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MITOCHONDRIAL PHYLOGEOGRAPHY OF MOOSE (*ALCES ALCES*) IN NORTH AMERICA

KRIS J. HUNDERTMARK,* R. TERRY BOWYER, GERALD F. SHIELDS, AND CHARLES C. SCHWARTZ

Alaska Department of Fish and Game, Kenai Moose Research Center, 43961 Kalifornsky Beach Road, Suite B, Soldotna, AK 99669, USA (KJH, CCS)
Institute of Arctic Biology and Department of Biology and Wildlife, University of Alaska Fairbanks, Fairbanks, AK 99775, USA (KJH, RTB, GFS)
Present address of KJH: Zoological Society of London, King Khalid Wildlife Research Centre, P.O. Box 61681, Riyadh 11575, Saudi Arabia
Present address of GFS: Department of Natural Sciences, Carroll College, Helena, MT 59601, USA
Present address of CCS: United States Geological Survey, Biological Resources Division, Forestry Sciences Laboratory, Montana State University, Bozeman, MT 59717, USA

Nucleotide variation was assessed from the mitochondrial control region of North American moose (*Alces alces*) to test predictions of a model of range expansion by stepping-stone dispersal and to determine whether patterns of genetic variation support the current recognition of 4 subspecies. Haplotypes formed a star phylogeny indicative of a recent expansion of populations. Values of nucleotide and haplotype diversity were low continent-wide but were greatest in the central part of the continent and lowest in peripheral populations. Despite low mitochondrial diversity, moose exhibited a high degree of differentiation regionally, which was not explained by isolation by distance. Our data indicate a pattern of colonization consistent with a large central population that supplied founders to peripheral populations (other than Alaska), perhaps through rare, long-distance dispersal events (leptokurtic dispersal) rather than mass dispersal by a stepping-stone model. The colonization scenario does not account for the low haplotype diversity observed in Alaska, which may be derived from a postcolonization bottleneck. Establishment of peripheral populations by leptokurtic dispersal and subsequent local adaptation may have been sufficient for development of morphological differentiation among extant subspecies.

Key words: *Alces alces*, Beringia, genetic diversity, leptokurtic dispersal, moose, mtDNA, subspeciation, Wisconsinan glaciation

The moose (*Alces alces*) is a recent immigrant to North America (from Asia through Beringia about 14,000–11,000 years ago), arriving shortly before the flooding of the Bering land bridge (Hundertmark et al. 2002). The process by which moose expanded their range across North America, however, is still in doubt. Moreover, the manner in which 4 North American subspecies were formed in conjunction with that expansion continues to be debated (Bubenik 1998a; Geist 1998).

Hundertmark et al. (1992) proposed a stepping-stone mode of colonization that, combined with serial founder effects, yielded a decreasing gradient of genetic diversity from the point of entry into North America (Alaska) to the eastern extent of the continent. Slatkin (1993) modeled scenarios of dispersal wherein a range expansion occurred very quickly under a stepping-stone model and time since expansion (τ) varied.

* *Correspondent: kris.hundertmark@zsl.org*

Isolation by distance was not apparent when τ was small but became more apparent as τ increased. Moose colonizing North America underwent a sudden population expansion in the early Holocene (Hundertmark et al. 2002), which may be sufficiently recent for such a mechanism to operate. Yet, under the scenario of recent expansion as modeled by Slatkin (1993), the genetic composition of pre- and postexpansion populations was similar, which is a different outcome than predicted by the hypothesis of serial founder events proposed by Hundertmark et al. (1992).

The 4 subspecies of moose recognized in North America (Hall 1981; Peterson 1955) inhabit Alaska and a portion of Yukon Territory (*A. a. gigas*), western Canada, to the Great Lakes (*A. a. andersoni*), the Rocky Mountains from northern Colorado to southern Alberta (*A. a. shirasi*), and eastern North America from the Great Lakes to the east coast (*A. a. americana*). Morphological (Bowyer et al. 1991; Peterson 1955) and behavioral (Bubenik 1998b; Molvar and Bowyer 1994) differences among these subspecies are apparent. Based on anecdotal evidence, Peterson (1955) concluded that, as recently as the early 20th century, moose were still in the process of expanding their range after the retreat of ice sheets from the Wisconsinan glaciation. Thus, despite recent colonization, regional populations of moose may have been isolated, causing divergence through genetic drift and subsequent selection for adaptations to particular habitat characteristics. Geist (1998) argued, however, that morphological variation in North American moose is clinal and is not a basis for subspecific recognition. Moreover, Cronin (1992) found no variation within North American moose for restriction fragment length polymorphisms of the mitochondrial genome and no support for subspecies.

