

## A MOLECULAR PERSPECTIVE ON THE HISTORICAL BIOGEOGRAPHY OF THE NORTHERN HIGH LATITUDES

ERIC WALTARI,\* JOHN R. DEMBOSKI, DAVID R. KLEIN, AND JOSEPH A. COOK

*Department of Biological Sciences, Idaho State University, Pocatello, ID 83209-8007, USA (EW, JAC)*

*Biological Sciences Department, California State Polytechnic University, 3801 West Temple Avenue, Pomona, CA 91768-4032, USA (JRD)*

*Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, AK 99775-7020, USA (DRK)*

*Present address of JAC: Museum of Southwestern Biology, University of New Mexico, Albuquerque, NM 87131-1091, USA*

Phylogeographic analyses of arctic organisms provide spatial and temporal frameworks for interpreting the role of climate change on biotic diversity in high-latitude ecosystems. Phylogenetic analyses based on 673 base pairs of the mitochondrial control region from 95 arctic hares (*Lepus arcticus*, *L. othus*, *L. timidus*) and 2 other *Lepus* species identified 6 strongly or moderately supported clades. The 3 arctic hare species are closely related, but phylogenetic discontinuities were found at the eastern and western boundaries of Beringia, the latter not previously identified as a species boundary. The locations of these discontinuities are congruent with previously described genetic breaks in Arctic plants, birds, and small mammals. Similarly, the finding of a Beringian clade corroborates previous studies identifying Beringia as a refugium. A coalescent view of a population on Seward Peninsula, Alaska (eastern Beringia), did not, however, provide a genetic signature of population expansion. In contrast, a Greenland population did show a signal of expansion.

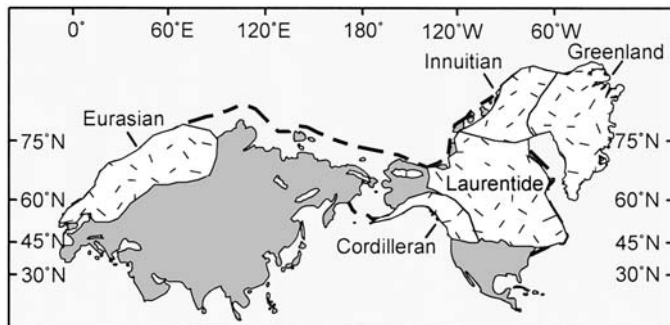
Key words: Arctic, Beringia, hares, *Lepus*, phylogenetics, phylogeography

Dramatic climate fluctuations in the Arctic, which have influenced continental ice sheet movement (Fig. 1), have contributed to the evolution and biogeography of many high-latitude organisms (Hewitt 1996, 2001, 2004; Weider and Hobæk 2000). To determine processes influencing patterns of biodiversity, both the evolutionary and geographical context for communities must be developed (Bermingham and Moritz 1998; Ricklefs and Schluter 1993). Because biotic complexity at high latitudes is decreased compared with lower latitudes, the Arctic could be a particularly suitable region for tracking responses to environmental changes and teasing apart the effects of abiotic events on the structure of extant diversity (Weider and Hobæk 2000). Phylogeographic analyses, which explore the relationship between gene genealogies and geography, are beginning to identify genetic structure within this vast region (Brunhoff et al. 2003; Fedorov and Goropashnaya 1999; Fedorov et al. 1999a, 1999b; Galbreath and Cook 2004; Holder et al. 2000; Wenink et al. 1996).

During periods of glacial expansion in the Pleistocene, many high-latitude organisms were displaced and confined to refugia. One of the best-known examples of Arctic refugia is the region known as Beringia, a largely unglaciated region of northwest North America and northeastern Asia (Figs. 1 and 2; Abbott and Brochmann 2003; Hultén 1937). This high-latitude refugium has been hypothesized to play a role in the diversification and evolution of high-latitude ecosystems (Hoffmann 1981; MacPherson 1965; Rand 1954).

During interglacial periods, populations are thought to have expanded from refugia, with lineages previously isolated in separate refugia often coming into contact to form geographic zones of sharp genetic discontinuity (Hewitt 1999, 2001; Manel et al. 2003). Thus, genetic discontinuities are closely associated with refugia, and many of these zones occur in deglaciated regions (Hewitt 1996). Other geologic events associated with deglaciation have erected new barriers. The Bering Strait, separating North America and Asia, for example, was created as sea levels rose with the melting of continental ice sheets and formed a barrier to genetic exchange for terrestrial arctic species approximately 10,000 years ago. Discovery of genetic structure does not immediately suggest that historical fragmentation in separate refugia is responsible, because genetic drift in the form of isolation by distance could produce a similar result (Irwin 2002). However, when genetic discontinuities, by definition occurring over small geographic distances, are spatially

\* Correspondent: walteric@isu.edu



**FIG. 1.**—The northern continents during the last glacial maximum. Dashed lines indicate seafloor exposed during glacial maximum, and names of ice sheets are labeled. Extent of ice sheets and glaciers is taken from Andersen and Borns (1994), Brigham-Grette (2001), Clark and Mix (2002), Dyke et al. (2002), Miller et al. (2002), the Paleoclimates from Arctic Lakes and Estuaries (PALE) Beringian Working Group (1999), and Siegert et al. (2001).

congruent across multiple taxa, historical fragmentation due to geologic events becomes a more likely cause than multiple, congruent stochastic events.

In this vein, phylogeographic studies examining the northern high latitudes have begun to identify genetic discontinuities that are common across multiple taxa (Fig. 2). For example, the Bering Strait has long been identified as a barrier dividing Palearctic and Nearctic biota (Wallace 1876). More recently, phylogeographic discontinuities in plants and mammals have been described at the Parry Channel and Mackenzie River in northern Canada (Abbott et al. 2000; Ehrich et al. 2000; Fedorov and Stenseth 2002; Fedorov et al. 2003; MacPherson 1965; Tremblay and Schoen 1999). In addition, the Kolyma and Omolon river systems in Far East Russia have been shown to separate lineages in birds and small mammals (Brunhoff et al. 2003; Fedorov et al. 1999a, 1999b, 2003; Galbreath and Cook 2004; Wenink et al. 1996). The generality of these discontinuities can be further tested by studying the comparative biogeography of additional taxa with similar Holarctic distributions.

