University of Wyoming, Laramie, WY 82071, USA (BRJ)
Montana Fish, Wildlife and Parks, Kalispell, MT 59901, USA (JRN)
North Dakota Game and Fish Department, Jamestown, ND 58401, USA (JRS)
Saskatchewan Ministry of Environment, Meadow Lake, Saskatchewan S9X 1Y5, Canada (RBT)
Wyoming Game and Fish Department, Sheridan, WY 82801, USA (TPT)

* *Correspondent: ndecesare@mt.gov*

Subspecies designations within temperate species' ranges often reflect populations that were isolated by past continental glaciation, and glacial vicariance is believed to be a primary mechanism behind the diversification of several subspecies of North American cervids. We used genetics and the fossil record to study the phylogeography of three moose subspecies (*Alces alces andersoni*, *A. a. gigas*, and *A. a. shirasi*) in western North America. We sequenced the complete mitochondrial genome (16,341 base pairs; *n* = 60 moose) and genotyped 13 nuclear microsatellites (*n* = 253) to evaluate genetic variation among moose samples. We also reviewed the fossil record for detections of all North American cervids to comparatively assess the evidence for the existence of a southern refugial population of moose corresponding to *A. a. shirasi* during the last glacial maximum of the Pleistocene. Analysis of mtDNA molecular variance did not support distinct clades of moose corresponding to currently recognized subspecies, and mitogenomic haplotype phylogenies did not consistently distinguish individuals according to subspecies groupings. Analysis of population structure using microsatellite loci showed support for two to five clusters of moose, including the consistent distinction of a southern group of moose within the range of *A. a. shirasi*. We hypothesize that these microsatellite results reflect recent, not deep, divergence and may be confounded by a significant effect of geographic distance on gene flow across the region. Review of the fossil record showed no evidence of moose south of the Wisconsin ice age glaciers ≥ 15,000 years ago. We encourage the integration of our results with complementary analyses of phenotype data, such as morphometrics, originally used to delineate moose subspecies, for further evaluation of subspecies designations for North American moose.

Key words: dynamics, evolution, genome, mitome, northwestern, Shiras, taxonomy, Yellowstone

In North America, contemporary patterns of differentiation within many northern species include the underlying signature of glacial cycles that occurred during the Pleistocene (Shafer et al. 2010). During the last glacial maximum (LGM; 19,000–26,500 years ago), the Laurentide and Cordilleran ice sheets covered much of northern North America (Yokoyama

© 2019 American Society of Mammalogists, www.mammalogy.org

Downloaded from https://academic.oup.com/jmammal/article-abstract/101/1/10/5648221 by The University of Montana user on 24 February 2020

et al. 2000; Clark et al. 2009). This forced species into ice-free refugia, including Beringia and portions of the conterminous United States (Shafer et al. 2010). The current interglacial period began 10,000–15,000 years ago and with the melting of the ice came recolonization of the formerly ice-covered land and, in some cases, reconnection of previously isolated populations (Dyke 2004). Contiguous contemporary distributions of species may obscure separation during the Pleistocene, but closer attention to intra-species differentiation (e.g., subspecies) can reflect past glacial vicariance. As such, researchers have used both genetic approaches and the fossil record for phylogeographic reconstruction of intraspecific evolution.

Intra-species taxonomy, such as the designation of subspecies, has been enriched through the use of molecular tools (Wilson et al. 1985; Avise et al. 1987). Genetics-based phylogeographic studies of North American biota have been used to assess subspecies assemblages across many mammalian taxa, including the red fox (*Vulpes vulpes*—Aubry et al. 2009), black bear (*Ursus americanus*—Puckett et al. 2015), and Gunnison's prairie dog (*Cynomys gunnisoni*—Sackett et al. 2014), among others. Also, with the advent of genetic tools have come efforts to improve the rigor and consistency with which subspecies designations are determined and evaluated. Fossil records have also been used to test hypotheses concerning glacial refugia across taxa. Typically, such studies use the distribution of fossils dated to a particular time period (e.g., the LGM) to identify refugial populations and locales when ice sheets dominated much of the landscape (Sommer and Nadachowski 2006; Provan and Bennett 2008). More recently, fossil data have served as an additional line of evidence to complement genetic analyses of species evolution (Hewitt 2000; Provan and Bennett 2008).

Large herbivores, such as those in the family Cervidae, have been described as model species for studying glacial vicariance and post-glacial recolonization given the range restrictions they faced during the Pleistocene and their subsequent ability to quickly recolonize (Latch et al. 2009). In temperate North America, there are four genera of extant cervid species, three of which contain a single species (moose [*Alces*]¸ elk [*Cervus*], and caribou [*Rangifer*]), while the fourth (*Odocoileus*) includes two species (mule deer [*O. hemionus*] and white-tailed deer [*O. virginianus*]). All five extant North American cervid species are further divided into subspecies, with strong phylogeographic support in some cases. For example, the distinction between woodland caribou (*R. tarandus caribou*) and multiple subspecies of barren-ground caribou (*R. t. groenlandicus*, *R. t. granti*) was explained by divergence within distinct glacial refugia in Beringia (barren-ground) and south of the ice sheet (woodland—Weckworth et al. 2012). This was complicated by evidence that *R. t. caribou* may have been further isolated in multiple refugia and diverged, but not in a manner corresponding to existing subspecies designations (Klütsch et al. 2012). In contrast, two other caribou subspecies, *R. t. groenlandicus* and *R. t. granti*, may not be genetically distinct (Weckworth et al. 2012). Likewise, morphological and genetic differences indicate a history of glacial separation that distinguishes the subspecies labeled as black-tailed deer (*O. h. sitkensis* and *O. h. columbianus*) from 11 different subspecies collectively labeled as mule deer. However, genetic comparisons are less supportive of distinctions among the 11 mule deer subspecies themselves (Latch et al. 2014).

Four subspecies of moose (*Alces alces*) have been described from North America, with reported differences in geographic distribution, pelage coloration, and cranial measurements (Peterson 1952). However, Geist (1998) indicated that morphological variation in North American moose was insufficient for subspecific designations. Furthermore, analyses of moose samples from across their North American distribution were shown to have identical mitochondrial DNA (hereafter mtDNA) haplotypes through analysis of restriction fragment length polymorphisms (Cronin 1992) and the cytochrome-*b* gene (Hundertmark et al. 2002). Analysis of major histocompatibility complex genes also documented low diversity among North American moose (Mikko and Andersson 1995). However, analysis of portions of the more variable mtDNA control region did reveal genetic differences, with some adherence to existing subspecies' geographical distributions (Mikko and Andersson 1995; Hundertmark et al. 2003). Although some authors have proposed that moose occurred in multiple North American refugia during Pleistocene glaciations (Peterson 1955; Kelsall and Telfer 1974), others using follow-up genetic analyses have rejected this notion and instead hypothesized that moose rapidly colonized most of post-glacial North America from Beringia (≲ 15,000 years ago—Cronin 1992; Hundertmark et al. 2003). Consequently, the observed genetic differences in North American moose are believed to reflect patterns of isolation during and since the LGM (Hundertmark et al. 2003). Although we focus here on North American moose populations, the taxonomy of moose across their global distribution also remains a subject of debate. Herein we follow Hundertmark (2016) and treat moose as a single species (*A. alces*), with further differentiation at the subspecific level.

In western North America, the range of moose is characterized by a north–south gradient of subspecies assignment, from *A. a. gigas* in the north of Alaska and the Yukon, to *A. a. andersoni* in west-central Canada, ending with *A. a. shirasi* at the southern range edge of the US Rocky Mountains (Fig. 1). Prior study of mtDNA has revealed *A. a. andersoni* to exhibit the highest degree of variation, compared to *A. a. gigas* with little variation, and *A. a. shirasi* with none (Hundertmark et al. 2003). However, sampling may have played at least some role in these results, given that samples of *A. a. shirasi* came from just a single jurisdiction (Colorado) within the subspecies' range. Here, we revisit genetic variation among these three subspecies using a wider suite of genomic data, and with specific attention to sampling more intensively in and around the range of *A. a. shirasi*. First described by Nelson (1914) from Yellowstone National Park, Wyoming, the phylogeography of *A. a. shirasi* in particular may be most informative to the study of glacial vicariance and moose in North America. Despite the designation of a unique moose taxon in this region and hypothesized occurrence of this subspecies of moose in a southern glacial refugium during the LGM, historical accounts consistently

Downloaded from https://academic.oup.com/jmammal/article-abstract/101/1/10/5648221 by The University of Montana user on 24 February 2020

