

# Mitochondrial Phylogeography of Moose (*Alces alces*): Late Pleistocene Divergence and Population Expansion

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**We examined phylogeographic relationships of moose (*Alces alces*) worldwide to test the proposed existence of two geographic races and to infer the timing and extent of demographic processes underpinning the expansion of this species across the Northern Hemisphere in the late Pleistocene. Sequence variation within the left hypervariable domain of the control region occurred at low or moderate levels worldwide and was structured geographically. Partitioning of genetic variance among regions indicated that isolation by distance was the primary agent for differentiation of moose populations but does not support the existence of distinct eastern and western races. Levels of genetic variation and structure of phylogenetic trees identify Asia as the origin of all extant mitochondrial lineages. A recent coalescence is indicated, with the most recent common ancestor dating to the last ice age. Moose have undergone two episodes of population expansion, likely corresponding to the final interstade of the most recent ice age and the onset of the current interglacial. Timing of expansion for the population in the Yakutia-Manchuria region of eastern Asia indicates that it is one of the oldest populations of moose and may represent the source of founders of extant populations in North America, which were colonized within the last 15,000 years. Our data suggest an extended period of low population size or a severe bottleneck prior to the divergence and expansion of extant lineages and a recent, less-severe bottleneck among European lineages. Climate change during the last ice age, acting through contraction and expansion of moose habitat and the flooding of the Bering land bridge, undoubtedly was a key factor in-**

**fluencing the divergence and expansion of moose populations.** © 2002 Elsevier Science (USA)

**Key Words:** Beringia; control region; ice ages; mtDNA; sudden-expansion model.

## INTRODUCTION

*Alces* is a monotypic genus: the sole extant species, *A. alces* (L.), has a circumboreal distribution (Geist, 1998). Origination of this genus likely occurred in Eurasia during the early Pleistocene (>1.5 million years ago; Thouveny and Bonifay, 1984), with a chronocline from *A. gallicus* to *A. latifrons*, and to *A. alces*, the prevailing view of speciation (Lister, 1987, 1993). Lister (1993) reported the earliest fossil remains in Eurasia attributable to *A. alces*, dated to approximately 100,000 years ago; furthermore, he placed the transition from *A. latifrons* to *A. alces* in Eurasia in the upper Pleistocene, perhaps 200,000 to 100,000 years ago. Guthrie (1995) implied a much later transition due to the paucity of fossil evidence for *A. alces* from the Pleistocene and the presence of its putative ancestor, *A. latifrons*, in Beringia as late as 35,000 years ago.

The date and demographic characteristics of the worldwide expansion of *A. alces* remain open to conjecture. Mikko and Andersson (1995) estimated time of divergence for moose of Sweden and North America at 350,000 to 165,000 years ago, based on variation in sequences of the mitochondrial control region. Furthermore, they documented low variability within a locus of the major histocompatibility complex (MHC) on both continents and similar amino acid motifs among MHC alleles. Those authors concluded that moose must have passed through a population bottleneck prior to the divergence of European and North American forms. Ellegren *et al.* (1996) also reported low variability in MHC in moose from Sweden, but used variation within highly polymorphic minisatellite loci as an index of the

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age of a presumptive bottleneck. They concluded that the levels of minisatellite variation that they observed could have been generated within a homogeneous population in as little as 10,000 to 50,000 years. Conversely, Hundertmark *et al.* (1992) documented moderate levels of allozyme diversity in moose from Alaska, which was not indicative of a severe bottleneck.

Factors affecting the expansion of moose across the northern hemisphere and the divergence of moose lineages were governed, to a large degree, by climate change in the late Pleistocene (Lister, 1993; Guthrie, 1995). The Wisconsinan (North America) and Weichselian (Europe) glaciations were the last extensive ice ages of the Pleistocene and were contemporaneous with the emergence of *A. alces*. Data from North America indicate that the Wisconsinan glaciation lasted from 120,000 to 8000 years ago, with two periods of maximum ice coverage occurring from 75,000 to 65,000 and from 23,000 to 18,000 years ago (Fulton *et al.*, 1986; Dyke and Prest, 1987). Northern Europe had a similar history, and Fennoscandia was completely covered with ice at glacial maxima (Lundqvist, 1986). Between the two periods of maximum extent of ice, an interstade that was characterized by a variable but generally warmer climate and retreating ice sheets occurred from approximately 60,000 to 30,000 years ago (Fulton *et al.*, 1986; Arkhipov *et al.*, 1986). During the most recent glacial maximum, sea level in the North Pacific Ocean was  $\leq 100$  m lower than that today and the Bering land bridge was exposed (Elias *et al.*, 1996), creating Beringia, the land mass connecting Asia and North America. Glacial coverage was much more extensive in North America than in Eurasia and created an effective barrier between Beringia and the remainder of the continent. The land bridge flooded approximately 11,000 years ago as glaciers melted (Elias *et al.*, 1996), effectively separating the two continents.

*A. alces* likely became established in North America during the final retreat of the Wisconsinan ice sheets (Geist, 1987; Bowyer *et al.*, 1991; Cronin, 1992; Guthrie, 1995). Cronin (1992) documented a lack of variation in mtDNA restriction fragments in North American moose and concluded that the population must be relatively young and that moose entered North America from Beringia in the late Wisconsinan. Bowyer *et al.* (1991) and Guthrie (1995) supported a post-Wisconsinan entry based on the paucity of modern moose in the fossil record in North America prior to 9000 years ago (Guthrie, 1990). Geist (1987), (1998) also favored a recent entry of moose to the New World and suggested that North American moose originated in Beringia from an ancestral population that also gave rise to the morphologically similar population in the Russian Far East (Magadan Oblast and vicinity). Whether North American moose arose in Beringia or migrated from Asia, a recent and common ancestry of moose in Alaska

and far-eastern Asia has been presumed, with some authors placing those populations within the same subspecies (Peterson, 1952; Kistchinski, 1974).