Variation in nucleotide sequences from a hypervariable domain of the mitochondrial DNA (mtDNA) control region was examined to detect population structure in North American moose. Specifically, geographic variation in the mitochondrial genome was examined to determine the circumstances under which moose expanded their range and to test whether geographic variation provides support for the current designation of 4 subspecies. Although mtDNA variation alone is not sufficient to identify subspecies, this marker is informative for assessing population history and levels of gene flow among regions (Avise et al. 1987). Maternal inheritance and lack of recombination in mtDNA facilitate examination of historical processes in lineages. Moreover, the fast mutation rates that characterize the control region allow recent processes to be studied. Different colonization scenarios likely would produce different patterns of mtDNA variation. For example, a stepping-stone scenario combined with serial founder events (Hundertmark et al. 1992) would produce a directional gradient of higher genetic diversity in Alaska to lower diversity in populations toward the end of the colonization pathway. Wavelike colonization characterized by founding events with large effective sizes would exhibit relatively homogeneous distributions of genetic variation, possibly combined with an isolation-by-distance pattern of diversity. The hypothesis that genetic variation of populations would decrease with distance from Alaska, where moose colonized North America via the Bering land bridge (Hundertmark et al. 2002), was tested.

Materials and Methods

Tissue samples were acquired from animals that were either harvested or handled by biologists for research or management purposes. Samples were collected from Alaska (n = 74), northwestern British Columbia (n = 11), Colorado (n = 19), central North America (n = 24), and eastern North America (n = 13). Samples from Alaska were divided geographically into ''mainland'' (*A. a. gigas*, n = 52) and ''southeastern'' (subspecies undetermined, n = 22) populations. The latter population was composed of moose from the southeastern panhandle of Alaska but did not include an isolated popu-

lation in Berners Bay that was established as a transplant of *A. a. gigas* (Burris and McKnight 1973). The population from British Columbia (*A. a. andersoni*) included samples from areas immediately east of the coastal mountain range separating southeastern Alaska from Canada. Samples from Colorado (*A. a. shirasi*) were collected from Jackson County, a translocated population founded by animals from up to 3 separate introductions from source populations in Utah and Wyoming (Duvall and Schoonveld 1988). The central North American population (*A. a. andersoni*) included samples from northeastern and north-central Minnesota, southwestern Ontario, Isle Royale, Michigan, northeastern North Dakota, and the Lake Winnipeg area of Manitoba. The eastern population (*A. a. americana*) was composed of samples from New Hampshire and New Brunswick.

Tissue samples (skeletal muscle, liver, kidney, or skin) were stored temporarily at −20°C or preserved in 100% ethanol as soon as possible after collection and archived at −80°C. A list of accession numbers of all specimens used in this study is available from the senior author. All tissue types were subjected to salt extraction for isolation of genomic DNA (Millar et al. 1988). mtDNA was isolated from nuclear DNA and RNA in 1 sample by means of a $CsCl_2$ density-gradient centrifugation (Sambrook et al. 1989) to verify the mitochondrial origin of the amplified sequences.

