ORIGINAL ARTICLE

Deep barriers, shallow divergences: reduced phylogeographical structure in the collared pika (Mammalia: Lagomorpha: *Ochotona collaris*)

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ABSTRACT

Aim Pikas (*Ochotona* spp.) are alpine specialists that show considerable morphological and genetic variation structured along latitudinal and elevational gradients. Recent studies of North American and Asian pikas have uncovered phylogeographical partitioning among separate mountain ranges and drainages, driven by Quaternary climate fluctuations. We tested the prediction that collared pikas (*O. collaris*) exhibit lower genetic diversity and less structure than other species of pika due to lower long-term habitat stability.

Location Eastern Beringia (Alaska and north-western Canada).

Methods We examined phylogeographical variation in the collared pika (*O. collaris*) throughout much of its known range using the mitochondrial cytochrome *b* gene. Population divergence and genetic diversity were compared within and between mountain ranges. Genetic diversity was contrasted with current and Pleistocene habitat fragmentation inferred from ecological niche models.

Results Low but significant differentiation was evident between most populations of *O. collaris*, with little haplotype sharing among populations and a single phylogeographical break separating a genetically distinct haplogroup in the south-eastern part of its range. Variation was mostly structured within populations (as opposed to between populations or mountain ranges). When within-species nucleotide diversity in *O. collaris*, *O. princeps* and *O. curzoniae* was compared, genetic diversity was found to decline with increasing latitude.

Main conclusions Populations of *O. collaris* are less geographically structured and exhibit less variability than populations of either *O. princeps* or *O. curzoniae*. This pattern is better explained by the amount of available Pleistocene habitat predicted for each species than by current predicted habitat or population discontinuity. Collared pikas are currently considered to be a common species at low risk of extinction, but their habitat specificity, population subdivision, and low genetic variation may have consequences for their continued viability in a warming world.

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INTRODUCTION

The latitudinal gradient in diversity is one of the oldest recognized patterns in biogeography (Turner & Hawkins, 2004), with more species, subspecies and genetic diversity found at lower latitudes (Wiens & Donoghue, 2004). Much of this pattern has been attributed to niche conservatism, the length of regional habitat occupancy, and the extent of habitable area over time (Martínez-Meyer *et al.*, 2004; Wiens & Donoghue, 2004). Large-scale Quaternary phenomena in northern regions, such as ice sheets covering much of northern North America during Pleistocene glacial cycles, have also

played a formative role in structuring diversity (Lessa *et al.*, 2003; Hewitt, 2004). Populations in areas glaciated during the Last Glacial Maximum (LGM) generally exhibit lower genetic diversity than those occupying unglaciated areas, especially those with a long history of occupation (Hewitt, 2000). However, many cold-adapted and alpine-adapted species exhibit inverse trends to their temperate relatives, with relatively greater genetic and species diversity at higher latitudes (Fedorov & Stenseth, 2002; Dalén *et al.*, 2004; Melo-Ferreira *et al.*, 2007; Shafer *et al.*, 2010).

Elevation also plays an important role in structuring diversity in montane species. Elevational gradients in temperature and area (both of which decrease at higher elevations; Körner, 2007) can isolate alpine populations and species into separate mountain regions, with the degree of isolation varying latitudinally. Abiotic barriers to dispersal between lower-latitude mountains are thought to be greater than those between higher-latitude mountains (Janzen, 1967), because the range of temperatures between habitable patches is much greater in tropical regions than in temperate zones. Thus, evolutionary processes affected by these barriers should have relatively greater impacts on montane biodiversity at lower latitudes. Biotic connections between mountain ranges have been further affected by Quaternary climate change. Montane glaciations shifted alpine habitat and climate to lower elevations during glacial maxima, allowing contact between previously disjunct interglacial populations (Knowles, 2000; Galbreath *et al.*, 2009). Connections between disjunct mountain ranges may be stronger at higher latitudes, where Quaternary glaciations were more extensive and elevational zonation is less extreme (Pielou, 1991; Körner, 2007).

Pikas (*Ochotona* spp.) are alpine specialists in which geographical variation is often structured along latitudinal and elevational gradients (Galbreath *et al.*, 2009). These small-bodied lagomorphs (Order Lagomorpha; rabbits, hares and pikas) are territorial, non-hibernating herbivores notable for their high intraspecific (Corbet, 1978) and low interspecific (Hoffmann & Smith, 2005; Smith, 2008) variation. They are thought to have originated and diversified in Asia (Erbajeva, 1994), and only two species of *Ochotona* live in North America: the well-studied American pika – *Ochotona princeps* (Richardson, 1828) – and the poorly known collared pika – *Ochotona collaris* (Nelson, 1893) (see Fig. 1). About half of the Asian pikas and both North American species are categorized as 'rock-dwelling', an ecotypic designation reflecting habitat preferences as well as a suite of life-history characteristics that separate them from meadow-dwelling pikas (see Smith, 2008). Rock-dwelling pikas maintain territories in rocky habitat, which provides protection from predators and affords important thermal stability (Millar & Westfall, 2010). Sensitivity to high temperatures is currently thought to restrict *O. princeps* to discontinuous sky islands in the Intermountain West (MacArthur & Wang, 1973; Smith, 1974; Beever *et al.*, 2003). During the Pleistocene, however, *O. princeps* was more widespread in North America (Kurtén & Anderson, 1980; Mead, 1987). Phylogeographical and
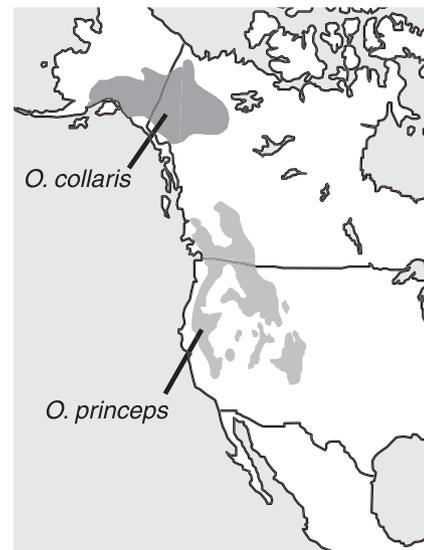
Figure 1 Approximate distributions of the two extant species of North American pika (*Ochotona collaris* and *O. princeps*) based on the literature (MacDonald & Jones, 1987; Smith & Weston, 1990; Hafner & Smith, 2010) and verified against localities from specimen records (Appendix S1).

ecological studies have revealed recent shifts to higher elevations and latitudes in this species (Galbreath *et al.*, 2009). Five major phylogroups occur in *O. princeps*, exhibiting differentiation influenced by LGM landscape features and broadly structured into separate mountain regions (Hafner & Sullivan, 1995; Galbreath *et al.*, 2009, 2010; Hafner & Smith, 2010).

Much less is known about the more northerly of the two North American species, the collared pika (*O. collaris*). In E. R. Hall's (1981) *Mammals of North America*, 36 distinct subspecies of *O. princeps* and several major biogeographical discontinuities were recognized. Hall's treatment of *O. collaris* was based on only 14 museum specimens and implied a geographically continuous distribution. In that work, *O. princeps* was represented by nearly six times as many specimens as *O. collaris* (2408 vs. 403, respectively; H.C.L., unpublished data). This lack of knowledge is not a trifling matter. Historical data for *O. princeps* populations have been used to track and model climate-mediated extirpation (Beever *et al.*, 2003, 2010), whereas such precise locality information is unavailable for nearly all *O. collaris* specimens collected during the 19th and 20th centuries. However, the disappearance of historically documented populations at the southern range margins has been noted (Swarth, 1936). Distinguishing climate-mediated extirpation from metapopulation dynamics (Franken, 2002; Morrison & Hik, 2008) may be important for understanding the effects of climate change on *O. collaris*.