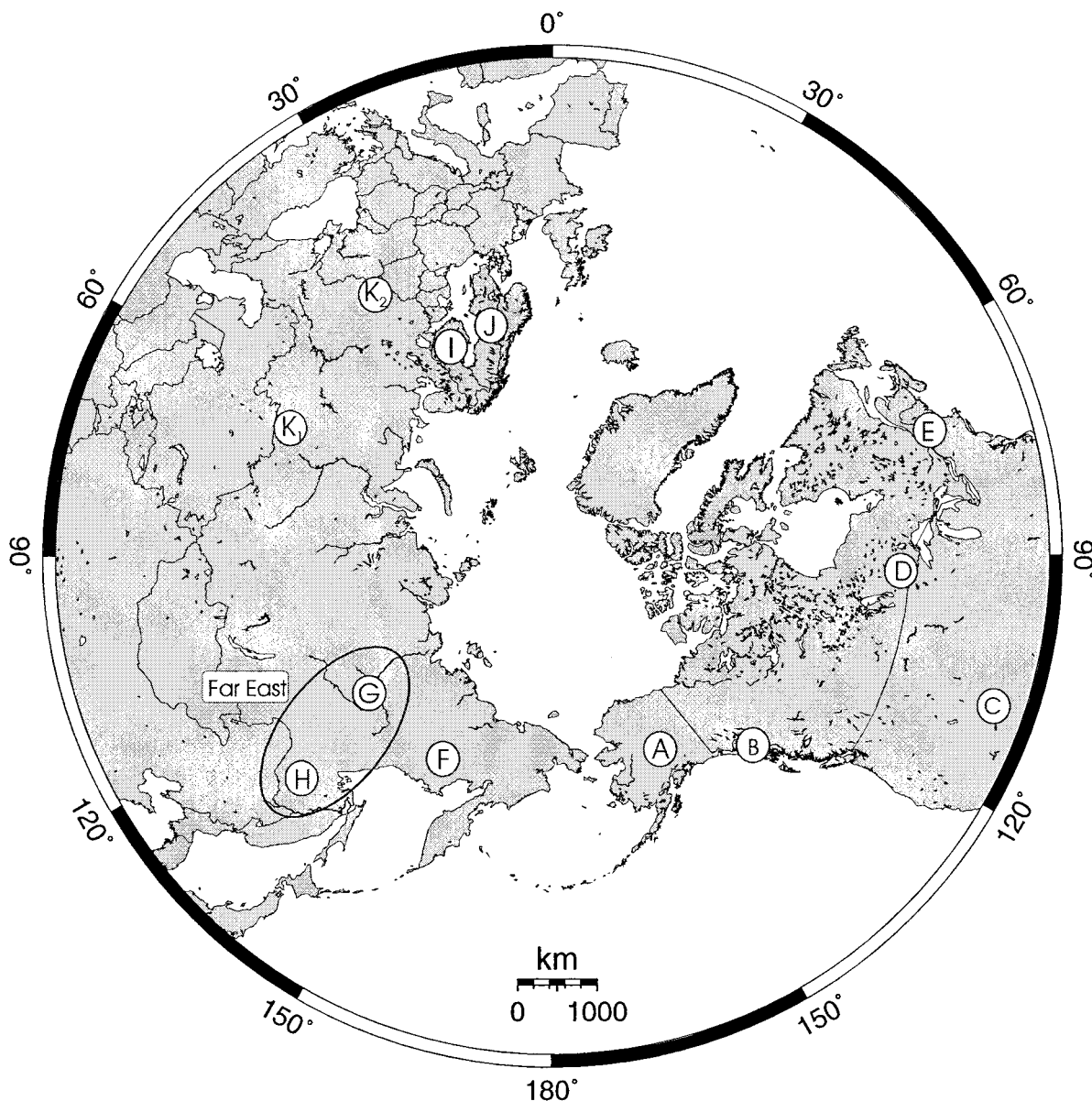
The early view of geographic variation in modern moose consisted of a western race (*A. a. alces*) inhabiting Europe and western Asia to the Yenisei River and an eastern race (*A. a. americana*) inhabiting eastern Asia and North America (Flerov, 1952). Although the eastern race had been subdivided into as many as seven subspecies (Peterson, 1952; Chernyavski and Domnich, 1989), Geist (1998) argued for recognition of only the original two subspecies because they represented a fundamental phylogenetic division within the species. Existence of distinct eastern and western races was supported by differences in morphology (Flerov, 1952; Peterson, 1952; Geist, 1987) and numbers of chromosomes. A karyotype of  $2n = 68$  typified European moose (Gustavson and Sundt, 1968), whereas  $2n = 70$  was characteristic of North American moose (Hsu and Benirschke, 1973). Moreover, the presence of a 75-bp repeat in the mitochondrial control region distinguished moose from Sweden and North America (Mikko and Andersson, 1995). Recently, the karyotype and form of mitochondrial indel associated with North American moose were documented in eastern Asia (Boeskorov, 1996, 1997; Udina *et al.*, 2002), further bolstering the notion of two races with ranges meeting in central Asia. Based primarily on karyotypes, Groves and Grubb (1987) defined eastern and western races of moose as semispecies, whereas Boeskorov (1997) proposed separate species status.

To better understand the history of moose in the late Pleistocene, we studied variation within the left hypervariable domain of the mitochondrial control region to discern phylogeographic patterns in populations worldwide. We chose the mitochondrial genome for those analyses because of its nonrecombining mode of transmission and its fast evolutionary rate (Avice *et al.*, 1987); moreover, the control region has been useful in constructing phylogenies of other deer species (Douzery and Randi, 1997; Polzeihn and Strobeck, 1998). We tested the hypotheses that (a) mtDNA variation supports two primary lineages of moose, (b) timing of divergence and expansion of extant moose lineages date to the most recent glacial period, (c) evidence of a late-Pleistocene bottleneck exists, and (d) moose occurring on either side of the Bering Sea (Magadan Oblast and North America) share a recent Beringian ancestry.

## MATERIALS AND METHODS

### *Tissue Collection and Population Identification*

Tissue samples were solicited from moose hunters and biologists from across North America, Europe, and Asia (Fig. 1). North American populations were (A) northern, interior, and southcentral Alaska ( $n = 62$ ),



**FIG. 1.** Sampling sites for moose used in this study. Multiple sampling sites contained within a circle were combined to form a single population for particular analyses. The perimeter of the map is 35° north latitude.

(B) southeastern Alaska and northwestern British Columbia ( $n = 23$ ), (C) Colorado ( $n = 19$ ), (D) central North America ( $n = 24$ ), and (E) eastern North America ( $n = 13$ ). Asian populations were (F) Magadan Oblast ( $n = 18$ ), (G) Yakutia ( $n = 7$ ), and (H) Eastern Asia ( $n = 7$ ), consisting of Amur Oblast, Khabarovsk Kray, and northwestern China. European populations were (I) Finland ( $n = 10$ ), (J) Sweden ( $n = 6$ ), and (K) eastern Europe ( $n = 3$ ), consisting of southern Urals ( $K_1$ ) and Smolensk ( $K_2$ ), Russia.