We studied the arctic hare complex, consisting of 3 nominal species distributed across the Holarctic in a disjunct manner (Fig. 3): *Lepus timidus* (Eurasia), *L. othus* (western Alaska), and *L. arcticus* (northern Canada and Greenland). The taxonomy of the complex has been debated. For example, some authors regard eastern Siberian hares as *L. othus* (Baker et al. 1983; Hoffmann 1993). Because of the difficulty of identifying these species with morphology, we follow Anderson (1974) and Best and Henry (1994) and initially identify these Asian populations as *L. timidus*. A molecular study examining evolutionary relationships among 11 species of *Lepus* reported limited differentiation among the arctic hare species but included only 4 specimens of these high-latitude species (Halanych et al. 1999). We further examined the phylogenetics of arctic hares with the use of the relatively fast-evolving control region of the mitochondrial genome and expanded the sampling regimen ( $n = 95$ ) of the 3 species.

We compare our results with those from other terrestrial arctic organisms to examine support for the following 3 previously described glaciation-induced genetic discontinuities (Fig. 2): Parry Channel (High Canadian Arctic refugium) in northern Canada; Mackenzie River in northwestern Canada; and Kolyma and Omolon rivers in Far East Russia. For a deglaciation-induced phylogeographic discontinuity, we explored genetic variation across the Bering Strait. To examine evidence for Canadian and eastern Beringian refugia, we calculated mismatch distributions and coalescent-based estimates of population size and growth rate in 2 arctic hare populations (Greenland and Seward Peninsula, Alaska).

## MATERIALS AND METHODS

We examined the mitochondrial control region and the threonine tRNA gene from 95 arctic hares (58 *L. othus*, 17 *L. timidus*, 20 *L. arcticus*), 2 *L. townsendii*, and 1 *L. coreanus* (Appendix I). *L. coreanus* was also included, and *L. townsendii* was designated as an outgroup species due to their close evolutionary association with arctic hares (Halanych et al. 1999; Koh et al. 2001; Pierpaoli et al. 1999).

Genomic DNA was isolated from museum skins, blood, or frozen tissues curated by museums listed in Appendix I. Methods for DNA extraction, polymerase chain reaction (PCR) amplification, and cycle sequencing followed those summarized in Halanych et al. (1999) and Lessa and Cook (1998). Two primer pairs were used for amplification and sequencing of the control region: LEPUS3' (5'-ATTGGDCAAGT-AGCATCTATCCTT-3') and DLOOP1R (5'-ATTTGACTTGGATG-GTCTATG-3'), and DLOOP2F (5'-CTATGTAATTCGTGCATTA-3') and TDKD (Slade et al. 1994). PCR products were sequenced in both directions with an automated sequencer (model 373, Applied Biosystems, Inc., Foster City, California). The resulting sequences, 473 bp of the control region, and the upstream 200 bp of the threonine tRNA and cytochrome-*b* genes were assembled and aligned by Sequence Navigator (version 1.01, Applied Biosystems). Sequences have been cataloged in GenBank with accession numbers AY422231–AY422328.

Phylogenies were estimated with MrBayes (version 2.01—Huelsenbeck and Ronquist 2001) and PAUP\* (version 4.0b8—Swofford 2002) under Bayesian (Lewis 2001; Shoemaker et al. 1999) and maximum likelihood (Swofford et al. 1996) frameworks, respectively. MODELTEST (version 1.0—Posada and Crandall 1998) was used to determine the optimal model of DNA substitution for Bayesian and maximum likelihood analyses. Nodal support for maximum likelihood topologies was estimated with 100 nonparametric bootstrap replicates (Felsenstein 1985), with identical haplotypes removed to minimize computation time.

Bayesian analyses were initiated with random starting trees, run for  $2 \times 10^6$  generations, and the Markov chain was sampled every 100 generations. Model parameters were estimated directly from the data, and 3 independent replicates were conducted to avoid entrapment in local optima (Huelsenbeck and Iennov 2002). Stationarity of the Markov chain was determined by plotting log likelihood values against number of generations, and trees sampled from generations preceding stationarity were discarded (Huelsenbeck and Ronquist 2001). The remaining data collected were used to estimate nodal support as posterior probabilities and a summary phylogeny.

To test for monophyly of each putative arctic hare species, we conducted likelihood ratio tests (Shimodaira and Hasegawa 1999) by enforcing monophyly of each species of arctic hare and comparing the constrained likelihood score with the tree with the best maximum likelihood score.