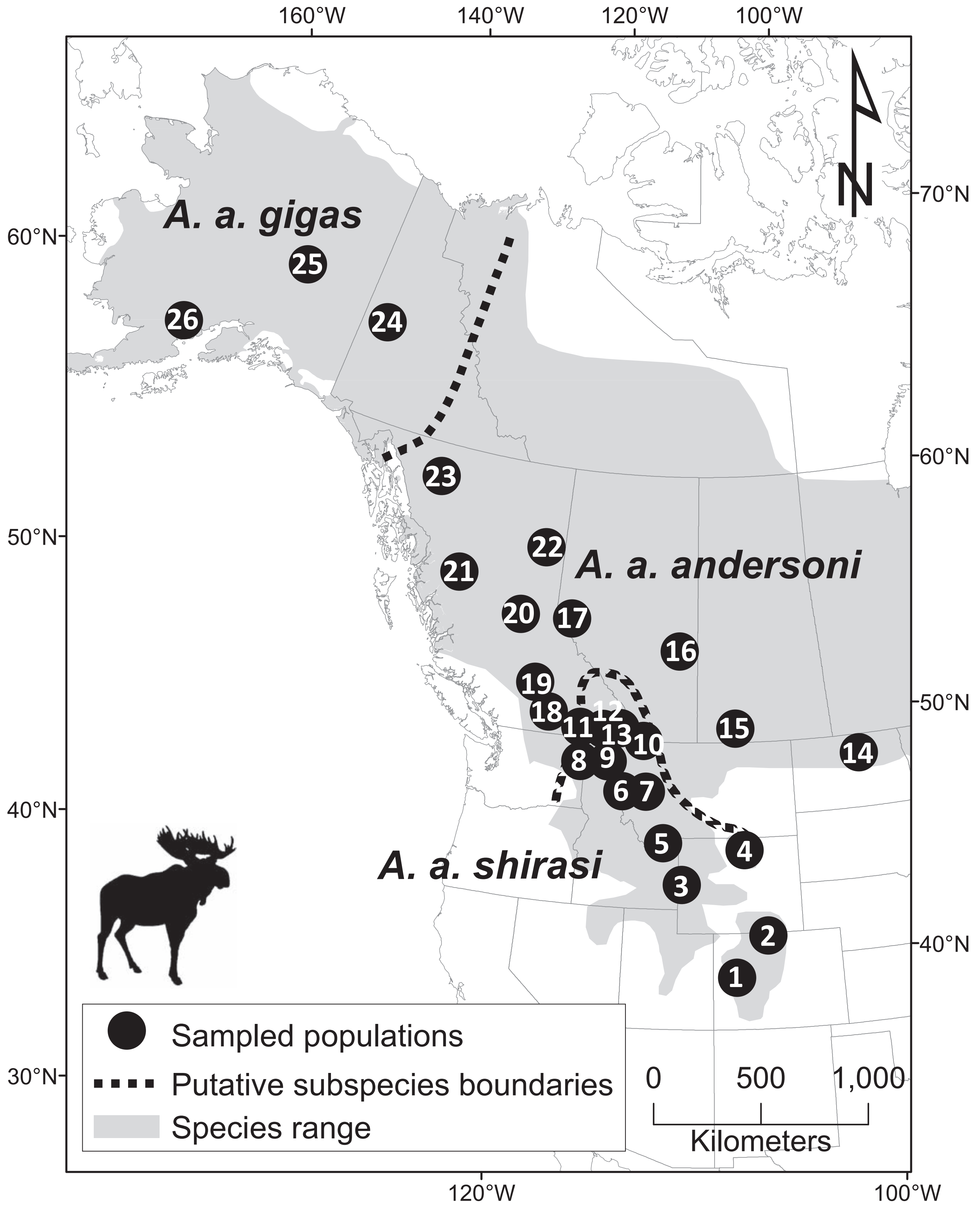

Fig. 1.—Centroid locations of 26 local populations of moose in western North America sampled for genetic material during 2004–2016 with respect to putative subspecies range boundaries. Populations 1 and 2 in Colorado and southcentral Wyoming were introduced from native lineages elsewhere in Wyoming.

Downloaded from https://academic.oup.com/jmammal/article-abstract/101/1/10/5648221 by The University of Montana user on 24 February 2020

suggest that most of the range of *A. a. shirasi* was colonized only recently, beginning in the mid– to late 1800s (Bergerud and Elliot 1986; Brimeyer and Thomas 2004; Toweill and Vecellio 2004; Wolfe et al. 2010; DeCesare et al. 2014). Thus, contrary to hypotheses that *A. a. shirasi* occurred in a southern refugium in the contiguous United States during and prior to the LGM (Peterson 1955; Kelsall and Telfer 1974), the apparent absence of moose in this southern landscape until the 19th century may suggest they are instead the result of a relatively recent range expansion from areas currently occupied by *A. a. andersoni*.

Morphological distinctions upon which the three western subspecies descriptions were based included differences in coloration, cranial morphometrics, and body size characteristics. *Alces a. gigas* are documented to be the largest specimens, rich in coloration, and with the greatest ratio of palate width to toothrow length (Peterson 1952). The *A. a. shirasi* subspecies designation comprise individuals with smaller hooves and a lighter coloration of the ears and along the back; no differences in skull measurements were detected (Nelson 1914). Later, Hall (1934) was unable to substantiate the differences in hoof measurements, but Peterson (1952) detected a proportionately greater flaring of the nasal aperture in a sample of 15 specimens of this subspecies relative to those elsewhere in North America. *Alces a. andersoni* have been found to be intermediate between the other subspecies in terms of both coloration and cranial proportions. Since the work of Peterson (1952), no follow-up analyses of cranial measurements or other morphometric evaluation has occurred. Trophy records document *A. a. shirasi* individuals to be of smaller average antler size than moose from elsewhere in North America, but proportionally similar in antler shape (Gasaway et al. 1987). Demographic research has also shown lower fecundity (e.g., twinning rates) in *A. a. shirasi* relative to northern populations (Ruprecht et al. 2016). Across the full suite of defining characteristics of these subspecies, some seem indicative of genetic adaptation (cranial proportions—Peterson 1952), while others may instead be indicators of the environment or diet (antler size and fecundity—Mayr 1956; Boertje et al. 2007; Herfindal et al. 2014; Kangas et al. 2017). For example, antler morphology is well known to be driven largely by environmental conditions and nutrition, with low additive genetic variation due to heritability in cervids (e.g., Kruuk et al. 2002).

In this study, we combined genetic analyses of the full mitochondrial genome and 13 neutral nuclear microsatellite loci to assess phylogeography and genetic differentiation of moose in western North America with the goal of understanding the evolutionary history of moose populations in this portion of the species' range. We also augmented our genetic analysis with data from the North American fossil record for moose and other cervids as an additional line of evidence to test whether glacial vicariance may have given rise to a distinct, southernmost subspecies of *Alces*, akin to the phylogeographic histories of other cervids.

Materials and Methods

Sample collection and DNA extraction.—We analyzed DNA from tissue and blood samples collected from dead (i.e., hunter-killed or opportunistically found) and live-captured moose during 2004–2016. Sample collection from live animals followed ASM guidelines (Sikes et al. 2016) and were approved by an institutional animal care and use committee (ACUC; Montana Fish, Wildlife & Parks' ACUC Permit # FWP12-2012 and University of Montana ACUC Permit # 059-09MHWB-122109). Samples were collected from Alaska, Alberta, British Columbia, Colorado, Montana, North Dakota, Saskatchewan, Washington, Wyoming, and the Yukon territory (Fig. 1). Yukon territory samples (UAM:Mamm:126589, UAM:Mamm:126545, and UAM:Mamm:126569) were provided by the University of Alaska Museum. The remaining samples were used destructively and not physically archived for future use. We stratified our sampling across the region to include a target of 10 individuals each within 26 local "populations" from Colorado to Alaska, which we defined according to geographic proximity, with increased sampling emphasis on populations near the hypothesized boundary between ranges of *A. a. shirasi* and *A. a. andersoni* in southern Alberta and British Columbia (Fig. 1; Table 1). In total, sampling included 255 animals from the ranges of three subspecies: *A. a. andersoni* ($N = 116$), *A. a. gigas* ($N = 23$), and *A. a. shirasi* ($N = 116$). Spatial locations of samples were mostly attributed to the centroids of wildlife management units, but precise locations were known for some samples. Genomic DNA was isolated using standard protocols for blood and tissue using the DNeasy Tissue Kit (Qiagen, Valencia, CA).

Mitochondrial genome diversity.—Genetic markers come with differences in terms of mutation rates, heredity, and neutral versus coding relationships to phenotype (Zink and Barrowclough 2008). Subspecies differentiation concerns broad-scale phylogeography over relatively long- and large-scales of time and space. We emphasized analyses of mtDNA to assess phylogeography of moose across western North America, with relevance for subspecies-level differentiation (Avise et al. 1987). Several sections of mtDNA (403–554 bp) have been studied in North American moose, each with differing results in terms of structure (Cronin 1992; Hundertmark et al. 2002, 2003). To minimize the effect of marker selection on patterns of variation found in mtDNA, we sequenced the entire mitochondrial genome (~16,500 bp—Knaus et al. 2011). Mitogenomic divergence has been recently used to study phylogeography in mammal species (killer whale, *Orcinus orca*—Morin et al. 2010; fisher, *Martes pennant*—Knaus et al. 2011]), and is particularly recommended when studying divergence that has occurred in evolutionarily contemporary time scales (Holocene; Knaus et al. 2011), such as is hypothesized for North American moose (Hundertmark et al. 2003).

We sequenced a spatially stratified subset of 63 samples to obtain entire mitochondrial DNA sequences (mitogenomes), which included one or more samples from 23 of the 26 local populations sampled in total. Shotgun sequencing was performed using 100 bp paired-end reads on an Illumina HiSeq 3000 operated by the Oregon State University Center for Genome Research and Biocomputing. Sequencing reads were groomed, clipped, and assembled de novo using the

Downloaded from https://academic.oup.com/jmammal/article-abstract/101/1/10/5648221 by The University of Montana user on 24 February 2020

Table 1.—Sample sizes of individual moose included in analysis of full mitochondrial genomes (N_{mtDNA}) and nuclear microsatellite loci ($N_{microsat}$) as well as mean and effective numbers of microsatellite alleles per locus, observed heterozygosity (H_O) and expected heterozygosity (H_E) for 26 populations of moose sampled in western North America, 2004–2016.