Tissue samples consisted of skeletal muscle, liver, kidney, skin, blood, or hair. Genomic DNA was extracted using standard protocols (Maniatis *et al.*,

1989). Mitochondrial DNA was isolated from nuclear DNA and RNA from one moose by means of a  $\text{CsCl}_2$  density-gradient centrifugation. That sample was used to verify the mitochondrial origin of amplified sequences.

#### *DNA Amplification*

Amplification and sequencing of samples were conducted in either of two laboratories (USA or Russia), with each laboratory having its own protocol. Protocol A used primers LGL283 (L15693, 5'—TACACTGG-TCTTGTA AAC—3') and ISM015 (H00068, 5'—ATG-GCCCTGTAGAAAGAAC—3'); PCR conditions consisted

of a 2-min soak at 94°C followed by 30 cycles of 94°C (15 s), 50°C (15 s), and 72°C (45 s) with a final extension period of 10 min at 72°C; cycle sequencing was conducted in both directions with fluorescing ddNTPs and final products were analyzed on an automated sequencer (ABI 373; PE Applied Biosystems). Protocol B used primers LmPro (L15766, 5'—GCCATCAACTC-CCAAAGCT—3') and TDKD (H00074, 5'—CCTGAAG-TAGGAACCAGATG—3'); PCR conditions consisted of a 1-min soak at 95°C followed by 30 cycles of 95°C (10 s), 62°C (20 s), and 74°C (20 s) with a final extension period of 5 min at 74°C; sequencing was conducted in both directions with the fmole DNA Cycle Sequencing System (Promega) and sequences were transcribed manually from autoradiograms. Primer binding sites were identified by strand and position, where H and L represent heavy and light strand, respectively, and the numerals represent the position of the 3' end of the primer relative to the appropriate strand of the bovine mitochondrial sequence (Anderson *et al.*, 1982). Both protocols amplified the left hypervariable domain of the control region by having a light-strand primer within the flanking tRNA<sup>Pro</sup> gene and a heavy-strand primer within the central conserved domain of the control region. Protocol A amplified a slightly longer fragment than protocol B. All sequences were aligned with the CLUSTAL V algorithm (Higgins *et al.*, 1992). Indels were aligned, whenever possible, to correspond with similar indels in other species of deer (Douzery and Randi, 1997). After alignment, the longer sequences were truncated to match the shorter sequences, and all sequences were truncated to remove the 3' end of tRNA<sup>Pro</sup>. Sequences of all haplotypes were submitted to GenBank and assigned accession numbers AF412224–AF412269, and AF414123.

### Data Analysis

**Phylogenetic relationships.** A neighbor-joining (Saitou and Nei, 1987) tree of haplotypes was computed with Kimura's (1980) two-parameter model of sequence evolution. A maximum-parsimony cladogram was constructed with a heuristic search, with gaps treated as a fifth state, and with any gap larger than one base treated as a single entity. PAUP\* version 4.0b4 (Swofford, 1999) was used to construct the neighbor-joining and parsimony trees, and confidence in those topologies was assessed by 1000 bootstrap replicates (Felsenstein, 1985). A maximum-likelihood phylogeny was computed with program PUZZLE version 4.0 (Strimmer and von Haesler, 1996), and the reliability of the topology was assessed by 10,000 quartet-puzzling steps. The Hasegawa–Kishino–Yano (Hasegawa *et al.*, 1985) model of sequence evolution was chosen for the maximum-likelihood approach. Trees were rooted with homologous sequences from elk (*Cervus elaphus*; Polziehn and Strobeck, 1998) and Chinese water deer (*Hydropotes inermis*; Douzery and Randi,

1997) that were obtained from GenBank and from caribou (*Rangifer tarandus*; this study). Evolutionary relationships among haplotypes also were inferred from a minimum-spanning tree constructed with ARLEQUIN version 2.0 (Schneider *et al.*, 2000). That method used a parsimony approach to connect each sequence to its closest neighbor, based on pairwise differences, and differed from traditional methods of tree construction by allowing extant haplotypes to occupy internal nodes.

**Inter- and intrapopulation variation.** Estimates of variability were computed with ARLEQUIN and MEGA2 (Kumar *et al.*, 2001). We expressed variation within populations as haplotype diversity ( $H$ ), nucleotide diversity ( $\pi$ ), number of segregating sites ( $S$ ), and average number of pairwise differences ( $d_s$ ) among haplotypes assuming a Kimura (1980) two-parameter model of sequence evolution. Genetic differentiation between populations was expressed as mean number of pairwise differences per site ( $d_{xy}$ ) and as pairwise  $\Phi_{ST}$ , which took into account variation in haplotype frequencies among populations and genetic distance based on nucleotide variation. Distribution of genetic variance within a hierarchical structure of population organization was expressed as  $\Phi$  statistics in a nested analysis of molecular variance (AMOVA; Weir and Cockerham, 1984; Excoffier *et al.*, 1992). We conducted AMOVA analysis with three groups representing current continental populations (Europe, Asia, and North America) and with two groups representing the putative eastern (Asia and North America) and western (Europe) lineages of moose. Statistical confidence in variance estimates was determined by comparing the observed  $\Phi$  statistics against a distribution of estimates generated from 10,000 permutations of data (Excoffier *et al.*, 1992). We used a Mantel test (Mantel, 1967) to determine whether pairwise values of  $\Phi_{ST}$  were related to geographic distances between populations; North America was considered a single population for that analysis with a geographic location in Alaska.

We estimated net pairwise divergence per base pair ( $d_A$ ), which is proportional to time since divergence ( $T$ ) of two populations. Divergence time was calculated as  $T = d_A/2\mu$ , where  $\mu$  is the rate of nucleotide substitution (Nei and Kumar, 2000). An appropriate rate had not been calibrated for moose, so we used two different estimates for divergence ( $2\mu$ ), each of which was specific to the left hypervariable domain of the control region. One estimate was 62.8% per million years derived for domestic cattle (Brady *et al.*, 1996), and the other was 78.5% per million years calculated for Bison (Burzynska *et al.*, 1999).

