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Historical biogeography of western heather voles (*Phenacomys intermedius*) in montane systems of the Pacific Northwest

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Quaternary climate fluctuations and topographical variation in the Pacific Northwest region of North America have interacted to affect the historical biogeography of biota in this region. High-elevation mammals have unique diversification patterns due to their isolation on mountaintops and potential for population growth and range expansion in lowland refugia that are available during glacial periods. We examined the phylogeographic structure, dates of lineage diversification, and historical demography of western heather vole (*Phenacomys intermedius*) populations across several mountain ranges in the Pacific Northwest. Our analysis of sequence variation in the mitochondrial control region using both maximum-likelihood and Bayesian methods identified 3 major geographically distinct lineages: an Oregon and California lineage, Washington lineage, and Northern and Interior lineage. Our estimate of divergence times using a Bayesian relaxed molecular-clock method revealed that diversification among these major lineages began ~1.8 million years ago (mya) in the early Pleistocene with the split of the Oregon and California lineage followed by the split of the Washington lineage and the Northern and Interior lineage ~1.5 mya. All 3 clades remain allopatric, suggesting that they did not share a common refugium during cold climatic intervals of the Pleistocene. Further diversification within each major clade occurred in the middle Pleistocene when populations in isolated mountain ranges became distinct. Demographic estimates from Bayesian skyline plots indicate that each of the 3 major clades has experienced population decline since the early Holocene, possibly due to the redistribution of populations into higher-elevation habitats that became restricted to mountaintops following continental and alpine deglaciation. DOI: 10.1644/09-MAMM-A-303.1.

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The interaction of historical climate changes with topographical variation can produce both intermittent lowland biogeographic refugia and high-altitude isolation of montane biota. The Pacific Northwest region of North America possesses a heterogeneous landscape that transects major mountain ranges and intervening lowland valleys and plateaus. Throughout the glacial cycles of the Pleistocene, between 2.58 million years ago (mya—Gibbard et al. 2010) and 11,700 years ago (Walker et al. 2009), the Pacific Northwest was exposed to a succession of about 20 glacial periods characterized by expansion and retraction of continental ice sheets and alpine glaciers. However, the timing of peak expansion of alpine glaciers varied across major mountain ranges (Porter et al. 1983; Thackray 2008) resulting in dynamic changes in the location of lowland ice-free zones that served as biotic refugia. Furthermore, periods of climate warming forced the redistribution of lowland biota into alpine zones that were isolated from one another by the topography of the Pacific Northwest.

Our understanding of the responses of North American plants and animals to Quaternary climate history has been enhanced by studies of genetic variation of populations across geographic space (Avise 2009). The genetic signatures of populations that resulted from historical vicariance and dispersal events developed through the interplay of 2 important evolutionary forces: genetic drift and mutation. When populations become isolated and small, genetic drift acts to reduce genetic diversity and thereby to promote differentiation among populations. By comparing both the genetic diversity of contemporary populations and the genealogical relationships among populations over geographic space, investigations of phylogeography can provide clues as to where and when isolation events and dispersal routes occurred.

Climate-mediated impacts on the structuring of genetic variation of mammal populations in the Pacific Northwest have been investigated in several low-elevation species (Arbogast and Kenagy 2001; Miller et al. 2006; Yang and Kenagy 2009; Zheng et al. 2003). One common pattern that has emerged from these studies is the apparent diversification of lineages during full glacial periods due to climate-driven shifts of populations into disjunct refugia. Another pattern is the major latitudinal shift in distribution and accompanying population expansion that follows the retraction of continental ice sheets (Lessa et al. 2003). However, mammals currently restricted to high-altitude environments experienced major elevation shifts due to these climate changes, which has led to different predictions on the outcomes of their genetic structuring and demographic changes. The expansion of alpine glaciers during cold climatic periods will force downslope shifts in populations from disjunct mountain ranges and possibly allow them to merge in common lowland refugia with greater habitat availability, which will lead to increases in population size. Moreover, upslope shift of populations into restricted mountaintops during warm periods will lead to smaller population sizes and facilitate significant lineage divergences due to founder effects (Wright 1942). Recent examples of the response of alpine specialists to these environmental scenarios demonstrate patterns that are consistent with these predictions (Carstens et al. 2005; Galbreath et al. 2009; Knowles 2001; Spaeth et al. 2009).

The western heather vole (*Phenacomys intermedius*) is distributed sparsely in alpine and subalpine habitat across several mountain ranges in the Pacific Northwest, including the Coastal Mountains of Canada, Rockies, Cascades, Olympics, and the Sierra Nevada (McAllister and Hoffmann 1988). The earliest known fossil records of this genus date back to the Pliocene/Pleistocene boundary ~2.5 (mya) and were discovered in both Siberia and Alaska (Repenning et al. 1987). Subsequently the purported ancestors of modern *P. intermedius* spread southward into Canada and the conterminous United States in the early Pleistocene (Repenning 2001). Several late-Pleistocene and early-Holocene fossils of *Phenacomys* have been found in wide-ranging lowland areas outside their contemporary distribution (Diveley 1999; Emslie 1986; Graham and Mead 1987; Grayson 1981; Karrow et al. 1995; Lundelius et al. 1983; Lyman 2008; Rensberger and Barnosky 1993; Rogers et al. 2000; Walker 1987; Fig. 1). Based on palynological studies, habitat likely to be suitable for *P. intermedius* was widespread throughout the Pacific Northwest lowlands during full glacial conditions (Barnosky 1985; Daniels et al. 2005; Thompson and Anderson 2000; Worona and Whitlock 1995). However, it is uncertain whether refugial locations of *P. intermedius* represented 1 large common refugium or disjunct refugia separated by physical barriers. Furthermore, a large portion of the contemporary distribution of *P. intermedius* occurs in high northern latitude areas that were glaciated by continental ice sheets during the last glacial maximum. The Cascades and Rockies represent the 2 major mountain axes that span nonglaciated and glaciated

