OF PIKAS AND PARASITES: HISTORICAL BIOGEOGRAPHY OF AN ALPINE HOST-PARASITE ASSEMBLAGE

A Dissertation

Presented to the Faculty of the Graduate School

of Cornell University

in Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

by

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January 2009

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Cornell University 2009

The goal of my dissertation research is to quantify the genetic consequences of Quaternary climatic oscillations for an alpine host-parasite assemblage. I focus on a mammalian alpine specialist (*Ochotona princeps*; American pika) that is distributed across North America's Intermountain West, and a suite of its obligate nematode (*Cephaluris*, *Labiostomum*, *Graphidiella*, *Murielus*) and cestode (*Schizorchis*) endoparasites.

In chapters one and two I reconstruct the population history of *O. princeps* to understand how climate-driven distributional shifts by the species have structured genetic diversity. Pika populations are partitioned into five non-overlapping mitochondrial lineages associated with different mountain ranges. Pulses of range expansion and contraction associated with past climatic oscillations have maintained lineage cohesion by promoting gene flow among sky island populations. Low-elevation basins may have helped maintain differentiation between lineages by limiting opportunities for genetic admixture, but two nuclear loci revealed genetic patterns consistent with recent gene flow across mitochondrial lineage boundaries. Thus, historical contact between lineages probably occurred, but it had different outcomes for different genetic loci. Northern lineages retain the genetic signature of glacial-age population expansion, likely associated with climate-driven shifts to lower elevations, and all lineages exhibit evidence of recent (post-Pleistocene) population decline. An ecological niche model suggested that continued climate warming could

dramatically reduce the distribution of American pikas, potentially eliminating a major mitochondrial lineage.

Chapter three builds on the phylogeographic framework established for *O. princeps* by testing for concordant population histories across several parasites. There is little phylogeographic congruence between the host and its parasites, suggesting independent evolutionary trajectories for the parasites. Indeed, close relationships between parasite populations associated with different host lineages indicate relatively extensive parasite gene flow across host lineage boundaries.

Finally, chapter four uses host-parasite comparative phylogeography to elucidate the unresolved origins of *O. princeps* and its northern sister taxon, *O. collaris*. Four parasite lineages show that populations associated with *O. collaris* are phylogenetically nested within populations from *O. princeps*. This repeated pattern strongly indicates that *O. collaris* arose from a low-latitude ancestor, and demonstrates the utility of parasites for revealing cryptic host history.

BIOGRAPHICAL SKETCH

Kurt Egan Galbreath was born on the south side of Chicago on November 15, 1975. This decidedly urban beginning contrasted sharply with the rural experience that influenced his formative years, after his family moved to the small town of Pecatonica, IL. Kurt enjoyed a comfortable and intellectually stimulating childhood, surrounded by loving family, excellent teachers, and a diverse network of friends. His world was expanded beyond the cornfields and country roads of his hometown through Boy Scout and family trips that crisscrossed the United States and ultimately took him as far as South Korea. These trips fostered an enthusiasm for exploration and discovery that later played a key role in determining the trajectory of his academic career.

After graduating from Pecatonica High School in 1993, Kurt pursued a bachelor's degree in biology at Illinois Wesleyan University in Bloomington, IL. There he conducted independent research with Given Harper (maternal control of hatching asynchrony in European starlings) and Susie Balser (environmental controls of life history characteristics of bdelloid rotifers), worked as a campus photographer for the university, played principal tuba in the concert band, and sang in multiple choirs. He participated in two influential summer internships, first working with Jim Thomas at the National Museum of Natural History on the biogeography of anamixid amphipods, and later with Patrick Gaffney at the University of Delaware's College of Marine Studies on the phylogeography of Atlantic horseshoe crabs. Most significantly, however, at Illinois Wesleyan he met Kimberly Fryzel, who became a lifelong partner on his journey.

Kurt completed his undergraduate work in 1997, but did not have immediate plans to attend graduate school. In 1998 he underwent an invasive operation to correct a severe spinal curvature. Though his body healed relatively quickly, the operation took a psychological toll that was more difficult to overcome. Graduate studies offered

a way forward, and when an offer came from Joseph Cook at the University of Alaska Fairbanks to pursue a master's degree in wildlife biology, he leapt at the opportunity to explore the North. In the summer of 1999, Kurt and Kimberly drove the Alaska Highway to Fairbanks, marveling at the long days and the breathtaking views. Kurt spent two joyful years in Alaska, studying the genetic consequences of Pleistocene glaciations for the tundra vole. He worked with the University of Alaska Museum's mammal collection, participating in several mammal collecting expeditions in Alaska and Siberia.

In 2001, Kurt completed his M.S., married Kimberly, and left his cabin in the woods for a research position at the US National Parasite Collection in Beltsville, MD. There he worked with Eric Hoberg and Benjamin Rosenthal on the historical biogeography of *Arostrilepis*, a group of tapeworms that parasitize many northern rodents. In 2002 he moved to Ithaca, NY, to pursue a Ph.D. at Cornell University under Kelly Zamudio. Kurt enjoyed many exciting opportunities for discovery in both the lab and field while at Cornell, but most of those experiences pale in comparison to the adventure that began on November 9, 2007 with the birth of his son, Ian Muir. With the completion of his dissertation, Kurt will move to Bellingham, WA, with his growing family, where he will conduct post-doctoral research at Western Washington University with Eric DeChaine on comparative phylogeography of arctic and alpine plants.

For my grandfather, Louis K. Davis

ACKNOWLEDGMENTS

Behind every dissertation lies a network of people and organizations that made it possible, and mine is no exception. Though their contributions too often go unrecognized, I want to take a moment to express my sincere gratitude to all those who helped me on this journey. First of all, I thank the members of my special committee for their role in directing, motivating, and refining my progress over the course of my graduate career. My advisor, Kelly Zamudio, was an exceptional mentor, giving me ample independence to pursue the questions that interested me, while offering timely words of wisdom when I was at risk of wandering off course. Kelly supported my work financially and intellectually, and by her example I learned a great deal about how to be a successful professional scientist. I am grateful that she opened the doors of her lab to me, despite my distinct lack of herpetological credentials!

Rick Harrison and Irby Lovette were steady advocates for me and my research from the moment I arrived at Cornell, and they enriched my time there in many ways. Both brought deep experience to my committee and greatly improved the scientific rigor of my research. They also offered abundant encouragement when my enthusiasm flagged. I owe Rick a particular debt of gratitude for reminding me of the essential importance of maintaining balance in life (a lesson that many graduate students need to hear often), and to Irby I am especially thankful for the contributions that he made to my research through his support of the mammal collection of the Cornell University Museum of Vertebrates (CUMV).

Finally, Eric Hoberg has been a friend and mentor for many years, but he also brought unrivaled parasitological knowledge to my committee, making this dissertation feasible. The work would have been impossible, or at least much less complete, without his contributions to unraveling the taxonomy of the parasite groups that I examined. Furthermore, my understanding of the biogeography and evolution of

northern organisms has benefited from the many long discussions on Beringia and the Arctic that we have enjoyed. As the curator of the US National Parasite Collection, Eric also graciously undertook the task of archiving the several thousand parasite specimens that resulted from the work.

The Zamudio lab provided a collegial and intellectually stimulating environment that fostered my academic growth. Lauren Chan and Jeanne Robertson, the senior graduate students in the lab when I arrived, gave me an enthusiastic welcome. I benefited greatly from their camaraderie, generosity, and thoughtful consideration of my research. Likewise, Anna Savage, Elizabeth Kuperberg, and Angie Stevenson offered friendship and valuable insights that improved my work. Christine Voyer and Angie Stevenson played a vital role in keeping the wet lab running smoothly. Without their help I undoubtedly would not have accomplished as much as I did. Jim Austin and Jonathan Richmond brought fresh perspectives to the Zamudio lab, and offered key insights that opened up productive avenues of research.

I was fortunate to have many friends and colleagues in Cornell's Ecology and Evolutionary Biology (EEB) community beyond those of the Zamudio lab. Pete McIntyre, Katie Wagner, Amy Parachnowitsch, and Mari Kimura were officemates that deserve special recognition for their encouragement, advice, and willingness to talk science for hours on end. Sarah Stockwell shared her appreciation of music and her expertise with computers. Dan Rabosky taught me to program in R and is a model of intellectual curiosity. Betty McGuire was a wonderful collaborator on the Mammalogy course that we taught together, and shared in my love of furry, milk-producing organisms. Harry Greene and I enjoyed many conversations about Beringia, glaciers, and a world where Pleistocene megafauna could still walk the plains of North America. Jill Anderson, Jason Andras, Laurelin Evanhoe, Paulo Llambias, Sarah and James Reilly, Tim Sackton, Andrea Townsend, Marissa Weiss, and many other friends

contributed to my time at Cornell in large and small ways, and I am grateful for their insights and support.

My research benefited from resources contributed by a widespread network of colleagues. Willy Bemis (Shoals Marine Lab) provided access to microscopy equipment for photographing parasites. Eric Waltari (American Museum of Natural History) donated data layers from climate simulations for the last glacial maximum, and Paul Allen (Laboratory of Ornithology) taught me about virtual Linux environments. Marjorie Matocq (Idaho State University) and Joseph Cook (University of New Mexico) provided support for fieldwork. David Hafner (New Mexico Museum of Natural History) and Kelly Agnew (University of California at Berkeley) offered critical data and insight into the world of pikas. Patricia Pilitt (US National Parasite Collection) contributed her time and parasitological expertise to help sort out some complicated taxonomic issues in nematodes. Several people provided important specimens, including Joseph Cook and Eric Hoberg, Alasdair Veitch and Richard Popko (Northwest Territories' Environment and Natural Resources) and Patrick Giraudoux, Francis Raoul, and Jean-Pierre Quéré (University of Franche-Comté), respectively. Many analyses were performed using resources of Cornell's Computational Biology Service Unit, which is partially funded by Microsoft.

Staff members of EEB provide the bedrock upon which all the work of the department's faculty and students is built, and as such their contributions were indispensable for the success of my dissertation. Linda Harrington, Janeen Orr, Rosie Brainerd, Alberta Jackson, DeeDee Albertsman, LuAnne Kenjerska, Patty Jordan, Carol Damm, Gary Oltz, John Howell, Brian Mlodinski, and Tim Larkin all played key rolls in keeping the department running smoothly, and at one time or another many of them rescued me from my own folly with grace and kindness. Steve Bogdanowicz kept the Evolutionary Genetics Core Facility humming like a well-oiled

machine and generously shared his molecular biology expertise. Kimberly Bostwick and Charles Dardia of the CUMV provided field equipment and other resources, and they cheerfully humored my interest in mammal collections.

My research was supported financially from a variety of sources, including a National Science Foundation (NSF) Graduate Research Fellowship and Doctoral Dissertation Improvement Grant (DEB 050604), Grant-in-Aid and Elizabeth Horner Award from the American Society of Mammalogists, Andrew Mellon Foundation grant, Sigma Xi Grant-in-Aid of Research, American Museum of Natural History Roosevelt Fund grant, and EEB research award. Indirect support of my work was provided by NSF grants to Eric Hoberg (DEB 0196095 and DEB 0415668) and Kelly Zamudio (DEB 0343526), and a National Institutes of Health grant to Patrick Giraudoux (EID TW001565-05). I received travel grants from the Cornell Graduate School that allowed me to attend several professional meetings.

Most of the specimens that I used in this research were collected over two field seasons, which would not have been possible without the efficient and professional assistance of wildlife agency employees of the ten states and two Canadian provinces in which I worked. Furthermore, my success in the field was greatly enhanced by the contributions of Amy Barroll and Kimberly Fryzel, who spent long hours huddled over pika specimens and data sheets. Their cheerful dispositions in the face of occasionally challenging circumstances helped keep me going.

I am deeply grateful to my family for their unwavering support. My parents, Jim and Beth, and my sister, Lee, have been tireless cheerleaders from the start, and they patiently listened to many rambling explanations of my research, no matter how poorly articulated. Gloin Scout was a steadfast companion from the beginning of my Cornell career to its whirlwind end, keeping me grounded with daily reminders of the simple joys of long walks. Kimberly Fryzel supported me with patience and strength

through a graduate program that involved many highs and lows. My success was a direct consequence of the grace and wisdom with which she met the challenges that my path set before us. I have been richly blessed to have had such a remarkable partner for this journey. Lastly, I am thankful to Ian Muir for providing daily epiphanies regarding life's marvelous complexities. My wish for him is that he, like his namesake, will continue to experience that same spark of discovery throughout his entire life.

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WHEN COLD IS BETTER: CLIMATE-DRIVEN ELEVATION SHIFTS YIELD COMPLEX PATTERNS OF DIVERSIFICATION AND DEMOGRAPHY IN AN ALPINE SPECIALIST (AMERICAN PIKA, *OCHOTONA PRINCEPS*)

Abstract.— The genetic consequences of climate-driven range fluctuation during the Pleistocene have been well-studied for temperate species, but cold-adapted (e.g., alpine, arctic) species that may have responded uniquely to past climatic events have received less attention. In particular, we have no a priori expectation for long-term evolutionary consequences of elevation shifts into and out of sky islands by species adapted to alpine habitats. Here we examined the influence of elevation shifts on genetic differentiation and historical demography in an alpine specialist, the American pika (*Ochotona princeps*). Pika populations are divided into five genetic lineages that evolved in association with separate mountain systems, rather than lineages that reflect individual sky islands. This suggests a role for glacial-period elevation shifts in promoting gene flow among high-elevation populations and maintaining regional cohesion of genetic lineages. We detected a signature of recent demographic decline in all lineages, consistent with the expectation that Holocene climate warming has driven range retraction in southern lineages, but unexpected for northern populations that presumably represent post-glacial expansion. An ecological niche model of past and future pika distributions highlights the influence of climate on species range and indicates that the distribution of genetic diversity may change dramatically with continued climate warming.

INTRODUCTION

For low-elevation temperate species, historical global cooling is generally a driver of range retraction and habitat fragmentation, and isolation by glacial-age barriers has contributed to intra- and interspecific genetic structuring in many taxa (Avise 2000; Hewitt 1999; Hewitt 2004a; Taberlet et al. 1998). Indeed, a major goal of historical biogeography and phylogeography is to infer the number and location of isolated refugia to which species were restricted during Pleistocene glacial periods (Waltari et al. 2007). However, glacial climatic conditions were not necessarily unfavorable or restrictive for all species. Many cold-adapted organisms, such as those found in alpine and arctic environments, experienced range expansion and increased gene flow during glacials (Hewitt 2000). Such species are especially sensitive to rising temperatures, and if we assume a high degree of niche conservatism (Holt 2003; Peterson et al. 1999; Wiens and Graham 2005), they may face a greater threat from contemporary climate warming than they did from past periods of climate cooling (Sala et al. 2000).

The genetic consequences of past glaciations for temperate taxa are well-studied (Avise 2000; Hewitt 2000), and these studies have led to refugial hypotheses (Hewitt 2004b) and models for understanding processes of post-glacial colonization (Hewitt 1996) that are a foundation for the field of phylogeography. For example, many temperate species responded to Pleistocene climatic fluctuations with latitudinal shifts into and out of southern refugia (Hewitt 2004b). Glacial periods were generally associated with range retraction caused by the expansion of ice sheets and periglacial tundra, and interglacials were associated with range expansion. Many species that fit this model, which we call here the ‘latitude shift’ model of range fluctuation, exhibit population genetic signatures of high diversity at low latitudes, low diversity at high latitudes, and recent (post-glacial) population growth.

A growing literature is now focusing on patterns of diversity at high latitudes (e.g., Fedorov and Stenseth 2002; Galbreath and Cook 2004; Weider and Hobaek 2003; Wickström et al. 2003) and elevations (e.g., Albach et al. 2006; DeChaine and Martin 2005; Haubrich and Schmitt 2007; Schönswetter et al. 2003), yet specific predictions regarding population genetic effects of glaciations for species in these extreme environments are lacking, and models based on temperate organisms may not always apply (Fedorov et al. 1999). For example, alpine species did not necessarily respond to climate change through large-scale latitudinal shifts. In North America's Intermountain West, expansion and contraction of species ranges proceeded through local movements along elevation gradients from and to scattered high-elevation patches of habitat (sky islands) (Guralnick 2007). This 'elevation shift' scenario differs from the latitude shift model in that the major period of range expansion was during glacial periods, while retraction occurred during interglacials.

The two models of range fluctuation should yield different genetic outcomes, but the genetic consequences of fluctuation under the elevation shift model has not been well-studied. The ideal taxon in which to test for these differences is one that permits a direct comparison of the two models among populations within a single species, thereby minimizing the influence of confounding life history differences inherent in interspecific comparisons. In this study we conduct an intraspecific comparison of the genetic effects of the two range fluctuation models in an alpine specialist. Our goal is to develop general predictions for the elevation shift model regarding climate's influence on the distribution of diversity and the genetic signature of demographic change.

We hypothesize that the evolutionary trajectories of alpine species that undergo climate-driven elevation shifts differ in at least two respects from those that responded to climate change through large-scale shifts in latitude. First, patterns of

genetic differentiation in species that fit the elevation shift model may be more strongly dictated by isolation atop interglacial sky islands than by restriction in glacial refugia. This hypothesis makes specific predictions regarding the number and distribution of divergent lineages, which should reflect the distribution of sky islands. Also, coalescence times among populations should be deepest in regions where warming has caused extensive population subdivision, which would permit old genotypes to persist in different population isolates without being lost via lineage sorting. Such population subdivision may be greatest at low latitudes or in regions where mountain ranges are separated by deep, wide valleys. Conversely, glacial-age isolation in low-latitude refugia should result in fewer major lineages, each associated with a different refugium and distributed widely across regions into which populations expanded post-glacially (e.g., interconnected mountain systems and shallow valleys). Highest diversity and deepest coalescence within lineages would be expected in refugial areas.

A second way in which effects of elevation shifts may differ from those of latitude shifts is in the genetic signatures of demographic fluctuations. Many latitudinally shifting species have undergone extensive post-glacial expansions, resulting in signatures of rapid population growth that date to the end of the Last Glacial Maximum (LGM) approximately 10,000 years ago (10 ka). In contrast, alpine species whose histories were dominated by elevation shifts should yield signatures of population expansion coincident with the rise of the LGM, followed by population retraction during the Holocene as climate warming caused optimal climatic envelopes to shift upslope and low-elevation populations went extinct.

North America's Intermountain West spans the western contiguous United States and southwestern Canadian provinces and presents an excellent landscape for examining the genetic consequences of the elevation shift model for alpine taxa. This

region is topographically complex, with high mountains and low intermountain basins that were heavily influenced by climatic oscillations of the Quaternary (Porter et al. 1983). During ice ages, continental ice sheets engulfed the northern latitudes (> 48° N), but glaciation was not extensive in the numerous mountain ranges distributed at lower latitudes. These southerly distributed mountains represent sky islands in which alpine species persisted during interglacials. Populations of cold-adapted species expanded from these islands as climate cooled and retreated to them as climate warmed. Thus we have the opportunity to directly compare the effects of latitude shifts at high latitudes, where species expanded northward following glacial retreat, and elevation shifts at low latitudes, where alpine species move in and out of sky islands.

We apply a phylogeographic framework to test hypotheses regarding the consequences of latitude shifts and elevation shifts for patterns of genetic differentiation and signatures of population fluctuation. We focus on the American pika (*Ochotona princeps*), an alpine specialist that is widespread throughout the Intermountain West and that has undergone both types of range fluctuations in different parts of its distribution. For low-latitude populations that likely underwent elevation shifts in response to Pleistocene climate fluctuations, we predict that 1) lineage diversity and depth is correlated with the distribution of interglacial sky islands, and 2) population expansion preceded the peak of the LGM (~ 21 ka), followed by population retraction as climate warmed. We also test the predictions of the latitude shift model for northern populations that were established during post-glacial colonization: 1) lineage distributions should reflect expansion from glacial-age refugia, and 2) populations should exhibit a recent (Holocene) signature of demographic expansion. To quantify climate-driven range fluctuation in *O. princeps* and to evaluate our interpretations of population connectivity and range changes from

an independent perspective (Kozak et al. 2008), we develop an ecological niche model (ENM) for pikas under LGM, current, and future climatic conditions using climate data and museum specimen records.

METHODS

Study organism

The American pika is an alpine specialist distributed across most of the major mountain ranges of the Intermountain West (Figure 1.1), where it is found almost exclusively in high-elevation talus habitats (Hafner 1994; Smith and Weston 1990). This small lagomorph is a good model for testing the genetic consequences of range fluctuation because its distribution includes post-glacially colonized populations in the north and isolated sky island populations in the south. As one of the few alpine mammals in North America with an extensive distribution, it offers a rare opportunity to compare patterns of diversity across a range of latitudinal, topological, and climatological contexts. Pikas are highly specialized in terms of thermal biology; with their low heat tolerance (Smith 1974) and limited capacity for physiological thermoregulation (MacArthur and Wang 1974), they are restricted to areas that fall within a narrow climatic envelope. Fossils indicate that populations were broadly distributed at lower elevations during the last glacial maximum (Grayson 2005; Hafner 1993; Mead 1987). During the Holocene, spanning the last 10,000 years, once widespread populations became isolated in fragmented alpine refugia. Climate warming was implicated in the recent extirpation of populations in the Great Basin (Beever et al. 2003; Grayson 2005) and because they are poor dispersers, pikas are unlikely to recolonize isolated habitat islands (Brown 1971). An earlier study based on allozymes explored some of the genetic consequences of this extreme specialization and found four major pika lineages distributed across separate mountain

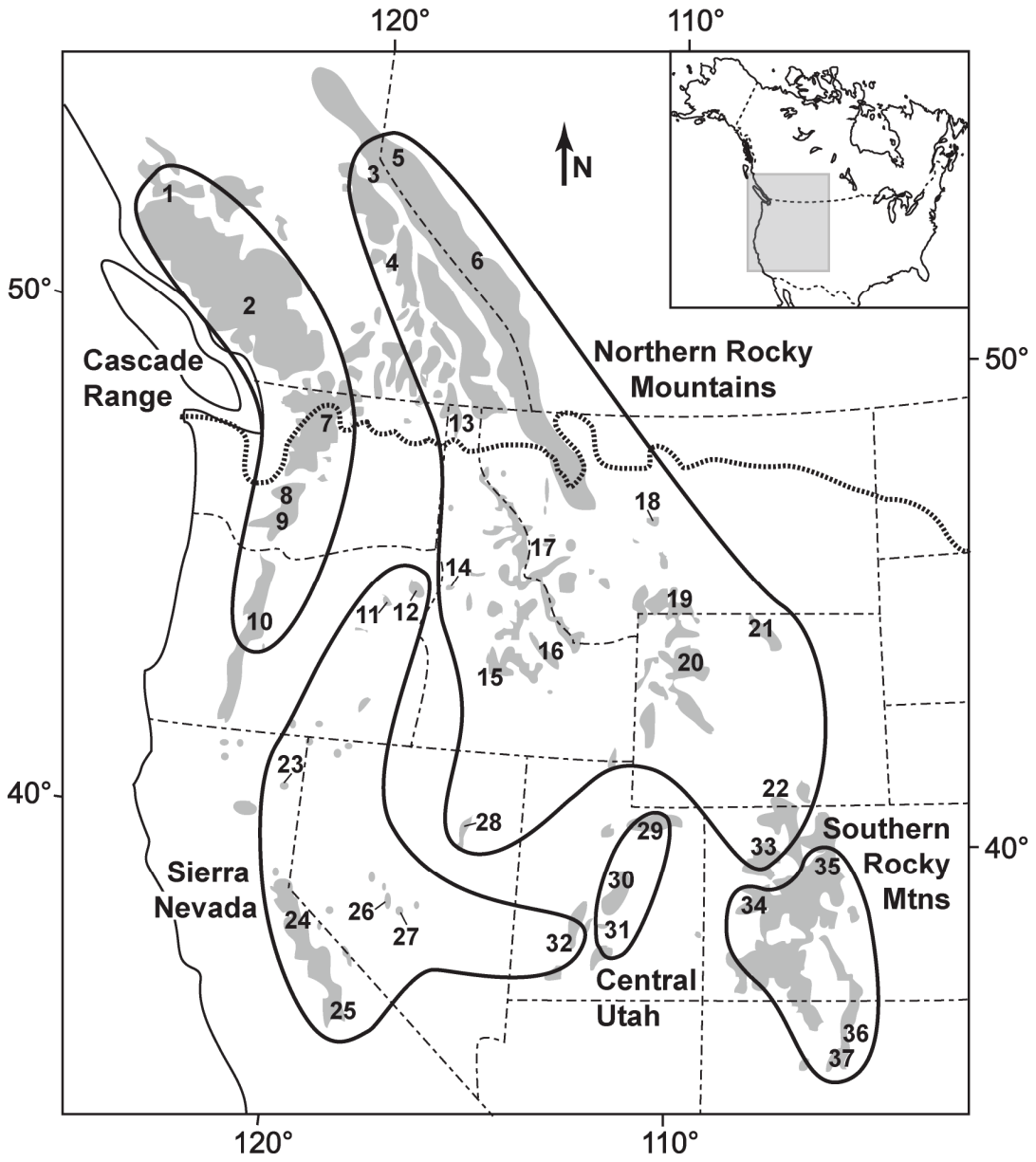

Figure 1.1. Distribution of *O. princeps* and major mtDNA lineages. Gray patches indicate the approximate distribution of American pikas (from Hafner 1993). Mitochondrial lineages are denoted by heavy lines, and the approximate southern margin of the continental ice sheet during the LGM is indicated by the broken line (from Porter et al. 1983). Numbers indicate sampling localities (see Figure 1.2; Appendix). The inset shows the position of the detail map in North America as a light gray box.

systems (Hafner and Sullivan 1995); however, that study was based on limited allozymic variation, precluding conclusive inferences about the origin and structure of this diversity.

Data collection

We obtained tissues (liver, kidney, spleen, or muscle) from 175 American pikas representing 37 localities (2 – 7 specimens per locality) distributed throughout the species' range (Figure 1.1; Appendix). Vouchers and tissue specimens for all individuals are archived at the Cornell University Museum of Vertebrates (Appendix). We extracted genomic DNA using Qiagen DNeasy extraction kits and sequenced a portion of mtDNA spanning the entire cytochrome b gene and part of the control region's D-loop (~1700 total base pairs) using primer pairs that we designed based on published mtDNA sequences for *O. princeps* (GenBank # AJ537415): PIKA01 (5'TGACTAATGATATGAAAAAYCAYCGTTG) / PIKA02 (5'AGGAGGTCTGGGGAGAATAGTACTA), PIKA03 (5'GACGCAGACAAAATYCCATTCCA) / PIKA04 (5'ATGGCCCTGAAGTAAGAACCAGATG). We amplified all gene fragments in 20 µl volumes, with final reagent concentrations of 1.5 mM $MgSO_4$, 0.5 µM primers, 0.4 mM dNTPs, 0. 5 U *Taq* polymerase, and approximately 5 ng/µl template DNA. All reactions included a 3 minute initial denaturation (92ºC), 30 cycles of 15 second denaturation (92ºC), 30 second annealing (60ºC), and 30 second extension (72ºC), and a final 10 minute extension (72ºC). All DNA fragments were sequenced in both directions. For outgroups we obtained sequences from GenBank representing four closely related *Ochotona* species, *O. hyperborea* (GenBank # AB053257), *O. alpina* (AF273009), *O. pallasi* (AF272990), and *O. collaris* (AF348080) as well as a single more deeply divergent species (*O. thomasi*; AF272987) (Yu et al. 2000).

Sequences were aligned via CLUSTALW (Thompson et al. 1994) using MEGA 3.1 (Kumar et al. 2004) and default parameters. Alignments were checked by eye, and indels removed from subsequent analyses. A region of ambiguous alignment in the D-loop was also excluded (positions 15429-15461 in the *O. princeps* mtDNA genome, GenBank #AJ537415), leaving 1668 bp. We tested the data for selective neutrality using a Tajima's *D* test, assessing significance using DnaSP 4.0 (Rozas et al. 2003) to produce a null distribution for *D* from 10,000 coalescent simulations of a population of large and constant size evolving neutrally under an infinite sites model of nucleotide substitution. To assess general patterns of diversity we calculated haplotype diversity (*h*) and nucleotide diversity (π) for major lineages using DnaSP. Sequences are deposited in GenBank (EU590920 - EU591094).

Phylogenetic analyses

To resolve the overall distribution of mtDNA lineages within *O. princeps*, we first collapsed the 1668 bp cytb/D-loop data set to include a single sequence representing each unique haplotype and identified the simplest nucleotide substitution model that best fit the data. Using the Akaike Information Criterion, MODELTEST 3.7 (Posada and Crandall 1998) selected the TVM + I + Γ model with rate matrix = (0.7246, 13.9637, 1.1419, 0.1700, 13.9637), proportion of invariant sites = 0.5323, and gamma shape parameter = 0.5951. We applied this model in a heuristic likelihood search conducted in PAUP* 4.0b10 (Swofford 2000). We also performed a Bayesian analysis using MRBAYES 3.0b4 (Huelsenbeck and Ronquist 2001) under the GTR + I + Γ substitution model, running 4 chains for 5 million generations, sampling every 100 generations, and discarding the first 10,000 samples as burn-in. To test for convergence, we repeated the analysis three times with different random seeds. We report posterior probabilities from a majority rule consensus of all the trees (*n* =

120,000) saved from the three Bayesian runs. We rooted all phylogenies with *O. thomasi*.

To test for differences in depth of coalescence for each pika lineage, we analyzed the mtDNA haplotype dataset using a Bayesian Markov Chain Monte Carlo method implemented in BEAST 1.4.7 (Drummond et al. 2002; Drummond and Rambaut 2006; Drummond and Rambaut 2007). This approach estimates marginal posterior probability distributions for model parameters (e.g., time to divergence for a given node), allowing differences among parameters to be assessed for significance. BEAUti 1.4.7, the analysis design utility for building BEAST analyses, implements the HKY and GTR models of nucleotide substitution (with or without invariant sites or a gamma shape parameter) so we used the GTR + I + Γ model, which best approximates the model selected by MODELTEST for the likelihood analysis. We fixed the mean substitution rate and assumed a strict molecular clock based on a likelihood ratio test of clock-like evolution (Felsenstein 1988) that failed to detect a significant difference between maximum likelihood phylogenies generated with and without a molecular clock ($p > 0.1$). We applied the Bayesian skyline plot tree prior (Drummond et al. 2005), which is based on the coalescent and appropriate for intraspecific genealogies. This prior requires fewer assumptions about the model of demographic history than other coalescent priors (e.g., constant population size, logistic growth, exponential growth). Recognizing that molecular clock estimates are often dubious, and lacking a clock calibration for pikas, we applied two different mutation rates (1.5 and 6 % per million years), which conservatively bracket the range of mtDNA rates proposed for other small mammals (Martin and Palumbi 1993). The analysis was run for 20 million steps, sampling every 1000 steps, and repeated three times to test for convergence. We discarded 2000 samples from each run as burn-in.

Results from the three runs were combined using LOGCOMBINER 1.4.7 from the BEAST package, and analyzed in TRACER 1.4 (Rambaut and Drummond 2004).

Demographic analyses

We assessed historical changes in the effective population size of each major lineage using multiple approaches. First, we calculated the F_s (Fu 1997) and R_2 (Ramos-Onsins and Rozas 2002) statistics, which have high power for revealing demographic growth under a model of sudden expansion (Ramos-Onsins and Rozas 2002). Large negative values of F_s and small positive values of R_2 are indicative of population growth. For each test we assessed significance by generating null distributions from 10,000 coalescent simulations of a neutrally evolving, large population of constant size in DnaSP 4.10 (Rozas et al. 2003). Second, we examined pairwise mismatch distributions (generated in DnaSP 4.10) for the signature of demographic expansion from a small ancestral population. A smooth, unimodal mismatch distribution is expected when populations undergo sudden expansions, assuming panmixia and an infinite-sites model of neutral nucleotide substitution (Rogers and Harpending 1992; Slatkin and Hudson 1991). In contrast, multimodal and ragged distributions suggest a stable or contracting population. Population subdivision can mask the effect of expansion by causing ragged distributions, suggesting that the observation of a smooth, unimodal mismatch distribution despite apparent subdivision is good evidence for a recent demographic expansion. However, the corollary to this is that multimodal distributions do not permit us to confidently reject the possibility of expansion when the assumption of panmixia is not met.

The F_s and R_2 statistics and mismatch distributions are based on summary statistics (number of segregating sites, distribution of haplotypes), and therefore fail to make use of all the historical information contained within a sample of DNA

sequences. To explore changes in demographic growth over the history of each major lineage, we employed the Bayesian skyline method (Drummond et al. 2005) implemented in BEAST 1.4.7. This coalescent-based approach estimates the posterior distribution for effective population size at intervals along a phylogeny, thereby allowing inferences of population fluctuations over time. We analyzed each major lineage and the complete dataset for *O. princeps* separately. As in the previous BEAST analysis, we selected the model of nucleotide substitution using MODELTEST 3.7 (Posada and Crandall 1998), applied the best approximation of the model available in BEAUti, fixed the mean substitution rate, and applied a strict molecular clock. We used default settings for skyline model (constant) and number of groups (10; 9 for the Cascades post-glacial dataset; see below). For most lineages, analyses were run for 10 million steps, sampling every 1000 steps and discarding 1000 samples as burn-in. For the full dataset and the Northern Rocky Mountains lineage the run length was 20 million steps with 2000 samples discarded. Analyses were repeated twice using different random seeds to test for convergence. After discarding the burn-in from each analysis, we combined results from multiple runs using LOGCOMBINER 1.4.7 and visualized skyline plots using TRACER 1.4 (Rambaut and Drummond 2004).

Ecological niche modeling

To obtain an independent perspective on the consequences of climate change for range fluctuations in *O. princeps* we developed an ENM for pikas based on current climate data. This model was projected onto climate reconstructions for both the LGM and a future time when atmospheric CO_2 concentrations are double their current levels. Assuming niche conservatism over time (Holt 2003; Peterson et al. 1999; Wiens and Graham 2005), ENMs permit inferences regarding past and potential future

pika distributions. The ENM projections at all time points were based on 19 bioclimatic parameters that are likely to be involved in determining species distributions, such as climatic extremes (e.g., precipitation of the driest quarter), annual averages (e.g., mean annual temperature), and seasonal variation (e.g., range of annual temperature).

We obtained bioclimatic data layers for current (1950-2000) conditions from the WorldClim database (http://worldclim.org/) at a spatial resolution of 2.5 minutes (Hijmans et al. 2005) and masked the data to include only 100° to 130° W and 30° to 60° N. Data on LGM climate were derived from separate datasets representing simulations run using two general circulation models, the Community Climate System Model (CCSM3) (Collins et al. 2006) and Model for Interdisciplinary Research on Climate (MIROC, v3.2) (Hasumi and Emori 2004). These data, originally produced at a spatial resolution of 2.8 degrees and available from the Paleoclimate Modelling Intercomparison Project Phase 2 (http://pmip2.lsce.ipsl.fr/), were statistically downscaled to 2.5 minutes by Robert J. Hijmans (Waltari et al. 2007). We used the CCSM3 and MIROC datasets to generate separate predictions for pika distributions during the LGM and averaged the two for the final result. Data layers for future climate were acquired from the WorldClim database. These data are based on simulations using the Community Climate Model 3 (CCM3) (Kiehl et al. 1998) of the National Center for Atmospheric Research for conditions under which atmospheric CO_2 levels reach 710 ppmv, or twice current levels (Govindasamy et al. 2003).

We constructed an ENM for pikas for current conditions using MAXENT (Phillips et al. 2006; Phillips and Dudik 2008), which implements a maximum entropy algorithm to model species distributions from data on species occurrence and environmental variables. To determine the probability of species occurrence for each point in a given geographic area, the algorithm calculates the probability distribution

that is the closest to uniform (i.e., maximum entropy), but for which the expected value of each environmental parameter is equal to its empirical mean as determined from sample points representing known species occurrence records. We used the Mammal Networked Information System (MaNIS; http://manisnet.org/) to obtain pika occurrence data points from 33 natural history collections. Records with text-only locality descriptions were georeferenced using a combination of the BioGeomancer workbench (Guralnick et al. 2006) and Google Earth™. This resulted in 140 presence records adequately spaced given the spatial resolution of the climate layers. We used 75% of these occurrence records to train the ENM, retaining 25% of the data at random for testing model performance. MAXENT tests the ENM by calculating the area under the receiver operating characteristic curve (AUC) for the training data, test data, and random prediction (Fielding and Bell 1997). If the ENM has good predictive power, the AUC for the test data should approach that of the training data, which itself should approach a value of 1. Conversely, a test AUC below 0.5 indicates that the ENM performs worse than a random prediction model. Given test data, MAXENT further calculates binomial probabilities for several common thresholds to test the hypothesis that the ENM predicts occurrence for test data no better than does a random prediction model.

Use of an ENM to explore past and future species distributions relies on two assumptions. The first is that the most important environmental parameters for determining the distribution of the species are included in the model. Though bioclimatic variables encompass a range of biologically meaningful parameters, other factors, such as interspecific interactions or geology could also be important and are not included in our model. For example, all living Nearctic pikas are restricted to cool, mesic, rocky habitat. Patches of appropriate talus habitat exist as a subset within the broader climatic envelope predicted by the WorldClim climatic variables, but can also

exist as isolated patches of microhabitat outside of that broad envelope (e.g., extant populations in lava flows in low-elevation, xeric sites in California and Idaho; Hafner 1993). The second assumption is that the niche that is being modeled has been relatively conserved through time (Peterson 2003). This assumption has been generally supported for a variety of species (Martinez-Meyer and Peterson 2006; Peterson et al. 1999; Wiens and Graham 2005), although there is some debate over whether or not the American pika's niche was more relaxed during the LGM than it is currently. Specifically, Hafner (1993) has debated with Mead (1987) and Mead and Spaulding (1995) as to whether fossil records of Nearctic pikas should be used as indicators of a rocky/talus niche. If the niche was broader during the LGM, our ENM will err on the side of predicting an overly restrictive habitat distribution; thus our inferences of range expansion would be conservative and under-predict the possible LGM species distribution.

RESULTS

Phylogeny and divergence

We identified 104 unique haplotypes among 175 cyt *b*/D-loop sequences. The distribution of nucleotide variation in these sequences matched expectations for true mtDNA. In the cyt *b* coding region, substitutions occurred primarily at third positions, and synonymous substitutions outnumbered nonsynonymous by a ratio of 11:1. The non-coding D-loop was more variable and included several indels of varying lengths. Two intervening tRNAs (tRNA-Thr, tRNA-Pro) were highly conserved. We did not detect positive selection in the data (Tajima's $D = 0.477$, $P = 0.76$).

Phylogenetic analyses were robust, with strong concordance between likelihood and Bayesian results. Multiple Bayesian analyses yielded nearly identical topologies and high posterior probabilities for relationships at all levels of the tree,

confirming that the American pika is monophyletic and sister to the collared pika, *O. collaris* (Figure 1.2). Our topology revealed five well-defined clades within *O. princeps* that differed by 1.6 – 2.7% net uncorrected sequence divergence. Lineages were genetically diverse, as indicated by relatively high haplotype and nucleotide diversity (Table 1.1), and no haplotypes were shared between populations. Geographic distributions of four lineages match the Cascade Range, Sierra Nevada, Northern Rocky Mountains, and Southern Rocky Mountains lineages previously identified in the allozyme study (Hafner and Sullivan 1995). These names reflect the core geographic distributions of each lineage, and we continue to use them here for consistency. A fifth lineage united several populations in central Utah that were previously unassigned and thought to represent sites of contact between other neighboring lineages (Figure 1.1; Hafner and Sullivan 1995). Hereafter, references to differentiated lineages refer specifically to these five major mtDNA clades unless otherwise noted. Geographic congruence between the mtDNA and allozymic lineages supports the contention that they are the result of extrinsic barriers to gene flow (Riddle 1996), rather than the consequence of intrinsic stochastic processes such as lineage sorting (Irwin 2002). High nodal support at the base of the pika phylogeny indicates that the Cascade Range lineage diverged first, followed by the Sierra Nevada lineage. In contrast, the three easternmost clades (Northern Rocky Mountain, Southern Rocky Mountain, Central Utah) form a poorly resolved but monophyletic group. Lineage coalescence times for each of the major clades are roughly concordant, given overlapping posterior probability distributions generated in BEAST (Table 1.2). They are also relatively deep, predating the LGM regardless of mutation rate.

