Limited phylogeographic structure and genetic variation in Alaska's arctic and alpine endemic, the Alaska marmot

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Alpine and arctic environments are thought to be more vulnerable to climate change than other lower-elevation and lower-latitude regions. Being both arctic and alpine distributed, the Alaska marmot (*Marmota broweri*) is uniquely suited to serve as a harbinger of the effects of climate change, yet it is the least-studied marmot species in North America. We investigated the phylogeography and genetic diversity of *M. broweri* throughout its known distribution in northern Alaska using the mitochondrial cytochrome *b* gene to better understand how post-Pleistocene changes and population fragmentation have structured genetic diversity. Our results show significant, although shallow, geographic structure among Alaska marmot populations. The diversity within and among populations is consistent with 2 phylogeographic hypotheses: Alaska marmots persisted in the eastern Brooks Range, Ray Mountains, and Kokrines Hills during the Pleistocene and have only recently expanded into the western Brooks Range; and the western Brooks Range served as a refugium as well and those populations have undergone a bottleneck resulting in reduced genetic variation in extant populations. Levels of mitochondrial deoxyribonucleic acid diversity are lower in *M. broweri* than in any other codistributed small mammal species and alpine mammal species with comparable data available. This is the 1st phylogeographic study of any marmot species and provides a baseline measure of the current structure and diversity within *M. broweri*.

Key words: Alaska marmot, alpine, arctic, Beringia, climate change, GEODIS, *Marmota broweri*, phylogeography

There are 14 currently-recognized species of marmots (Rodentia: Sciuridae; *Marmota*) ranging across the Holarctic. With 1 exception (woodchuck, *Marmota monax*), marmots are largely restricted to alpine habitats, living on sky islands isolated by ecological barriers in lower-elevation ecosystems. Their restricted distributions and dependence on high-elevation habitats make alpine marmots particularly vulnerable to climate change and the subsequent contraction or expansion of their preferred habitat (Griffin et al. 2008; Inouye et al. 2000). For these reasons, marmots have been called harbingers of changes occurring in alpine regions around the world (Krajick 2004). In a review of ecological and evolutionary effects of recent climate change, Parmesan (2006:637) concluded that, ''[r]ange-restricted species, particularly polar and mountaintop species, show severe range contractions and have been the first groups in which entire species have gone extinct due to recent climate change.'' As shrublines and treelines in the Northern Hemisphere move rapidly northward and upslope (Overpeck et al. 1997; Sturm

et al. 2001), marmots and other alpine-obligate species must shift their distributions accordingly or adapt to their new environments if they are to survive the changes in their habitat. A recent study of the effects of 100 years of climate change on small mammal species in Yosemite National Park demonstrated an average 500-m upward shift in the distributions of half of the species surveyed (Moritz et al. 2008). These authors also found that while low-elevation species have expanded their ranges upslope, the ranges of high-elevation species contracted. Like most other marmot species, Alaska marmots (*M. broweri*) occur at or near mountain peaks where talus and rock outcrops provide shelter, and nearby alpine meadows provide forage. However, the Alaska marmot occurs primarily in the Brooks Range of northern Alaska, the northernmost mountain range in North America (Gunderson et
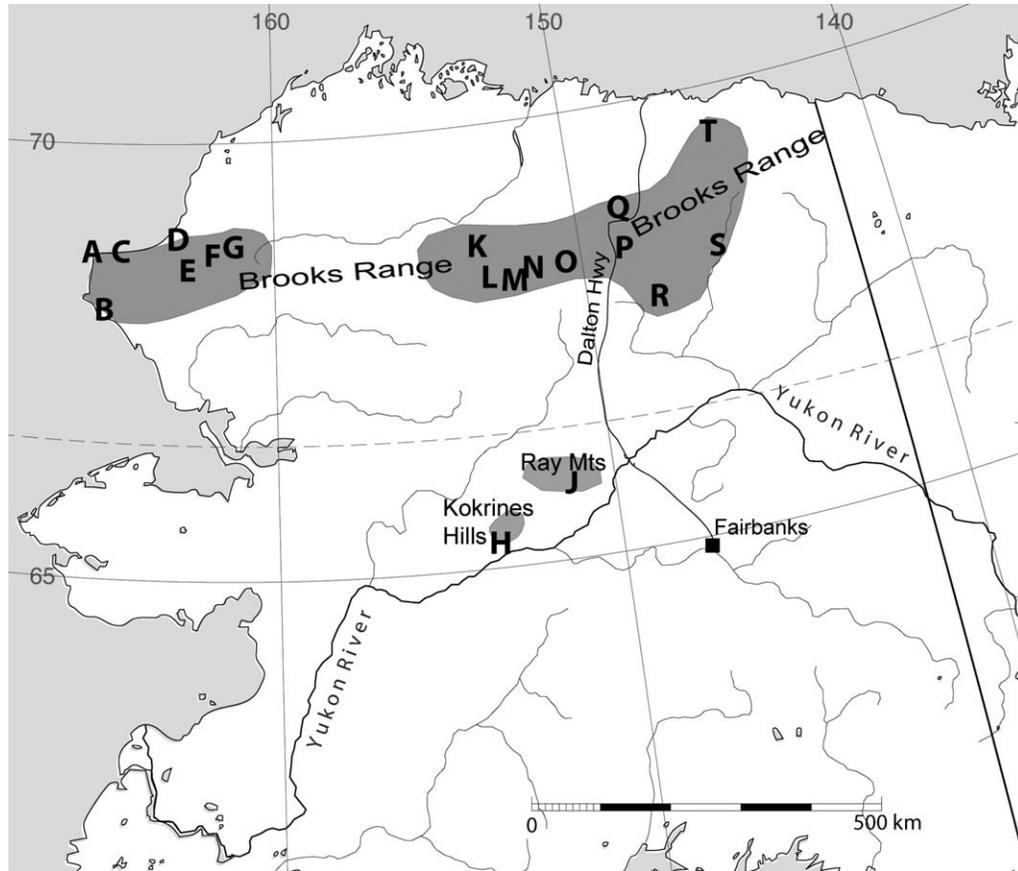
FIG. 1.—The distribution of *Marmota broweri* (shaded gray, modified from Gunderson et al. 2009) and the localities sampled for this study (letters). The letters correspond to specimens listed in Appendix I.

al. 2009). Along with its congener, the black-capped marmot (*M. camtschatica*) of eastern Russia, the Alaska marmot is the most northerly distributed alpine mammal; its distribution cannot shift northward and it already occupies the highest elevations available (Gunderson et al. 2009). Furthermore, *M. broweri* may face competition from the northward expansion of hoary marmots (*M. caligata*), which have recently been found to occur in near parapatry with and directly south of the southernmost Alaska marmot populations (Gunderson et al. 2009). To understand how Alaska marmots, and other alpine-restricted species, will be affected by rapidly accelerating climate change, and to assess the conservation status of this vulnerable species, we need to understand the current genetic diversity and phylogeographic structure among extant populations. This study is the first of its kind for any species of marmot and is focused on the most remotely distributed and least-studied marmot in the world. It provides an initial characterization of population structure, geographic history, and genetic diversity of *M. broweri*—a mammal uniquely amenable to studying, and presumably uniquely vulnerable to the effects of climate change.