**Historic demography.** We tested for the presence of historic population bottlenecks with Tajima's  $D$  statistic (Tajima, 1989a,b). Historic demographic expansions were detected by examination of frequency distribu-

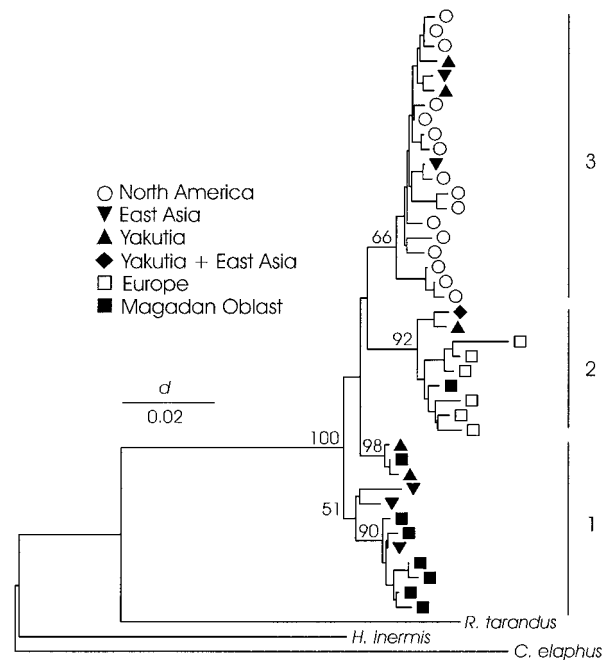
tions of pairwise differences of sequences (mismatch distributions) within continental or regional assemblages (Slatkin and Hudson, 1991; Rogers and Harpending, 1992). Concordance of our data with the distribution underlying the sudden-expansion model of Rogers (1995) was assessed by means of a least-squares approach (Schneider and Excoffier, 1999) implemented by ARLEQUIN. For distributions that did not differ significantly ( $P > 0.05$ ) from the expectations of the sudden-expansion model, we estimated  $\tau$  (an estimate of the mode of the mismatch distribution), which is an index of time since expansion expressed in units of mutational time (Slatkin and Hudson, 1991). Confidence intervals for  $\tau$  were estimated from 5000 bootstrap replicates. We transformed values of  $\tau$  to estimates of time since expansion with the equation  $\tau = 2\mu t$ , where  $\mu$  is the mutation rate for the sequence, and  $t$  is the time since expansion. We used the same estimates of mutation rates described previously for divergence estimates.

## RESULTS

### Sequence Variation

The section of the control region that we analyzed was 442 to 518 nucleotides in length, with the first nucleotide in the moose control region corresponding to nucleotide position 15,792 in the bovine mitochondrial genome (Anderson *et al.*, 1982). Variation in length was attributable to two length mutations: a single insertion in six Eurasian haplotypes at position 510 and the 75-bp indel. The fragment comprising the large indel was absent in all North American moose and 4 Asian moose from Yakutia and eastern Asia, yielding the shorter sequence. There were 4 variable sites within the large indel: 3 transitions and 1 transversion. The transversion occurred in all moose from eastern Europe and in 3 of 18 moose from Magadan Oblast. The first 365 nucleotides of the sequence (excluding the large indel) comprised the left hypervariable domain of the control region. Excluding indels, there were 47 polymorphic sites within the fragment that we analyzed: 46 transitions and 1 transversion. That transversion was confined to one individual from eastern Europe. No site had more than two bases segregating.

We identified 16 haplotypes among 141 individual moose sampled from North America. Twenty-five different haplotypes were detected among 51 Eurasian moose: 19 in 32 Asian moose and 6 in 19 European moose. No haplotypes were shared among continents, and there was little sharing of haplotypes among populations within continents. Among all haplotypes,  $\pi = 0.025$  and  $d_x = 10.64$ . The number of pairwise differences between haplotypes ranged from 1 to 23 (1 to 25 if indels were included). Among all individuals,  $H = 0.919$ ,  $\pi = 0.018$ , and  $d_x = 8.05$ .

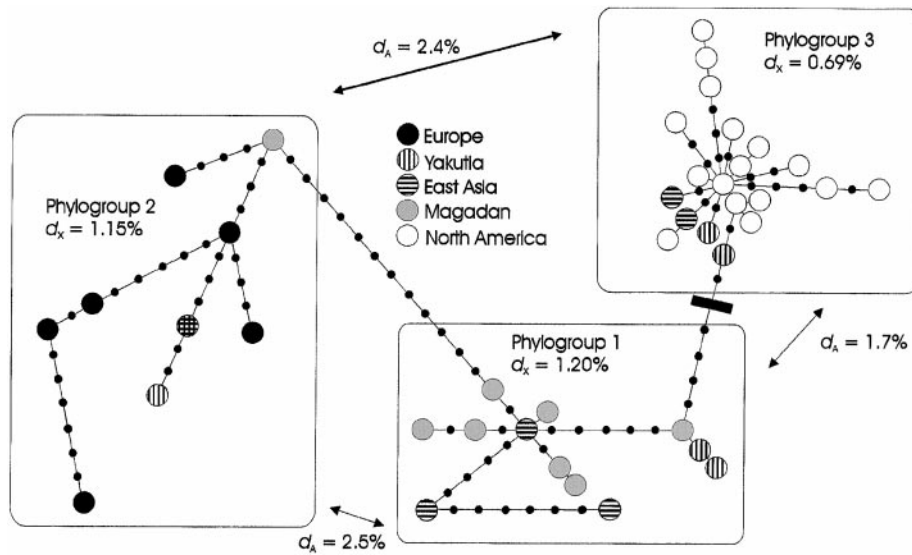


**FIG. 2.** The neighbor-joining tree representing phylogenetic relationships among control region haplotypes for moose worldwide. Bootstrap support for major branches are indicated at nodes. Phylogroup membership is indicated at the right.

### Phylogenetic Relationships

The neighbor-joining tree exhibited a shallow branching structure with four clades that we grouped into three phylogroups (Fig. 2) corresponding roughly to continental associations. Asian haplotypes occurred in each clade, and one phylogroup was exclusively Asian (phylogroup 1). European (phylogroup 2) and North American (phylogroup 3) haplotypes were confined to separate phylogroups. The maximum-likelihood and parsimony trees (not shown) formed nearly identical topologies except that the maximum-likelihood tree joined the two clades comprising phylogroup 1 with 50% bootstrap support. Phylogroups 1 and 2 were characterized by the presence of the 75-bp insertion. Absence of that fragment was a reliable marker for membership in phylogroup 3, because haplotype composition of that phylogroup was identical in all trees even though the indel was used as an informative character in the parsimony analysis only. One European haplotype exhibited an unusually long branch (Fig. 2); that haplotype occurred in all moose from Sweden. In pairwise comparisons among all haplotypes, the Swedish haplotype was ranked as most divergent from 32 of the remaining 40 haplotypes.