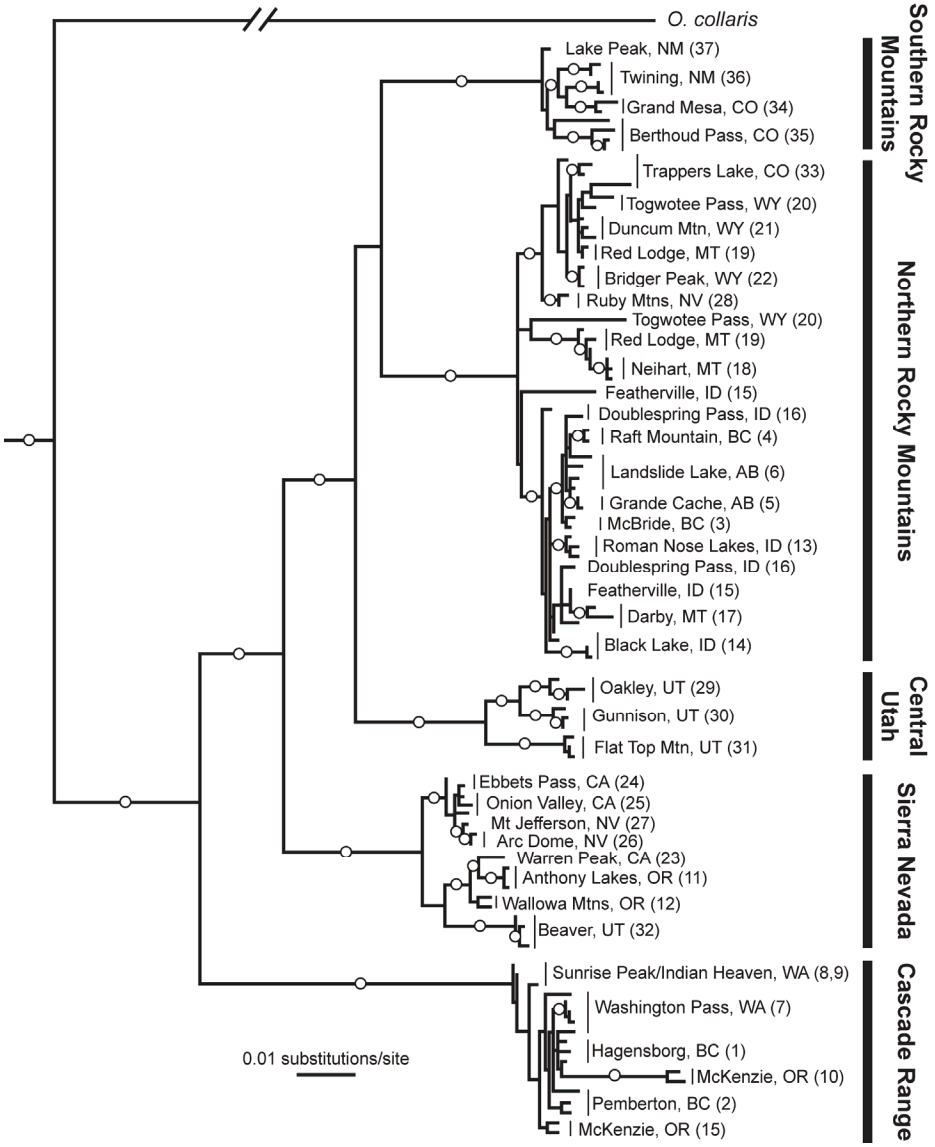

Figure 1.2. Maximum likelihood phylogeny based on mtDNA haplotypes. Outgroups, with the exception of *O. collaris*, have been pruned to improve clarity. Open circles on branches indicate Bayesian posterior probabilities >95%. Locality numbers in parentheses cross-reference with Figure 1.1 and Appendix.

Table 1.1. Summary statistics, results of tests of population expansion, and the substitution model applied to the Bayesian skyline analysis of demographic history. Number of individuals (*N*), haplotype diversity (*h*), percent nucleotide diversity (π), mean number of pairwise nucleotide differences (k), Fu's F_s, and the Ramos and Rozas R_2 statistic.

Lineage	*N*	*h*	π (%)	k	F_s [a]	R_2 [a]	Skyline model
Southern Rocky Mountains	20	0.905	0.939	15.67	2.299	0.132	HKY + I
Northern Rocky Mountains	79	0.986	1.266	20.94	-8.483	0.079	HKY + I + G
Northern Rocky Mountains (post-glacial)	25	0.937	0.517	8.57	-0.305	0.108	HKY + I
Northern Rocky Mountains (non-glaciated)	54	0.984	1.355	22.51	-3.633	0.096	HKY + I + G
Central Utah	15	0.914	1.397	23.30	3.850	0.194	HKY + I
Sierra Nevada	35	0.943	1.098	18.31	0.833	0.136	GTR + I
Cascade Range	26	0.966	0.843	13.97	-1.785	0.099	GTR + I + G
Cascade Range (post-glacial)	10	0.911	0.453	7.56	1.394	0.165	HKY + I
Cascade Range (non-glaciated)	16	0.942	0.977	16.18	-0.064	0.141	GTR + I
Total	175	0.992	4.285	70.40	-5.028	0.110	GTR + I + G

[a] None of the F_s or R_2 statistics were significantly different from expectations based on the null model of constant population size.

Table 1.2. Coalescence times for lineages. The mean and 95% highest probability density (HPD) interval of posterior probability distributions generated by BEAST are given in million year (My) units for both 1.5%/My and 6%/My mutation rates.

	1.5%/My		6%/My	
Lineage	mean	95% HPD	mean	95% HPD
Cascade Range	0.876	0.604 - 1.153	0.219	0.151 - 0.288
Sierra Nevada	0.857	0.615 - 1.113	0.214	0.154 - 0.278
Central Utah	0.957	0.691 - 1.253	0.239	0.173 - 0.313
Southern Rocky Mountains	0.619	0.451 - 0.813	0.154	0.113 - 0.203
Northern Rocky Mountains	0.926	0.720 - 1.153	0.232	0.180 - 0.288

Historical demography

The Cascade Range and Northern Rocky Mountains lineages both extend from historically unglaciated regions with sky islands into post-glacially colonized areas (Figure 1.1). These two lineages therefore provide a direct comparison between elevation shift and latitude shift range fluctuation dynamics. We conducted separate demographic analyses on the post-glacial (Cascade Range populations 1 and 2; Northern Rocky Mountains populations 3, 4, 5, 6, 13) and non-glaciated (all other Cascade Range and Northern Rocky Mountains populations) portion of the range of these lineages, in addition to the total analyses including all populations.

Tests of sudden demographic expansion based on summary statistics did not reveal evidence of population growth in any lineage. In all cases, F_s and R_2 statistics failed to reject the null hypothesis of constant population size (Table 1.1). Likewise, pairwise mismatch distributions were ragged and in many cases strongly multimodal (Figure 1.3), reflecting substantial genetic structure that does not match the expectation for a population that underwent sudden expansion from a small effective population size. Mismatch distributions for post-glacial populations in both Cascade and Northern Rocky Mountains lineages are slightly more unimodal than those of other population sets, but recent range expansion along the northern montane axes has apparently not resulted in a strong signature of demographic expansion.

Bayesian skyline plots revealed a more complex demographic history. Skyline plots for all lineages showed a negative trend in effective population size during the most recent time interval (Figure 1.4). In the Sierra Nevada, Central Utah, and Southern Rocky Mountains lineages, this decline follows a period of relative stability. In contrast, the two northern lineages (Cascade Range and Northern Rocky Mountains) exhibit a clear signature of population expansion in the period preceding

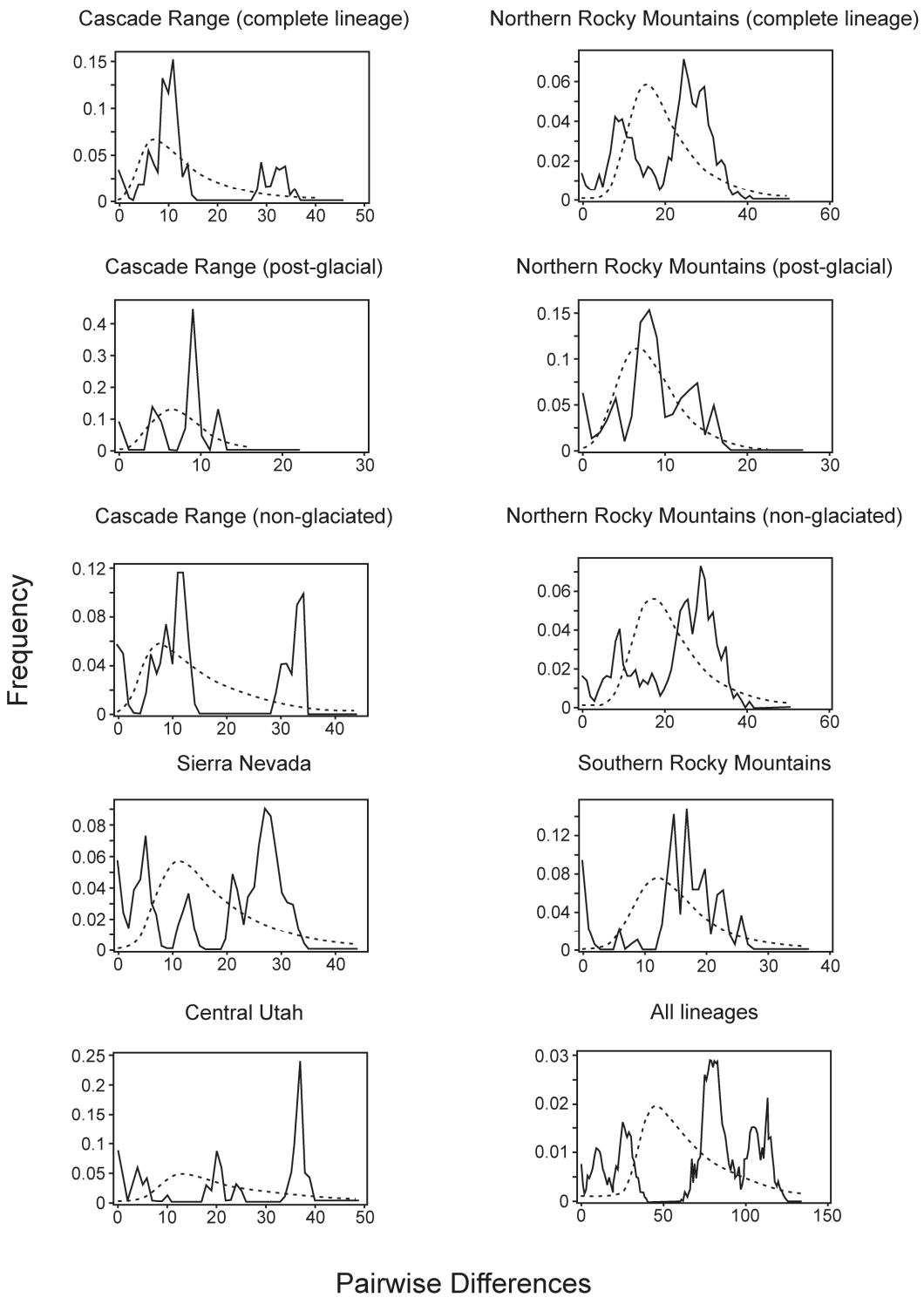

Figure 1.3. Mismatch distributions for all major lineages and lineage subsets. Solid lines indicate the observed frequency of pairwise nucleotide differences between sequences, and dashed lines represent the expected distribution based on a model of sudden population expansion (Rogers and Harpending 1992).

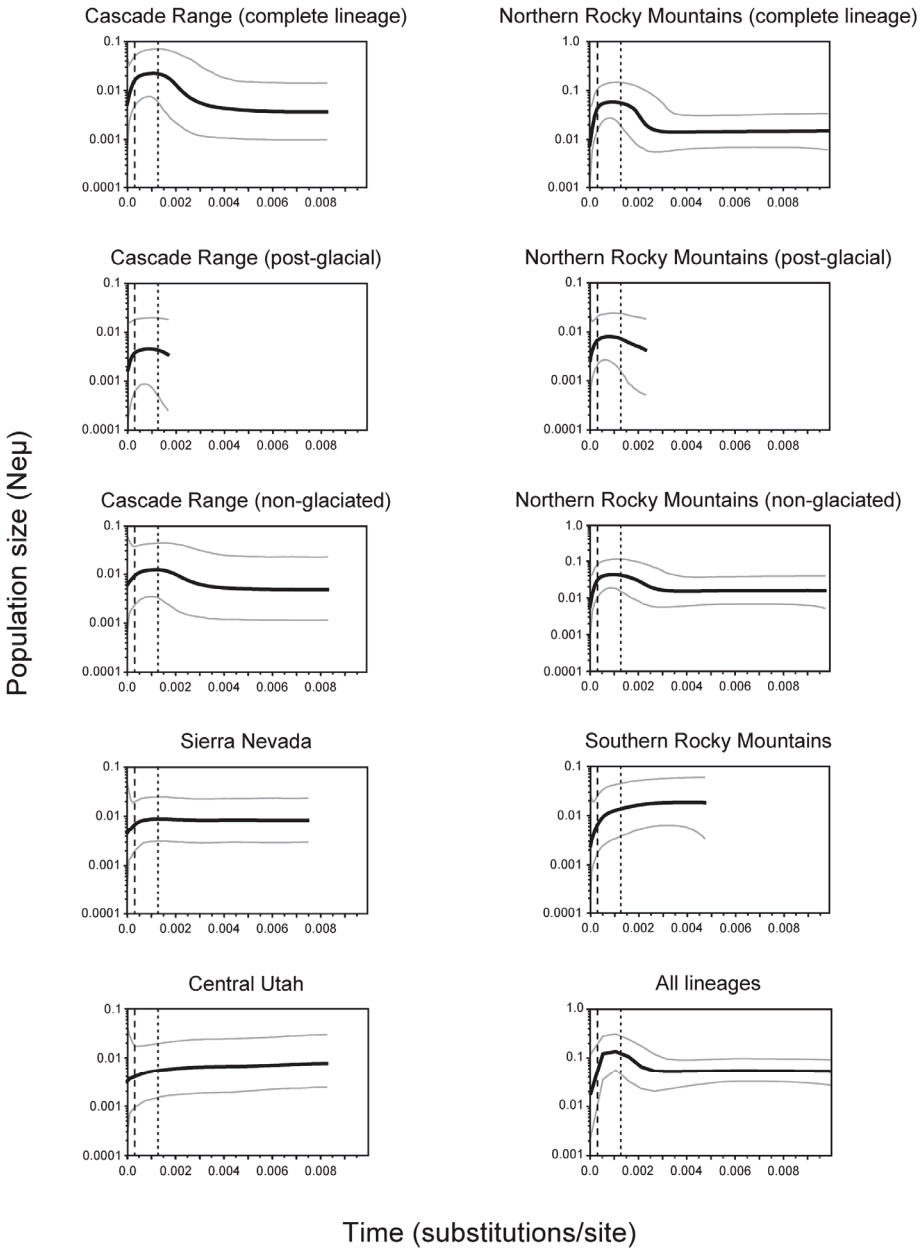

Figure 1.4. Bayesian skyline plots for all major lineages and lineage subsets showing effective population size plotted as a function of time. Note that the time scale begins with the present on the left and is given in substitutions/site, which can be converted to units of time via a molecular clock calibration. Black lines indicate the median value of effective population size; gray lines denote the 95% highest posterior probability interval. The position of the LGM (21 ka) is indicated based on a 1.5% (dashed vertical lines) or 6% (dotted vertical lines) mutation rate. The horizontal axis has been scaled to show the same interval (0 – 0.01 substitutions/site) for all plots, requiring the "All lineages" plot to be truncated. The discarded portion showed no evidence of change in effective population size.

the recent decline. Application of the 1.5%/my and 6%/my mutation rates to assess the timing of population expansion and contraction indicates that expansion preceded the LGM, and contraction followed it (Figure 1.4). Under the assumption that the peak in population size for the two lineages coincides with the LGM, we calculate an average mutation rate estimate of approximately 4.8%/my.

Ecological niche models

The ENM for *O. princeps* had excellent predictive power for species occurrence under current conditions. The AUC for the receiver operating characteristic curve of the test data (0.92) was substantially better than that of a random model (0.5) and close to that of the training data (0.975). Also, test data were predicted significantly better by the ENM than by a random model for all eleven thresholds tested by MAXENT ($P << 0.001$).

For all three time periods, we report the logistic output of the ENM (Figure 1.5), which represents the probability of species occurrence. Rather than apply a single arbitrary threshold, we present contours for four ranges of occurrence probability ranging from 0.1 to 1. At the lower limit of this range, the area of suitable habitat predicted by the model encompassed 99% of all training data, suggesting that this is a relatively conservative threshold for predicting occurrence. The prediction for current climatic conditions is largely consistent with the known distribution of *O. princeps*, suggesting that pikas occupy talus microhabitat within most areas with suitable climatic conditions found throughout the Intermountain West. Results for the LGM show a dramatic expansion of suitable climatic conditions across the region, especially in the Cascade Range, Northern Rocky Mountains, and Sierra Nevada Mountains. Under future climatic conditions the projected species distribution is greatly restricted, with particular losses in the Sierra Nevada and throughout the southwestern portion of

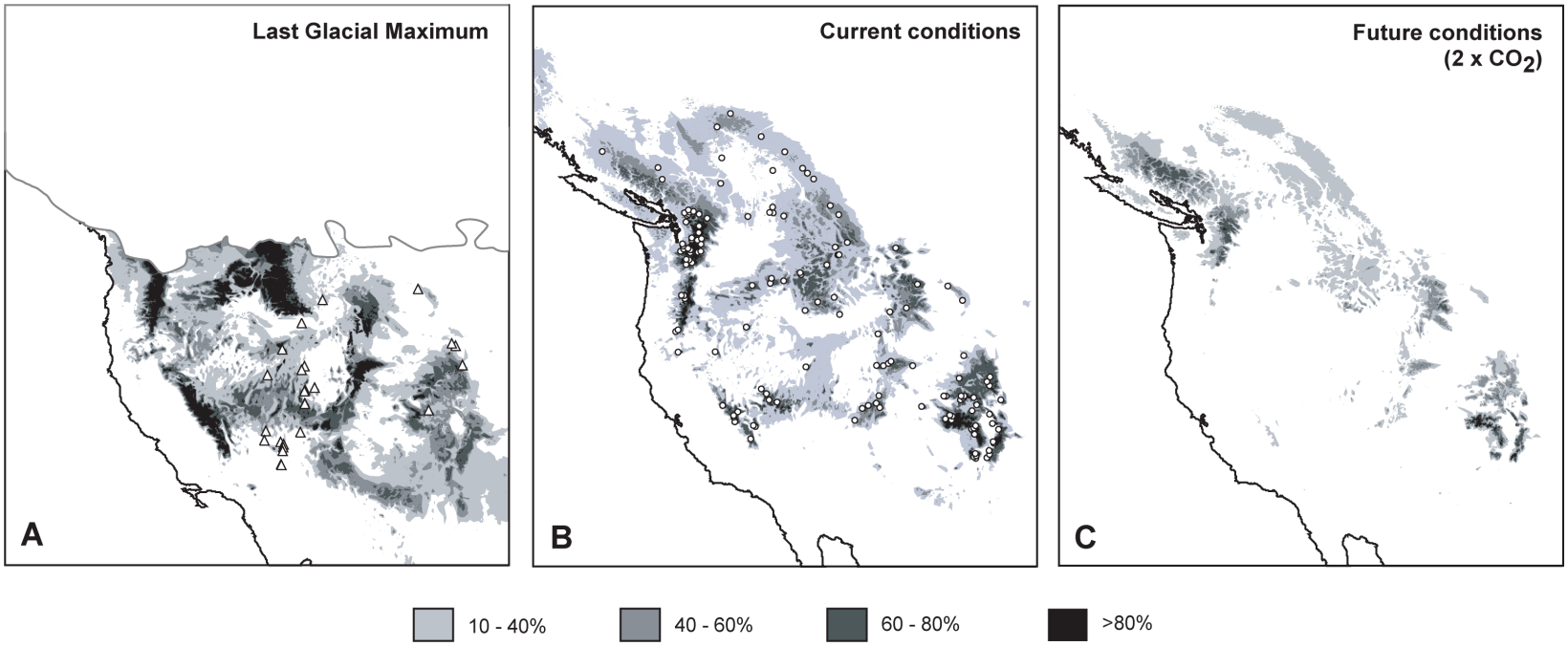

Figure 1.5. Modeled pika distributions for: A) LGM; B) current; and C) future climatic conditions. Darker areas indicate higher probabilities of occurrence. The area shown in all maps is 103° to 130° W and 30° to 60° N. Note that the continental margin depicted on the LGM map differs from the others because sea levels were lower during glacial periods. On the LGM map, the approximate extent of the continental ice sheet is masked in white. Open triangles indicate fossil pika localities that date to the Late Pleistocene (Grayson 2005; Hafner 1993; Mead 1987). On the current conditions map, open circles indicate localities used to train and test the ENM.

the species range. An ENM based solely on populations of the Sierra Nevada lineage (not shown) was not appreciably different from predicted LGM, current, or future distributions of that lineage, indicating that this more southern form that is found throughout sky islands in the Great Basin desert is apparently not more adapted to xeric or warmer conditions than pikas found elsewhere.

DISCUSSION

Range fluctuation and genetic differentiation

Our data show that the current distribution of American pikas was shaped by both latitude shift and elevation shift range fluctuation dynamics. Following the LGM, suitable habitat at high latitudes only became available with the retreat of the North American continental ice sheets. The northernmost populations therefore reflect recent post-glacial expansion (Figure 1.1), which we predicted should result in phylogeographic patterns consistent with the latitude shift model, i.e., lineage distributions that reflect extensions from refugial sources and relatively low genetic diversity. Our data confirm these predictions. Post-glacial expansion occurred along two major mountain axes, the Cascade/Coastal Range and Northern Rocky Mountains. Post-glacial populations of the two genetic lineages associated with these mountain ranges are closely related to pikas from the northern portion of the non-glaciated range (Figure 1.1), and exhibit decreased genetic diversity relative to more southerly distributed populations (Table 1.1). Both lineages apparently followed retreating glaciers northward from non-glaciated southern (refugial) areas, yielding elongate post-glacial distributions.

Below the lower limit of the LGM continental ice sheets, the distribution of pika populations has largely been determined by climate-driven shifts up and down elevation gradients. Fossils dating to the LGM demonstrate that pika populations were

established at lower elevations and latitudes when climate was cooler (Hafner 1993; Mead 1987) and subsequent climate warming during the Holocene has caused range retraction to isolated sky islands (Grayson 2005). Warming during past interglacials was similar to or more extreme than that of the Holocene (Petit et al. 1999), suggesting that the extent of contemporary population fragmentation may be similar to that of past interglacial periods. Population expansion and retraction from and to sky islands is consistent with our elevation shift model of range fluctuation, and was probably the major pattern of climate-driven change across much of the non-glaciated portion of the American pika's distribution.

We predicted that because elevation shifts are associated with persistent sky island populations and ephemeral low-elevation populations, isolation in sky islands during interglacials would represent the major driver of lineage diversification. Lineages would therefore be most numerous at low latitudes where fragmentation is extensive and climate warming presumably had the greatest influence. Our data reject this prediction, instead suggesting that elevation shifts at local scales (i.e., individual sky island populations) helped to maintain genetic cohesion at regional scales. We show that *O. princeps* is subdivided into relatively few major lineages, most of which are distributed widely across specific mountain systems (Figure 1.1). Each lineage includes multiple populations that are isolated from one another under current conditions. In addition, estimated coalescence times (Table 1.2) for southern lineages (Sierra Nevada, Central Utah, Southern Rocky Mountains) are not significantly older than times estimated for northern lineages (Cascade Range, Northern Rocky Mountains), indicating that latitudinal variation in interglacial warming and population fragmentation did not result in deeper genetic structure at lower latitudes.

Our results indicate that gene flow during glacial periods contributed significantly to the overall pattern of diversification in pikas. Although population

fragmentation during interglacials caused shallow differentiation at fine spatial scales, as indicated by the current lack of shared haplotypes among populations, range expansion facilitated by cooler climates during glacial periods largely erased these signatures of local differentiation. Periods of climate cooling caused previously fragmented populations within mountain systems to coalesce into regional units, permitting gene flow that maintained genetic cohesion of populations representing each major lineage. The projected distribution of suitable habitat during the LGM corroborates this interpretation (Figure 1.5). Under climatic conditions that prevailed during the LGM, the ENM indicates that areas with high probability of species occurrence were widespread throughout the Intermountain West, suggesting ample opportunity for expansion and contact between once-isolated populations.

Gene flow within lineages certainly increased during glacial periods, but so too did opportunities for gene flow among lineages. Indeed, the results of the ENM analysis of LGM distribution indicate that areas of suitable habitat were largely continuous among lineages (Figure 1.5); likewise, allozymic data revealed mixtures of alleles from neighboring lineages (Hafner and Sullivan 1995). What, then, has prevented breakdown of lineage boundaries? Full-glacial barriers such as mountain glaciers and pluvial lakes that subdivided the Intermountain West may have helped limit gene flow, but interglacial climate warming likely also played an important role in maintaining differentiation. Contact between neighboring lineages probably occurred in low-elevation populations that were also probably the first to be extirpated during elevation shifts caused by post-glacial climate warming (Grayson 2005). For the signature of gene flow to be maintained after the loss of these contact populations, alleles from one lineage would have had to introgress rapidly and deeply into neighboring lineages, reaching populations that would survive interglacial climatic

conditions. Thus, lineage boundaries are maintained by an interaction between glacial-age population expansion and interglacial range retraction.

Life history characteristics of pikas may have further limited the degree of introgression between lineages, even during periods of favorable conditions. Dispersal by pikas is limited to juveniles, which are typically philopatric, though individuals may occasionally disperse 2-3 kilometers (Peacock 1997; Tapper 1973). Adult pikas aggressively defend their territories from conspecifics, reducing opportunities for successful colonization by dispersers (Smith and Ivins 1983). Populations are distributed across talus habitat patches of varying sizes, which act as metapopulations where small populations undergo extinction and recolonization. Though large "mainland" populations may conserve genetic variation (Peacock and Smith 1997), pikas exhibit low heterozygosity (Smith and Weston 1990), suggesting the effect of inbreeding due to small effective population sizes. Under such conditions, introgressed alleles at low frequencies would be vulnerable to loss via lineage sorting. Thus, we would not predict extensive and rapid genetic admixture between lineages even if glacial-age contact was relatively extensive.

Together, range expansion and retraction along elevation gradients have made an important contribution to the distribution of phylogeographic patterns in *O. princeps*. Evidence that lineages were in place well before the LGM (Table 1.2) shows that cyclical elevation shifts have helped to maintain both intra-lineage cohesion and inter-lineage differentiation through multiple climatic oscillations. This suggests that elevation shifts can play an important role in shaping the evolutionary trajectories of species over relatively long time periods, acting both to inhibit (Dynesius and Jansson 2000) and to promote (Lister 2004) differentiation depending on characteristics of landscape and life history.

Range fluctuation and historical demography

The results of our demographic analyses contradict our predictions based on the latitude shift and elevation shift models of range fluctuation. We expected a signature of recent growth for post-glacially colonized populations, and growth followed by decline for populations that underwent a pulse of LGM expansion and Holocene retraction. However, tests of sudden expansion failed to detect evidence of growth in post-glacially colonized populations (Table 1.1; Figure 1.3), and Bayesian skyline plots revealed a universal pattern of recent decline for post-glacial and non-post-glacial populations alike (Figure 1.4). Furthermore, southern lineages that underwent range expansion during the LGM (based on the fossil record; Grayson 2005; Hafner 1993; Mead 1987) do not retain the genetic signature of that expansion.

Our data suggest that latitude alone may be a better predictor of demographic history for alpine organisms, independent of the model of range fluctuation. Northern pika lineages exhibit unambiguous signatures of expansion leading into the LGM and decline after it, while the three southern lineages present the contrasting pattern of relative stability before decline (Figure 1.4). The cause of this latitudinal effect is not readily apparent. Disregarding post-glacial populations, both fossil evidence and the results of our ENM analysis (Figure 1.5) indicate that pika populations had undergone range expansion across all available latitudes by the time of the LGM. Why then is there no genetic evidence of pre-LGM growth in low-latitude lineages?

The disparity may lie in a difference in the extent of LGM expansion at high and low latitudes. If the magnitude of demographic growth necessary to leave a genetic signature is particularly large, and that threshold was achieved by the northern lineages but not the southern, we might expect the contrasting demographic results. The projected LGM distribution of pikas provides some evidence of this; expansion of areas with high probability of species occurrence was greater at northern latitudes than

at southern (Figure 1.5). Alternatively, a difference in the extent of population extinction during Holocene warming could also account for the different demographic signatures. Currently, there is a negative correlation between the minimum elevation of pika populations and latitude (Figure 1.1; Hafner 1993) indicating that extinction of low-elevation populations in the south has been more extensive than in the north. Thus, even if LGM expansion was widespread at low latitudes, the genetic signature of that expansion may have been extinguished with the loss of low-elevation populations. If the deleterious effect of climate warming at higher latitudes was less severe, patterns of genetic diversity that reflect the history of expansion would have a higher probability of being retained.

Because *O. princeps* is sensitive to high temperatures, contemporary climate warming is expected to cause the reduction or extinction of populations at lower elevations and latitudes, and our demographic analysis suggests that the effect of population decline since the LGM can already be detected at the genetic level (Figure 1.4). Evidence that the major pika lineages have persisted through climatic oscillations in the past offers optimism that barring complete extirpation of a major genetic lineage, the species-wide pool of genetic diversity should not be greatly diminished by ongoing climate change. However, our projection of the distribution of *O. princeps* under simulated future climatic conditions suggests that the Sierra Nevada lineage may be at risk of extinction if anthropogenic climate change continues unabated; the ENM predicted that nearly all patches of suitable habitat in the southwestern part of the Intermountain West will be lost if atmospheric CO_2 levels double (Figure 1.5).

Even if complete lineage extinction does not occur, the distribution of diversity across the region will change considerably if local population extinction continues. For example, loss of Great Basin populations since the end of the last glacial

maximum has already restricted the Sierra Nevada lineage to a small number of relictual populations scattered between California and Utah (Figure 1.3; Grayson 2005). Ongoing extinction of Great Basin populations threatens to extinguish these relicts (Beever et al. 2003), causing the loss of potential sources for recolonization of the region during future periods of climate cooling. Their disappearance would yield a dramatic range reduction with important implications for the long-term distribution of the Sierra Nevada lineage.

Demographic retraction, population fragmentation, and lineage extinction can affect the evolutionary potential of American pikas (Myers and Knoll 2001), reducing the likelihood that the species will adapt to future environmental perturbations (Mace and Purvis 2008). Furthermore, the evidence for climatic effects on pika populations highlights the sensitivity of alpine communities to ongoing climate change (Sala et al. 2000), and offers a warning that other alpine species may be at risk. Our results suggest that species with distributions restricted to southwestern sky islands may be of particular concern. Continued efforts to characterize patterns of diversity and genetic effects of climate warming for other alpine organisms are necessary to provide policy makers with general predictions for the future of alpine communities in the Intermountain West (Sutherland et al. 2006).

Conclusions

Our goal was to quantify the genetic consequences of range fluctuation for alpine species, with a specific focus on understanding the effects of elevation shifts for species that fluctuate around sky islands in response to climatic oscillations. We found that for the alpine specialist *O. princeps*, interglacial isolation on sky islands is not the major driver of lineage diversification. Instead, patterns of differentiation are determined by an interaction between gene flow caused by glacial expansion, and

extinction caused by interglacial retraction. We also found that although all pika lineages exhibit a genetic signature of recent population decline, probably reflecting range retraction during the Holocene, lineages at high latitudes retained a longer record of historical demographic change than did lineages at low latitudes.

These results suggest that specific predictions for the genetic effects of the elevation shift model of range fluctuation may be more difficult to identify than for the latitude shift model, but two general patterns emerge. First, contemporary isolation is not a good predictor of either lineage diversity or major historical barriers to gene flow. For alpine specialists, lineage cohesion within mountain systems should be expected due to periodic episodes of gene flow among sky island populations during glacial periods. Second, alpine specialists with narrow thermal tolerances may be expected to exhibit a recent signature of population decline, regardless of their deeper history of range fluctuation. Though further tests of these predictions in other alpine specialists are necessary, this study offers a first step toward developing a general model for understanding the genetic effects of climate-driven range fluctuations in alpine organisms.

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CHAPTER TWO

ISOLATION AND INTROGRESSION IN THE INTERMOUNTAIN WEST: INCONGRUENCE AT MITOCHONDRIAL AND NUCLEAR LOCI ILLUMINATES A COMPLEX BIOGEOGRAPHIC HISTORY FOR THE AMERICAN PIKA (*OCHOTONA PRINCEPS*)

Abstract.— We studied how Pleistocene climatic fluctuations have shaped the distribution of diversity at mitochondrial (mtDNA) and nuclear loci in the American pika (*Ochotona princeps*), an alpine specialist distributed across North America's Intermountain West. We accumulated mtDNA sequence data (~560 – 1700 bp) from 232 pikas representing 64 localities, and sequenced two nuclear introns (MGF, ~550 bp, $N = 148$; PRKCI, ~660 bp, $N = 139$) from a subset of individuals. To assign mtDNA sequences to major pika lineages we conducted a neighbor joining phylogenetic analysis, and we calculated divergence times among the lineages using a Bayesian Markov Chain Monte Carlo approach. Relationships among nuclear alleles were explored with minimum spanning networks, and each lineage was tested for the signature of isolation by distance. Finally, we conducted coalescent simulations of alternative models of population history to test for congruence between nuclear and mtDNA responses to Pleistocene glacial cycles. We found that: 1) all individuals could be assigned to one of five allopatric mtDNA lineages, 2) lineages are associated with separate mountain provinces, 3) lineages originated from at least two rounds of differentiation, 4) nuclear and mtDNA markers exhibited phylogeographic congruence, 5) a signature of isolation by distance was detected from all three loci only in the Northern Rocky Mountains lineage, and 6) inter-lineage introgression at nuclear loci has occurred since the origin of the lineages. Pika populations associated with different mountain systems have followed separate but not completely

independent evolutionary trajectories through multiple glacial cycles. Range expansion associated with climate cooling (i.e., glaciations) promoted genetic admixture among populations within mountain ranges. It also permitted periodic contact between lineages associated with different mountain systems, resulting in introgression at nuclear but not mtDNA loci.

INTRODUCTION

North America's Intermountain West spans the western contiguous United States and southwestern Canadian provinces. The geology (Porter et al. 1983) and biology (Heusser 1983; Spaulding et al. 1983) of this topographically complex landscape was strongly influenced by climatic oscillations of the Quaternary. During glacial periods, continental ice sheets engulfed the northern latitudes, while mountain glaciers and pluvial lakes subdivided the south. Glaciers and lakes retreated during interglacials in the face of climate warming and aridification, the effects of which were more pronounced at lower elevations and latitudes. These climate-driven effects had extensive biogeographic consequences for the biota of western North America (DeChaine and Martin 2005a; DeChaine and Martin 2006; e.g., Graham et al. 1996; Grayson 2000; Knowles 2001), particularly for alpine species that are especially sensitive to climate (Galbreath, Chapter 1; Sala et al. 2000).

For species that live on mountain slopes, climatic shifts can result in elevational displacement of optimal niche envelopes (Guralnick 2007), altering the range and connectivity of available habitat and consequently influencing population distribution, size, and potential for gene-flow. Species are likely to have tracked these niche shifts if they exhibit strong niche conservatism through time, a characteristic that has been observed in a range of taxa, but may not be universal (Martinez-Meyer and Peterson 2006; Peterson et al. 1999; Wiens and Graham 2005). For cold-adapted

species with low niche flexibility, rising temperatures during interglacials restricted distributions to montane sky islands. Conversely, past climate cooling likely allowed the ranges of these species to expand as they tracked local climatic optima to lower elevations. Climatic oscillations during the Pleistocene therefore probably caused repeated episodes of population fragmentation and coalescence for alpine organisms, providing alternating opportunities for genetic differentiation and extensive gene flow.

Phylogeographic studies are contributing to our understanding of the genetic consequences of climate-induced range shifts across elevational gradients in the Intermountain West (e.g., Brunsfeld et al. 2001; Carstens and Richards 2007; Dobes et al. 2004a; Good et al. 2003; Marlowe and Hufford 2008; Spellman et al. 2007). For example, several species exhibit concordant genetic breaks across arid, low-elevation basins such as the Columbia and Wyoming Basins, indicating that isolation associated with warm interglacial climates drove allopatric differentiation across these barriers (Carstens et al. 2005; Carstens and Richards 2007; DeChaine and Martin 2005a; DeChaine and Martin 2006). Though range expansion out of montane sky islands during glacial periods is likely, the evidence suggests that this expansion did not result in range-wide panmixia in alpine species. Large genetic distances between relictual populations in adjacent mountain ranges imply that for some taxa differentiation has persisted through multiple glacial cycles (DeChaine and Martin 2005a; DeChaine and Martin 2006; Nielson et al. 2001). Thus, it would appear that while range expansion associated with cool glacial-age environments may have promoted gene flow within mountain ranges, thereby maintaining regional cohesion of separate genetic lineages, it did not necessarily lead to extensive gene flow between ranges (Galbreath, Chapter 1).

Most of the studies demonstrating that barriers to gene flow persisted through repeated climatic cycles have been based on a single genetic locus, typically from the

DNA of either mitochondria (mtDNA) or chloroplasts (e.g., Brunsfeld et al. 2001; Carstens et al. 2005; DeChaine and Martin 2006; Dobes et al. 2004b; Good and Sullivan 2001). The small effective population size and elevated mutation rate of these cytoplasmic genomes make them useful indicators of phylogeographic structure (Avise 2000; Zink and Barrowclough 2008). However, single-locus studies provide little insight into evolutionary processes (e.g., gene flow) that may have played a greater role in determining patterns of diversity at nuclear DNA (ncDNA) loci. In the Intermountain West, periodic bouts of range expansion during Pleistocene glaciations represent repeated opportunities for contact between isolated populations of alpine species. Though these periods of contact may not have degraded boundaries of neighboring cytoplasmic lineages due to rapid sorting of haplotypes after contact ended (i.e., low frequency introgressive haplotypes would have a low probability of persistence within small population isolates; Galbreath, Chapter 1), introgression at ncDNA loci across historical contact zones is possible. This must be tested to determine the degree to which regional populations have evolved independently or in concert.