More recently, collared pikas have been the subject of several ecological and behavioural studies in a limited portion of their range (MacDonald & Jones, 1987; Franken & Hik, 2004; Smith *et al.*, 2004; Morrison & Hik, 2007, 2008; Trefry & Hik, 2009a). These studies suggest that *O. collaris* is highly

philopatric but is subjected to repeated extinction and recolonization events between habitat patches within a site (Franken, 2002; Franken & Hik, 2004). Little to no morphological differentiation has been described in *O. collaris*, but geographical variation in call structure has been described (Trefry & Hik, 2009b). Fossil data indicate that *O. collaris* was present in Pleistocene Beringia, the ice-free corridor connecting North America and Asia (Guthrie, 1973; Weston, 1981). Guthrie (1973) hypothesized the allopatric divergence of *O. collaris* from a common ancestor from the American pika in separate LGM refugia. While the Nearctic species may have diverged in allopatry, recent studies suggest that their divergence pre-dated the Pleistocene (Lanier & Olson, 2009; Galbreath et al., 2010).

We have three objectives for this study: (1) to characterize the phylogeographical structure of *O. collaris* in order to test whether the lack of infraspecific taxonomic diversity actually reflects a lack of geographical structure; (2) to compare the observed diversity and differentiation within *O. collaris* to phylogeographical patterns recently inferred in other species of pika (Ci et al., 2009; Galbreath et al., 2009); and (3) to test whether habitat availability (contemporary and historical) underlies putative differences in phylogeographical structure and genetic diversity.

MATERIALS AND METHODS

Sampling strategy and study area

Field collecting was conducted from 2005 to 2008, with samples of heart, kidney or muscle tissue retained in buffer or subsampled directly into cell lysis solution (PureGene; Gentra Systems, Valencia, CA) in the field. Additional tissues were subsampled from specimens archived in the University of Alaska Museum Mammal Collection (http://arctos.database.museum/). A map showing the collection localities of all specimens included in this study is provided in Fig. 2, with additional information in Table 1 and Appendix S1 in the Supporting Information.

Logistical constraints are partly responsible for the lack of research on collared pikas, because many of the regions they inhabit are inaccessible by road. This is exacerbated in north-western Canada, where few museum specimens of mammals have been collected since the advent of modern molecular techniques (meaning that most specimens are not associated with fresh tissues). In order to increase the geographical range of our sampling, we included samples from historical museum specimens (defined here as specimens that were not originally preserved for the purpose of consumptive DNA extraction), which are being used increasingly in phylogeographical and phylogenetic studies (Roberts et al., 2011). While not truly ancient, these sources of degraded DNA are vulnerable to many of the same risks as ancient DNA (Olson & Hassanin, 2003). Samples from the 27 historically collected museum specimens included in this study ranged from 30 to 120 years old.

Laboratory methods

We amplified 1140 bp of the mitochondrial cytochrome *b* (cyt *b*) gene from 148 modern (vouchered fresh tissue) samples using the primers CB-HLF1 and CB-HLR1 (Appendix S1) and sequenced the resulting product using a combination of those primers and CB-HLiF2 and CB-HLiR2 (Lanier & Olson, 2009). Despite its limitations as a uniparentally inherited single-linkage marker, mitochondrial DNA (mtDNA) is valuable for investigating phylogeographical and demographic structure, and has the additional advantage of being more tractable when working with degraded material (thereby allowing for larger sample sizes). We recognize that it provides a single estimate of the phylogeographical history that may differ from those obtained from other unlinked loci due to lineage sorting or other biological processes. DNA extraction and amplification followed Lanier & Olson (2009).

For the historical specimens, samples of dried soft tissue (*c.* 0.5–5 mm^3) were removed from skulls or skeletons and washed in 100% ethanol at room temperature overnight, 70% ethanol for 3 h at 55 °C, sterile distilled water (sdH$_2$O) at room temperature for 3 h, and sdH$_2$0 overnight at room temperature. Larger samples were extracted using the PureGene Kit (Gentra Systems, Minneapolis, MN) following the manufacturer's Animal Tissue Protocol (omitting the addition of RNAse). Additional proteinase K was added every 24 h until tissues appeared to be fully digested. The extracted DNA was resuspended in 50 μL DNA Hydration Solution and diluted 1:10 for polymerase chain reaction (PCR). For smaller samples (< 1 mm^3), the QIAamp DNA Micro Kit (Qiagen, Valencia, CA) was used following the manufacturer's Tissue Sample Protocol. The extracted DNA was resuspended in buffer and diluted 1:10 for PCR.

Specialized protocols (Appendix S1) were followed to minimize the possibility of contamination of DNA extracted from degraded museum samples. All extractions and PCR set-ups were conducted in the University of Alaska Museum's Ancient DNA Lab inside a permanently PCR-free building. The complete cyt *b* gene was reconstructed from degraded material by amplifying nine overlapping PCR products to yield fragments of 215–272 bp with 2–3× coverage over most of the gene (Appendix S2). Amplifications were conducted in 15-μL reaction volumes using 7.5 μL GoTaq Green Mastermix (Promega, Madison, WI), 1.2 μL diluted template, 3.9 μL ultrapure water, and 1.2 μL of each primer (see Appendix S1). Amplification followed Lanier & Olson (2009), with 44 cycles of denaturing, annealing and extension, with annealing temperatures in the range 48–55 °C. Each PCR was performed along with extraction negatives, PCR negatives, and a positive historical DNA control. This approach was generally successful regardless of sample age; a linear regression indicated no relationship between year of collection and PCR success for historical material ($P = 0.1711$).
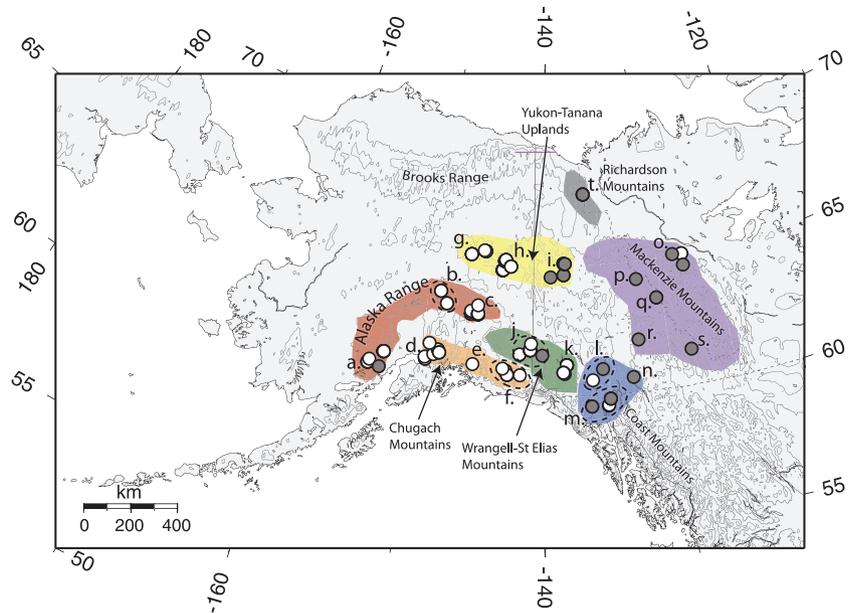
Figure 2 Localities of collared pika (*Ochotona collaris*) populations in this study (detailed in Table 1). The major mountain ranges and overall topography of the region is shown behind samples. The majority of the Canadian localities are represented by historical DNA samples from museum specimens (grey circles) whereas fresh material (white circles) was available for most Alaskan localities.

Table 1 Summary of collared pika (*Ochotona collaris*) samples used in this study. Mountain region, population identifier (a–t; Fig. 2) and name, total number of individuals (n_{tot}), and number of those samples from historical DNA sources (n_a). All samples are from Alaska or north-western Canada.

