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# REFUGIAL ORIGINS OF REINDEER (*RANGIFER TARANDUS* L.) INFERRED FROM MITOCHONDRIAL DNA SEQUENCES

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*Abstract.*—The glacial-interglacial cycles of the upper Pleistocene have had a major impact on the recent evolutionary history of Arctic species. To assess the effects of these large-scale climatic fluctuations to a large, migratory Arctic mammal, we assessed the phylogeography of reindeer (*Rangifer tarandus*) as inferred from mitochondrial DNA (mtDNA) sequence variation in the control region. Phylogenetic relationships among haplotypes seem to reflect historical patterns of fragmentation and colonization rather than clear-cut relationships among extant populations and subspecies. Three major haplogroups were detected, presumably representing three separate populations during the last glacial. The most influential one has contributed to the gene pool of all extant subspecies and seems to represent a large and continuous glacial population extending from Beringia and far into Eurasia. A smaller, more localized refugium was most likely isolated in connection with ice expansion in western Eurasia. A third glacial refugium was presumably located south of the ice sheet in North America, possibly comprising several separate refugial populations. Significant demographic population expansion was detected for the two haplogroups representing the western Eurasian and Beringian glacial populations. The former apparently expanded when the ice cap retreated by the end of the last glacial. The large continuous one, in contrast, seems to have expanded by the end of the last interglacial, indicating that the warm interglacial climate accompanied by marine transgression and forest expansion significantly confined population size on the continental mainland. Our data demonstrate that the current subspecies designation does not reflect the mtDNA phylogeography of the species, which in turn may indicate that morphological differences among subspecies have evolved as adaptive responses to postglacial environmental change.

*Key words.*—Caribou, glacial and interglacial refugia, mitochondrial DNA, phylogeography, population expansion, *Rangifer tarandus*, recolonization, reindeer.

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The glacial-interglacial cycles of the upper Pleistocene have had a major influence on the evolutionary history of species distributed in Arctic and sub-Arctic (Chapin and Körner 1994; Hewitt 1996). Recent speciation events have been attributed to rapid allopatric diversification in isolated refugia during periods of Pleistocene ice expansion (e.g., MacPherson 1965; Avise and Walker 1998; Bernatchez and Wilson 1998). Similarly, isolation during the last glacial—the Wisconsin in North America and the Weichselian in Eurasia—was important in shaping the intraspecific genetic variability currently seen in many species (e.g., Fedorov et al. 1999; Holder et al. 1999; Brunner et al. 2001). Notably, there were pivotal differences between the North American and Eurasian parts of Arctic during the last glacial. In North America ice cover was extensive and isolation in glacial refugia was significant for genetic differentiation of Arctic species (e.g., Tremblay and Schoen 1999; Ehrich et al. 2000; Holder et al. 2000). Beringia, encompassing the Bering land bridge as well as parts of Yukon, Alaska, and eastern Siberia, served as the most important refugium (Pielou 1991). In contrast, the extent of the glacial in the Eurasian Arctic was much more limited. This region was characterized by large continuous areas of tundra extending throughout much of Siberia and central Eurasia (Andersen and Borns 1997), potentially supporting large populations of Arctic species.

*Rangifer tarandus*, called reindeer in Eurasia and caribou in North America, is a highly migratory species (Banfield 1961). As such, it is a typical representative for the fauna of large mammals in Arctic and sub-Arctic (cf. Mech 1970; Gardner et al. 1986). At the time of the last glacial maximum, reindeer were found south to the ice sheets both in Eurasia (Kurten 1968) and North America as well as in Beringia (Kurtén and Anderson 1980). By retreat of the ice, a rapid recolonization of the emerging habitat presumably took place (e.g., Ukkonen 1993), subsequently leading to the current circumpolar distribution in the northern part of the Holarctic region.

Across this vast area, several subspecies have been described (Fig. 1). These can be classified into three major ecological groups—the woodland form, continental tundra form, and high Arctic island form, each showing distinct morphological characteristics. The continental tundra form, typically carrying long and rangy antlers, are represented by the Eurasian tundra reindeer (*R. t. tarandus*), the Alaska caribou (*R. t. grantii*), and the Canadian barrenground caribou (*R. t. groenlandicus*). The woodland form, including the Eurasian forest reindeer (*R. t. fennicus*) and the North American woodland caribou (*R. t. caribou*), is characterized by a larger body size, long legs, and short and heavy antlers. In fact, 30–40% of the females in the woodland form are naturally antlerless (Banfield 1961), which may indicate that the antler anatomy is an adaptation to woodland conditions. The Arctic ecotype, characterized by a small body size and a short rostrum, comprises the Svalbard reindeer (*R. t. platyrhynchus*), the Peary caribou (*R. t. pearyi*), and the extinct form previously found on eastern Greenland (*R. t. eogroenlandicus*). Small size reduces the surface:volume ratio of the animal, which may point toward a selective pressure for small body

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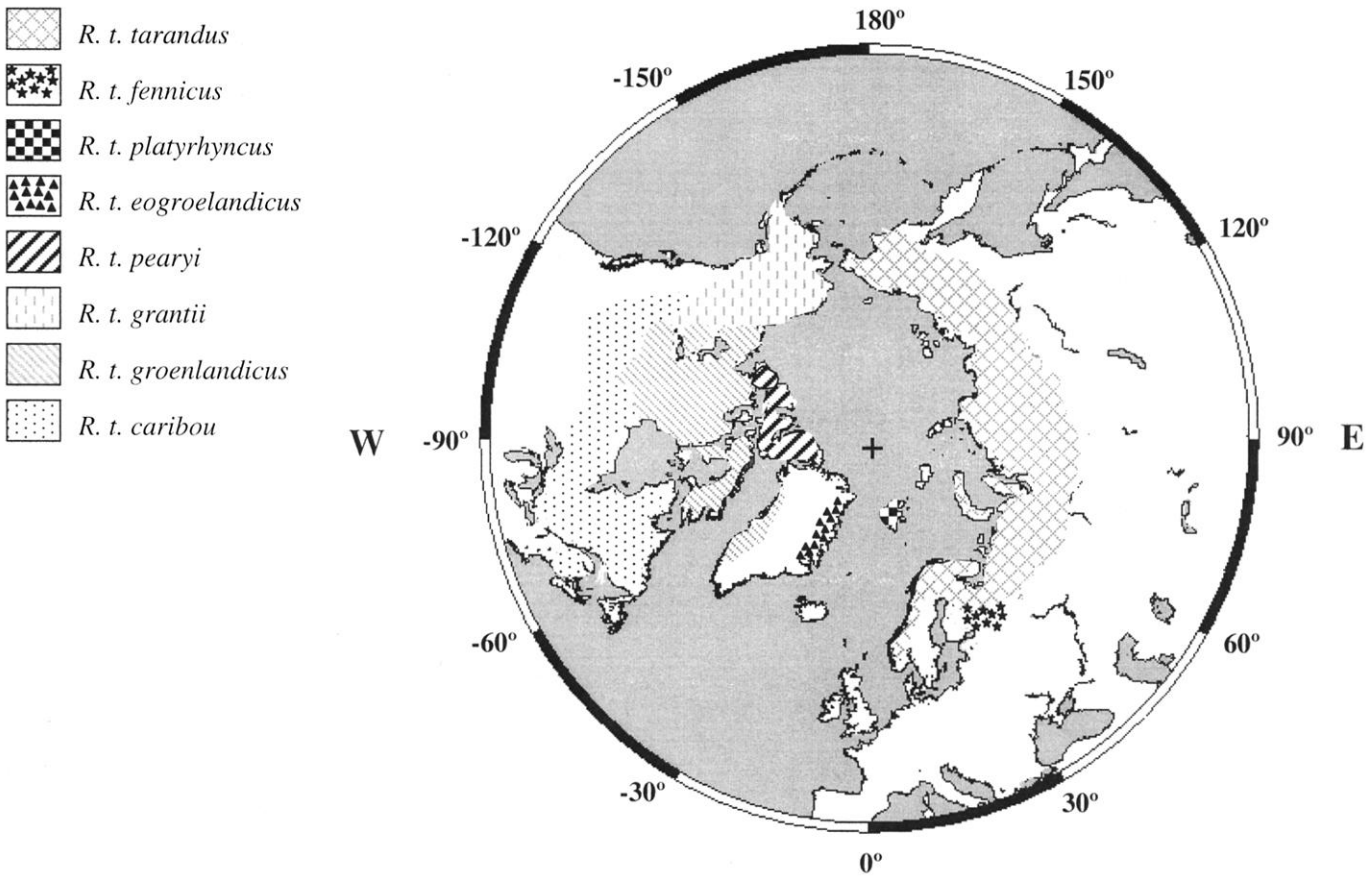


FIG. 1. Distribution map for the different subspecies of reindeer (*Rangifer tarandus* L.). Although additional subspecies have been suggested, these eight constitute the conventional taxonomic classification of the species. Eastern and western longitudes are indicated and the north pole is represented by a +.

size under the extreme environmental conditions on the Arctic islands.