Here we study the American pika (*Ochotona princeps*), an alpine specialist that is widespread throughout the Intermountain West, as a model for examining the historical biogeography of the region and the consequences of paleoenvironmental change for patterns of mtDNA and ncDNA diversity in alpine organisms. First, we apply a traditional comparative approach, reconstructing the phylogeographic history of *O. princeps* by comparing patterns of mtDNA and ncDNA diversity within pikas and between pikas and other alpine taxa to test for congruent signatures of isolation and gene flow. Second, we use coalescent simulations to assess the probability of alternative models of population history given our data, with a specific focus on testing the hypothesis that glacial-age contact among pika populations has resulted in

recent (e.g., last glacial) gene flow at nuclear loci despite early (e.g., mid to late Pleistocene) mitochondrial isolation (Galbreath, Chapter 1).

METHODS

Study organism

American pikas are small lagomorphs found almost exclusively in alpine talus habitats (Hafner 1994; Smith and Weston 1990) in most of the major mountain ranges of the western United States and southwestern Canada (Figure 2.1). With low heat tolerance (Smith 1974) and limited capacity for physiological thermoregulation (MacArthur and Wang 1974) pikas are extremely sensitive to local climate. Fossils dated to ~10 – 30 thousand years ago (KyBP) show that pika populations were more broadly distributed at lower elevations during the last glacial maximum than they are today (Hafner 1993; Mead 1987). Since then, previously widespread populations have fragmented into alpine sky islands. Recent extirpation of populations in the Great Basin has been attributed to climate warming (Beever et al. 2003; Grayson 2005), and because they are poor dispersers, pikas are unlikely to recolonize isolated habitat islands (Brown 1971).

In a previous study of mitochondrial (mtDNA) variation in *O. princeps* we showed that the phylogeographic structure of the species reflects both deep vicariance and shallow population-level processes (Galbreath, Chapter 1). Populations belong to five divergent and non-overlapping mtDNA lineages (Cascade Range – CR; Sierra Nevada – SN; Northern Rocky Mountains – NRM; Southern Rocky Mountains – SRM; Central Utah – CU). Four of these lineages are distinguishable by allozymic variation (Hafner and Sullivan 1995), suggesting deep histories of differentiation for both mtDNA and ncDNA. Lineages are associated with separate mountain systems, indicating that glacial-period elevational shifts permitted gene flow within, but not

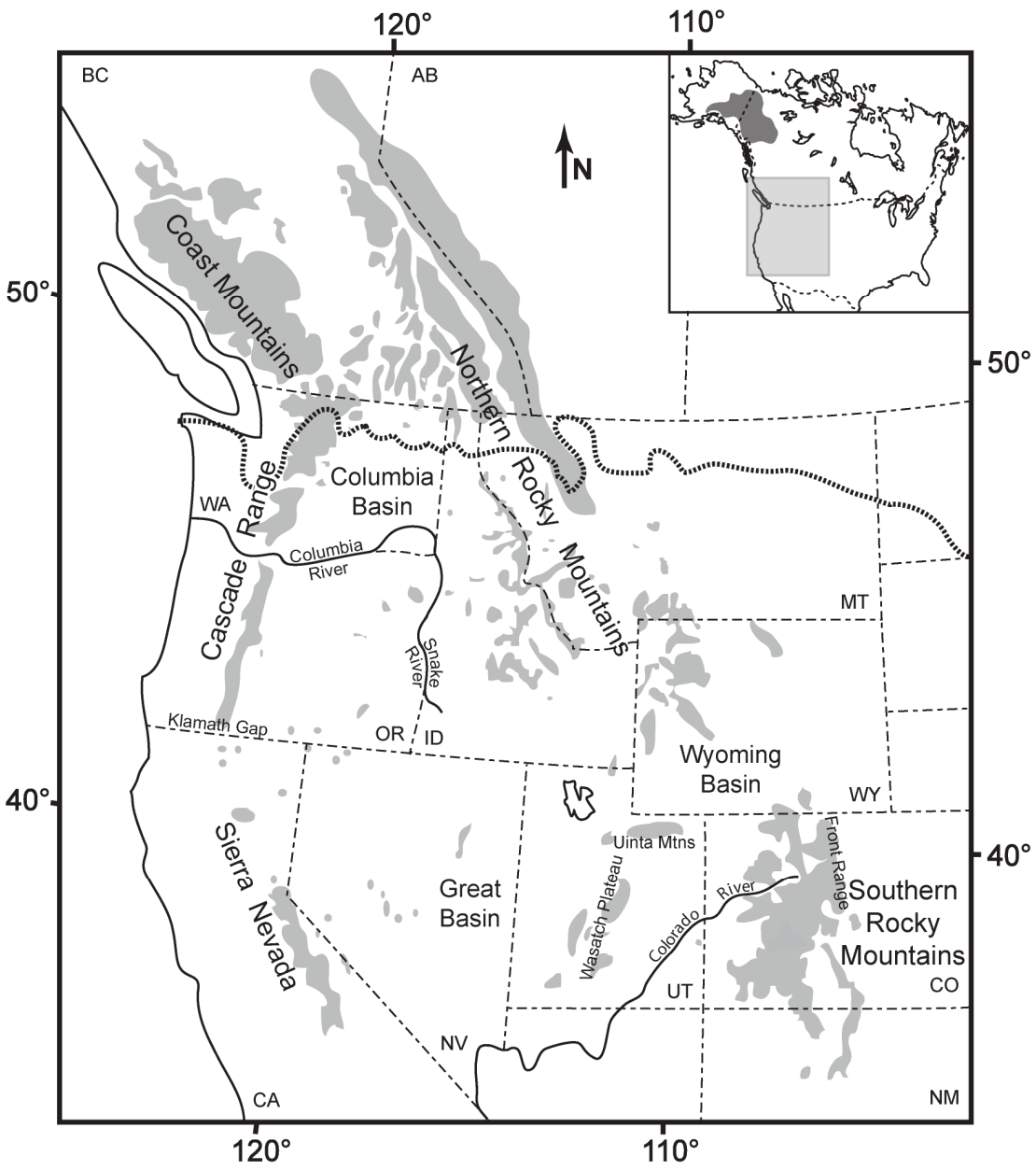

Figure 2.1. Distribution of American pikas and major landscape features of the Intermountain West. The approximate range of *O. princeps* is shown as gray patches on the large map (adapted from Hafner 1993). A heavy dotted line marks the maximum southern extent of the Cordilleran ice sheet during the last glacial maximum. The inset shows the position of the detail map in North America as a light gray box, and the approximate distribution of *O. collaris*, the sister species of *O. princeps*, as a dark gray patch. State and province names are abbreviated as follows: AB = Alberta, BC = British Columbia, CA = California, CO = Colorado, ID = Idaho, MT = Montana, NM = New Mexico, NV = Nevada, OR = Oregon, UT = Utah, WA = Washington, WY = Wyoming.

between, mountain ranges. These cyclical episodes of expansion likely maintained lineage cohesion through multiple cycles of climate change despite periodic range retraction and isolation during interglacials (Galbreath, Chapter 1).

Data collection

For our previous study we collected cytochrome b (cytb) and D-loop sequences (~1700 total bp) from 175 individuals representing 37 localities (GenBank # EU590920 - EU591094; Appendix). For the current study, we added to these data a set of partial mtDNA sequences (~560 bp of the D-loop) from an additional 57 pika specimens representing 52 localities. Twenty-seven of these localities were not included in the previous mtDNA study. We refer to the two datasets as the partial (560 bp) and full (1700 bp) datasets. The specimens used to create the partial and full datasets were drawn from collections that were made during different time periods (1987-1992 and 2004-2005, respectively) and sequencing was performed in independent labs. Including individuals from the same populations but different collections allowed us to check for consistency between datasets. For the partial mtDNA dataset, genomic DNA was extracted from tissues (kidney) using a standard phenol-chloroform extraction method (Hillis et al. 1996). A portion of the mtDNA D-loop was amplified using primers L15774t and H16498 (Shields and Kocher 1991) via protocols described in Hillis et al. (1996), and sequenced on an ABI 337 automated sequencer.

To obtain an independent perspective on population history we sequenced two nuclear introns from a subset of the individuals represented in the mtDNA dataset. We used A and B primers described in Matthee *et al*. (2004) to amplify and sequence introns from the mast cell growth factor (MGF; ~550 bp; $N = 148$) and protein kinase C iota (PRKCI; ~660 bp; $N = 139$) loci. Annealing temperatures were: MGF-A/B,

48ºC; PRKCI-A/B, 55ºC. Polymerase chain reaction conditions for all amplifications followed the methods of (Galbreath, Chapter 1). Sequences are deposited in GenBank (partial mtDNA: EU568283 – EU568340; MGF: EU591095 - EU591291; PRKCI: EU591292 - EU591463). Vouchers and tissue samples of specimens are archived in the mammal collections of the Cornell University Museum of Vertebrates, New Mexico Museum of Natural History, and Humboldt State University (Appendix).

Electropherograms of nuclear loci showed distinctive overlapping peaks at heterozygous nucleotide positions. Allelic sequences were easily determined if only a single site was heterozygous or if an indel permitted overlapping sequences to be traced individually across the electropherogram. In cases where these two methods were not applicable, we either cloned individuals using Invitrogen's Topo TA cloning kit (sequencing four clones per individual), or inferred allelic sequences using the program PHASE (Stephens and Scheet 2005; Stephens et al. 2001). PHASE applies a Bayesian approach to infer haplotypes from multi-allelic loci while accounting for recombination. We ran the algorithm five times, keeping the results of the run that yielded the best goodness-of-fit to an approximate coalescent model. Independent runs from different starting seeds produced equivalent results, with high probabilities of non-singleton base calls (> 0.9) for all MGF sequences and all but two PRKCI sequences. The final MGF and PRKCI datasets included two sequences per individual, whether they were heterozygous or homozygous, to allow for proper estimates of allele frequencies.

Phylogeographic analyses

We previously resolved the relationships among the five major mtDNA lineages (Galbreath, Chapter 1). To determine relationships between the partial mtDNA sequences and these lineages, we combined the overlapping portions of the

partial and full datasets and aligned them using CLUSTALW (Thompson et al. 1994) in MEGA 3.1 (Kumar et al. 2004). Alignments were checked by eye and indels removed. We conducted a neighbor-joining analysis of the combined data in PAUP* 4.0b10 (Swofford 2000) using maximum likelihood distances calculated under the TVM + I + Γ model of nucleotide substitution (rate matrix = 1.0000, 11.0256, 0.4944, 0.4944, 11.6851; gamma shape parameter = 0.5149; proportion of invariant sites = 0.5566), which was selected using AIC in Modeltest 3.8 (Posada 2006; Posada and Crandall 1998). We used the collared pika, *O. collaris*, the sister taxon of the American pika (Formozov et al. 2006; Lissovsky et al. 2007; Niu et al. 2004), to root the phylogeny (GenBank # AF348080).

To estimate lineage divergence times, we collapsed the full mtDNA dataset to haplotypes and analyzed it using a Bayesian Markov Chain Monte Carlo method implemented in BEAST 1.4.7 (Drummond et al. 2002; Drummond and Rambaut 2006; Drummond and Rambaut 2007). BEAST estimates marginal posterior probability distributions for model parameters (e.g., time to divergence for a given node), providing a statistical framework for testing differences among parameters. We used the GTR + I + Γ model of nucleotide substitution, which is implemented in BEAUti 1.4.7, the design utility for building BEAST analyses, and best approximates the model selected by MODELTEST for mtDNA sequence data. A likelihood ratio test of clock-like evolution (Felsenstein 1988) failed to detect a significant difference between maximum likelihood phylogenies generated with and without a molecular clock ($p > 0.1$), so we fixed the mean mutation rate and assumed a strict molecular clock. Because we were working with intraspecific data, a coalescent-based tree prior was appropriate. However, most coalescent priors require simplifying assumptions about the demographic history of a species (e.g., constant population size, logistic growth, exponential growth) that are unrealistic for pikas (Galbreath, Chapter 1).

Therefore we applied the Bayesian skyline plot tree prior (Drummond et al. 2005), which requires fewer assumptions than other coalescent priors. We estimated divergence times for the split between *O. collaris* and *O. princeps* and for four internal nodes in the ingroup (A-E in Figure 2.2) based on a rough substitution rate estimate of 4.8% for pikas (Galbreath, Chapter 1). The analysis was run for 20 million steps, sampling every 1000 steps after a burn-in of 2 million steps. We repeated the analysis three times to test for convergence. For the final parameter estimates we combined the results of the three runs using LOGCOMBINER 1.4.7 from the BEAST package, and analyzed them in TRACER 1.4 (Rambaut and Drummond 2004).

Preliminary phylogenetic analyses of the nuclear loci revealed low levels of sequence divergence and poor resolution of relationships, suggesting that ancestral sequences may be retained and few mutational steps separate alleles. In such cases, a fully dichotomous phylogenetic tree may not provide the best description of relationships among sequences. To assess patterns of differentiation in PRKCI and MGF we used TCS 1.21 (Clement et al. 2000) to generate unrooted minimum spanning networks, in which internal nodes can represent existing sequences and branching relationships need not be fully dichotomous.

We quantified the extent of glacial-age gene flow within lineages by testing the full mtDNA, PRKCI, and MGF datasets for evidence of isolation by distance among populations. Mantel tests of non-random associations between matrices of pairwise inter-population geographic and genetic (Φst) distances were performed using IBDWS 3.15 (Jensen et al. 2005). We applied the K2P substitution model for calculating Φst, and assessed significance with 5000 randomizations. Geographic distances were calculated using Geographic Distance Matrix Generator 1.2.2 (Ersts 2008).

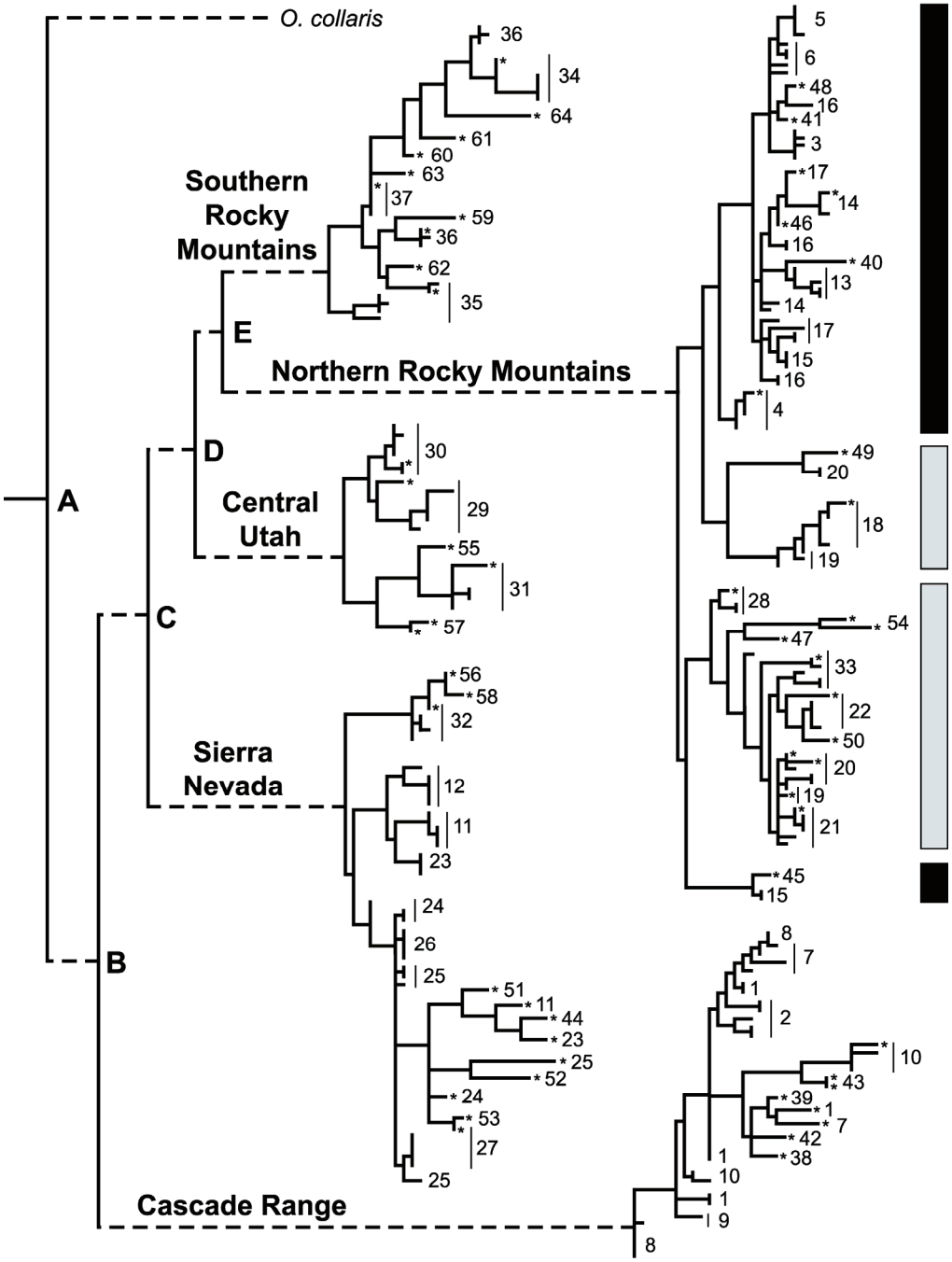

Figure 2.2. Composite of the neighbor joining phylogeny for each mtDNA lineage and the phylogenetic backbone depicting relationships among lineages inferred previously (Galbreath, Chapter 1). Dashed branches indicate uninformative branch lengths. Locality numbers at tips cross-reference with Figure 2.5 and Appendix. Nodes A-E are referenced in the text. Heavy bars adjacent to the Northern Rocky Mountains phylogeny indicate subclades associated with NRM-NW (black) and NRM-SE (gray) phylogroups.

Testing models of population history

We used coalescent simulations to test alternative models of population history within a statistical framework, an approach that has been described thoroughly elsewhere (Carstens and Knowles 2007; DeChaine and Martin 2006; Knowles 2001). In brief, we 1) developed models in the form of phylogenies depicting expected relationships among major pika lineages under a variety of diversification scenarios, 2) calculated 500 coalescent genealogies for each model, 3) simulated nucleotide evolution along the branches of each genealogy to produce matrices of simulated DNA, 4) reconstructed genealogies from the simulated data, and 5) calculated test statistics from each reconstructed genealogy to produce a distribution that could be compared to empirical data. We designed two types of population models to test different plausible historical responses to Pleistocene climatic oscillations.

Models derived from mtDNA diversification – The history of diversification revealed by phylogenetic analysis of mtDNA provides a reasonable starting point for developing testable population models. In this study we show that the five mtDNA lineages of pikas originated through at least two rounds of isolation, possibly during sequential interglacial periods (see Results). Our molecular clock analysis dates the basal split, from which were derived the CR and possibly SN lineages, to approximately 1.3 million years ago. The three eastern lineages (CU, SRM, and NRM) have their origins in a second round of diversification dated to approximately 800 KyBP. These dates substantially predate the fossil record for *O. princeps* in the Intermountain West (Hafner 1993; Mead 1987), and we consider them to be an upper bound on the age of the extant pika lineages. To test this early origin scenario against the possibility of a more recent origin for the lineages, we generated three population models that differed in timing of lineage diversification but maintained the two-divergence tree structure (Figure 2.3). Due to ambiguity regarding the timing of

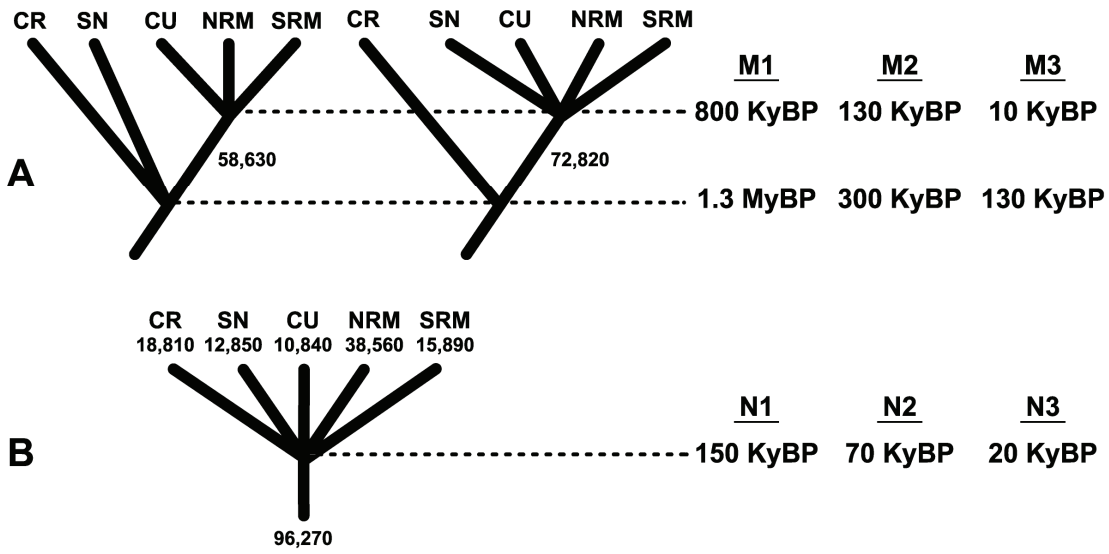

Figure 2.3. Models of population history for coalescent simulations. The two major components of the models are timing of lineage diversification and effective population size. Diversification times varied by model and are listed for each node under the appropriate model number (M1, M2, …). Effective population sizes were held constant for all analyses, and are indicated as a number adjacent to the appropriate branch. The two types of models that we tested are A) those based on a history of two divergences and B) those based on a history of glacial-age gene flow.

differentiation of the SN lineage, we tested separate models for each timing scenario in which the Sierra Nevada lineage was derived from either the first or second divergence event. Results were essentially identical for these alternatives, so we only report findings from the former (i.e., early divergence of CR and SN lineage with subsequent diversification of three eastern lineages). The first model (M1) was based on the deep history of diversification suggested by the molecular clock analysis. Two alternative models posited more recent lineage origins and included divergence events timed to coincide with consecutive interglacial periods: model M2 included an early split at 300 KyBP (pre-Illinoian interglacial) and later split at 130 KyBP (Sangamonian interglacial), and model M3 represented splits at 130 KyBP and 10 KyBP (Holocene interglacial).

Models derived from expectations of glacial-age gene flow – Deep phylogeographic structure at the mtDNA locus implies isolation across lineage boundaries, even during glacial periods when range expansion and inter-lineage contact may have been extensive. If the population histories of the nuclear loci reflect that of the mtDNA, then we would expect all three loci to exhibit patterns of genetic variation consistent with one or more of models M1 – M3. However, if climate cooling during glacials permitted gene flow at nuclear loci despite mtDNA isolation, we would predict the distribution of nuclear diversity to reflect relatively recent subdivision from a single glacial-age population. To test if the nuclear data are more consistent with a deep history of isolation or a recent history of glacial-age gene flow, we constructed 3 population models in which lineages are derived from single ancestral populations associated with the Illinoian (150 KyBP; model N1), 1st Wisconsinan (70 KyBP; model N2), and 2nd Wisconsinan (20 KyBP; model N3) glacial periods (Figure 2.2).

Each branch of the population models requires an estimate of effective population size (N_e) for coalescent calculations. We estimated θ (effective population size scaled by mutation rate) for each lineage and internal branch using the maximum likelihood formulation of the isolation with migration coalescent model implemented in IM (Hey and Nielsen 2004). This was accomplished by setting the maximum divergence time prior in IM to zero, causing the program to treat the complete set of input data as though it came from a single population. We used subsets of the full mtDNA and nuclear datasets for these analyses to allow manageable computation times. Estimates of θ for individual lineages were based on random samples of a maximum of 50 sequences per locus (if a lineage was represented by fewer than 50 sequences, all were included in the analysis). For θ estimates of ancestral populations represented by internal branches in a population model, fifteen sequences were chosen at random from each descendant lineage and pooled.

IM assumes that molecular markers evolve neutrally and are free of recombination. We tested for neutrality of all three molecular markers using DNASP v4.10.4 (Rozas et al. 2003) to perform Tajima's *D* test and found no evidence of selection at any locus (in all cases $p > 0.1$). We also used DNASP to perform a four-gamete test of recombination on both nuclear markers (Hudson and Kaplan 1985). Taken in their entirety, both MGF and PRKCI datasets showed evidence of recombination. However, when sequences associated with each mtDNA lineage were analyzed separately, only the SRM PRKCI data retained a signature of recombination. Therefore, we used all three loci to estimate θ in separate analyses of the CR, SN, CU, and NRM lineages. Estimates of θ for the SRM and internal branches were based on only mtDNA data to avoid breaking the assumption of no recombination. For each locus we used the infinite sites model of nucleotide substitution unless the data did not meet the requirements of that model, in which case we applied the HKY model.

IM runs included 20 chains with a two-step heating scheme. Heating parameters were calculated from preliminary runs using the adaptive two-step heating mode. Multiple runs of varying lengths (1 to 20 million generations) produced very similar estimates of theta for all populations. The minimum effective sample size (ESS) for all analyses was 76, suggesting that parameters were estimated based on reasonably independent observations. To calculate N_e from estimates of θ we used a mtDNA substitution rate estimate for pikas of 4.8%/My (Galbreath, Chapter 1) and a generation time of 2 years (Peacock 1997). Lacking substitution rates for MGF and PRKCI, for results based on all three loci we used the mean value of the mtDNA mutation rate scalar provided by IM to calculate N_e.

We performed coalescent simulations of population models using Mesquite v2.5 (Maddison and Maddison 2008) based on the 75-sequence data subset used to calculate N_e for the ancestral population from which all descendant lineages are derived. Each model was a graphic representation of the population history to be tested, with branch lengths measured in generations and branch widths measured in number of individuals (i.e., N_e). We scaled branch widths of models built for mtDNA simulations down by half to account for maternal inheritance of mtDNA in mammals, which results in an effective population size of approximately half that of the full population given that the sex ratio of American pikas is typically close to parity (Smith and Weston 1990). All models were anchored with *O. collaris* represented by a single individual to ensure proper rooting of reconstructed phylogenies. The divergence time between *O. princeps* and *O. collaris* was deep enough (2 MyBP) to ensure complete lineage sorting between the two species, thereby negating any potential influence of the outgroup on calculations of the test statistic (see below). Next, we simulated nucleotide evolution along the branches of coalescent trees built within the constraints of the models, using an appropriate model of nucleotide

substitution for each genetic marker determined via AIC in Modeltest 3.8 (Posada 2006; Posada and Crandall 1998). Models were as follows: mtDNA – TVM + I + G, base frequencies (A: 0.3005, C: 0.3323, G: 0.1179, T: 0.2493), rate matrix (1.1631, 20.1829, 1.7880, 0.2151, 20.1829), gamma shape parameter (0.7428, 4 categories), proportion of invariant sites (0.6552), scaling factor ($8x10^{-7}$); PRKCI – HKY + G, base frequencies (A: 0.3004, C: 0.1645, G: 0.2043, T: 0.3308), gamma shape parameter (0.133, 4 categories), Ti/Tv ratio (2.9058), scaling factor ($8x10^{-8}$); MGF – GTR, base frequencies (A: 0.3059, C: 0.1666, G: 0.196, T: 0.3315), rate matrix (0.0001, 8.7946, 0.0001, 1.9967, 3.5823), scaling factor ($5x10^{-8}$). There are no formal guidelines for determining an appropriate scaling factor, which compensates for the fact that branch lengths of coalescent trees are measured in generations rather than substitutions per site. To ensure that the characteristics of the simulated data were as realistic as possible, we tested various scaling factors for each marker until mean sequence divergences within lineages were approximately equivalent between empirical and simulated data.

We simulated 500 data matrices for each model and reconstructed genealogies from each matrix in PAUP* using heuristic parsimony searches with 10 random addition replicates, TBR branch swapping, and maxtrees set to 100 (Carstens and Richards 2007). Each search produced majority rule consensus trees, from which we calculated *s* (Slatkin and Maddison 1989), a measure of the discord between the reconstructed gene tree and the assignment of individuals into separate mtDNA lineages. If lineage assignment is treated as a character that is mapped parsimoniously onto the gene tree, the value of *s* is the number of observed character state changes. For example, given five defined lineages, the *s* value for a molecular marker that showed reciprocal monophyly for each set of individuals assigned to these lineages would be 4. Higher values of *s* for a specific locus would indicate that sequences are

paraphyletic with respect to their lineage associations, a possible indication of either gene flow or incomplete lineage sorting. A histogram of *s* values based on the simulations produced a null distribution reflecting the distribution of discord expected for a specific genetic marker and population model. By comparing the *s* value of the empirical data (calculated using the same majority-rule parsimony approach) to the null distribution, we assessed the probability of observing the empirical data given the model. This approach offers a statistical framework for evaluating alternative models of population history while accounting for both the stochasticity of the coalescent and the uncertainty of tree reconstruction (Knowles and Maddison 2002).

RESULTS

Phylogeography

Comparison of the 57 partial mtDNA sequences acquired for this study to the full mtDNA dataset collected previously (Galbreath, Chapter 1) revealed 52 new haplotypes, suggesting that ample unsampled diversity remains in American pikas. However, the deep phylogeographic structure of this diversity appears to have already been captured by the full dataset; all sequences were unambiguously assigned to one of the five major mtDNA lineages by the neighbor-joining analysis (Figure 2.2). Given our extensive geographic sampling across the distribution of *O. princeps* it is unlikely that additional deeply divergent lineages remain to be identified. In general, variation in the partial mtDNA sequence data was consistent with expectations based on the full mtDNA dataset. Four sequences matched haplotypes identified previously, and temporally separated sequences (representing the partial and full datasets) from the same population usually clustered together. Two exceptions were apparent in the SN and CR lineages, where sequences from the partial mtDNA dataset clustered with each other rather than with full mtDNA sequences from the same populations. We

suspect that this is an artifact introduced by differences in the sequencing protocols used to collect these data, though we can not reject the possible influence of demographic effects in the years separating the two samples. This should be explored further with additional sampling from archived specimens collected concurrently with the specimens represented by the partial dataset.

Divergence time estimates for mtDNA lineages reveal a deep and complex history of diversification (Table 2.1). Highest probability densities (95%) for divergence times associated with the youngest lineages (nodes D and E; Figure 2.2) overlapped broadly, implying nearly simultaneous differentiation of the NRM and SRM and CU lineages. Relative timing of differentiation of the SN lineage (node C) is unclear. We cannot reject the possibility that this lineage diverged independently or in concert with either the CR lineage (node B) or the three eastern lineages (nodes D/E). However, we can reject the hypothesis that all five pika lineages underwent simultaneous isolation and diversification. The origin of the CR lineage significantly predated diversification of the NRM, SRM, and CU lineages. Likewise, the split between *O. collaris* and *O. princeps* significantly predated subsequent diversification within American pikas.

The ncDNA loci exhibited substantially less variation (MGF = 4.6% and PRKCI = 9.4% variable sites) than the mtDNA D-loop (25.5%). MGF and PRKCI yielded 27 and 52 unique alleles (excluding indels), respectively, and a combination of cloning and direct sequencing showed no evidence of multiple gene copies at either locus. Minimum spanning networks of the ncDNA markers reveal few clearly monophyletic groups of individuals associated with specific mtDNA lineages. Nevertheless, some ncDNA patterns were similar to the mtDNA results (Figure 2.4). Sequences from populations assigned to the SN mtDNA lineage formed a monophyletic cluster in both ncDNA networks. Cascade Range populations also were

Table 2.1. Divergence time estimates for major nodes in the pika phylogeny (A-E). The mean and 95% highest probability density (HPD) interval of posterior probability distributions generated by BEAST are given in million year (My) units based on a mutation rate of 4.8%/My. Nodes A-E are identified in Figure 2.2.

Node (descendant lineage)	mean	95% HPD
A (*O. collaris* / *O. princeps*)	2.146	1.706 – 2.625
B (Cascade Range)	1.334	1.092 – 1.598
C (Sierra Nevada)	0.997	0.821 – 1.188
D (Central Utah)	0.804	0.658 – 0.954
E (Rocky Mountains lineages)	0.730	0.592 – 0.879

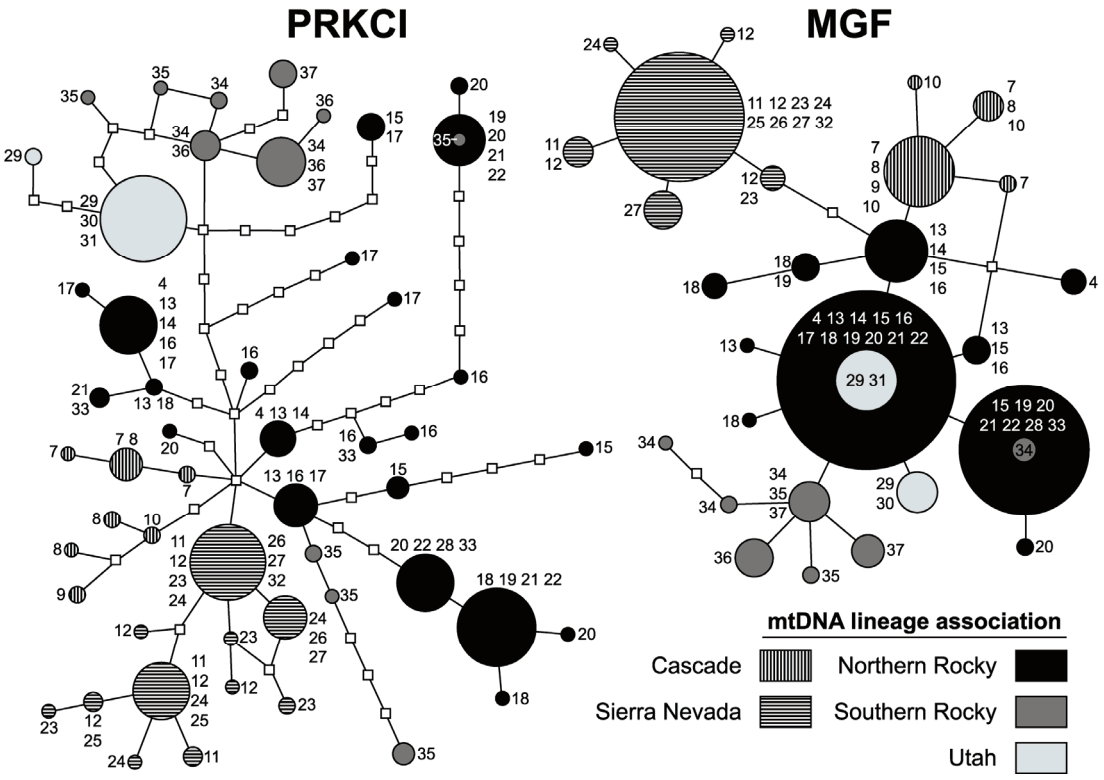

Figure 2.4. Minimum spanning networks for all individuals sequenced for MGF and PRKCI. Circles represent unique alleles and the size of each circle reflects the allele's relative frequency. Numbers next to circles indicate the populations of origin for each allele. Fill color or pattern reflects the mtDNA lineage association for individuals from which each allele was identified. Overlapping circles denote alleles associated with multiple mtDNA lineages. Lines connecting nodes indicate one mutational step, and small open squares represent unsampled hypothetical alleles.

closely related, though they did not form unambiguously monophyletic clades. Both ncDNA markers indicated a close relationship among populations from the SRM, NRM, and CU lineages. Populations of the NRM and SRM lineages shared an allele at both the MGF and PRKCI loci, and NRM and CU populations shared an MGF allele.

Genetic variation is spatially structured at deep and shallow scales. The pooled small and large datasets of mtDNA sequences show that individuals representing different major lineages were never found in the same population, and each mtDNA haplotype we recovered was unique to a specific population. Nuclear alleles also were partitioned into separate subsets of populations belonging to different mtDNA lineages. With the exception of the few shared alleles mentioned above, ncDNA alleles associated with one mtDNA lineage were not found in populations representing another. However, many ncDNA alleles were shared among populations within lineages.

Comparative analysis of the geographic distribution of variation in the three loci revealed little evidence of congruent genetic structure within most lineages; however, the NRM lineage was an exception. This lineage is partitioned into genetically distinct northwestern (populations 3, 4, 5, 6, 13, 14, 15, 16, 17, 40, 41, 45, 46, 48; designated NRM-NW) and southeastern (populations 18, 19, 20, 21, 22, 28, 33, 47, 49, 50, 54; designated NRM-SE) regions, though neither is reciprocally monophyletic at any locus. Both of these regional clusters are represented by two mtDNA subclades that are associated only with either northwestern or southeastern populations (Figure 2.5). This pattern of differentiation holds at the nuclear loci as well. If we consider the distribution of widespread alleles (i.e., alleles found in more than one population), we find for the PRKCI locus that only two of ten widespread alleles are found in populations associated with both phylogroups, and both of these alleles are very rare, having been retrieved from only two individuals each (Table 2.1).

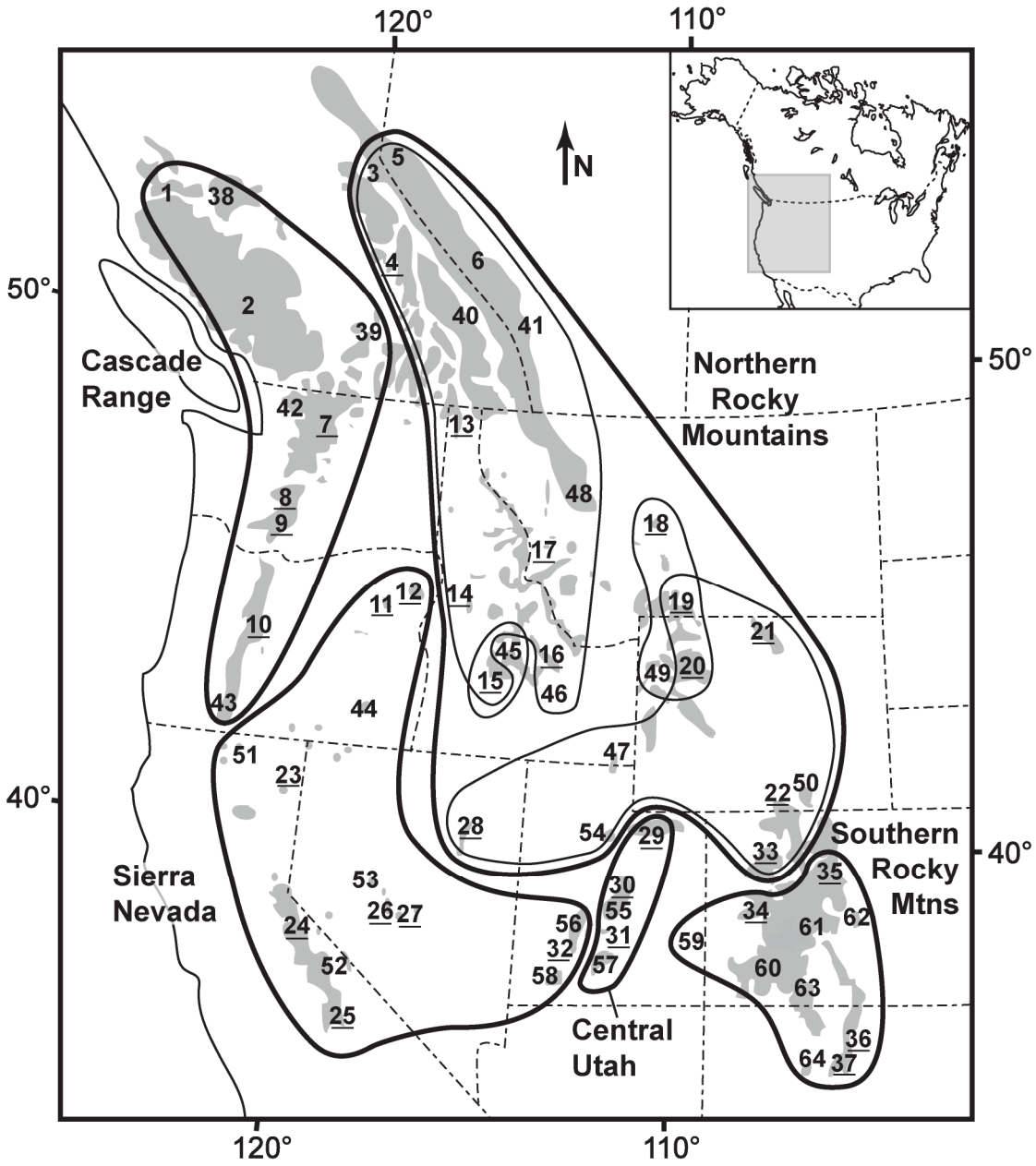

Figure 2.5. Distribution of five major mtDNA lineages. Numbers indicate sampling localities (see Figure 2.2, Appendix) and underscores denote localities from which all three loci (mtDNA, MGF, PRKCI) were sequenced. Mitochondrial subclades associated with the NRM-NW and NRM-SE phylogroups within the Northern Rocky Mountains lineage are denoted by thin lines.