The Alaska marmot currently occurs in alpine areas of northern Alaska, primarily in the Brooks Range but also in central Alaska in the Ray Mountains and Kokrines Hills (Gunderson et al. 2009; Fig. 1). Both molecular and morphological analyses suggest

that *M. broweri* is more closely related to Asian marmot species (subgenus *Marmota*) than to its nearest North American congener, the hoary marmot (*M. caligata*; Cardini et al. 2005; Steppan et al. 1999). Two hypotheses have been proposed for the origin of *M. broweri* in North America. Rausch and Rausch (1971) suggested that the Alaska marmot was left behind in Alaska as an ancestral marmot crossed the Bering Land Bridge and radiated across Asia and Europe. Hoffmann and Nadler (1968) and Hoffman et al. (1979) proposed that *M. broweri* originated in Asia and crossed back into North America during the Pleistocene. Steppan et al. (1999) were unable to reject either hypothesis using statistical methods available at the time but suggested that the 2nd was more likely. Both of these scenarios place *M. broweri* in the ice-free region of Beringia at the end of the Pleistocene.

Beringia was a huge land area that remained ice-free during the repeated glacial advances and retreats that characterized the Pleistocene (Hopkins 1967). It extended from eastern Siberia through central Alaska and into western Yukon Territory and served as a refugium, separated from North America and Asia by glaciers and ice sheets, and as a dispersal corridor allowing the dispersal of flora and fauna between the 2 continents. Many studies have focused on Beringia's role as a refugium, shaping the present diversity and distribution of arctic species. Phylogeographic structure observed in many mammal species indicates that Beringia has served as a source for colonization of

TABLE 1.—Primer sequences used in PCR and sequencing reactions.

Name	Sequence (5′–3′)
F1	CTCACCGTTGTTATTCAACTA
AGR1	GGGATTTTGTCTGAGTCAGA
AGF1	CAAAGCCACTCTAACACGAT
R3AG	GGTTTACAAGGCCAGGGTAATG
R4AG	TGTGGGCAACTGATGAGAAA
F4AG	ATCCAAATCTTTACCGGACT
R5AG	TGACCTCAGGGGAGGACATA
F5AG	CTACGGCTCATATACCTACTC
R6AG	TAGGGCTGCGATGATAAAGG
R7AG	ATCAGGGTCTCCCAGAAGGT
F6AG	AATCCCCTTTCACCCGTACT
R8AG	GAGAAGATTAGGGCTAGGACTC
F7AG	TACACCCGCAAACCCTCTAA
R9AG	AGATTGTCCTCCGATTCAGGT
F8AG	TCGACCATTAAGCCAATGTG

recently deglaciated areas (Eddingsaas et al. 2004; Fedorov and Goropashnaya 1999; Galbreath and Cook 2004; Hundertmark et al. 2002; Waltari et al. 2007). We predict this common historical pattern of persistence in Beringia with subsequent expansion into previously glaciated areas to be found in *M. broweri* and for the genetic diversity within and among marmot populations to exhibit a pattern of divergence in isolation resulting from their fragmented and insular-like distribution.

MATERIALS AND METHODS

Sampling.—We collected 24 Alaska marmot specimens from 8 localities from June through September 2005–2007. All fieldwork was carried out in accordance with guidelines approved by the American Society of Mammalogists (Sikes et al. 2011) and with the approval of the Institutional Animal Care and Use Committee of the University of Alaska Fairbanks. We obtained fresh tissue subsamples from the 6 existing museum specimens with such material available. An additional 27 dry tissue samples were obtained from museum study skins or dried residual tissue left on cranial or skeletal material. These degraded samples included the 1st *M. broweri* specimens ever collected as well as the type specimen collected in 1931 (Hall and Gilmore 1934). Of the 27 degraded tissue sample extractions, 4 did not yield amplification products, leaving a total sample size of 53 individuals (Appendix I). This represents 47% of all *M. broweri* museum specimens, collected from across the distribution of Alaska marmots with 19 of 22 known localities represented, including 7 localities newly documented with voucher specimens in our previous work (Gunderson et al. 2009; Fig. 1). The 3 localities not included were considered redundant due to their proximity to other localities within the data set. We understand this sample may be of limited statistical power, but it is the best representation of *M. broweri* currently available. Given the significant financial and logistical challenges involved in collecting specimens or noninvasive material of Alaska marmots, this is likely to remain the case well into the future.

Molecular methods.—Deoxyribonucleic acid (DNA) was extracted from fresh or frozen tissue samples using the PureGene Genomic DNA Purification Kit (Gentra Systems, Minneapolis, Minnesota). The resulting template was diluted into a 1:10 working solution for use in polymerase chain reaction (PCR). From fresh tissue extractions we amplified and sequenced the entire length of the mitochondrial cytochrome *b* gene (1,140 base pairs) in 2 overlapping segments using the primer pairs F1/AGR1 and AGF1/R3AG (all primers were designed by the authors; Table 1).

Deoxyribonucleic acid extractions from samples of study skins and dried tissue were performed in the Ancient DNA Laboratory at the University of Alaska Museum (a PCR-free building), a facility designed specifically for procedures with high risks of contamination. Each sample of approximately 20 mg of tissue was digested in 600 μl of cell lysis solution (PureGene Genomic DNA Purification Kit, Gentra Systems, Minneapolis, Minnesota), 20 μl of proteinase K (20 mg/ml), and 30 μl dithiothreitol (100 mM) for 24–48 h, with agitation in a dry heat block at 55°C. For those samples that remained undigested after 24 h, 20 μl of additional proteinase K was added and the samples were allowed to digest for another 24 h. After digestion, the extractions proceeded according to the PureGene Genomic DNA Purification Kit protocol for DNA purification from fresh tissue. Each extraction included a negative control to test for contamination from the extraction procedure. All PCR and other downstream reactions were conducted in a lab in a separate building. Due to the degraded nature of the DNA extracted from the skin and dried tissue samples, we amplified and sequenced the entire length of cytochrome *b* in 7 overlapping segments using the following primer pairs: F1/R4AG, F4AG/R5AG, F5AG/R6AG, AGF1/R7AG, F6AG/R8AG, F7AG/R9AG, and F8AG/R3AG (Table 1). Volumes and concentrations of reagents used in these amplifications were as follows: 1 μl of undiluted DNA template, 1 μl each of primers (10 μM), 2.5 μl of 10× Promega reaction buffer, 1 μl of MgCl$_2$ (25 mM), 0.5 μl of deoxyribonucleotide triphosphates (10 mM), 0.25 μl of Promega GoTaq polymerase (5 U/μl), and 17.75 μl of H$_2$O for a total reaction volume of 25 μl. The reactions were conducted with the following cycling parameters: 94°C for 3 min, then 40 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min. The extraction negatives were run along with DNA extracts; each PCR reaction also included a negative control to determine if any contaminating DNA was introduced during the PCR protocol as well as a positive control to verify that the PCR reaction occurred as expected.