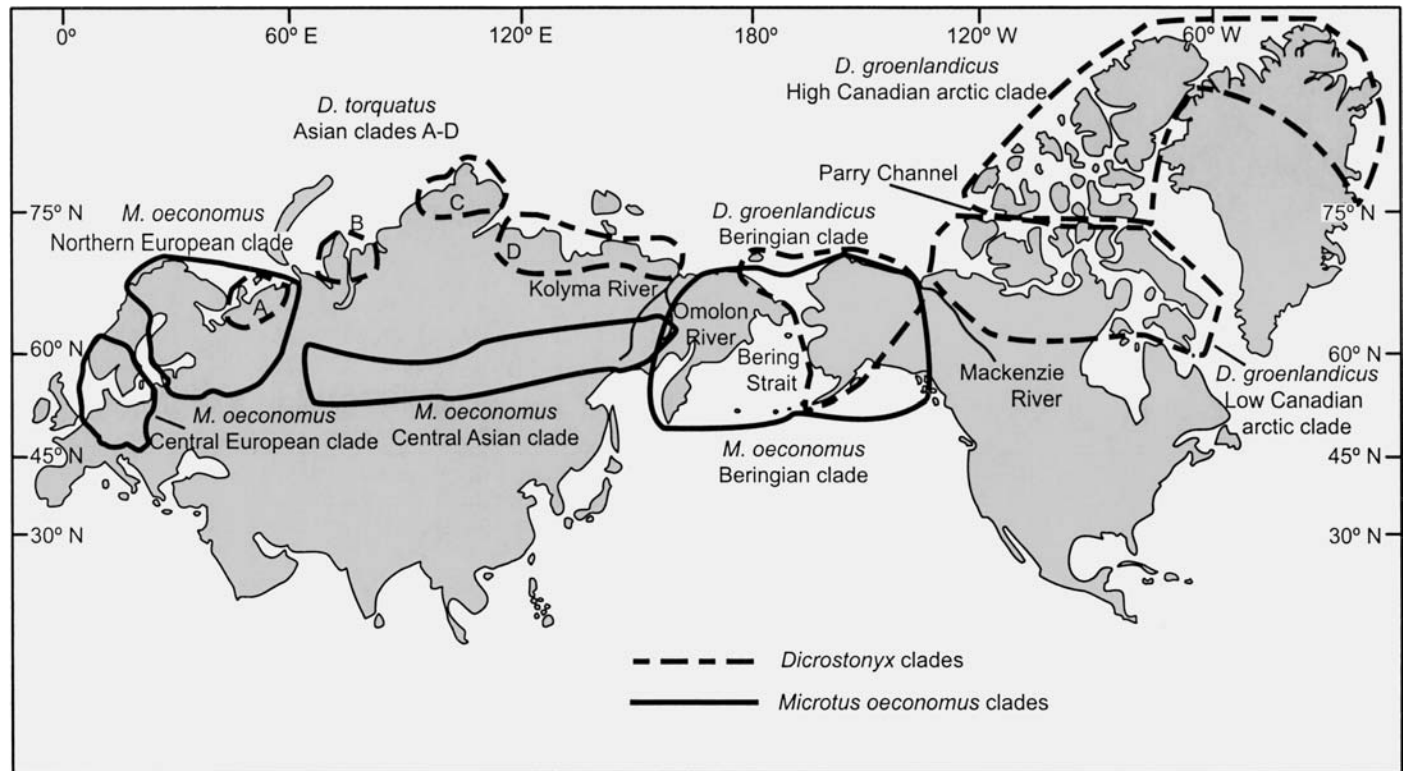


FIG. 2.—Distribution of some small mammal phylogeographic clades and common genetic discontinuities in the Holarctic (from Brunhoff et al. 2003; Fedorov and Stenseth 2002; Fedorov et al. 1999a; Galbreath and Cook 2004).

We further examined 2 arctic hare populations for genetic signatures suggestive of population fluctuation to see whether these populations are located in potential refugia. The 2 populations were from Nome, Alaska ( $n = 15$ ), a subset of the Seward Peninsula hares, and Nansen Land, Greenland ( $n = 15$ ). With the use of FLUCTUATE (Kuhner et al. 1998), a program that uses coalescent techniques, effective female population sizes  $N_{ef}$  were estimated under the constraint of constant population size, and under a model allowing population growth or decline with the equation  $\theta_f = 2N_{ef}\mu$ , where  $\mu$  is the mutation rate. This model assumes no selection or migration between populations. The latter assumption is likely correct given the geographic distance between our 2 study populations, and to test neutrality of the control region, we estimated Tajima's  $D$  (Tajima 1993). The mutation rate for the control region in hares (12.4%/million years) was obtained from Pierpaoli et al. (1999). FLUCTUATE was also used to estimate the exponential growth rate scaled in terms of the number of mutations,  $g$ . Changes in effective population sizes under growth and no-growth models and positive estimates of  $g$  significantly different from 0 would suggest recent population size increase, indicative of a recent colonization. Insignificant growth estimates and changes in effective population size, and increased genetic diversity are indicative of a historically stable population, suggesting that the region was a potential refugium. The shapes of the mismatch distributions were analyzed with ARLEQUIN (Schneider et al. 2000) as sudden population size changes generate unimodal distributions (Rogers and Harpending 1992).

## RESULTS

Alignment of DNA sequences was unambiguous, with no insertions or deletions observed among the arctic hares and *L. coreanus*. One insertion and 2 deletions were observed when

these sequences were compared with *L. townsendii* sequences. Of the 98 hares examined, 39 haplotypes were observed (see Appendix I). The Hasegawa–Kishino–Yano (HKY; Hasegawa et al. 1985) model of DNA substitution incorporating rate heterogeneity was determined to be the most appropriate for our data (HKY+ $\Gamma$ ). This model was used for both Bayesian and maximum likelihood analyses.

Bayesian and maximum likelihood analyses produced identical topologies (Fig. 4), but with differing levels of nodal support for some clades. Given that a Bayesian posterior probability of  $\geq 0.80$  has been suggested to indicate strong support (Whittingham et al. 2002), we define 6 major arctic hare clades with moderate (0.75–0.80 posterior probability) or strong support ( $>0.80$  posterior probability). However, there is a complicated relationship between maximum likelihood bootstrap values and Bayesian branch support (Huelsenbeck et al. 2002; Whittingham et al. 2002), and some of the 6 described clades have low maximum likelihood bootstrap support. Within the arctic hares, all *L. arcticus* haplotypes form a moderately supported (0.78 posterior probability) clade (Figs. 3 and 4: Canadian Clade). All *L. othus* haplotypes form a weakly supported clade (0.70 posterior probability) but form a more strongly supported clade (0.81 posterior probability) when grouped with 1 *L. timidus* from the Omolon River (Figs. 3 and 4: Beringian Clade). Individual *L. timidus* appear throughout the phylogeny, with some *L. timidus* haplotypes weakly grouping with *L. othus* and *L. arcticus* ( $<0.70$  posterior probability). *L. timidus* displays more structure than other arctic