The remaining eight widespread alleles are split into NRM-NW and NRM-SE alleles, four for each group. At the MGF locus the pattern is similar, though unlike the PRKCI locus, there is a very widespread and high-frequency allele (MGF allele A; Table 2.1) that partially obscures the overall signal of differentiation. Other than this high-frequency allele there are four widespread MGF alleles. Of these, two are associated only with NRM-NW populations, one with NRM-SE populations, and one primarily with NRM-SE populations, but with a low frequency (4.4%) presence in population 15. Randomization analyses (10,000 replicates), in which alleles are randomly drawn from populations at occurrence rates identical to the empirical data, show extremely low probabilities of observing geographically partitioned alleles as described above by random chance (PRKCI: $p < 0.001$; MGF: $p = 0.007$). An R script used to perform this randomization analysis is available from the corresponding author.

The SN lineage represents an example in which apparent mtDNA structure is not reflected at nuclear loci. Three distinct mtDNA clades are evident (Figure 2.3). Analyses based solely on the large dataset (Galbreath, Chapter 1) indicate strong support and geographic partitioning into northern, central, and eastern clades, though the clustering behavior of the partial D-loop sequences from the small dataset obscures this phylogeographic pattern in the pooled data analysis presented here (Figure 2.3). At the PRKCI and MGF loci, there is no clear signature of allelic partitioning into separate geographic regions (Figure 2.4), with the distribution of widespread alleles not departing from expectations based on random sampling (PRKCI: $p = 0.5961$; MGF: $p = 0.2131$).

Only the NRM lineage exhibited a significant correlation between geographic and genetic distances at all three loci (Table 2.2), indicative of isolation by distance. We detected evidence of isolation by distance at the mtDNA and PRKCI loci of the SN and CU lineages, respectively, though the extremely small sample size (Table 2.2),

Table 2.2. Results of Mantel tests of isolation by distance.

	Lineage	*N* (# popns)	r	*p* (H_o: r ≤ 0)
mtDNA:	CR	6	0.158	0.280
	SN	8	0.354	0.046
	CU	3	-0.387	0.660
	NRM	16	0.443	< 0.001
	SRM	4	0.250	0.245
PRKCI:	CR	4	0.371	0.482
	SN	8	-0.145	0.743
	CU	3	0.938	<0.001
	NRM	13	0.261	0.027
	SRM	4	-0.038	0.373
MGF:	CR	4	-0.547	0.924
	SN	8	-0.184	0.734
	CU	3	-0.830	> 0.999
	NRM	13	0.440	0.001
	SRM	4	-0.264	0.878

and few alleles (Figure 2.4) present in the CU lineage suggest that this significant result is not very robust.

Finally, unambiguous assignment of all populations on the basis of mtDNA sequences allows us to assess the distribution of allozymic diversity (Hafner and Sullivan 1995) for *O. collaris* and the five genetic lineages of *O. princeps* (Table 2.3). The distinctiveness of *O. collaris* is evident, with three fixed alleles and one major (highest frequency, but not fixed) allele that are unique to collared pikas. None of the *O. princeps* lineages are as deeply differentiated. Only the SN (one unique fixed allele) and NRM (two unique major alleles) lineages have major or fixed alleles that are not shared by other lineages. There is no evidence of allozymic substructuring within the NRM lineage to match that observed in the sequence data.

Coalescent simulations

The minimum value possible for the test statistic *s* given five lineages and one outgroup is 5. Because lineage assignments were defined by the mtDNA phylogeny, the *s* value for the empirical mtDNA data was 5, meaning that we could only reject population models that resulted in higher values of *s* (i.e., greater paraphyly among lineages). This is evident in the failure of mtDNA simulations to reject models representing relatively deep origins for the pika lineages; only models involving very recent lineage origins (M3 and N3) were rejected (Table 2.4). In contrast, both PRKCI and MGF had higher empirical *s* values (8 and 11, respectively), permitting better resolution of the model that best fit the observed data. Indeed, only model N2, based on a history of gene flow among lineages during the 1st Wisconsinan glaciation approximately 70 KyBP, was not rejected by simulations of one or both markers.

Table 2.3. Allelic frequencies for variable allozymic loci (summarized from Hafner and Sullivan 1995) and combined sample sizes (in parentheses) for five genetic lineages of *Ochotona princeps* (Southern Rocky Mountains, Northern Rocky Mountains, Cascade Range, Sierra Nevada, and Central Utah) relative to *O. collaris* (samples from the Alaska Range, Alaska) and two sites of past secondary contact (Mt. McLoughlin, southern Oregon; Aquarius Plateau, southern Utah). Major alleles are indicated in bold.

Locus		*O. collaris*	Southern Rocky	Northern Rocky	Cascade	Mt. McLoughlin	Sierra Nevada	Aquarius Plateau	Central Utah
		(40)	(285)	(347)	(97)	(6)	(135)	(13)	(65)
ME2	a	-	0.112	-	-	-	-	-	-
	b	**0.975**	**0.775**	0.196	-	-	**0.881**	-	-
	c	0.025	0.112	**0.804**	**1**	1	0.119	1	**1**
PEPB	a	-	-	-	-	0.417	**1**	0.077	-
	b	**1**	**0.958**	**1**	**1**	0.583	-	0.923	**1**
	c	-	0.035	-	-	-	-	-	-
	d	-	0.007	-	-	-	-	-	-
PEPA	a	-	-	-	0.134	-	-	-	-
	b	**1**	**0.993**	0.104	**0.866**	1	**1**	1	**1**
	c	-	0.007	**0.888**	-	-	-	-	-
	d	-	-	0.009	-	-	-	-	-
PEPC	a	-	0.1	**0.941**	**1**	-	-	-	0.108
	b	-	**0.9**	0.059	-	1	**1**	1	**0.892**
	c	**1**	-	-	-	-	-	-	-

Table 2.3 (continued).

Locus		*O. collaris*	Southern Rocky	Northern Rocky	Cascade	Mt. McLoughlin	Sierra Nevada	Aquarius Plateau	Central Utah
6PGD	a	**1**	-	-	-	-	-	-	-
	b	-	-	**0.543**	-	-	-	-	-
	c	-	**1**	0.457	**1**	1	**1**	1	**1**
αGPD	a	**0.975**	-	-	-	-	-	-	-
	b	-	-	-	-	-	0.038	-	-
	c	-	**1**	**1**	**1**	1	**0.963**	1	**1**
	d	0.013	-	-	-	-	-	-	-
	e	0.013	-	-	-	-	-	-	-
ADA	a	-	-	-	-	-	-	0.038	-
	b	-	**0.965**	**0.882**	**1**	1	**0.852**	0.962	**1**
	c	-	-	0.072			0.148		
	d	**1**	-						
	e	-	0.035	0.056					

Table 2.4. Results of coalescent simulations. The *P* value represents the probability of observing a value of *s* equal to or more extreme than the *s* value calculated from the empirical data, given simulations on a specific model of population history.

H_0 Model	Locus	*P* value
M1 – Two divergence events:	mtDNA	> 0.998
	MGF	< 0.002
1.3 Mya and 800 Kya	PRKCI	< 0.002
M2 – Two divergence events:	mtDNA	> 0.998
	MGF	0.038
300 Kya and 130 Kya	PRKCI	0.004
M3 – Two divergence events:	mtDNA	0.022
	MGF	0.034
130 Kya and 10 Kya	PRKCI	0.01
N1 – Illinoian gene flow:	mtDNA	> 0.998
	MGF	0.06
150 Kya	PRKCI	0.02
N2 – 1st Wisconsinan gene flow:	mtDNA	0.968
	MGF	0.404
70 Kya	PRKCI	0.202
N3 – 2nd Wisconsinan gene flow:	mtDNA	0.034
	MGF	0.002
20 Kya	PRKCI	0.004

DISCUSSION

Origins and introgression

Ochotona princeps is a member of a large, predominantly Old World genus that includes 30 species (Hoffmann and Smith 2005). The genus is of Old World origin (Kurtén and Anderson 1980), and only two species are currently found in North America: the American pika, *O. princeps*, and the collared pika, *O. collaris*, which occurs in Alaska and northern Canada (Figure 2.1). The genus *Ochotona* has a relatively deep history in North America (Mead and Grady 1996). Fossils of an extinct, large species (*O. whartoni*) indicate its continued existence in Alaska beginning in the Irvingtonian (1.6 – 0.3 MyBP) and extending into the mid-Wisconsinan (Mead and Grady 1996). Mead and Grady (1996) speculated that during the early Rancholabrean (0.3 myBP – 11,000 yBP), *O. whartoni* migrated from Alaska south and east into Ontario, Canada, where fossils record their persistence into the early Holocene (~9 KyBP). Fossils of a smaller form consistent with *O. princeps* and *O. collaris* are first known from the Irvingtonian (700 – 500 KyBP) of eastern North America, and indicate a continued but geographically restricted presence in the Appalachian Mountains throughout the Rancholabrean until at least 30 KyBP (Mead and Grady 1996). Fossils that are both morphologically and geographically consistent with modern *O. princeps* do not appear until approximately 33 KyBP (Hafner 1993; Mead 1987).

The shallow fossil record of *O. princeps* in the Intermountain West underestimates the arrival of pikas in the region, as both mtDNA and ncDNA demonstrate that the species was established across its current range well before the last glacial period (2nd Wisconsinan). Assuming that the major mtDNA lineages evolved in association with the mountain ranges in which they are currently found, calculated lineage divergence times place pikas in the Intermountain West since the

mid-Pleistocene (Table 2.1). We note, however, that these single-locus estimates should be considered approximate, and may over-estimate the true history of divergence (Carstens and Knowles 2007).

Additional support for an earlier arrival of pikas in the Intermountain West is apparent in mtDNA and allozymic evidence that lineage diversification has proceeded through at least two glacial/interglacial cycles. Divergence of the CR mtDNA lineage significantly predates the origin of the three eastern lineages (Table 2.1), indicating multiple rounds of range fragmentation that probably occurred during separate periods of warm climate. The allozymic data of Hafner and Sullivan (1995) corroborate this pattern and demonstrate introgressive hybridization of previously isolated lineages. In the previous study of allozymic variation, eight populations exhibited admixture of alleles from neighboring lineages. Inspection of the allozymic data in light of population assignment based on mtDNA leads us to conclude that allelic mixtures in six of these populations (4 of which are assigned to the previously unrecognized CU lineage) resulted from fixation of shared, ancestral alleles rather than gene flow. However, two populations (localities 43 and 57; Mt. McLoughlin and Aquarius Plateau, respectively) likely represent secondary introgressive contact. At one locus (PEBB), both populations exhibit a mixture of fixed alleles from neighboring genetic lineages (Table 2.3). The Mt. McLoughlin population (characterized by a CR mtDNA haplotype; Figure 2.5) is fixed for the CR allele at the ME2 locus and the SN allele at the PEPC locus (Table 2.3). The Aquarius Plateau population (characterized by a CU haplotype; Figure 2.5) also possesses an allele at the ADA locus that is unique to all Nearctic pikas, and may have resulted from the "rare allele phenomenon," which has been invoked to explain the high incidence of novel or otherwise rare electrophoretic variants in natural hybrid zones (e.g., Bradley et al. 1993; Hoffman and Brown 1995).

The distribution of PRKCI and MGF alleles may also reflect secondary contact and introgression between lineages that had already diverged in allopatry. Shared alleles between the NRM and SRM lineages at both ncDNA loci are likely indicators of recent (e.g., last glacial) north to south gene flow given their relatively widespread distributions in the NRM, but not SRM. Furthermore, their restricted presence in the SRM lineage is limited to populations situated along the transition zone between the lineages (Figure 2.4, 2.5). An alternative history may be responsible for the MGF allele that is shared between the NRM and CU lineages. The allele is relatively widespread and of high frequency in both lineages, suggesting that it is old (Watterson and Guess 1977). Coupled with the finding that CU populations retain ancestral allozymic alleles, this suggests that incomplete lineage sorting is a more parsimonious explanation for the shared allele than recent gene flow.

Comparative phylogeography

The distribution of genetic diversity in *O. princeps* is highly structured across the Intermountain West at both deep and shallow scales. Congruence between allozymic (Hafner and Sullivan 1995) and mtDNA lineages, and partial congruence at the MGF and PRKCI loci, demonstrate that extrinsic barriers to gene flow are likely responsible for phylogeographic structure (Riddle 1996), rather than intrinsic processes that act independently on different loci (Irwin 2002). This inference is further strengthened by congruent phylogeographic patterns in co-distributed organisms (Arbogast and Kenagy 2001; Hafner and Riddle 2005; Riddle 1996; Riddle and Hafner 2006; Riddle et al. 2000), as the distributions of some pika lineages closely match patterns of genetic structure identified in other montane or boreal organisms. Regional alpine biotas may have to some extent evolved in concert, responding to paleoenvironmental events in similar ways. Here we assess the relative contributions

of major biogeographic features of the Intermountain West to genetic differentiation in *O. princeps* and other alpine organisms (Figure 2.1).

Over 150 species of plants, fungi, invertebrates, and vertebrates exhibit disjunct distributions in the Cascade Range and Northern Rocky Mountains (Nielson et al. 2001). The intervening Columbia Basin in eastern Washington is an effective barrier to gene flow between the mountain ranges under current climatic conditions, and may have been unsuitable for dispersal during glacial periods as well (Carstens and Richards 2007). Though there is evidence that ephemeral dispersal corridors allowed some taxa to circumnavigate the Columbia Basin during recent glacials (Carstens et al. 2005; Carstens and Richards 2007), the most common phylogeographic pattern among these alpine species is one of long term isolation between populations in the coastal (Cascade and Coastal Ranges) and Rocky Mountain cordilleras. This is evident in diverse taxa, including plants (Albach et al. 2006), mammals (Demboski and Cook 2001), and amphibians (Carstens et al. 2005; Nielson et al. 2001). The distributions of the CR and NRM pika lineages are consistent with such a history of isolation. Secondary contact between lineages may have occurred to the north following the recession of continental ice sheets (Figure 2.1), but the signature of such contact would likely be obliterated during glacial advances.

The CR lineage is bounded to the south by the Klamath Gap of southern Oregon. This physiographic break in the coastal mountain chain, which lies between the southern terminus of the Cascade Range (locality 43) and Mount Shasta (locality 51), marks a major biogeographic discontinuity between the Cascade and Sierra Nevada mountain ranges. Merriam (1899) noted that Mount Shasta shared much of its mammalian fauna with the Sierra Nevada, but had relatively few species in common with the Cascades. More recently, studies of several plant and animal species show phylogeographic breaks in central and southern Oregon (Brunsfeld et al. 2001; see

reviews by Soltis et al. 1997), though not always congruent with the Klamath Gap. Also, allozymic evidence for gene flow between the SN and CR lineages at locality 43 suggests that the gap has been a somewhat permeable barrier, permitting some historical contact between neighboring populations.

A third important biogeographic barrier for alpine species in the Intermountain West is the Wyoming Basin, which forms a major rift along North America's Rocky Mountain axis. As with the Columbia Basin, this lowland area has restricted gene flow for many species associated with montane environments (e.g., Albach et al. 2006; DeChaine and Martin 2004; DeChaine and Martin 2005a; DeChaine and Martin 2005b; Demboski and Cook 2001; Wilson et al. 2005), and we would predict a similar pattern in pikas. However, our data do not implicate the basin as a major driver of either inter- or intra-lineage diversification as it is spanned by the southeastern phylogroup of the NRM lineage (Figure 2.5). Also, fossils dating to the Pleistocene–Holocene boundary are scattered across the basin (Mead 1987), demonstrating that *O. princeps* bridged the gap between mountain ranges during the last period of climate cooling.

Differentiation between phylogroups within the NRM lineage contrasts with low phylogeographic structure apparent in other pika lineages (Figure 2.5). This pattern indicates that though pulses of glacial-age gene flow among populations maintained NRM lineage cohesion through deep time, gene flow within the lineage during the last major glaciation was probably not widespread. Evidence of isolation by distance at all three loci is consistent with this interpretation (Table 2.2). Dispersal was likely restricted between NRM-NW and NRM-SE populations. The distribution of the two NRM phylogroups coincides with the division between northern and central physiographic provinces of the Rocky Mountains (Brouillet and Whetstone 1993). Isolation during the last glacial maximum may have been promoted by mountain

glaciers that were particularly extensive in the Rocky Mountains northwest of the Wyoming Basin (Brouillet and Whetstone 1993; Porter et al. 1983), and subsequent range retraction during Holocene climate warming would have prevented gene flow after these glaciers receded. Post-glacial expansion into the Canadian Rockies originated from NRM-NW populations restricted along the western slopes of the Rocky Mountain cordillera in central Idaho, mirroring the northern expansion of the Cascade lineage from a geographically restricted glacial-age distribution (Figure 2.5).

Pikas show phylogeographic patterns not reported for other species. The deep genetic break between the NRM and SRM lineages falls across the relatively contiguous Southern Rocky Mountains of Colorado (Figure 2.5). This genetic discontinuity is concordant with a transition zone in pika vocalizations (Somers 1973); contact occurs here between populations representing distinct northern and southern dialects that may reflect underlying genetic differences. Indeed, if vocalizations play a role in mate choice, call variation could contribute to differentiation by inhibiting mate recognition and gene flow, though this hypothesis remains to be tested. Landscape features of the Southern Rocky Mountains that might have contributed to differentiation include the Colorado River, which separates the northern and southern lineages, and glaciers in Colorado's Front Range (Figure 2.6; Porter et al. 1983). However, the evidence indicating deep origins for the major pika lineages would suggest that these explanations may be too simplistic to account for the full history of isolation. For example, a major barrier elsewhere (e.g., Wyoming Basin) could have been involved in the initial divergence of the two lineages, which subsequently shifted to their current distribution due to changes in availability of habitat. Under the scenario of a recent expansion across the Wyoming Basin from the north, we would predict population admixture and gene flow between the NRM and SRM lineages at localities 22, 33, and 50 (Figure 2.5); however, neither DNA sequence (this study) nor

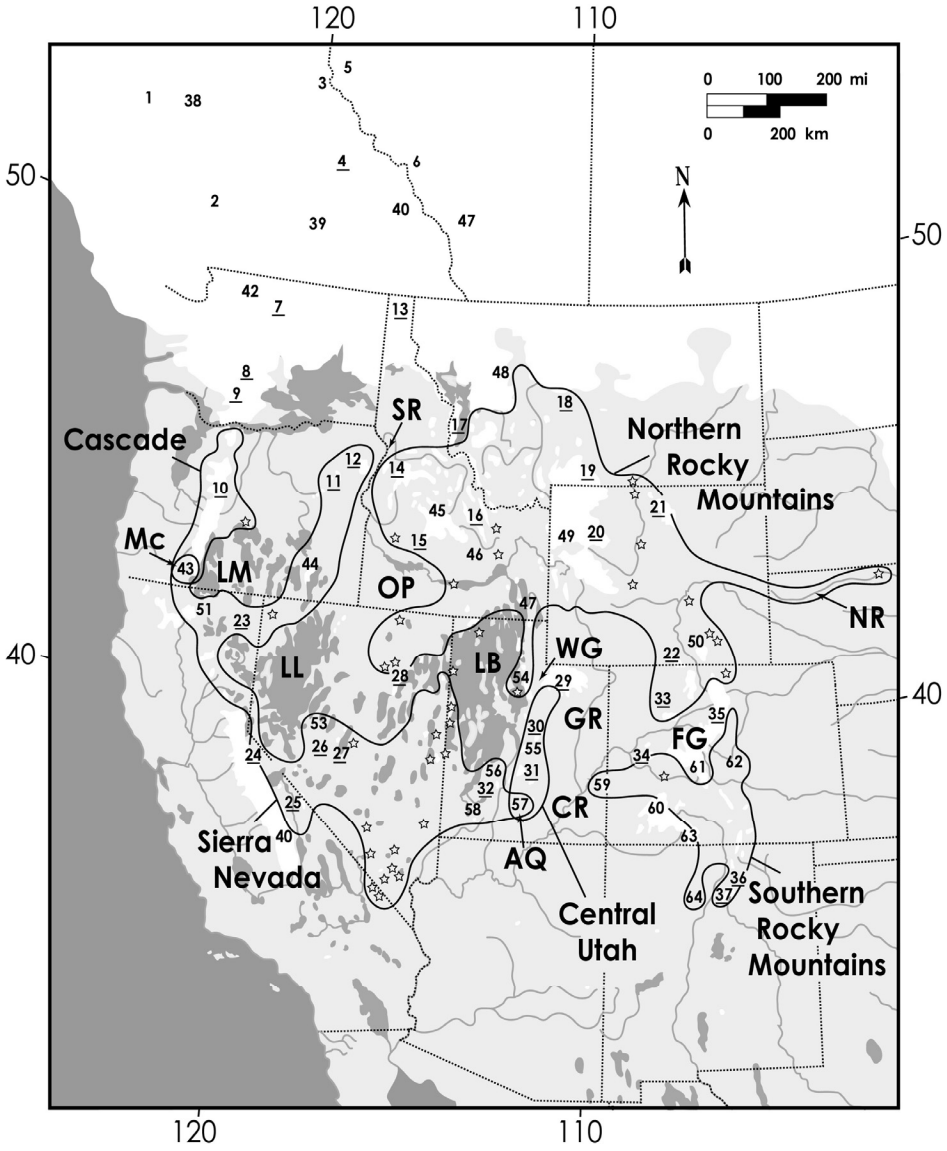

Figure 2.6. Inferred full-Wisconsinan distribution of five genetic lineages of *O. princeps* (Cascade Range, Sierra Nevada, Central Utah, Northern Rocky Mountains, and Southern Rocky Mountains) based on current lineage distributions (this study), fossil sites, and late-Pleistocene landscape features (glaciers, pluvial lakes). White = glaciers. Dark gray = rivers, pluvial lakes, and ocean. Numbers indicate genetic sampling localities. Stars indicate Wisconsinan extralimital fossil sites. Sites of likely Wisconsinan-age secondary contact: Mt. McLoughlin, OR (Mc), Aquarius Plateau, UT (AQ), Wasatch-Uinta glaciers, UT (WG), and Front Range glacier and upper Colorado River, CO (FG). Putative full-Wisconsinan barriers: Lake Modoc, OR (LM; Harney Basin), Lake Lahonton, NV (LL), Lake Bonneville, UT (LB), Colorado River, UT (CR), Green River, UT and WY (GR), Snake River, ID (SR), and Owyhee Plateau, ID (OP). The Niobrara Valley, WY and NE (NR) probably served as a full-glacial dispersal corridor.

allozymic (Hafner and Sullivan 1995) data indicate introgression at these localities. More extensive population-level sampling throughout the Southern Rockies will be necessary to resolve the history of lineage range shifts and introgression that resulted in the current phylogeographic pattern.

The distribution of pikas throughout the southwestern portion of the species range is relictual and highly fragmented; therefore, we would predict deep phylogeographic structure among several narrowly distributed lineages. Indeed, even during glacial conditions the region was subdivided by huge pluvial lakes that were probable barriers to dispersal. Lake Bonneville, which spanned the eastern portion of the Great Basin, inundated over 51,000 km^2 of western Utah at its maximum extension ~17 – 15 KyBP (Morrison 1991; Oviatt et al. 1992). Lake Lahontan, lying across the northwestern third of the Great Basin, was a patchwork of interconnected small lakes in valleys that may have likewise restricted dispersal by terrestrial organisms (Smith and Street-Perrott 1983). However, deep phylogeographic structure is only apparent in the region between the CU and SN lineages. While the restricted distribution of the CU lineage is consistent with the expectation of small, divergent lineages, the widespread distribution of the SN lineage is not (Figure 2.5). Substructure within the SN lineage is relatively weak, especially in comparison to the NRM clade. Differentiation of mtDNA subclades may have been promoted by the presence of the pluvial lakes during recent glacials (Figure 2.2, 2.6), and a significant correlation between genetic and geographic distances is consistent with such a history of restricted gene flow (Table 2.2), but we detected no corresponding ncDNA signature of isolation.

A lack of phylogeographic structure in the SN lineage may reflect a complex history of large scale range retraction and expansion associated with population extinction and recolonization. The extensive fossil record in the southwestern United

States reveals that although pikas were distributed throughout the region during the Late Pleistocene, they vanished from low elevations as climate warmed during the Holocene (Grayson 2005). Ecological niche modeling suggests that suitable habitat for pikas may largely disappear from southwestern mountain ranges if atmospheric greenhouse gas concentrations continue to rise (Galbreath, Chapter 1), and recolonization of the region would likely require a return to glacial conditions. If large scale range fluctuations have been a common feature of the biogeographic history of southwestern pikas, extensive genetic admixture throughout the distribution of the lineage would be expected.

Testing population histories for mtDNA and ncDNA

Our comparative phylogeographic analysis showed remarkable geographic congruence between patterns of diversity revealed by nuclear (allozymic and sequence) and mtDNA data. However, the comparative approach offers a weak test of temporal congruence in the history of differentiation in each of these markers. If all molecular markers had produced identical phylogenetic topologies, it would be reasonable to infer that the same sequence of events led to the observed phylogeographic patterns, but our analyses of two ncDNA loci yielded dissimilar topologies that also differed from the mtDNA tree. While this incongruence could reflect different marker histories, it could also be a consequence of the stochastic nature of lineage sorting and effective population size differences among loci. By using a coalescent approach to simulate possible genealogies for different models of population history, we were able to test whether or not the distribution of variation in the three molecular markers reflects the same population history.

Results of the coalescent simulations indicate that gene flow among lineages at the ncDNA loci during the 1st Wisconsinan glaciation (model N2) best explains the

observed variation (Table 2.4). Population models that involved deeper histories of lineage isolation were rejected because those histories allowed sufficient time for lineages to reach reciprocal monophyly at both loci. Simulations based on mtDNA also failed to reject model N2; however, this was the case for all models with divergence times old enough to yield reciprocal monophyly among lineages. Because lineage identity was defined based on reciprocally monophyletic clades in the mtDNA phylogeny, simulated test statistic values could not be less than the observed test statistic for the mtDNA data. Thus, coalescent simulations based on mtDNA could reject shallow population histories, but could not determine a lower bound on lineage divergence times. Given the deep history of separate, sequential divergence events evident in mtDNA (Table 2.1), the true history for the mtDNA locus probably involves isolation that predates the 1st Wisconsinan. Therefore, we conclude that the distribution of diversity at the mtDNA and ncDNA loci reflect different histories of isolation and gene flow.

A history involving long term isolation at the mtDNA locus and more recent gene flow at ncDNA loci is reasonable and fully compatible with the results of the comparative phylogeographic analysis. At the mtDNA locus, we detected no evidence of secondary introgression between lineages. Conversely, PRKCI, MGF, and the allozymic data of Hafner and Sullivan (1995) all exhibit signatures of glacial-age gene flow between lineages. Furthermore, though we are cautious not to place too much confidence in the inferred timing of ncDNA introgression, we note that gene flow during the 1st Wisconsinan would allow time for the partitioning of alleles associated with phylogeographic congruence that we observed at both deep (between lineages) and intermediate (within the NRM lineage) scales, with subsequent (2nd Wisconsinan) gene flow between the NRM and SRM lineages at populations 34 and 35.

Different processes could be responsible for incongruent histories of gene flow at mtDNA and ncDNA markers. Male-biased dispersal could promote ncDNA admixture without causing mtDNA introgression, but there is no empirical evidence that male pikas are more likely to disperse than females (Smith and Ivins 1983; M. Peacock, pers. comm.). Alternatively, historical introgression of both mtDNA and ncDNA may have occurred during periods of inter-lineage contact, but the signature of that gene flow was only retained in the ncDNA. Differences in genomic effective population sizes may be sufficient to explain such a history. With lineage sorting times approximately four times greater than mtDNA, ncDNA loci are much more likely to have retained low frequency alleles introduced from neighboring lineages during intermittent periods of contact.

Our use of coalescent simulations to test for congruent histories among independent loci represents a novel approach to addressing the question of whether phylogeographic incongruence between loci is a consequence of recent introgression, incomplete sorting of ancestral polymorphisms, or a combination of the two. We were able to reject the alternatives of very recent gene flow and very old isolation to account for paraphyly at ncDNA loci, demonstrating statistical support for a more complex history that would be difficult to resolve using traditional comparative phylogeographic methods. This highlights the potential of statistical phylogeographic methods to enrich historical perspectives by allowing explicit tests of hypotheses developed from comparative phylogeographic studies (Knowles and Maddison 2002).

Coalescent simulations offer a useful tool for testing phylogeographic hypotheses while taking into account both inherent stochasticity of the coalescent and error in gene tree reconstruction; however, we recognize that the method also has limitations. In particular, simulations are only informative if the model upon which they are based is biologically realistic, which generally comes at the cost of making a

model more parameter rich (Knowles 2004). Each parameter estimate introduces error that can decrease the accuracy of the final result, but too few parameters may lead to oversimplification that fails to capture the most important aspects of population history. For example, our coalescent simulations assumed panmixia within lineages, a condition that certainly does not reflect the population structure of *O. princeps* throughout its entire history. A more realistic model might include cycles of intra-lineage population fragmentation and coalescence in association with climate warming and cooling. Such a history could lead to retention of alleles over longer time periods than expected under a history of panmixia, suggesting that timing estimates derived from our simulations may be too shallow. However, because this change to the population model would presumably affect all loci similarly, the relative timing of specific events (e.g., diversification of mtDNA lineages vs. gene flow at ncDNA loci) should be minimally affected, even if the absolute timing is incorrect. Thus, our major finding of incongruence between loci should be relatively robust.

Conservation implications

Ochotona princeps is an indicator of healthy alpine ecosystems, and may provide early warnings of biotic effects of climate change at high elevations (Krajick 2004). With the future of some pika populations in question (Beever et al. 2003), our study provides important baseline data on the distribution of genetic diversity. We show that the five major pika lineages have independent evolutionary trajectories at multiple loci and should probably be considered distinct evolutionarily significant units (sensu Moritz 1994) for management purposes. Evidence for substructure within lineages, as well as apparent isolation among populations (e.g., no shared mtDNA haplotypes) may warrant management at finer scales, though this requires better resolution of genetic structure and connectivity at intermediate levels. Future research

should investigate variation among the major lineages that may be relevant to targeted conservation efforts. For example, differences in phylogenetic relationships (Galbreath, Chapter 1; Hafner and Sullivan 1995), vocalizations (Somers 1973), and parasite assemblages (Galbreath, Chapter 3) could have implications for the success of strategies such as population translocation.

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CHAPTER 3

EPISODIC HOST-SWITCHING DRIVES PARASITE DIVERSIFICATION IN A PIKA-PARASITE ASSEMBLAGE

Abstract.— Host-parasite co-differentiation, in which parasite populations diverge in response to host population divergence, is widely viewed as a major driver of parasite diversification, yet few empirical examples of strict co-differentiation exist. Instead, host-parasite co-evolutionary histories usually reflect complex mosaics of co-differentiation, host-switching, lineage extinction and other co-evolutionary events. The episodic host-switching model of parasite diversification accounts for this complexity by suggesting that diversification is a consequence of oscillations between periods of environmental disruption that create novel host-parasite interactions and periods of environmental stability that promote co-differentiation. We tested the phylogeographic predictions of the strict co-differentiation and episodic host-switching models using the American pika (*Ochotona princeps*) and a suite of its endoparasitic helminths (*Cephaluris*, *Labiostomum*, *Graphidiella*, *Murielus*, *Schizorchis*). Genetic structure in parasites is not congruent with the distribution of major host lineages, arguing against a history of strict co-differentiation. As predicted by the episodic host-switching model, parasite dispersal among host lineages probably occurred during major environmental perturbations associated with glacial/interglacial transitions. Range expansion, population growth, and dispersal by parasites are associated. Population fragmentation under current relatively stable conditions has initiated local co-differentiation in the host and parasites at shallow scales.

INTRODUCTION

Parasites represent an exceptionally diverse component of the biosphere. Approximately half of known species can be classified as parasites (Poulin and Morand 2000), and molecular studies are revealing cryptic species complexes that suggest current estimates of parasite diversity are too low (Dobson et al. 2008). Though biologists recognize that this extraordinary diversity exists, we understand little about its origins. Diversification of parasite lineages, especially those that are strongly host-specific, is widely viewed as a consequence of, or at least associated with, host diversification. This perspective has deep roots in parasitology (Brooks and McLennan 1993; Klassen 1992) and predicts that the evolutionary histories of the associated taxa should mirror one another, resulting in co-speciation and yielding phylogenetic congruence between hosts and parasites (Page 2003). Such a straightforward prediction is relatively easy to test, and the growing database of co-phylogenetic studies demonstrates that congruence between host and parasite phylogenies is a rarity (e.g., Brant and Gardner 2000; Brooks and Ferrao 2005; Hoberg et al. 2001; Hoberg and Klassen 2002; Huyse and Volckaert 2005; Zarlenga et al. 2006). Indeed, it is not universal even among the phthirapteran chewing lice (Gomez-Diaz et al. 2007; Johnson et al. 2002; Johnson et al. 2003), some of which have been described as models of co-speciation (Hafner et al. 2003; Hafner and Page 1995).

Until recently, studies of parasite diversification have primarily focused on inter-specific relationships. However, the development of molecular tools and their application to the field of phylogeography (Avise 2000) has prompted expanding interest in exploring host-parasite relationships below the species level (Anderson et al. 1998; Criscione et al. 2005). Phylogeography explores the interface of micro- and macroevolutionary history (Riddle and Hafner 2004), and thus provides key insight

into the processes that initiate diversification. Furthermore, the history of differentiation of host and parasite lineages may be less likely to be obscured by lineage extinction at intra-specific scales due to the shorter time periods involved.

Parasites would seem to be ideal targets for phylogeographic studies, particularly within the context of comparative phylogeography, which seeks to uncover general patterns of genetic structure among co-distributed organisms to reveal broad population effects of extrinsic historical processes (Arbogast and Kenagy 2001; Bermingham and Moritz 1998). Possibly because the strict co-speciation model of diversification makes essentially the same prediction that comparative phylogeography does (i.e., congruent phylogeographic structure between associated taxa), the concept has been embraced by researchers interested in host-parasite comparative phylogeography (e.g., Gomez-Diaz et al. 2007; Nieberding et al. 2004; Nieberding and Olivieri 2007; Whiteman and Parker 2005). This is exemplified by the suggestion that genetic patterns apparent in host-specific parasites, but not the hosts themselves, reflect cryptic host history rather than indicating that parasites may have evolutionary trajectories that are at least in part independent from their hosts (Nieberding et al. 2004; Nieberding and Olivieri 2007).

Though both comparative phylogeography and the co-differentiation model make similar predictions, the interpretation of underlying causal mechanisms differs (Note that to accommodate intra-specific diversification, we will hereafter use the term ‘co-differentiation’ in place of ‘co-speciation’, but the concept is the same). In comparative phylogeographic studies, congruent genetic structure among co-distributed taxa is typically seen as a consequence of external historical factors (e.g., climate change, vicariance) that affected multiple organisms in a similar way. Conversely, the co-differentiation model assumes that the strength of the host-parasite interaction is such that parasite differentiation occurs as a consequence of host

differentiation. In the extreme view, parasites represent the equivalent of an organellar genome, potentially providing insight into host history (Nieberding and Olivieri 2007), but not offering an independent perspective on general biotic effects of historical vicariance or paleoenvironmental change.

Do host-parasite comparative phylogeographic studies show patterns consistent with co-differentiation? Though the field is still young, available data suggest that as in the case of inter-specific co-phylogeny studies, perfect phylogeographic congruence between host and parasite populations is rare (e.g., Criscione and Blouin 2007; Gomez-Diaz et al. 2007; Nieberding et al. 2004; Perkins 2001; Whiteman et al. 2007; Wickström et al. 2003). In most cases, additional co-evolutionary events must be invoked (e.g., parasite dispersal among divergent host populations, which is analogous to host-switching from one host species to another at inter-specific scales), in order to reconcile incongruent patterns of host and parasite genetic structure. Thus, the explanatory power of strict co-differentiation for understanding intra-specific diversification in parasites may be limited.

As an alternative to strict co-differentiation, Hoberg and Brooks (2008) proposed the episodic host-switching (EHS) model as a general framework for parasite diversification. This hypothesis seeks to accommodate both co-differentiation and parasite dispersal (Brooks 1988; Hoberg and Brooks 2008), as well as other co-evolutionary events that can yield incongruent host-parasite lineage associations (e.g., parasite lineage extinction, 'missing the boat', and independent differentiation of either parasite or host lineages; Brooks and McLennan 1993; Paterson and Banks 2001; Paterson and Gray 1997). The EHS model is predicated on the idea that rather than tracking hosts per se, parasites track specific resources that are provided by a set of potential hosts. Thus, apparent host-specificity and parasite dispersal among hosts is mediated by opportunities for contact with potential hosts that possess the appropriate

resource. The model proposes that parasite diversification has occurred as a consequence of oscillations between 1) periods of environmental disruption that promote range changes and novel species' interactions, triggering parasite dispersal events among host lineages, and 2) periods of environmental stability that permit isolated host and parasite lineages to co-differentiate (Hoberg and Brooks 2008). Global episodes of environmental change (e.g., climatic cycles) provide a mechanism for cycling between the stages over the course of Earth's history.

The EHS model makes several predictions for host-parasite assemblages that show overall phylogenetic incongruence between hosts and parasites. First, members of the assemblage are expected to have undergone periodic range shifts historically. Second, evidence of dispersal among host lineages by parasites should be linked to range shifts (by host, parasite, or both) that resulted in new host-parasite associations. Third, range changes should be a consequence of environmental perturbations. Fourth, co-differentiation between host and parasite lineages should be evident if isolation of populations has been maintained during periods of relative environmental stability. These predictions hold across a broad range of temporal scales. Though the EHS model was originally formulated from observations based on deep historical events and phylogenetic relationships, it is also applicable to the shallow temporal scales and intra-specific patterns explored via phylogeographic methods. In this study we use a phylogeographic approach to test the predictions of the co-differentiation and EHS models.

METHODS

Study system

We studied the American pika (*Ochotona princeps*) and a suite of its endoparasitic helminths. *Ochotona princeps* is a small lagomorph that lives in alpine

environments of North America's Intermountain West (Hafner 1994; Smith and Weston 1990). The species is sensitive to climate, with low tolerance for high temperatures (Smith 1974), and a limited ability to thermoregulate (MacArthur and Wang 1974). This sensitivity played a role in historical range fluctuations during climatic oscillations of the Quaternary, as evidenced by fossils showing that pika populations were more broadly distributed at low elevations during the last glacial period than they are today (Grayson 2005; Hafner 1993; Mead 1987).