The minimum-spanning tree (Fig. 3) was the most parsimonious arrangement of haplotypes, assuming only one loss or gain of the large indel. That tree shows phylogroups 2 and 3 connected to a central phylogroup 1. Net sequence divergence between those groups



**FIG. 3.** A minimum-spanning tree of worldwide moose haplotypes. Filled and open circles indicate haplotypes that we analyzed, whereas small black dots indicate presumptive intermediate haplotypes, assuming a change of one substitution per step. The black bar indicates the branch on which the deletion of the 75-bp fragment occurred.

ranged from 1.7 to 2.5%, whereas mean pairwise differences within groups ranged from 0.69 to 1.20% (Fig. 3); phylogroup 3 exhibited the lowest level of within-group variation. Phylogroups 1 and 2 were equally divergent from phylogroup 3, suggesting some degree of homoplasmy. Asian haplotypes within phylogroup 3 occurred in Yakutia and eastern Asia. Haplotypes sampled from Magadan Oblast, which was the closest Asian population geographically to North America, were associated with phylogroups 1 and 2, but not phylogroup 3. Phylogroup 3 exhibited a star-like structure indicative of recent or rapid expansion. Based on divergence rates of 62.8 and 78.5% per million years, we estimated divergence of phylogroups 1 and 3 at approximately 27,000 and 21,500 years ago, respectively. Similarly, our estimates for divergence of phy-

logroup 2 and phylogroup 1 were approximately 38,000 and 30,000 years ago.

#### Inter- and Intrapopulation Variation

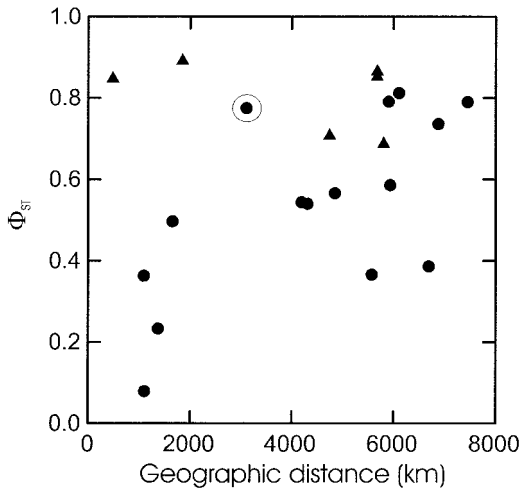
Pairwise comparisons of  $\Phi_{ST}$  indicated significant ( $P < 0.05$ ) differentiation between populations except for the comparison between Yakutia and eastern Asia (Table 1). For further analyses, those populations were combined to form a population that we termed Far East (Fig. 1). Measures of population divergence generally were greatest between populations of North America and Europe and least between populations of North America and Asia (Table 1). Pairwise estimates of  $\Phi_{ST}$  between populations were related significantly to geographic distance ( $r = 0.37$ ,  $P = 0.023$ ; Fig. 4). That relatively low correlation, however, was influ-

**TABLE 1**  
**Indices of Population Differentiation for Moose**

	North America	Finland	Eastern Europe	Sweden	East Asia	Yakutia	Magadan Oblast
North America		0.78	0.75	0.84	0.49	0.49	0.74
Finland	2.8		0.38	0.82	0.60	0.57	0.78
Eastern Europe	2.4	0.3		0.89	0.43	0.41	0.73
Sweden	4.2	1.3	1.6		0.70	0.72	0.84
East Asia	0.7	1.7	1.5	3.3		0.08 <sup>a</sup>	0.34
Yakutia	0.7	1.3	1.4	3.0	0.2 <sup>a</sup>		0.47
Magadan Oblast	2.3	2.8	2.6	4.1	0.6	1.2	

*Note.* Values above the diagonal are pairwise  $\Phi_{ST}$ , and those below the diagonal are average net pairwise divergence ( $d_A$ ) between populations. All values assume a Kimura (1980) two-parameter model of sequence evolution and differ significantly ( $P < 0.05$ ) from zero except where noted.

<sup>a</sup>  $P > 0.05$ .



**FIG. 4.** Plot of pairwise comparisons of  $\Phi_{ST}$  values versus geographic distance between moose populations. Comparisons involving the population from Sweden are indicated by triangles. The circled observation is the comparison between North America and its nearest Asian population, Magadan Oblast.

enced to a large degree by the Swedish population, which showed a high degree of divergence from all populations regardless of geographic distance (Fig. 4). When that population was removed from the analysis, the relationship between  $\Phi_{ST}$  and geographic distance improved ( $r = 0.67$ ,  $P = 0.011$ ). The comparison between North America and Magadan Oblast was notable in that analysis (Fig. 4), with a large amount of differentiation for the moderate geographic separation.

Asian populations of moose exhibited the greatest nucleotide diversity, followed by European and North American populations (Table 2). Moose from Asia also exhibited more segregating sites than those in North America or Europe and had the greatest haplotype diversity. When Asian moose were segregated into two populations, Far East exhibited much more diversity than did Magadan Oblast. Moose from Europe were the next most diverse, but much of the heterogeneity therein related to the highly divergent haplotype from

Sweden. The levels of nucleotide diversity in moose of eastern Europe and Finland were similar to those in Magadan Oblast and North America (Table 2).

Analysis of molecular variance demonstrated a high degree of structure at all levels of organization (Table 3). The index of differentiation among groups ( $\Phi_{CT}$ ) was greater for the three-group comparison (Asia vs Europe vs North America) than for the two-group comparison (Europe vs Asia + North America) and was associated with a corresponding decrease in differentiation among populations within groups ( $\Phi_{SC}$ ; Table 3). If the two-race hypothesis were correct, we would have expected the opposite result.