From morphological and historical data, Banfield (1961) suggested that the three ecotypes originated in three or more isolated refugia during the last glacial. Following his interpretation, the continental tundra forms originated in Beringia and possibly in central Europe north of the Alps. The Arctic Island forms may have survived in tundra refugia north to the continental ice sheets in Arctic Canada and/or on northern Greenland. Finally, the forest or woodland forms probably survived in temperate refugia south to the continental ice sheets.

A number of previous studies assessed refugial origins of reindeer. Røed et al. (1991) reported a major dichotomy in allele frequencies at the locus coding for serum transferrin between the North American woodland caribou and various subspecies of the tundra form. The observation supported the conventional view that woodland and tundra caribou survived the Wisconsin in separate refugia south and north of the continental ice sheet, respectively (cf. Banfield 1961). In contrast, Cronin (1992) did not find any phylogenetic differentiation in mitochondrial restriction fragment length polymorphisms (RFLPs) between woodland and Alaskan caribou and concluded that the populations had diverged recently from a common ancestor. Contradictory variability patterns have been reported also for the Arctic ecotype. Røed et al. (1986) found distinct similarities at the transferrin locus between Svalbard reindeer and Peary caribou, suggesting a common origin of the high Arctic forms (Røed et al. 1986). Gravlund et al. (1998), however, showed that Svalbard reindeer had a slightly smaller mitochondrial DNA (mtDNA) genetic distance to Eurasian mainland populations than to the high Arctic forms on Greenland and the Canadian Arctic islands and suggested a diphyletic origin of the Arctic ecotype.

Here we report on all three ecotypes as represented by all extant subspecies sampled across the circumpolar distribution range of the species. We assess the phylogeography of the species as revealed from sequence variability patterns in the mtDNA control region. The locations of possible glacial refugia and postglacial recolonization of the Holarctic are discussed in light of the phylogeographic patterns.

## MATERIALS AND METHODS

### *Sampling*

Blood samples were obtained from nine free-ranging populations and five domestic herds, covering most of the species distribution range in North America and Eurasia (Table 1). If possible, poorly sampled areas were supplied with sequences from Genbank. A total of 14 sequences were imported (Table 1), mainly representing the widely distributed woodland caribou of which our own material had been sampled from a limited area.

TABLE 1. Occurrence of haplotypes at the different sampling sites. Haplotype frequency is indicated in parentheses.

| Geographic origin | Subspecies | Sample size | Haplotypes present[1] | Genbank accession numbers |
|---|---|---|---|---|
| Hardangervidda, Norway | *R. t. tarandus* (Tar) | 15 | TarN-Fen1 (8), TarNR1 (6), TarN104 (1) | AY178672, AY178683, AY178673 |
| Snøhetta, Norway | *R. t. tarandus* (Tar) | 13 | TarN101 (9), TarN102 (2), TarN103 (1), TarN-Fen1 (1) | AY178669, AY178670, AY178671, AY178672 |
| Norwegian domestic | *R. t. tarandus* (Tar) | 15 | TarNR1 (3), TarNR2 (2), TarN31 (2), TarN-Fen1 (2), TarN2 (1), TarN3 (1), TarN5 (1), TarN7 (1), TarN8 (1), TarN11 (1) | AY178683, AY178729, AY178731, AY178672, AY178730, AY178674, AY178724, AY178725, AY178726, AY178719 |
| Russian domestic | *R. t. tarandus* (Tar) | 18 | TarR1 (4), TarR2 (4), TarR3 (3), TarR4 (3), TarNR1 (2), TarNR2 (2) | AY178679, AY178727, AY178680, AY178728, AY178683, AY178729 |
| Southeastern Finland | *R. t. fennicus* (Fen) | 13 | Fen1 (11), TarN-Fen1 (2) | AY178682, AY178672 |
| Svalbard | *R. t. platyrhynchus* (Pla) | 12 | Pla-Car1 (7), Pla32 (5) | AY178689, AY178688 |
| Quebec, Canada | *R. t. caribou* (Car) | 16 | Pla-Car1 (2), Car-Pea1 (1), Car3 (1), Car5 (1), Car6 (1), Car14 (1), Car19 (1), Car20 (1), Car21 (1), Car24 (1), Car25 (1), Car27 (1), Car31 (1), Car34 (1), Car38 (1) | AY178689, AY178702, AY178711, AY178712, AY178722, AY178723, AY178713, AY178690, AY178703, AY178692, AY178693, AY178695, AY178694, AY178681, AY178691 |
| North West Territories, Canada | *R. t. groenlandicus* (Gro) | 11 | Gro20 (1), Gro23 (1), Gro25 (1), Gro26 (1), Gro55 (1), Gro56 (1), Gro59 (1), Gro64 (1), Gro67 (1), Gro68 (1), Gro 71 (1) | AY178706, AY178707, AY178708, AY178709, AY178700, AY178701, AY178696, AY178699, AY178697, AY178698, AY178710 |
| Alaska | *R. t. grantii* (Gra) | 12 | Gra102 (2), Gra104 (2), Gra101 (1), Gra103 (1), Gra899 (1), Gra903 (1), Gra904 (1), Gra905 (1), Gra907 (1), Gra951 (1) | AY178676, AY178678, AY178675, AY178677, AY178715, AY178716, AY178717, AY178718, AY178720, AY178721 |
| Peary Islands, Canadian Archipelago | *R. t. peayri* (Pea) | 12 | Pea83 (3), Pea22 (2), Pea24 (2), Car-Pea1 (1), Pea23 (1), Pea53 (1), Pea88 (1), Pea90 (1) | AY178687, AY178704, AY178684, AY178702, AY178685, AY178705, AY178686, AY178714 |
| Imported from Genbank: | *R. t. groenlandicus* (Gro) | 2 | Gro7 (1), Gro101 (1) | AF096446, AF096414 |
| (unknown population origin) | *R. t. caribou* (Car) | 11 | Car401 (4), Car301 (2), Car25 (2), Car203 (1), Car204 (1), Car302 (1) | [AF096417, AF096418 AF096429, AF096442], [AF096420, AF096438], [AF096421, AF096427], AF096422, AF096440, AF096419 |

[1] Each haplotype is given a three-letter code according to subspecies origin (cf. column 2). Norwegian and/or Russian origin of *tarandus* (Tar) haplotypes is indicated by capital letters N and R, respectively.

[2] Genbank accession numbers that represent the same haplotype are given within brackets [ ].

### *DNA Extraction, Polymerase Chain Reaction Amplification, and DNA Sequencing*

DNA from all blood samples was extracted according to a standard phenol:chloroform protocol (Sambrook et al. 1989). A 470-bp region of the mtDNA control region adjacent to the *tRNA$^{pro}$* gene was amplified using primers 5′-AATAGCCCCAC TATCAGCACCC-3′ (L15394; originally designed for hartebeest [*Alcelaphus buselaphus*] and targeting the *tRNA$^{pro}$* gene; cf. Flagstad et al. 2000) paired with 5′-TATGGCCCTGAAGTAAGAACCAG-3′ (H15947; "mammalian" primer targeting CSB-D; cf. Southern et al. 1988). Thirty-five cycles of amplification with 40 sec at 94°C, 40 sec at 60°C, and 40 sec at 72°C were preceded by a 4-min predenaturation step at 94°C and followed by an additional 7-min extension step at 72°C. Amplifications were performed in 25-μl volumes containing 1.35 mM $MgCl_2$, 200 μM of each dNTP, 5 pmol of each primer, and 0.5 units of AmpliTaq DNA polymerase (Applied Biosystems, Foster City, CA).

DNA sequencing was carried out using a ThermoSequenase kit for $[\gamma\text{-}^{33}P]$ATP-labeled dideoxy-nucleotides following standard procedures as recommended by the manufacturer (Amersham Pharmacia, Uppsala, Sweden).

### *Data Analysis*

Sequences were aligned by eye using the sequence editor software SeqApp provided by D. G. Gilbert (http://iubio.bio.indiana.edu/soft/molbio/seqap). No indels were found among the reindeer sequences, but inclusion of moose (*Alces alces*) as an outgroup imposed three indels between the two species. The final alignment is available on request.