Seven helminth parasite species have been described from *O. princeps*, representing five distinct genera that include tapeworms (*Schizorchis*), oxyurid nematodes (*Cephaluris*, *Labiostomum*), and strongylid nematodes (*Graphidiella*, *Murielus*). The genus *Labiostomum* is further divided into two sub-genera, *L.* (*Labiostomum*) and *L.* (*Eugenuris*). To reduce confusion caused by discussing different taxonomic ranks, we will hereafter refer to these two subgenera and the other parasite genera as the major parasite lineages associated with pikas.

Species diversity in *Ochotona* (Hoffmann and Smith 2005) and each of the parasite lineages (Hoberg 2005; Quentin 1975; Rausch and Smirnova 1984; Seesee 1973) is greatest in the Old World, implying a Palearctic origin for them all. Furthermore, the parasite lineages that we focus on in this study are restricted to pikas (Grundmann and Lombardi 1976; Rausch and Smirnova 1984) and presumably arrived in North America with one or more early pika colonists. Only one other pika species presently occurs in North America in addition to *O. princeps*. *Ochotona collaris*, likely the sister to the American pika (Formozov et al. 2006; Lissovsky et al. 2007; Niu et al. 2004; Rausch and Ritter 1973), is restricted to Alaska and northwestern Canada. The two species probably diverged in isolation when continental ice sheets sundered a widespread ancestral population during a glacial period (Guthrie

1973). Though their distributions have expanded toward each other since the last glacial maximum, they currently remain separated by several hundred kilometers.

Several characteristics make *O. princeps* and its helminth parasites an excellent system for testing the predictions of the two parasite diversification models. First, because the parasites are associated only with pikas, we have confidence that patterns of parasite diversity have not been influenced by dispersal mediated by other hosts. Only the life cycle of *Schizorchis* includes an intermediate host, probably an oribatid mite (Guan and Lin 1988), which seems an unlikely long-distance dispersal agent. The close association between North American pikas and the major parasite lineages probably dates back to their entry into the Nearctic, indicating a shared history that has spanned their co-tenure in North America. Second, American pikas exhibit strong phylogeographic structure as a consequence of isolation in separate mountain systems (Galbreath, Chapter 1, Chapter 2; Hafner and Sullivan 1995). Lineages are associated with the Northern Rocky Mountains (NRM), Southern Rocky Mountains (SRM), Cascade Range (CR), Sierra Nevada (SN), and Central Utah (CU) (Figure 3.1), providing a framework for testing the hypothesis of co-differentiation. Isolation is complete during interglacial periods when warm and dry climates force pika populations to retreat to fragmented sky islands. Periodic contact and limited gene flow among pika lineages has occurred during glacial periods when pika populations expanded to lower elevations (Galbreath, Chapter 2; Hafner and Sullivan 1995). Though insufficient to break down genetic lineage boundaries, such contact may have provided opportunities for parasite dispersal among lineages as posited by the EHS model. Third, over the course of the Quaternary, the Intermountain West has been strongly influenced by periods of rapid climate change (e.g., glacial/interglacial transitions) alternating with periods of relative climatic stability (e.g., interglacial periods). Divergence time estimates place *O. princeps* in the region through several

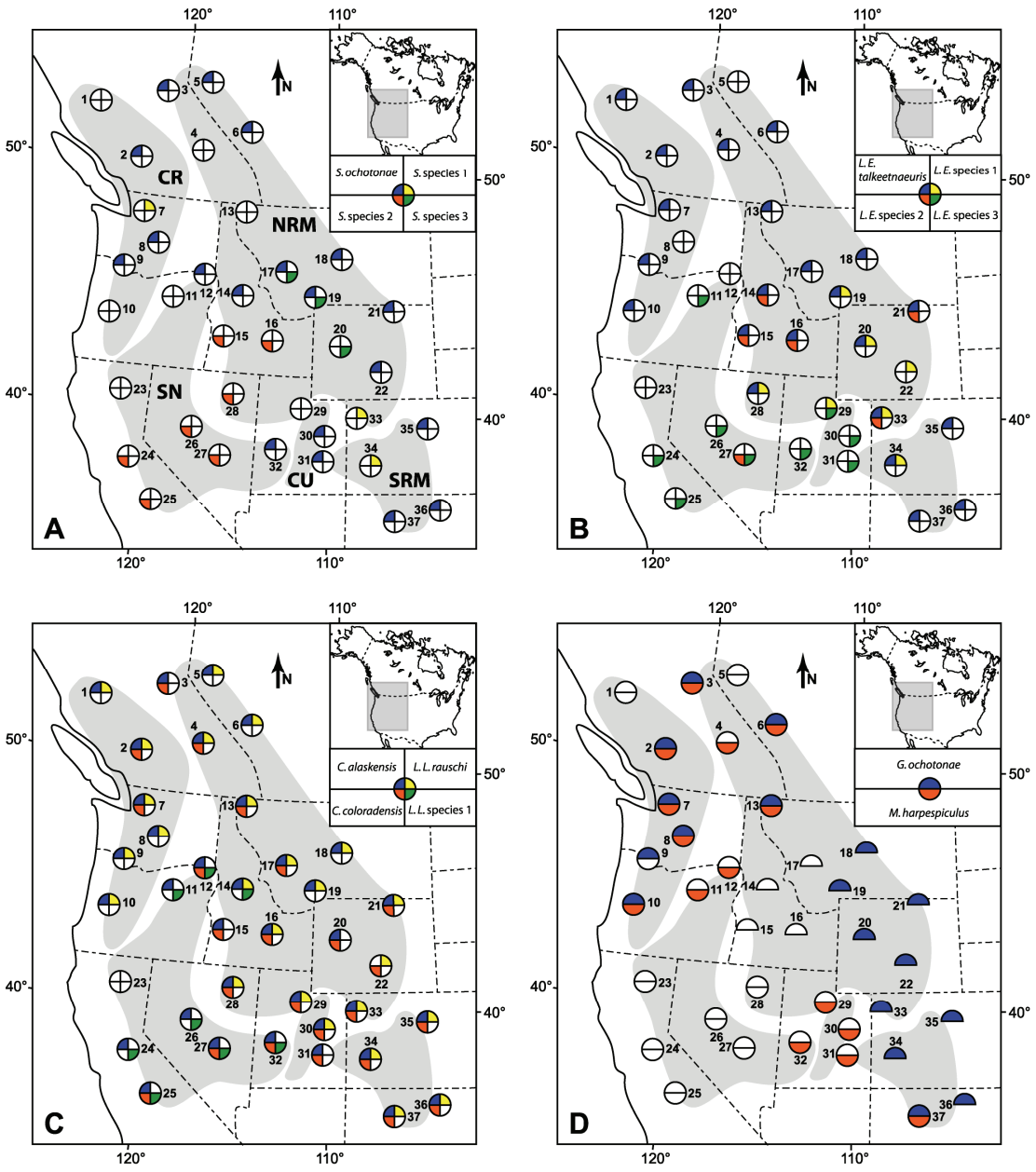


Figure 3.1. Geographic distributions of parasite species. Numbers denote sampling localities and the adjacent segmented circles indicate presence (colored) or absence (white) of each parasite species. Gray patches on the primary map show the distribution of major host lineages, which are identified in map A: Northern Rocky Mountains (NRM), Southern Rocky Mountains (SRM), Cascade Range (CR), Sierra Nevada (SN), Central Utah (CU). A gray box in the inset shows the position of the primary map relative to North America. A key below each inset indicates the color and circle segment that denotes each species. The missing lower half of several circles in map D indicates that due to an error in sampling, those localities were not screened for *M. harpespiculus*.

cycles of climatic change (Galbreath, Chapter 2), establishing a context for testing two predictions of the EHS model; i.e., that episodes of environmental perturbation lead to parasite dispersal and periods of stability lead to co-differentiation. Thus, neither model of parasite diversification is favored from the outset, but requisite conditions for both are met.

Data collection

We obtained endoparasites from 222 *O. princeps* specimens representing 36 localities distributed across the Intermountain West. Field collections were conducted under the auspices of the Cornell University Museum of Vertebrates (CUMV) and in consultation with local wildlife managers. Pikas were immediately necropsied in the field and parasites were preserved in 70% ethanol, providing high quality material for both DNA extraction and morphological identification. We subsampled individual parasites to obtain tissue for DNA extraction while retaining diagnostic structures for morphological identification. Tissue samples were removed from the middle section of nematodes, preserving the anterior and posterior end of each individual as a voucher, as well as representative samples of intact individuals. From tapeworms we removed five to ten posterior proglotids for DNA extraction, preserving the majority of the strobila and scolex as a voucher. Voucher and tissue samples from all host specimens are archived at the CUMV and helminth specimens are archived at the United States National Parasite Collection (USNPC).

We identified all parasites to species using methods appropriate to the taxon. We cleared nematode specimens in phenol-alcohol and prepared them as temporary mounts, which we examined using interference contrast microscopy. Tapeworms were cleared in xylene, stained with haematoxylin, and mounted in Canada balsam. Species identifications were based on original species descriptions and revisions, as well as

comparisons to the following types and vouchers from the United States National Parasite Collection: *S. caballeroi*, holotype: 39024; *S. ochotonae*, syntype: 37056; *S. ryzhikovi*, holotype: 77472, paratype: 77473; *S. yamashitai*, holotype: 59876, voucher: 77471; *L. coloradensis*, 39443; *E. utahensis*, syntype: 73259; *C. alaskensis*, vouchers: 73536, 73538.

We purified genomic DNA from tissues using either phenol chloroform extractions or Qiagen DNeasy kits. A section of the mitochondrial (mtDNA) cytochrome oxidase subunit I (COI; 369 bp) was PCR amplified from specimens of *Cephaluris* (N = 165 individuals), *L.* (*Eugenuris*) (N = 123), *L.* (*Labiostomum*) (N = 86), *M. harpespiculus* (N = 43), and *G. ochotonae* (N = 72) using primers COI-F and COI-R (McDonnell et al. 2000). From *Schizorchis* individuals (N = 135), we amplified a region of mtDNA spanning a portion of both the large and small ribosomal subunits (rRNA; ~815 bp) using primers Hym16sF and Hym12sR (von Nickisch-Rosenegk et al. 2001). Conditions for all PCR reactions are described elsewhere (Galbreath, Chapter 1), except that the annealing temperature for all parasite primer sets was 50°C. We sequenced PCR products in both directions on either an ABI 3100 or 3730 capillary sequencer using Big Dye Terminator chemistry. COI sequences were aligned by eye. Several indels were evident in the rRNA dataset, so we aligned these data with CLUSTALW (Thompson et al. 1994), using MEGA 3.1 (Kumar et al. 2004) and default parameters. We checked the alignment by eye and removed indels. To explore interspecific variation in genetic diversity, we calculated haplotype and nucleotide diversity for each species using DNASP 4.10.4 (Rozas et al. 2003). We also used DNASP to test each species for selective neutrality by calculating Tajima's D statistic and assessing significance based on coalescent simulations of 1000 neutrally evolving populations. Unless otherwise noted, redundant haplotypes were removed from sequence datasets for subsequent analyses.

Phylogeography

We used two methods to assess phylogeographic structure. First we applied a Bayesian phylogenetic approach using MRBAYES 3.0b4 (Huelsenbeck and Ronquist 2001) to reconstruct relationships within each genus represented in our data. The best model of nucleotide substitution for each dataset was chosen using DT-MODSEL (Table 3.1; Minin et al. 2003), and analyses included five chains that were each run for five million steps. Sampling took place every 100 steps and the first 10,000 samples were discarded as burn-in. We repeated analyses three times from different starting seeds to confirm topological convergence, and we assessed support for relationships based on nodal posterior probabilities. Nematode phylogenies were rooted using one representative sequence from the most closely related lineage(s) available in the dataset. Thus, the pinworms (*Cephaluris*, *L.* [*Labiostomum*], *L.* [*Eugenuris*]) provided outgroups for each other, as did the trichostrongyles (*Graphidiella*, *Murielus*). We rooted the *Schizorchis* tree with *Hymenolepis diminuta* (GenBank #AF314223).

Phylogeny reconstruction methods generally assume that existing sequences occupy tip positions in a tree rather than ancestral nodes, and branching is strictly bifurcating. However, at the population level both ancestral and descendant haplotypes may be present and a single haplotype may give rise to multiple descendant haplotypes. We therefore used TCS 1.21 (Clement et al. 2000) to construct gene genealogies (minimum spanning networks; MSNs) for all species based on the statistical parsimony method described by Templeton et al. (1992). We set the probability of parsimony to 99% to conservatively infer parsimonious relationships and avoid over-interpreting relationships between more distantly related haplotypes that may be better reconstructed via phylogenetic methods.

Table 3.1. Summary statistics and model selection for molecular data from parasites of *O. princeps*. The first column lists major genera (or subgenera) and morphospecies identified from each. The remaining columns list the number of specimens sequenced (N), number of haplotypes (N_H), haplotype diversity (h), percent nucleotide diversity (π), effective population size scaled by per generation per sequence mutation rate (μN_e) with lower (-CL) and upper (+CL) 95% confidence limits, Tajima's D statistic, Fu's F_s statistic, the R_2 statistic, and the nucleotide substitution models used in Bayesian phylogenetic (major lineage) and skyline (individual species) analyses. *Ohbayashinema* is not listed because it was not included in the molecular dataset.

	N	N_H	h	π (%)	μN_e	-CL	+CL	D	F_s	R_2	Model
Schizorchis											HKY+G
S. ochotonae	94	41	0.96	1.60	13.71	9.49	20.34	-0.59	-4.74	0.08	HKY+I+G
S. species 1	9	4	0.69	2.08	8.32	3.83	22.49	-1.52	6.32	0.28	HKY
S. species 2	19	8	0.81	2.40	10.08	5.78	18.69	0.80	4.49	0.17	HKY+I
S. species 3	13	4	0.62	0.29	1.19	0.42	3.32	0.17	1.49	0.16	HKY
Cephaluris											HKY+G
C. alaskensis	118	56	0.98	3.94	38.41	26.65	55.71	-0.37	-15.32**	0.09	HKY+I
C. coloradensis	47	29	0.98	1.95	19.22	12.48	29.91	-0.64	-12.45**	0.09	HKY+I
L. (*Eugenuris*)											GTR+G
L. (*E.*) *talkeetnaeuris*	76	22	0.92	0.98	8.32	4.81	14.18	-0.60	-7.54*	0.08	HKY+G
L. (*E.*) species 1	16	7	0.86	1.10	3.71	1.54	8.95	0.79	0.30	0.18	HKY
L. (*E.*) species 2	7	6	0.95	2.49	15.86	6.43	49.57	0.13	0.00	0.16	GTR
L. (*E.*) species 3	24	9	0.88	1.08	4.96	2.35	10.01	0.20	-0.28	0.14	GTR
L. (*Labiostomum*)											HKY+G
L. (*L.*) *rauschi*	68	31	0.96	1.53	12.92	7.98	20.75	-0.32	-13.45**	0.09	HKY [a]
L. (*L.*) species 1	17	5	0.62	0.26	2.43	0.88	6.57	-1.52*	-1.41	0.13	HKY
Murielus											GTR+I
M. harpespiculus	43	30	0.95	4.22	40.36	27.65	60.06	-0.46	-4.59	0.12	HKY+I+G

Table 3.1 (continued).

	N	N_H	*h*	π (%)	μN_e	-CL	+CL	*D*	F_s	R_2	Model
Graphidiella											HKY
G. ochotonae	72	29	0.94	1.44	13.09	8.16	21.06	-1.10	-11.09**	0.068	HKY

[a] DT-MODSEL chose the HKY+I model for the Bayesian skyline analysis of *L.* (*L.*) *rauschi*, but inclusion of the invariant sites parameter caused terminal errors in BEAST. We therefore removed the parameter for this specific analysis.

* Significant at α = 0.05

** Significant at α = 0.005

Demography

Effective population size (N_e) plays an important role in determining the distribution of genetic diversity in parasite populations (Criscione and Blouin 2005; Nadler 1995), and may offer insight into differences in phylogeographic structure observed in parasites that share the same host. We obtained relative estimates of long-term N_e for each parasite species using IM (Hey and Nielsen 2004), which performs Markov chain Monte Carlo simulations of gene genealogies to estimate demographic parameters. Each species was analyzed separately based on a maximum of 50 representative DNA sequences, which were selected at random from the full sequence dataset if a greater number of individuals had been sequenced from the species. Species were treated as a single population by setting the population divergence time to zero (flag –j5), and simulations ran for 10 million generations following a 100 thousand generation burn-in. Each analysis included 10 chains which followed a two-step heating scheme (h1 = 0.05, h2 = 0.7). We applied the infinite sites model of nucleotide substitution if it was consistent with the data, but in most cases the data required the HKY model.

We used several methods to quantify recent changes in N_e undergone by parasite species, including tests of recent expansion based on summary statistics as well as a coalescent-based approach. Species were analyzed separately under all methods based on complete sequence datasets rather than just unique haplotypes. First, we calculated F_s (Fu 1997) and R_2 (Ramos-Onsins and Rozas 2002) statistics, which have been shown to be sensitive to demographic growth under a model of sudden expansion (Ramos-Onsins and Rozas 2002). We assessed significance for both statistics using DnaSP 4.10.4 (Rozas et al. 2003) to generate null distributions from 1,000 coalescent simulations of a neutrally evolving, large population of constant size.

Significant departures of the observed test statistic from the simulated data could be indicative of recent population expansion.

Second, we used DnaSP to calculate pairwise mismatch distributions. Under a model of sudden expansion from a small ancestral population, mismatch distributions are expected to have a smooth, unimodal shape (Rogers and Harpending 1992; Slatkin and Hudson 1991). Ragged or multi-modal distributions are indicative of long-term population stability or decline. These expectations are based on an assumption of panmixia, which is presumably not met by either pikas or their parasites given their highly fragmented alpine distributions. Population fragmentation would be expected to increase the raggedness of a mismatch distribution, suggesting that a smooth curve despite fragmentation should strengthen an inference of expansion. However, if population subdivision is extensive, a ragged or multi-modal distribution will not allow the hypothesis of demographic expansion to be confidently rejected.

Finally, we used BEAST 1.4.8 (Drummond et al. 2002; Drummond and Rambaut 2007) to generate Bayesian skyline plots (Drummond et al. 2005). Rather than test for a single demographic event (e.g., recent population growth), skyline plots allow fluctuations in effective population size to be assessed over time. Furthermore, because skyline plots are generated by sampling from the coalescent, they take into account stochastic variation in the evolutionary process that is ignored by demographic tests that rely on summary statistics.

For the skyline analyses, we selected a separate model of nucleotide substitution for each species using DT-MODSEL (Table 3.1; Minin et al. 2003). Lacking information on whether or not nucleotide evolution in the parasites is clock-like, we fixed the mean substitution rate but applied a relaxed, uncorrelated lognormal molecular clock (Drummond et al. 2006). Each analysis used the constant Bayesian skyline tree prior (10 groups) with default priors for model parameters. We ran the

model for 50 million generations and sampled trees and parameters every 100 generations. Skyline plots were generated in TRACER 1.4 (Rambaut and Drummond 2004) after discarding five million generations as burn-in. For all datasets that produced informative results (i.e., skyline plots that did not simply reflect the prior distribution), we ran a second analysis (10 million generations) to confirm the initial result.

RESULTS

Collections and species identifications

Morphological examination of collected endoparasitic helminths revealed 15 morphospecies and demonstrated that all parasite species known to be associated with *O. princeps* were present in our sample. Our collections prompted a taxonomic reevaluation of several pinworm species previously reported from pikas, necessitating two named species (*L.* [*L.*] *coloradensis* and *L.* [*E.*] *utahensis*) to be reduced as junior synonyms of *L.* (*E.*) *talkeetnaeuris* (see Hoberg et al. submitted for a complete discussion). *Labiostomum* (*E.*) *talkeetnaeuris* and *L.* (*L.*) *rauschi* had previously been thought to be restricted to *O. collaris*, but we found both to be widespread throughout the range of *O. princeps*. In addition, we detected several morphologically distinct groups of *L.* (*Labiostomum*), *L.* (*Eugenuris*), and *Schizorchis* that probably represent undescribed species (Table 3.1); these will be described elsewhere. Lastly, from one locality in the Cascade Range (9) we identified a small number of nematodes representing an unknown species of the genus *Ohbayashinema*, a strongylid previously known only from three species associated with Eurasian pikas. Limited sampling precludes its inclusion in our phylogeographic analysis, and it will be excluded from further discussion unless specifically mentioned. However, its discovery demonstrates the importance of comprehensive biotic surveys for a full

understanding of historical biogeography, particularly when components of a biota are rare or relictual.

Our sampling showed that four of the major parasite lineages associated with American pikas are distributed widely across most of the host's range (Figure 3.1), contrasting sharply with the apparent absence of the two strongylid genera, *Murielus* and *Graphidiella*, from many southwestern populations (Figure 3.1d). The distributions of different species of *L*. (*Labiostomum*), *L*. (*Eugenuris*), and *Schizorchis* also distinguish southwestern populations from the remainder of the pika distribution. In each of these parasite lineages, a single species is primarily associated with southwestern populations and the remaining species are distributed across the Cascade Range and Rocky Mountain cordilleras (Figure 3.1a-c). There is no general concordance between parasite species boundaries and those of the major pika lineages, however. All but one species was associated with two or more host lineages.

Molecular data

All DNA sequences exhibited characteristics consistent with expectations for true mtDNA. In the protein coding COI region sequenced from the nematodes, substitutions were concentrated at first and third codon positions, and *Schizorchis* rRNA sequences showed a deficiency in cytosine (~ 12%) and excess of thymine (~ 38%) similar to that observed in other tapeworms (von Nickisch-Rosenegk et al. 2001). We detected no evidence of heteroplasmy or numts (e.g., overlapping peaks in electropherograms) and little evidence of directional selection. Tajima's *D* tests indicated that one species exhibited a weakly significant signature of selection (*L.* [*L.*] species 2), but the remaining 13 tests were non-significant (Table 3.1). Haplotype diversity was universally high within all species, showing that species are not dominated by a small number of high-frequency haplotypes. In contrast, nucleotide

diversity varied considerably among lineages, though there was no obvious taxonomic or geographic pattern among species with high or low diversity.

Phylogeographic structure

Phylogenetic analyses confirmed the results of the morphological examination, showing that all morphospecies represent genetically distinct, and in most cases unambiguously monophyletic, lineages (Figure 3.2). In the most speciose lineages, *Schizorchis* and *L*. (*Eugenuris*), relationships among species were generally well-resolved. In contrast, phylogenetic resolution within species was relatively poor, though small clusters of well-supported nodes were evident (see e.g., *S. ochotonae*, *G. ochotonae*, *M. harpespiculus*; Figure 3.2a, f, g).

To illustrate intra-specific relationships, we present a composite phylogeny that incorporates MSNs onto the phylogenetic framework provided by the Bayesian analysis (Figure 3.2). In this way we establish a phylogenetic context for unlinked networks, while retaining information regarding fine scale relationships among haplotypes separated by few mutations. In general, species are represented by many haplotypes of roughly equal frequency, and haplotypes are rarely shared among more than two populations. Notable exceptions include widespread, high-frequency haplotypes of *L*. (*E*.) *talkeetnaeuris*, *L*. (*L*.) *rauschi*, and *G. ochotonae* (Figure 3.2b, c, f). Approximately 16% of all nematode haplotypes were shared among two or more localities. In contrast, only around 5% of *Schizorchis* haplotypes were shared, suggesting a higher level of geographic structuring in the tapeworms. Of the haplotypes that are shared, most are geographically clustered, though *L*. (*L*.) *rauschi* again provides an exception. In this species, one haplotype is found in two localities that span nearly the full *O. princeps* distribution (1 and 22; Figure 3.2), but not in intervening localities.

Figure 3.2. Composite Bayesian phylogenies and MSNs for *Schizorchis* (A), *L*. (*Eugenuris*) (B), *L*. (*Labiostomum*) (C), *Cephaluris* (D, E), *Graphidiella* (F), and *Murielus* (G). Open white circles on tree branches indicate Bayesian posterior probability support of 95% or greater for the branch. Branch lengths are uninformative and outgroups have been removed for clarity. In the MSNs, circles represent unique haplotypes and small squares represent inferred, but unsampled, haplotypes. Lines separating haplotypes indicate a single mutational step. Relative size of circles reflects haplotype frequency, and numbers adjacent to circles identify the host population(s) from which each haplotype was collected. The lineage identity of the host population(s) is denoted by each circle's fill pattern or color: SN (spots), CR (stripes), NRM (black), SRM (dark grey), CU (light grey). The phylogeny for *Cephaluris* was split for clarity, and the arrows at the root of each sub-tree indicate how they connect.

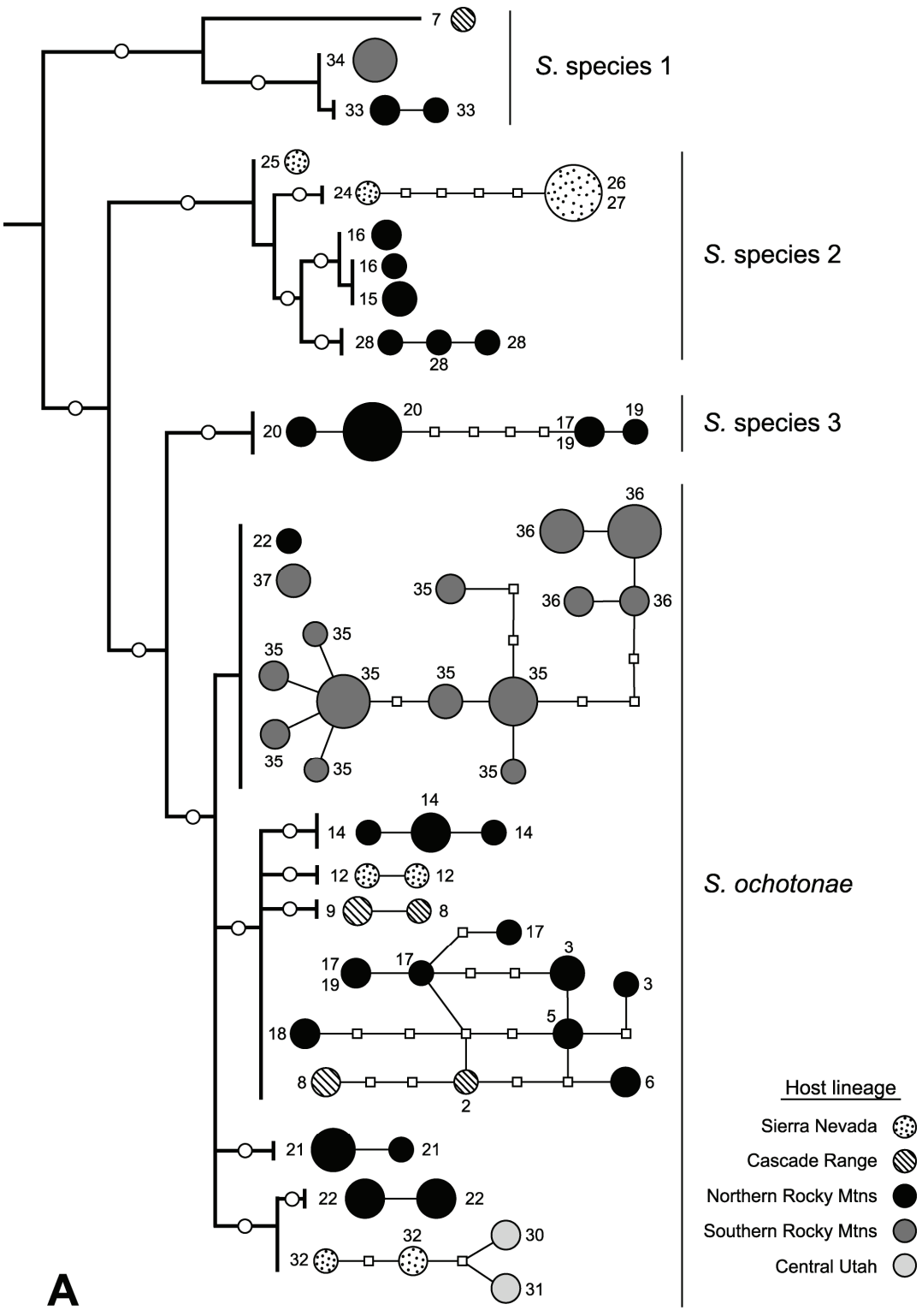

S. species 1
S. species 2
S. species 3
S. ochotonae
A
Host lineage
Sierra Nevada
Cascade Range
Northern Rocky Mtns
Southern Rocky Mtns
Central Utah

Figure 3.2 (continued).

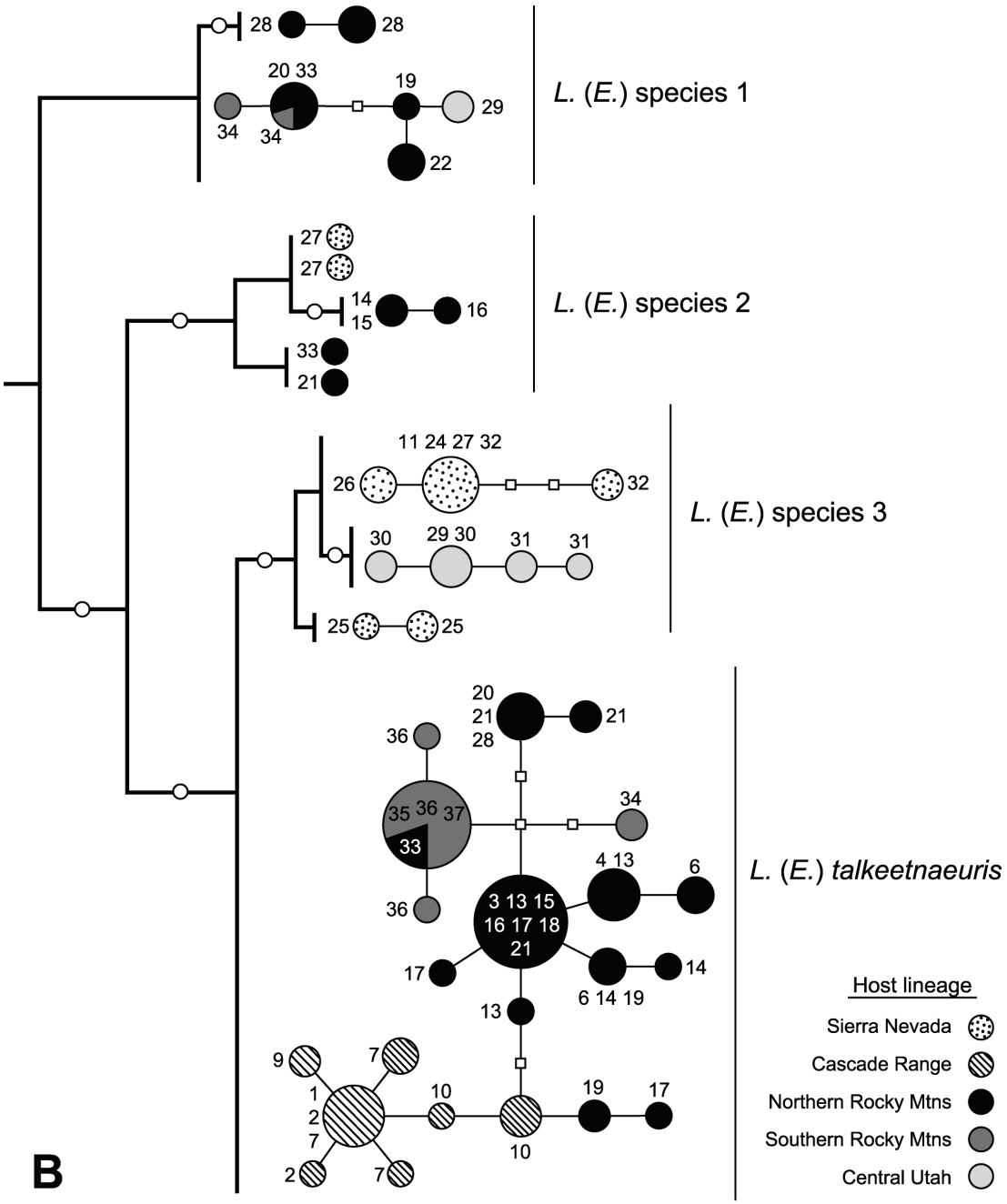


Figure 3.2 (continued).

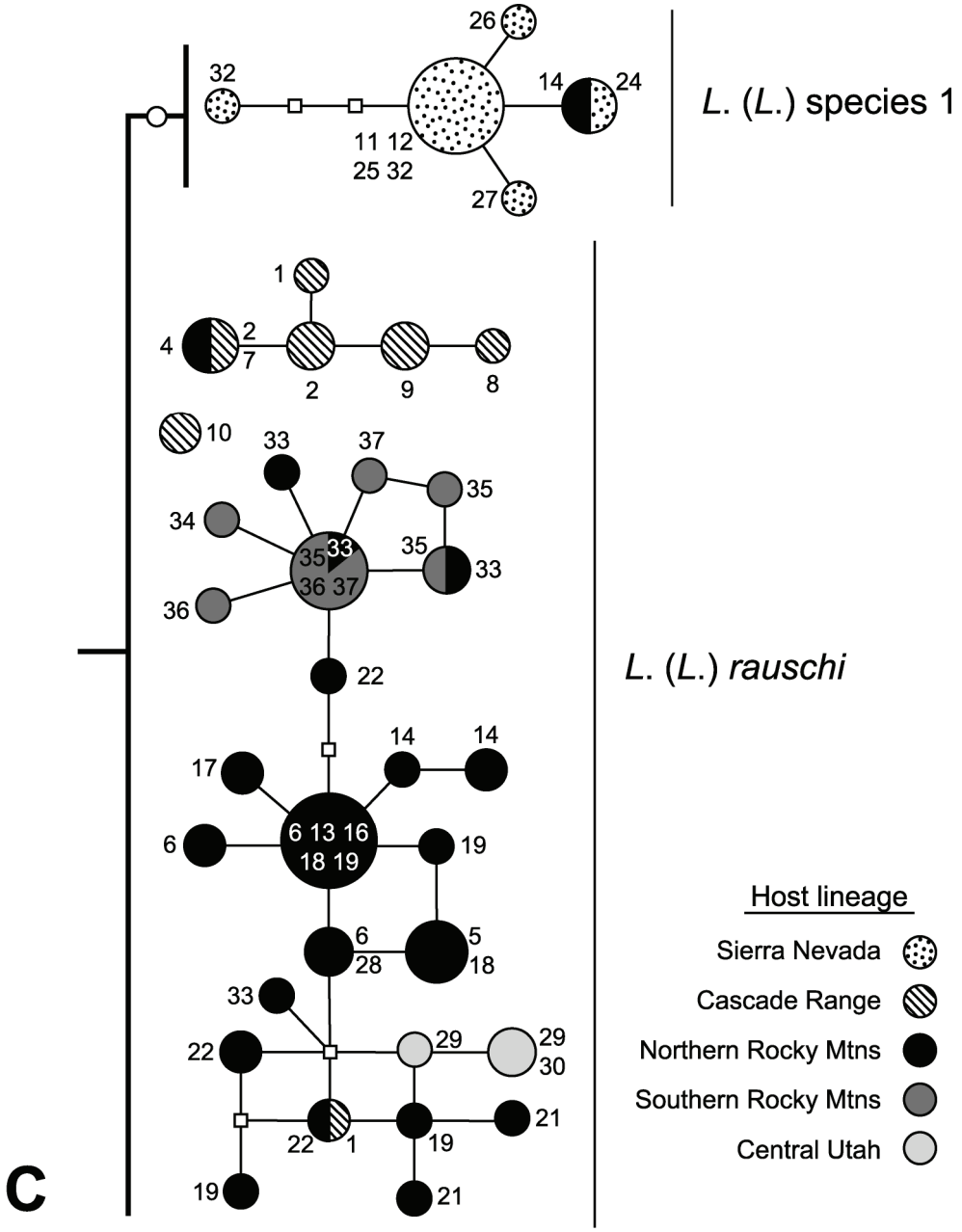


Figure 3.2 (continued).

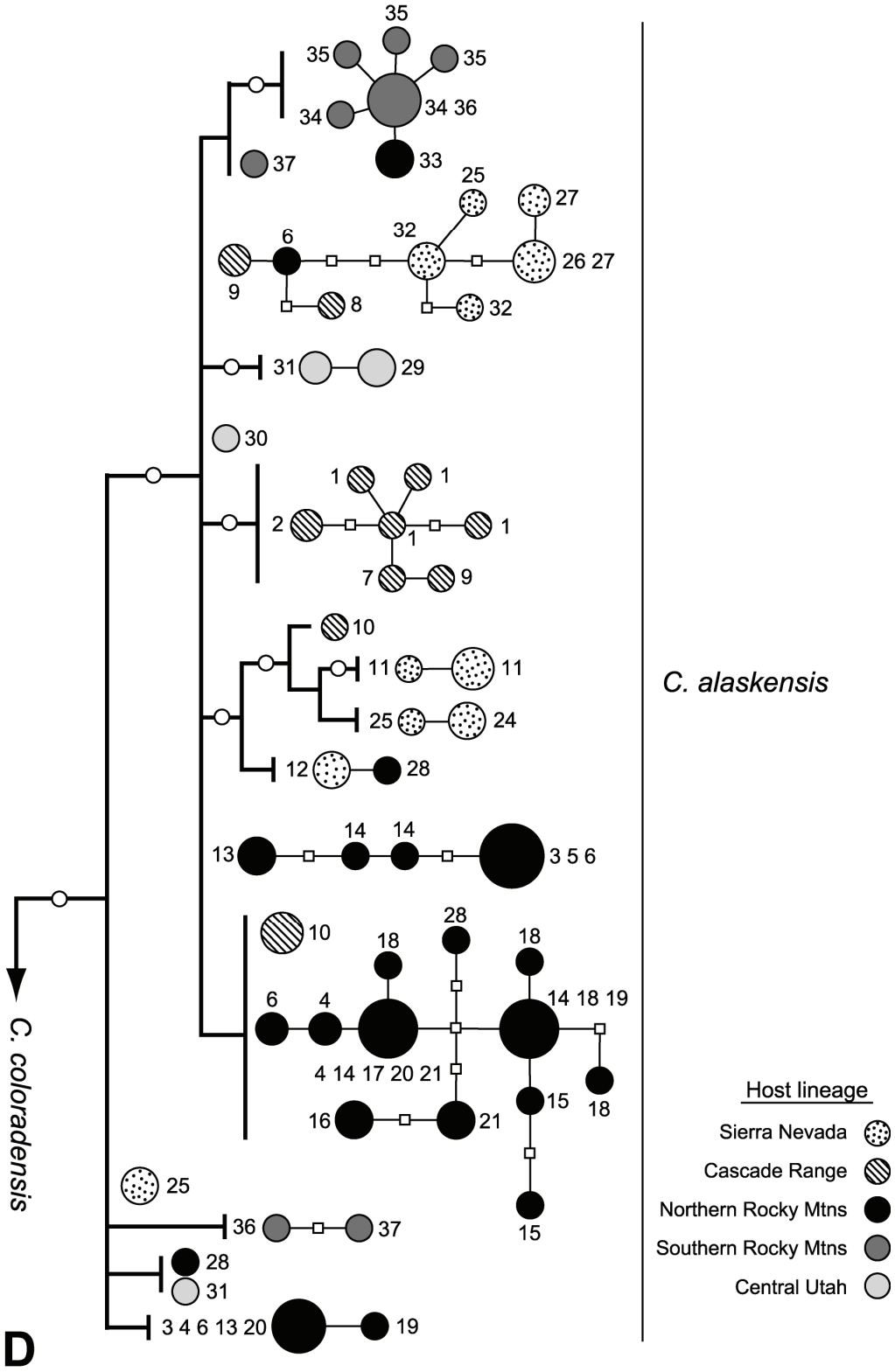


Figure 3.2 (continued).