#### Historic Demography

We detected no signature of historic bottlenecks; values of Tajima's  $D$  were nonsignificant ( $P > 0.05$ ) for all populations (Table 2). The mismatch distribution for moose worldwide exhibited two distinct waves, which we attributed to temporally distinct expansions in Eurasia and North America (Fig. 5). Mismatch distributions for North America, Eurasia, Magadan Oblast, and Far East did not differ significantly ( $P > 0.05$ ) from expectations under the sudden-expansion model and therefore were suitable for analysis of demographic patterns. The mismatch distribution for Europe differed significantly from the sudden-expansion model ( $P = 0.038$ ). North America and Magadan Oblast showed evidence of recent expansions, whereas the Far East expansion was older. Estimates of  $\tau$  indicated that Eurasian moose underwent an expansion that was 4.2 times older than that undergone by moose in North America. Relative ages of expansion for populations in Far East and Magadan Oblast (as multiples of North America) were 3.1 and 0.1, respectively. The 95% confidence intervals of  $\tau$  did not overlap in comparisons between Eurasia and North America or between Far East and Magadan Oblast (Fig. 5), indicating significantly different times since expansion for those populations.

Our estimates of time of expansion for Eurasian

**TABLE 2**

#### Measures of Intrapopulation Variability for Moose Based on Grouping at the Continental Level

Population/region	$N$	No. haplotypes	Haplotype diversity ( $H$ )	Nucleotide diversity ( $\pi$ )	Segregating sites ( $S$ )	Mean pairwise differences ( $d_i$ )	Tajima's $D$
North America	141	16	0.86 (0.02)	0.007 (0.004)	19	3.0 (1.6)	-0.29
Asia	32	19	0.94 (0.03)	0.019 (0.010)	39	9.6 (4.5)	0.08
Magadan Oblast	18	8	0.84 (0.07)	0.010 (0.006)	23	5.0 (2.6)	-0.84
Far East	14	12	0.98 (0.03)	0.021 (0.011)	33	11.0 (5.3)	0.39
Europe	19	6	0.74 (0.07)	0.010 (0.006)	18	5.3 (2.7)	0.31
Eastern Europe	3	2	0.67 (0.31)	0.008 (0.007)	6	4.0 (2.7)	0.00
Finland	10	3	0.38 (0.18)	0.004 (0.003)	8	2.2 (1.3)	-0.92
Sweden	6	1	0.00	0.00	0	0.0	0.00

Note. Values in parentheses are variances of the estimates. Mutations within the indel were ignored.

TABLE 3

**Analysis of Molecular Variance for Moose Based on Grouping Sequences by Three Continents (North America, Asia, and Europe) and by Populations within Continents**

Groups	Source of variation	Percentage of variance	$\Phi$	$P$
Europe vs Asia vs North America	Among groups	61.5	$\Phi_{CT} = 0.62$	<0.0001
	Among populations/within groups	21.6	$\Phi_{SC} = 0.56$	<0.0001
	Within populations	16.9	$\Phi_{ST} = 0.83$	<0.0001
Europe vs Asia + North America	Between groups	58.1	$\Phi_{CT} = 0.58$	0.009
	Among populations/within groups	28.8	$\Phi_{SC} = 0.69$	<0.0001
	Within populations	13.1	$\Phi_{ST} = 0.87$	<0.0001

*Note.* Probability values refer to variance components and  $\Phi$  statistics and were generated by comparing observed values against the distribution of 10,000 permutations.

moose, based on the two rate estimates, were approximately 59,000 and 47,000 years ago, whereas our estimates for North American moose were approximately 14,000 and 11,000 years ago (Table 4). Expansion of the Far East population occurred early in the expansion of Eurasian moose, but the moose population in Magadan Oblast was of very recent origin.

### DISCUSSION

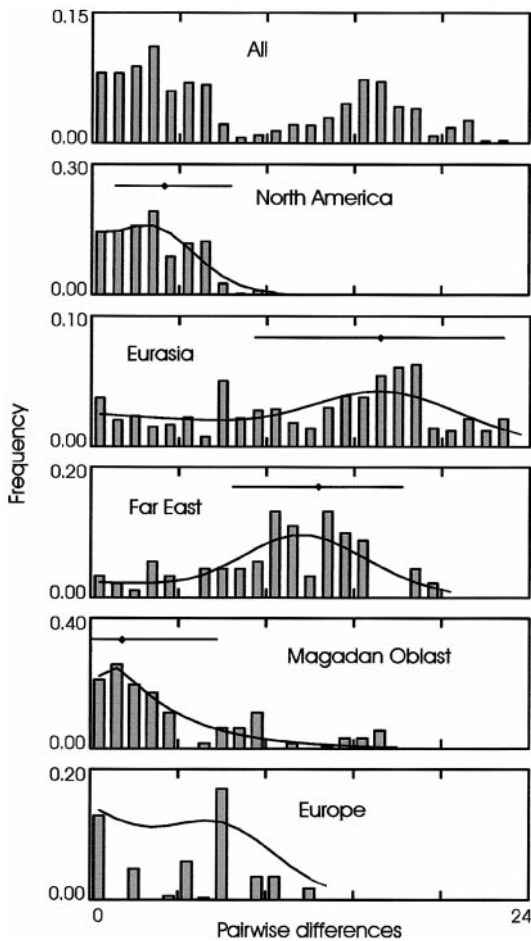
Despite their extensive distribution throughout the northern part of the Northern Hemisphere, moose exhibit a distinct lack of diversity within the control region compared to other species of large, hoofed mammals. Nucleotide diversity for reindeer (*R. tarandus*), a species with a distribution similar to that of moose, was 3.42% (Gravlund *et al.*, 1998), compared to our estimate for moose of 1.8%. Nucleotide diversity for the central African antelope, kob (*Kobus kob*), was 4.6%, with a maximum intrapopulation diversity of 5.72% (Birungi and Arctander, 2000), greatly exceeding our estimates for moose. Moreover, nucleotide diversity across the range of moose was less than or equal to that of four species of African bovids sampled from only portions of their ranges (Arctander *et al.*, 1996b), and the greatest level of intrapopulation diversity in moose (2.1% in Far East) was less than that measured in 16 of 27 populations in three species of bovids distributed across southern Africa (Arctander *et al.*, 1996a). Those comparisons indicate a demographic history of moose populations consisting of low effective size and recent expansion.

Our data indicate an extensive expansion of moose populations at the end of the late Pleistocene in Eurasia from which all extant populations were derived. The three phylogroups that we documented diverged within a short period; divergence of phylogroups 1 and 2 likely occurred first, followed by the splitting of phylogroups 1 and 3. Expansion dates estimated from mismatch distributions corresponded generally with dates of divergence of the three phylogroups in the gene tree and support the hypothesis of Guthrie (1995) that mod-

ern moose flourished only recently. The paraphyly of Asian haplotypes with respect to European and North American haplotypes suggests Asia as the region of origin for all extant lineages of moose. Subsequently formed populations in Europe and North America have not been separated long enough from the founding Asian lineages, however, to achieve reciprocal monophyly.