An appropriate model of nucleotide substitution was selected using the hierarchical test approach implemented in MODELTEST (ver. 3.06; Posada and Crandall 1998). An extended HKY-85 model (Hasegawa et al. 1985), including a gamma-distributed rate variation across sites (Yang 1993) and a certain proportion of invariable sites, was selected as the most optimal model. Phylogenetic relationships among the different haplotypes were estimated from a Bayesian approach as implemented in MRBAYES 3.0 (Huelsenbeck and Ronquist 2001), using moose as an outgroup. Two different analyses were performed, one allowing branch lengths to evolve unconstrained and the other constraining branch lengths according to a molecular clock. One million Metropolis-coupled Markov chain Monte Carlo steps were run in each of the analyses, using two Markov chains. Two chains allow one of them to be heated, which improves the efficiency when searching for the most optimal tree. A phylogenetic tree was saved every 100 generations and consensus trees were estimated from the trees sampled after the stationary distribution had been reached, in our case 9500 trees. In addition to reconstruction of phylogenetic trees, a minimum spanning network (MSN) was constructed using the statistical parsimony approach described by Templeton et al. (1992) as implemented in TCS version 1.13 (Clement et al. 2000).

The major groups as revealed from the phylogenetic analyses were tested for sudden population expansion using the mismatch distribution approach (Slatkin and Hudson 1991; Rogers and Harpending 1992) as implemented in ARLEQUIN version 2000 (Schneider et al. 2000). The mismatch distribution is the distribution of the observed number of differences between pairs of haplotypes. This distribution is usually multimodal in samples drawn from populations that are in demographic equilibrium, as it reflects the highly stochastic shape of gene trees. In contrast, haplotypes drawn from a population that has undergone a recent demographic expansion usually have a unimodal distribution of pairwise differences (Slatkin and Hudson 1991; Rogers and Harpending 1992). The approach implemented in ARLEQUIN assumes a stepwise expansion model, reflecting a sudden population growth from $N_0$ to $N_1$ $t$ generations ago, after which the population rapidly reaches demographic equilibrium. Such a model may be particularly useful when studying populations that has been affected by dramatic environmental fluctuations and probably fits well to species affected by the glacial-interglacial cycles of the upper Pleistocene.

Under the stepwise expansion model a general nonlinear least-square approach, based on the mean and variance of pairwise differences, is used to estimate three demographic parameters: $\theta_0 = 2\mu N_0$, $\theta_1 = 2\mu N_1$, and $\tau = 2\mu t$, where $\mu$ is the mutation rate for the whole haplotype. The validity of the assumed model is tested from a parametric bootstrapping approach (Schneider & Excoffier 1999), where the sum of square deviance (SSD) between the observed and expected mismatch distribution is used as a test statistic. The expected distribution is obtained by simulating $B$ samples around the estimated demographic parameters, using a coalescent algorithm modified from Hudson (1990). If the validity of the model is confirmed, the time since the putative expansion event can be estimated from $\tau(= 2\mu t)$. $\tau$ represents the expected number of differences between two randomly drawn haplotypes at time $t$ since the expansion. We assumed a divergence rate of 16% per million years, which is similar to that of other large mammals for the left domain of the *D-loop* (Brown et al. 1979; George & Ryder 1986; Vigilant et al. 1991). Simple rearrangement gives,

$$t = (\tau \text{ differences per haplotype}) \div \{[(0.16 \times 470) \text{ differences per haplotype}] \div 1 \text{ million years}\}, \quad (1)$$

where 470 represents the number of base pairs sequenced. Confidence intervals (95%) were estimated from the same parametric bootstrap approach (10,000 replicates) that was used to test the validity of the stepwise expansion model; that is, simulating values of the three demographic parameters using a coalescent algorithm.

An analysis of molecular variance (AMOVA) was used to examine the amount of genetic variability partitioned within and among populations as well as among groups of populations (Excoffier et al. 1992; Weir 1996). To assess the significance of the various factors that could affect partitioning of genetic variability, populations were grouped according to a set of different grouping criteria: subspecies designation, ecotype characteristics, geographic distribution, and phylogenetic affinity.

## RESULTS

### *Phylogenetic Relationships*

Sixty-five variable sites, comprising 58 transitions and 7 transversions, were found among the reindeer sequences. In

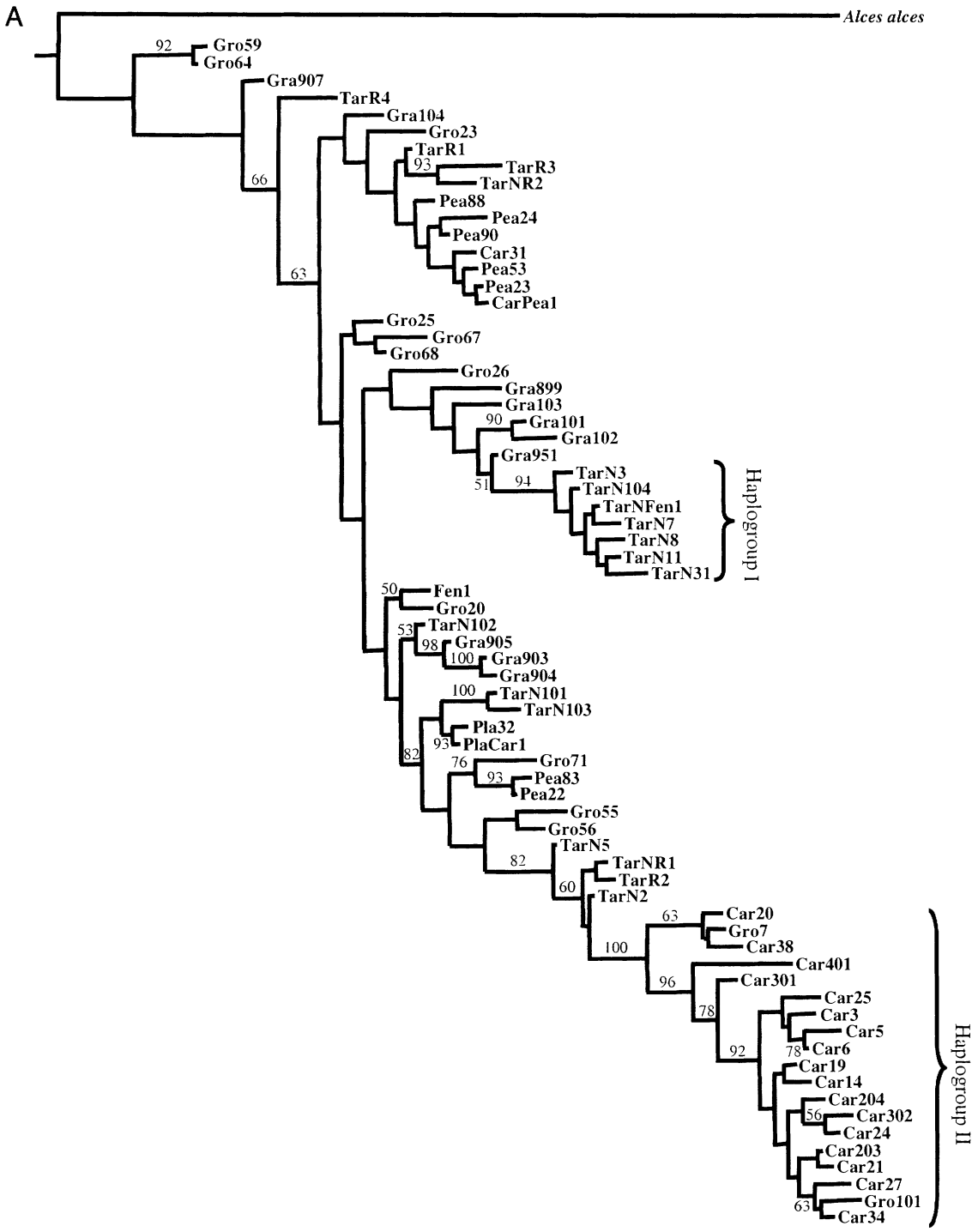


FIG. 2. Bayesian inference of the intraspecific phylogeny of reindeer (see Table 1 for haplotype abbreviations) under the HKY-85 model of nucleotide substitution allowing a gamma-distributed rate variation across sites and a certain proportion of invariable sites. Moose (*Alces alces*) was used as an outgroup. (A) Branch lengths are unconstrained with an exponentially distributed prior. Support values are indicated for nodes that were supported in 50% or more of 9500 sampled trees (burnin = 500). The arithmetic mean of the marginal likelihood of the sampled trees is −2068.32. (B) Branch lengths are constrained to evolve according to a molecular clock under a coalescence model with an exponentially distributed prior for the coalescence parameter, θ. Support values are indicated as in (A). The arithmetic mean of the marginal likelihood of 9500 sampled trees is −2044.71. The displayed consensus tree represents an average of the sampled trees, which explains why the tree does not appear strictly ultrametric.