Subspecies, a priori	ID	Location	N_{mtDNA}	$N_{microsat}$	Alleles	Effective alleles	H_O	H_E
A. a. shirasi	1	CO	0	5	2.6	1.96	0.54	0.46
	2	CO, WY	2	10	2.93	2.06	0.46	0.48
	3	WY	2	10	2.67	1.95	0.47	0.42
	4	WY	2	10	2.67	1.81	0.42	0.39
	5	MT	2	10	2.73	1.93	0.41	0.42
	6	MT	0	4	3	2.3	0.7	0.52
	7	MT	3	10	3.33	2.2	0.47	0.5
	8	WA	2	10	4.07	2.69	0.51	0.59
	9	MT	2	10	3.73	2.49	0.56	0.53
	10	AB, MT	7	13	4.47	3.01	0.58	0.6
	11	BC	0	3	2.87	2.4	0.76	0.53
	12	BC, MT	2	10	3.87	2.72	0.57	0.56
	13	BC, MT	1	11	4.13	2.72	0.62	0.59
A. a. andersoni	14	ND	4	8	4.47	3.21	0.57	0.62
	15	MT, SK	6	12	5.07	3.4	0.68	0.66
	16	AB	3	10	4.87	3.28	0.67	0.65
	17	AB	4	10	4.6	3.18	0.69	0.62
	18	BC	1	12	4.87	2.91	0.58	0.63
	19	BC	1	10	3.6	2.39	0.6	0.53
	20	BC	1	9	4.07	2.64	0.55	0.59
	21	BC	2	11	4.73	3.08	0.62	0.62
	22	BC	2	10	4.27	3.03	0.68	0.61
	23	BC	4	22	5.93	3.47	0.67	0.65
A. a. gigas	24	YK	3	3	2.73	2.3	0.69	0.53
	25	AK	2	10	4.2	2.82	0.63	0.61
	26	AK	2	10	3.73	2.62	0.61	0.59

software ABySS (Simpson et al. 2009—Galaxy Version 1.9.0.0) on a local Galaxy server (Giardine et al. 2005; Goecks et al. 2010). Scaffolds were then aligned in the software Sequencher (Genecodes) to a reference mitochondrial genome (NC_020677) from a Eurasian moose that was 16,417 bp. However, as our moose were from North America, they lacked 76 bp (a single indel plus a 75 bp indel) present in the control region in Eurasian moose (Hundertmark et al. 2002) making the sequence data recovered for our mitogenomes 16,341 bp in length when aligned to the reference. We then trimmed off the last 135 bp of the control region sequence in our mitogenomes because many of the samples had long strings of unresolved base pairs in this portion. We believe the portion that we trimmed was negligible in assessing population variation as we did not observe polymorphic sites in the last 135 bp in the samples that did yield data. We were successful at obtaining mitogenomes for 60 of 63 samples.

We estimated the number of polymorphic sites, haplotype diversity, nucleotide diversity, and mean nucleotide differences for both the full mitogenome and the control region specifically using DnaSP (Rozas et al. 2017). We then tested for geographic partitioning among groups using a hierarchical analysis of molecular variance (AMOVA—Excoffier et al. 1992) in the software Arlequin 3.5.2.2 (Excoffier and Lischer 2010). This analysis tested a priori hypothesized groupings consistent with currently recognized subspecies, as well as alternative hypotheses that lumped different combinations of subspecies into larger groups (Table 2). An AMOVA divides total variance into variance components according to differences among groups (Φ_{CT}), among populations within groups (Φ_{SC}), and within populations (Φ_{ST}). The most likely geographic subdivisions are significantly different from random distributions and have maximum among-group variance (Φ_{CT} values). The optimal genetic subdivisions in our evaluation of subspecies and alternative groupings will maximize the between-group variance (Φ_{CT}) compared to the within-group component (Φ_{SC}).

Phylogenetic relationships among mitogenome haplotypes were analyzed using Bayesian methods. We used the software jModelTest 0.1.1 (Posada 2008) to identify HKY+G as the best substitution model for moose mitogenomes based on the Bayesian Information Criterion. A Bayesian maximum clade credibility tree was created using BEAST v1.8.4 (Drummond et al. 2012) under a strict clock model, HKY+G substitution model, default optimization schedule, MCMC chain-length of 100 million, sampling every 10,000 generations and discarding the first 10% of samples. We analyzed results from BEAST in Tracer v1.7 (Rambaut et al. 2018) and all effective sample sizes (ESS) were > 8,000, indicating length of MCMC was appropriate. The phylogenetic trees we estimated using the software BEAST were summarized in TreeAnnotator v1.8.4 and subsequently viewed and stylized in FigTree v1.4.3 (Rambaut 2016). The tree was rooted with a Eurasian moose mitogenome from Kazakhstan (Hassanin et al. 2012—GenBank accession NC_020677). We also estimated a minimum spanning tree haplotype network from mitogenome haplotypes using the randomized minimum spanning tree method (Paradis 2018).

Given the different rates of mutation across mtDNA genes, we partitioned out the highly variable control region for separate analysis in which mutation rates can be used to infer a

Downloaded from https://academic.oup.com/jmammal/article-abstract/101/1/10/5648221 by The University of Montana user on 24 February 2020

Table 2.—Results from hierarchical analysis of molecular variance (AMOVA) testing three hypothesized groupings consistent with currently recognized subspecies and groupings of such, where total variance is divided into variance components according to differences among groups (Φ_{CT}), among populations within groups (Φ_{SC}) and within populations (Φ_{ST}). The optimal grouping (Model C) is significantly different from random distributions (P [Φ_{CT}]) and has maximum among-group variance (ΦCT values).

Model	Hypothesized groupings	Φ_{SC}	Φ_{ST}	Φ_{CT}	% Among groups	P (Φ_{CT})
A	[*gigas*, *andersoni*] [*shirasi*]	0.437	0.463	0.046	4.58	0.136
B	[*gigas*] [*andersoni*] [*shirasi*]	0.408	0.473	0.109	10.93	0.029
C	[*gigas*] [*andersoni*, *shirasi*]	0.417	0.545	0.220	22.05	0.013

molecular clock. This partitioning resulted in a reduction of haplotypes from 37 to 22 among all moose. Phylogenetic relationships among the unique haplotypes were analyzed using both Bayesian and Maximum Likelihood methods. Analysis with jModelTest 0.1.1 identified TrN+I+G (Tamura and Nei 1993) as the best substitution model based on Bayesian Information Criterion. A Bayesian maximum clade credibility tree was calculated using the same methods and criteria as the full mitogenome phylogeny. We also created a maximum likelihood tree with the software MEGA7 (Kumar et al. 2016), both assessing relationships with 500 bootstraps as well as employing the molecular clock option. For consistency with previous mtDNA control region moose phylogenetic studies (e.g., Niedziałkowska 2017) we used mutation rates of 3.14×10^{-7} and 3.93×10^{-7} substitutions per site per year (Bradley et al. 1996 and Burzyńska et al. 1999, respectively). Control region trees were rooted with the same Kazakhstan moose as the mitogenome phylogeny. Lastly, population expansion can promote low levels of diversity among haplotypes over large areas (Avise 2000). We used Arlequin to calculate Tajima's D and Fu's F_S tests of neutrality (Tajima 1989; Fu 1997), where significantly negative values may indicate recent population expansion. Fu and Li's (1993) D^* and F^* statistics distinguish background selection from population growth or range expansion when compared with Fu's F_S. If F_S is significant and D^* and F^* statistics are not, population growth or range expansion is supported.

Microsatellite diversity.—Mitochondrial DNA are typically used for broad-scale questions concerning speciation and phylogeography. However, it has also become common practice to complement such analyses with other genetic markers, such as nuclear microsatellites, which mutate at faster rates and thus can reveal more contemporary patterns of gene flow and isolation (Zink and Barrowclough 2008). With adequate sample sizes, microsatellites can detect subtle and recent genetic divergence, which is often more appropriate for objectives such as designating units for population management rather than for studying historical speciation or phylogeography. With these caveats in mind, we also assessed contemporary genetic structure using 13 microsatellite markers from nuclear DNA. Microsatellites have been commonly used to assess genetic structure among ungulate populations and species, including moose (Wilson et al. 2003, 2015). We amplified 13 microsatellites used previously on moose and other ungulates: RT5, RT9, RT24, RT30 (Wilson et al. 1997); BM203, BM2830, BM888, BM1225, BL42 (Bishop et al. 1994); FCB193 (Buchanan and Crawford 1993); MAP2C (Moore et al. 1992); and T156, T193 (Jones et al. 2002). We used program MICRO-CHECKER 2.2.3 to check for null alleles (van Oosterhout et al. 2004), and we used program GENEPOP 4.7 (Raymond and Rousset 1995) to check for linkage disequilibrium. We were successful at obtaining microsatellite genotypes for 253 of 255 samples.