The expansion of moose in Magadan Oblast and North America most likely occurred after the last glacial maximum. Nonetheless, the lack of close relationship between those two populations is notable and unexpected. If the wave of moose that colonized North America left remnant populations in its path, a close relationship between Magadan Oblast and North America would be expected. Perhaps genetic drift caused the differences that we demonstrated, but the estimate for expansion of the Magadan Oblast population indicates that that population is recent and is not descended directly from the wave that colonized North America. Thus, we conclude that the noted morphological similarity between moose from Magadan Oblast (*A. a. burturlini*) and Alaska (*A. a. gigas*) resulted from convergent evolution. Gravlund *et al.* (1998) documented a similar instance of polyphyletic origins of morphologically similar populations. In that instance, three subspecies of small-bodied, high-arctic reindeer (*R. tarandus*) were shown to have arisen from two different lineages. Furthermore, a common Beringian ancestry for North American and Siberian moose was not supported by paleontological data (Sher, 1987; Guthrie, 1990), which indicate that moose were not present in northeastern Asia or Beringia in great numbers much earlier than 9000 years ago. Consequently, we reject the hypotheses of Geist (1987, 1998) and Cronin (1992) for place of origin of the moose population in North America.

Of the populations that we sampled, Far East was the Eurasian population most closely related to North America as a whole and is the best candidate as the source for the colonizers of North America. Our estimates of the timing of that colonization were similar to estimates of a colonization of North America by hu-



**FIG. 5.** Mismatch distributions for worldwide, continental, or regional assemblages of moose, based on the 442-bp segment of the control region. The abscissa represents number of pairwise differences and the ordinate represents the proportion of observations. The vertical bars are the observed distribution of mismatches and the smoothed line represents the expected distribution under the sudden-expansion model of Rogers (1995) as modified by Schneider and Excoffier (1999). For those continental or regional distributions that met the assumptions of the sudden-expansion model, a horizontal bar that shows the mean and 95% confidence interval for 5000 bootstrapped estimates of  $\tau$ , the mode of the distribution, is provided.

mans based on control region variation (Bonatto and Salzano, 1997). Remarkably, studies place the origin of aboriginal human populations of North America in the same region as that for moose (Neel *et al.*, 1994; Bonatto and Salzano, 1997).

The presence of *A. latifrons* in eastern Beringia 35,000 years ago (Harington, 1978; Guthrie, 1990) indicated that species existed either parapatrically with *A. alces* in Asia during the late Pleistocene or disappeared at the time that *A. alces* flourished in Asia. In either instance, we note that the most recent confirmed dates for *A. latifrons* in North America coincide with our estimate for the date of expansion of Eurasian moose. Beringia probably was the last refuge for *A. latifrons*, but *A. alces* eventually replaced it.

Assuming that *A. alces* existed in Eurasia for the last 100,000 years, the limited diversity and recent divergence of lineages that we have documented must be interpreted as evidence of either an extended period at low effective population size or a bottleneck that constrained the genetic diversity of modern moose. Our estimate of the timing of that occurrence is more recent than the estimate of 350,000–165,000 years ago (Mikko and Andersson, 1995) and is consistent with the conclusion of Ellegren *et al.* (1996) that moose in Sweden could have generated the observed level of genetic diversity within 50,000 years. Our divergence estimates were more in line with the paleontological record, as the estimate of Mikko and Andersson (1995) markedly predates the earliest known evidence of *A. alces* (Lister, 1993). Certainly, divergence of a single marker can predate the divergence of taxa that carry it. If that were true in this instance, either a multiregional origin of moose combined with substantial gene flow or a large effective population size at the *A. latifrons*-*A. alces* transition would be suggested. Given the high degree of female philopatry exhibited by moose (Hundertmark, 1998), we believe that the multiregional explanation is unlikely. Furthermore, the paucity of sequence variation present in modern moose argues against a large historic effective size.

The primary reason for the difference in estimates of divergence between Mikko and Andersson (1995) and this study was the faster mutation rates that we used in our calculations. We selected those rates because they applied only to the left hypervariable domain of the control region (the range of our data) and were derived from taxa closely related to moose. Moreover, the estimates that we used were well within the bounds of short-term rates estimated for humans, which can be as high as 110% per million years (Lundstrom *et al.*, 1992). Nonetheless, dates assigned to historic events based on rates of genetic divergence are subject to much variation and should be interpreted with caution. Yet, application of a more conservative rate of 33% per million years (Ward *et al.*, 1991) still placed our oldest estimate for coalescence of moose worldwide within the last 85,000 years.

#### *Historic Effective Population Size*

Our inability to detect a bottleneck was informative but may not indicate the true demographic history of moose. Tajima's *D* tests for an expected divergence of *S* and  $\pi$  caused by bottlenecks (Tajima, 1989b), but is thought to have low statistical power (Hartl and Clark, 1997; Nei and Kumar, 2000). If a population went through a severe bottleneck such that virtually all variation was purged, Tajima's *D* likely would not detect that event. Moreover, a population that existed at low effective size for a long period might not express such a signature. Thus, we have no conclusive evidence

TABLE 4

**Estimates of  $\tau$  for the Mismatch Distributions of Moose Populations Based on Variation within the Left Hypervariable Domain of the Control Region and Associated Estimates of Expansion Times Based on Two Different Divergence Rates**

$\tau$	$0.628 \times 10^{-6}$		$0.785 \times 10^{-6}$	
	Expansion time (years ago)	95% CI	Expansion time (years ago)	95% CI
North America	3.87	13,942	11,154	4,035–23,345
Eurasia	16.40	59,083	47,266	24,498–67,441
Far East	12.30	44,312	35,450	21,039–48,419
Magadan Oblast	0.36	1,297	1,038	0–17,869

*Note.* Confidence intervals were constructed using 5000 bootstrapped data sets.

of an historic bottleneck, but cannot reliably discount its existence.

Some insight may be gained into historic demographics by estimating historic effective population size ( $N_e$ ). According to Neigel and Avise (1986), the effective size of a population can be predicted with respect to the phylogenetic relationships among regional populations. When one population is paraphyletic with respect to another, there is a high probability that the clades diverged between  $N_e$  and  $4N_e$  generations ago. That model required an estimate of the time of founding of the daughter populations, which we could not estimate for Europe. Nonetheless, that event must have occurred after the divergence of phylogroups 1 and 2, which was approximately 34,000 years ago. If we use that estimate, however, and a generation time of 7 years, we estimate  $N_e$  within a range of 1214 to 4856 female moose. For North America and Asia, we used the midpoint of our estimates of the expansion of North America (~13,000 years ago) and estimated a range for  $N_e$  of 464 to 1856 females. Those numbers suggest that both regions were founded by a small effective number of female moose.