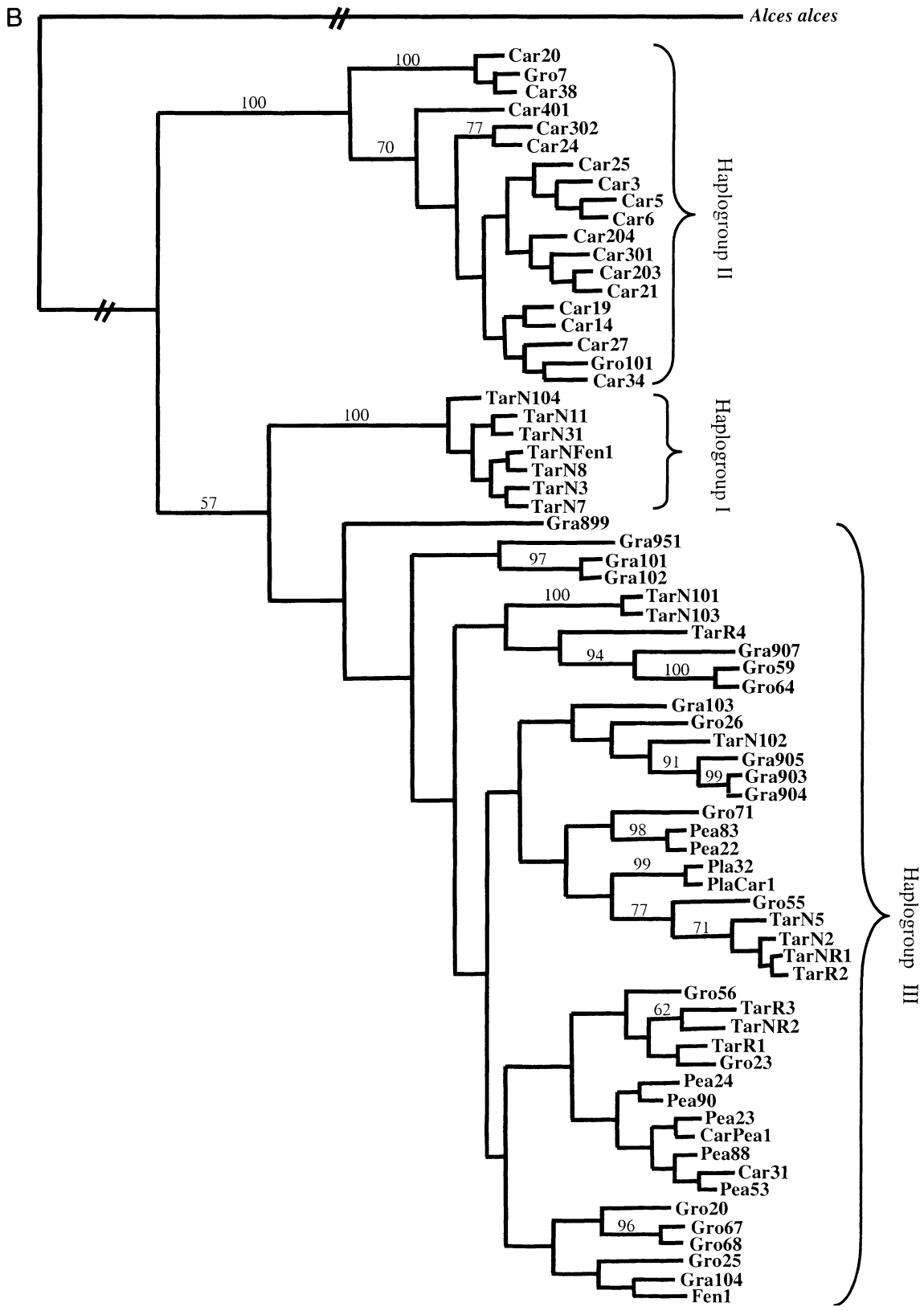


FIG. 2. Continued.

addition, a 7-bp inversion was found in one individual. Among the 150 individuals of reindeer included in the study, 70 haplotypes were detected, of which 50 were found only once (Table 1).

The unconstrained Bayesian phylogeny (Fig. 2A) appears poorly structured, with many unsupported (< 50%) or moderately supported groupings. Nevertheless, the tree comprises two highly supported, relatively large clades, each of them comprising samples collected from a geographically distinct area (haplogroups I and II). Haplogroup I exclusively consists of haplotypes found among Fennoscandian individuals. Haplotypes belonging to this group were not represented among the Russian domestic herds, but was found in a previous study in three of 23 individuals from the Taimyr Peninsula (Gravlund et al. 1998). Haplogroup II comprises haplotypes found among the North American woodland caribou together with two haplotypes found in two individuals of barrenground origin (Gro7 and Gro101). The remaining parts of the tree are characterized by haplotypes that have a very limited affinity to counterparts of the same subspecies or geographic region. Yet, a few small clades with reasonable support reflect geographically well-defined regions. For example, both haplotypes found on Svalbard are contained within one clade. Notably, though, this clade is not population specific: the most common haplotype found on Svalbard is identical to the only haplotype found more than once in Quebec.

The clock-constrained tree (Fig. 2B) shows a higher likelihood than the unconstrained tree and seems to have a more distinct phylogenetic structure. In addition to the strongly supported haplogroups I and II, a third haplogroup (haplogroup III) is indicated, comprising all haplotypes not contained within haplogroups I and II. Although the three haplogroups appear reciprocally monophyletic, haplogroup III was supported in only 16% of the 9500 sampled trees. Such a weakly supported clade may indicate recent divergence among the putative ancestral populations represented by the three haplogroups, which in turn suggests that there has been insufficient time for unambiguous lineage sorting. Alternatively, the poor support could indicate that haplogroup III in fact comprises a paraphyletic assemblage, as indicated from the unconstrained tree. However, because the clock-constrained tree showed the highest likelihood, it should reflect the most reliable phylogeny of the species. Thus, it seems reasonable to assume that the present gene pool may have its origin from three separate ancestral populations.

The existence of the same three haplogroups is indicated also from the MSN (Fig. 3). Haplogroup I displays a clearly defined central haplotype, TarN-Fen, which was represented in all Fennoscandian populations. This haplotype was found in 13 of 20 individuals belonging to this group, while the remaining six haplotypes were found in one or two individuals only. Haplogroup II seems to comprise three subgroups. The same subgroups is also recognized in the phylogenetic trees with moderate to high support. Haplogroup III constitutes a wide subnetwork of cross-linked haplotypes located between haplogroups I and II in the network.

### *Analysis of Molecular Variance*

The AMOVA (Table 2) shows that there is no relationship between the current subspecies designations and differentiation at the mtDNA level. Similarly, weak relationships were found when grouping the populations according to geographic distribution or ecotype characteristics. The only convincing figures appeared when populations were grouped in various ways according to the main haplogroups as suggested from the phylogenetic analyses (Figs. 2, 3). When considering the two northerly distributed haplogroups (I and III) as a single entity, 33% ($P = 0.09$) of the total variability is explained at the group level. An increased and significant proportion (42%; $P < 0.001$) is assigned to the group level when pooling the two North American haplogroups (II and III). The highest proportion, however, is obtained when treating the three groups as separate entities. Almost 50% ($P < 0.001$) of the total variability is now explained at the group level, supporting the actual existence of three main mtDNA lineages.

### *Population Expansion*

The mismatch distributions of haplogroups I and III are clearly unimodal (Fig. 4A, C), a pattern compatible with a historical population expansion. In contrast, haplogroup II shows a multimodal mismatch distribution (Fig. 4B), suggesting stable population size through time or that this haplogroup comprised several subgroups as indicated from the MSN (Fig. 3). The average number of nucleotide differences is much higher for haplogroup III as compared to that of haplogroup I, indicating that the ancestral populations of the two haplogroups expanded at different times. Accordingly, the putative expansion of haplogroup I was dated to 15,000 years ago (95% confidence interval: 0–26,000 years ago), whereas that of haplogroup III was estimated to have occurred approximately 115,000 years ago (95% confidence interval: 87,000–142,000 years ago).

## Discussion

### *Glacial and Interglacial Refugia*

As shown in the phylogenetic trees and the MSN (Figs. 2, 3), there is only limited congruence between the different clades and the present-day geographic distribution of haplotypes, suggesting that the phylogeny of the sequences reflects historical patterns of fragmentation and colonization rather than clear-cut relationships among extant populations. Three main haplogroups are recognized (Figs. 2B, 3) and approximately 50% of the total sequence variability is assigned to variation among these groups (Table 2). These results point toward the existence of three separate glacial populations during the Weichselian/Wisconsin.