Mountain region	ID	Population	n_{tot}	n_a
Alaska Range	a	Southwest Alaska Range	17	1
	b	Denali	5	
	c	Central Alaska Range	23	
Chugach Mountains	d	Anchorage	10	
	e	Thompson Pass	1	
	f	Eastern Chugach	13	
Yukon-Tanana Uplands	g	White Mountains	12	
	h	Yukon-Charley Rivers NPP	38	
	i	Ogilvie Mountains	7	7
Wrangell-St Elias	j	Wrangell Mountains	10	1
	k	Kluane	14	
Coast Mountains	l	Whitehorse	2	1
	m	British Columbia	7	5
	n	Canol Road	1	1
Mackenzie Mountains	o	Northwest Territories	4	2
	p	Bonnet Plume Lake	1	1
	q	Keele Lake	2	2
	r	Ross River	1	1
	s	Little Hyland River	1	1
Richardson Mountains	t	Horn Lake	4	4

All PCR products were purified as described previously (Lanier & Olson, 2009). Sequencing products were purified with Sephadex G-50 (Princeton Separation, Freehold, NJ) fine clean up. Sequencing was conducted on an ABI 3100 Genetic Analyzer (Applied Biosystems, Carlsbad, CA) in the Institute of Arctic Biology's Core Facility for Nucleic Acid Analysis at the University of Alaska Fairbanks. Sequences were visually verified, edited and aligned in Sequencher 4.6 (Gene Codes Corp., Ann Arbor, MI), with additional *post-hoc* sequence verification (see Appendix S1). All sequences have been deposited in GenBank (accessions EU549736–EU549743 and JQ624421–JQ624583).

Phylogenetic analyses

Tree-building methodologies have a long history in phylogeographical studies and can reveal information about the genetic distances between haplotypes and the statistical support for those relationships. The Akaike information criterion (AIC) was used in jModeltest 0.1.1 (Posada, 2008) to select the best-fit model of nucleotide substitution. Phylogenetic trees were constructed in MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003); analyses were allowed to proceed for 10 million generations, sampling every 1000 generations. The first 200 trees were discarded as burn-in using a plot of log likelihood ($-\ln L$) against generation as a guide.

We inferred intraspecific relationships in our data set by constructing statistical parsimony haplotype networks in tcs 1.21 (Clement *et al.*, 2000). These methods can be particularly useful for revealing geographical structure when phylogenetic trees are poorly resolved and when ancestral haplotypes (sequences identical to those that gave rise to daughter sequences, both of which can be found in a contemporaneous sample) are present in the data set (Posada & Crandall, 2001).

Population assignment

Populations were assigned based on minimum geographical proximity between sampling localities and maximum distance between clusters of samples (Table 1, Fig. 2). Seven isolated localities represented by fewer than four individuals were used only in the regional comparisons. Based on levels

of population differentiation from other phylogeographical studies of Alaskan mammals using mtDNA (e.g. Fedorov *et al.*, 2003; Weksler *et al.*, 2010), further population subdivision is unlikely to present an unrecognized bias in our analyses.

Population genetic analyses

To examine the geographical distribution of genetic diversity, we calculated a series of standard population genetic summary statistics in DNASP 5 (Librado & Rozas, 2009). These statistics allow comparisons of genetic diversity within *O. collaris*, as well as with other studies, although they distil variation down to several metrics disregarding information on the genealogy of alleles. We tested for evidence of demographic fluctuations such as post-Pleistocene expansion (Hewitt, 2004) and/or recent population decline (similar to those that have been described for *O. princeps*; Galbreath *et al.*, 2010). We used R_2 (Ramos-Onsins & Rozas, 2002), Fu's (1997) F_S, and Fay & Wu's (2000) H to assess historical demographic change. F_S and R_2 have the most power to detect population growth (Ramos-Onsins & Rozas, 2002). Fay & Wu's (2000) H contrasts low-frequency and intermediate-frequency alleles, making it sensitive to population decline and population subdivision (Zeng *et al.*, 2006). Negative values of H and F_S and small positive values of R_2 can be indicative of sudden population expansion, whereas positive values of F_S and H indicate population contraction (Zeng *et al.*, 2006). Significance was calculated using 10,000 coalescent simulations in DNASP. To contrast pairwise genetic differences with those expected under a null model of sudden demographic expansion (Harpending *et al.*, 1998), mismatch distributions were calculated in DNASP for each mountain region and for the entire species. To look at subdivision among populations and mountain ranges, we used an analysis of molecular variance (AMOVA; Excoffier *et al.*, 1992) with population pairwise Φ_{ST} statistics calculated in ARLEQUIN 3.1 (Excoffier *et al.*, 2005). Significance was tested with 1000 permutations in ARLEQUIN.

Ecological niche modelling

We constructed species distribution models (SDMs) using the maximum entropy algorithm implemented in MAXENT 3.3 (Phillips *et al.*, 2006) to test the predicted relationship between habitat availability and genetic diversity in *O. collaris*, the American pika (*O. princeps*), and the plateau pika – *Ochotona curzoniae* (Hodgson, 1857). This allowed us to examine some underlying factors (latitude, current predicted habitat, predicted LGM habitat) that may contribute to diversity in collared pikas, although it is important to note that while SDMs are useful tools, predicted habitat does not necessarily reflect actual occurrence. Locality data for collared pikas consisted of 160 unique georeferenced localities (see Appendix S2). Locality data for American pikas and the meadow-dwelling plateau pikas were obtained from the literature (Ci *et al.*, 2009; Galbreath *et al.*, 2009) and supplemented with additional georeferenced locality data from the Mammal Networked Information System (MaNIS; included in Appendix S2). Models were built for each species using randomly subdivided training and testing data sets (90% and 10%, respectively). Distributions were modelled for each species under current climatic conditions using the 19 bioclimatic variables in the WorldClim data set (Hijmans *et al.*, 2005) and projected onto climate layers approximating the LGM (21,000 years ago; hereafter 21 ka) created by the Paleoclimate Modeling Intercomparison Project Phase II (PMIP2; Braconnot *et al.*, 2007). Model robustness was cross-validated with 50 separate runs per species with points randomly allocated to either the training set or the testing set. The relationship between habitat availability and genetic diversity was examined by calculating the correlation across species between current nucleotide diversity (π) on a population level and habitat patch size for each species. Because of the difficulties associated with confidently assigning a current population to an ancestral LGM locality, correlation coefficients were calculated using a randomized association between population and habitat patch but keeping species identity constant. Randomizations were performed 10,000 times each, and the mode of each correlation coefficient was used to compare the results. These results were contrasted with the relationship between latitude and nucleotide diversity, which was calculated using Spearman's rank correlation coefficient test for each species individually and for all populations combined.

RESULTS

We recovered moderate diversity in *O. collaris*, with 79 unique haplotypes from 173 individuals (haplotype diversity = 0.983; Table 2). Of those samples, 27 were from historical specimens. In the whole data set, there were 115 polymorphic sites, of which 47 were singleton mutations. Historical data matched the overall profile for the rest of the data set, with 21 haplotypes and 48 variable sites. Per-site nucleotide diversity (π) was 0.0070 for the entire data set and 0.0067 for the historical samples. The general time-reversible model (GTR) with a proportion of invariant sites (I) and a gamma rate parameter (Γ) was selected as the best-fit model of evolution.

A strongly supported, geographically circumscribed clade was recovered in the southern region of the range of *O. collaris*, in the Wrangell-St Elias range and Coast Mountains (Figs 2 & 3; populations k–n as well as individuals from populations j and f). Genealogical relationships among the other samples were unresolved (hereafter referred to as the 'Beringian haplotypes', to distinguish them from the Wrangell-St. Elias haplogroup; Fig. 4). Phylogeographical-level results lacked broad-scale resolution for much of the range of the collared pika. Most samples formed a basal polytomy (Fig. 3), a pattern supported by the general lack of strong structure in the haplotype network (Fig. 4).