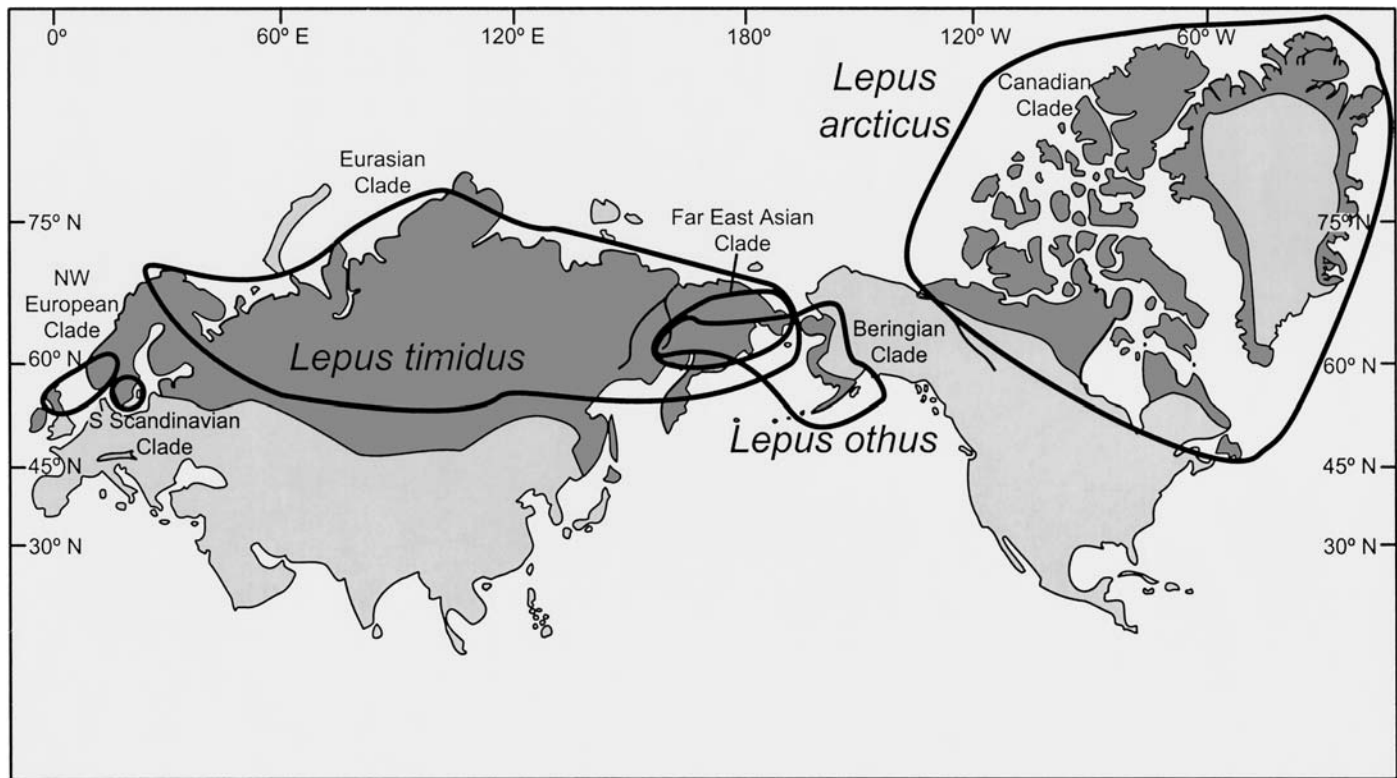


FIG. 3.—Arctic hare distribution and phylogeographic clades identified on the basis of mitochondrial sequence variation.

hares, including a widespread clade with hares from Chukotka and Magadan in Far East Russia, Pechora Bay in northwestern Russia, and Kalix in northern Sweden (Figs. 3 and 4: Eurasian Clade), a clade with 1 Chukotka individual and the remaining Omolon River hares (Figs. 3 and 4: Far East Asian Clade), and 2 European clades (Figs. 3 and 4: Southern Scandinavian and Northwest European Clades). In addition, *L. coreanus* appears nested within the arctic hares (Fig. 4). Tests examining the monophyly of each arctic hare species found that phylogenies constraining *L. othus*, *L. arcticus*, and *L. timidus* were not significantly worse than the phylogeny with the best likelihood score ( $P = 1.00, 1.00, \text{ and } 0.097$ , respectively).

In our analysis of arctic hare populations from Seward Peninsula, Alaska, and Greenland, neither population significantly deviated from neutrality (Seward Peninsula,  $P = 0.14$ ; Greenland,  $P = 0.76$ ). Seward Peninsula hares showed a population growth rate significantly greater than 0 ( $299.8 \pm 296.0$ ; Table 1). Similarly, the effective female population size under size constancy was lower than the population size under a model allowing growth. The growth rate of Greenland hares was not significant ( $23.9 \pm 54.2$ ; Table 1), and the 2 effective female population size estimates were similar. In both populations, mismatch distribution analyses did not reject a sudden expansion model.

## DISCUSSION

*Arctic phylogeography.*—Examination of the phylogeography of Holarctic terrestrial biota provides the opportunity to address 3 issues: the nature and extent of biotic interchange

between continents during glacial maxima, recolonizations in response to climate change, and the effects of large-scale habitat fragmentation on genetic diversity and, ultimately, on speciation. Previous phylogeographic studies have identified genetic discontinuities across a variety of taxa. We compare these findings with those from arctic hares from across the Holarctic to examine congruence in genetic discontinuities among different Arctic taxa, which would provide evidence that abiotic factors such as continental ice sheets and glacial refugia have been involved in shaping these genetic relationships.