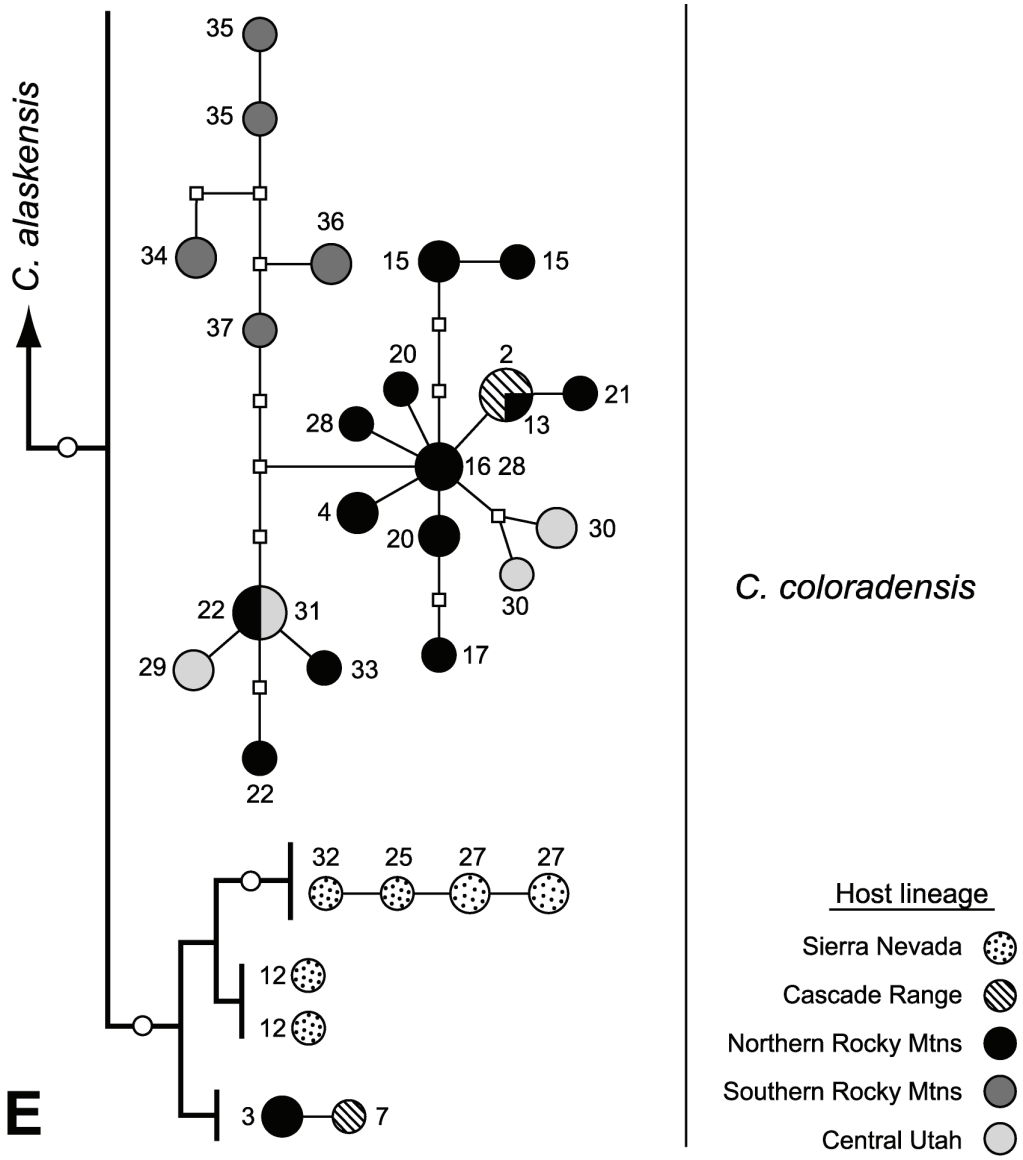


Figure 3.2 (continued).

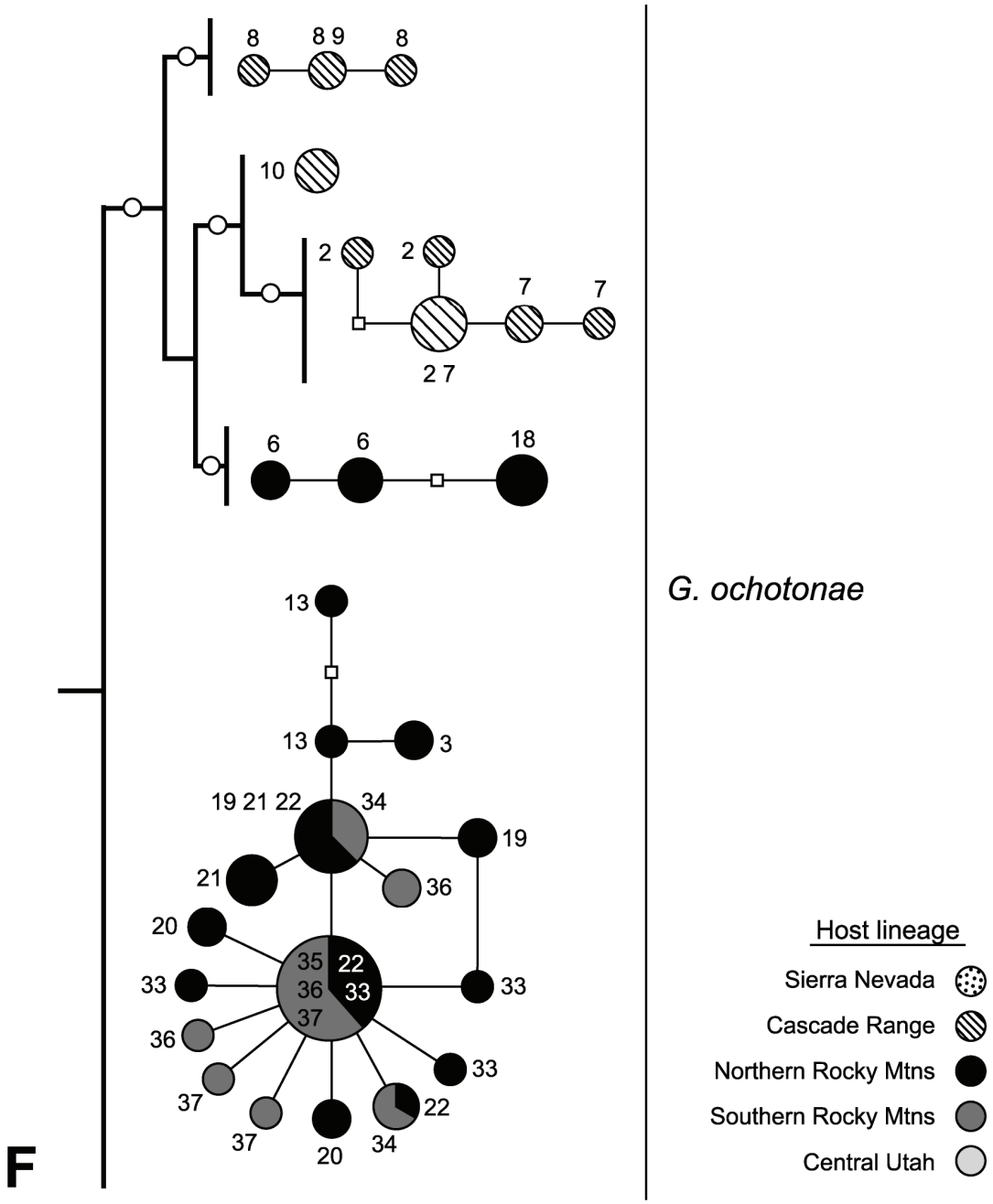
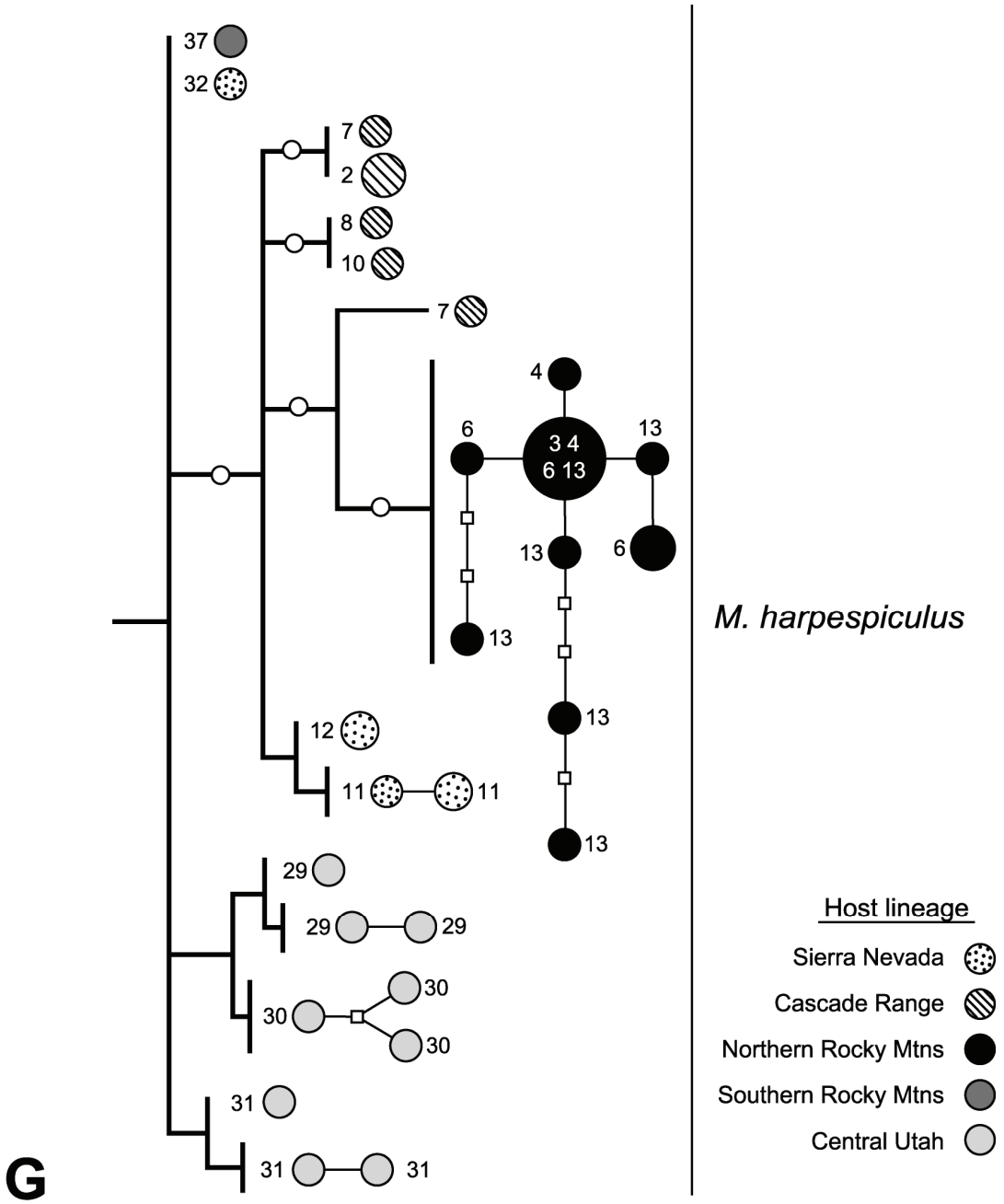


Figure 3.2 (continued).



Strong concordance between pika genetic lineages and intra-specific genetic structure in parasites is not evident. Though some parasite haplotypes associated with specific host lineages form unambiguously monophyletic clades within parasite species (e.g., *S.* species 2 – NRM cluster, *L.* [*E.*] species 3 – CU cluster; *M. harpespiculus* – NRM cluster; Figure 3.2a, b, g), the overall pattern is one of paraphyly with respect to host lineages. Also, haplotypes of six species are shared between two different host lineages. Sharing between the NRM and SRM lineages is most extensive (7 haplotypes), followed by NRM and CR (3 haplotypes), NRM and SN (1 haplotype), and NRM and CU (1 haplotype).

Demography

Estimates of N_e scaled by mutation rate were relatively consistent across most parasite species (Table 3.1), but values for two species differed from the rest. Barring the possibilities of either substantially faster molecular evolution or fewer generations per year relative to other parasites, the pinworm *C. alaskensis* and strongylid *M. harpespiculus* appear to have significantly higher N_e than most of the other species that we examined. However, without reliable estimates of either mutation rates or generation times for each species, we chose not to calculate absolute values for population size.

Tests of recent demographic change revealed varied results, though some general patterns were evident. The R_2 statistic did not identify demographic expansion in any species (Table 3.1). In contrast, Fu's F_s test detected a signature of recent expansion in five species (*C. alaskensis*, *C. coloradensis*, *L.* [*E.*] *talkeetnaeuris*, *L.* [*L.*] *rauschi*, and *G. ochotonae*). Pairwise mismatch distributions were consistent with a recent history of population growth in *C. coloradensis*, *L.* [*E.*] *talkeetnaeuris*, and *L.* [*L.*] *rauschi*, but *C. alaskensis* and *G. ochotonae* produced strongly bimodal

distributions that would not suggest expansion from a small ancestral population of low diversity (Figure 3.3). *Labiostomum* (*L.*) species 1 had a shallow unimodal mismatch distribution, but the F_s and R_2 statistics did not detect expansion.

Bayesian skyline plots provided additional perspective on the history of demographic change for several species (Figure 3.4). Consistent with the F_s tests, *C. coloradensis*, *L.* (*L.*) *rauschi*, *L.* (*E.*) *talkeetnaeuris*, and *G. ochotonae* all exhibited signatures of recent population growth. *Cephaluris alaskensis* and *M. harpespiculus* showed evidence of long-term stability in population size, and two tapeworms, *S. ochotonae* and *S.* species 2, exhibited signatures of recent and precipitous population decline. Six Bayesian skyline analyses yielded uninformative results in the form of skyline plots that did not differ substantially from the prior distribution. We do not present these here.

DISCUSSION

Co-differentiation

If strict co-differentiation was the dominant process driving diversification within the major parasite lineages associated with American pikas, we would expect host and parasite phylogeographic structure to be strongly concordant. We find little evidence of concordance (Figure 3.2), suggesting that alternative processes have been involved in determining the complex pattern of relationships between host lineages and parasite diversity. Though parasite dispersal between host lineages is one common cause of incongruence between host and parasite phylogenies, there are a variety of other processes that can produce incongruent relationships (Page 1993; Page 1994). For example, if a peripheral isolate of a host population diverges in allopatry, but a parasite fails to successfully colonize the new host isolate, the parasite is said to have 'missed the boat' and thus the opportunity for co-differentiation will have been missed

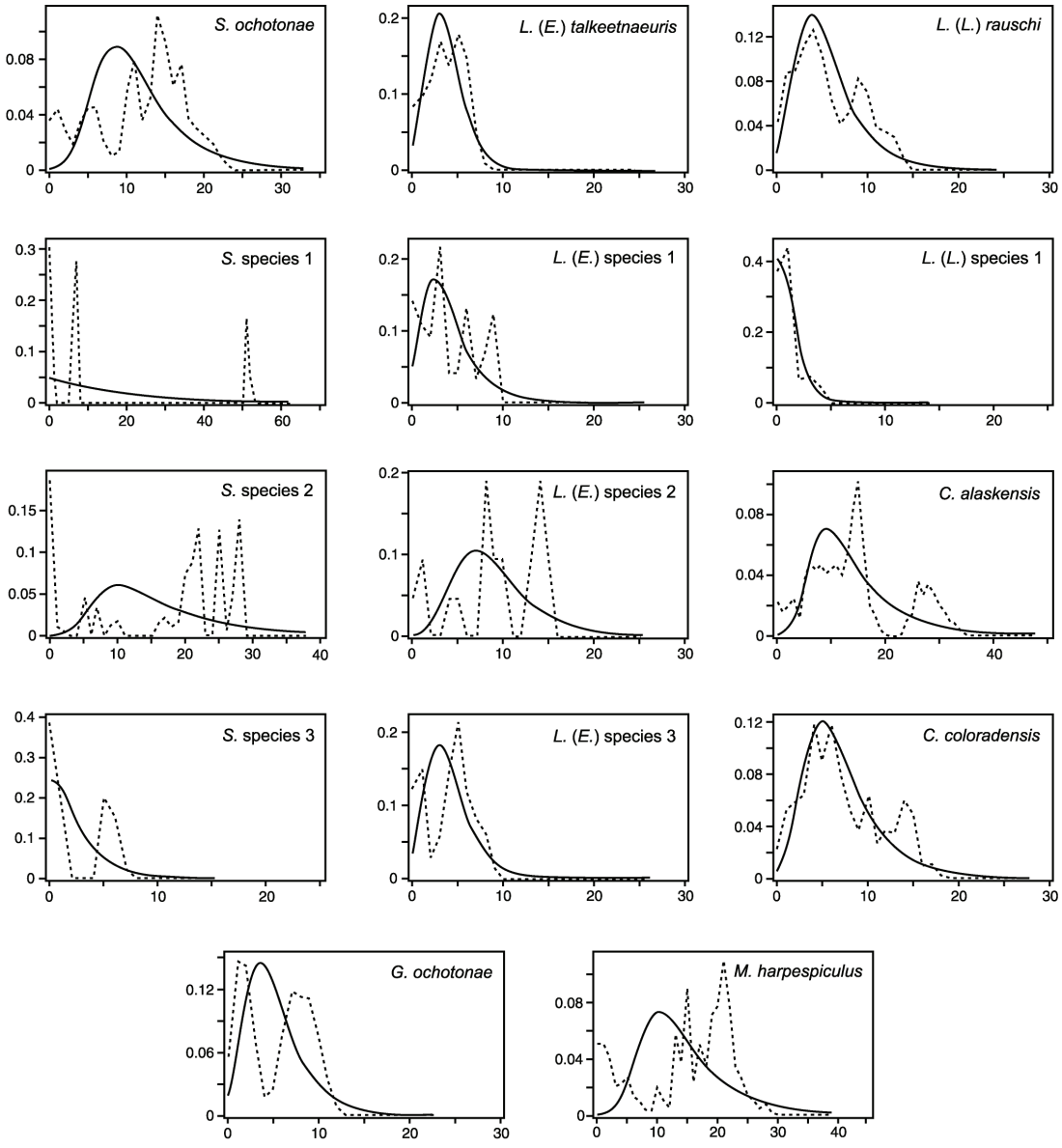


Figure 3.3. Mismatch distributions for all parasite species. A dashed line indicates the observed frequency of pairwise nucleotide differences between sequences, and a solid line represents the expected distribution based on a model of sudden population expansion (Rogers and Harpending 1992).

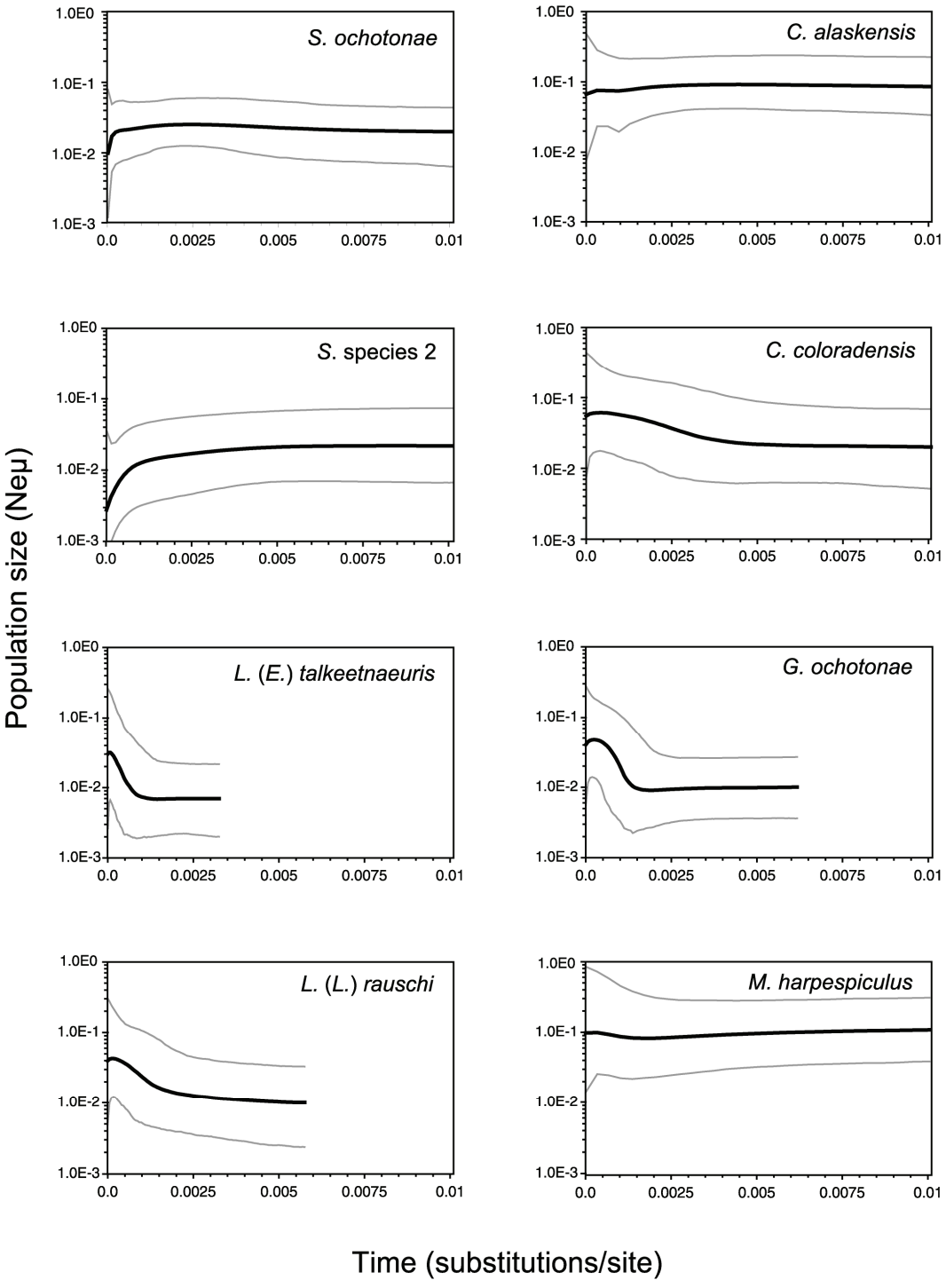


Figure 3.4. Bayesian skyline plots for parasite species showing effective population size scaled by mutation rate and plotted as a function of time. A heavy black line indicates the median value of effective population size. Gray lines denote the 95% highest posterior probability interval. The horizontal axis has been scaled to show the same interval (0 – 0.01 substitutions/site) for all plots.

(Paterson and Gray 1997). A similar pattern might result if a parasite associated with a specific host lineage independently goes extinct. Also, differentiation of a parasite lineage without concurrent host differentiation can result in complex host-parasite relationships, particularly in combination with other co-evolutionary events.

In the case of pikas and their parasites, missing the boat or lineage extinction could be invoked to explain, for example, the apparent absence of *Graphidiella* and *Murielus* from many southwestern populations. However, neither these scenarios nor independent parasite differentiation can parsimoniously account for the repeated pattern of geographically widespread parasite species associated with multiple pika lineages (Figure 3.1). Alternatively, if these species were associated with the ancestor of *O. princeps* before diversification began in the mid-Pleistocene (Galbreath, Chapter 2), their widespread occurrence could be explained by a failure to co-differentiate (Paterson and Banks 2001). From a micro-evolutionary perspective, this would result if insufficient time has elapsed for lineage sorting to complete (Rannala and Michalakis 2003). Time to lineage sorting is a function of N_e, and indeed, estimates of N_e for the parasites (Table 3.1) are probably much greater than they are for the hosts (Galbreath, Chapter 2). Without good estimates of per generation mutation rate it is impossible to calculate absolute values of N_e with confidence, but rough approximations based on a mutation rate of 1%/My and 1 generation/year suggest that parasite N_e may be 1-3 orders of magnitude greater than that of the pikas. Parasite lineage sorting periods are therefore likely to be relatively long compared to those of their hosts.

Incomplete lineage sorting might explain the general lack of reciprocal monophyly among parasite clades associated with different host lineages. However, it is less convincing as an explanation for the persistence of extraordinarily shallow relationships between parasite haplotypes that span host lineage boundaries. Twelve

haplotypes representing six parasite species are shared between well-differentiated host lineages. If they were present in the ancestral population (i.e., before host lineage separation), their antiquity might be reflected in higher frequencies relative to more recently derived haplotypes (Watterson and Guess 1977). However, shared haplotypes did not occur at higher frequencies than non-shared haplotypes (Figure 3.2). Older haplotypes are also expected to be widespread (Watterson and Guess 1977), but most of the shared haplotypes are restricted to narrow geographic ranges that overlap boundaries between adjacent host lineages, indicating recent gene flow rather than long-term persistence. Lastly, the high haplotype diversity apparent in most parasite species (Table 3.1), and the occurrence of novel haplotypes in post-glacially colonized populations established within the past 10 Ky (localities 1, 2, 3, 4, 5, and 6; Figure 3.2), demonstrate that new diversity is acquired relatively rapidly through mutation and ancient diversity may not be retained.

Shared haplotypes are most often observed between the NRM and SRM host lineages (7 haplotypes representing four parasite species). Though the NRM and SRM mtDNA phylogroups of pikas are reciprocally monophyletic, alleles at nuclear loci that are shared between NRM and SRM populations indicate that recent contact and gene flow between the two lineages has occurred (Galbreath, Chapter 2). Such contact likely created opportunities for parasite gene flow as well. Other zones of contact may have existed between populations of the SN/CU lineages, and SN/CR lineages (Galbreath, Chapter 2; Hafner and Sullivan 1995), and a pair of parasite haplotypes that are shared between northern populations associated with the CR and NRM lineages suggest contact across the northern part of the pika's range. It is evident that despite a general history of isolation that has driven differentiation among host lineages, periodic contact has permitted parasite dispersal and gene flow to occur.

Episodic host-switching

The EHS model proposes that parasite dispersal among host lineages is a consequence of changes in species distributions that are initiated by major environmental disturbances (Hoberg and Brooks 2008). This describes the likely driver of parasite dispersal among pika lineages very well. Though pika populations are restricted to high alpine islands under interglacial (e.g., current) climatic conditions, the fossil record shows that past environmental disturbance in the form of climate cooling initiated pika range expansion into low-elevation basins that separate the major mountain ranges of the Intermountain West (Grayson 2005; Hafner 1993; Mead 1987). This expansion permitted contact between previously isolated populations (Galbreath, Chapter 2; Hafner and Sullivan 1995), which produced opportunities for both host and parasite gene flow. In pikas, the signature of historical introgression is retained at nuclear but not mitochondrial loci, probably due to differences in relative N_e between the two genomes (Galbreath, Chapter1, Chapter 2). Specifically, the small N_e of mtDNA likely contributed to rapid sorting of introgressive haplotypes after contact between populations ended, helping to maintain pika mtDNA lineage boundaries. In contrast, the relatively large N_e of parasites may allow longer lineage sorting periods, resulting in genetic patterns more similar to those seen at pika nuclear loci.

Demographic growth by pika and parasite populations did not necessarily accompany range expansion, but it seems to have been associated with parasite dispersal among host lineages. Only the two northern pika lineages (NRM and CR) retain the genetic signature of demographic growth from the last glacial period (Galbreath, Chapter 1). Likewise, we detected strong evidence of growth from only four parasite species (Table 3.1, Figure 3.4). In addition to *L.* (*L.*) species 1, which exhibited mixed demographic results (unimodal mismatch distribution but non-

significant F_s test), these species represent the majority of parasites that share haplotypes between host lineages. Thus, range expansion, population growth, and parasite dispersal appear to be linked.

Though not all host lineages exhibit a signature of demographic growth associated with range expansion, all show evidence of population decline during the recent range retraction phase that has accompanied post-glacial climate warming (Galbreath, Chapter 1). Of the parasite datasets that yielded informative Bayesian skyline results, only the two *Schizorchis* species showed strong evidence of recent demographic decline (Figure 3.4). Population decline may have begun in the four nematodes that showed clear signatures of earlier population growth, but it did not match the strong post-glacial decline evident in the host lineages (Galbreath, Chapter 1). This may reflect a lag in the parasite's response to demographic change in the host, but it also may indicate that the parasites have responded independently to the same history of environmental change. Indeed, the range of demographic responses that we detected in different parasite species (expansion, decline, stability) suggests that parasite population history can not be assumed to track host history with perfect fidelity.

The second stage of the EHS model, following environmental disruption and parasite dispersal, is that of environmental stability and co-differentiation (Hoberg and Brooks 2008). As conditions stabilize, the frequency and magnitude of population range shifts decrease and a new biotic landscape is established. Isolated populations, some of which may include newly formed host-parasite associations, initiate new evolutionary trajectories. The Holocene period, which began with the close of the Wisconsinan Glaciation (~10 Kya) and extends to the present, is one such period of environmental stability. The arrival of the Holocene was marked by a period of rapid climate warming that caused pikas to retreat to isolated sky islands (Grayson 2005;

Hafner 1993). Since that time, a relatively stable climate has maintained the highly subdivided pika distribution. Isolation among pika populations is clearly evident in the distribution of their mtDNA haplotype variation. In a study on the distribution of genetic variation across 64 populations, no mtDNA haplotypes were shared by multiple populations (Galbreath, Chapter 2). Though some parasite haplotypes are shared among populations, providing evidence of recent gene flow, most (82%) are not shared, reflecting local differentiation. Thus, pikas and parasites are co-differentiating as a consequence of shared isolation in refugial sky islands. Co-adaptation between the host and parasites is not assumed, though neither is it precluded. Co-differentiation will continue until another environmental perturbation initiates a new round of population range shifts and parasite dispersal.

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CHAPTER FOUR

RETURN TO BERINGIA: PARASITES REVEAL CRYPTIC COLONIZATION HISTORY OF NORTH AMERICAN PIKAS

Abstract.— Host-parasite comparative phylogeography (HPCP) represents a new and potentially powerful approach to address questions regarding host history using genealogical information from parasites. To date most HPCP studies have focused on a two-way comparison between a single host and parasite, yet for parasites to reveal novel aspects of host history, multiple parasite lineages must independently corroborate patterns not seen in the host itself. We studied comparative phylogeographic patterns in five host-specific parasites of the Collared pika (*Ochotona collaris*) and American pika (*O. princeps*) of North America to determine whether the southern species (*O. princeps*) was descended from the northern, or vice versa. Four out of five parasite phylogenies revealed that southern diversity was paraphyletic with respect to northern populations, as predicted under the hypothesis of a southern origin. Four additional parasite lineages that are restricted to *O. princeps* further corroborate this biogeographic inference. As the most taxonomically comprehensive HPCP analysis to date, this study demonstrates the power of HPCP for resolving aspects of host history that are not revealed by characteristics of the host itself.

INTRODUCTION

It has long been recognized that parasites can be excellent indicators of host phylogeny, historical biogeography, and ecology (Manter 1966). The study of host-parasite co-evolution has its origins in the late 19th century (Klassen 1992), but it was transformed by the introduction of rigorous systematic methods (Hennig 1966) and

their application to reconstructing co-phylogenetic relationships between hosts and parasites (Brooks 1981). Recent decades have seen vigorous growth in the field of co-evolutionary research fostered by continued development of co-phylogenetic methods and the recognition of ample conceptual overlap between host-parasite and comparative biogeographic approaches (Brooks and McLennan 1991; Brooks et al. 2001; Charleston 1998; Huelsenbeck et al. 2000; Page 1994; Wojcicki and Brooks 2004).

The rise of the field of phylogeography (i.e., the study of geographic distributions of genealogical lineages within and among closely related species; Avise 2000; Avise et al. 1987), coupled with the recognition that phylogeographic comparisons among multiple, co-distributed taxa offer a powerful approach for reconstructing regional or community-level biogeographic histories ('comparative phylogeography'; Arbogast and Kenagy 2001; Riddle 1996; Zink 1996), has inevitably raised interest in using co-phylogenetic methods to address population-level phylogeographic questions. The list of host-parasite comparative phylogeographic (HPCP) studies is growing (Criscione et al. 2005; Nieberding and Olivieri 2007), as parasites would seem to be ideal targets for comparative phylogeographic studies. They certainly are co-distributed with their hosts across at least a portion of the host's range, and to the extent that host specificity has been maintained over a specific timescale of interest, parasite population history is predicted to reflect host history.

Of particular interest is the potential for parasites to offer insight into host population history, especially history that has left no signature on the host itself (Hoberg 1995). For example, phylogeographic structure evident in parasites but not hosts may reveal cryptic isolation events that once sundered host populations (e.g., Nieberding et al. 2004; Wickström et al. 2003). However, if a single host clade is compared to a single parasite clade, as is the case with most HPCP studies published

to date (but see Criscione and Blouin 2004; Whiteman et al. 2007), it is impossible to know if phylogeographic patterns found in one taxon and not the other are general patterns reflecting the history of the assemblage or a consequence of taxon-specific processes (e.g., parasite isolation without host isolation). The solution to this problem is to incorporate into the HPCP analysis at least one additional taxon, representing a phylogenetically independent perspective on the history in question (the 'Threes Rule'; Brooks and McLennan 2002). Here we demonstrate the power of a multi-parasite HPCP analysis for resolving aspects of host history that are not revealed by study of the host alone. In the first such study of its scope, we test the predictions of competing biogeographic hypotheses for nine parasite lineages that represent independent perspectives on the same host history.

Study system: Of pikas and parasites

Pikas (genus *Ochotona*) are small lagomorphs that originated in the Palearctic and colonized the Nearctic via the Bering Land Bridge, as evidenced by their greater species diversity in Eurasia (28 extant species) than in North America (2 extant species) (Hoffmann and Smith 2005) and by paraphyly of Eurasian species with respect to those in North America (Niu et al. 2004; Yu et al. 2000). The North American Collared pika (*O. collaris* – found in Alaska and adjacent Canadian provinces; Figure 4.1) and American pika (*O. princeps* – distributed across North America's Intermountain West) are probably sister taxa (Formozov et al. 2006; Lissovsky et al. 2007; Niu et al. 2004; Rausch and Ritter 1973), suggesting that they evolved from a common ancestor that crossed the Bering Land Bridge and underwent subsequent speciation. The ancestor of *O. collaris* and *O. princeps* presumably crossed the Bering Land Bridge during a glacial period, and following glacial retreat its range expanded to lower latitudes. Based on this history and the geographic distributions of

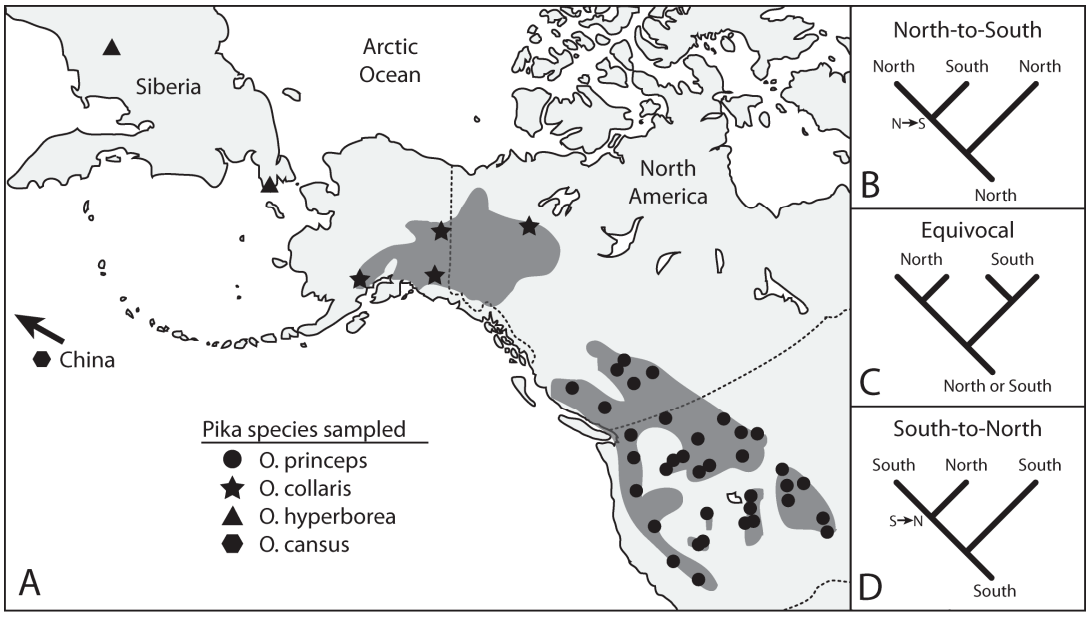


Figure 4.1. Species distributions, sampling localities, and phylogenetic predictions of biogeographic hypotheses for the history of North American pikas. On the map (A), dark grey patches indicate approximate species distributions for *O. princeps* and *O. collaris*, which are distinguished by the shape of the symbols used to identify sampling localities (see key). The side-bar (B, D) shows the different patterns of phylogenetic relationships between northern (associated with *O. collaris*) and southern (associated with *O. princeps*) populations that are predicted by different biogeographic hypotheses (see text). Reciprocal monophyly (C) between populations does not discriminate between hypotheses.

the two species, it is generally assumed that the northern species, *O. collaris*, represents the ancestral Beringian population, and *O. princeps* evolved from a southern extension of the ancestral range (Rausch and Smirnova 1984). However, there is little evidence that *O. collaris* arose from a lineage that has persisted in the North since the original colonization of Beringia. Indeed, pikas that are morphologically consistent with *O. collaris* and *O. princeps* (fossils of the two species are essentially indistinguishable) appear much earlier in the fossil record at low latitudes (~ 850 kyBP) than at high latitudes (< 300 kyBP) (Mead 1987; Mead and Grady 1996). We must therefore consider the alternative hypothesis that the ancestral northern population did not persist after pikas colonized low latitudes, but that *O. collaris* has its origins in a northward expansion by the southern ancestor of *O. princeps*. Under either scenario, speciation probably occurred when advances of continental ice isolated populations in northern and southern refugia (Guthrie 1973).

The two hypotheses differ primarily in the ancestor-descendant relationship between northern and southern populations, and thus they make different phylogenetic predictions. The North-to-South (N-S) hypothesis assumes that North American colonization proceeded in stepwise fashion from Siberia to Alaska and eventually to lower latitudes. Such a history could result in northern (*O. collaris*) populations being paraphyletic with respect to those in the south (*O. princeps*; Figure 4.1). Conversely, if extant northern populations were derived from a southerly distributed ancestor (South-to-North; S-N) we would predict southern paraphyly. Under either scenario, northern and southern populations could be reciprocally monophyletic given sufficient time for lineage sorting to occur since speciation. Evidence from mitochondrial (Galbreath, Chapter 1) and allozymic (Hafner and Sullivan 1995) data suggest that Collared and American pikas are reciprocally monophyletic, neither supporting nor refuting either hypothesis. However, a diverse assemblage of host-specific

endoparasites associated with pikas offers additional independent tests of the host's biogeographic history in North America.