Before cycle sequencing, all PCR products were purified with Exo-SAP-IT (USB, Cleveland, Ohio) according to the manufacturer's instructions. Purified PCR products were cycle sequenced using BigDye Terminator (Perkin-Elmer, Boston, Massachusetts) and the same primers used in PCR. Sequencing was performed on an ABI 3100 (Applied Biosystems, Foster City, California) sequencer. All gene segments were bidirectionally sequenced.

Data analysis.—DNA sequences were aligned and assembled with reference to a *M. broweri* cytochrome *b* sequence

obtained from GenBank (AF143918) and checked by eye with SEQUENCHER 4.7 (Gene Codes, Ann Arbor, Michigan). All new sequences have been deposited in GenBank (FJ438931-FJ438934, FJ438936, JN024574-JN024621).

Phylogenetic analyses under the maximum likelihood (ML) and maximum parsimony (MP) criteria were performed with PAUP* 4.0b10 (Swofford 2003). Heuristic MP and ML tree searches were conducted using stepwise addition of 100 random addition sequences with the tree bisection–reconnection branch-swapping algorithm. For the ML analysis a model of nucleotide substitution (GTR+G) and associated parameters were estimated using MODELTEST 3.7 and selected on the basis of the Akaike information criterion (Posada and Buckley 2004, Posada and Crandall 1998). Bootstrap values of nodal support were generated for the ML tree from 1,000 replicates. Bayesian inference was implemented with MRBAYES 3.1.2 (Huelsenbeck and Ronquist 2001) using 2 runs of 4 chains (3 heated) for 10 million generations (sampling every 1,000 with a burn-in of 2,000) to produce posterior probabilities for the most probable tree topology. *Marmota caudata*, *M. menzbieri*, and *M. monax* sequences obtained from GenBank (AF143923-143925, AF143931, AF143932-143934) were used to root trees in all analyses.

Standard molecular diversity statistics were calculated using Arlequin 2.0 (Schneider et al. 2000) for combined regional samples from the western Brooks Range (sample sites A–G, referred to hereafter as West), eastern Brooks Range (sample sites K–T, referred to hereafter as East), and southern mountain ranges (Kokrines Hills and Ray Mountains, sample sites H and J, referred to hereafter as South). Smaller values of π (nucleotide diversity) can indicate that a population has recently expanded into that area, whereas larger values can indicate that a population has persisted for longer periods of time. We used Arlequin to conduct an analysis of molecular variance (AMOVA), run the Ewens–Watterson test for selective neutrality (Watterson 1978), calculate Tajima's D (Tajima 1989) and Fu's F_S (Fu 1997), and plot mismatch distributions to test for population expansion in each of the 3 regions (East, West, and South) of the Alaska marmot's known distribution as well as in the entire sample (Rogers and Harpending 1992). Arlequin uses coalescent simulations under the infinite-sites model to test the significance of Tajima's D and Fu's F_S. DnaSP (Librado and Rozas 2009) was used to estimate the R_2 neutrality test (Ramos-Onsins and Rozas 2002) and to test the significance of R_2 using coalescent simulations under the infinite-sites model. In the absence of selection, negative values of Tajima's D and unimodal mismatch distributions are indicative of population expansion, whereas positive values of Tajima's D are suggestive of population contraction. Significantly low values of Fu's F_S and R_2 would reject the null hypothesis of population stability in support of population expansion. We used Harpending's raggedness index (rg; Harpending 1994) to test for a departure from the sudden expansion model for the mismatch distributions. Lower values of rg are expected under a population growth model. We defined regions as populations and compared

within-population molecular variance among the 3 regions and in a grouping of South and East populations separate from the West.

The phylogeographic structure of *M. broweri* was further explored with a haplotype network constructed using TCS 1.21 (Clement et al. 2000) and through a modified nested-clade phylogeographic analysis using the program GEODIS 2.0 (Posada et al. 2000). In a standard nested clade analysis the haplotype network is nested according to the published nesting rules of Templeton et al. (1987) and Templeton and Sing (1993) into a clade hierarchy of 1-step clades, 2-step clades, etc., until the entire network is contained in a single clade. Then the geographic association of clades is tested through the calculation of clade distances (D_c) and nested clade distances (D_n) and inferences about the biological meaning of those statistics are made using an inference key provided with the program. Our use of GEODIS is limited to the calculation of those statistics and tests of their statistical significance; we do not make use of the inference key to draw biological conclusions from these statistics.

Clade distance, D_c, is the mean geographic distance of all individuals in a clade, in kilometers, from that clade's geographic center (Templeton et al. 1995). When considering a single haplotype as a clade, D_c is the mean distance of each individual with that haplotype from the geographic center of that haplotype. A statistically significant value of D_c indicates that the geographic spread of individuals within a clade, or with a given haplotype, is smaller (significantly small D_c) or larger (significantly large D_c) than would be expected by chance. Nested clade distance, D_n, is the mean geographic distance of all individuals within a clade from the center of the next higher-level nested clade (Templeton et al. 1995). This is where our analysis differs from a traditional nested clade analysis. For our modified nested clade analysis, the haplotype network was not nested according to the published nesting rules. We calculated clade distances (D_c) as described by Templeton et al. (1995), but all nested clade distances (D_n) were calculated using the geographic center of the total cladogram as the next higher-level nesting clade. This design allowed each clade of interest to be tested for geographic association in relation to the entire cladogram, not just in relation to the next higher-level nesting clade. In this manner the geographic distribution of each clade is tested for statistically significant geographic structure in reference to the entire geographic distribution of *M. broweri*. Statistically significant values of D_n indicate that individuals within a clade are nearer to (significantly small D_n) or farther from (significantly large D_n) the geographic center of *M. broweri* than would be expected by chance. We used this method to provide statistical significance to the phylogeographic associations observed in the phylogenetic tree and haplotype network. Because we modified the nested design, we made no use of the inference key provided with GEODIS (Posada et al. 2000; Templeton et al. 1995). The null hypothesis of no geographic structure was tested by comparing observed clade distances with expected distances obtained from 10,000

TABLE 2.—Summary of molecular diversity and neutrality test statistics by region and for the total sample. Number of individuals (N), number of haplotypes (H), number of segregating sites (S), mean number of pairwise differences (k), percent nucleotide diversity (π), Tajima's D, Fu's F_S, R_2, and Harpending's raggedness index (rg). All of the test statistics fail to reject the null hypothesis. Letters in parentheses correspond to localities in Fig. 1.