The left hypervariable domain of the control region was examined because this region evolves at an extremely fast rate and is useful for reconstructing phylogenies in cervids (Douzery and Randi 1997) and for intraspecific population studies (Avise et al. 1987). The polymerase chain reaction (PCR) was used to amplify a double-stranded, 554–base pair fragment of mtDNA. Primers used were LGL283 (5′-TACACTGGTCTTGTAAAC-3′—Bickham et al. 1996) located within $tRNA^{Thr}$ and primer ISM015 (5′-ATGGCCCTGTAGAAAGAAC-3′—R. Purdue, pers. comm.) located within the central conserved domain of the control region. That fragment contained part of $tRNA^{Thr}$, t-RNA^{Pro}, and part of the control region. The reaction mix contained 10 mM Tris–HCl, pH 8.3, 50 mM KCl, 2.5 mM $MgCl_2$, 0.2 mM deoxynucleotide triphosphates (dNTPs), 10 μM each primer, and 0.5 U DNA polymerase (AmpliTaq, Applied Biosystems, Foster City, California). Cycling conditions were a 2-min soak at 94°C followed by 30 cycles of 94°C (15 s) denaturation, 50°C (15 s) annealing, and 72°C (45 s) extension, followed by 1 extension period of 10 min at 72°C. PCR products were observed on a 6% agarose gel. Primers, dNTPs, and polymerase were separated from successful PCR amplifications after precipitation in polyethylene glycol. Purified PCR products were cycle sequenced (both directions) with fluorescing dideoxynucleotide triphosphates. Nucleotide composition of the final products was determined using an automated sequencer (ABI 373, Applied Biosystems) with standard protocols supplied by the manufacturer. Sequences were aligned with the Clustal V algorithm (Higgins et al. 1992) and edited with Sequence Navigator software (Applied Biosystems). The control region was identified by comparison with published sequences from other cervids (Douzery and Randi 1997). Haplotypes described in this study were deposited in GenBank (accession nos. AF412224–AF412250).

Relationships among haplotypes were assessed with a neighbor-joining phylogeny (Saitou and Nei 1987) using Kimura's 2-parameter distance measure (Kimura 1980) computed with MEGA2 software (Kumar et al. 2001). A minimum-spanning tree was generated with a distance-parsimony approach to examine potential evolutionary pathways among haplotypes (Excoffier and Smouse 1994). This tree was constructed from genetic distances (Kimura 1980) with Arlequin 2.0 software (Schneider et al. 2000) and differed from the neighbor-joining tree by allowing haplotypes to be placed at internal nodes rather than restricting their positions to branch tips. Haplotypes of moose from Eurasia (Hundertmark et al. 2002) were used to determine the basal node of the minimum-spanning tree for North America.

Estimates of variability of haplotype sequences within and among populations also were computed with Arlequin. Sequence variation among individuals and within populations was expressed as haplotype diversity (H: probability that 2 randomly chosen individuals will have different haplotypes), mean number of nucleotide differences in pairwise comparisons (d_X), nucleotide diversity (π: probability that 2 randomly chosen individuals will differ at a nucleotide site—Nei and Kumar 2000), and total number of variable nucleotide sites within a

group (S). Degree of structuring among populations was measured as Φ_{ST} (an analog of Wright's F_{ST}) as estimated from a hierarchical analysis of molecular variance (AMOVA—Excoffier et al. 1992; Weir and Cockerham 1984) performed in Arlequin. A Mantel test (Mantel 1967) was used to determine whether significant correlation existed between genetic distances and geographic distances among populations. Coancestry coefficients (Reynolds et al. 1983) were used as genetic distances between populations. Arlequin was used to generate 1,000 sets of bootstrapped data to determine the probability of observing a correlation coefficient less than or equal to that computed from original data.

We conducted searches of on-line databases via GenBank and European Molecular Biology Laboratory to identify homologous sequences from moose with known locality information to compare with sequences generated for this study. A single haplotype from a moose collected in Banff National Park, Alberta, Canada (GenBank accession no. AF016951—Polziehn and Strobeck 1998), was identified. Mikko and Andersson (1995) listed polymorphic sites for the control region of 19 Canadian and 30 Swedish moose, although no locality information was assigned to individual haplotypes. Their samples from Canada were collected in 5 national parks in the Rocky Mountains, 1 park in central Saskatchewan, and 1 park in Newfoundland. With the exception of the sample from the Newfoundland park (*A. a. americana*), all samples came from the range of *A. a. andersoni*, including its contact zone with *A. a. shirasi*. Our sequences were compared with the table of polymorphic sites (Mikko and Andersson 1995, table 2), and by assuming that all other sites were identical to our consensus sequence, an alignment was generated. The sequences of Mikko and Andersson (1995) were not included in most analyses due to the lack of specific locality data; however, they were treated as an independent sample to determine whether our sampling efforts were successful in describing existing variation.