At least 17 genera of endoparasitic helminths (roundworms, tapeworms, flukes) parasitize pikas. Only six of these (*Murielus*, *Ohbayashinema*, *Graphidiella*, *Labiostomum*, *Cephaluris*, *Schizorchis*) are known to occur in the North American pikas, and of these six, one tapeworm (*Schizorchis*) and two pinworm (*Cephaluris*, *Labiostomum*) genera parasitize both pika species. The trichostrongyloids *Murielus*, *Ohbayashinema*, and *Graphidiella* appear to be restricted to *O. princeps*. The genus *Labiostomum* is subdivided into the subgenera *L.* (*Labiostomum*) and *L.* (*Eugenuris*), which we treat as separate units of analysis in this study. To minimize confusion when discussing groups that represent different taxonomic ranks, we will refer to these two subgenera and all other parasite genera as the 'major parasite lineages' associated with pikas. Each of the parasite lineages have greater species diversity in Eurasian pikas (Hoberg 2005; Quentin 1975; Rausch and Smirnova 1984; Seesee 1973), indicating a Palearctic origin for the parasites as well as the hosts. Indeed, *Schizorchis* may be sister to a parasite of hares and rabbits (*Mosgovoyia*), suggesting an ancient association with Lagomorpha dating to the origin of the major lagomorph families in Central Asia (Rausch and Smirnova 1984). Furthermore, in North America none of the major parasite lineages are known to parasitize host species other than pikas (Grundmann and Lombardi 1976), and in Eurasia most reports of non-ochotonid hosts have been shown to be erroneous (e.g., Rausch and Smirnova 1984). Thus, it is reasonable to assume that host-specificity has been maintained in North America since the common ancestor of *O. collaris* and *O. princeps* crossed the Bering Land Bridge, and barring additional unknown trans-Beringian colonizations by pikas, all parasite lineages presumably entered the Nearctic with that ancestral colonization. Finally, of the major parasite lineages, only *Schizorchis* requires an intermediate host to complete

its life cycle, probably an oribatid mite (Guan and Lin 1988). Gene flow by parasites is therefore likely to be entirely mediated by pika dispersal.

Here we use the parasites of North American pikas as independent historical indicators to test the competing biogeographic hypotheses regarding the origins of *O. collaris* and *O. princeps* (N-S vs. S-N). We focus on *Schizorchis*, *Cephaluris*, *L.* (*Labiostomum*), and *L.* (*Eugenuris*) because they are shared between both pika species. For these lineages we use molecular phylogenetic methods to 1) determine the minimum number of successful North American colonizations accomplished by parasites (representing the number of independent tests of host history in North America since colonization), 2) identify paraphyletic relationships that shed light on centers of origin for the two living North American pikas, and 3) test the hypothesis that isolation between northern and southern populations of parasites occurred simultaneously.

METHODS

Data collection

For an earlier study of comparative genetic structure between *O. princeps* and its helminth endoparasites, we sampled parasites from 222 *O. princeps* specimens representing 36 localities distributed across the host's range (Figure 4.1; Galbreath, Chapter 3). For that study, we collected mtDNA sequence data (COI; 369 bp) from specimens of *Cephaluris* (N = 165 individuals), *L.* (*Eugenuris*) (N = 123), and *L.* (*Labiostomum*) (N = 86); from *Schizorchis* individuals (N = 135), we collected sequence data from the small and large mtDNA ribosomal subunits (rRNA; ~815 bp). Here we add to these data homologous DNA sequences representing parasite material collected from three additional hosts.

To examine the relationship between parasite populations associated with *O. princeps* and *O. collaris*, we acquired parasite specimens from 13 *O. collaris* individuals representing 4 localities distributed across Alaska and Canada's Northwest Territories (Figure 4.1). We also sampled 4 *O. hyperborea* specimens (Northern pika; 2 localities; Siberia) and 2 *O. cansus* specimens (Gansu pika; 1 locality; Sichuan, China), providing a deeper context for understanding relationships between Old and New World parasite lineages. In most cases, hosts were either immediately frozen or necropsied in the field. We preserved parasites in 70% ethanol, providing high quality specimens for both molecular and morphological work.

We purified genomic DNA from specimens of *Cephaluris* ($N = 12$ individuals), *L.* (*Eugenuris*) ($N = 9$), *L.* (*Labiostomum*) ($N = 7$), and *Schizorchis* ($N = 11$) using Qiagen DNeasy kits, and sequenced the homologous mtDNA regions (COI from nematodes; rRNA from *Schizorchis*) that we had previously studied from parasites associated with *O. princeps*. See Galbreath (Chapter 3) for primers and reaction conditions. We aligned COI sequences by eye. Several indels were evident in the rRNA dataset, so we aligned these data with CLUSTALW (Thompson et al. 1994), using MEGA 3.1 (Kumar et al. 2004) and default parameters. We checked the alignment by eye and removed indels, yielding a final rRNA dataset 805 bp in length. Unless otherwise noted, we removed redundant haplotypes from datasets for subsequent analyses.

Parasites were identified to species using methods appropriate for each taxon. Detailed methods and lists of comparative material used for identifications are described elsewhere (Galbreath, Chapter 3; Hoberg et al. submitted). Vouchers and frozen tissues for host and parasite specimens are archived in appropriate research collections (hosts – University of Alaska Museum, Museum of Southwestern Biology,

Cornell University Museum of Vertebrates; parasites – United States National Parasite Collection).

Phylogenetic analyses

We reconstructed phylogenetic relationships among haplotypes in separate analyses for each parasite lineage. To root the nematode phylogenies we included one representative sequence from each of the other major pinworm lineages. We used *Hymenolepis diminuta* (GenBank #AF314223) as the outgroup in analyses of *Schizorchis*. First, we conducted maximum parsimony searches in PAUP* 4.0b10 (Swofford 2000) with TBR branch swapping and 1000 random addition replicates, summarizing the most parsimonious topologies in a strict consensus tree. Second, we performed a Bayesian analysis using MRBAYES 3.0b4 (Huelsenbeck and Ronquist 2001), after selecting an appropriate model of nucleotide substitution using AIC in MRMODELTEST (Nylander 2004). Bayesian analyses included five chains and were run for five million steps, sampling every 100 steps. We discarded the first one million steps as burn-in. Analyses were repeated four times from different random seeds to ensure convergence on essentially identical topologies. The final topology for each dataset was produced by generating a majority rule consensus tree of the 160,000 samples retained from the four analyses.

Paraphyletic relationships between northern (i.e., associated with *O. collaris*) and southern (i.e., associated with *O. princeps*) populations allowed us to discriminate between biogeographic hypotheses. For each case in which one population was shown by phylogenetic analyses to be paraphyletic with respect to the other, we tested the null hypothesis that the data could have been retrieved from a history in which the putatively paraphyletic population was in reality monophyletic. Failure to reject the null hypothesis would represent failure to reject other phylogenetic patterns that could

indicate alternative conclusions (i.e., reciprocal monophyly – biogeographic history inconclusive; inverse paraphyletic relationship – opposite biogeographic inference; Figure 4.1).

We used two approaches to test the null hypothesis. First, for a given instance of paraphyly, we used constraint filters in PAUP to calculate the proportion of trees retained by the Bayesian phylogenetic analysis in which the paraphyletic relationship was not evident (Carstens et al. 2005). Second, we used parametric bootstrapping (Goldman 1993; Hillis et al. 1996), which involves simulation of many sequence datasets under the null phylogenetic hypothesis to produce a null distribution for a test statistic that can be compared to the empirical data. The test statistic was the difference in log likelihood scores between maximum likelihood phylogenies computed with and without topological constraints representing the null hypothesis. To allow for manageable computation times we pruned datasets by arbitrarily removing one sequence out of every sequence pair that differed by less than 1% (uncorrected distance), and we included only the least divergent outgroup. We used AIC in MODELTEST 3.7 (Posada and Crandall 1998) to select a model of nucleotide evolution based on a phylogeny constrained to fit the null hypothesis. This model was then used for maximum likelihood searches in PAUP to identify the best constrained and unconstrained phylogenies from the empirical data, while simultaneously conducting likelihood estimation of parameter values. If a pruned dataset was too large to estimate both the topology and model parameters simultaneously, we identified appropriate parameter values by running multiple iterations of parameter estimation in MODELTEST and likelihood tree searches in PAUP, updating the input tree and parameter values with the results of the last iteration until parameter values and tree topology stabilized. We used MESQUITE 2.5 (Maddison and Maddison 2008) to simulate 500 datasets under the nucleotide model and parameters selected for the

constrained phylogeny. The difference in log likelihood between constrained and unconstrained maximum likelihood tree searches on these simulated datasets performed using PAUP provided the null distribution of the test statistic.

If the parasite communities of North American pikas were separated in the same event that initiated speciation of *O. princeps* and *O. collaris*, we would expect divergence times to be essentially simultaneous for pairs of northern and southern populations of the different parasite lineages. Incongruent divergence times could be an indicator that assembly of the parasite assemblage was staggered over time, or that periodic gene flow between northern and southern populations has occurred in some parasite species and not others. To test the relative timing of divergence among parasite lineages, we employed the approximate Bayesian computation approach implemented in the program MSBAYES (Hickerson et al. 2007). Unlike other coalescent-based methods for determining population divergence times, this approach estimates all parameters simultaneously for multiple population pairs representing different taxa that may share a common history of vicariance. Population pairs may or may not be reciprocally monophyletic. The method is based on a hierarchical coalescent model in which hyper-parameters that describe the full set of population pairs (e.g., number of possible divergence times, mean divergence time) are estimated while accounting for uncertainty and stochastic variation in taxon-specific sub-parameters (e.g., current and ancestral effective population size, divergence time) (Hickerson et al. 2006). The analysis involves three main steps: 1) calculate summary statistics for each population pair from the empirical data (e.g., mean pairwise distance among sequences within populations, net distance between populations), 2) simulate DNA sequence datasets for population pairs using parameters drawn at random from the hyper-prior and sub-prior parameter distributions, and calculate summary statistics from the simulated data, 3) generate the posterior distribution for hyper-parameters

using an acceptance/rejection algorithm to compare empirical and simulated summary statistic vectors.

We used MSBAYES to analyze only the pinworm lineages for simultaneous divergence. We chose not to include *Schizorchis* in the analysis because the model implemented in MSBAYES assumes that mutation rates are equal across taxa (Hickerson et al. 2006). While we cautiously make this assumption for the nematodes, which share life history characteristics and for which we sequenced identical mtDNA fragments, it is unlikely to hold true for a different gene region from a species representing another ancient branch of the Tree of Life. For this analysis, sequences were drawn from complete sequence datasets rather than just haplotypes, and we limited each population to 50 individuals, selecting sequences at random if a larger sample was available. MSBAYES implements the HKY model of sequence evolution and requires estimates of base frequencies and transition:transversion ratio for each population pair, which we calculated using PAUP. We ran 500,000 simulations, sampling 1000 of these to construct the posterior probability distribution. For the summary statistics used to generate the posterior probability distribution in the final acceptance/rejection step we selected mean pairwise differences among sequences between populations, mean pairwise distances among sequences within southern populations, number of segregating sites within southern populations normalized for sample size, and the denominator of Tajima's *D* for southern populations (Hickerson et al. 2007). Most summary statistics based on both northern and southern populations could not be calculated because some northern populations were represented by a single individual or haplotype.

RESULTS

Species identifications

Morphological examination of parasites revealed that our collections from *O. collaris* included all parasite species previously reported from that host (Table 4.1). In addition to known species, we identified two morphologically distinct groups that may represent undescribed species; these will be described elsewhere. We detected several examples of new host records relative to previous reports, particularly in *O. hyperborea* and *O. cansus* (Table 4.1). Of particular interest was *Labiostomum* (*E.*) *talkeetnaeuris*, which was previously considered to be restricted to *O. collaris* but we also identified in *O. hyperborea* from eastern Siberia.

Molecular data and phylogenetic analyses

All sequences exhibited characteristics consistent with expectations for true mtDNA. In the protein coding COI region sequenced from the nematodes, substitutions were concentrated at first and third codon positions, and *Schizorchis* rRNA sequences showed a deficiency in cytosine (~ 12%) and excess of thymine (~ 38%) similar to that observed in other tapeworms (von Nickisch-Rosenegk et al. 2001). We detected no evidence of heteroplasmy or numts (e.g., overlapping peaks in electropherograms).

Maximum parsimony and Bayesian analyses yielded largely congruent phylogenies for all major parasite lineages (Figure 4.2). *Cephaluris* was divided into two deeply divergent clades, which we treated as separate major lineages for analyses (hereafter *Cephaluris* A and B). Lineages varied in depth and phylogenetic resolution, with *Cephaluris* A, *L.* (*Eugenuris*), and *Schizorchis* exhibiting substantial substructure, while *Cephaluris* B and *L.* (*Labiostomum*) formed shallow, poorly resolved clades. Most species and undescribed morphotypes formed monophyletic

Table 4.1. Parasite species identified in this study from *O. collaris*, *O. hyperborea*, and *O. cansus*, or in our previous study of *O. princeps* (Galbreath, Chapter 3). Species denoted by an asterisk (*) were previously reported for these hosts but not detected in our collections; a plus sign (+) denotes new host records for described species. Numerical notations (e.g., species 1, etc.) represent currently undescribed species-level taxa as determined based on integrated morphological and molecular data.

	O. princeps	***O. collaris***	***O. hyperborea***	***O. cansus***
Schizorchis	*S. ochotonae* *S.* species 1 *S.* species 2 *S.* species 3	*S. caballeroi*	+*S. mongoliensis* **S. altaica* **S. yamashitai* **S. ryzhikovi*	+ *S. yamashitai*
Cephaluris	*C. alaskensis* *C. coloradensis*	*C. alaskensis* *C. coloradensis* *C.* species 1	+*C.* cf. *coloradensis*	+*C.* cf. *hashmi*
***L.* (*Eugenuris*)**	+*L.* (*E.*) *talkeetnaeuris* *L.* (*E.*) species 1 *L.* (*E.*) species 2 *L.* (*E.*) species 3	*L.* (*E.*) *talkeetnaeuris*	+*L.* (*E.*) *talkeetnaeuris*	none
***L.* (*Labiostomum*)**	+*L.* (*L.*) *rauschi* *L.* (*L.*) species 1	*L.* (*L.*) *rauschi*	*L.* (*L.*) species 2	+*L.* (*L.*) cf. *akhtari*
Murielus	*M. harpespiculus*	none	**M. harpespiculus*	+*M.* cf. *tjanschaniensis*
Graphidiella	*G. ochotonae*	none	none	none
Ohbayashinema	*O.* species 1	none	none	+*O. ochotoni*

Figure 4.2. One of the parsimonious trees identified for A) *Cephaluris* A, B) *Cephaluris* B, C) *Eugenuris*, D) *Labiostomum*, and E) *Schizorchis*. The inset adjacent to the *Cephaluris* A phylogeny shows the Bayesian result for relationships among major clades (denoted by grey boxes with numbers), and a question mark (?) specifies the disputed node on the parsimony tree. On all trees, black branches indicate relationships retained by strict consensus of all most parsimonious trees, and grey branches indicate nodes that were collapsed in the strict consensus tree. Open circles on branches indicate Bayesian posterior probabilities > 0.95, and stars highlight the position of haplotypes associated with *O. collaris*. Scale bars reflect branch lengths of five substitutions. Host associations (in parentheses) and either species' names or temporary clade designations are given next to major clades that could be distinguished morphologically.

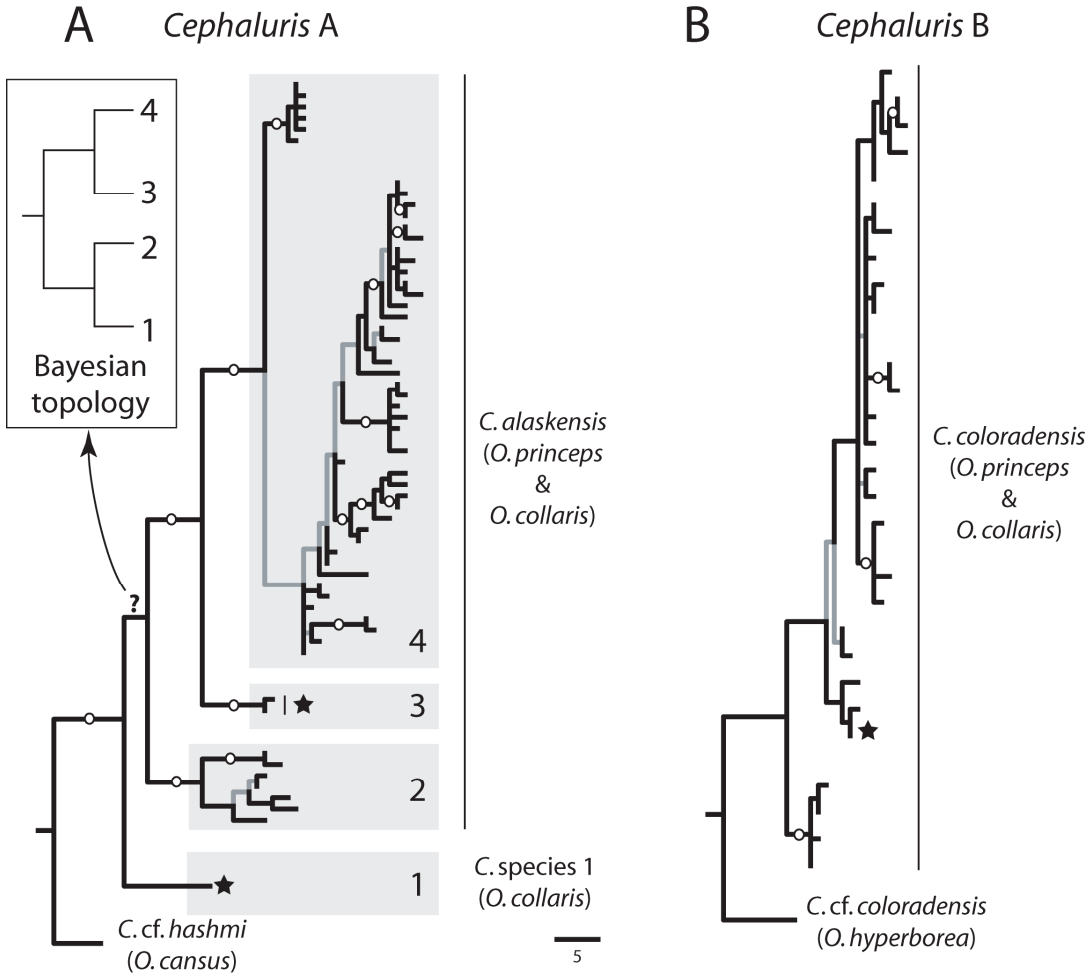

A
Cephaluris A
4
3
2
1
Bayesian topology
?
4
3
2
1
C. alaskensis (O. princeps & O. collaris)
C. species 1 (O. collaris)
C. cf. hashmi (O. cansus)
5
B
Cephaluris B
C. coloradensis (O. princeps & O. collaris)
C. cf. coloradensis (O. hyperborea)

Figure 4.2 (continued).

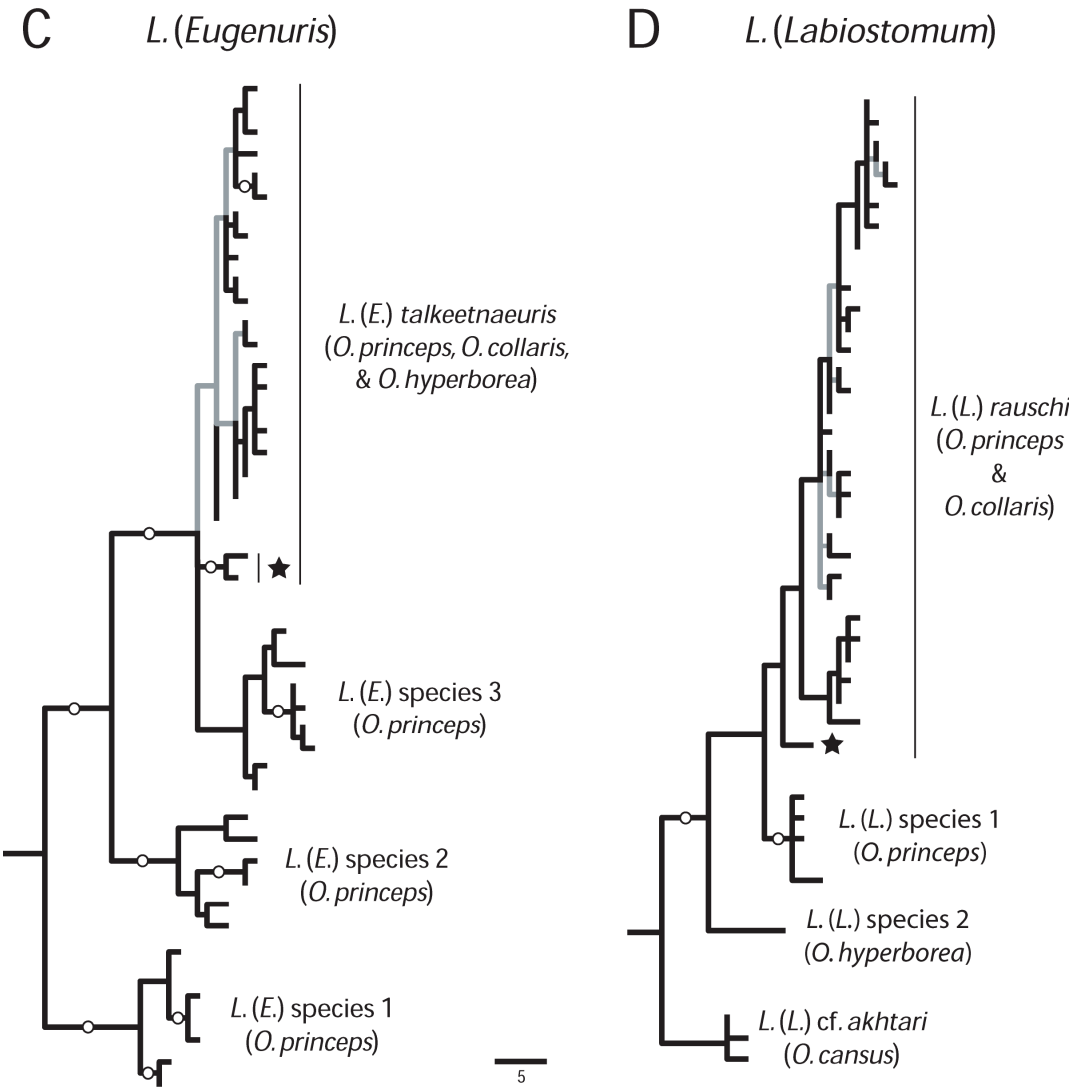
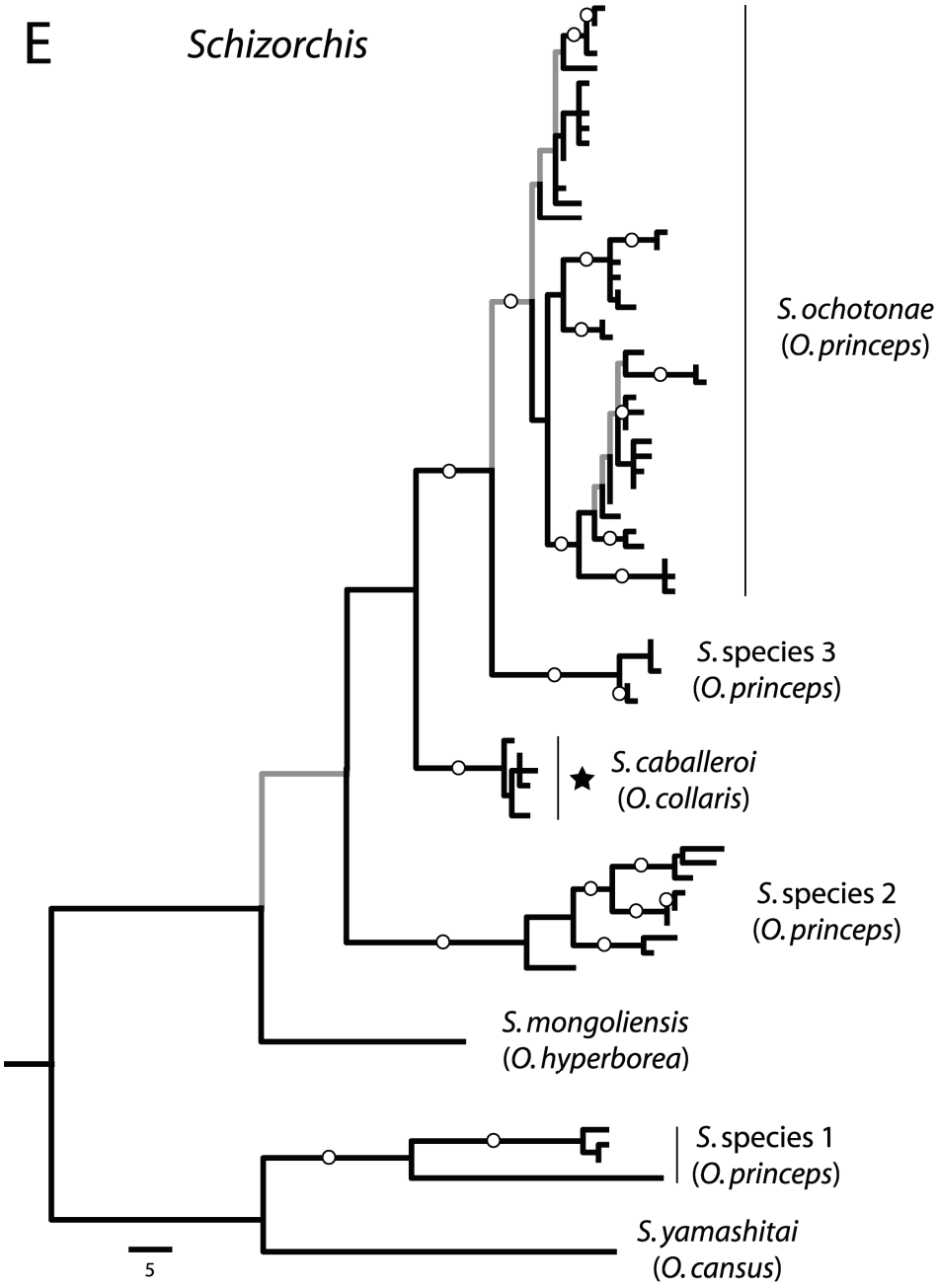


Figure 4.2 (continued).



clades based on the molecular data, demonstrating congruence between morphological and molecular structure (Figure 4.2).

Because all the major parasite lineages are presumed to be of Old World origin, at least one trans-Beringian colonization event is assumed for each and Palearctic species are expected to be phylogenetically basal relative to Nearctic taxa. Thus, we inferred single colonizations of North America for *L*. (*Eugenuris*) and *L*. (*Labiostomum*), as well as for the three genera from which we did not collect molecular data (*Murielus*, *Graphidiella*, *Ohbayashinema*). Unexpectedly, the COI sequence from a specimen of *L*. (*Eugenuris*) from *O. hyperborea* in Siberia was identical to those of specimens from *O. collaris*, and nested within the crown of the Nearctic *L*. (*Eugenuris*) clade (Figure 4.2c). This implies very recent (e.g., last glacial) dispersal from eastern to western Beringia. Morphological and molecular data showed that like *Cephaluris*, *Schizorchis* was divided into two deeply divergent sub-lineages (Figure 4.2e). Each of the *Cephaluris* and *Schizorchis* sub-lineages were anchored by basal splits between Nearctic and Palearctic taxa, suggesting that North America was colonized by two *Cephaluris* and two *Schizorchis* species. Only one of the *Schizorchis* sub-lineages was shared by both *O. princeps* and *O. collaris* in North America; the other was restricted to *O. princeps*. In total we identified nine North American colonization events involving independent parasite lineages, and one secondary dispersal from Alaska into Siberia.

Phylogenies of *Cephaluris* B, *L*. (*Eugenuris*), *L*. (*Labiostomum*), and *Schizorchis* all revealed that parasite populations associated with American pikas are paraphyletic with respect to those from Collared pikas (Figure 4.2b-e). Bayesian posterior probability tests of the null hypothesis that parasites associated with *O. princeps* are in fact monophyletic indicated that the evidence for paraphyly is relatively robust (Table 4.2). Only the analysis of *L*. (*Labiostomum*) failed to reject the

Table 4.2. Models applied and results of the Bayesian (Bayes) and parametric bootstrapping (PB) phylogenetic hypothesis tests.

Hypothesis	$Model_{Bayes}$	P_{Bayes} [a]	$Model_{PB}$	P_{PB} [b]
Tests of biogeographic hypotheses				
Monophyly of southern *Cephaluris* A	HKY + Γ	< 0.001	GTR + Γ	< 0.002
Monophyly of northern *Cephaluris* A	HKY + Γ	< 0.001	GTR + Γ	< 0.002
Monophyly of southern *Cephaluris* B	HKY + Γ	0.002	HKY + I + Γ	0.268
Monophyly of southern *L.* (*Eugenuris*)	GTR + Γ	< 0.001	GTR + Γ	0.006
Monophyly of southern *L.* (*Labiostomum*)	HKY + I + Γ	0.080	GTR + I + Γ	0.110
Monophyly of southern *Schizorchis*	GTR + I + Γ	0.026	HKY + I + Γ	0.136
Tests of alternative *Cephaluris* A topologies				
Bayesian topology	HKY + Γ	0.86	GTR + Γ	0.96
Parsimony topology	HKY + Γ	0.095	GTR + Γ	0.15

[a] P_{Bayes} = proportion of retained trees from the Bayesian analysis that match the phylogenetic hypothesis

[b] P_{PB} = proportion of the simulated test statistic distribution that is greater than or equal to the empirical test statistic

null hypothesis at $\alpha = 0.05$. Tests using parametric bootstrapping were more conservative, only rejecting the null hypothesis for *L*. (*Eugenuris*). The *Cephaluris* A lineage is unique in that it includes two strongly differentiated clades associated with both *O. collaris* and *O. princeps* (Figure 4.2a). Monophyly of these northern and southern pairs was strongly rejected by both Bayesian posterior probability tests and parametric bootstrapping (Table 4.2). Furthermore, *Cephaluris* A represents a notable exception to the general congruence observed between results of the parsimony and Bayesian phylogenetic analyses. Maximum parsimony identified a sister relationship between *C. alaskensis* and an undescribed species associated with Collared pikas (Figure 4.2a), but the Bayesian phylogeny showed that only the basal *C. alaskensis* clade was sister to the undescribed northern species, and together these clades were sister to the remainder of *C. alaskensis* (Figure 4.2a, inset). We tested both of these phylogenetic hypotheses using Bayesian posterior probabilities and parametric bootstrapping, and though neither hypothesis was clearly rejected, both methods of hypothesis testing indicated higher probabilities for the topology retrieved by the Bayesian phylogenetic analysis than that identified by maximum parsimony (Table 4.2).

For the test of simultaneous divergence between northern and southern populations of pinworms, we used the results of the phylogenetic analyses to guide the selection of population pair sequences. Relationships between northern and southern haplotypes were not fully resolved for *Cephaluris* B, *Eugenuris*, and *Labiostomum*, so we drew population samples from individuals representing the least inclusive, strongly supported clade that included all northern and some southern individuals. We could not apply this strategy to the *Cephaluris* A lineage because it included two independent clades from both northern and southern hosts. Though the true phylogeny of the lineage remains in doubt, we based our selection of population pairs on the two

north/south divergences implied by the Bayesian tree topology (Figure 4.2a, inset) because it was better supported by the topology hypothesis tests than the parsimony topology. Thus, the analysis included 5 population pairs in total, and we were unable to reject the hypothesis of simultaneous divergence. The Bayesian posterior estimate of hyper-parameter Ψ (the number of possible divergence times) did not differ significantly from 1 (median, 95% quantile; 1.00, 1.00 – 1.02).

DISCUSSION

Historical biogeography of North American pikas

The conventional explanation for the origin of *O. princeps* and *O. collaris* is that their common ancestor colonized Beringia during the Late Pleistocene (~125 – 11 kyBP), dispersing to lower latitudes via the Coastal and Rocky Mountain cordilleras (Guthrie 1973; Rausch and Smirnova 1984), and undergoing speciation when glacial-age expansion of continental ice sheets isolated northern and southern refugial populations. This scenario offers a parsimonious explanation for the distribution and origin of the two species, as well as their strong morphological (Weston 1981; Youngman 1975) and behavioral (Broadbrooks 1965; Kawamichi 1981) similarities. However, recent studies suggest that the history of isolation between the species predates the Late Pleistocene, implying an earlier arrival in North America by their common ancestor (Galbreath, Chapter 1, Chapter 2; Hafner and Sullivan 1995; Niu et al. 2004).

An earlier arrival by pikas is consistent with the fossil record. The earliest record of *Ochotona* in North America (*O. spanglei*) is from deposits of Late Miocene or Early Pliocene age in Oregon (Shotwell 1956). While this may represent an early trans-Beringian colonization by a pika species that later went extinct (Shotwell 1956), it could also be a first record of a lineage that persisted in the Nearctic throughout the

Pliocene and Pleistocene (Mead and Grady 1996) to become the ancestor of *O. princeps* and *O. collaris*. Furthermore, Pleistocene records of another extinct species (*O. whartoni*) in Alaska and Canada (Guthrie and Matthews 1971) and a pika in eastern North America that is morphologically consistent with both *O. collaris* and *O. princeps* (Mead and Grady 1996) indicate that the history of diversification and dispersal by pikas, especially at lower latitudes, is deeper and more complex than predicted by a simple model of recent trans-Beringian colonization and glacial isolation.

Our HPCP analysis further illuminates the complex history of North American pikas. We identified five parasite lineages that are shared by both *O. princeps* and *O. collaris* and that represent separate colonizations of North America, offering independent perspectives on the biogeographic history of North American pikas since colonization. In four of the five lineages, haplotypes associated with the northern host, *O. collaris*, are nested within the phylogeny of parasites from *O. princeps* (Figure 4.2b-e), demonstrating that the northern parasite assemblage was largely, if not entirely, derived from southern ancestral stock. Because each of the parasites has an obligate association with pikas, dispersal and colonization by the parasites must be mediated by the pika hosts. Thus, phylogeographic evidence from the parasites offers support for a host history consistent with the S-N hypothesis (Figure 4.1), in which the ancestral population of trans-Beringian migrants disappeared from high latitudes, but was subsequently re-established by northward expansion of southern populations.

Might hosts and parasites have contrasting biogeographic histories? In the absence of evidence for multiple trans-Beringian colonizations by pikas, we assume that all North American parasite lineages arrived with the common ancestor of *O. princeps* and *O. collaris*. If that ancestral pika persisted in Beringia to the present day, it would be necessary to invoke multiple independent parasite extinction events in the

North, followed by secondary contact and parasite exchange between northern and southern host populations. Furthermore, northern extinctions of one sub-lineage of *Schizorchis* (Figure 4.2e) and three nematodes (*Murielus, Graphidiella, Ohbayashinema*) that are absent from *O. collaris* but present in *O. princeps* also must be invoked to reconcile the N-S hypothesis (Table 4.1) (Hoberg 2005). The S-N hypothesis presents a much more parsimonious scenario. If the ancestral Beringian population of pikas did not persist through the Quaternary, extinction of ancestral parasite populations is explained by host extinction and parasite re-colonization of the North accompanied host re-colonization. The four parasite lineages that are absent from *O. collaris* likely 'missed the boat' during re-colonization, which is prone to happen if founder populations are small and isolated, just as bottlenecking due to founder effect can lead to a loss of genetic diversity at the leading edge of expanding populations (Hewitt 1996).

Cephaluris A is the only parasite lineage shared by *O. collaris* and *O. princeps* that fails to unambiguously meet the phylogenetic predictions of either the N-S or S-N biogeographic hypotheses. Furthermore, uncertainty regarding relationships among clades within the lineage casts doubt on the biogeographic interpretation of the phylogeny. It is evident that the lineage consists of two northern and two southern clades, and neither the northern pair nor the southern pair are sister, implying two separate north/south divergence events (Figure 4.2a). Neither Bayesian nor parsimony tree topologies discriminate between the two biogeographic hypotheses based on parsimonious reconstruction of dispersal events. Both topologies require two dispersals between northern and southern populations, regardless of the center of origin for the lineage (Figure 4.3). However, the two trees differ in their implications for the relative timing of dispersal events. The parsimony phylogeny indicates that dispersals occurred sequentially (e.g., during consecutive interglacial periods),

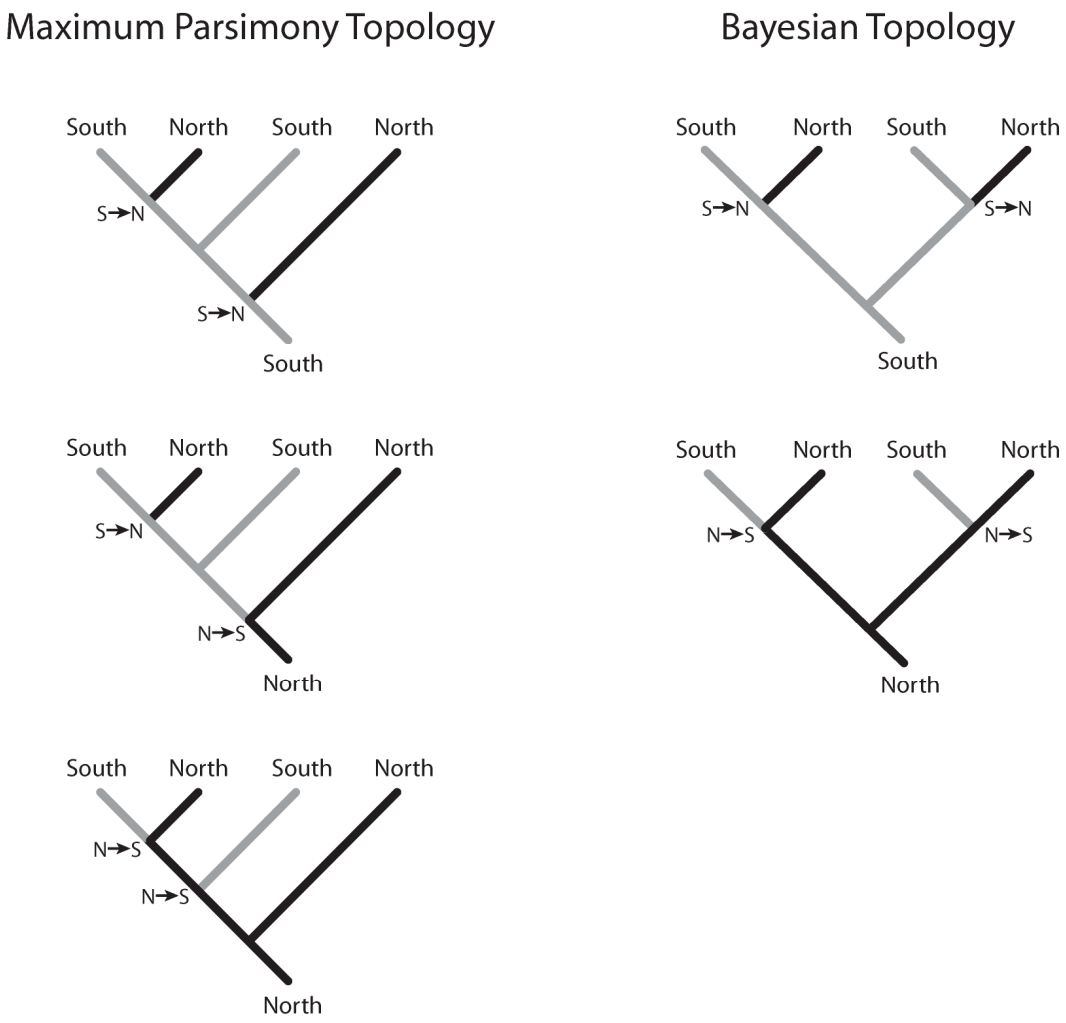


Figure 4.3. Alternative parsimonious reconstructions of biogeographic history for *Cephaluris* A based on tree topologies retrieved from maximum parsimony and Bayesian analyses. Nodes associated with dispersal/divergence events between northern and southern hosts are marked. Black branches are associated with northern hosts and grey branches are associated with southern hosts. Note that two dispersal/divergence events are implied regardless of topology or geographic position of the ancestral population.

whereas the Bayesian tree suggests that they could have occurred simultaneously. This distinction is important, as sequential dispersals by parasites would require at least two episodes of large-scale range expansion by pikas between high and low latitudes. In contrast, simultaneous dispersal by the two lineages only requires environmental conditions to have been optimal for large-scale pika dispersal one time (e.g., concurrently with dispersal inferred for the other major parasite lineages). Indeed, the fact that we failed to reject the hypothesis of simultaneous divergence between northern and southern parasite populations suggests that colonization by host and parasites occurred during a single episode of range expansion. The Bayesian topology may therefore be a better representation of the true history of diversification in the *Cephaluris* A lineage, though additional molecular data will be necessary to test this hypothesis. If the Bayesian tree is correct, it implies that *C. alaskensis* is paraphyletic. Comprehensive morphometric analyses of *C. alaskensis* and the putative undescribed species in *Cephaluris* A, which differ in number of caudal papillae, a species-level diagnostic character for *Cephaluris*, will help to resolve this unexpected result.