Table 2 Population genetic summary statistics for collared pikas (*Ochotona collaris*) in north-western North America: n = number of samples, S = number of segregating sites, h = number of haplotypes, H_d = haplotype diversity, π = per-site nuclear diversity, k = mean number of pairwise nuclear differences, Ramos-Onsins and Rozas's R_2, Fu's F_S, and Fay and Wu's H. Diversity statistics are not shown separately for populations with < 4 samples (l, n, p–s), but those samples were included in regional comparisons. Test statistics significant at the $P < 0.05$ level (tested with 10,000 coalescent simulations) are shown in bold.

Population	n	S	h	H_d	π	k	R_2	F_S	H
Alaska Range	45	29	16	0.91	0.0046	5.170	0.085	−2.097	1.666
a. Southwest Alaska Range	17	6	5	0.772	0.002	1.779	0.144	0.114	−1.191
b. Denali	5	11	5	1	0.0052	5.676	0.203	−1.113	0.4
c. Central Alaska Range	23	13	6	0.771	0.003	3.905	0.148	2.131	1.265
Chugach Mountains	24	24	9	0.859	0.0065	7.225	0.146	1.889	3.318
d. Anchorage	10	13	6	0.889	0.005	5.333	0.188	0.541	1.778
f. Eastern Chugach	13	13	3	0.653	0.006	6.692	0.257	**7.499**	−0.115
Yukon-Tanana Uplands	57	32	22	0.936	0.0054	6.053	0.091	−4.382	2.847
g. White Mountains	12	2	2	0.53	0.001	1.061	0.265	2.535	−0.424
h. Yukon-Charley	38	28	15	0.902	0.005	5.677	0.096	−1.609	−4
i. Ogilvie Mountains	7	6	5	0.905	0.0028	3.143	0.245	−0.612	0.952
Wrangell-St Elias	24	31	15	0.942	0.0067	7.471	0.111	−2.656	−1.355
j. Wrangell Mountains	10	24	6	0.778	0.006	6.778	0.127	1.121	2.222
k. Kluane	14	10	9	0.934	0.003	3.474	0.156	−2.981	1.013
Coast Mountains	10	12	7	0.933	0.0037	4.156	0.175	−1.19	1.422
m. British Columbia	7	12	5	0.905	0.0039	4.381	0.18	0.039	2.429
Mackenzie Mountains	9	27	9	1	0.0058	6.500	**0.073**	−4.01	**5.5**
o. Northwest Territories	4	17	4	1	0.009	8.833	**0.12**	0.188	**5**
Richardson Mountains									
t. Horn Lake	4	8	3	0.833	0.0037	4.167	0.227	1.225	−0.333
All *O. collaris*	173	115	79	0.983	0.007	8.374	**0.035**	**−58.54**	

Most populations were significantly differentiated from one another based on Φ_{ST} (Appendix S3). Populations from the Northwest Territories (population o) were not significantly different from those in the Ogilvie Mountains (population i). Haplotype sharing between populations was also low: only four haplotypes were shared between populations and two of these were also shared between mountain regions. The AMOVA (Table 3) further supports this pattern, with the majority of variation partitioned within (48.78%) or among (32.69%) populations within a range, and relatively little variation partitioned among mountain ranges (18.53%).

The mismatch distribution, R_2 and Fu's F_S all indicate past population expansion in *O. collaris* (Table 2). This contrasts with our findings of demographic stability within most groups at the mountain-range and population level. A statistically significant R_2 was only found in the Mackenzie Mountains (and within them the Northwest Territories; population o). Values of Fu's F_S indicated demographic decline in the eastern Chugach (population f), but neither of the other summary statistics indicated population decline in this region.

DISCUSSION

Phylogeography of *O. collaris*

One major phylogeographical discontinuity is evident within *O. collaris*. A well supported (> 0.90 posterior probability) clade is found in the Wrangell-St Elias range and Coast Mountains. This haplogroup extends from north-western British Columbia into south-western Yukon Territory and is parapatric with the Beringian haplotypes in south-central Alaska (in southern Wrangell-St Elias National Park and Preserve). Differentiation based on Φ_{ST} between the two major haplogroups (Fig. 4) ranged from 0.57 to 0.81 and exceeded most of the within-group values. Subdivision may have resulted from the isolation of Pleistocene populations in ice-free cryptic refugia, separate from those in central Beringia (as described in Pielou, 1991; Loehr et al., 2006; Stewart et al., 2010). The discovery of regional cryptic endemism supports this hypothesis. Phylogeographical studies of singing voles (*Microtus miurus*; Weksler et al., 2010) have revealed a highly divergent mitochondrial lineage in the Wrangell Mountains. In addition, a divergent deer mouse lineage (*Peromyscus* sp.; Lucid & Cook, 2007) has been reported from this region. Neither study incorporated dense geographical sampling, so the geographical extents of these lineages are unknown.

In collared pikas, the nucleotide divergence (0.6%) is less than that described at the same locus for singing voles or deer mice (4.2% and 4.32%, respectively), which might be a reflection of a slower mutation rate in collared pikas. Alternatively, this pattern might result from secondary contact between haplogroups isolated in separate Beringian and sub-Laurentide refugia during the LGM. For several species, the Wrangell-St Elias region represents a contact zone between divergent (but not locally endemic) lineages. Widespread phylogroups in brown lemmings (*Lemmus trimucronatus*;
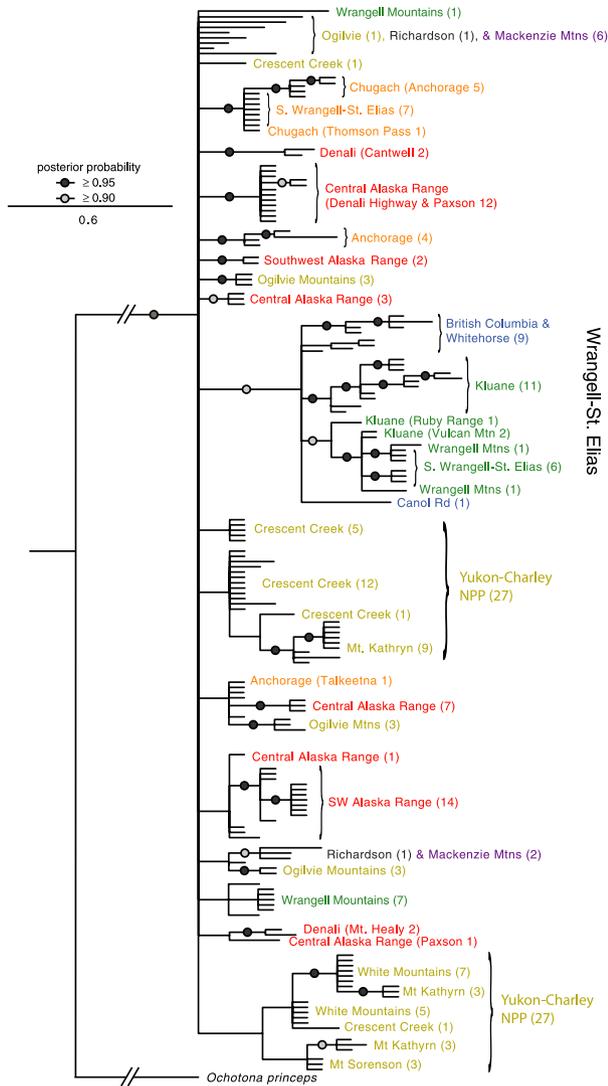
Figure 3 Majority-rule consensus phylogenetic tree and locality information for all *Ochotona collaris* (Alaska and north-western Canada) samples, with the branch leading to the outgroup *O. princeps* shortened 6.5×. Nodes with posterior probability ≥ 0.90 are indicated with grey circles; the number of samples is shown in parentheses.

Fedorov *et al.*, 2003) and ermine (*Mustela erminea*; Fleming & Cook, 2002) come into contact in Wrangell-St Elias, although different refugial histories have been proposed for each species. The presence of contact zones for multiple species may be indicative of a shared phylogeographical history of vicariance and isolation. Because Beringian and southern Canadian haplogroups both occur in this region (inflating the estimate of θ_π), the results of Fu's F_S for this region may reflect population subdivision instead of demographic decline.