Region	N	H	S	k	π (%)	D	F_S	R_2	rg
Eastern Brooks Range (K–T)	30	8	20	5.710	0.516	0.42	2.579	0.1406	0.0848
Western Brooks Range (A–G)	16	2	1	0.125	0.011	−1.16	−0.700	0.2421	0.5781
Kokrines Hills and Ray Mountains (H, J)	7	3	17	5.238	0.459	−1.36	3.656	0.2460	0.4354
Total sample	53	12	24	4.643	0.420	−0.27	0.352	0.0983	0.0948

random chi-squared permutations. To eliminate any bias resulting from the sampling of family groups, we removed all but 1 of the individuals that were collected from the same burrow. Of the samples from museum specimens we did not collect ourselves, we removed redundant individuals that were collected by the same collector on the same date from a single locality. This left a sample size of 43 for this analysis.

RESULTS

Among the 53 individual specimens from 19 localities we recovered 12 unique haplotypes. There were 24 polymorphic sites within the total 1,140 base pairs of the cytochrome *b* gene, consisting of 22 inferred transitions and 2 transversions. The mean number of pairwise differences for the entire sample was 4.64 and the average uncorrected pairwise distance (*p*-distance) between individuals was 0.42%, with a range of 0.0–1.4%. The Ewens–Watterson test for selective neutrality was nonsignificant ($P = 0.785$). Molecular diversity statistics for each region and the total sample are summarized in Table 2.

The optimal ML tree with branch lengths and nodal support values is shown in Fig. 2. Both MP and Bayesian inference produced the same optimal tree topology as the ML analysis. Four main haplotype clades were recovered, although only clade IV was well supported by both ML bootstrap values and Bayesian posterior probabilities. Clade IV contained individuals from 3 localities in the eastern and southern extremes of the Alaska marmot's distribution. Individuals from the South were also found in clades II and III, suggesting higher levels of diversity in the South. The 2 most common haplotypes were geographically segregated into West and East/South regions. A single individual from the East (Tolugak Lake) had a unique haplotype that grouped among the samples from the West.

Nucleotide diversity estimates (π) for each region (Table 2) also indicate more diversity in populations from the East and from the South than in populations from the West. A mismatch distribution plot could not reject the null hypothesis of recent population expansion in any of the 3 regional samples or the total sample. Tajima's D values are negative for the regional samples from the West and South and for the whole data set, but slightly positive for the East samples (Table 2). These results suggest a recent population expansion in the western Brooks Range and in the southern mountain ranges and perhaps a population contraction in the eastern Brooks Range, although none of the values is statistically significant. Fu's F_S and R_2 tests failed to reject the null hypothesis of population

stability, suggesting that a refugium for Alaska marmots may have existed in the western Brooks Range as well.

Results of our AMOVA show that 71% of variation is found within regions (East, West, and South) and that the East + South versus West division explained 31% of variation. The South versus East split accounts for −1.85% of variation (negative due to shared haplotypes between the 2 regions). The haplotype network estimation yielded a single most parsimonious network (Fig. 3) with a maximum of 16 mutational steps inferred between haplotypes. The network shows more clearly the short branch lengths found between clades I, II, and III. The network also illustrates the higher levels of diversity found in samples collected from the East (8 haplotypes from 10 localities) and in the South (3 haplotypes from 2 localities) than was found in samples from the West (2 haplotypes from 7 localities). Our modified nested clade analysis revealed significant geographic association of haplotypes and higher-level clades. The null hypothesis of no geographic association was rejected for clade III (Table 3) and the total cladogram ($P < 0.001$). The geographic distance statistics further support a pattern of population structure within *M. broweri*—clade distance (D_c) and nested clade distance (D_n) values were significant for haplotypes 1–4, 8, 9, and 11 and for clades I–IV (Table 3).

DISCUSSION

The results from our modified nested clade analysis provide a statistically significant rejection of the null hypothesis of no phylogeographic structure within *M. broweri*. Alaska marmot populations have diverged in isolation, presumably due to their restriction to sky island habitats, limited dispersal abilities, and the glacial history of central and northern Alaska. Overall levels of genetic diversity within *M. broweri* are low compared with other small mammals distributed within Alaska (Brunhoff et al. 2003; Lanier 2010; Weksler et al. 2010) and marmot populations of the western Brooks Range show less diversity than those of the eastern Brooks Range and southern ranges of the Ray Mountains and Kokrines Hills.