*Glaciation-induced genetic discontinuities: High Canadian Arctic.*—Populations of the arctic dryad (*Dryas integrifolia*, Rosaceae—Tremblay and Schoen 1999) and collared lemming (*Dicrostonyx groenlandicus*—Fedorov and Stenseth 2002) in the High Canadian Arctic islands north and east of the Parry Channel appear divergent from the Low Canadian Arctic populations (Fig. 2), whereas northern Greenland populations of purple saxifrage (*Saxifraga oppositifolia*, Saxifragaceae) display elevated molecular diversity (Abbott et al. 2000). Additionally, a number of mammals in the High Canadian Arctic show distinct morphological variation (MacPherson 1965). The discontinuities found in these studies support the hypothesis of a High Canadian Arctic refugium (MacPherson 1965; Pielou 1991).

Our arctic hare phylogeny does not show a genetic discontinuity between hares of Greenland and Banks Island, Northwest Territories, Canada, although our population-level analyses suggest that Greenland hares have not experienced recent population expansion. In addition, the grouping of

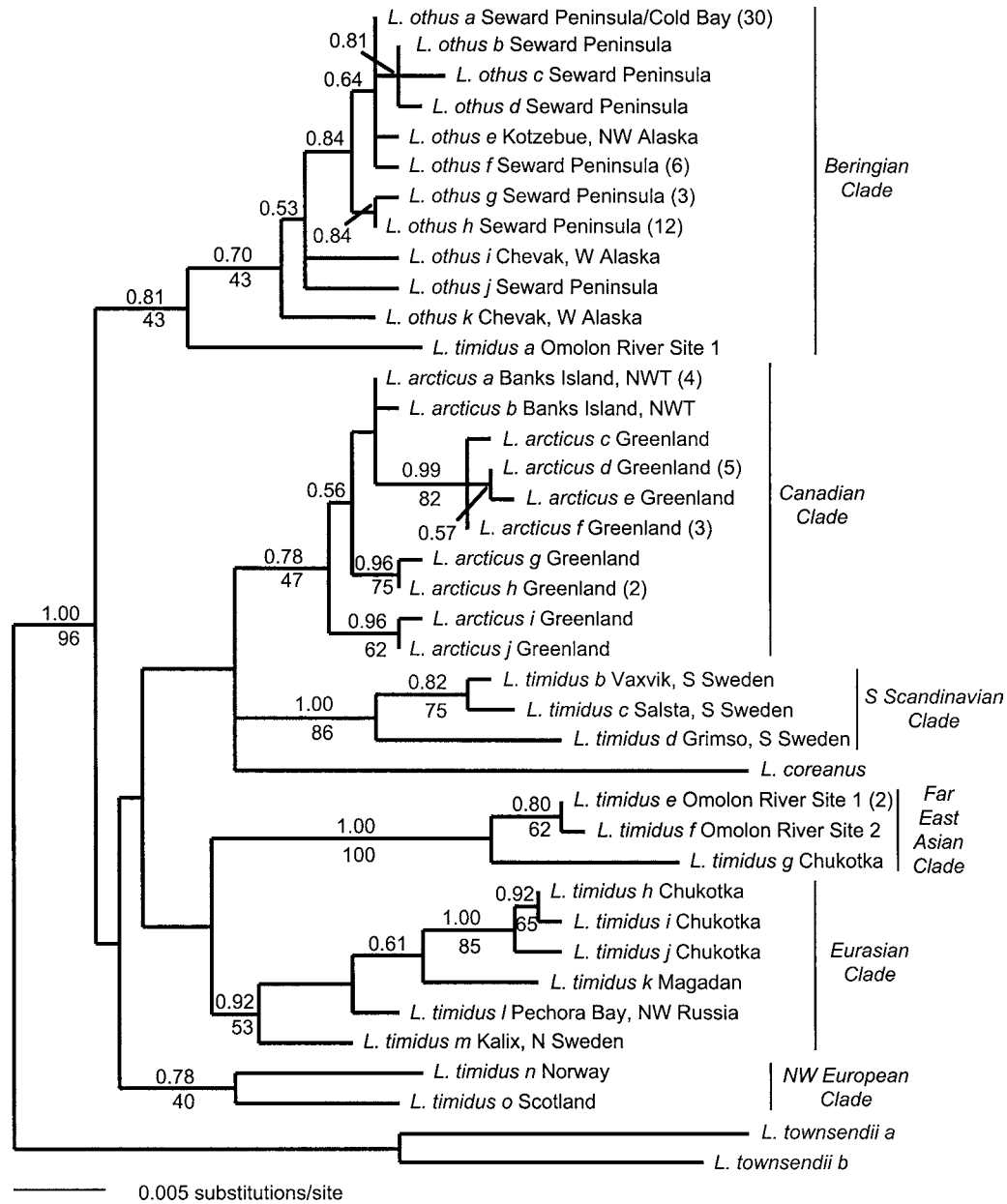


FIG. 4.—Phylogeny of arctic hares (*L. arcticus*, *L. othus*, *L. timidus*) and 2 closely related species (*L. coreanus*, *L. townsendii*), on the basis of 673 base pairs of the mitochondrial control region and threonine tRNA estimated with both Bayesian and maximum likelihood methods. Numbers above branches are Bayesian posterior probabilities, and numbers below branches are values from maximum likelihood bootstrap analysis. Bayesian posterior probabilities for 3 clades are indicated with diagonal lines for purposes of space. Numbers in parentheses indicate number of individuals representing a particular haplotype. Species names followed by italic letters indicate haplotype name (see Appendix I).

*L. othus* and *L. arcticus* into 2 clades (Fig. 3) is congruent with reports of divergence between Canadian and Beringian collared lemmings (*Dicrostonyx groenlandicus*—Fedorov and Stenseth 2002), ptarmigan (*Lagopus mutus*—Holder et al. 2000), and *Daphnia* (Weider and Hobæk 2003), suggesting separate refugia for these lineages. Geological studies (Andersen and Borns 1994; Dyke et al. 2002; Vincent 1992) and paleontological findings of pre-Illinoian ptarmigan and lemming fossils and Wisconsin mammoth and musk ox fossils on Banks Island (Harrington 1990) also support the hypothesis of a separate

refugium northeast of Beringia along the margins of the Laurentide and Innuitian ice sheets (Fig. 1).