Strong population differentiation is expected in pikas given their extreme philopatry (Peacock & Ray, 2001; Morrison & Hik, 2008). Low haplotype sharing in *O. collaris* indicates a high degree of geographical partitioning within

populations. Low nucleotide diversity and high haplotype diversity provide evidence of a strong population bottleneck followed by population growth. The large proportion of silent substitutions is consistent with this pattern (Grant & Bowen, 1998). This pattern is also likely to translate to low resolution of phylogeographical relationships as was observed on all but local levels (Figs 3 & 4). While not unexpected for this scale of divergence (e.g. Weksler *et al.*, 2010), this has important implications when co-distributed or closely related species are compared (i.e. actual subdivision in collared pikas may be difficult to detect because too few mutations have occurred). Strong past population growth followed by population subdivision can result in a pattern of localized differentiation with little broad-scale geographical partitioning (e.g. *Lepus timidus*; Melo-Ferreira *et al.*, 2007). Population expansion in collared pikas may have been facilitated by widespread glaciation in southern Alaska and north-western Canada during the LGM. Collared pikas are commonly found in early successional habitats (rocky substrates with herbaceous plants and reduced woody vegetation) along glacial margins. Dispersal across glaciers may also be important, although less is known about dispersal success. Whereas individuals dwelling on talus slopes will only venture a few metres into meadows (Morrison *et al.*, 2004), collared pikas living on nunataks have been observed venturing several kilometres onto glaciers (Krajick, 1998). Glacially mediated dispersal might be reflected in the distribution of lineages in Wrangell-St Elias National Park and Preserve (NPP). The Wrangell-St Elias clade was better represented in the eastern Chugach region (population f) of southern Wrangell-St Elias NPP than in the northern regions of the park (in the Wrangell Mountains; population j). The ice fields of Wrangell-St Elias NPP are known to harbour nunatak populations of pika (Murray & Murray, 1969; Cook & MacDonald, 2003), which may contribute to gene flow into this region. Future studies of pika gene flow across ice fields may be interesting from both biogeographical and conservation perspectives.

Comparison with other pikas

Although the number of described subspecies in American pikas (see above) may be upwardly biased, within-species divergence in *O. princeps* differed significantly from that in collared pikas (*O. collaris*). Historical factors (habitat availability and time since population expansion) are likely to have played a strong role in shaping the geographical distribution of diversity (or lack thereof) in collared pikas. *Ochotona princeps* is composed of five well-supported mtDNA phylogroups, with uncorrected sequence divergence ranging from 1.6% to 2.7% (Galbreath *et al.*, 2009). Only two distinct phylogeographical groups were evident in *O. collaris*, with divergence of 0.6%, less than half the distance separating most phylogroups in *O. princeps*. Greater divergences have also been described in the plateau pika (*O. curzoniae*; Ci *et al.*, 2009) from the Tibetan Plateau. Whereas the majority of genetic variance was explained by within-
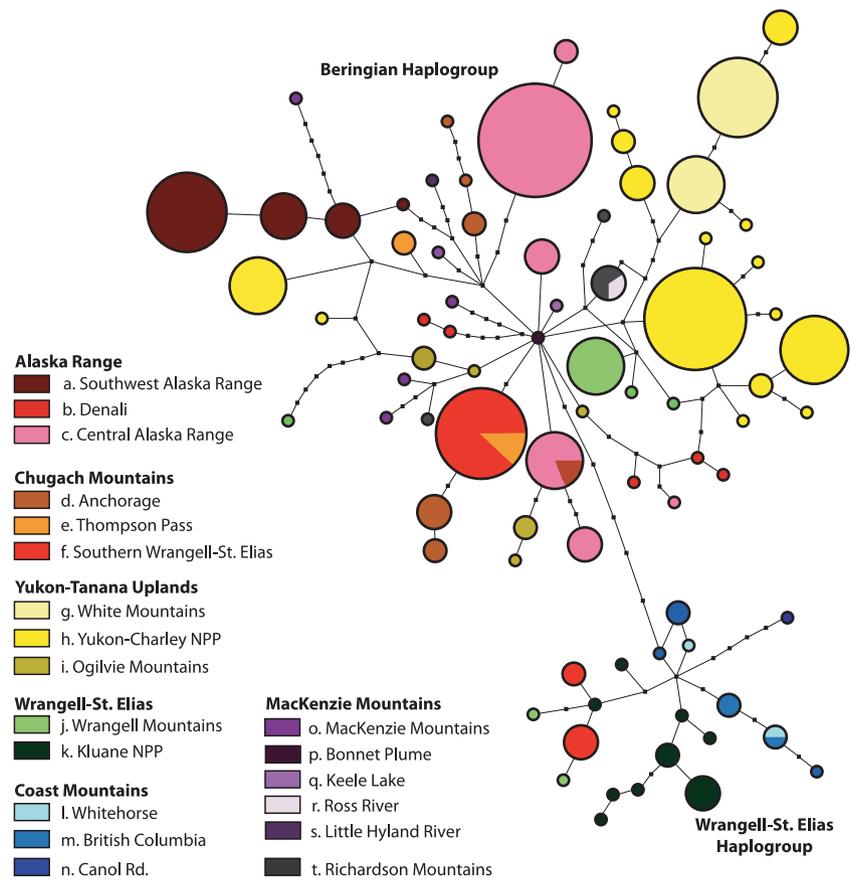
Figure 4 Statistical parsimony network of the haplotypes found in *Ochotona collaris*. The Beringian haplogroup is separated by five mutations from the Wrangell–St Elias clade (below). Nodes are proportional to the number of individuals sharing a haplotype (smallest circles correspond to one individual). Square nodes represent unsampled (inferred) haplotypes. Each branch is equal to one mutation. The SPLITSTREE network (not shown) recovered similar structure.

population differences in *O. collaris*, among-group structuring explained the majority of the variance in *O. curzoniae* (Ci *et al.*, 2009). Populations of collared pikas are less geographically structured and more closely related to one another than are populations of either the American pika or the plateau pika.

Nucleotide diversity across all three species declined at higher latitudes (Fig. 5; $\rho = -0.60$, $P = 0.001$), broadly fitting the 'southern diversity, northern purity' model (described by Hewitt, 1996, 2004), whereby successive range contractions during glaciation and post-glacial expansion events combined to purge diversity from northern populations. While the relationship between nucleotide diversity and latitude was marginally significant in American pikas

($\rho = -0.67$, $P = 0.048$), it was not significant for either the collared pika ($\rho = -0.22$) or the plateau pika ($\rho = 0.35$). The lack of a strong pattern in population-level diversity in any of these species could result from the increased genetic drift at their distributional limits (Herrera & Bazaga, 2007) via low population size, low survivorship, and/or recent colonization or recolonization (Hampe & Petit, 2005). Furthermore, climate-mediated latitudinal shifts in diversity could lead to a parabolic distribution of within-species diversity, as diversity will be reduced along the leading edge by coloniza-

Table 3 Analysis of molecular variance (AMOVA) comparing genetic variance of collared pikas (*Ochotona collaris*) among mountain ranges (Alaska and north-western Canada), within populations within ranges, and within populations.