The largest and most influential origin to the current gene pool of the species is represented by haplogroup III (Figs. 2B, 3). All North American samples except for those of the southerly distributed woodland caribou (plus two *groenlandicus* specimens) belong to this haplogroup (Fig. 5), pointing toward a Beringian origin. Importantly, though, paleobotanical evidence suggests the existence of extensive areas of tundra throughout Siberia and Central Europe during the Weichselian (Andersen and Borns 1997). Moreover, fossils of reindeer from this time have been found at many sites in Siberia as well as in Central and Western Europe (Germonpre 1986; Kirsche et al. 1993; Andersen and Borns 1997; Iacumin

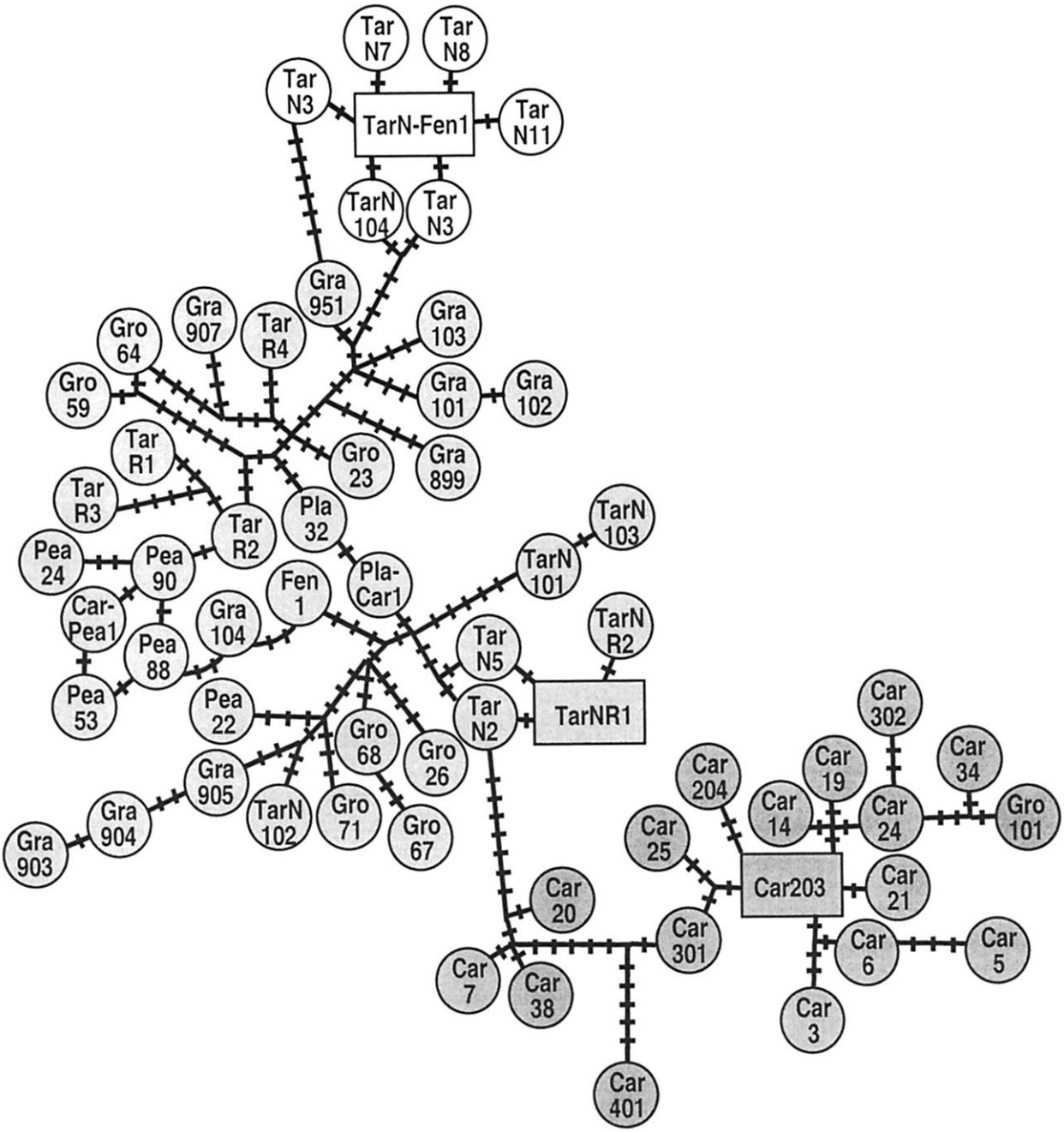


FIG. 3. Minimum spanning network (MSN) as estimated from statistical parsimony (Templeton et al. 1992). Mutations are represented as bars. Haplogroups I, II, and III as revealed from the ML tree are indicated with white, dark gray, and lighter gray, respectively. Haplotypes estimated to be the most likely common ancestor within each of the three haplogroups are displayed as squares. As recommended by Clement et al. (2000), sequences with one or more ambiguous sites were not included in the analysis.

et al. 2000). These figures may suggest that haplogroup III represents an ancestral glacial population ranging across vast areas of tundra in Eurasia extending into North America across the Beringian land bridge (Elias et al. 1996). Such a scenario is supported by the large contingent of Eurasian haplotypes that also falls into haplogroup III (Fig. 5). The large sequence diversity (Fig. 3) and unimodal distribution of sequence differences (Fig. 4) suggest that the population remained large and continuous after an initial demographic expansion.

The expansion event itself was dated to approximately 115,000 years ago. Molecular dating based on a single locus must obviously be interpreted with caution (e.g., Hillis et al. 1996). Nevertheless, it is noteworthy that our data suggest a sudden expansion during the transitional stage between the last interglacial and the Weichselian/Wisconsin, a period when global climate was characterized by rapid cooling (e.g., Hooghiemstra et al. 1992; Berger et al. 1996). During the interglacial, tundra habitat was very restricted on mainland Eurasia and North America (Sher 1991; Andersen and Borns

TABLE 2. Analysis of molecular variance based on several possible groupings of the populations examined. Proportion of the total genetic variation explained at the individual and population levels are given in parentheses in their respective columns.

| Grouping criteria | Groups of populations | Fixation index | | | Proportion of total variation assigned to groups | Significance level of grouping (*P*) |
|---|---|---|---|---|---|---|
| | | Individuals $\Phi_{ST}$ | Population $\Phi_{SC}$ | Group $\Phi_{CT}$ | | |
| Subspecies | [*tarandus*] [*fennicus*] [*platyrhyncus*] [*pearyi*] [*caribou*] [*groenlandicus*] [*grantii*] | 0.369 (63%) | 0.373 (37%) | −0.005 | 0 | 0.52 |
| Ecotype | [*tarandus, groenlandicus, grantii*] [*platyrhyncus, pearyi*] [*fennicus, caribou*] | 0.370 (63%) | 0.369 (37%) | 0.002 | 0.2 | 0.43 |
| Geographic distribution I | [*tarandus, fennicus, platyrhyncus*] [pearyi, grantii, groenlandicus, caribou] | 0.374 (63%) | 0.365 (36%) | 0.014 | 1.4 | 0.34 |
| Geographic distribution II | [*platyrhyncus*] [Norwegian *tarandus*] [*fennicus*, Russian *tarandus*] [*pearyi, grantii, groenlandicus*] [*caribou*] | 0.381 (62%) | 0.295 (26%) | 0.122 | 12.2 | 0.02 |
| Northern vs. southern haplogroups | [haplogroup I, haplogroup III] [haplogroup II] | 0.567 (43%) | 0.355 (24%) | 0.328 | 32.8 | 0.09 |
| Eurasian vs. North American haplogroups | [haplogroup I] [haplogroup II, haplogroup III] | 0.733 (27%) | 0.539 (31%) | 0.419 | 41.9 | <0.001 |
| Three main haplogroups | [haplogroup I] [haplogroup II] [haplogroup III] | 0.708 (29%) | 0.428 (22%) | 0.490 | 49.0 | <0.001 |

1997), implying that reindeer populations surviving under tundra conditions on the continental mainland were confined to relatively small and isolated refugia. The estimated coalescence time suggests a single refugial origin of haplogroup III by the time of the last interglacial. Paleoecological data (Sher 1991) may indicate that such a refugium have been located in eastern Siberia. This line of evidence suggests that the Asian part of the Beringian mainland was not dramatically affected by forest expansion and sea transgressions during this period. Moreover, it has been suggested that this part of Siberia most likely provided a strictly tundra-adapted lemming species with suitable habitat during the warm interglacials (Agadjanyan 1976; Fedorov and Goropashnaya 1999). Built on these lines of evidence, our data may suggest that the ancestral population represented by haplogroup III expanded rapidly from a small interglacial refugium when tundra habitat spread during the transitional stage between interglacial and glacial.