We summarized samples belonging to each sampled local population according to the mean number of alleles per locus, the number of alleles per locus scaled by abundance, observed heterozygosity (H_O), and expected heterozygosity (H_E). We used the software GenAlEx, version 6.5 (Peakall and Smouse 2006), to estimate the relationship between individual genetic and Euclidean geographic distances. We used these same data to evaluate spatial autocorrelation among distance classes (bins of samples located within 50 and 200 km of each other). We next calculated group-based substructure by estimating two matrices of genetic distance (F_{ST} and Nei's unbiased genetic distance among pre-defined groups—Nei 1978). We plotted the centroid of each group and calculated Euclidean distance among groups. We next compared the matrix of Euclidean distance to the two matrices of genetic distance (Nei's unbiased estimate of genetic distance and F_{ST}) with a Mantel correlation. We also used F_{ST} population pairwise comparisons from microsatellite data to assess gene flow among sampled populations and visualized this genetic distance matrix with a multidimensional scaling (MDS) plot.

To evaluate genetic structure among the 26 a priori local groupings, we performed a principal coordinates analysis of microsatellite allele frequencies at all loci among populations using the software GenAlEx (Peakall and Smouse 2006). Next, we conducted a clustering analysis of these samples using the software Structure 2.3.2 (Pritchard et al. 2000) to identify the likely number of clusters, K, with an individual-based Bayesian algorithm. This approach assigned individuals to clusters by minimizing Hardy–Weinberg proportions and linkage disequilibrium between loci in each cluster. We assessed all values of K from 1 to 10 with 10 replicates each using 1,000,000 iterations for each run following a burn-in period of 100,000. We used an admixture model, considered allele frequencies as correlated, and excluded sampling location priors. The most supported value of K was estimated both by calculating the maximum likelihood value ($\ln[P(X|K)]$) and using the ΔK method (Evanno et al. 2005). We then plotted the population assignment probabilities for each individual across all supported values of K to explore spatial patterns of population structure.

Comparison of the fossil record among Cervidae.—We queried the Quaternary Faunal Mapping Project (FAUNMAP) database of documented mammal fossil occurrences for

Downloaded from https://academic.oup.com/jmammal/article-abstract/101/1/10/5648221 by The University of Montana user on 24 February 2020

extant North American cervid species (*Alces alces*, *Cervus elaphus*, *Odocoileus* spp., and *Rangifer tarandus*—Graham and Lundelius 2010). We removed records with questionable species identifications and used the minimum estimated age for each sample to identify a conservative age of deposition. We then filtered the data to capture only those specimens that represented occupation during or prior to the LGM, which has been estimated to span 19,000–26,500 years ago (Clark et al. 2009). Although the LGM represents the peak of the late Wisconsin glacial expanse, estimates of the timing of the first ice-free corridors between southern and northern refugia were not until ≲ 13,000 years ago (Dyke 2004). Here, we present summary of the fossil data filtered to include only those records with minimum ages ≥ 15,000 years ago as a conservative metric of species distributions prior to ice-free connectivity among refugia. We also assessed the sensitivity of our results to this cutoff by summarizing data with alternate values from 11,000–20,000 years ago and found no differences with respect to detections of *Alces alces*. We then spatially mapped these records to characterize the locality and quantity of cervid specimens deposited during or before periods of glacial separation (Dyke 2004).

Results

Mitogenome diversity.—Mitogenomic results included 132 polymorphic sites (0.81% of the total genome) and 37 distinct haplotypes (GenBank accession numbers MK644889–MK644928). Haplotype diversity was 0.951, nucleotide diversity was 0.00101, and mean nucleotide differences were 13.733. Of these 37 distinct mitogenomic haplotypes, seven were represented by multiple moose and 30 by single individuals only. Notably, one mitogenomic haplotype (no. 39; Fig. 2) was shared by 11 different individuals spanning five different jurisdictions (Colorado, Wyoming, Montana, Washington, and northeast British Columbia) and within the putative ranges of both *A. a. shirasi* and *A. a. andersoni*. Our analyses included 716 bp of the control region specifically, which included 28 (3.9%) polymorphic sites and which accounted for 21.2% of the total mitogenome variation. Control region haplotype diversity was 0.918, nucleotide diversity was 0.00721, and mean nucleotide differences were 4.864.

AMOVAs were used to evaluate the mitogenomic support for a small set of a priori groupings of moose in correspondence to subspecies distinctions. AMOVA results did not support Model A, characterizing a single distinction between moose currently within the range of *A. a. shirasi* and those sampled further north ($P = 0.14$; 5% of mitogenomic variance explained; Table 2). Model B distinguished among each of the three putative subspecies (*shirasi*, *andersoni*, *gigas*), and was statistically significant ($P = 0.029$), explaining 11% of the mitogenomic variation. However, the best model, Model C, combined *A. a. shirasi* and *A. a. andersoni* into one group but kept *A. a. gigas* as a second group and was also significant ($P = 0.013$), explaining 22% of the total mitogenomic variation (Table 2).

Mitogenomic haplotype phylogenies showed a relatively early divergence between a small sample of three individuals (from northwest British Columbia and western Yukon) and the remaining 57 individuals, including some in close spatial proximity to these three (Fig. 2). Within the larger group, some additional clades were supported, including a group of Alaska-only individuals; however, no subspecies was reciprocally monophyletic. *Alces a. shirasi* samples were not different from those of *A. a. andersoni* (Fig. 2). A minimum spanning tree haplotype network supported the same distinction of several clades from the phylogenetic tree (Supplementary Data SD1), including the distinct grouping of some samples from Alaska and others east of the Continental Divide in North Dakota, Saskatchewan, and eastern portions of Montana and Alberta, though many other individuals sampled in these same areas were not grouped similarly. A phylogenetic time tree using only control region sequences supported largely the same tree topology as that with full mitogenomes, but with some nodes compressed (Supplementary Data SD2). Estimates of divergence times from control region data indicated divergence of the clade of individuals from northwest British Columbia and western Yukon approximately 20,000–25,000 years ago and subsequent divergence of all remaining clades post-LGM < 13,000–17,000 years ago (Supplementary Data SD2). Both Tajima's D ($D = -1.52$, $P = 0.033$) and Fu's F_S ($F_S = -7.79$, $P = 0.042$) were significantly negative while D^* ($P > 0.1$) and F^* ($P > 0.1$) were not, indicating evidence of population expansion across our sampling range.

Microsatellite diversity.—Genetic distance among individuals was significantly correlated with Euclidean distance ($P < 0.001$; Mantel test), but relatively little individual genetic variance was explained by distance ($r^2 = 0.03$). However, group-based structure was also significantly explained by Euclidean distance, as measured both with Nei's unbiased genetic distance ($P < 0.001$, $r^2 = 0.33$) and F_{ST} ($P < 0.001$, $r^2 = 0.19$), suggesting distance explained as much as 33% of the variance in group structure. We detected positive spatial autocorrelation among individuals up to distances of 700 km.

Principal coordinates analysis using the 26 a priori populations showed that 34.2% and 19.8% of the variation (54.0% in total) could be explained by coordinates 1 and 2, respectively. Plots of populations along these coordinates showed two distinct groups separated by coordinate 1 (Fig. 3). A group of six populations on the southern edge of the distribution of *A. a. shirasi* (i.e., southern Montana, Wyoming and Colorado) were separated from all others to the north along coordinate 1. Within the northern populations, multiple additional subgroups were evident, and the clustering of these was coincident with a priori subspecies definitions, with grouping of populations of *A. a. shirasi*, *A. a. andersoni*, and *A. a. gigas* (Fig. 3). Bayesian clustering analysis also showed strong support for two groups, southern and northern, as evidenced by the strong peak in ΔK at $K = 2$ (Fig. 4A). However, gradual improvement in log probability of data provided support for a range of values of $K = 2–5$. Visual assessment of the proportion of membership (q) for each individual for values of

Downloaded from https://academic.oup.com/jmammal/article-abstract/101/1/10/5648221 by The University of Montana user on 24 February 2020