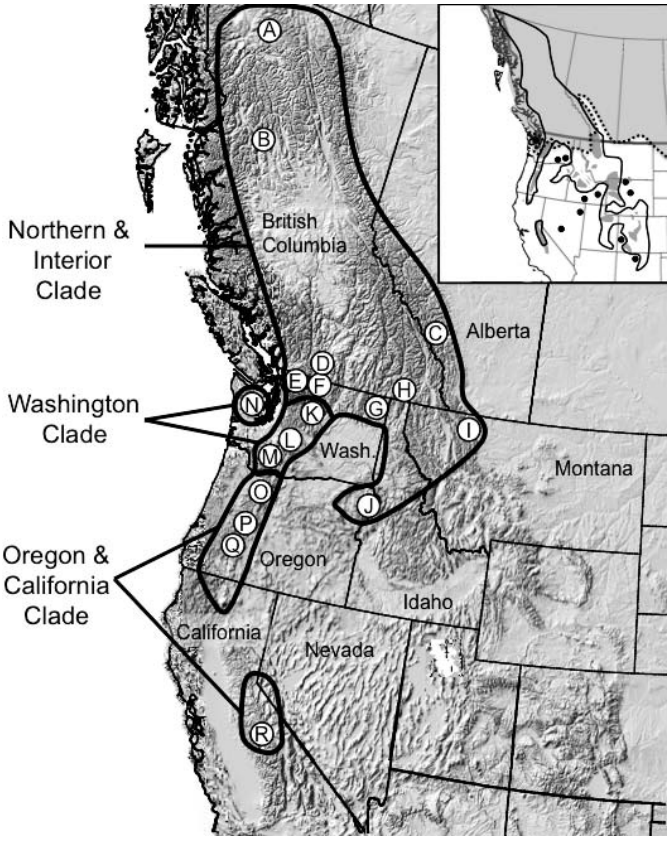

FIG. 1.—Geographic map of western heather vole (*Phenacomys intermedius*) sampling localities (A–R, Appendix I) in the Pacific Northwest. Also identified is the geographic extent of each of the 3 major clades as defined in Fig. 2. The inset shows the entire contemporary distribution of *P. intermedius* in dark outline (redrawn from locality data in Hall [1981], Nagorsen [2005], and Verts and Carraway [1998]), approximate margins of the Cordilleran and Laurentide Ice Sheets (~14,000 years before present) as the shaded area north of the dotted line (Siegert 2001), and major alpine glaciers as shaded areas south of the ice sheets (Porter et al. 1983). Late-Pleistocene and Holocene fossil localities outside the modern heather vole range are indicated by black circles.

areas and might have served as important northward dispersal routes for *P. intermedius* following the retraction of continental ice sheets.

To assess the impacts of late-Quaternary climate change and geological features on the historical biogeography of *P. intermedius* across montane systems of the Pacific Northwest, we obtained samples throughout several montane regions from northern British Columbia, Canada, southward to California. Our objective was to incorporate phylogenetic inference analyses, relaxed molecular-clock dating computations, and historical demographic estimations into a phylogeographic framework to test the hypotheses that contemporary isolated populations were forced into a series of disjunct refugia separated by topographical barriers during full glacial conditions in the Pleistocene rather than into a common refugium; dispersal into northerly areas that were previously occupied by continental ice sheets occurred from multiple refugial sources; postglacial shifts of elevation into isolated

mountain ranges resulted in lineage divergence; and major lineages are experiencing declines in population since the end of the Pleistocene due to their upslope range shift and reoccupation of restricted montane regions.

Materials and Methods

Sampling.—Our analysis is based on 89 samples of *P. intermedius* from 18 localities representing the northern and western portions of the geographic range, with a special emphasis on isolated montane populations of the Pacific Northwest (Appendix I; Fig. 1). The samples consisted of 36 frozen tissues from the liver of specimens collected between 1999 and 2008, which were deposited in the Burke Museum, University of Washington (UWBM), and the Museum of Vertebrate Zoology, University of California (MVZ). The remaining 53 samples were obtained from skins of museum study specimens collected between 1925 and 2000, which were deposited in the UWBM; the Slater Museum of Natural History, University of Puget Sound (PSM); and the Department of Fisheries and Wildlife, Oregon State University (OSUFW; Appendix I). Research was conducted in adherence with guidelines of the American Society of Mammalogists (Gannon et al. 2007).

Laboratory methods.—We extracted whole genomic DNA from fresh liver or spleen tissue using the prescribed protocol of DNeasy Tissue Kit (Qiagen, Valencia, California). For samples from museum study skins genomic DNA was extracted following a protocol developed by Mullen and Hoekstra (2008), which included an ethanol wash every 3 h for 24 h to remove salts and polymerase chain reaction (PCR) inhibitors that may have been used in preserving museum skins. For these samples we also undertook several steps to avoid contamination. First, we used a new sterilized razor blade for the removal of snippets from each specimen. Second, we performed ethanol washes in a separate room from where PCR amplifications were performed to avoid contamination from PCR amplicons. Next, we divided our samples into smaller subsets and staggered the dates of extractions and PCR amplifications for each subset. We also checked for contamination by checking for PCR products from negative-control extractions and negative-control PCR master mixes with gel electrophoresis. Finally, we repeated DNA extractions for 5 random individuals using skin snippets from another part of the study specimen and compared their sequences with original samples.

We amplified a 325-base pair (bp) sequence of the mitochondrial control region using the PCR with primers RTVL16000 (5′-GTCAACACCCAAAGCTGACA-3′) and RtvInt1r (5′-GTTGGTTTCACGGAGGATGG-3′—Miller et al. 2006). PCR mixtures totaled 25 μl and followed slightly different protocols for frozen tissue and museum study skin samples. For the frozen tissue samples each reaction mixture contained 14.9 μl of nuclease-free H_2O, 2.5 μl of 10X PCR buffer, 1 μl of 25 mM $MgCl_2$, 0.2 mM of each deoxynucleoside triphosphate, 2.5 μl of each 1 X primer, 0.125 μl of JumpStart Taq DNA polymerase (Sigma, St. Louis, Missouri),