Haplogroup I seems to have been established as a result of a rather different evolutionary history, characterized by high degrees of recent isolation. Because haplotypes belonging to this group were not represented among any of the North American samples, a pure Eurasian origin is indicated. The starlike appearance of the haplogroup in the MSN with many short branches radiating from a common, central haplotype suggests a recent origin in a small refugium, which was probably isolated in connection with ice expansion in Eurasia during the Weichselian. The current distribution gradient of haplotypes belonging to this group (Fennoscandia: ~40% of the examined individuals; Russia: 7.3%; North America: 0%; Fig. 5) may suggest a location in Western Europe. Notably, reindeer lived on the western coast of Norway as late as 30,000 years ago (Valen et al. 1996), showing that small and isolated refugia have existed in Western Europe in close connection to the expanding ice cap.

In North America another distinct and geographically well-defined refugial area is indicated by the existence of haplogroup II (Fig. 2). Virtually all haplotypes belonging to this group are found among the southerly distributed woodland caribou (Fig. 5), suggesting that this subspecies has its origin in one or several refugia located south to the Wisconsinian ice sheet. Our data (Figs. 2B, 3) suggest that this haplogroup may comprise three different subgroups, which in turn may indicate the existence of three separate glacial refugia. Such a subdivision of the ancestral population is compatible with the multimodal mismatch distribution observed among these sequences (Fig. 4B). Importantly, though, a similar genetic signature would also be produced if these haplotypes originated in a single glacial population that was large enough for ancestral polymorphism to be retained.

### *Subspecies Origin and Postglacial Recolonization of the Holarctic*

As seen from the AMOVA (Table 2), the current subspecies designations are not compatible with the differentiation at the mtDNA level. In fact, 0% of the variability was explained at the subspecies level, strongly suggesting that the morphological differences among extant subspecies did not evolve in separate glacial refugia. A possible exception to this is the North American woodland caribou, which seems to have its origin in a subspecies-specific refugium south to the continental ice sheet.

The large Eurasian glacial population, possibly ranging across extensive areas of tundra from Beringia to Central Europe, has clearly been the most influential source to the present gene pool of reindeer. All current populations are to some extent affected by this origin (Fig. 5). As ice cover retreated by the end of the Weichselian/Wisconsin, representatives from this population appear to have recolonized exposed habitat on the continental mainland in North America, Siberia, and Fennoscandia. The North American tundra forms (*grantii* and *groenlandicus*) almost exclusively comprise haplotypes of such an origin (Fig. 5). The Eurasian tundra form (*tarandus*), on the other hand, appears to have a diphyletic origin, as the putatively small and isolated Eurasian refugium also contributed to its gene pool (Fig. 5).

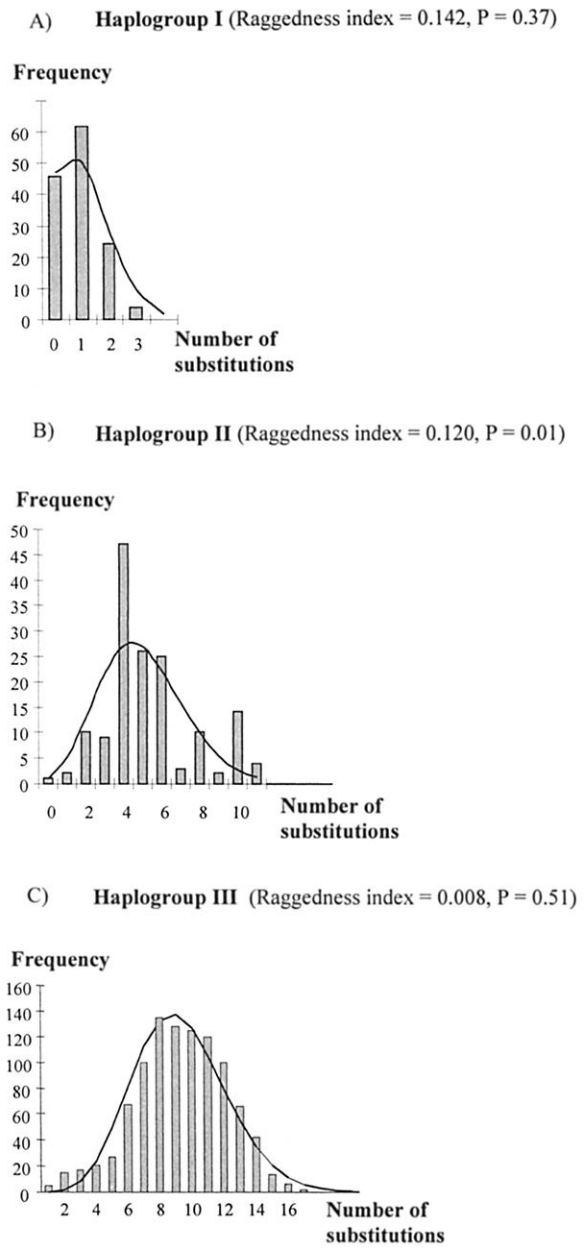


FIG. 4. Observed (columns) and expected (line) mismatch distributions given a null distribution compatible with sudden population expansion for (A) haplogroup I: (B) haplogroup II; and (C) haplogroup III. Harpending's raggedness index (Rogers and Harpending 1992) and significance levels for deviance from the expected mismatch distributions are indicated for each of the haplogroups.

These results largely support the classical interpretation of refugial origins based on morphological and historical data (Banfield 1961), which suggests that the continental tundra forms (*groenlandicus*, *grantii*, and *tarandus*) originated in Beringia and possibly in refugia in Central Europe north of the Alps. For the Eurasian forest reindeer (subspecies *fennicus*) the classical interpretation (Banfield 1961) invokes a separate temperate refugial origin, a scenario that is not supported from our data. Rather, a similar diphyletic origin as for *tarandus* is suggested (Fig. 5). Accordingly, adaptation to woodland conditions for this subspecies seems to be a recent phenomenon, which may have taken place in connection with postglacial forest expansion.

As briefly mentioned above, the North American woodland caribou (subspecies *caribou*) likely originated in one or several refugia south of the continental ice sheet, which is in accordance to the classical view suggested by Banfield (1961). This subspecies is highly dominated by haplotypes belonging to haplogroup II, although a few individuals display haplotypes apparently originating from Beringia (Fig. 5). It seems that colonization attempts from the north to some extent have been buffered against the caribou populations already present in the southern areas. Prolonged isolation may also have led to adaptive differences between the two lineages (cf. Templeton et al. 1986), as indicated from the conspicuous adaptations to forest habitat seen in the woodland caribou (Banfield 1961; Cumming 1992). Although the existence of haplotypes of northern origin in the more southerly distributed populations and vice versa indicates recent events of gene flow, the limited mixture of haplotypes may suggest some degree of reproductive isolation.

Similar to the North American tundra forms, the Arctic forms (*pearyi*, *eogroenlandicus*, and *platyrhyncus*) exclusively comprise haplotypes originating from the large Beringian-Eurasian glacial population represented by haplogroup III (Fig. 5). This indicates a common glacial origin on the continental mainland of all three Arctic subspecies. Banfield (1961) suggested a more peripheral islandic origin north of the continental ice sheets, a scenario that is not supported from our data. Rather, the haplotypes representing the Arctic subspecies (*pearyi* [Pea] and *platyrhyncus* [Pla]) are well integrated at internal positions in the MSN (Fig. 3), suggesting that these haplotypes did not evolve separately from those of the Beringian population. Consequently, tundra refugia on the Arctic islands may not have been large enough to support viable populations of reindeer over extensive periods of time. However, such islandic refugia have been found to support populations of smaller arctic species such as the rock ptarmigan (Holder et al. 1999), the collared lemming (Ehrich et al. 2000), and the widespread arctic plant *Dryas integrifolia* (Tremblay and Schoen 1999). It may therefore be premature to rule out the possibility that these refugia also supported small populations of reindeer. If, after all, the species was able to survive within these islandic refugia throughout the Wisconsin, gene flow from Beringia seems to have been significant, swamping any distinct genetic pattern.