The phylogenetic tree we recovered further supports population structure across the range of *M. broweri*. Although our sample included only 7 individuals from the southernmost populations (Kokrines Hills and Ray Mountains), within those samples we found 3 different haplotypes recovered in 3 of the 4 major clades (Fig. 2). Measures of molecular diversity and
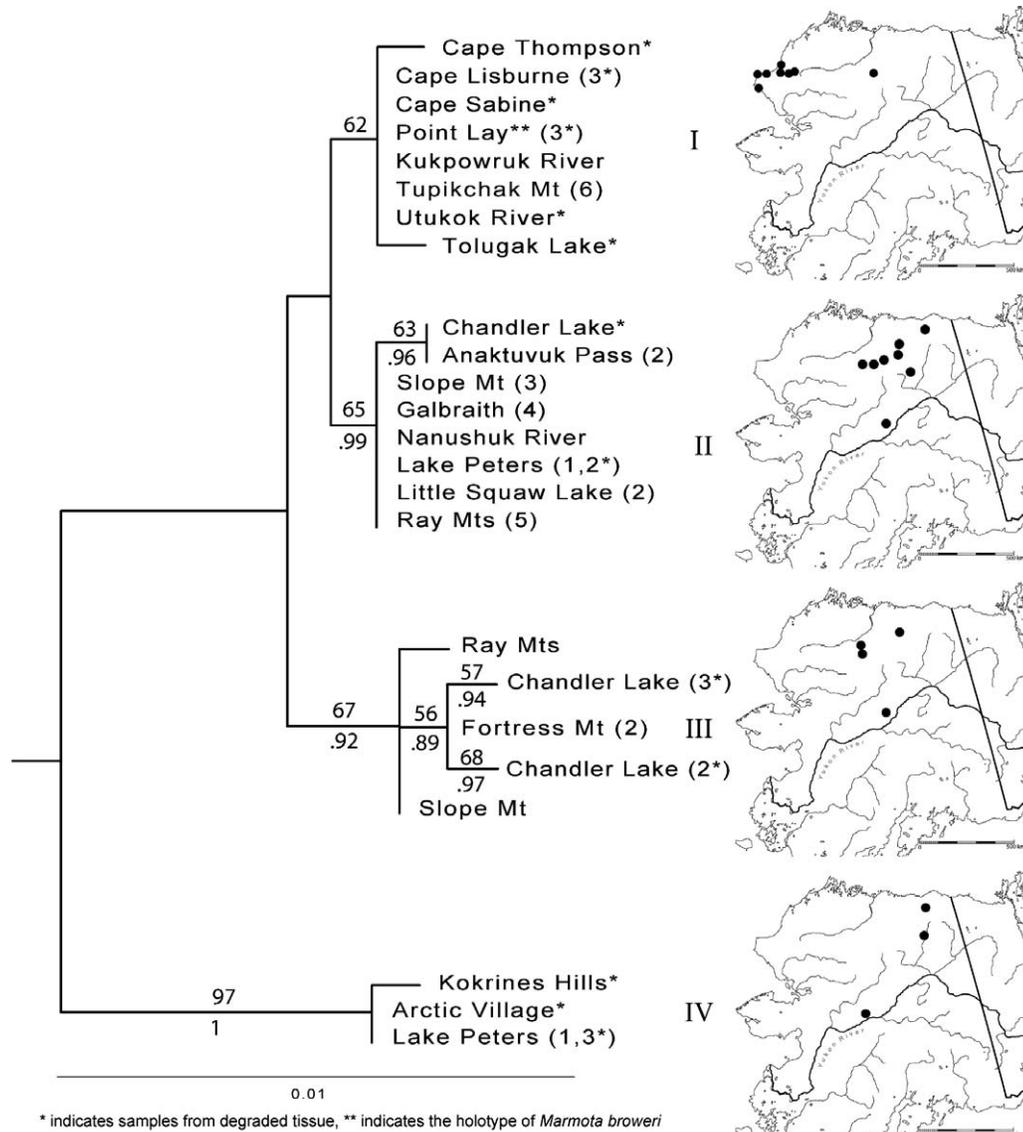
FIG. 2.—Maximum likelihood phylogram and clade distribution. Terminal labels correspond to the collecting locality of individuals. Numbers in parentheses indicate the number of samples with a haplotype. Bootstrap support values greater than 50 are shown above clade branches. Posterior probabilities greater than 0.50 are shown below clade branches. Clade numbers correspond to Table 4 and Fig. 3.

the AMOVA results also indicate higher levels of genetic variation in these southern and eastern Brooks Range populations than in populations from the western Brooks Range. The higher levels of diversity found in the southern and eastern regions of the Alaska marmot's distribution could be the result of marmots having persisted there longer than in the genetically less diverse western Brooks Range. Both the Kokrines Hills and the Ray Mountains were nearly ice-free during the glacial cycles of the Pleistocene and would have been available to Alaska marmots (Brigham-Grette 2001; Clark and Mix 2002; Kaufman and Manley 2004). During that time large portions of the Brooks Range were periodically covered by glaciers (Kaufman and Manley 2004), which would have limited the distribution and dispersal of marmots. Areas of the eastern Brooks Range may have remained unglaciated or contained nunataks (ice-free areas within a glacier) of sufficient size to allow marmots to persist

there (Kaufman and Manley 2004). These results are consistent with a recent range expansion into the western Brooks Range from the east and south as habitat and dispersal corridors became available after the Last Glacial Maximum.

The lack of genetic diversity in marmots of the western Brooks Range could also be the result of a population bottleneck within a refugium in that region. The statistical tests for a signature of a recent population expansion produced conflicting results. Although the Harpending's raggedness index failed to reject the null hypothesis of population expansion for the mismatch distributions of the western Brooks Range, Fu's F_S and R_2 did not reject a hypothesis of population stability. A negative value of Tajima's D in the western populations is suggestive of recent expansion; however, that value was not statistically significant. The western Brooks Range is described as having been nearly
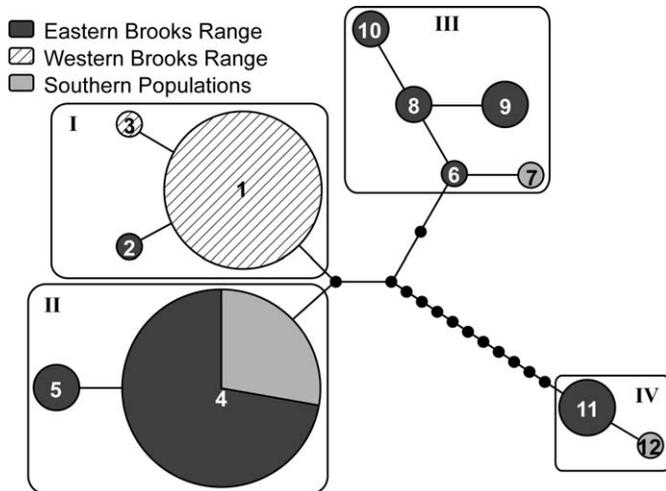
Fig. 3.—The most parsimonious haplotype network. Circles are proportional to the number of individuals with that haplotype. Haplotype and clade numbers correspond to Table 4 and Fig. 2.

Table 3.—Results of the nested clade analysis. Significant values are in bold ($P < 0.05$). Significantly small and significantly large values of D_c and D_n are indicated by *S and *L, respectively. Clade numbers correspond to those in Fig. 3.

Haplotypes			Higher-level clades			χ^2-statistic
Clade	D_c	D_n	Clade	D_c	D_n	probability
1	**66.93*S**	**358.13*L**				
2	0	**440.76*L**				
3	0	137.3	I	**139.91*S**	338.91	0.1266
4	**116.03*S**	260.05				
5	11.77	**119.22*S**	II	**112.99*S**	231.66	0.0535
6	0	242.91				
7	0	316.15				
8	0	**87.95*S**				
9	**0*S**	**84.26*S**				
10	0	84.26	III	**60.45*S**	**117.39*S**	**0.0035**
11	**56.35*S**	**391.66*L**				
12	0	357.61	IV	275.51	**379.19*L**	0.3371

ice-free, except for high mountain valleys, during the Late Wisconsinan glaciation (Kaufman and Manley 2004). With suitable habitat available, the persistence of Alaska marmots in the western Brooks Range through the late Pleistocene is plausible. Under this scenario, the lack of diversity in the western populations could be the result of a recent population bottleneck.