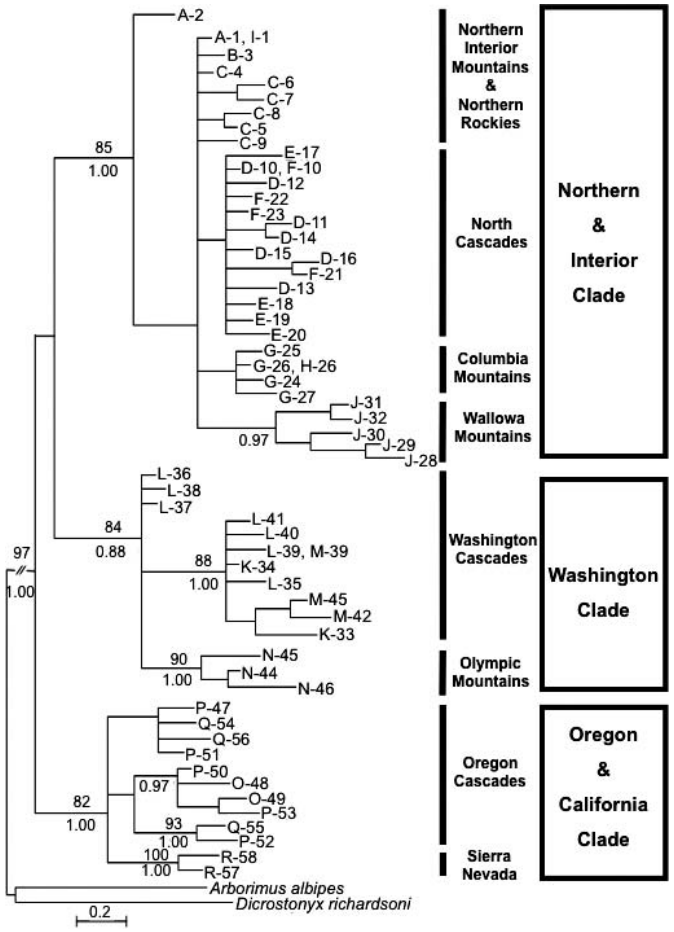

Fig. 2.—Bayesian gene tree based on 325 base pairs of the mitochondrial control region of haplotypes of *Phenacomys intermedius* sampled from the northern and western portions of the species' range. The phylogeny inferred with maximum-likelihood methods yielded an essentially identical topology. Numbers above nodes represent maximum-likelihood bootstrap percentages > 80 and those below represent Bayesian posterior probabilities > 0.85. Sampling localities (A–R) and haplotype numbers (1–58), as shown in Appendix I, are indicated at branch tips.

and 1 μl of genomic DNA. We performed PCR amplifications in a GeneAmp PCR System 9700 thermocycler (Applied Biosystems, Foster City, California). The cycle conditions included a denaturing step at 94°C (10 min), followed by 30 cycles (1 min at 94°C, 30 s at 50°C, and 1 min at 72°C) with a final extension period of 5 min at 72°C. Because DNA from study skins was presumed to be somewhat degraded, we modified the PCR master mix to improve PCR amplification by adding 2.5 μl of 10X bovine serum albumin, 2 μl of 25 mM $MgCl_2$, 0.5 μl of JumpStart Taq DNA polymerase, and 9.9 μl of genomic DNA, and by not adding the 14.9 μl of nuclease-free H_2O. We also increased the number of amplification cycles to 40 during PCR. We treated all PCR products with ExoSapIT (USB Corp., Cleveland, Ohio) to remove unincorporated nucleotides and primers. Sequencing reactions for PCR products of each sample were performed separately for each forward and reverse primer. Sequencing reaction mixtures of 10 μl for each sample included 1 μl of PCR

product, 7.5 μl of nuclease-free H_2O, 0.5 μl of the Big Dye Terminator Cycle Sequencing Ready Reaction Mix (Applied Biosystems), 0.5 μl of the Big Dye 5X Sequencing Buffer, and 0.5 μl of primer. Samples were run on an ABI 3100 Genetic Analyzer (Applied Biosystems) for sequencing of nucleotides. Sequences were aligned using SEQUENCHER 4.6 (Gene Codes Corp., Ann Arbor, Michigan) and deposited in GenBank under accession numbers GQ903588–GQ903676.

Phylogenetic analyses.—We used both maximum-likelihood and Bayesian inference methods to reconstruct phylogenetic relationships of haplotypes. We determined that the Hasegawa–Kishino–Yano (Hasegawa et al. 1985) model of nucleotide substitution was the best-fitting model for our phylogenetic analyses by examining Akaike's information criteria corrected for small sample size differences (ΔAIC_c) and Akaike weights in MODELTEST (version 3.7—Posada and Crandall 1998). Other parameters estimated were as follows: base frequencies (A = 0.3547, C = 0.0722, G = 0.2312, T = 0.3419); proportion of invariable sites (I) = 0.6089; and gamma shape parameter = 0.5954. We reconstructed the maximum-likelihood phylogeny using PHYML 2.4 (Guindon and Gascuel 2003) with 1,000 bootstrap replicates to evaluate nodal support for phylogenetic clades (Felsenstein 1985). The Bayesian inference phylogenetic reconstructions were performed with MRBAYES 3.1 (Ronquist and Huelsenbeck 2003) using Markov chain Monte Carlo sampling. Runs were set for 5,000,000 generations, with 1 cold and 3 heated chains and with trees sampled every 100 generations. The 1st 25% of sampled trees were discarded as burn-in after visual inspection revealed that these chain samples had not converged on stable likelihoods (stationarity). Outgroup sequences were obtained from GenBank from 1 *Arborimus albipes* (accession DQ198850) and 1 *Dicrostonyx richardsoni* (AF192739).