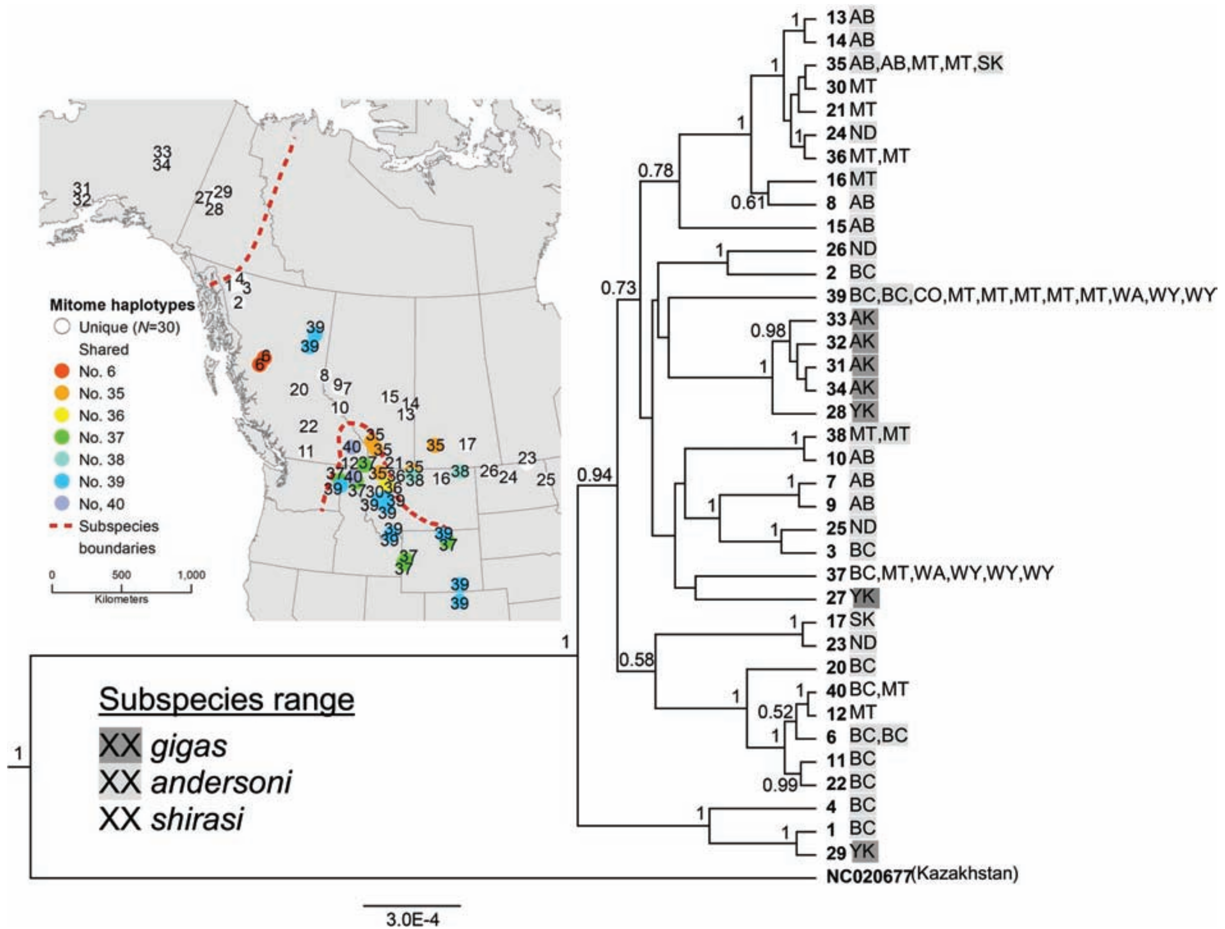

Fig. 2.—Bayesian maximum clade credibility tree showing 37 mitochondrial genome haplotypes from 60 moose and an inset of sample locations according to haplotype number, sampled in western North America, 2004–2016, and including a Eurasian moose mitogenome from Kazakhstan (Hassanin et al. 2012—GenBank accession NC_020677). In the tree, each haplotype is followed by a sequence of highlighted (by subspecies) and labeled (by state or provincial abbreviation) squares signifying the location where each sample was collected. In the inset, haplotypes unique to a single individual are colored white, while the seven haplotypes found in multiple moose are colored by haplotype number.

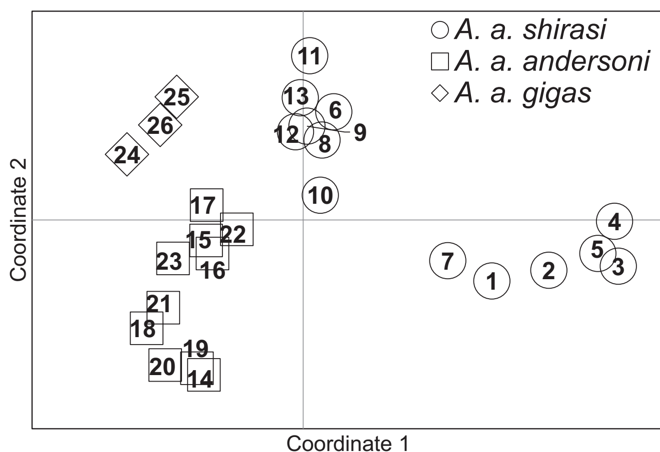

Fig. 3.—Locations of 26 local populations of moose, labeled by sampling location (see Fig. 1), relative to the first two components of a principal coordinates analysis of allele frequencies at 13 microsatellite loci, sampled from three subspecies in western North America, 2004–2016.

$K = 2–5$ showed spatial clustering of two groups, a northern and southern group (Fig. 4B), similar to that revealed by component 1 in the PCoA. Weaker support was shown for subgroups in the Yukon and Alaska, in the northern Rocky Mountains of northern Montana and southern Alberta and British Columbia, and in western British Columbia. Although the distinction between northern and southern moose at $K = 2$ was supported by these analyses, there remained uncertainty in the assignment probabilities of individuals to each of these groups across all populations (Fig. 4B). Lastly, an MDS plot of genetic distance (F_{ST}) among populations was generally supportive of these clustering results, indicating relatively lower gene flow among southern populations in Colorado, Wyoming, and southwest Montana and between them and other populations to the north (Supplementary Data SD3).

Comparison of the fossil record among Cervidae.—Southern refugial (i.e., ≥ 15,000 years before present) populations were detected by the presence of numerous fossil remains of *Cervus*, *Odocoileus*, and *Rangifer*, supporting certain subspecies

Downloaded from https://academic.oup.com/jmammal/article-abstract/101/1/10/5648221 by The University of Montana user on 24 February 2020

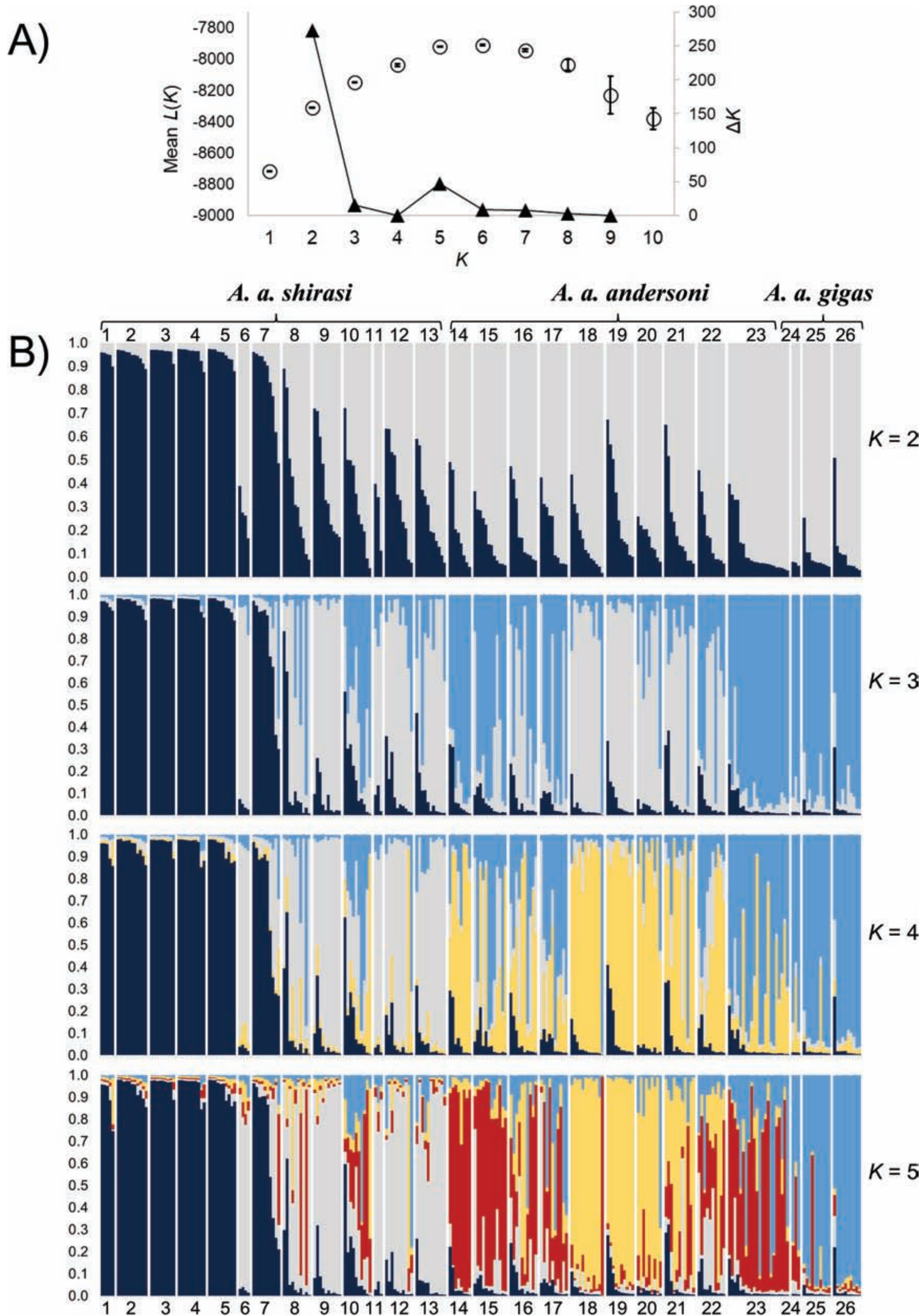

Fig. 4.—A) Plots of both the log probability of data (left y-axis) and Δ*K* (right y-axis) as a function of the number of putative groupings (*K*); and B) individual cluster probabilistic assignments (y-axis) for *K* = 2, 3, 4, and 5 groupings from Bayesian cluster analyses of 13 microsatellite loci for 253 moose individuals sampled across 26 sampling locations (x-axis) in western North America, 2004–2016.