Neither hypothesis—a population expansion into the western Brooks Range or a refugium in the west followed by a bottleneck—can be rejected by our results from the current data set. Further sampling of the western Brooks Range and the addition of nuclear loci could clarify the geographic history of *M. broweri*.

No other mammal species shares the unique restricted distribution of the Alaska marmot in northern Alaska. Arctic ground squirrels (*Spermophilus parryii*) are ecologically similar but have a much more expansive distribution than Alaska marmots. The ranges of the 2 species overlap in the Brooks Range and the Ray Mountains. Eddingsaas et al. (2004) found significant geographic structure among populations of *S. parryii* across its distribution. However, most of the divergence in *S. parryii* was among southern populations. They found a single well-supported clade of arctic ground squirrels from the Brooks Range, presumably isolated from southern populations by the invasion of boreal forests as glaciers retreated, and concluded that the Brooks Range populations could be the result of a recent population expansion due to an increase in suitable habitat. A more exhaustive study of *S. parryii* supports a distinct arctic lineage, and suggests it is a result of isolation north of Brooks Range during glacial maxima (Galbreath et al. 2011). As Alaska marmots and Arctic ground squirrels exhibit similar ecological requirements, a shared pattern of persistence north of the Brooks Range or expansion into the Brooks Range after deglaciation may be likely. Alternatively, Eddingsaas et al. (2004) hypothesized that arctic ground squirrels may have persisted in a northern Canadian refugium and expanded westward into the Brooks Range following the expansion of suitable habitat. This Canadian arctic refugium has been proposed for the collared lemming and rock ptarmigan as well (Fedorov and Stenseth 2002; Holder 1999). The possible expansion of Alaska marmots across the Brooks Range could be the result of dispersal from northwestern Canada. However, Alaska marmots are not known to occur in Canada at present and arctic ground squirrels are absent from the northern Canadian archipelago. A refugium in that area is unlikely for either species. Eddingsaas et al. (2004) included only 7 *S. parryii* specimens from 3 localities within the distribution of *M. broweri*. An increase in sampling effort for each of these species would better elucidate their respective, and possibly shared, geographic histories.

The measures of nucleotide diversity found in *M. broweri* are lower than those in other Beringian small mammals for which data are available, including singing voles (*Microtus miurus*), tundra voles (*M. oeconomus*), and collared pikas (*Ochotona collaris*; Brunhoff et al. 2003; Lanier 2010; Weksler et al. 2010). A recent phylogeograhic study of the collared pika, another alpine specialist and Beringian denizen, found that *O. collaris* populations exhibit low levels of genetic variation and a shallow branching pattern similar to that in *M. broweri*, and that among pika species, those occurring at higher latitudes show less genetic variation (Lanier 2010). Without any comparable published studies on other marmot species, we cannot test whether a pattern of lower diversity at higher latitudes might exist among marmots. However, a microsatellite study of the lower-latitude alpine marmot (*M. marmota*) describes that species as exhibiting levels of genetic variation comparable with numerous other mammal species ($H_O = 0.63$, Goossens et al. 2001) and a similar study showed that yellow-bellied marmots (*M. flaviventris*) of the Great Basin region exhibit even higher microsatellite variation ($H_O = 0.73$, Floyd et al. 2005).

American pikas (*O. princeps*) are known to exhibit lower levels of diversity at the northern margins of their distribution in the Rocky Mountains, due to recent colonization of

previously glaciated areas, while maintaining higher levels of diversity at lower latitudes in the Great Basin region, where suitable habitat persisted below (in both latitude and elevation) the advancing ice (Galbreath et al. 2009). Within Beringia, Alaska marmots had no vast southern refugium to retreat to during glacial maxima (Kaufman and Manley 2004). They would have been limited to the available habitat at lower elevations and within the ice-free alpine areas of central Alaska and perhaps parts of the Brooks Range. A pattern of repeated extirpation, retreat to relatively few suitable alpine refugia, and subsequent recolonization during interglacials would serve to reduce overall genetic diversity and limit detectable population structure in *M. broweri* to only the most recent subdivisions (Hewitt 2004).

Current climate warming is affecting the distributions of species, with a general trend of northward (in the Northern Hemisphere) and upslope range shifts occurring within the last 100 years (Moritz et al. 2008; Parmesan and Yohe 2003). Alpine and arctic regions are more dramatically affected by climate change than other regions and the species adapted to those ecosystems are considered more vulnerable to population declines or extinction (Hansen et al. 2006; Krajick 2001, 2004; Parmesan 2006; Walther et al. 2002). Recent declines or extirpations of populations have been documented in pika and marmot species (Beever et al. 2003; Griffin et al. 2008; Morrison and Hik 2007; Wei-Dong and Smith 2005), including a decline in Alaska marmot abundance and elevational range at Lake Peters in the eastern Brooks Range, 50 years after an initial survey (Gunderson et al. 2009). The habitat of the Alaska marmot, specifically in the Brooks Range, is shrinking due to an increased abundance and upslope migration of shrubs and trees (Hinzman et al. 2005; Serreze et al. 2000; Sturm et al. 2001; Tape 2010), which will likely further restrict gene flow among isolated marmot populations and increase their vulnerability to extirpation. As inhabitants of the highest elevations in the northernmost mountains in North America, Alaska marmots will have to adapt to their changing environment if they are to survive. A recent increase in the population size of yellow-bellied marmots (*M. flaviventris*) from one locality in Colorado, linked to earlier emergence from hibernation and a longer growing season, illustrates the complexity of variables affected by recent climate change and some possible phenotypic and phenological consequences marmots may experience (Ozgul et al. 2010). The data to make any determination about status of Alaska marmot populations or how they will respond to their changing environment do not yet exist. Studies such as this one, and our previous work on *M. broweri* (Gunderson et al. 2009), provide a baseline measure of current distribution and genetic diversity from which species can be monitored, specifically for changes resulting from the rapidly changing climate.

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LITERATURE CITED

BEEVER, E. A., P. F. BRUSSARD, AND J. BERGER. 2003. Patterns of apparent extirpation among isolated populations of pikas (*Ochotona princeps*) in the Great Basin. Journal of Mammalogy 84:37–54.

BRIGHAM-GRETTE, J. 2001. New perspectives on Beringian Quaternary paleogeography, stratigraphy, and glacial history. Quaternary Science Reviews 20:15–24.

BRUNHOFF, C., K. E. GALBREATH, V. B. FEDOROV, J. A. COOK, AND M. JAAROLA. 2003. Holarctic phylogeography of the root vole (*Microtus oeconomus*): implications for late Quaternary biogeography of high latitudes. Molecular Ecology 12:957–968.