To examine temporal and geographic extent of the colonization, we obtained records of fossils from North America that had radiometric dates (A. Dyke, in litt.; Harington, in press). Histograms of occurrence of dated fossils were created for 4 contiguous regions of North America: >130°W longitude, 130–110°W longitude N of 52°N latitude combined with 110–80°W longitude, 130–110°W longitude S of 52°N latitude, and <80°W longitude. These regions approximated the ranges of *A. a. gigas*, *A. a. andersoni*, *A. a. shirasi*, and *A. a. americana*, respectively.

Results

Sequence characteristics.—The amplified fragment of the control region was 470 nucleotides in length and was characterized by 20 variable sites (Table 1). All substitutions were transitions, and no deletions or insertions were detected. Sixteen haplotypes were identified among 142 North American moose. Although nucleotide diversity was low among individuals (π = 0.0069, d_X = 3.23) and haplotypes (π = 0.010, d_X = 4.87), haplotype diversity was high throughout the sampling area (H = 0.86).

Phylogenetic relationships.—The consensus neighbor-joining tree exhibited little phylogenetic structure and was highly polytomous. No monophyly was detected within the tree relative to subspecies designation (Fig. 1). Twelve of 16 haplotypes identified in this study were found within the range of *A. a. andersoni*; furthermore, the haplotype obtained from GenBank resulted in the addition of a 13th haplotype (P) to that group (Fig. 1). The minimum-spanning tree (Fig. 2) indicated a starlike structure with haplotypes occurring on short branches radiating from a central haplotype. The longest branch (A–G) was 6 substitutions in length, as measured from the central node (haplotype A). The root of the tree was haplotype J, which was unique to central North America (Table 1).

Population variation and structure.—Mitochondrial diversity varied among the North American populations. Populations from within the range of *A. a. andersoni* (central North America and British Columbia) exhibited the greatest diversity, whereas populations from mainland Alaska, southeastern Alaska, and eastern North America showed less variation. Samples from Colorado were monomorphic for a unique haplotype (Table 2).

TABLE 1.—Variable nucleotide sites within the left hypervariable domain of the mitochondrial control region in North American moose (*Alces alces*). The nucleotide positions are numbered relative to the first nucleotide of the control region sequence and consist of 470 sites. Haplotype A is listed as the reference and variable sites in other haplotypes are indicated. Identical sites are indicated by a dot. The numbers of each haplotype occurring in the populations sampled are indicated in columns at the right.

Haplotype	Position (1122222233333334444 / 81602258800122460225 / 60824871203816750895)	Population: Mainland Alaska	Population: Southeastern Alaska	Population: British Columbia	Population: Colorado	Population: Central North America	Population: Eastern North America
A	TCGTCTCATAACTTTCAGCC	21				1	
B	.............C......	31	8	1			
C	....T.TGC.......G...		13	1			
D	....T..GC.......G...		1				
E	...C....C...C.......			6			
F	C.....T....T......T.			1			
G	..A.T.TGC.......G...			1			
H	...C..T.......C.....			1			
I	......T.....C......T				19		
J	...............T.A..					6	
K	........CGG.........					1	
L	...............T....					9	
M	.T.............T....					1	
N	C..........T..C...T.					6	
O	.....C..............						12
P	...C.C..............						1
Total		52	22	11	19	23	13

Matrices of geographic and genetic distances were not significantly correlated ($r = 0.22$, $P = 0.29$). Nonetheless, haplotypes segregated strongly on a geographic basis. Thirteen of 16 haplotypes were restricted to 1 population. Haplotype B occurred in >2 populations (Table 1) primarily because it occurred in a zone where 3 populations (Alaska, southeastern Alaska, and British Columbia) intersected and not because it was widespread outside Alaska. Overall, Φ_{ST} from AMOVA was 0.61 ($P < 0.0001$), indicating a high degree of population structure.