Downloaded from https://academic.oup.com/jmammal/article-abstract/101/1/10/5648221 by The University of Montana user on 24 February 2020

distinctions that correspond to populations isolated for long periods of time by glacial ice (Supplementary Data SD4). In contrast to this evidence of southern occurrence of other cervid species, only two cases of fossil remains of moose were detected with minimum ages of 15,000 years before present. Both moose fossils came from archeological sites in the western Yukon and were dated with minimum ages of 20,780 and 30,500 years before present.

Discussion

Our analyses of genetic variability and structure do not support the notion of long-standing vicariance among the three subspecies of moose in western North America. Instead, our results indicate a generally low degree of genetic diversity within the mitogenome of moose across western North America, and mitogenomic signatures were not diagnosably distinct indicators of subspecies. Our assessment of the fossil record also failed to detect evidence of moose occupation of a southern refugium during the last glacial maximum (Supplementary Data SD4). These findings contrast with those from other North American cervids, such as caribou and mule deer, for which genetic analyses (as well as our analysis of the fossil record) have supported and clarified the role of glacial vicariance in giving rise to existing subspecific distinctions (Cronin 1992; Weckworth et al. 2012).

Review of previous genetic studies of North American moose phylogeography show inference is sensitive to the particular portion of mtDNA sequenced. Some regions of mtDNA have yielded only a single haplotype continent-wide (Cronin 1992; Hundertmark et al. 2002), whereas others, including the control region, have shown modest variation (Mikko and Andersson 1995; Hundertmark et al. 2003). Here, we used whole mitochondrial genome analysis to similarly focus on the mitochondrial genetic material but provide a more complete depiction of variability. Upgrading mtDNA analyses from short regions to the full mitogenome has improved the resolution of genetic structure and taxonomy for several species, including killer whales, fisher, and speartooth sharks (*Glyphis glyphis*—Morin et al. 2010; Knaus et al. 2011; Feutry et al. 2014). In our case, results from the full mitogenome generally supported the conclusions of Cronin (1992) and Hundertmark et al. (2003) of a single post-Pleistocene colonization of North America by moose based on the paucity of variation and lack of a deep divergence among clades in mtDNA. Our results also uphold the control region as an area of focused variability within the mitochondrial genome, and re-analysis of our data using only the control region subset did not substantively affect results (Supplementary Data SD2).

We found no evidence of moose in a southern refugium during the LGM. Previous fossil record queries elsewhere in Eurasia have indeed shown evidence of moose occupation of several regions during the LGM, yet records of moose in such areas have been found in lower frequency than those of other mammals (Sommer and Nadachowski 2006; Niedziałkowska 2017). Despite the potential for false absences in fossil record data, the results shown here (Supplementary Data SD4) are generally in agreement with our and others' genetic analyses for both moose and other cervid species (Cronin 1992; Weckworth et al. 2012; Latch et al. 2014).

Microsatellite data did reveal a more recent signature of isolation and genetic drift for moose in western North America (Figs. 3 and 4; Supplementary data SD3). The algorithm implemented in the software Structure for determining the appropriate number of underlying populations can be affected when there is a strong effect of isolation by distance within a species (Schwartz and McKelvey 2009). Indeed, we did see evidence for clinal patterns of genetic variation, as indicated by significant isolation by distance (IBD) tests at the levels of both individuals and groups. This effect of IBD could partially drive the consistent grouping of southernmost moose in the distribution across levels of K. However, this grouping can also be biogeographically explained as the result of recolonization and isolation of small populations of moose in the western US during late 19th and 20th centuries. For example, the genetic divide between southern and northern groupings is found in west-central Montana. While moose currently occupy a continuous distribution throughout western Montana (Nadeau et al. 2017), maps of moose range earlier in the 20th century show a wide swath of unoccupied habitat aligning geographically with our observed genetic discontinuity (Stevens 1971).

Moose faced near-extirpation in Montana in 1900 and numbered an estimated 300 individuals in 1910 after 10 years of protection (DeCesare et al. 2014). Populations in Idaho and Wyoming suffered similar declines and range reductions (Brimeyer and Thomas 2004; Toweill and Vecellio 2004). Subsequent protections led to expansion of moose in the 20th century, which showed an apparent pattern of recolonization from distinct northern and southern population sources (Stevens 1971). Even as recently as the 1970s, a large gap in the distribution of moose remained in west-central Montana, separating those in southern Montana, Wyoming, and Colorado, from those to the north (Stevens 1971). History would indicate that the southernmost populations of *A. a. shirasi* were founded from relatively few individuals after the declines of the late 1800s and experienced low gene flow with northern populations until distribution gaps were filled as these populations expanded over the past 50 years. Our results support these notions. Similarly, Bergerud and Elliot (1986) reviewed the colonization of British Columbia by moose, noting that much of the province was not occupied until 1910–1940. This recent colonization of much of British Columbia may similarly underlie the findings of relatively distinct population units, at $K = 4–5$, in our microsatellite results including portions of western and southeastern British Columbia (Fig. 4B). Overall, the contemporary population structure of moose detected with microsatellite analyses likely reflects the relatively recent (i.e., < 100–200 years before present) colonization and recolonization of animals in the western United States and Canada from a limited distribution of source populations that may have undergone significant genetic drift due to small population sizes prior to their expansion.

Downloaded from https://academic.oup.com/jmammal/article-abstract/101/1/10/5648221 by The University of Montana user on 24 February 2020

It has been shown that these three western North American moose subspecies can be discriminated according to antler size, with *A. a. shirasi* smallest, *A. a. andersoni* intermediate, and *A. a. gigas* largest (Gasaway et al. 1987). Broad-scale variation in such traits is driven by differences in genetics, environment, and age distribution among ungulate populations (Monteith et al. 2018; Quéméré et al. 2018), but disentangling the relative effects of each can be challenging (Kruuk and Hadfield 2007). Moose antler size has been shown to vary with habitat type and population density among populations in Alaska (Bowyer et al. 2002; Schmidt et al. 2007), although Bowyer et al. (2002) posited a role of genetics as well. In studies of moose in Scandinavia, Herfindal et al. (2014) found both genetic structure and environmental conditions to be drivers of north–south variation in body mass of moose in Norway, whereas Kangas et al. (2017) found that genetic structure did not explain the similar north–south spatial cline in mandible shape of moose in Finland. In a study of antler size in red deer (*Cervus elaphus*), Kruuk et al. (2002) found that, while antler size was heritable, genetic effects on antler size were ultimately driven by environmental covariances and the nutritional state of individuals. Furthermore, Monteith et al. (2009) found nutritional effects manifested during gestation to strongly explain differences in body and antler growth among white-tailed deer from disparate populations. For moose in western North America, our results suggest only limited and recent genetic distinctiveness, thereby pointing towards environmental differences as the primary driver of reduced antler sizes, over genetic differences. This conclusion also is supported by the findings of Ruprecht et al. (2016) showing an increase in the fecundity of female moose with latitude that generally corresponds to that of antler size.

For the purpose of evaluating genetic versus environmental drivers of body and antler size, moose on Isle Royale may also serve as a case study with parallels to the smallest of our studied subspecies, *A. a. shirasi*. Moose colonized Isle Royale from mainland Ontario in the early 1900s, not long after the apparent colonization by *A. a. shirasi* of the US Rocky Mountains in the mid-1800s. Moose on Isle Royale are characterized by notably smaller body and antler sizes than representative populations from any subspecies in North America, including both *A. a. shirasi* and populations of *A. a. andersoni* in immediate proximity to Isle Royale on mainland Ontario (Peterson et al. 2011; Mills and Peterson 2013). Thus, this population displays evidence that reduction in mean body and antler size can occur over the relatively short time period of 100 years. While moose on Isle Royale also show reduced genetic diversity relative to mainland populations (Wilson et al. 2003; Sattler et al. 2017), reductions in body and antler sizes have been attributed to nutrient limitation on the island caused by five to 10 times higher population densities relative to the mainland population (Peterson et al. 2011; Mills and Peterson 2013). For the purposes of understanding genetic and phenotypic differences of *A. a. shirasi*, the Isle Royale example supports the notions that: 1) observed differences in body and antler size seen in *A. a. shirasi* could be caused, at least in part, by differences in environmental conditions; and 2) that such differences can manifest over relatively short (100 years) time periods.

Our phylogeographic study of moose subspecies in western North America did not show them as genetically divergent according to the mitochondrial genome. While not supportive of the subspecies distinctions akin to mtDNA for other North American cervids, these results are not definitive with regards to taxonomy. The means by which to standardize or quantify differentiation for subspecies taxonomy remains an active area of research and discussion (Patten and Remsen 2017; Patton and Conroy 2017). It is commonly held that both morphological and genetic differences should support taxonomic designations (Patton and Conroy 2017). Although mtDNA have become a standard genetic marker for such questions, patterns of differentiation from mtDNA do not always mirror those of morphology or the functional DNA upon which phenotype is based (Padial et al. 2010; Pedraza-Marrón et al. 2019). Thus, further study of subspecies designations and distributions of North American moose is warranted, ideally integrating analyses of morphology with both neutral and functional genetics (e.g., Wilting et al. 2015).