CARDINI, A., R. S. HOFFMANN, AND R. W. THORINGTON, JR. 2005. Morphological evolution in marmots (Rodentia, Sciuridae): size and shape of the dorsal and lateral surfaces of the cranium. Journal of Zoological Systematics and Evolutionary Research 4:258–268.

CLARK, P. U., AND A. C. MIX. 2002. Ice sheets and sea level of the last glacial maximum. Quaternary Science Reviews 21:1–7.

CLEMENT, M., D. POSADA, AND K. A. CRANDALL. 2000. TCS: a computer program to estimate gene genealogies. Molecular Ecology 9:1657–1660.

EDDINGSAAS, A. A., B. K. JACOBSEN, E. P. LESSA, AND J. A. COOK. 2004. Evolutionary history of the arctic ground squirrel (*Spermophilus parryii*) in Nearctic Beringia. Journal of Mammalogy 85:601–610.

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. B., AND N. C. STENSETH. 2002. Multiple glacial refugia in the North American Arctic: inference from phylogeography of the collared lemming (*Dicrostonyx groenlandicus*). Proceedings of the Royal Society of London B Biological Sciences 269:2071–2077.

FLOYD, C. H., D. H. VAN VUREN, AND B. MAY. 2005. Marmots on Great Basin mountaintops: using genetics to test a biogeographic paradigm. Ecology 86:2145–2153.

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

GALBREATH, K. E., AND J. A. COOK. 2004. Genetic consequences of Pleistocene glaciations for the tundra vole (*Microtus oeconomus*) in Beringia. Molecular Ecology 13:135–148.

GALBREATH, K. E., J. A. COOK, A. A. EDDINGSAAS, AND E. G. DECHAINE. 2011. Diversity and demography in Beringia: multilocus tests of paleodistribution models reveal the complex history of arctic ground squirrels. Evolution 65:1879–1896.

GALBREATH, K. E., D. J. HAFNER, AND K. R. ZAMUDIO. 2009. When cold is better: climate-driven elevation shifts yield complex patterns of diversification and demography in an alpine specialist (American pika, *Ochotona princeps*). Evolution 63:2848–2863.

GOOSSENS, B., L. CHIKHI, P. TABERLET, L. P. WAITS, AND D. ALLAINÉ. 2001. Microsatellite analysis of genetic variation among and within Alpine marmot populations in the French Alps. Molecular Ecology 10:41–52.

GRIFFIN, S. C., M. L. TAPER, R. HOFFMAN, AND L. S. MILLS. 2008. The case of the missing marmots: are metapopulation dynamics or range-wide declines responsible? Biological Conservation 141:1293–1309.

GUNDERSON, A. M., JACOBSEN B. K., AND L. E. OLSON. 2009. Revised distribution of the Alaska marmot, *Marmota broweri*, and confirmation of parapatry with hoary marmots. Journal of Mammalogy 90:859–869.

HALL, E. R., AND R. M. GILMORE. 1934. *Marmota caligata broweri*, a new marmot from northern Alaska. Canadian Field-Naturalist 48:57–59.

HANSEN, J., M. SATO, R. RUEDY, K. LO, D. W. LEA, AND M. MEDINA-ELIZADE. 2006. Global temperature change. Proceedings of the National Academy of Sciences 103:14288–14293.

HARPENDING, H. 1994. Signature of ancient population growth in a low resolution mitochondrial DNA mismatch distribution. Human Biology 66:591–600

HEWITT, G. 2004. Genetic consequences of climatic oscillations in the Quaternary. Philosophical Transactions of the Royal Society of London B 359:183–195.

HINZMAN, L. D., ET AL. 2005. Evidence and implications of recent climate change in northern Alaska and other arctic regions. Climatic Change 72:251–298.

HOFFMANN, R. S., J. W. KOEPPL, AND C. F. NADLER. 1979. The relationships of the amphiberingian marmots (Mammalia: Sciuridae). Occasional Papers of the Museum of Natural History, The University of Kansas 83:1–56.

HOFFMANN, R. S., AND C. F. NADLER. 1968. Chromosomes and systematics of some North American species of the genus *Marmota* (Rodentia: Sciuridae). Experientia 24:740–742.

HOLDER, K. R. 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.

HOPKINS, D. M. 1967. The Bering land bridge. Stanford University Press, Stanford, California.

HUELSENBECK, J. P., AND F. RONQUIST. 2001. MRBAYES: Bayesian inference of phylogeny. Bioinformatics 17:754–755.

HUNDERTMARK, K. J., G. F. SHIELDS, I. G. UDINA, R. T. BOWYER, A. A. DANILKIN, AND C. C. SCHWARTZ. 2002. Mitochondrial phylogeography of moose (*Alces alces*): late Pleistocene divergence and population expansion. Molecular Phylogenetics and Evolution 22:375–387.

INOUYE, D. W., B. BARR, K. ARMITAGE, AND B. D. INOUYE. 2000. Climate change is affecting altitudinal migrants and hibernating species. Proceedings of the National Academy of Sciences 97:1630–1633.

KAUFMAN, D. S., AND W. F. MANLEY. 2004. Pleistocene maximum and Late Wisconsinan glacier extents across Alaska, USA. Pp. 9–27 in Quaternary glaciations—extent and chronology, part II: North America. Developments in Quaternary science. Vol. 2B (J. Ehlers and P. L. Gibbard, eds.). Elsevier, Amsterdam, The Netherlands.

KRAJICK, K. 2001. Arctic life, on thin ice. Science 291:424.

KRAJICK, K. 2004. All downhill from here? Science 303:1600–1602.

LANIER, H. C. 2010. Phylogeography, ecogeographic variation, and evolutionary history of the collared pika (*Ochotona collaris*). Ph.D. dissertation, University of Alaska, Fairbanks.

LIBRADO, P., AND J. ROZAS. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451–1452.

MORITZ, C., J. L. PATTON, C. J. CONROY, J. L. PARRA, G. C. WHITE, AND S. R. BEISSINGER. 2008. Impact of a century of climate change on small-mammal communities in Yosemite National Park, USA. Science 322:261–264.

MORRISON, S. F., AND D. S. HIK. 2007. Demographic analysis of a declining pika *Ochotona collaris* population: linking survival to broad-scale climate patterns via spring snowmelt patterns. Journal of Animal Ecology 76:899–907.

OVERPECK, J. K., ET AL. 1997. Arctic environmental change of the last four centuries. Science 278:1251–1256.

OZGUL, A., ET AL. 2010. Coupled dynamics of body mass and population growth in response to environmental change. Nature 466:482–485.