The sequences from Canada reported by Mikko and Andersson (1995) were composed of 7 haplotypes, 4 of which were identical to haplotypes described in this study (A, F, J, and O), and 2 others were represented in the minimum-spanning tree as presumed intermediate haplotypes (small dots adjacent to haplotypes E and I in Fig. 2). The 7th haplotype (not shown) represented a new sequence and was positioned 2 steps from the central haplotype (A). The sample from Alberta, Canada (Polziehn and Strobeck 1998), possessed haplotype O and was the only sample with a confirmed locality outside eastern United States with that haplotype.

Paleontological data.—With the exception of 1 specimen from Yukon Territory, Canada, dated at 32,250 years ago (not shown), all fossil remains identified as *A. alces* were <15,000 years old, although age distribution varied geographically (Fig. 3). All specimens >8,000 years old occurred within the range of *A. a. gigas*. For the 3 southern subspecies, only 1 specimen (7,484 years old, presumably *A. a. andersoni*) was >5,110 years old.

DISCUSSION

Templeton (1998) identified 3 factors that led to spatial structuring of genetic varia-

FIG. 1.—A consensus neighbor-joining phylogeny of the control region for North American moose (*Alces alces*) based on the 2-parameter distance measure of Kimura (1980). Only groups occurring in at least 50% of 1,000 bootstrap replicates are shown. Haplotype labels correspond to those in Table 1. Distribution of haplotypes among putative North American subspecies is indicated by filled circles. The open circle indicates placement of GenBank accession no. AF016951 (Polziehn and Strobeck 1998). Individuals with haplotypes B, C, and D also occurred in southeastern Alaska but are not shown because of their uncertain taxonomic status.

FIG. 2.—Minimum-spanning tree of mitochondrial haplotypes of North American moose (*Alces alces*). Labeled circles indicate haplotypes identified in this study shown in Table 1, and dots indicate presumed intermediate haplotypes based on a most parsimonious arrangement of mutations. Alternate, equally parsimonious pathways are indicated by broken lines. Size of circles is proportional to occurrence of haplotypes, and partitions within circles indicate geographic distribution of haplotypes.

tion: population fragmentation; restricted gene flow, notably isolation by distance; and range expansion. The starlike phylogeny of haplotypes indicated recent expansion of a single population rather than temporal and geographic isolation of 4 separate populations as postulated by Peterson (1955). Furthermore, we know of no historical events that could have sundered populations of North American moose for long periods after post–ice age colonization. Therefore, we reject fragmentation of an existing population as an explanation for subspeciation of moose in North America.

Consistent with the prediction of Slatkin (1993), genetic relationships among regions did not resemble an equilibrium state caused by isolation by distance. Haplotype composition of peripheral populations of moose in North America, however, differed from that of populations occupying the center of the continent. That result was not expected under the Slatkin (1993) model. Therefore, we conclude that differentiation seen in our samples was not generated via isolation by distance as a function of τ under the Slatkin (1993) model of stepping-stone expansion. We hypothesize that few individuals were involved in founding populations of North American moose, thereby leading to the notable lack of variation in mtDNA detected within most populations.

A mechanism that may explain the observed phylogeographic pattern is rare, long-distance (i.e., leptokurtic) dispersal (Hewitt 1996). Under this concept, the few members of the preexpansion population that are successful long-distance dispersers

TABLE 2.—Parameters indicating intrapopulation level diversity of mitochondrial DNA (mtDNA) for North American populations of moose (*Alces alces*). Subspecific designations for populations follow Hall (1981).

Population	Subspecies	*n*	Number of haplotypes/ number of unique haplotypes	Haplotype diversity ($H \pm SE$)	Nucleotide diversity ($\pi \pm SE$)	Variable sites (S)
Mainland Alaska	*A. a. gigas*	52	2/0	0.49 ± 0.03	0.0010 ± 0.0010	1
Southeastern Alaska	Indeterminate	22	3/1	0.54 ± 0.07	0.0062 ± 0.0038	6
Colorado	*A. a. shirasi*	19	1/1	0	0	0
Central North America	*A. a. andersoni*	24	6/5	0.76 ± 0.05	0.0052 ± 0.003	10
British Columbia	*A. a. andersoni*	11	6/4	0.73 ± 0.14	0.0087 ± 0.005	13
Eastern North America	*A. a. americana*	13	2/1	0.15 ± 0.13	0.00033 ± 0.0005	1