Source of variation	Variance component	% of variation	P
Among mountain ranges	0.845	18.53	< 0.0001
Among populations within ranges	1.491	32.69	< 0.0001
Within populations	2.225	48.78	0.0078
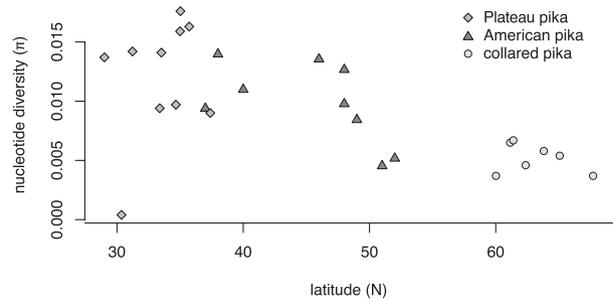
Figure 5 Nucleotide diversity for the major regional groups in the Asian plateau pika (*Ochotona curzoniae*; Ci *et al.*, 2009), the American pika (*O. princeps*; Galbreath *et al.*, 2009), and the collared pika (*O. collaris*; this study), plotted against the average latitude for each region.

tion dynamics and along the lagging edge by localized extinctions (Hewitt, 2004; Hampe & Petit, 2005). Although summary statistic approaches do not indicate significant population fluctuations in populations or lineages of either North American pika species (Galbreath *et al.*, 2009; this study), decline and extirpation have been documented in lower-latitude populations of *O. princeps* (Grayson & Livingston, 1993; Beever *et al.*, 2003). Multiple populations of *O. curzoniae* showed evidence of rapid range expansion, which appears to have occurred during the last interglacial (Ci *et al.*, 2009).

While the absolute extent (in km²) of suitable habitat is predicted to be similar for each of the three species, predicted habitat availability at the LGM differs considerably (Fig. 6). Although the SDM predicts habitat suitability and does not account for patch occupancy, it offers a relative (as opposed to realized) comparison of population connectivity

and habitat availability. Certain biological features may also exacerbate the differences. Plateau pikas are meadow dwelling and actual habitat occupancy is far greater for meadow-dwelling pikas than for rock-dwelling species (Smith, 2008). This is likely to translate to larger population sizes at both time-scales, an effect that would be consistent between the Recent and LGM. When levels of diversity were compared across the three species, there was no correlation between current predicted habitat and genetic diversity. However, models of LGM habitat availability were positively correlated with within-species genetic diversity ($r = 0.35$), suggesting that the amount of available Pleistocene habitat may help to explain the diversity gradient in pikas. Strong bottlenecks associated with low habitat availability during Pleistocene glaciations may have occurred multiple times in collared pikas, purging genetic variation as the species was repeatedly limited to small habitat patches (Fig. 6a).
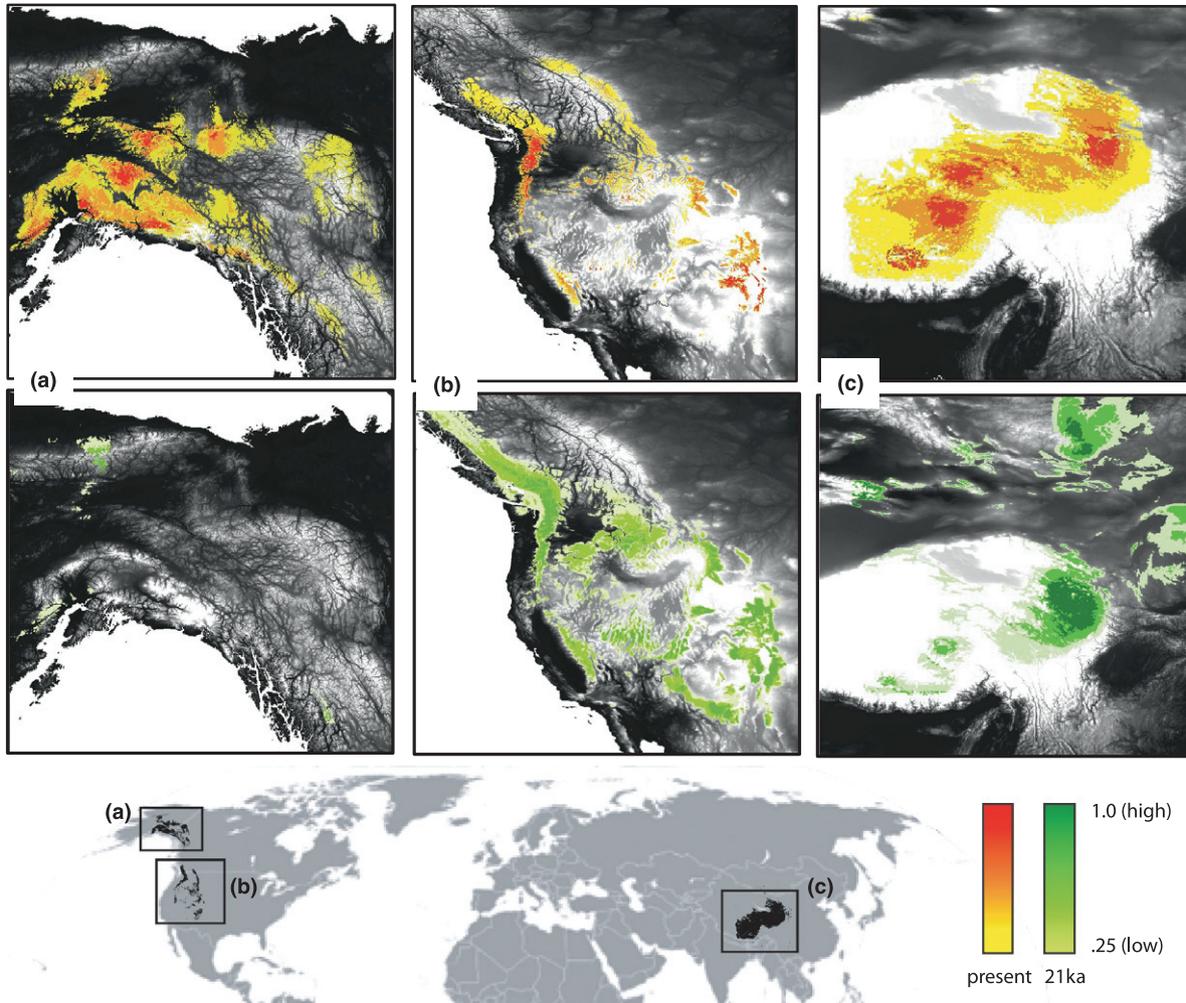
Figure 6 Ecological niche models of suitable habitat for (a) the collared pika (*Ochotona collaris*), (b) the American pika (*O. princeps*), and (c) the plateau pika (*O. curzoniae*), based on current climate (top row) and climate projections from the Last Glacial Maximum, 21 ka (bottom row); terrestrial elevation range is shown in background from low (dark) to high (light).

Implications and future directions

'Rear-edge' populations (those occurring at the lower latitudinal or elevational limits of a species range; *sensu* Hampe & Petit, 2005) may be disproportionally important for conservation and evolution because they contain the oldest lineages and are better adapted to warmer climates. The southernmost collared pika populations show reduced genetic diversity but no statistically significant signal of population decline. This might reflect a lag between census and effective population sizes, as older alleles may not have had time to be purged by genetic drift. Despite documented declines along the southern margin of *O. princeps* (Beever *et al.*, 2003), mtDNA summary statistics do not suggest population decline (Galbreath *et al.*, 2009). The only long-term study of any collared pika population documented climate-mediated decline at a site in the south-western Yukon Territory (Morrison & Hik, 2007). Previously documented populations in the Coast Mountains at Atlin Lake (Swarth, 1936) and White Pass (H.C.L., unpublished data) were not observed during subsequent surveys. Determining whether these patterns correspond to actual declines (climate-mediated or otherwise) or metapopulation dynamics (Morrison & Hik, 2008) will be critical for predicting the effects of climate change on *O. collaris*.

CONCLUSIONS

Here, we show that the amount and degree of phylogeographical structure is greater for pika species that occur in temperate mountain regions, probably because these species had access to more Pleistocene habitat during cold phases, more extreme bottlenecks occurred, and/or geographical subdivision has been greater in temperate regions. While both current and historical habitat constrained differentiation within *O. collaris*, thereby minimizing observed infraspecific variation, the differential in diversity is better explained by the suitable habitat at the LGM. An inferred Pleistocene bottleneck followed by a rapid population expansion with subsequent subdivision has resulted in decreased phylogeographical structure in the collared pika. The lack of described subspecies in *O. collaris* is therefore not just a difference in the amount of historical research: it is also the result of historical factors that have acted to diminish genetic and phenotypic diversity throughout the species' range. *Ochotona collaris* exhibits similar phylogeographical structure to other co-distributed mammals, but with lower genetic distance between phylogroups. The IUCN currently considers *O. collaris* to be a species of Least Concern (Smith & Johnston, 2008), but the high population isolation and low observed genetic and phenotypic variation in collared pikas may compromise their ability to adapt to a rapidly changing climate regime (Reed, 2004; Willi *et al.*, 2006; Millar & Westfall, 2010).