Divergence dating.—We estimated the time to the most recent common ancestor of all haplotypes and several monophyletic clades using BEAST 1.5.2 (Drummond and Rambaut 2007). BEAST brings together several complementary evolutionary models (i.e., substitution models, insertion-deletion models, demographic models, tree-shape priors, relaxed clock models, and node-calibration models) into a single coherent framework for evolutionary inference. Based on our MODELTEST results, we used an Hasegawa–Kishino–Yano substitution model with a gamma rate distribution and proportion of invariant sites. We also determined from a likelihood ratio test (Felsenstein 1981) that a relaxed molecular-clock model with an uncorrelated lognormal tree prior best accounted for the varying rates of evolution across lineages in our data set. To calibrate the tree we added several outgroup taxa belonging to higher taxonomical levels and used estimated fossil dates to constrain node heights and topological features. These node constraints included (F1) the earliest fossil representing the subfamily Arvicolinae, which was found 5.5 mya (95% confidence interval [95% *CI*] = 5.0–6.0 mya) in East Asia from the early Pliocene (Chaline et al. 1999; Jin and Zhang 2005); and (F2) the earliest fossil representing the tribe Phenacomyini, found in the early Pleistocene 2.4 mya (95% *CI* = 2.1–2.6 mya—Repenning et al. 1987; Fig. 3) We obtained the following genetic sequences of outgroup taxa representing these higher-order groups from GenBank (with accession numbers): *Peromyscus maniculatus* (GQ860675) as the representative outgroup taxon from the family Cricetidae; *Eothenomys chinensis* (FJ483847), *Microtus mexicanus* (AF251260), *Myodes glareolus* (Y07543), and *Lemmus sibiricus* (AF355450) as outgroup taxa belonging to the subfamily Arvicolinae; and *Arborimus albipes* (DQ198850) as the representative outgroup taxon belonging to the supergenus Phenacomyini. In addition, we sequenced 1 specimen of *Microtus montanus* (GU394082) to include with the Arvicolinae group. We used a Yule tree prior for this analysis because our data set included several species-level taxa. Therefore, we used a reduced data set for our ingroup sequences that included only a few individuals from each of the 3 major clades as determined by our maximum-likelihood and Bayesian inference phylogenetic analyses. This data set included 4 individuals from the Northern and Interior clade and 3 each from the Washington clade and the Oregon and California clade (see letters a–j in Appendix I). We also included 1 sequence from subclades identified in the phylogenetic analysis (Wallowa, Olympic, and the Sierra Nevada) as part of the reduced data set. The analysis was run 5 times with different random seeds, each for 10 million generations with samples logged every 10,000 generations, and also run for 50 million generations to test for convergence. The initial 10% of samples was discarded as burn-in after visual inspection of the trace revealed that these samples had not reached stationarity. We combined results from each run in LOGCOMBINER 1.5.2 from the BEAST package and analyzed them in TRACER 1.5 (Rambaut and Drummond 2007). Effective sample size values for all parameters exceeded 200.

Molecular diversity and demographic analyses.—We computed haplotype (h) and mean nucleotide (π) diversities (Nei 1987) for each clade using ARLEQUIN (Excoffier et al. 2005) for comparisons of genetic diversity. We also examined the demographic history of each major clade using Fu's F_s test for neutrality (Fu 1997). Large and negative F_s values suggest an excess of rare alleles, which can be an indication of population growth or selection. We also investigated patterns of historical demographic expansion by examining the distribution of pairwise differences (mismatch distributions) for each clade (Rogers and Harpending 1992) using ARLEQUIN. In general, populations undergoing recent and sudden expansion exhibit a Poisson-shaped mismatch distribution, and populations in equilibrium tend to have ragged distributions (Slatkin and Hudson 1991). We assessed the statistical significance of these distributions with sum of squared distances and Harpending's raggedness index (Harpending 1994).

We also explored demographic history of each major lineage using Bayesian skyline plots (Drummond et al. 2005) in BEAST. This coalescent-based inference method uses a Markov chain Monte Carlo sampling procedure with gene sequence data to estimate a posterior distribution of effective population size through time. We used divergence date

estimates of time to the most recent common ancestor from our divergence analysis as root age priors for each of the major lineages so that we could model changes in effective population size against a timescale of years rather than expected substitutions per site. We also used the Hasegawa–Kishino–Yano substitution model with a gamma distribution and proportion of invariant sites, along with a relaxed clock model with an uncorrelated lognormal prior. We forced coalescent intervals into 5 groups for each lineage because test runs for higher group numbers did not converge as well. We used default settings for the remaining model parameter priors. We performed 5 runs with different random seeds, sampled every 10,000 generations, and discarded 10% of the samples as burn-in for each major lineage. We varied the length of runs for each lineage with the Northern and Interior clade set at 100 million generations, the Washington clade set at 80 million generations, and the Oregon and California clade set at 40 million generations. Effective sample size values for all parameters in each run exceeded 200. We combined results for each lineage in LOGCOMBINER and produced skyline plots in TRACER.

Results

Phylogenetic inferences and estimates of divergence dates.—Both the maximum-likelihood and Bayesian phylogenetic inferences reveal 3 major clades (Fig. 2), each associated with distinct geographic areas: a Northern and Interior clade represented by populations in the Northern Rockies, Wallowa Mountains, Cascades of northern Washington and southern British Columbia, and the northern interior mountains of British Columbia (Fig. 1, localities A–J); a Washington clade represented by populations in the southern Washington Cascades and the Olympic Mountains (K–N); and an Oregon and California clade represented by populations in the Oregon Cascades and the Sierra Nevada of California (O–R).

Within each major clade we found 1 well-supported subclade associated with a geographically isolated mountain range, as follows. Within the Northern and Interior clade the isolated Wallowa Mountains of eastern Oregon (J) contain a lineage with high Bayesian nodal support (Fig. 2). Within the Washington clade the lineage in the isolated Olympic Mountains (N) shows strong nodal support. Within the Oregon and California clade the disjunct population in the Sierra Nevada (R) shows strong nodal support.

The estimation of time to the most recent common ancestor (Fig. 3; Table 1) indicates that the tribe Phenacomyini diverged from its sister lineage in the subfamily Arvicolinae ~5.37 mya. The estimated mean time to the most recent common ancestor for the split of the genera *Phenacomys* and *Arborimus* was ~2.45 mya. This analysis estimated an early-Pleistocene origin of ~1.8 mya for the Oregon and California clade and an early-Pleistocene divergence of ~1.54 mya between the Washington clade and Northern and Interior clade. Sublineage diversification of isolated mountain populations (Wallowa, Olympic, and Sierra Nevada) occurred in the middle Pleistocene within each of the 3 major clades.

Molecular diversity and demographic analyses.—All 3 clades had high haplotype diversity and low nucleotide diversity (Table 2), and no haplotype was shared among the 3 regions occupied by the major clades. Only 4 haplotypes were found at >1 sampling locality. The significantly negative Fu's F_s for the Northern and Interior clade suggests that this clade experienced recent rapid expansion. This is corroborated by the mismatch distributions, which were unimodal for this clade and did not possess a statistically significant Harpending's raggedness index or sum of squared deviation. The multimodal mismatch distributions and nonsignificant Fu's F_s values for the other 2 major clades suggest population stability; however, this conflicts with the nonsignificant Harpending's raggedness index and sum of squared deviation of the mismatch distribution for both clades. This lack of correspondence could be due to the more conservative test based on pairwise sequence differences (mismatch distribution) as opposed to the Fu's F_s (Ramos-Onsins and Rozas 2002).