In a qualitative assessment of demographics, we note that Magadan Oblast and North America have moderate to high  $H$  and low  $\pi$ , a signature of rapid demographic expansion from a small effective population size, and Far East has large  $H$  and large  $\pi$ , a characteristic of a stable population. Conversely, Europe was characterized by relatively low  $H$  and low  $\pi$ , a signature of a recent bottleneck (Avise, 2000). Excoffier and Schneider (1999) modeled the effects of bottlenecks on mismatch distributions, and the distribution that we observed for Europe was similar to the results of one of their simulations. In that example, Excoffier and Schneider (1999) imposed a recent bottleneck on a population that had previously undergone sudden expansion. They demonstrated that such a scenario would cause rejection of the sudden-expansion model by the least-squares test even when an expansion had occurred. Consequently, European moose may

have suffered a recent reduction in effective size after a late-Pleistocene expansion. Historical accounts of moose in Fennoscandia (Markgren, 1974) indicated a marked decline in moose numbers beginning in the 15th century; furthermore, intense exploitation of moose by humans in the 18th and 19th centuries led to near extirpation of that species in Norway, Sweden, and Finland. A slow recovery was not completed until the mid-20th century when commercial forest activities created abundant habitat for moose, and extirpation of predators allowed maximal population growth (Markgren, 1974). Therefore, such a scenario is plausible.

#### *Characteristics of Moose Lineages*

The structure of the gene tree and the similar magnitude of difference of pairwise  $\Phi_{ST}$  estimates between Europe–Asia and North America–Asia indicated that mitochondrial markers offered no support for two primary (eastern and western) lineages of moose; rather, those data are more consistent with an isolation-by-distance process of divergence. That conclusion also was supported by AMOVA results; the three-lineage scenario maximized variation among groups and minimized variation within groups.

The haplotype found in moose from Sweden was the only exception to the isolation-by-distance model. We hypothesize that the divergence of that haplotype was a result of drift associated with isolation. A migration corridor existed from western Europe directly into southern Sweden because of lower sea levels in the late Pleistocene (Taberlet *et al.*, 1998). That corridor and a second path through Finland were proposed by Markgren (1974) as routes by which moose colonized Sweden and Norway. Taberlet *et al.*, (1998) documented a floral and faunal suture zone across central Norway and Sweden that supports the existence of those routes for other species also. Thus, the haplotype from Sweden may represent a lineage of moose that colonized the region from the south, perhaps from a

different glacial refugium than the one from which other lineages in Scandinavia arose.

The chromosomal differences noted between eastern and western moose must be of recent origin, occurring some time after the expansion of Eurasian moose but prior to the divergence of phylogroups 1 and 3. Nonetheless, those chromosomal differences may cause reproductive isolation in moose in central Siberia, which warrants further study. Presence or absence of the 75-bp repeat was not indicative of an eastern–western division because we documented both forms of the indel in moose from Asia. Furthermore, the amount of variation within the repeat and the absence of the repeat in the most recently derived lineage lead us to conclude that the presence of the additional 75-bp fragment is the ancestral form in moose. Ultimately, that fragment originated as an insertion (Douzery and Randi, 1997), but the deletion of that fragment in moose from phylogroup 3 is the explanation most consistent with our data.

#### *Paleoecological Factors Affecting Phylogeography of Moose*

The glacial cycles of the Pleistocene had a dramatic effect on population structure of northern species (Hewitt, 1996, 2000; Bernatchez and Wilson, 1998; Taberlet *et al.*, 1998). Species inhabiting areas that recently were covered by Pleistocene ice sheets exhibit reduced levels of genetic diversity and shallow depth of clades compared with species further to the south (Bernatchez and Wilson, 1998). Mechanisms affecting the structure of those populations include latitudinal shifts of habitats, geographic barriers, number and location of refugia, and dispersal ability of the species (Hewitt, 1996, 2000). Moose represent an excellent illustration of these effects on a species that (1) is adapted to a habitat (boreal forest) that underwent large range shifts associated with climate change (Anderson and Brubaker, 1993), (2) has the potential for long-distance dispersal but normally exhibits a high degree of female philopatry (Hundertmark, 1998), and (3) utilized a route (Bering land bridge) to spread to North America that now is an effective barrier to gene flow.

The range of suitable habitat for moose in Eurasia in the late Pleistocene would have moved north and south with cooling and warming trends, even though Asia was relatively ice-free (Guthrie, 1995). Moreover, the east–west orientation of mountain ranges in Eurasia would have imposed limits to the southern spread of habitats, likely causing reductions in their size. The process of latitudinal shifts of range can reduce genetic heterogeneity in animal species restricted to affected habitats (Hewitt, 1996). The first cold phase of the most recent glacial period, which occurred from about 75,000 to 65,000 years ago according to data from North America (Fulton *et al.*, 1986), provides the nec-

essary conditions for reduction of population size in moose in Eurasia. That period saw extensive ice coverage in northern Europe (Forsström and Punkari, 1997), and the extent of ice coverage in Siberia was greater than that during the most recent glacial maximum (Arkhipov *et al.*, 1986), which would have had a profound effect on the distribution of boreal forest. The relatively mild climate of the most recent interstade (approximately 60,000 to 30,000 years ago) most certainly was accompanied by expansion of Eurasian boreal forest and, subsequently, moose populations. Our estimates of expansion of Eurasian moose and the divergence of phylogroups 1 and 2 coincide with that period. The most recent cold period, 23,000 to 18,000 years ago (Dyke and Prest, 1987), saw another contraction of habitat and populations and provided a mechanism for separate refugia in Asia and the subsequent divergence of phylogroups 1 and 3. Finally, climatic warming of the current interglacial caused the northward spread of boreal forest in Eurasia, allowing moose to enter Scandinavia by multiple pathways and to colonize North America via Beringia prior to the flooding of the land bridge 11,000 years ago (Elias *et al.*, 1996). The narrow temporal window during which moose could have passed across the land bridge restricted opportunities for gene flow between Asian and North American populations and likely was responsible for the star-like phylogeny of moose haplotypes from North America.

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