In summary, four out of five parasite lineages (*Cephaluris* B, *L.* [*Eugenuris*], *L.* [*Labiostomum*], *Schizorchis*) shared between Collared pikas and American pikas exhibit phylogenies consistent with the S-N biogeographic hypothesis. American pikas also harbor four parasite lineages that are entirely absent from Collared pikas, a pattern that is also best explained by the S-N hypothesis. One shared parasite lineage (*Cephaluris* A) fails to discriminate between either of the biogeographic hypotheses. Thus, evidence from eight of nine independent parasite lineages points to a southern origin for the northern parasite assemblage, strongly implying that *O. collaris*, itself, was derived from a northern isolate of *O. princeps*. No parasite phylogenies support the N-S hypothesis.

This result has important implications for understanding the complimentary roles of Beringia and temperate North America in structuring biotic diversity across the Nearctic. Beringia has served as both a continental crossroads (Hoffmann 1984; Hopkins 1959; Hopkins 1967) and as a center of diversification (Sher 1999; Sher 1984), and in both capacities it is viewed as a source of new diversity for North America. During the Quaternary, the flow of species across the land bridge was almost entirely from west to east (Guthrie and Matthews 1971; Kurtén 1966; Kurtén and Anderson 1980; Waltari et al. 2007), with continental ice sheets and arctic environments blocking temperate North American species from crossing to the west (Hoffmann 1981). Northward range expansion from low latitudes is usually considered in the context of post-Pleistocene population responses to glacial retreat, which generally result in genetically homogeneous populations that will presumably be pushed southward once again with the onset of the next glacial advance (Hewitt 1996; Hewitt 1999; Hewitt 2004; Taberlet et al. 1998).

Though the colonization of North America from Asia by the ancestral *Ochotona* fits the traditional Beringian narrative, our finding that pikas returned to Beringia from temperate latitudes and persisted there, undergoing speciation and contributing to the evolution of a diverse North American parasite fauna (up to 18 species descended from 9 ancestral parasites; Table 4.1), contrasts markedly with the perspective that Beringia is primarily a source for North American colonization. Furthermore, the indication that at least one parasite lineage (*L*. [*Eugenuris*]) recently crossed from eastern to western Beringia raises the possibility of Late Pleistocene dispersal by Collared pikas into Asia, although only the ancestral eastward colonization is reflected in current phylogenetic hypotheses for *Ochotona* (Niu et al. 2004; Yu et al. 2000). North American pikas are alpine specialists and are therefore highly adapted for cold environments (MacArthur and Wang 1974; Smith 1974; Smith

2008), which may have facilitated dispersal from low to high latitudes. In light of our findings, histories of dispersal and diversification in other North American species associated with montane environments should be reviewed (e.g., Albach et al. 2006; Conroy and Cook 2000) and assessed for the potential to use the HPCP approach to enrich biogeographic inferences.

Host-parasite comparative phylogeography

This study represents the most comprehensive phylogeographic assessment to date of a parasite assemblage, and it demonstrates the potential for using HPCP to resolve host biogeographic histories that are otherwise intractable. The strength of this approach is particularly evident in consideration of potential weaknesses of our study. For example, our phylogenetic reconstructions are based on a relatively small fragment of a single DNA locus, which yielded limited support for specific nodes in trees and may have contributed to the non-significant results of parametric bootstrapping. From such data, conclusions drawn from a single parasite lineage would be weak due to the potential influence of stochastic error in phylogenetic reconstruction. However, support for a given biogeographic hypothesis increases with each additional taxon that meets the predictions of the hypothesis. Though individually weak, concordance among the biogeographic perspectives offered by four independent parasite phylogenies, and further corroboration from four parasite taxa restricted to *O. princeps*, suggests that the over-all pattern is quite robust.

Comprehensive sampling of diversity is clearly a critical aspect of HPCP studies, as incomplete sampling can strongly influence conclusions, and current taxonomic frameworks for parasites may be insufficient indicators of species-level diversity, much less intraspecific diversity (Brooks and Hoberg 2000). Our sampling of parasites from *O. princeps* was extensive and may represent a complete survey of

that host for both known and unknown species of the focal helminth genera (Figure 4.1, Table 4.1). Sampling of *O. collaris* was less thorough by comparison. Though future work should examine material from additional northern populations, we are cautiously optimistic that our sampling of species-level parasite diversity from Collared pikas is relatively complete for two reasons. First, sampling localities were representative of the full host range (Figure 4.1). Second, most of the current range of *O. collaris* was buried under ice during the last glacial maximum (Hamilton et al. 1986), and therefore was colonized only recently during post-glacial expansion from a restricted Beringian population. Range retraction and expansion associated with a single glacial-age refugium may have had a homogenizing influence on the parasite assemblage, breaking up pockets of locally endemic faunas and spreading species widely among host populations (cf. Coope 2004; Coope 1979).

If we did fail to detect some parasite diversity from *O. collaris*, our major conclusions could be weakened but probably would not be invalidated. An undetected parasite haplotype could fall into one of three phylogenetic positions: 1) within or sister to an existing clade of *O. collaris* parasites, 2) not sister to other *O. collaris* parasites, but still nested within the phylogeny of parasites from *O. princeps*, or 3) basal to the *O. princeps* parasites. The first of these scenarios would not have any effect on the conclusions of our study, and neither would the second as long as the overall pattern continued to be one of southern paraphyly with respect to northern populations. If only one new sub-clade was detected, scenario 3 would likely result in an equivocal biogeographic inference much like that represented by the *Cephaluris* A lineage, but it would not result in support for the competing biogeographic hypothesis. Only the discovery of many, deeply divergent and currently unknown sub-clades could reverse our conclusions. Finally, the fact that we detected congruent histories

across multiple parasite lineages again reduces the likelihood that the observed pattern is simply a consequence of stochastic factors (e.g., sampling bias).

To date, advocates of the use of parasites in comparative phylogeographic contexts have focused primarily on the search for phylogeographic congruence between hosts and parasites (Criscione et al. 2005; Nieberding and Olivieri 2007; Whiteman and Parker 2005), which would presumably reflect co-differentiation due to extrinsic barriers to gene flow. Under the assumption that co-differentiation is the dominant cause of parasite genetic structure, phylogeographic structure observed in parasites but not hosts reflects population fragmentation that failed to leave a genetic signature in the host (Nieberding et al. 2004; Nieberding and Olivieri 2007). This emphasis on co-differentiation has led some to conclude that HPCP studies should focus on parasites with vertical (parent-to-offspring) transmission, small effective population sizes, high mutation rates, and short generation times, thereby maximizing the likelihood of congruence between parasite and host genealogies (Nieberding and Olivieri 2007; Whiteman and Parker 2005).

We argue that this perspective is limiting, in part because very few parasites meet the proposed criteria. More importantly, a focus on strict co-differentiation restricts the types of questions that an investigator can ask. In the current study, incongruence between host and parasite phylogenies provided the key to testing competing hypotheses. Had all the parasite lineages been phylogenetically congruent with their hosts (i.e., northern and southern sister lineages), we would have learned nothing new. Rather than seek co-differentiation for its own sake, investigators should carefully define the specific hypotheses that they wish to test, and the phylogenetic, biogeographic, demographic, or population genetic predictions that follow. This is essentially the same procedure that should be applied in any comparative phylogeographic analysis (Riddle and Hafner 2006). Characteristics of parasites that

may affect their utility for testing the hypotheses should be considered (e.g., host specificity over the time period of interest), but the qualities of the ideal study organism will vary with the question being asked.

An effective HPCP research program is truly an interdisciplinary effort, minimally incorporating expertise in molecular methods, phylogeographic analyses, natural history, and taxonomy. Taxonomic knowledge is incomplete for many parasite groups (Brooks and Hoberg 2000) and the use of molecular methods to explore parasite diversity has great potential to detect new species (Anderson et al. 1998; McManus and Bowles 1996). However, molecular data alone are insufficient to adequately characterize species diversity (Hansen et al. 2007), which is fundamental to a research endeavor that revolves around exploring geographic patterns of biotic diversity. Our discovery of morphologically and genetically distinct clades that may represent up to nine new species (Table 4.1) illustrates the potential for HPCP studies to address questions regarding both host-parasite population histories and gaps in the taxonomic record, but it also highlights the importance of incorporating appropriate taxonomic expertise into the research program. Integration of HPCP approaches with comprehensive biotic inventories presents a powerful model for addressing questions regarding the biogeography and diversification of regional biotas (e.g., Beringian Coevolution Project; Cook et al. 2005; Hoberg et al. 2003).

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APPENDIX

Museum catalog numbers, collection localities, and molecular markers sequenced for *Ochotona princeps* specimens. CUMV = Cornell University Museum of Vertebrates; NMMNH = New Mexico Museum of Natural History; HSU = Humboldt State University. Molecular datasets include cytb/D-loop (full mtDNA dataset), D-loop (partial mtDNA dataset), and the nuclear markers MGF and PRKCI. Localities 38 through 64 were georeferenced based on locality descriptions using the Biogeomancer Workbench (http://www.biogeomancer.org/).

Museum catalog #	Latitude	Longitude	Dataset
1. Hagensborg, BC			
CUMV 20726	N 52° 12’ 56.6”	W 126° 21’ 47.5”	cytb/D-loop
CUMV 20729	N 52° 13’ 11.5”	W 126° 22’ 7.0”	cytb/D-loop
CUMV 20735	N 52° 12’ 41.8”	W 126° 21’ 50.9”	cytb/D-loop
CUMV 20741	N 52° 12’ 53.7”	W 126° 21’ 54.2”	cytb/D-loop
CUMV 20742	N 52° 13’ 3.7”	W 126° 21’ 40.1”	cytb/D-loop
NMMNH 978			D-loop
2. Pemberton, BC			
CUMV 20745	N 50° 34’ 57.7”	W 123° 1’ 40.0”	cytb/D-loop
CUMV 20746	N 50° 35’ 14.9”	W 123° 1’ 51.5”	cytb/D-loop
CUMV 20748	N 50° 34’ 56.9”	W 123° 2’ 3.3”	cytb/D-loop
CUMV 20585	N 50° 34’ 46.9”	W 123° 1’ 57.3”	cytb/D-loop
CUMV 20584	N 50° 34’ 42.0”	W 123° 1’ 56.3”	cytb/D-loop
3. McBride, BC			
CUMV 20590	N 53° 21’ 32.8”	W 120° 6’ 49.3”	cytb/D-loop
CUMV 20712	N 53° 21’ 55.1”	W 120° 7’ 29.5”	cytb/D-loop
CUMV 20715	N 53° 21’ 29.9”	W 120° 7’ 23.5”	cytb/D-loop
CUMV 20716	N 53° 20’ 53.7”	W 120° 8’ 8.9”	cytb/D-loop
CUMV 20717	N 53° 20’ 54.5”	W 120° 8’ 2.8”	cytb/D-loop
4. Raft Mountain, BC			
CUMV 20769	N 51° 43’ 3.0”	W 119° 51’ 44.6”	cytb/D-loop, MGF, PRKCI
CUMV 20756	N 51° 43’ 4.3”	W 119° 51’ 43.2”	cytb/D-loop, MGF, PRKCI
CUMV 20770	N 51° 43’ 15.8”	W 119° 51’ 30.7”	cytb/D-loop, MGF, PRKCI
CUMV 20759	N 51° 43’ 25.8”	W 119° 51’ 13.5”	cytb/D-loop, MGF, PRKCI
CUMV 20772	N 51° 43’ 27.4”	W 119° 51’ 10.5”	cytb/D-loop, MGF, PRKCI
NMMNH 953			D-loop
5. Grande Cache, AB			
CUMV 20719	N 54° 4’ 26.1”	W 119° 24’ 10.4”	cytb/D-loop
CUMV 20589	N 54° 4’ 38.2”	W 119° 25’ 7.8”	cytb/D-loop
CUMV 20722	N 54° 4’ 57.9”	W 119° 25’ 41.2”	cytb/D-loop
CUMV 20723	N 54° 5’ 15.9”	W 119° 25’ 50.4”	cytb/D-loop
CUMV 20724	N 54° 5’ 14.0”	W 119° 25’ 57.3”	cytb/D-loop

Museum catalog #	Latitude	Longitude	Dataset
6. Landslide Lake, AB			
CUMV 20591	N 52° 2’ 13.9”	W 116° 30’ 56.2”	cytb/D-loop
CUMV 20696	N 52° 2’ 14.2”	W 116° 30’ 56.7”	cytb/D-loop
CUMV 20697	N 52° 3’ 37.3”	W 116° 31’ 17.2”	cytb/D-loop
CUMV 20698	N 52° 3’ 39.7”	W 116° 31’ 4.3”	cytb/D-loop
CUMV 20699	N 52° 3’ 28.8”	W 116° 32’ 29.1”	cytb/D-loop
7. Washington Pass, WA			
CUMV 20284	N 48° 30’ 34.3”	W 120° 38’ 1.9”	cytb/D-loop, MGF, PRKCI
CUMV 20287	N 48° 30’ 29.5”	W 120° 37’ 56.2”	cytb/D-loop, MGF, PRKCI
CUMV 20288	N 48° 30’ 26.2”	W 120° 37’ 49.9”	cytb/D-loop, MGF, PRKCI
CUMV 20293	N 48° 30’ 34.3”	W 120° 37’ 59.5”	cytb/D-loop, MGF
CUMV 20295	N 48° 30’ 25.3”	W 120° 37’ 49.9”	cytb/D-loop, MGF, PRKCI
NMMNH 1028			D-loop
8. Sunrise Peak, WA			
CUMV 20300	N 46° 19’ 49.7”	W 121° 44’ 48.4”	cytb/D-loop, MGF, PRKCI
CUMV 20302	N 46° 19’ 48.7”	W 121° 44’ 52.6”	cytb/D-loop, MGF
CUMV 20303	N 46° 19’ 47.0”	W 121° 44’ 52.8”	cytb/D-loop, MGF, PRKCI
CUMV 20304	N 46° 19’ 45.7”	W 121° 44’ 54.8”	cytb/D-loop, MGF, PRKCI
9. Indian Heaven, WA			
CUMV 20307	N 46° 3’ 8.8”	W 121° 45’ 32.5”	cytb/D-loop, MGF
CUMV 20310	N 46° 3’ 10.8”	W 121° 45’ 27.9”	cytb/D-loop, MGF, PRKCI
10. McKenzie Pass, OR			
CUMV 21152	N 44° 14’ 40.2”	W 121° 49’ 18.8”	cytb/D-loop, MGF
CUMV 20628	N 44° 14’ 43.9”	W 121° 49’ 20.1”	cytb/D-loop, MGF
CUMV 20630	N 44° 14’ 46.5”	W 121° 49’ 38.0”	cytb/D-loop, MGF
CUMV 20631	N 44° 15’ 39.6”	W 121° 48’ 32.5”	cytb/D-loop, MGF
CUMV 20632	N 44° 15’ 51.4”	W 121° 48’ 53.8”	cytb/D-loop, MGF, PRKCI
NMMNH 862			D-loop
11. Anthony Lakes, OR			
CUMV 20637	N 44° 56’ 41.1”	W 118° 14’ 37.1”	cytb/D-loop, MGF
CUMV 20577	N 44° 56’ 19.1”	W 118° 13’ 27.0”	cytb/D-loop, MGF, PRKCI
CUMV 20638	N 44° 56’ 52.8”	W 118° 12’ 39.2”	cytb/D-loop, MGF, PRKCI
CUMV 20642	N 44° 56’ 48.0”	W 118° 12’ 34.4”	cytb/D-loop, MGF, PRKCI
CUMV 20576	N 44° 56’ 49.8”	W 118° 12’ 35.7”	cytb/D-loop, MGF, PRKCI
NMMNH 1049			D-loop
12. Wallowa Mtns., OR			
CUMV 21154	N 45° 3’ 20.9”	W 117° 17’ 7.5”	cytb/D-loop, MGF, PRKCI
CUMV 20575	N 45° 3’ 39.6”	W 117° 16’ 37.9”	cytb/D-loop, MGF, PRKCI
CUMV 20648	N 45° 3’ 38.9”	W 117° 16’ 37.3”	cytb/D-loop, MGF, PRKCI
CUMV 20574	N 45° 3’ 27.9”	W 117° 16’ 59.4”	cytb/D-loop, MGF, PRKCI
CUMV 20649	N 45° 3’ 15.2”	W 117° 17’ 2.4”	cytb/D-loop, MGF, PRKCI

Museum catalog #	Latitude	Longitude	Dataset
13. Roman Nose Lakes, ID			
CUMV 20270	N 48° 37’ 54.3”	W 116° 34’ 13.7”	cytb/D-loop, MGF, PRKCI
CUMV 20062	N 48° 37’ 51.0”	W 116° 35’ 19.0”	cytb/D-loop, MGF, PRKCI
CUMV 20278	N 48° 37’ 52.1”	W 116° 35’ 24.1”	cytb/D-loop, MGF, PRKCI
CUMV 20279	N 48° 37’ 46.1”	W 116° 35’ 29.5”	cytb/D-loop, MGF, PRKCI
CUMV 20282	N 48° 37’ 48.5”	W 116° 35’ 38.5”	cytb/D-loop, MGF, PRKCI
14. Black Lake, ID			
CUMV 20255	N 45° 11’ 13.2”	W 116° 34’ 30.8”	cytb/D-loop, PRKCI
CUMV 20260	N 45° 11’ 11.8”	W 116° 34’ 58.3”	cytb/D-loop, MGF, PRKCI
CUMV 20262	N 45° 10’ 50.7”	W 116° 34’ 5.7”	cytb/D-loop, MGF, PRKCI
CUMV 20263	N 45° 10’ 47.3”	W 116° 34’ 5.6”	cytb/D-loop, MGF, PRKCI
CUMV 20268	N 45° 10’ 31.9”	W 116° 34’ 4.8”	cytb/D-loop, MGF, PRKCI
NMMNH 624			D-loop
15. Featherville, ID			
CUMV 20248	N 43° 36’ 55.9”	W 115° 26’ 7.1”	cytb/D-loop, MGF, PRKCI
CUMV 20249	N 43° 36’ 55.8”	W 115° 26’ 6.3”	cytb/D-loop, MGF, PRKCI
CUMV 20251	N 43° 36’ 57.0”	W 115° 26’ 8.3”	cytb/D-loop, MGF, PRKCI
CUMV 20253	N 43° 36’ 52.1”	W 115° 26’ 8.7”	cytb/D-loop, MGF, PRKCI
CUMV 20254	N 43° 36’ 52.7”	W 115° 26’ 12.4”	cytb/D-loop, MGF, PRKCI
16. Doublespring Pass, ID			
CUMV 20241	N 44° 18’ 41.6”	W 113° 53’ 45.9”	cytb/D-loop, MGF, PRKCI
CUMV 20243	N 44° 18’ 25.8”	W 113° 53’ 59.7”	cytb/D-loop, MGF, PRKCI
CUMV 20244	N 44° 18’ 25.7”	W 113° 54’ 1.5”	cytb/D-loop, MGF, PRKCI
CUMV 20246	N 44° 18’ 19.0”	W 113° 54’ 27.5”	cytb/D-loop, MGF, PRKCI
CUMV 20247	N 44° 18’ 18.8”	W 113° 54’ 13.8”	cytb/D-loop, MGF, PRKCI
17. Darby, MT			
CUMV 20237	N 46° 2’ 3.9”	W 114° 17’ 0.4”	cytb/D-loop, MGF, PRKCI
CUMV 20238	N 46° 2’ 5.8”	W 114° 16’ 59.6”	cytb/D-loop, MGF, PRKCI
CUMV 20239	N 46° 1’ 47.5”	W 114° 17’ 32.2”	cytb/D-loop, MGF, PRKCI
CUMV 20240	N 46° 2’ 1.0”	W 114° 17’ 7.9”	cytb/D-loop, MGF, PRKCI
NMMNH 594			D-loop
18. Neihart, MT			
CUMV 20210	N 46° 56’ 4.9”	W 110° 37’ 28.3”	cytb/D-loop, MGF, PRKCI
CUMV 20056	N 46° 56’ 6.7”	W 110° 37’ 29.7”	cytb/D-loop, MGF, PRKCI
CUMV 20211	N 46° 57’ 59.0”	W 110° 37’ 45.2”	cytb/D-loop, MGF, PRKCI
CUMV 20214	N 46° 57’ 50.2”	W 110° 38’ 2.6”	cytb/D-loop, MGF, PRKCI
CUMV 20216	N 46° 56’ 51.2”	W 110° 39’ 47.0”	cytb/D-loop, MGF, PRKCI
CUMV 20217	N 46° 57’ 23.7”	W 110° 38’ 41.9”	cytb/D-loop, MGF, PRKCI
NMMNH 570			D-loop
19. Red Lodge, MT			
CUMV 20223	N 45° 0’ 22.3”	W 109° 28’ 16.6”	cytb/D-loop, MGF, PRKCI
CUMV 20224	N 45° 0’ 21.2”	W 109° 28’ 16.0”	cytb/D-loop, MGF, PRKCI
CUMV 20226	N 44° 59’ 52.4”	W 109° 30’ 38.2”	cytb/D-loop, MGF, PRKCI

Museum catalog #	Latitude	Longitude	Dataset
19. Red Lodge, MT (continued)			
CUMV 20230	N 44° 59’ 49.4”	W 109° 30’ 26.9”	cytb/D-loop, MGF, PRKCI
CUMV 20236	N 44° 59’ 51.1”	W 109° 30’ 42.3”	cytb/D-loop, MGF, PRKCI
NMMNH 561			D-loop
20. Togwotee Pass, WY			
CUMV 20180	N 43° 45’ 9.5”	W 110° 2’ 33.7”	cytb/D-loop, MGF, PRKCI
CUMV 20181	N 43° 45’ 9.9”	W 110° 2’ 34.7”	cytb/D-loop, MGF, PRKCI
CUMV 20186	N 43° 42’ 42.9”	W 110° 2’ 38.7”	cytb/D-loop, MGF, PRKCI
CUMV 20189	N 43° 42’ 21.2”	W 110° 1’ 34.5”	cytb/D-loop, MGF, PRKCI
CUMV 20190	N 43° 42’ 20.2”	W 110° 1’ 36.7”	cytb/D-loop, MGF, PRKCI
NMMNH 500			D-loop
NMMNH 503			D-loop
21. Duncum Mtn., WY			
CUMV 20194	N 44° 54’ 34.4”	W 107° 51’ 30.1”	cytb/D-loop, MGF, PRKCI
CUMV 20198	N 44° 51’ 27.3”	W 107° 50’ 40.0”	cytb/D-loop, MGF, PRKCI
CUMV 20199	N 44° 51’ 28.1”	W 107° 50’ 40.1”	cytb/D-loop, MGF, PRKCI
CUMV 20201	N 44° 52’ 56.0”	W 107° 51’ 33.2”	cytb/D-loop, MGF, PRKCI
CUMV 20208	N 44° 55’ 23.9”	W 107° 52’ 7.3”	cytb/D-loop, MGF, PRKCI
NMMNH 537			D-loop
22. Bridger Peak, WY			
CUMV 20165	N 41° 10’ 40.3”	W 107° 0’ 50.5”	cytb/D-loop, MGF, PRKCI
CUMV 20166	N 41° 10’ 40.1”	W 107° 0’ 57.3”	cytb/D-loop, MGF, PRKCI
CUMV 20168	N 41° 10’ 39.2”	W 107° 0’ 58.4”	cytb/D-loop, MGF, PRKCI
CUMV 20169	N 41° 10’ 37.4”	W 107° 0’ 57.4”	cytb/D-loop, MGF, PRKCI
CUMV 20170	N 41° 10’ 38.6”	W 107° 0’ 52.0”	cytb/D-loop, MGF, PRKCI
CUMV 20177	N 41° 11’ 27.4”	W 107° 2’ 4.0”	cytb/D-loop, MGF, PRKCI
NMMNH 474			D-loop
23. Warren Peak, CA			
CUMV 20680	N 41° 22’ 43.1”	W 120° 12’ 44.7”	cytb/D-loop, MGF, PRKCI
CUMV 20681	N 41° 22’ 41.9”	W 120° 12’ 44.3”	cytb/D-loop, MGF, PRKCI
CUMV 20682	N 41° 22’ 11.7”	W 120° 14’ 19.8”	cytb/D-loop, MGF, PRKCI
NMMNH 847			D-loop
24. Ebbets Pass, CA			
CUMV 20594	N 38° 32’ 45.0”	W 119° 48’ 58.3”	cytb/D-loop, MGF, PRKCI
CUMV 20683	N 38° 32’ 40.0”	W 119° 48’ 59.2”	cytb/D-loop, MGF, PRKCI
CUMV 20684	N 38° 32’ 40.2”	W 119° 49’ 0.1”	cytb/D-loop, MGF, PRKCI
NMMNH 833			D-loop
25. Onion Valley, CA			
CUMV 20685	N 36° 46’ 49.9”	W 118° 19’ 33.5”	cytb/D-loop, PRKCI
CUMV 20593	N 36° 46’ 49.7”	W 118° 19’ 35.1”	cytb/D-loop, MGF, PRKCI
CUMV 20686	N 36° 46’ 49.3”	W 118° 19’ 35.1”	cytb/D-loop, MGF, PRKCI
CUMV 20687	N 36° 46’ 48.5”	W 118° 19’ 35.1”	cytb/D-loop, MGF, PRKCI
NMMNH 826			D-loop

Museum catalog #	Latitude	Longitude	Dataset
26. Arc Dome, NV			
CUMV 20312	N 38° 50’ 13.2”	W 117° 20’ 59.6”	cytb/D-loop, MGF, PRKCI
CUMV 20313	N 38° 52’ 38.4”	W 117° 21’ 12.3”	cytb/D-loop, MGF, PRKCI
CUMV 20314	N 38° 52’ 37.1”	W 117° 20’ 58.8”	cytb/D-loop, MGF, PRKCI
CUMV 20315	N 38° 52’ 38.5”	W 117° 20’ 57.6”	cytb/D-loop, MGF, PRKCI
27. Mt. Jefferson, NV			
CUMV 20316	N 38° 43’ 30.8”	W 116° 55’ 33.9”	cytb/D-loop, MGF, PRKCI
CUMV 20317	N 38° 43’ 31.5”	W 116° 55’ 34.4”	cytb/D-loop, MGF, PRKCI
CUMV 20318	N 38° 44’ 23.7”	W 116° 55’ 26.2”	cytb/D-loop, MGF, PRKCI
CUMV 20319	N 38° 44’ 24.1”	W 116° 55’ 26.3”	cytb/D-loop, PRKCI
CUMV 20320	N 38° 43’ 31.4”	W 116° 55’ 32.5”	cytb/D-loop, MGF, PRKCI
CUMV 20321	N 38° 43’ 34.3”	W 116° 55’ 31.6”	cytb/D-loop, MGF, PRKCI
CUMV 20322	N 38° 43’ 37.5”	W 116° 55’ 27.5”	cytb/D-loop, MGF, PRKCI
NMMNH 250			D-loop
28. Ruby Mtns., NV			
CUMV 20688	N 40° 34’ 57.2”	W 115° 23’ 32.0”	cytb/D-loop, MGF, PRKCI
CUMV 20689	N 40° 34’ 54.2”	W 115° 23’ 33.7”	cytb/D-loop, MGF, PRKCI
CUMV 20692	N 40° 35’ 13.5”	W 115° 23’ 23.4”	cytb/D-loop, MGF
NMMNH 257			D-loop
29. Oakley, UT			
CUMV 20573	N 40° 45’ 53.1”	W 111° 0’ 58.2”	cytb/D-loop, MGF, PRKCI
CUMV 20654	N 40° 45’ 43.5”	W 111° 0’ 43.2”	cytb/D-loop, MGF, PRKCI
CUMV 20655	N 40° 45’ 24.6”	W 111° 2’ 19.5”	cytb/D-loop, MGF, PRKCI
CUMV 20572	N 40° 45’ 27.2”	W 111° 2’ 14.3”	cytb/D-loop, MGF, PRKCI
CUMV 20658	N 40° 45’ 27.6”	W 111° 2’ 31.7”	cytb/D-loop, MGF, PRKCI
NMMNH 281			D-loop
30. Gunnison, UT			
CUMV 20678	N 39° 3’ 45.6”	W 111° 31’ 11.5”	cytb/D-loop, MGF, PRKCI
CUMV 20571	N 39° 3’ 47.9”	W 111° 31’ 8.3”	cytb/D-loop, MGF, PRKCI
CUMV 21155	N 39° 6’ 35.8”	W 111° 28’ 11.2”	cytb/D-loop, MGF, PRKCI
CUMV 20570	N 39° 6’ 42.7”	W 111° 28’ 16.1”	cytb/D-loop, MGF, PRKCI
CUMV 20660	N 39° 6’ 53.2”	W 111° 28’ 23.2”	cytb/D-loop, MGF, PRKCI
NMMNH 310			D-loop
31. Flat Top Mtn., UT			
CUMV 20569	N 38° 26’ 25.4”	W 111° 28’ 58.6”	cytb/D-loop, MGF, PRKCI
CUMV 20663	N 38° 26’ 26.3”	W 111° 28’ 51.7”	cytb/D-loop, MGF, PRKCI
CUMV 20666	N 38° 26’ 27.1”	W 111° 28’ 56.7”	cytb/D-loop, MGF, PRKCI
CUMV 20668	N 38° 26’ 44.6”	W 111° 28’ 37.7”	cytb/D-loop, MGF, PRKCI
CUMV 20669	N 38° 26’ 42.9”	W 111° 28’ 39.4”	cytb/D-loop, MGF, PRKCI
NMMNH 1217			D-loop
32. Beaver, UT			
CUMV 20567	N 38° 23’ 16.3”	W 112° 23’ 59.7”	MGF, PRKCI
CUMV 20671	N 38° 22’ 55.3”	W 112° 24’ 3.5”	cytb/D-loop, MGF, PRKCI

Museum catalog #	Latitude	Longitude	Dataset
32. Beaver, UT (continued)			
CUMV 21156	N 38° 22’ 59.8”	W 112° 24’ 0.8”	cytb/D-loop, MGF, PRKCI
CUMV 21157	N 38° 23’ 13.0”	W 112° 24’ 6.6”	cytb/D-loop, MGF
CUMV 20677	N 38° 23’ 14.1”	W 112° 24’ 10.4”	cytb/D-loop, MGF
NMMNH 235			D-loop
33. Trappers Lake, CO			
CUMV 20154	N 39° 58’ 41.8”	W 107° 15’ 13.3”	cytb/D-loop, MGF
CUMV 20155	N 39° 58’ 42.5”	W 107° 15’ 11.6”	cytb/D-loop, MGF, PRKCI
CUMV 20156	N 39° 58’ 15.8”	W 107° 15’ 26.0”	cytb/D-loop, MGF, PRKCI
CUMV 20157	N 39° 58’ 17.4”	W 107° 15’ 27.1”	cytb/D-loop, MGF, PRKCI
CUMV 20158	N 39° 58’ 17.6”	W 107° 15’ 28.3”	cytb/D-loop, MGF, PRKCI
NMMNH 405			D-loop
34. Grand Mesa, CO			
CUMV 20142	N 39° 2’ 46.2”	W 108° 4’ 10.6”	cytb/D-loop, MGF, PRKCI
CUMV 20145	N 39° 2’ 48.9”	W 108° 4’ 6.3”	cytb/D-loop, MGF, PRKCI
CUMV 20146	N 39° 2’ 49.1”	W 108° 4’ 5.3”	cytb/D-loop, MGF, PRKCI
CUMV 20149	N 39° 2’ 39.7”	W 108° 4’ 17.2”	cytb/D-loop, MGF, PRKCI
CUMV 20152	N 39° 2’ 48.9”	W 108° 4’ 5.6”	cytb/D-loop, MGF, PRKCI
NMMNH 368			D-loop
35. Berthoud Pass, CO			
CUMV 20125	N 39° 48’ 8.6”	W 105° 46’ 54.6”	cytb/D-loop, MGF, PRKCI
CUMV 20127	N 39° 47’ 59.9”	W 105° 47’ 19.2”	cytb/D-loop, MGF, PRKCI
CUMV 20128	N 39° 48’ 0.0”	W 105° 47’ 18.5”	cytb/D-loop, MGF, PRKCI
CUMV 20131	N 39° 48’ 6.1”	W 105° 46’ 47.4”	cytb/D-loop, MGF, PRKCI
CUMV 20137	N 39° 48’ 2.6”	W 105° 47’ 23.7”	cytb/D-loop, MGF, PRKCI
NMMNH 436			D-loop
36. Twining, NM			
CUMV 20114	N 36° 36’ 44.0”	W 105° 30’ 4.7”	cytb/D-loop, MGF, PRKCI
CUMV 20115	N 36° 36’ 44.5”	W 105° 30’ 4.0”	cytb/D-loop, MGF, PRKCI
CUMV 20116	N 36° 37’ 12.2”	W 105° 30’ 5.4”	cytb/D-loop, MGF, PRKCI
CUMV 20121	N 36° 36’ 52.0”	W 105° 30’ 5.2”	cytb/D-loop, MGF, PRKCI
CUMV 20124	N 36° 36’ 57.7”	W 105° 29’ 59.5”	cytb/D-loop, MGF, PRKCI
NMMNH 729			D-loop
37. Lake Peak, NM			
CUMV 20323	N 35° 47’ 42.6”	W 105° 46’ 31.3”	cytb/D-loop, MGF, PRKCI
CUMV 20325	N 35° 47’ 44.2”	W 105° 46’ 30.7”	cytb/D-loop, MGF, PRKCI
CUMV 20329	N 35° 47’ 48.8”	W 105° 46’ 32.0”	cytb/D-loop, MGF, PRKCI
CUMV 20334	N 35° 47’ 55.2”	W 105° 46’ 27.4”	cytb/D-loop, MGF, PRKCI
CUMV 20335	N 35° 47’ 48.3”	W 105° 46’ 32.1”	cytb/D-loop, MGF, PRKCI
NMMNH 711			D-loop
38. Little Itcha Mtns., BC			
NMMNH 1000	?	?	D-loop

Museum catalog #	Latitude	Longitude	Dataset
39. Westwold, BC			
NMMNH 934	N 50° 21’ 48.6”	W 119° 55’ 15.6”	D-loop
40. Golden, BC			
NMMNH 917	N 51° 2’ 54.0”	W 117° 9’ 11.0”	D-loop
41. Buller Mtn., AB			
NMMNH 894	N 50° 54’ 17.9”	W 115° 18’ 53.7”	D-loop
42. Glacier, WA			
NMMNH 1011	N 48° 51’ 23.6”	W 121° 40’ 22.2”	D-loop
43. Mt. McLoughlin, OR			
NMMNH 1211	N 42° 26’ 42.7”	W 122° 18’ 29.6”	D-loop
NMMNH 1212	N 42° 26’ 42.7”	W 122° 18’ 29.6”	D-loop
44. Steens Mtn., OR			
NMMNH 871	N 42° 40’ 55.0”	W 118° 33’ 40.4”	D-loop
45. Stanley, ID			
NMMNH 642	N 44° 3’ 59.3”	W 114° 45’ 52.9”	D-loop
46. Arco, ID			
NMMNH 663	N 43° 24’ 11.0”	W 113° 36’ 17.3”	D-loop
47. Copenhagen Basin, ID			
NMMNH 670	N 42° 19’ 19.7”	W 111° 33’ 54.0”	D-loop
48. Ovando, MT			
NMMNH 602	N 47° 13’ 36.9”	W 113° 11’ 44.4”	D-loop
49. Teton Pass, WY			
NMMNH 517	N 43° 32’ 27.2”	W 110° 56’ 7.1”	D-loop
50. Centennial, WY			
NMMNH 454	N 41° 22’ 14.5”	W 106° 17’ 43.4”	D-loop
51. Mt. Shasta, CA			
NMMNH 854	N 41° 21’ 37.6”	W 122° 10’ 52.9”	D-loop
52. White Mtns., CA			
NMMNH 819	N 37° 29’ 28.7”	W 118° 11’ 4.4”	D-loop
53. Desatoya Mtns., NV			
HSU 5150	N 39° 24’ 57.8”	W 117° 45’ 44.8”	D-loop
54. Cottonwood, UT			
NMMNH 299	N 40° 39’ 29.8”	W 111° 38’ 37.7”	D-loop
NMMNH 301			D-loop

Museum catalog #	Latitude	Longitude	Dataset
55. Richfield, UT			
NMMNH 331	N 38° 39’ 49.7”	W 111° 39’ 32.2”	D-loop
56. Monroe, UT			
NMMNH 1857	N 38° 32’ 37.4”	W 112° 5’ 2.5”	D-loop
57. Aquarius Plateau, UT			
NMMNH 881	N 37° 58’ 26.8”	W 111° 32’ 13.6”	D-loop
NMMNH 882	N 37° 58’ 26.8”	W 111° 32’ 13.6”	D-loop
58. Cedar City, UT			
NMMNH 222	N 37° 45’ 51.4”	W 112° 47’ 56.8”	D-loop
59. Geyser Pass, UT			
NMMNH 348	N 38° 29’ 34.9”	W 109° 15’ 7.2”	D-loop
60. Ouray, CO			
NMMNH 1062	N 37° 58’ 45.4”	W 107° 36’ 59.3”	D-loop
61. Garfield, CO			
NMMNH 380	N 38° 34’ 38.2”	W 106° 19’ 29.6”	D-loop
62. Pikes Peak, CO			
NMMNH 683	N 38° 52’ 12.0”	W 105° 4’ 12.0”	D-loop
63. Cumbres Pass, CO			
NMMNH 1201	N 37° 1’ 38.1”	W 106° 26’ 15.2”	D-loop
64. Jemez Mtns., NM			
NMMNH 1891	N 35° 49’ 55.1”	W 106° 28’ 17.1”	D-loop