REFERENCES

Beever, E.A., Brussard, P.F. & Berger, J. (2003) Patterns of apparent extirpation among isolated populations of pikas (*Ochotona princeps*) in the Great Basin. *Journal of Mammalogy*, **84**, 37–54.

Beever, E.A., Ray, C., Mote, P.W. & Wilkening, J.L. (2010) Testing alternative models of climate-mediated extirpations. *Ecological Applications*, **20**, 164–178.

Braconnot, P., Otto-Bliesner, B., Harrison, S. *et al.* (2007) Results of PMIP2 coupled simulations of the Mid-Holocene and Last Glacial Maximum – Part 1: experiments and large-scale features. *Climate of the Past*, **3**, 261–277.

Ci, H.X., Lin, G.H., Cai, Z.Y., Tang, L.Z., Su, J.P. & Liu, J.Q. (2009) Population history of the plateau pika endemic to the Qinghai-Tibetan Plateau based on mtDNA sequence data. *Journal of Zoology*, **279**, 396–403.

Clement, M., Posada, D. & Crandall, K.A. (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, **9**, 1657–1660.

Cook, J.A. & MacDonald, S.O. (2003) Small mammal inventory of Alaska's National Parks and Preserves: Wrangell-St. Elias National Park and Preserve. *NPS Annual Report 2002–2003*, pp. 1–32. National Park Service, Idaho State University, Moscow, ID.

Corbet, G.B. (1978) *The mammals of the Palaearctic Region: a taxonomic review*. British Museum (Natural History), London and Cornell University Press, Ithaca, NY.

Dalén, L., Fuglei, E., Hersteinsson, P., Kapel, C.M.O., Roth, J.D., Samelius, G., Tannerfeldt, M. & Angerbjörn, A. (2004) Population history and genetic structure of a circumpolar species: the arctic fox. *Biological Journal of the Linnean Society*, **84**, 79–89.

Erbajeva, M.A. (1994) Phylogeny and evolution of Ochotonidae with emphasis on Asian ochotonids. *Rodent and Lagomorph Families of Asian Origins and Diversification: Proceedings of Workshop WC-2, 29th International Geological Congress, Kyoto, Japan* (ed. by Y. Tomida, C.K. Li and T. Setoguchi), pp. 1–13. National Science Museum Monographs no. 8, Tokyo.

Excoffier, L., Smouse, P.E. & Quattro, J.M. (1992) Analysis of molecular variance inferrred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479–491.

Excoffier, L., Laval, G. & Schneider, S. (2005) Arlequin version 3.0: an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online*, **1**, 47–50.

Fay, J.C. & Wu, C.-I. (2000) Hitchhiking under positive Darwinian selection. *Genetics*, **155**, 1405–1413.

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

Fedorov, V.B., Goropashnaya, A.V., Jaarola, M. & Cook, J.A. (2003) Phylogeography of lemmings (*Lemmus*): no evidence for postglacial colonization of Arctic from the Beringian refugium. *Molecular Ecology*, **12**, 725–731.

Fleming, M.A. & Cook, J.A. (2002) Phylogeography of endemic ermine (*Mustela erminea*) in southeast Alaska. *Molecular Ecology*, **11**, 795–807.

Franken, R.J. (2002) *Demography and metapopulation dynamics of collared pikas (Ochotona collaris) in the southwest Yukon*. Master's Thesis. University of Alberta, Edmonton.

Franken, R.J. & Hik, D.S. (2004) Influence of habitat quality, patch size and connectivity on colonization and extinction dynamics of collared pikas *Ochotona collaris*. *Journal of Animal Ecology*, **73**, 889–896.

Fu, Y.-X. (1997) Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, **147**, 915–925.

Galbreath, K.E., Hafner, D.J. & Zamudio, K.R. (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.

Galbreath, K.E., Hafner, D.J., Zamudio, K.R. & Agnew, K. (2010) Isolation and introgression in the Intermountain West: contrasting gene genealogies reveal the complex biogeographic history of the American pika (*Ochotona princeps*). *Journal of Biogeography*, **37**, 344–362.

Grant, W.A.S. & Bowen, B.W. (1998) Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. *Journal of Heredity*, **89**, 415–426.

Grayson, D.K. & Livingston, S.D. (1993) Missing mammals on Great Basin mountains: Holocene extinctions and inadequate knowledge. *Conservation Biology*, **7**, 527–532.

Guthrie, R.D. (1973) Mummified pika (*Ochotona*) carcass and dung pellets from Pleistocene deposits in interior Alaska. *Journal of Mammalogy*, **54**, 970–971.

Hafner, D.J. & Smith, A.T. (2010) Revision of the subspecies of the American pika, *Ochotona princeps* (Lagomorpha: Ochotonidae). *Journal of Mammalogy*, **91**, 401–417.

Hafner, D.J. & Sullivan, R.M. (1995) Historical and ecological biogeography of Nearctic pikas (Lagomorpha: Ochotonidae). *Journal of Mammalogy*, **76**, 302–321.

Hall, E.R. (1981) *The mammals of North America*, 2nd edn. Wiley, New York.

Hampe, A. & Petit, R.J. (2005) Conserving biodiversity under climate change: the rear edge matters. *Ecology Letters*, **8**, 461–467.

Harpending, H.C., Batzer, M.A., Gurven, M., Jorde, L.B., Rogers, A.R. & Sherry, S.T. (1998) Genetic traces of ancient demography. *Proceedings of the National Academy of Sciences USA*, **95**, 1961–1967.

Herrera, C.M. & Bazaga, P. (2007) Adding a third dimension to the edge of a species' range: altitude and genetic structuring in mountainous landscapes. *Heredity*, **100**, 275–285.

Hewitt, G. (2000) The genetic legacy of the Quaternary ice ages. *Nature*, **405**, 907–913.

Hewitt, G.M. (1996) Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society*, **58**, 247–276.

Hewitt, G.M. (2004) Genetic consequences of climatic oscillations in the Quaternary. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **359**, 183–195.

Hijmans, R.J., Cameron, S.E., Parra, J.L., Jones, P.G. & Jarvis, A. (2005) Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology*, **25**, 1965–1978.

Hoffmann, R.S. & Smith, A.T. (2005) Order Lagomorpha. *Mammal species of the world* (ed. by D.E. Wilson and D.M. Reeder), pp. 185–211. Johns Hopkins University Press, Baltimore, MD.

Janzen, D.H. (1967) Why mountain passes are higher in the tropics. *The American Naturalist*, **101**, 233–249.

Knowles, L.L. (2000) Tests of Pleistocene speciation in montane grasshoppers (genus *Melanoplus*) from the sky islands of western North America. *Evolution*, **54**, 1337–1348.

Körner, C. (2007) The use of 'altitude' in ecological research. *Trends in Ecology and Evolution*, **22**, 569–574.

Krajick, K. (1998) Nunataks: icebound islands of life. *National Geographic*, **194**, 60–71.

Kurtén, B. & Anderson, E. (1980) *Pleistocene mammals of North America*. Columbia University Press, New York.

Lanier, H.C. & Olson, L.E. (2009) Inferring divergence times within pikas (*Ochotona* spp.) using mtDNA and relaxed molecular dating techniques. *Molecular Phylogenetics and Evolution*, **53**, 1–12.

Lessa, E.P., Cook, J.A. & Patton, J.L. (2003) Genetic footprints of demographic expansion in North America, but not Amazonia, during the Late Quaternary. *Proceedings of the National Academy of Sciences USA*, **100**, 10331–10334.