The median estimates from the Bayesian skyline plots indicate that the populations of all 3 major lineages have rapidly declined following a long period of relative stability. This decline occurred after the last glacial maximum and at the beginning of the warming period in the early Holocene (Fig. 4).

Discussion

The interaction between climate-mediated shifts in geographic distribution during the Pleistocene and variation in topography associated with regional occurrence of mountains has played a strong role in the phylogeographic structure of western heather voles in the Pacific Northwest. The initial southward range expansion of *Phenacomys* along the Pacific Coastal region into the conterminous United States (~1.8 mya) from its Arctic origins in Alaska and northeastern Siberia occurred during a warm interglacial period (Repenning 1990). Repeated intervals of glaciation through the remainder of the Pleistocene could have resulted in range expansions and contractions and subsequent secondary contact between diverging lineages in lowland refugia or in recolonized high-elevation regions. However, the geographic distinctiveness of the 3 contemporary lineages suggests that barriers were persistent throughout these repeated cycles of climate change and did not allow secondary contact to occur.

We can attempt to identify geographic barriers that prevented secondary contact between the 3 major lineages. The Columbia River Gorge lies between the Oregon and California clade and the Washington clade and appears to be an obvious barrier. Less obvious is why populations belonging to the Northern and Interior clade did not mix with populations belonging to the other clades in lowland refugia or in recolonized alpine sites. Fossil records of *Phenacomys* indicate that populations existed in lowland regions of both western and eastern Washington during the late Pleistocene and early Holocene (Karrow et al. 1995; Lyman 2008; Rensberger and Barnosky 1993; Fig. 1). Palynological studies also indicate the presence of habitats (parkland tundra and steppe) during full glacial conditions that

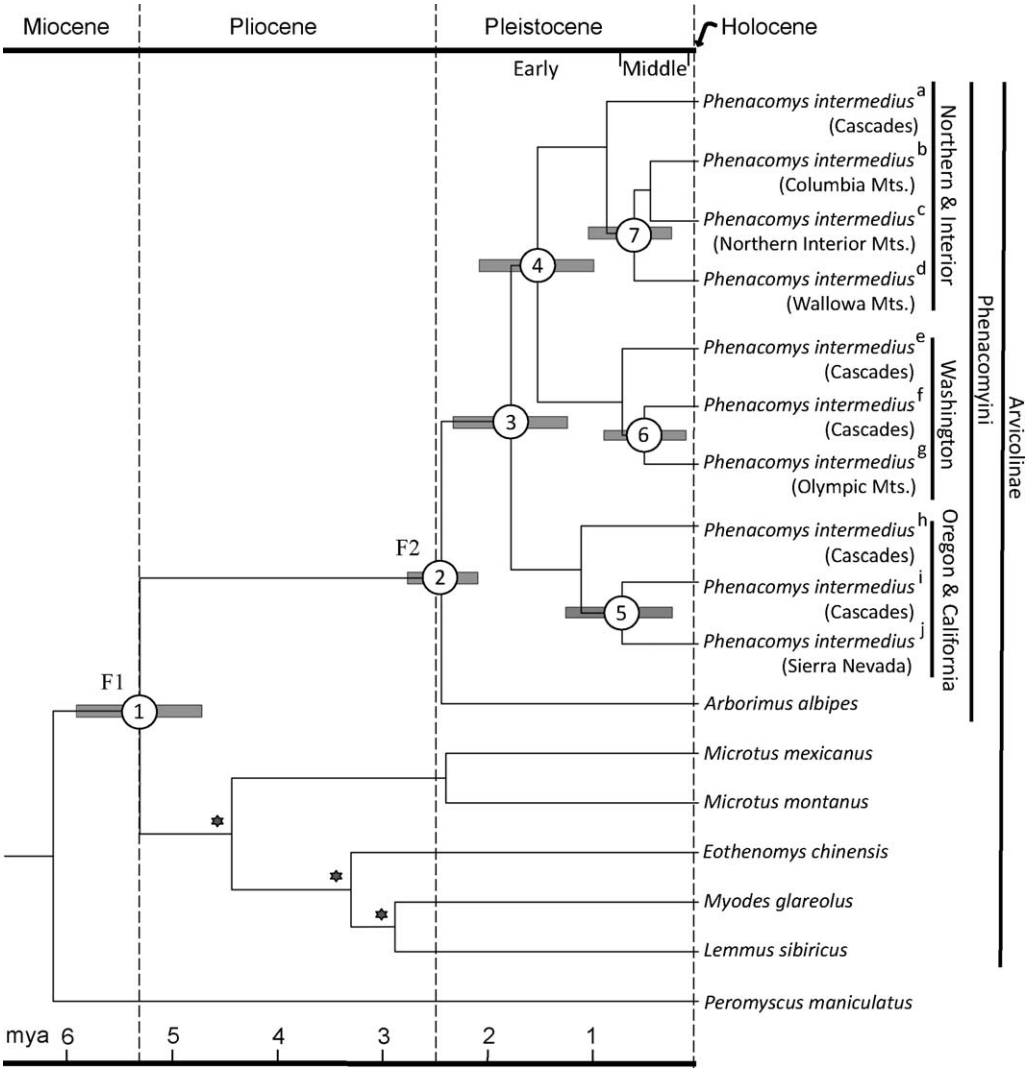

Fig. 3.—Bayesian tree with divergence dates from the relaxed molecular-clock analysis of haplotypes of *Phenacomys intermedius*. Circled numbers refer to estimated divergence dates, and gray horizontal bars indicate 95% credibility intervals (Table 1). Specimen numbers of *Phenacomys* indicated by letters a–j are provided in Appendix I, and outgroup sequence numbers and fossil calibration dates, indicated by F1 and F2, are provided in the ''Materials and Methods.'' All nodes possessed posterior probability support > 0.95 except for those indicated by gray stars.