**Glaciation-induced genetic discontinuities: Omolon and Kolyma Rivers.**—In eastern Asia, a common phylogeographic discontinuity along the Omolon and Kolyma rivers, regarded as the western boundary of Beringia, has been found in arctic vertebrates (Fig. 2). Fedorov and colleagues (Fedorov and Stenseth 2002; Fedorov et al. 1999a, 1999b, 2003) reported a genetic discontinuity between major clades of collared lemmings and true lemmings at the mouth of the

**TABLE 1.**—Genetic estimates, including  $\theta_f$ , a parameter incorporating population size and mutation rate, of 2 arctic hare populations.

Population	Seward Peninsula, Alaska	Nansen Land, Greenland
Sample size	15	15
Haplotypes	5	8
Nucleotide diversity		
$\bar{X}$	0.0023	0.0081
<i>SE</i>	0.0016	0.0046
$\theta_f$ (constant size)	0.0137	0.0276
$N_{ef}$ (constant size)	142,771	287,708
$\theta_f$ (growth)	0.0317	0.0318
$N_{ef}$ (growth)	330,104	331,042
Growth rate		
$\bar{X}$	299.8	23.9
95% <i>CI</i>	3.8–595.8	–30.3–78.1

Kolyma River, downstream of the Omolon River. In true lemmings, the break at the Kolyma River marks the border between *Lemmus sibiricus* and *L. trimucronatus* (Fedorov et al. 1999b, 2003). Similarly, a genetic discontinuity along the Omolon River divides Eurasian tundra vole (*Microtus oeconomus*) lineages from Beringian lineages (Brunhoff et al. 2003; Galbreath and Cook 2004). In addition, a genetic discontinuity in the dunlin (*Calidris alpina*) is thought to be located at the Kolyma River (Wenink et al. 1996). Our data also indicate a genetic discontinuity in arctic hares along the Omolon River, with these *L. timidus* samples found in 2 distinct clades in the phylogeny (Figs. 3 and 4). These concordant patterns suggest a common historical barrier to gene flow in this region, with hypothesized eastern Siberian montane glaciers the most likely candidates (Fig. 1; Glushkova 2001; Heiser and Roush 2001; Paleoclimates from Arctic Lakes and Estuaries [PALE] Beringian Working Group 1999).

*Glaciation-induced genetic discontinuities: Mackenzie River.*—Another genetic discontinuity is along the Mackenzie River in northwestern Canada, near the eastern boundary of Beringia. This river marks a major break in the collared lemming, *D. groenlandicus*, and is hypothesized to be the boundary at which populations expanding from Beringia and a Canadian Arctic refugium came into contact (Figs. 2 and 3; Ehrich et al. 2000; Fedorov and Stenseth 2002). In arctic hares, the Mackenzie River marks the western boundary of *L. arcticus*. Our molecular evidence that *L. othus* and *L. arcticus* have different phylogenetic histories (Fig. 4), in combination with their distributional gap, supports evidence from other taxa that the Mackenzie River is a boundary between populations expanding from 2 separate glacial refugia.

*Deglaciation-induced genetic discontinuities: Bering Strait.*—The Bering Strait now forms a significant physical barrier between the Asian and American biotas, although many terrestrial arctic species have distributions that span it due to the relatively recent flooding of the Bering Land Bridge at the end of the Pleistocene. As a result, the genetic structure of

lemmings (Fedorov and Stenseth 2002; Fedorov et al. 1999a, 1999b, 2003), shrews (Demboski and Cook 2003), tundra voles (Brunhoff et al. 2003; Galbreath and Cook 2004), and northern red-backed voles (Cook et al. 2004) show no discontinuities at the Bering Strait, reflecting the strong historical connections across Asia and North America in Beringia. Our data partially agree with these studies because the Beringian arctic hare clade spans the Bering Strait, but the Strait is the eastern boundary of the Eurasian and Far East Asian Clades (Fig. 3).

*Demographic estimates and implications for Pleistocene refugia.*—Our demographic estimates of Alaskan *L. othus* and Greenland *L. arcticus* populations centered in hypothesized Pleistocene refugia do not provide evidence for a historically stable refugial population on the Seward Peninsula in eastern Beringia (Table 1). Rather, this population shows evidence of recent expansion, with relatively low molecular diversity. In contrast, coalescent-based estimates of the arctic hare population in northern Greenland indicate that this population was historically stable (Table 1). These data further support the existence of a High Canadian Arctic refugium (MacPherson 1965; Pielou 1991). Although recent reconstructions of the North American ice sheets during the last glacial maximum hypothesize that much of the Canadian Arctic was covered by the Innuitian ice sheet, there is also evidence that some coastlines in the High Canadian Arctic were ice-free (Fig. 1; Clark and Mix 2002; Dyke et al. 2002; Miller et al. 2002). The body of evidence for a Beringian refugium is considerable (see, e.g., Abbott and Brochmann 2003; Pielou 1991); however, our eastern Beringian arctic hare population (*L. othus*) does not appear to be historically stable. Increased sampling for population genetic analyses is needed to examine molecular evidence for these refugia.

*Arctic hare systematics.*—The fossil record for arctic hares dates back only to the last interglacial period (Eemian or Sangamonian; 120,000–130,000 years ago) in Europe, with fossils common from the last glacial period (Kurtén 1968). In North America, arctic hare remains have also been found dating to the last interglacial (120,000–130,000 years ago; Kurtén and Anderson 1980), with many fossils found from the last glacial period (Wisconsin) in central Alaska and the Yukon Territory (Cinq-Mars 1979; Dixon et al. 1983; Morlan 1979; Weber et al. 1981). These localities are outside the current range of arctic hares, suggesting a recent range retraction in western North America. In eastern Russia, no *L. timidus* fossils dated prior to the Holocene have been found (Averianov 1998; Hopkins et al. 1982).