Whereas each of these North American moose subspecies appear only weakly diverged from one another, our microsatellite analyses do indicate a level of genetic distinctiveness of moose within the southern edge of this range. This raises the question of whether these populations of moose and the genetic diversity carried by them are worthy of additional conservation attention. We would generally argue against such attention, applying our results to several lines of reasoning: 1) across all 13 microsatellite loci examined, moose within this southernmost region (Colorado, Wyoming, and southern Montana) did not display any unique alleles relative to those further north, suggesting that genetic diversity in this region is only a subset of that found elsewhere in western North America; 2) such a result from microsatellite data can indicate subtle and recent divergence, such as has also been seen in moose when comparing two populations on either side of a highway (Wilson et al. 2015), and does not necessarily indicate evolutionarily significant divergence such as could be detected with mtDNA; 3) conservation of genetic uniqueness due to isolation and drift, such as is likely in this case, may be at the detriment of the species as whole (sensu Weeks et al. 2016); and 4) moose currently occupy a wide swath of continuous range across this genetic boundary, which suggests that natural maintenance of genetic connectivity should reduce, rather than intensify, any current degree of genetic isolation or associated demographic risks.

Acknowledgments

We thank the British Columbia Habitat Conservation Trust Foundation, Montana Fish, Wildlife and Parks, Pope and Young Club, and Wyoming Governor's Big Game License Coalition, for providing funds to complete this project. We thank the many state and provincial agencies, universities, agency staff, and hunters, for collecting and providing genetic samples. We also thank J. Gude, D. S. Rogers, and two anonymous reviewers, for helpful reviews of this manuscript.

Downloaded from https://academic.oup.com/jmammal/article-abstract/101/1/10/5648221 by The University of Montana user on 24 February 2020

Supplementary Data

Supplementary data are available at *Journal of Mammalogy* online.

Supplementary Data SD1.—Minimum spanning tree haplotype network showing 37 mitochondrial genome haplotypes from 60 moose sampled in western North America, 2004–2016.

Supplementary Data SD2.—Phylogenetic trees based on 22 control region-only haplotypes from 60 moose sampled across western North America, 2004–2016. Numeric mitogenome haplotypes are shown at branch tips. Approximate years before present (BP) are shown along x-axis for both mutation rates. Numbers at the branch nodes represent: A) posterior probabilities (above; only values above 0.50 are shown) and percentage of support from 500 bootstraps (below; only values above 50% shown); B) 95% credible intervals for divergence times assuming a mutation rate of 3.14×10^{-7} substitutions per year; and C) 95% credible intervals for divergence times assuming a mutation rate of 3.93×10^{-7} substitutions per year.

Supplementary Data SD3.—Multidimensional scaling plot based on pairwise FST estimates of genetic distance for microsatellite data among 26 sampled populations of moose, western North America, 2004–2016.

Supplementary Data SD4.—Quantities and locations of fossil remains in the FAUNMAP database for four North American cervid genera, and for which minimum age estimates were ≥ 15,000 years before present.

Literature Cited

Aubry, K. B., M. J. Statham, B. N. Sacks, J. D. Perrine, and S. M. Wisely. 2009. Phylogeography of the North American red fox: vicariance in Pleistocene forest refugia. Molecular Ecology 18:2668–2686.

Avise, J. C. 2000. Phylogeography: the history and formation of species. Harvard University Press. Cambridge, Massachusetts.

Avise, J. C., et al. 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Annual Review of Ecology and Systematics 18:489–522.

Bergerud, A. T., and J. P. Elliot. 1986. Dynamics of caribou and wolves in northern British Columbia. Canadian Journal of Zoology 64:1515–1529.

Bishop, M. D., et al. 1994. A genetic linkage map for cattle. Genetics 136:619–639.

Boertje, R. D., et al. 2007. Ranking Alaska moose nutrition: signals to begin liberal antlerless harvests. Journal of Wildlife Management 71:1494–1506.

Bowyer, R. T., K. M. Stewart, B. M. Pierce, K. J. Hundertmark, and W. C. Gasaway. 2002. Geographical variation in antler morphology of Alaskan moose: putative effects of habitat and genetics. Alces 38:155–165.

Bradley, D. G., D. E. MacHugh, P. Cunningham, and R. T. Loftus. 1996. Mitochondrial diversity and the origins of African and European cattle. Proceedings of the National Academy of Sciences of the United States of America 93:5131–5135.

Brimeyer, D. G., and T. P. Thomas. 2004. History of moose management in Wyoming and recent trends in Jackson Hole. Alces 40:133–144.

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

Burzyńska, B., W. Olech, and J. Topczewski. 1999. Phylogeny and genetic variation of the European bison *Bison bonasus* based on mitochondrial DNA D-loop sequences. Acta Theriologica 44:253–262.

Clark, P. U., et al. 2009. The Last Glacial Maximum. Science 325:710–714.

Cronin, M. A. 1992. Intraspecific variation in mitochondrial DNA of North American cervids. Journal of Mammalogy 73:70–82.

DeCesare, N. J., T. D. Smucker, R. A. Garrott, and J. A. Gude. 2014. Moose status and management in Montana. Alces 50:35–51.

Drummond, A. J., M. A. Suchard, D. Xie, and A. Rambaut. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Molecular Biology and Evolution 29:1969–1973.

Dyke, A. S. 2004. An outline of North American deglaciation with emphasis on central and northern Canada. Developments in Quaternary Sciences 2:373–424.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology 14:2611–2620.

Excoffier, L., and H. E. Lischer. 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10:564–567.

Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491.

Feutry, P., P. M. Kyne, R. D. Pillans, X. Chen, G. J. Naylor, and P. M. Grewe. 2014. Mitogenomics of the Speartooth Shark challenges ten years of control region sequencing. BMC Evolutionary Biology 14:232.

Fu, Y. X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. Genetics 147:915–925.

Fu, Y. X., and W. H. Li. 1993. Statistical tests of neutrality of mutations. Genetics 133:693–709.

Gasaway, W. C., D. J. Preston, D. J. Reed, and D. D. Roby. 1987. Comparative antler morphology and size of North American moose. Swedish Wildlife Research Supplement 1:311–325.

Geist, V. 1998. Deer of the world: their evolution, behaviour and ecology. Stackpole Books. Mechanicsburg, Pennsylvania.

Giardine, B., et al. 2005. Galaxy: a platform for interactive large-scale genome analysis. Genome Research 15:1451–1455.

Goecks, J., A. Nekrutenko, and J. Taylor; Galaxy Team. 2010. Galaxy: a comprehensive approach for supporting accessible, reproducible, and transparent computational research in the life sciences. Genome Biology 11:R86.

Graham, R. W., and E. L. Lundelius. 2010. FAUNMAP II: new data for North America with a temporal extension for the Blancan, Irvingtonian and early Rancholabrean. www.ucmp.berkeley.edu/faunmap. Accessed 9 March 2017.

Hall, E. R. 1934. Mammals collected by T. T. and E. B. McCabe in the Bowron Lake region of British Columbia. University of California Press. Berkeley, California.

Hassanin, A., et al. 2012. Pattern and timing of diversification of Cetartiodactyla (Mammalia, Laurasiatheria), as revealed by a comprehensive analysis of mitochondrial genomes. Comptes Rendus Biologies 335:32–50.

Downloaded from https://academic.oup.com/jmammal/article-abstract/101/1/10/5648221 by The University of Montana user on 24 February 2020

Herfindal, I., H. Haanes, E. J. Solberg, K. H. Røed, K. A. Høgda, and B. E. Sæther. 2014. Moose body mass variation revisited: disentangling effects of environmental conditions and genetics. Oecologia 174:447–458.

Hewitt, G. 2000. The genetic legacy of the Quaternary ice ages. Nature 405:907–913.

Hundertmark, K. J. 2016. *Alces alces*. In: IUCN 2018. The IUCN Red List of Threatened Species. Version 2018.1. www.iucnredlist.org. Accessed 30 August 2018.

Hundertmark, K. J., R. T. Bowyer, G. F. Shields, and C. C. Schwartz. 2003. Mitochondrial phylogeography of moose (*Alces alces*) in North America. Journal of Mammalogy 84:718–728.

Hundertmark, K. J., F. G. Shields, R. T. Bowyer, and C. C. Schwartz. 2002. Genetic relationships deduced from cytochrome-b sequences among moose. Alces 38:113–122.

Jones, K. C., K. F. Levine, and D. Banks. 2002. Characterization of 11 polymorphic tetranucleotide microsatellites for forensic applications in California elk (*Cervus elaphus canadensis*). Molecular Ecology Notes 2:425–427.

Kangas, V.-M., S. Rytkönen, L. Kvist, T. Käpylä, T. Nygrén, and J. Aspi. 2017. Geographic cline in the shape of the moose mandible: indications of an adaptive trend. Journal of Mammalian Evolution 24:233–241.

Kelsall, J. P., and E. S. Telfer. 1974. Biogeography of moose with particular reference to western North America. Le Naturaliste Canadien 101:117–130.

Klütsch, C. F., M. Manseau, and P. J. Wilson. 2012. Phylogeographical analysis of mtDNA data indicates postglacial expansion from multiple glacial refugia in woodland caribou (*Rangifer tarandus caribou*). PLoS ONE 7:e52661.

Knaus, B. J., R. Cronn, A. Liston, K. Pilgrim, and M. K. Schwartz. 2011. Mitochondrial genome sequences illuminate maternal lineages of conservation concern in a rare carnivore. BMC Ecology 11:10.

Kruuk, L. E., and J. D. Hadfield. 2007. How to separate genetic and environmental causes of similarity between relatives. Journal of Evolutionary Biology 20:1890–1903.

Kruuk, E. B., J. Slate, J. M. Pemberton, S. Brotherstone, F. Guinness, and T. Clutton-Brock. 2002. Antler size in red deer: heritability and selection but no evolution. Evolution 56:1683–1695.