might have been able to support widespread populations of *Phenacomys* in Washington (Barnosky 1985; Heusser 1983). The contemporary presence of only Washington clade populations in the southern portion of the Washington Cascades suggests that upslope movement into these mountains occurred only from western refugial populations. The Columbia River runs along the eastern side of the Cascades in Washington and might have prevented recolonization of this portion of the Cascades by refugial populations from eastern Washington. However, the contemporary presence of only Northern and Interior clade populations in the North Cascades region of northern Washington and southern British Columbia suggests that dispersal into this region occurred from an eastern refugial source, possibly by populations from eastern Washington. The phylogeographic break between the Northern and Interior clade and the Washington clade in the Washington Cascades occurs over a distance of only 50 km and might have been maintained by increasing restriction of dispersal during recent warming in the Holocene combined with short neoglacial advances of montane glaciers in the Glacier Peaks region (Burke and Birkeland 1983; Thompson Davis et al. 2009). For the Oregon Cascades and Sierra Nevada populations no obvious barriers are apparent in the intervening Great Basin region that historically

Table 1.—Estimates of divergence dates for key splits in *Phenacomys* as shown in Fig. 3.

Node	Diverged taxa or phylogeographic lineages	Divergence dates[a] (mya)
1	Phenacomyini/other Arvicolinae	5.37 (4.75–5.95)
2	*Phenacomys*/*Arborimus*	2.45 (2.11–2.78)
3	Oregon and California/*Phenacomys*	1.80 (1.25–2.35)
4	Northern and Interior/Washington	1.54 (1.00–2.09)
5	Sierra Nevada/Oregon Cascades	0.74 (0.25–1.27)
6	Olympic/Washington Cascades	0.49 (0.12–0.90)
7	Wallowa/Northern Interior and Columbia	0.63 (0.26–1.05)

[a] Mean dates estimated in millions of years before present (mya) with 95% higher posterior density in parentheses.

Table 2.—Haplotype distribution, molecular diversity, and demographic parameters for 3 major clades of *Phenacomys intermedius*.

Clade	No. individuals	No. haplotypes	Haplotype diversity[a]	Nucleotide diversity[a]	Fu's F_s	Sum of squared deviation	Harpending's raggedness index
Northern and Interior	45	32	0.973 ± 0.013	0.020 ±0.001	−20.021[b]	0.003[c]	0.009[c]
Washington	27	14	0.934 ± 0.026	0.019 ± 0.002	−1.880	0.009[c]	0.017[c]
Oregon and California	17	12	0.956 ± 0.033	0.022 ± 0.002	−2.237	0.026[c]	0.041[c]

[a] Mean ± *SD*.
[b] $P < 0.05$.
[c] $P > 0.05$.

would have separated them from populations in the Wallowa Mountains. Based on fossil and pollen records, the Great Basin supported populations of *Phenacomys* and possibly large areas of their habitat (Diveley 1999; Grayson 1981; Thompson and Anderson 2000). The maintenance of geographic distinctiveness among major lineages of *Phenacomys* in the Pacific Northwest could be a product of the high degree of niche conservatism in this species that resulted from its Arctic origins