Taxonomic summaries of the arctic hare complex, based primarily on morphological evidence, have recognized 3 species (Flux and Angermann 1990; Hoffmann 1993; Ramos 1999), 2 species (*L. timidus* and *L. arcticus*—Baker et al. 1983), or just a single species (*L. timidus*—Dixon et al. 1983; Rausch 1963). Baker et al. (1983) hypothesized a single morphological break in western Siberia, with hares in Chukotka, Far East Russia, more similar to North American hares. Consequently, Baker et al. (1983) suggested that *L. othus*, *L. arcticus*, and Far East Russian *L. timidus* are conspecific but distinct from other *L. timidus*. Anderson

(1974), in contrast, determined that *L. othus* is distinct from *L. arcticus* and *L. timidus*, with breaks at the Bering Strait and the Mackenzie River.

A molecular study of 11 species of *Lepus* worldwide concluded that arctic hares exhibited minimal genetic divergence relative to levels of divergence among other species (Halanych et al. 1999). Our study of the rapidly evolving control region found considerable genetic variation (up to 5% uncorrected sequence divergence) among arctic hares. We have identified genetic discontinuities among arctic hare clades at the Omolon River in eastern Russia and at the Bering Strait (Figs. 3 and 4) and found that *L. othus* and *L. arcticus* separate into identifiable clades. Although some authors include Chukotkan, tundra-associated hares within *L. othus* (Baker et al. 1983; Hoffmann 1993), our phylogenetic clades suggest that Chukotkan hares are more closely related to *L. timidus* (Fig. 4), a finding consistent with Anderson (1974) and Best and Henry (1994). The single Siberian hare on the Omolon River that clusters with *L. othus* is striking, however, and partially supports the Baker et al. (1983) hypothesis that *L. othus* is trans-Beringian. Nonetheless, because this and other Omolon River hares are associated with the taiga and >400 km west of the Chukotka tundra, more extensive sampling is needed to clarify the distribution of these species.

Shimodaira–Hasegawa tests did not reject monophyly of each arctic hare species. This molecular perspective supports the existing taxonomy but also identifies a genetic discontinuity in *L. timidus* at the Kolyma and Omolon Rivers, as well as additional genetic structure in this species in western Europe. In addition, placement of a Korean hare (*L. coreanus*) within the arctic hares suggests that identity and relationships of East Asian species of *Lepus* warrant additional investigation.

These geographic clades paint a complex picture of arctic hare evolution. In some respects, distinct genetic clades are similar to those from other phylogeographic studies, with identifiable Beringian, Canadian Arctic, and western European clades (Figs. 2 and 3). Similarly, the Beringian and Far East Asian Clades border on the Omolon and Kolyma Rivers in a region of genetic discontinuity identified in other Arctic complexes. However, these molecular data do not resolve relationships among the 6 clades. In addition, the Omolon River is not a complete genetic break in arctic hares because the Eurasian Clade includes individuals on both sides of the Omolon River (Figs. 3 and 4).

The arctic hare complex shows a relative lack of genetic and morphological variation compared with Holarctic lemmings (Fedorov and Stenseth 2002; Fedorov et al. 2003) and tundra voles (Brunhoff et al. 2003; Galbreath and Cook 2004). This shallower structure could be due to recent expansion across the Holarctic, although in the fossil record, hares appear at roughly the same time in both Europe and North America, with no clear ancestral region (Averianov 1998; Hopkins et al. 1982; Kurtén 1968; Kurtén and Anderson 1980). Another possible cause is limited, historical gene flow occurring over multiple glacial–interglacial cycles.

Our analyses of arctic hare phylogeography have detected genetic discontinuities that corroborate similar phylogeographic

studies of lemmings (Ehrich et al. 2000; Fedorov and Stenseth 2002; Fedorov et al. 1999a, 1999b, 2003), voles (Brunhoff et al. 2003; Galbreath and Cook 2004), birds (Holder et al. 2000; Wenink et al. 1996), plants (Abbott et al. 2000; Tremblay and Schoen 1999), and invertebrates (Weider and Hobæk 2003). Although these studies have uncovered seemingly concordant patterns, there are still relatively few biogeographic and phylogeographic studies on arctic taxa. Given the obvious importance of historic and contemporary climate change on structuring the high-latitude biota, it is clear that this region needs more attention. In addition, further study with multiple, independent loci and a more complete fossil record is needed to determine whether these geographical concordances are historically concordant. Our results, particularly of the little-understood Omolon and Kolyma contact zone, also support the idea that additional emphasis should be placed on the study of Beringia and its environs (Weider and Hobæk 2000).

#### ACKNOWLEDGMENTS

We thank J. Dallas, F. Gonzalez, P. Groves, N. Larter, J. Patton, D. Rogers, C.-G. Thulin, and the many hunters and trappers for collection of specimens and tissue samples. We thank K. Galbreath and A. Runck for insightful discussions. G. Hewitt and R. S. Hoffmann provided useful comments on the submitted manuscript. This research followed American Society of Mammalogists animal care and use guidelines (Animal Care and Use Committee 1998). Funding was provided by the National Science Foundation (DEB 0196095 and 9981915), National Institutes of Health NCRR (1P20RR016448-01), and NBS Research Work Order 13 through the Alaska Cooperative Fish and Wildlife Research Unit.

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Submitted 3 July 2003. Accepted 3 October 2003.

Associate Editor was Eric A. Rickart.

## APPENDIX I

*Specimens examined*.—Specimens were obtained from the following institutions: University of Alaska Museum (UAM); Monte L. Bean Life Science Museum, Brigham Young University (BYU); Museum of Vertebrate Zoology (MVZ). GenBank numbers begin with AY. Sample size in parentheses follows specific name. Localities are followed in parentheses by specimen, haplotype as shown in Fig. 4, and GenBank numbers. Individuals used in the population comparisons are indicated with an asterisk.