Librado, P. & Rozas, J. (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, **25**, 1451–1452.

Loehr, J., Worley, K., Grapputo, A., Carey, J., Veitch, A. & Coltman, D.W. (2006) Evidence for cryptic glacial refugia from North American mountain sheep mitochondrial DNA. *Journal of Evolutionary Biology*, **19**, 419–430.

Lucid, M.K. & Cook, J.A. (2007) Cytochrome-*b* haplotypes suggest an undescribed *Peromyscus* species from the Yukon. *Canadian Journal of Zoology*, **85**, 916–919.

MacArthur, R.A. & Wang, L.C.H. (1973) Physiology of thermoregulation in the pika, *Ochotona princeps*. *Canadian Journal of Zoology*, **51**, 11–16.

MacDonald, S.O. & Jones, C. (1987) *Ochotona collaris*. *Mammalian Species*, **281**, 1–4.

Martínez-Meyer, E., Peterson, A.T. & Hargrove, W.W. (2004) Ecological niches as stable distributional constraints on mammal species, with implications for Pleistocene extinctions and climate change projections for biodiversity. *Global Ecology and Biogeography*, **13**, 305–314.

Mead, J.I. (1987) Quaternary records of the pika, *Ochotona*, in North America. *Boreas*, **16**, 165–171.

Melo-Ferreira, J., Boursot, P., Randi, E., Kryukov, A., Suchentrunk, F., Ferrand, N. & Alves, P.C. (2007) The rise and fall of the mountain hare (*Lepus timidus*) during Pleistocene glaciations: expansion and retreat with hybridization in the Iberian Peninsula. *Molecular Ecology*, **16**, 605–618.

Millar, C.I. & Westfall, R.D. (2010) Distribution and climatic relationships of the American pika (*Ochotona princeps*) in the Sierra Nevada and Western Great Basin, U.S.A.; periglacial landforms as refugia in warming climates. *Arctic, Antarctic, and Alpine Research*, **42**, 76–88.

Morrison, S., Barton, L., Caputa, P. & Hik, D.S. (2004) Forage selection by collared pikas, *Ochotona collaris*, under varying degrees of predation risk. *Canadian Journal of Zoology*, **82**, 533–540.

Morrison, S.F. & Hik, D.S. (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.

Morrison, S.F. & Hik, D.S. (2008) When? Where? And for how long? Census design considerations for an alpine lagomorph, the collared pika (*Ochotona collaris*). *Lagomorph biology: evolution, ecology, and conservation* (ed. by P.C. Alves, N. Ferrand and K. Hackländer), pp. 103–113. Springer, Berlin.

Murray, B.M. & Murray, D.F. (1969) Notes on mammals in alpine areas of the northern St. Elias Mountains, Yukon Territory, and Alaska. *Canadian Field-Naturalist*, **83**, 331–338.

Olson, L.E. & Hassanin, A. (2003) Contamination and chimerism are perpetuating the legend of the snake-eating cow with twisted horns (*Pseudonovibos spiralis*). A case study of the pitfalls of ancient DNA. *Molecular Phylogenetics and Evolution*, **27**, 545–548.

Peacock, M.M. & Ray, C. (2001) Dispersal in pikas (*Ochotona princeps*): combining genetic and demographic approaches to reveal spatial and temporal patterns. *Dispersal* (ed. by J. Clobert, E. Danchin, A.A. Dhondt and J.D. Nichols), pp. 43–56. Oxford University Press, New York.

Phillips, S.J., Anderson, R.P. & Schapire, R.E. (2006) Maximum entropy modeling of species geographic distributions. *Ecological Modelling*, **190**, 231–259.

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

Posada, D. (2008) jModeltest: phylogenetic model averaging. *Molecular Biology and Evolution*, **25**, 1253–1256.

Posada, D. & Crandall, K.A. (2001) Intraspecific gene genealogies: trees grafting into networks. *Trends in Ecology and Evolution*, **16**, 37–45.

Ramos-Onsins, S.E. & Rozas, J. (2002) Statistical properties of new neutrality tests against population growth. *Molecular Biology and Evolution*, **19**, 2092–2100.

Reed, D.H. (2004) Extinction risk in fragmented habitats. *Animal Conservation*, **7**, 181–191.

Roberts, T.E., Lanier, H.C., Sargis, E.J. & Olson, L.E. (2011) Molecular phylogeny of treeshrews (Mammalia: Scandentia) and the timescale of diversification in Southeast Asia. *Molecular Phylogenetics and Evolution*, **60**, 358–372.

Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**, 1572–1574.

Shafer, A.B.A., Cullingham, C.I., Côté, S.D. & Coltman, D.W. (2010) Of glaciers and refugia: a decade of study sheds new light on the phylogeography of northwestern North America. *Molecular Ecology*, **19**, 4589–4621.

Smith, A.T. (1974) The distribution and dispersal of pikas: influences of behavior and climate. *Ecology*, **55**, 1368–1376.

Smith, A.T. (2008) The world of pikas. *Lagomorph biology: evolution, ecology and conservation* (ed. by P.C. Alves, N. Ferrand and K. Hackländer), pp. 89–102. Springer, Berlin.

Smith, A.T. & Johnston, C.H. (2008) *Ochotona collaris*. *IUCN Red List of Threatened Species*, version 2011.2. Available at: http://www.iucnredlist.org/details/41257/0 (downloaded on 10 November 2011).

Smith, A.T. & Weston, M.L. (1990) *Ochotona princeps*. *Mammalian Species*, **352**, 1–8.

Smith, A.T., Li, W. & Hik, D.S. (2004) Pikas as harbingers of global warming. *Species*, **41**, 4–5.

Stewart, J.R., Lister, A.M., Barnes, I. & Dalén, L. (2010) Refugia revisited: individualistic responses of species in space and time. *Proceedings of the Royal Society B: Biological Sciences*, **277**, 661–671.

Swarth, H.S. (1936) Mammals of the Atlin Region, northwestern British Columbia. *Journal of Mammalogy*, **17**, 398–405.

Trefry, S.A. & Hik, D.S. (2009a) Eavesdropping on the neighbourhood: collared pika (*Ochotona collaris*) responses to playback calls of conspecifics and heterospecifics. *Ethology*, **115**, 928–938.

Trefry, S.A. & Hik, D.S. (2009b) Variation in pika (*Ochotona collaris*, *O. princeps*) vocalizations within and between populations. *Ecography*, **33**, 784–795.

Turner, J.R.G. & Hawkins, B.A. (2004) The global diversity gradient. *Frontiers of biogeography: new directions in the geography of nature* (ed. by M.V. Lomolino and L.R. Heaney), pp. 171–190. Sinauer, Sunderland, MA.

Weksler, M., Lanier, H.C. & Olson, L.E. (2010) Eastern Beringian biogeography: historical and spatial genetic structure of singing voles (*Microtus miurus*) in Alaska. *Journal of Biogeography*, **37**, 1414–1431.

Weston, M.L. (1981) The *Ochotona alpina* complex: a statistical re-evaluation. *Proceedings of the World Lagomorph Conference* (ed. by C. Meyers and C. D. MacInnes), pp. 73–89. Guelph University Press, Guelph, ON.

Wiens, J.J. & Donoghue, M.J. (2004) Historical biogeography, ecology and species richness. *Trends in Ecology and Evolution*, **19**, 639–644.

Willi, Y., Van Buskirk, J. & Hoffmann, A.A. (2006) Limits to the adaptive potential of small populations. *Annual Review of Ecology, Evolution, and Systematics*, **37**, 433–458.

Zeng, K., Fu, Y.-X., Shi, S. & Wu, C.-I. (2006) Statistical tests for detecting positive selection by utilizing high-frequency variants. *Genetics*, **174**, 1431–1439.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Specimen information and degraded DNA amplification.
Appendix S2 Point localities used in the ecological niche models.
Appendix S3 Collared pika population differentiation based on Φ_{ST}.

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