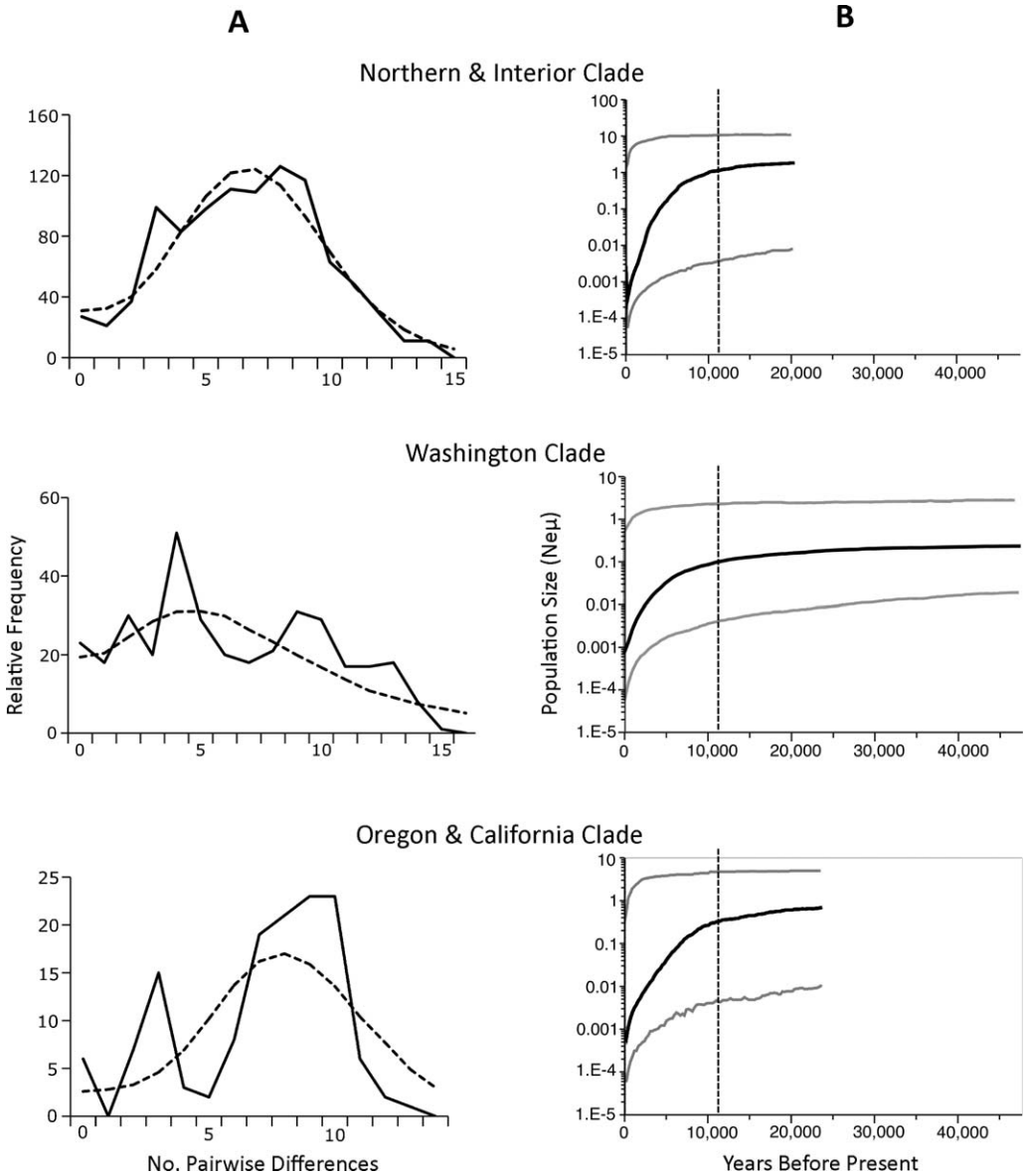

Fig. 4.—Demographic history of the 3 major clades of *Phenacomys intermedius*. A) Mismatch distributions are represented by solid lines for observed distributions of pairwise nucleotide differences between sequences and dashed lines for expected distribution under a sudden expansion model (Rogers and Harpending 1992). B) Bayesian skyline plots, in which central line indicates the median value for the log effective population size (effective population size times number of generations), and the upper and lower lines represent 95% range of posterior density. Dashed vertical line represents the Pleistocene–Holocene boundary (11,700 years before present), following Walker et al. (2009).

and restriction in modern and historical periods to cold environments. This could have played a role in the ability of *Phenacomys* to disperse across topographical barriers and persist through rapid environmental change. In contrast, *Microtus longicaudus*, another high-elevation arvicoline, possesses a wider niche breadth and was able to disperse across prominent geographic features during postglacial movements, which allowed its divergent lineages to come into secondary contact (Spaeth et al. 2009).

Postglacial routes for range expansion of western heather voles into northern areas previously occupied by continental ice sheets appear to have occurred along an interior axis rather than a coastal or Cascade Mountain axis. Examination of our data shows minimal northward expansion of Washington clade populations in comparison to Northern and Interior clade populations. In fact, only populations belonging to the Northern and Interior clade reoccupied areas previously overlaid by continental ice sheets, including the North Cascades. This spatial distribution suggests an interior dispersal route along or near the Rocky Mountain axis. The earlier retreat of the Laurentide Ice Sheet, as opposed to the Cordilleran Ice Sheet, during late-Pleistocene deglaciation (Siegert 2001) could have played a role in allowing northward expansion by interior refugial populations and the prevention of major northward expansion by the more-coastal Washington refugial populations. This pattern of asynchronous northward expansion by divergent lineages has been found in several low-elevation boreal mammals (Arbogast 1999; Arbogast et al. 2001; Demboski et al. 1999) and in another high-elevation arvicoline with similar habitat associations, the water vole (*Microtus richardsoni*—Carstens et al. 2005). The American pika (*Ochotona princeps*), however, displays a more symmetrical northward expansion between Cascade and Rocky Mountain lineages despite occupying similar high-elevation mountains in western North America (Galbreath et al. 2009). This demonstrates that climate-mediated movements did not necessarily occur in all assemblages of mammals but were likely more individualistic and dependent on each species' ecological breadth (Grayson 2006; Lawlor 1998).

Within the smaller geographic scale of each major clade we also found significant substructuring of populations of *P. intermedius* associated with shifts of elevation into isolated montane systems. The results from our divergence dating analyses indicate that the initial divergence of these sublineages began during the middle Pleistocene. As with the case for the 3 major clades, these sublineages persisted in distinct refugia despite repeated range expansions and contractions during glacial cycles associated with the remainder of the Pleistocene. Barriers that prevented mixing of lineages might have resulted from various prominent geographic features. The divergence between the Olympic Mountain and Washington Cascade populations possibly was reinforced by repeated advances of the Puget Lobe of the Cordilleran Ice Sheet during full glacial periods (Easterbrook 1986). The divergence between populations in the Wallowa Mountains and the rest of the populations in the Northern and Interior clade likely was due to the presence of the Snake River, whereas the divergence between populations in the Oregon Cascades and the Sierra Nevada could have been reinforced mostly by postglacial changes of vegetation in intervening regions from cool subalpine parkland to present-day mesic forests (Briles et al. 2005; Daniels et al. 2005; Hakala and Adam 2004; West 2004). Furthermore, lineage divergence also appears to be dependent on sufficient time for genetic drift to sort shared ancestral variation. None of the populations that recolonized northerly areas, which were covered by continental ice sheets, were shown to be divergent.

Results from our Bayesian skyline plots reveal population declines in all 3 major lineages of heather voles since the beginning of the Holocene. This pattern is also shared by pikas (Galbreath et al. 2009) and likely reflects the reduced amount of habitat in restricted mountain environments following postglacial upslope movements from more expansive lowland refugia. This pattern contrasts with those of low-elevation temperate mammals, which commonly show signatures of postglacial demographic expansion resulting from geographic expansion into large areas of recently available habitat (Lessa et al. 2003; Runck and Cook 2005). This pattern also might help explain why some of the more southerly interior populations, such as those that existed in the Great Basin Desert, are no longer extant. Nevertheless, some of our demographic analyses indicate that the Northern and Interior clade experienced rapid population growth. Although these results conflict with the outcome of the Bayesian Skyline Plots, they are consistent with the observation that this clade is the only major lineage to experience major geographic expansion since the end of the Pleistocene and the only case of substantial long-distance haplotype sharing (northern British Columbia and Montana; Fig. 2).

We have shown that the combination of major climatic fluctuations and topographical variation found within a regional montane system has had an important impact on the genetic variation of an alpine mammal, the western heather vole, in the Pacific Northwest. *P. intermedius* supports some of our predictions for high-elevation species, such as postglacial decline in population size and differentiation of populations restricted to isolated mountain ranges. However, in some ways *P. intermedius* showed similar patterns to those of low-elevation species; for example, the separation of refugial populations into distinct geographical regions and the asynchronous northward expansion by divergent lineages into areas previously glaciated by continental ice sheets. Studying the extreme environments and restricted ranges of alpine organisms is useful for understanding the tolerances of temperate mammals to strong environmental heterogeneity.

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APPENDIX I

List of 89 specimens of *Phenacomys intermedius* from 18 localities used in our analyses. Museum acronyms are defined in the ''Materials and Methods.'' mtDNA = mitochondrial DNA. BC = British Columbia; AB = Alberta; WA = Washington; MT = Montana; OR = Oregon; CA = California.