establish new populations during range expansion. Because of the distance of the dispersal, founding individuals have an opportunity to saturate the available habitat with descendants before the area is reached via normal range expansion, thereby promoting genetic homogeneity in founded populations. Simulations revealed that such populations have lower genetic diversity compared with populations founded by a traditional stepping-stone mode (Ibrahim et al. 1996). Moose exhibit patterns of movements necessary to facilitate genetic structure among populations, including a high degree of female philopatry and the ability to disperse long distances (Hundertmark 1998). Similarly, Geist (1987a) postulated that evolutionary change in moose could occur during rapid colonization of a region, which he believed was an important determinant of existing levels of morphological diversity.

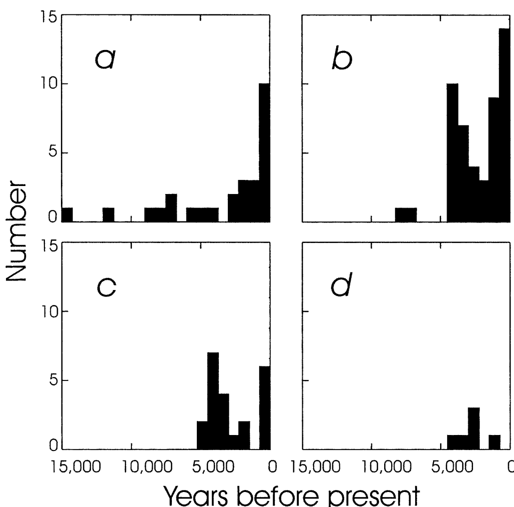

FIG. 3.—Distribution of estimated ages of paleontological remains of moose (*Alces alces*) in North America. Geographic locations of remains are grouped according to ranges of currently recognized subspecies: a) *A. a. gigas*, b) *A. a. andersoni*, c) *A. a. americanus*, and d) *A. a. shirasi*. One sample dated to 32,250 years ago within the range of *A. a. gigas* is not shown.

Our genetic data are consistent with a recent colonization of North America by moose (Hundertmark et al. 2002), rapid expansion of a centrally located population, and further range expansion characterized by small numbers of founders for populations on the periphery of the range. This scenario is concordant with paleontological evidence indicating a paucity of fossils >7,500 years old outside Alaska and Yukon Territory. Rapid morphological change must have followed colonization because paleontological evidence indicated that moose colonizing Alaska were larger than modern moose (Guthrie 1984). Attributes for which differences exist currently between forest-dwelling moose of Canada and the lower United States and tundra-dwelling moose of Alaska include body size, antler

size, pelage coloration, group size, and mating behavior (Bowyer et al. 1991, 1997, in press; Gasaway et al. 1987; Molvar and Bowyer 1994; Peek et al. 1974; Peterson 1955). Peterson (1955) also reported differences in size of palate among North American subspecies. We hypothesize that these differences resulted from natural selection acting on moose in North America since colonization, causing rapid evolutionary change. A period of rapid change combined with range expansion and restricted gene flow between source and sink populations are factors that could contribute to formation of subspecies without concomitant divergence of mtDNA lineages. Thus, the lack of diversity of restriction fragment length polymorphisms documented by Cronin (1992) for North American moose is consistent with this scenario.