A common North American origin for the Arctic forms is particularly well supported for *eogroenlandicus* and *pearyi* as these subspecies shared a common haplotype (Gravlund et al. 1998). When the ice retreated, colonizers appear to have migrated across the Canadian archipelago and eventually reached eastern Greenland. Reconstruction of the colonization route toward Svalbard and subsequent founding of this population appear more puzzling. Our data suggest a direct connection between Quebec and Svalbard, as the most common haplotype found on Svalbard is identical to the only haplotype found more than once in Quebec. The connection between these two extremes in the species distribution is surprising, and may solely be attributed to random haplotype survival. However, a similar pattern was observed by Røed et al. (1991), suggesting that some basic feature of coloni-

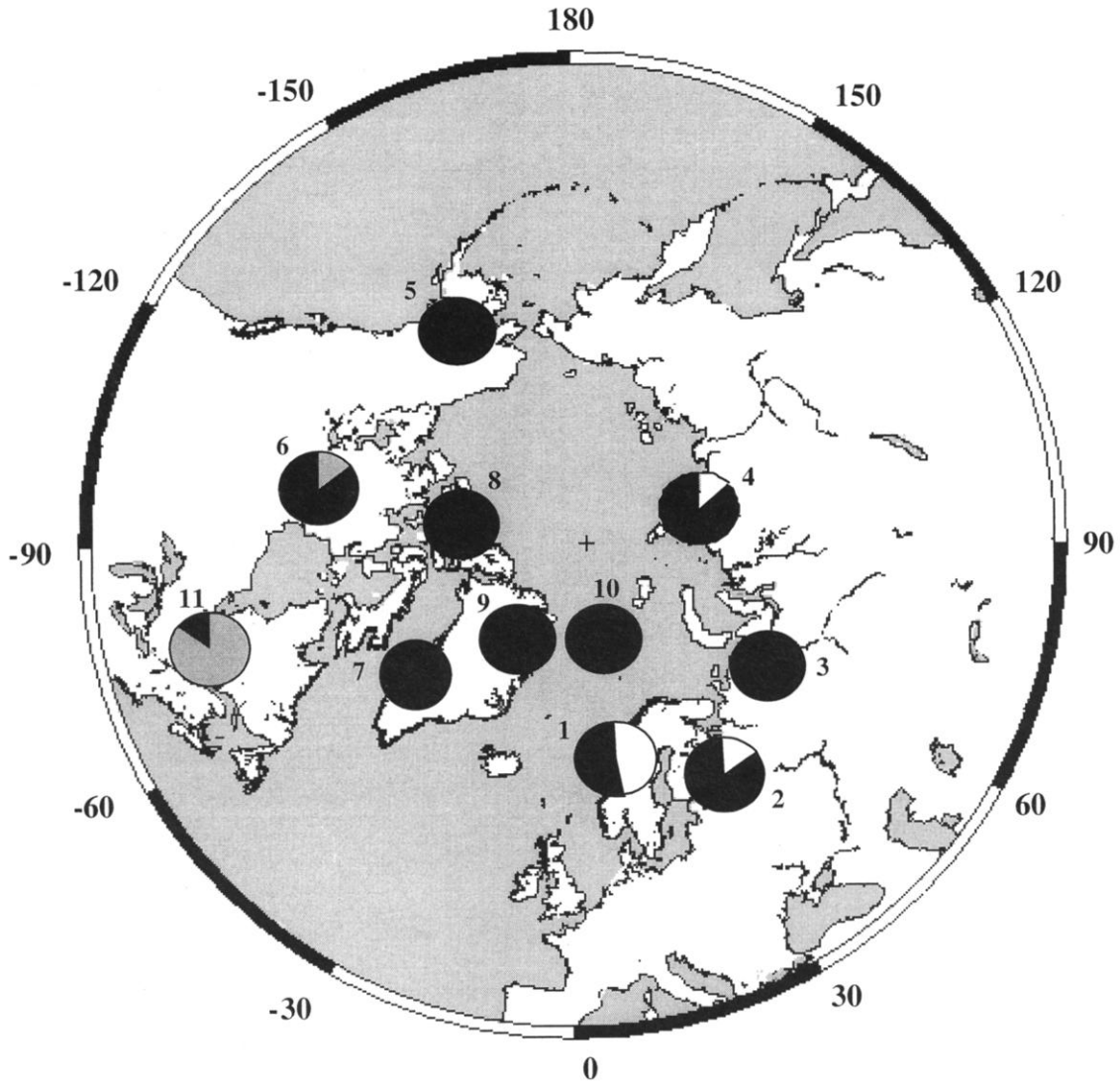


FIG. 5. Geographic distribution of the three main haplogroups; haplogroup I (white), haplogroup II (gray), and haplogroup III (black). Numbers correspond to the following geographic locations: (1) Norway (*tarandus*); (2) southeastern Finland (*fennicus*); (3) western Russia (*tarandus*); (5) Alaska (*grantii*); (6) North West Territories (*groenlandicus*); (8) Peary Islands (*pearyi*); (10) Svalbard (*platyrhyncus*); and (11) Quebec (*caribou*). To cover as much as possible of the species' distribution, three sampling localities from Gravlund et al. (1998) were included: (4) Taymyr Peninsula (*tarandus*); (7) western Greenland (*groenlandicus*); and (9) eastern Greenland (*eogroenlandicus*).

zation after the Wisconsin is responsible for the observed genetic patterns. Moreover, one haplotype is shared also between Peary and Quebec, providing further support to a true connection between geographically and ecologically very distinct entities. Alternatively, convergence could explain the observation. However, given the very limited number of haplotypes shared among populations, most of them among geographically adjacent populations, convergence per se seems to be a rare event.

These results may suggest a North American colonization route toward Svalbard. When the ice retreated by the end of the Wisconsin, individuals from the periphery of the Beringian distribution would presumably follow the edge of the ice sheet, attempting to colonize new areas of favorable habitat. Such a scenario would lead to a directed movement of colonizers toward the edges of the present circumpolar distribution range, that is, Svalbard to the north and Quebec and adjacent regions to the south. However, it is not possible from our data to rule out an eastern colonization route with Franz Josef's land (between Svalbard and Eurasia) as a stepping stone, especially because the same haplotype was also found at the Taimyr Peninsula in northern Russia (Gravlund et al. 1998). Yet, because common haplotypes among geographically distant localities are likely to originate from a single dispersal event (Avise et al. 1987), the strong affinity between the Arctic forms and the woodland form to the south may favor the North American alternative.

### *Conclusion*

This study suggests that a combination of glacial and interglacial effects have been important in shaping the recent

evolutionary history of reindeer. A large and continuous glacial population in Beringia extending far into Eurasia appears as the most influential origin to the current gene pool of the species. Notably, our data indicate a much more limited distribution in the same area during the last interglacial, suggesting that unfavorable environmental conditions on the continental mainland as perceived by the species itself may have been more significant during interglacial periods. Two additional refugial areas appear to have existed during the Weichselian/Wisconsin. A small and isolated refugium seems to have arisen in connection with ice expansion in western Eurasia. Another distinct and geographically well-defined refugial area, most likely comprising several subrefugia, was probably located south to the extensive North American continental ice sheet. In contrast to what has been found for several smaller Arctic species (Holder et al. 1999; Tremblay and Schoen 1999; Ehrich et al. 2000), our data did not support the classical view that populations of reindeer survived the Wisconsin in islandic refugia north of the North American ice sheet (Banfield 1961). Finally, our data demonstrates that the current subspecies designation does not reflect the mtDNA phylogeography of the species, which in turn may indicate that morphological differences among extant subspecies have evolved as adaptive responses to postglacial environmental change.

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## Literature Cited

Agadjanyan, A. K. 1976. The history of collared lemmings in the Pleistocene. Pp. 379–388 *in* V. L. Kontrimavichus, ed. Beringia in the Cenozoic Era. 6th ed. Academy of Sciences of USSR, Vladivostok. In English, 1984. Amerind Publishing, New Dehli.

Andersen, B. G., and H. W. Borns Jr. 1997. The Ice Age world: an introduction to Quaternary history and research with emphasis on North America and Europe during the last 2.5 million years. Scandinavian University Press, Oslo.

Avise, J. C., and D. Walker. 1998. Pleistocene phylogeeographic effects on avian populations and the speciation process Proc. R. Soc. Lond. B. 265:457–463.

Avise, J. C., J. Arnold, R. M. Ball, E. Bermingham, T. Lamb, J. E. Neigel, C. A. Reeb, and N. C. Saunders. 1987. Intraspecific phylogeography: the mitochondrial-DNA bridge between population-genetics and systematics. Ann. Rev. Ecol Syst. 18: 489–522.

Banfield, A. W. F. 1961. A revision of the reindeer and caribou, genus *Rangifer*. Bull. Natl. Mus. Canada 177. Biol. Ser. 66.

Berger, W. H., T. Bickert, M. K. Yasuda, and G. Wefer. 1996. Reconstruction of atmospheric $CO_2$ from ice-core data and the deep-sea record of Ontong Java plateau: the Milankovitch chron. Geol. Rundsch. 85:466–495.