PARMESAN, C. 2006. Ecological and evolutionary responses to recent climate change. Annual Review of Ecology, Evolution, and Systematics 37:637–669.

PARMESAN, C., AND G. YOHE. 2003. A globally coherent fingerprint of climate change impacts across natural systems. Nature 421:37–42.

POSADA, D., AND T. R. BUCKLEY. 2004. Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. Systematic Biology 53:793–808.

POSADA, D., AND K. A. CRANDALL. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14:817–818.

POSADA, D., K. A. CRANDALL, AND A. R. TEMPLETON. 2000. GEODIS: a program for the cladistic nested analysis of the geographical distribution of genetic haplotypes. Molecular Ecology 9:487–488.

RAMOS-ONSINS, S. E., AND J. ROZAS. 2002. Statistical properties of new neutrality tests against population growth. Molecular Biology and Evolution 19:2092–2100.

RAUSCH, R., AND V. RAUSCH. 1971. The somatic chromosomes of some North American marmots (Sciuridae), with remarks on the relationships of *Marmota broweri* Hall and Gilmore. Mammalia 35:85–101.

ROGERS, A. R., AND H. HARPENDING. 1992. Population growth makes waves in the distribution of pairwise genetic differences. Molecular Biology and Evolution 9:552–569.

SCHNEIDER, S., D. ROESSLI, AND L. EXCOFFIER 2000. Arlequin ver 2.000: a software for population genetics data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

SERREZE, M. C., ET AL. 2000. Observational evidence of recent change in the northern high-latitude environment. Climatic Change 46:159–207.

SIKES, R. S., W. L. GANNON, AND THE ANIMAL CARE AND USE COMMITTEE OF THE AMERICAN SOCIETY OF MAMMALOGISTS. 2011. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. Journal of Mammalogy 92:235–253.

STEPPAN, S. J., ET AL. 1999. Molecular phylogeny of the marmots (Rodentia: Sciuridae): tests of evolutionary and biogeographic hypotheses. Systematic Biology 48:715–734.

STURM, M., C. RACINE, AND K. TAPE 2001. Increasing shrub abundance in the arctic. Nature 411:546–547.

SWOFFORD, D. L. 2003. PAUP* Phylogenetic analysis using parsimony (*and other methods). Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts.

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

TAPE, K. D. 2010. The changing arctic landscape. University of Alaska Press, Fairbanks.

TEMPLETON, A. R., R. E. BOERWINKLE, AND C. F. SING. 1987. A cladistic analysis of phenotypic associations with haplotypes inferred from restriction endonuclease mapping. I. Basic theory and an analysis of alcohol dehydrogenase activity in *Drosophila*. Genetics 117:343–351.

TEMPLETON, A. R., E. ROUTMAN, AND C. A. PHILIPS. 1995. Separating population structure from population history: a cladistic analysis of the geographical distribution of mitochondrial DNA haplotypes in the tiger salamander, *Ambystoma tigrinum*. Genetics 140:767–782.

TEMPLETON, A. R., AND C. F. SING. 1993. A cladistic analysis of phenotypic association with haplotypes inferred from restriction endonuclease mapping. IV. Nested analyses with cladogram uncertainty and recombination. Genetics 134:659–669.

WALTARI, E., E. P. HOBERG, E. P. LESSA, AND J. A. COOK. 2007. Eastward ho: phylogeographical perspectives on colonization of hosts and parasites across the Beringian nexus. Journal of Biogeography 34:561–574.

WALTHER, G., ET AL. 2002. Ecological responses to recent climate change. Nature 416:389–395.

WATTERSON, G. A. 1978. The homozygosity test of neutrality. Genetics 88:405–417.

WEI-DONG, L., AND A. T. SMITH. 2005. Dramatic decline of the threatened Ili pika *Ochotona iliensis* (Lagomorpha: Ochotonidae) in Xinjiang, China. Oryx 39:30–34.

WEKSLER, M., H. C. LANIER, AND L. E. OLSON. 2010. Eastern Beringian biogeography: historical and spatial genetic structure of singing voles in Alaska. Journal of Biogeography 37:1414–1431.

APPENDIX I

Museum specimens included in this study. Site letters correspond to localities in Fig. 1. UAM = University of Alaska Museum, MVZ = Museum of Vertebrate Zoology, KU = University of Kansas Museum of Natural History, MSB = Museum of Southwestern Biology, USNM = National Museum of Natural History, Smithsonian. Frozen tissue = fresh frozen tissue, skin = dried study skin, dried tissue = dried residual tissue left on the skeleton.

Site	Catalogue number	Locality	Latitude (°N)	Longitude (°W)	Sample type
A	UAM 12613-5	Cape Lisburne	68.8827	166.2201	Skin
B	UAM 7014	Cape Thompson	68.1493	165.4700	Skin
C	MVZ 123895	Cape Sabine	68.9142	164.6325	Skin
D	MVZ 51654-5, 51675 (holotype)	Point Lay	69.7575	163.0511	Skin
E	UAM 87313	Kukpowruk River	68.9486	162.8909	Frozen tissue
F	UAM 87300-1, -4,-6, -10, -12	Tupikchak Mt.	68.8795	161.8271	Frozen tissue
G	UAM 13425	Utukok River	69.0662	161.1370	Skin
H	UAM 15044	Kokrines Hills	64.9494	154.8527	Skin
J	UAM 87303, -5, -7-9, 100000	Ray Mountains	65.7060	151.1098	Frozen tissue
K	UAM 78513, 79182	Fortress Mt.	68.5689	152.9395	Frozen tissue
L	KU 43227, MSB 137646-7, -9, 141158, USNM 305036	Chandler Lake	68.2000	152.7529	Dried tissue
M	USNM 583154-5	Anaktuvuk Pass	68.1431	151.7391	Frozen tissue
N	USNM 290275	Tolugak Lake	68.2818	151.4742	Skin
O	UAM 79245	Nanushuk River	68.2778	150.6743	Frozen tissue
P	UAM 85514, 86397, -9	Galbraith	68.5171	149.4552	Frozen tissue
	UAM 35015	Galbraith	68.4846	149.4253	Frozen tissue
Q	UAM 85224-6, 85760	Slope Mt.	68.7354	149.0397	Frozen tissue
R	UAM 87311, -4	Little Squaw Lake	67.5576	148.1174	Frozen tissue
S	MSB 136465	Arctic Village	68.1269	145.5411	Dried tissue
T	KU 50417-8	Lake Peters	69.3189	145.0080	Skin
	MSB 85688, 85707, 141153	Lake Peters	69.3189	145.0080	Dried tissue
	UAM 85847-8	Lake Peters	69.3189	145.0080	Frozen tissue