*Lepus arcticus* (20).—CANADA: Northwest Territories: Banks Island (UAM47252, *L. arcticus* a, AY422246), (UAM47253, *L. arcticus* a, AY422247), (UAM47254, *L. arcticus* a, AY422248), (UAM47255, *L. arcticus* a, AY422249), (UAM47257, *L. arcticus* b, AY422250); GREENLAND: Nansen Land (\*UAM67719, *L. arcticus* i, AY422231), (\*no number, *L. arcticus* g, AY422232), (\*no number, *L. arcticus* c, AY422233), (\*no number, *L. arcticus* d, AY422234), (\*no number, *L. arcticus* h, AY422235), (\*no number, *L. arcticus* e, AY422236), (\*no number, *L. arcticus* j, AY422237), (\*no number, *L. arcticus* d, AY422238), (\*no number, *L. arcticus* d, AY422239), (\*no number, *L. arcticus* h, AY422240), (\*no number, *L. arcticus* d, AY422241), (\*no number, *L. arcticus* f, AY422242), (\*no number, *L.*

*arcticus f*, AY422243), (\*no number, *L. arcticus d*, AY422244), (\*no number, *L. arcticus f*, AY422245).

*Lepus othus* (58).—ALASKA (western): Chevak (UAM10521, *L. othus i*, AY422252), (UAM10870, *L. othus k*, AY422253); ALASKA: Cold Bay (UAM42143, *L. othus a*, AY422251); ALASKA (northwest): Kotzebue (UAM45545, *L. othus e*, AY422254); ALASKA: Seward Peninsula (\*UAM5959, *L. othus d*, AY422255), (\*UAM5960, *L. othus c*, AY422256), (UAM5961, *L. othus a*, AY422257), (UAM15851, *L. othus g*, AY422258), (UAM15852, *L. othus b*, AY422259), (UAM15853, *L. othus j*, AY422260), (UAM71986, *L. othus a*, AY422261), (UAM72014, *L. othus a*, AY422262), (\*UAM72015, *L. othus a*, AY422263), (\*UAM72016, *L. othus a*, AY422264), (UAM72018, *L. othus f*, AY422265), (UAM72019, *L. othus a*, AY422266), (UAM72020, *L. othus h*, AY422267), (UAM72021, *L. othus a*, AY422268), (\*UAM66665, *L. othus a*, AY422269), (UAM66666, *L. othus a*, AY422270), (UAM66667, *L. othus a*, AY422271), (\*UAM66668, *L. othus h*, AY422272), (UAM66669, *L. othus a*, AY422273), (UAM66670, *L. othus h*, AY422274), (UAM66671, *L. othus g*, AY422275), (UAM63104, *L. othus h*, AY422276), (\*UAM66672, *L. othus f*, AY422277), (UAM73076, *L. othus a*, AY422278), (UAM73077, *L. othus a*, AY422279), (UAM73078, *L. othus a*, AY422280), (UAM73079, *L. othus h*, AY422281), (UAM73080, *L. othus a*, AY422282), (UAM73081, *L. othus a*, AY422283), (UAM73082, *L. othus h*, AY422284), (\*UAM53322, *L. othus f*, AY422285), (\*UAM53520, *L. othus a*, AY422286), (\*UAM53737, *L. othus h*, AY422287), (UAM75260, *L. othus g*, AY422288), (UAM77105, *L. othus a*, AY422289), (UAM77099, *L. othus a*, AY422290), (\*UAM77098, *L. othus a*, AY422291), (UAM77107, *L. othus h*, AY422292), (\*UAM77102, *L. othus a*, AY422293), (\*UAM77103, *L. othus a*,

AY422294), (\*UAM58607, *L. othus f*, AY422295), (UAM77097, *L. othus h*, AY422296), (UAM77100, *L. othus a*, AY422297), (UAM77104, *L. othus a*, AY422298), (UAM66675, *L. othus h*, AY422299), (UAM66676, *L. othus a*, AY422300), (UAM66677, *L. othus h*, AY422301), (UAM66673, *L. othus a*, AY422302), (UAM66674, *L. othus f*, AY422303), (UAM77093, *L. othus h*, AY422304), (UAM77101, *L. othus a*, AY422305), (UAM77108, *L. othus f*, AY422306), (UAM77096, *L. othus a*, AY422307), (\*UAM77106, *L. othus a*, AY422308).

*Lepus timidus* (17).—NORWAY (southern): Ringebu (UAM61331, *L. timidus n*, AY422310); RUSSIA (Far East): Chukotka Peninsula (UAM23260, *L. timidus j*, AY422317), (no number, *L. timidus h*, AY422322), (no number, *L. timidus i*, AY422323), (no number, *L. timidus g*, AY422325); RUSSIA (Far East): Magadan (no number, *L. timidus k*, AY422324); RUSSIA: Omolon River Site 1 (UAM80433, *L. timidus e*, AY422318), (UAM80434, *L. timidus e*, AY422319), (UAM80435, *L. timidus a*, AY422320); RUSSIA: Omolon River Site 2 (UAM80537, *L. timidus f*, AY422321); RUSSIA (northwest): Pechora Bay (UAM61330, *L. timidus l*, AY422314); SCOTLAND: Vicinity of Aberdeen (no number, *L. timidus o*, AY422315), (no number, *L. timidus o*, AY422316); SWEDEN (southern): Grimso (UAM63887, *L. timidus d*, AY422312); SWEDEN (northern): Kalix (UAM61332, *L. timidus m*, AY422311); SWEDEN (southern): Salsta (UAM63916, *L. timidus c*, AY422313); SWEDEN (southern): Vaxvik (UAM63915, *L. timidus b*, AY422309).

*Lepus coreanus* (1).—SOUTH KOREA: Kangwon Province (MVZ134375, *L. coreanus*, AY422326).

*Lepus townsendii* (2).—UTAH: Sevier County (BYU15986, *L. townsendii b*, AY422328); UTAH: Summit County (BYU15993, *L. townsendii a*, AY422327).