Bernatchez, L., and C. C. Wilson. 1998. Comparative phylogeography of nearctic and palearctic fishes. Mol. Ecol. 7:431–452.

Brown, W. M., M. George Jr.,and A. C. Wilson. 1979. Rapid evolution of animal mitochondrial DNA. Proc. Nat. Acad. Sci. USA 76:1967–1971.

Brunner, P. C., M. R. Douglas, A. Osinov, C. C. Wilson, and L. Bernatchez. 2001. Holarctic phylogeography of Arctic charr (*Salvelinus alpinus* L.) inferred from mitochondrial DNA sequences. Evolution 55:573–586.

Chapin, F. S., and C. Körner. 1994. Arctic and alpine biodiversity: patterns, causes, and ecosystem consequences. Trends Ecol. Evol. 9:45–47.

Clement, M., D. Posada, and K. A. Crandall. 2000. TCS: a computer program to estimate gene genealogies. Mol. Ecol. 9:1657–1660.

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

Cumming, H. G. 1992. Woodland caribou: facts for forest managers. Forest. Chron. 68:481–489.

Ehrich, D., V. B. Fedorov, N. C. Stenseth, C. J. Krebs, and A. Kenney. 2000. Phylogeography and mitochondrial DNA (mtDNA) diversity in North American collared lemmings (*Dicrostonyx groenlandicus*). Mol. Ecol. 9:329–337.

Elias, S. A., S. K. Short, C. H. Nelson, and H. H. Birks. 1996. Life and times of the Bering land bridge. Nature 382:60–63.

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.

Fedorov, V. B., and A. V. Goropashnaya. 1999. The importance of ice ages in diversification of Arctic collared lemmings (*Dicrostonyx*): evidence from the mitochondrial cytochrome b region. Hereditas 130:301–307.

Fedorov, V., A. V. Goropashnaya, G. H. Jarrell, and K. Fredga. 1999. Phylogeographic structure and mitochondrial DNA variation in true lemmings (*Lemmus*) from the Eurasian Arctic Biol. J. Linn. Soc. 66:357–371.

Flagstad, Ø., P. O. Syvertsen, N. C. Stenseth, J. E. Stacy, I. Olsaker, K. H. Røed, and K. S. Jakobsen. 2000. Genetic variability in Swayne's hartebeest, an endangered antelope of Ethiopia. Cons. Biol. 14:254–264.

Gardner, C. L., W. B. Ballard, and R. H. Jessup. 1986. Long distance movement by an adult wolverine. J. Mamm. 67:603.

George, M., Jr.,and O. A. Ryder. 1986. Mitochondrial DNA evolution in the genus *Equus*. Mol. Biol. Evol. 3:535–546.

Germonpre, M. 1986. Lower Weichselian at the Bos van A, Zemst (Brabant, Belgium). Lutra 29:320–324.

Gravlund, P., M. Meldgaard, S. Pääbo, and P. Arctander. 1998. Polyphyletic origin of the small-bodied, high-arctic subspecies of tundra reindeer (*Rangifer tarandus*). Mol. Phylogenet. Evol. 10:151–159.

Hasegawa, M., K. Kishino, and T. Yano. 1985. Dating the human-ape splitting by a molecular clock of mitochondrial DNA. J. Mol. Evol. 22:160–174.

Hewitt, G. M. 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. Biol. J. Linn. Soc. 58: 247–276.

Hillis, D. M., C. Moritz, and B. K. Mable. 1996. Molecular systematics. Sinauer Associates, Sunderland, MA.

Holder, K., R. Montgomerie, and V. L. Friesen. 1999. A test of the glacial refugium hypothesis using patterns of mitochondrial and nuclear DNA sequence variation in rock ptarmigan (*Lagopus mutus*). Evolution 53:1936–1950.

———. 2000. Glacial vicariance and historical biogeography of rock ptarmigan (*Lagopus mutus*) in the Bering region. Mol. Ecol. 9:1265–1278.

Hooghiemstra, H., H. Stalling, C. O. C. Agwu, and L. M. Dupont. 1992. Vegetational and climatic changes at the northern fringe of the Sahara 250,000–5000 years BP: evidence from four marine pollen records located between Portugal and the Canary Islands. Rev. Palaeobot. Palynol. 74:1–53.

Hudson, R. R. 1990. Gene genealogies and the coalescent process. Pp. 1–44 *in* D. J. Futayama and J. D. Antonovics, eds. Oxford surveys in evolutionary biology. 1990. Oxford Univ. Press, Oxford, U.K.

Huelsenbeck, J. P., and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17:754–755.

Iacumin, P., V. Nikolaev, and M. Ramigni. 2000. C and N stable isotope measurements on Eurasian fossil mammals, 40 000 to 10 000 years BP: herbivore physiologies and palaeoenvironmental reconstruction. Palaeogeogr. Palaeocl. 163:33–47.

Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitution through comparative studies of nucleotide sequences. J. Mol. Evol. 16:111–120.

Kirsche, W., W. Richter, and W. D. Heinrich. 1993. Microstructure of fossil mammalian bones from the Rixdorfer Horizon of Weichsel-Kaltzeit at Niederlehme near Konigs Wusterhausen (Brandengurg). Anat Anz. 175:177–184.

Kurtén, B. 1968. Pleistocene mammals of Europe. Aldine, Chicago.

Kurtén, B., and E. Anderson. 1980. Pleistocene mammals of North America. Columbia Univ. Press, New York.

MacPherson, A. H. 1965. The origin of diversity in mammals of the Canadian arctic tundra. Syst. Zool. 14:153–173.

Mech, L. D. 1970. The wolf: the ecology and behavior of an endangered species. Univ. of Minnesota Press, Minneapolis.

Pielou, E. C. 1991. After the Ice Age: the return of life to glaciated North America. Univ. of Chicago Press, Chicago.

Posada, D., and K. A. Crandall. 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14:817–818.

Rogers, A. R., and H. Harpending. 1992. Population growth makes waves in the distribution of pairwise genetic differences. Mol. Biol. Evol. 9:552–569.

Røed, K. H., H. Staaland, E. Broughton, and D. C. Thomas. 1986. Transferrin variation in caribou (*Rangifer tarandus* L.) on the Canadian Arctic islands. Can. J. Zool. 64:94–98.

Røed, K. H., M. A. D. Ferguson, M. Crête, and T. A. Bergerud. 1991. Genetic variation in transferrin as a predictor for differentiation and evolution of caribou from eastern Canada. Rangifer 11:65–74.

Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Schneider, S., and L. Excoffier. 1999. Estimation of past demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: application to human mitochondrial DNA. Genetics 152:1079–1089.

Schneider, S., D. Roessli, and L. Excoffier. 2000. Arlequin ver. 2000: a software for population genetics data analysis. Genetics and Biometry Laboratory, Univ. of Geneva, Switzerland.

Sher, A. V. 1991. Problems of the last interglacial in Arctic Siberia. Quat. Intern. 10–12:215–222.

Slatkin, M., and R. R. Hudson. 1991. Pairwise comparisons of mitochondrial DNA sequences in stable and exponentially growing populations. Genetics 129:555–562.

Southern, S. O., P. J. Southern, and A. E. Dizon. 1988. Molecular characterization of a cloned dolphin mitochondrial genome. J. Mol. Evol. 28:53–60.

Templeton, A. R., H. Hemmer, G. Mace, U. S. Seal, W. M. Shields, and D. S. Woodruff. 1986. Local adaptation, coadaptation and population boundaries. Zoo Biol. 5:115–125.

Templeton, A. R., K. A. Crandall, and C. F. Sing. 1992. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping and DNA sequence data. III. Cladogram estimation. Genetics 132:619–633.

Tremblay, N. O., and D. J. Schoen. 1999. Molecular phylogeography of *Dryas intergrifolia*: glacial refugia and postglacial recolonization. Mol. Ecol. 8:1187–1198.

Ukkonen, P. 1993. The postglacial history of the Finnish mammalian fauna. Annal. Zool. Fenn. 30:249–264.

Valen, V., J. Mangerud, E. Larsen, and A. K. Hufthammer. 1996. Sedimentology and stratigraphy in the cave Hamnsundhelleren, western Norway. J. Quat. Sci. 11:185–201.

Vigilant, L., M. Stoneking, H. Harpending, K. Hawkes, and A. C. Wilson. 1991. African populations and the evolution of human mitochondrial DNA. Science 253:1503–1507.

Weir, B. S. 1996. Genetic data analysis. II. Methods for discrete population genetic data. Sinauer Associates, Inc., Sunderland, MA.

Yang, Z. 1993. Maximum likelihood estimation of phylogeny from DNA sequences when substitution rates differ over sites. Mol. Biol. Evol. 10:1396–1401.

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