The current genetic structure of moose populations in North America may reflect limitations imposed by the movement corridor available to the colonizing population. Colonizing moose entering North America through Beringia would have had limited options for dispersal to the southern portions of the continent. Dispersal would have been constrained by the Laurentide and Cordilleran ice sheets of the Wisconsinan ice age. These ice sheets formed a barrier between eastern Beringia (Alaska) and the remainder of the continent until approximately 14,000 years ago (Dyke and Prest 1987), which was contemporaneous with the earliest estimate of entry by moose into North America (Hundertmark et al. 2002). Subsequently, a corridor was formed along the eastern slope of the Rocky Mountains as the ice sheets retracted (Dyke and Prest 1987). Moose following that corridor would have traveled south along the eastern front of the mountains to the present location of the contact zone between *A. a. andersoni* and *A. a. shirasi* in southern Alberta. Expansion to the east then would have been possible until moose reached the area of the Great Lakes. Until at least 8,000 years ago, this area was a series of large proglacial lakes (e.g., Ojibway, Agassiz, and Algonquin—Dyke and Prest 1987) that would have hindered further expansion to the east unless moose traversed a route south of these lakes. We hypothesize that by exploiting habitat south of the lakes, moose migrated to the eastern part of the continent and that these populations became isolated from populations in central North America as climatic warming made habitation south of the lakes unsuitable (Renecker and Hudson 1990; Telfer 1984). Peterson (1955) reported absence of moose in the region between the Great Lakes and Hudson Bay until recently, which would have restricted gene flow between populations to the east (*A. a. americana*) and west (*A. a. andersoni*) of this area. Lack of shared haplotypes between our samples from eastern and central North America indicate that these populations have not experienced high levels of gene flow historically and may have come into secondary contact only recently. A similar isolating mechanism could have operated in the Rocky Mountains, restricting gene flow between *A. a. shirasi* and *A. a. andersoni*.

The retreating ice sheets and subsequent growth of successional shrubs likely facilitated the eastward colonization of North America by moose at the start of the Holocene. Moose make extensive use of such seral vegetation, which provides important forages (Peek 1974, 1998). Likewise, aquatic plants, which offer a source of sodium for moose (Belovsky and Jordan 1981), probably were associated with retreating glaciers, thereby enhancing these areas for moose. Finally, the boreal forest was expanding northward after the Wisconsinan ice age, and moose would have encountered productive habitats, especially in areas undergoing succession after fire (Loranger et al. 1991; Peek et al. 1976; Weixelman et al. 1998). These successional stages undoubtedly hastened the eastward expansion by moose and offer an ecological mechanism for the genetic patterns we documented.

The only region of North America that was not well represented in our samples was the Rocky Mountains of Canada. Nonetheless, data describing moose inhabiting this area obtained from other studies (Mikko and Andersson 1995; Polziehn and Strobeck 1998) indicate that our sampling encompassed much of the variation present in this region. Furthermore, the high haplotype diversity represented by those data indicates that the Rocky Mountains region of southern Canada had a level of genetic diversity similar to that observed in British Columbia and central North America. This outcome further supports our contention that *A. a. andersoni* represents a diverse set of haplotypes and that it likely retained a relatively high effective population size during colonization.

Taxonomic implications.—The concept of subspecies is subjective and controversial. Mayr (1970) defined the term subspecies as a group of phenotypically similar populations inhabiting a geographic subdivision of the range of a species. Avise and Ball (1990) adopted a more restrictive definition that requires phylogenetic distinction among subspecies, specifically monophyly. They also emphasized that phylogenetic differences should consist of multiple, independent genetic traits. Moreover, Cronin (1993) argued that designation of subspecies should rely on analysis of multiple criteria rather than merely on analysis of genetic data. Yet, mtDNA is an informative locus for intraspecific phylogeography (Avise et al. 1987) and can provide insights into population history. Our data indicate a pattern of genetic structure among regional moose populations caused by lack of gene flow and are consistent with some degree of isolation of populations in the past. Moreover, these data are consistent with morphological characteristics used by Peterson (1955) to describe subspecies of moose in North America. Evidence from other taxa has indicated that small effective sizes of populations established in a similar manner have led to extensive and rapid differentiation (see Hewitt 1996). Although mtDNA haplotypes of North American moose did not exhibit reciprocal monophyly with respect to currently recognized subspecies, monophyly is merely an indicator of long-term separation of populations and is a function of effective population size and time since separation (Neigel and Avise 1986). If rapid evolutionary change derives from dispersal and colonization of new range (Geist 1987b; Hewitt 1996), then the differentiation necessary for subspecies may occur before attainment of reciprocal monophyly for a genetic marker. Although further investigations incorporating nuclear loci, particularly in contact zones, may be necessary to achieve a final conclusion, there is evidence of restriction in gene flow among regional populations of moose in the past, which is consistent with the distribution of 4 subspecies of moose in North America.

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