Kumar, S., G. Stecher, and K. Tamura. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for Bigger Datasets. Molecular Biology and Evolution 33:1870–1874.

Latch, E. K., J. R. Heffelfinger, J. A. Fike, and O. E. Rhodes, Jr. 2009. Species-wide phylogeography of North American mule deer (*Odocoileus hemionus*): cryptic glacial refugia and postglacial recolonization. Molecular Ecology 18:1730–1745.

Latch, E. K., D. M. Reding, J. R. Heffelfinger, C. H. Alcalá-Galván, and O. E. Rhodes, Jr. 2014. Range-wide analysis of genetic structure in a widespread, highly mobile species (*Odocoileus hemionus*) reveals the importance of historical biogeography. Molecular Ecology 23:3171–3190.

Mayr, E. 1956. Geographical character gradients and climatic adaptation. Evolution 10:105–108.

Mikko, S., and L. Andersson. 1995. Low major histocompatibility complex class II diversity in European and North American moose. Proceedings of the National Academy of Sciences of the United States of America 92:4259–4263.

Mills, K. J., and R. O. Peterson. 2013. Moose antler morphology and asymmetry on Isle Royale National Park. Alces 49:17–28.

Monteith, K. L., R. A. Long, T. R. Stephenson, V. C. Bleich, R. T. Bowyer, and T. N. Lasharr. 2018. Horn size and nutrition in mountain sheep: can we handle the truth? Journal of Wildlife Management 82:67–84.

Monteith, K. L., L. E. Schmitz, J. A. Jenks, J. A. Delger, and R. T. Bowyer. 2009. Growth of male white-tailed deer: consequences of maternal effects. Journal of Mammalogy 90:651–660.

Moore, S. S., W. Barendse, K. T. Berger, S. M. Armitage, and D. J. Hetzel. 1992. Bovine and ovine DNA microsatellites from the EMBL and GENBANK databases. Animal Genetics 23:463–467.

Morin, P. A., et al. 2010. Complete mitochondrial genome phylogeographic analysis of killer whales (*Orcinus orca*) indicates multiple species. Genome Research 20:908–916.

Nadeau, M. S., et al. 2017. Status and trends of moose populations and hunting opportunity in the western United States. Alces 53:99–112.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590.

Nelson, E. W. 1914. Description of a new subspecies of moose from Wyoming. Proceedings of the Biological Society of Washington 27:71–74.

Niedziałkowska, M. 2017. Phylogeography of European moose (*Alces alces*) based on contemporary mtDNA data and archaeological records. Mammalian Biology 84:35–43.

Padial, J. M., A. Miralles, I. De la Riva, and M. Vences. 2010. The integrative future of taxonomy. Frontiers in Zoology 7:16.

Paradis, E. 2018. Analysis of haplotype networks: the randomized minimum spanning tree method. Methods in Ecology and Evolution 9:1308–1317.

Patton, J. L., and C. J. Conroy. 2017. The conundrum of subspecies: morphological diversity among desert populations of the California vole (*Microtus californicus*, Cricetidae). Journal of Mammalogy 98:1010–1026.

Patten, M. A., and J. V. Remsen, Jr. 2017. Complementary roles of phenotype and genotype in subspecies delimitation. The Journal of Heredity 108:462–464.

Peakall, R. O. D., and P. E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Resources 6:288–295.

Pedraza-Marrón, C. D. R., et al. 2019. Genomics overrules mitochondrial DNA, siding with morphology on a controversial case of species delimitation. Proceedings. Biological Sciences 286:20182924.

Peterson, R. L. 1952. A review of the living representatives of the genus *Alces*. Royal Ontario Museum. Toronto, Ontario, Canada.

Peterson, R. L. 1955. North American moose. University of Toronto Press. London, Ontario, Canada.

Peterson, R. O., J. A. Vucetich, D. Beyer, M. Schrage, and J. Räikkönen. 2011. Phenotypic variation in moose: the island rule and the moose of Isle Royale. Alces 47:125–133.

Posada, D. 2008. jModelTest: phylogenetic model averaging. Molecular Biology and Evolution 25:1253–1256.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959.

Downloaded from https://academic.oup.com/jmammal/article-abstract/101/1/10/5648221 by The University of Montana user on 24 February 2020

Provan, J., and K. D. Bennett. 2008. Phylogeographic insights into cryptic glacial refugia. Trends in Ecology & Evolution 23:564–571.

Puckett, E. E., P. D. Etter, E. A. Johnson, and L. S. Eggert. 2015. Phylogeographic analyses of American black bears (*Ursus americanus*) suggest four glacial refugia and complex patterns of postglacial admixture. Molecular Biology and Evolution 32:2338–2350.

Quéméré, E., et al. 2018. Between-population differences in the genetic and maternal components of body mass in roe deer. BMC Evolutionary Biology 18:39.

Rambaut, A. 2016. FigTree v1.4.3. https://tree.bio.ed.ac.uk/software/figtree/. Accessed 1 May 2018.

Rambaut, A., A. J. Drummond, D. Xie, G. Baele, and M. A. Suchard. 2018. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. Systematic Biology 67:901–904.

Raymond, M., and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity 86:248–249.

Rozas, J., et al. 2017. DnaSP 6: DNA sequence polymorphism analysis of large data sets. Molecular Biology and Evolution 34:3299–3302.

Ruprecht, J. S., et al. 2016. Reproduction in moose at their southern range limit. Journal of Mammalogy 97:1355–1365.

Sackett, L. C., et al. 2014. Evidence for two subspecies of Gunnison's prairie dogs (*Cynomys gunnisoni*), and the general importance of the subspecies concept. Biological Conservation 174:1–11.

Sattler, R. L., J. R. Willoughby, and B. J. Swanson. 2017. Decline of heterozygosity in a large but isolated population: a 45-year examination of moose genetic diversity on Isle Royale. PeerJ 5:e3584.

Schwartz, M. K., and K. S. McKelvey. 2009. Why sampling scheme matters: the effect of sampling scheme on landscape genetic results. Conservation Genetics 10: 441.

Schmidt, J. I., J. M. Ver Hoef, and R. T. Bowyer. 2007. Antler size of Alaskan moose *Alces alces gigas*: effects of population density, hunter harvest and use of guides. Wildlife Biology 13:53–65.

Shafer, A. B., C. I. Cullingham, S. D. Côté, and D. W. Coltman. 2010. Of glaciers and refugia: a decade of study sheds new light on the phylogeography of northwestern North America. Molecular Ecology 19:4589–4621.

Sikes, R. S., and the Animal Care and Use Committee of the American Society of Mammalogists. 2016. 2016 Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. Journal of Mammalogy 97:663–688.

Simpson, J. T., K. Wong, S. D. Jackman, J. E. Schein, S. J. Jones, and I. Birol. 2009. ABySS: a parallel assembler for short read sequence data. Genome Research 19:1117–1123.

Sommer, R. S., and A. Nadachowski. 2006. Glacial refugia of mammals in Europe: evidence from fossil records. Mammal Review 36:251–265.

Stevens, D. R. 1971. Shiras moose. Pp. 89–95 in Game management in Montana (T. W. Mussehl and F. W. Howell, eds.). Montana Fish and Game Department. Helena, Montana.

Tajima, F. 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123:585–595.

Tamura, K., and M. Nei. 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10:512–526.

Toweill, D. E., and G. Vecellio. 2004. Shiras moose in Idaho: status and management. Alces 40:33–43.

van Oosterhout, C., W. F. Hutchinson, D. P. Wills, and P. Shipley. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4:535–538.

Weckworth, B. V., M. Musiani, A. D. McDevitt, M. Hebblewhite, and S. Mariani. 2012. Reconstruction of caribou evolutionary history in Western North America and its implications for conservation. Molecular Ecology 21:3610–3624.

Weeks, A. R., J. Stoklosa, and A. A. Hoffmann. 2016. Conservation of genetic uniqueness of populations may increase extinction likelihood of endangered species: the case of Australian mammals. Frontiers in Zoology 13:31.

Wilson, A. C., et al. 1985. Mitochondrial DNA and two perspectives on evolutionary genetics. Biological Journal of the Linnean Society 26:375–400.

Wilson, R. E., S. D. Farley, T. J. McDonough, S. L. Talbot, and P. S. Barboza. 2015. A genetic discontinuity in moose (*Alces alces*) in Alaska corresponds with fenced transportation infrastructure. Conservation Genetics 16:791–800.

Wilson, P. J., et al. 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., 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.

Wilting, A., et al. 2015. Planning tiger recovery: understanding intraspecific variation for effective conservation. Science Advances 1:e1400175.

Wolfe, M. L., K. R. Hersey, and D. C. Stoner. 2010. A history of moose management in Utah. Alces 46:37–52.

Yokoyama, Y., K. Lambeck, P. De Deckker, P. Johnston, and L. K. Fifield. 2000. Timing of the Last Glacial Maximum from observed sea-level minima. Nature 406:713–716.

Zink, R. M., and G. F. Barrowclough. 2008. Mitochondrial DNA under siege in avian phylogeography. Molecular Ecology 17:2107–2121.

Submitted 18 September 2018. Accepted 4 October 2019.

Associate Editor was Duke Rogers.

Downloaded from https://academic.oup.com/jmammal/article-abstract/101/1/10/5648221 by The University of Montana user on 24 February 2020