Locality	State or Canadian province	Mountain range	Latitude	Longitude	Museum no.[a]	GenBank accession no.	Tissue source	mtDNA haplotype no.
A	B	Northern Interior Mts.	58.22	−129.78	UWBM 50918c	GQ903588	Skin	1
A	BC	Northern Interior Mts.	57.80	−129.95	UWBM 50924	GQ903589	Skin	2
B	BC	Northern Interior Mts.	54.78	−127.37	UWBM 64172	GQ903590	Skin	3
C	AB	Northern Rockies	51.41	−116.23	UWBM 64156	GQ903591	Skin	4
C	AB	Northern Rockies	51.41	−116.23	UWBM 64159	GQ903592	Skin	5
C	AB	Northern Rockies	51.41	−116.23	UWBM 64160	GQ903593	Skin	6
C	AB	Northern Rockies	51.41	−116.23	UWBM 64162	GQ903594	Skin	7
C	AB	Northern Rockies	51.41	−116.23	UWBM 64164	GQ903595	Skin	5
C	AB	Northern Rockies	51.41	−116.23	UWBM 64165	GQ903596	Skin	8
C	AB	Northern Rockies	50.50	−115.06	UWBM 64171	GQ903597	Skin	9
D	BC	Cascades	49.13	−120.76	UWBM 48441	GQ903598	Skin	10
D	BC	Cascades	49.13	−120.76	UWBM 48443	GQ903599	Skin	11
D	BC	Cascades	49.15	−120.90	UWBM 48455	GQ903600	Skin	12
D	BC	Cascades	49.15	−120.90	UWBM 48456	GQ903601	Skin	13
D	BC	Cascades	49.16	−120.92	UWBM 48463	GQ903602	Skin	14
D	BC	Cascades	49.16	−120.92	UWBM 48466	GQ903603	Skin	13
D	BC	Cascades	49.10	−120.80	UWBM 48476	GQ903604	Skin	15
D	BC	Cascades	49.10	−120.80	UWBM 48477	GQ903605	Skin	13
D	BC	Cascades	49.10	−120.80	UWBM 48488	GQ903606	Skin	16
E	WA	Cascades	48.85	−121.69	UWBM 76638a	GQ903607	Liver	17
E	WA	Cascades	48.95	−121.64	UWBM 76659	GQ903608	Liver	17
E	WA	Cascades	48.95	−121.64	UWBM 76662	GQ903609	Liver	17
E	WA	Cascades	48.95	−121.64	UWBM 76666	GQ903610	Liver	17
E	WA	Cascades	48.95	−121.64	UWBM 76676	GQ903611	Liver	17
E	WA	Cascades	48.86	−121.84	UWBM 18705	GQ903612	Skin	18
E	WA	Cascades	48.78	−121.07	UWBM 27661	GQ903613	Skin	19
E	WA	Cascades	48.85	−121.58	UWBM 41891	GQ903614	Skin	20
E	WA	Cascades	48.85	−121.58	UWBM 41892	GQ903615	Skin	17
F	WA	Cascades	48.71	−120.68	UWBM 74878	GQ903616	Liver	21
F	WA	Cascades	48.71	−120.68	UWBM 76754	GQ903617	Liver	10
F	WA	Cascades	48.71	−120.68	UWBM 80415	GQ903618	Liver	10
F	WA	Cascades	48.71	−120.68	UWBM 80416	GQ903619	Liver	22
F	WA	Cascades	48.71	−120.68	UWBM 80417	GQ903620	Liver	23
F	WA	Cascades	48.52	−120.66	UWBM 80854	GQ903621	Liver	10
G	WA	Columbia Mts.	48.95	−117.09	UWBM 34353	GQ903622	Skin	24
G	WA	Columbia Mts.	48.81	−117.14	UWBM 76600b	GQ903623	Liver	25
G	WA	Columbia Mts.	48.92	−117.09	UWBM 76613	GQ903624	Liver	26
G	WA	Columbia Mts.	48.84	−117.14	UWBM 78129	GQ903625	Liver	27
H	BC	Columbia Mts.	49.03	−117.14	UWBM 50954	GQ903626	Skin	26
I	MT	Northern Rockies	48.70	−113.52	UWBM 50979	GQ903627	Skin	1
J	OR	Wallowa Mts.	45.18	−117.32	OSUFW 05051	GQ903628	Skin	28
J	OR	Wallowa Mts.	45.18	−117.32	OSUFW 05052	GQ903629	Skin	29
J	OR	Wallowa Mts.	45.22	−117.37	OSUFW 05056	GQ903630	Skin	30
J	OR	Wallowa Mts.	45.28	−117.42	OSUFW 05057d	GQ903631	Skin	31
J	OR	Wallowa Mts.	45.20	−117.14	UWBM 60816	GQ903632	Skin	32
K	WA	Cascades	48.08	−120.86	UWBM 32610	GQ903633	Skin	33
K	WA	Cascades	47.70	−121.23	UWBM 32715	GQ903634	Skin	34
L	WA	Cascades	46.87	−121.52	UWBM 30388	GQ903635	Skin	35
L	WA	Cascades	46.96	−121.88	UWBM 30407f	GQ903636	Skin	36
L	WA	Cascades	46.77	−121.72	UWBM 30408	GQ903637	Skin	37
L	WA	Cascades	46.89	−121.70	UWBM 30409	GQ903638	Skin	35
L	WA	Cascades	46.92	−121.57	UWBM 31236	GQ903639	Skin	38
L	WA	Cascades	46.69	−121.49	UWBM 56462	GQ903640	Skin	39
L	WA	Cascades	47.01	−121.46	UWBM 60865	GQ903641	Skin	40
L	WA	Cascades	46.92	−121.82	UWBM 60987	GQ903642	Skin	36
L	WA	Cascades	46.92	−121.82	UWBM 73506	GQ903643	Skin	35
L	WA	Cascades	47.12	−121.07	UWBM 73808	GQ903644	Liver	40

APPENDIX I.—Continued.

Locality	State or Canadian province	Mountain range	Latitude	Longitude	Museum no.[a]	GenBank accession no.	Tissue source	mtDNA haplotype no.
L	WA	Cascades	46.87	−121.52	UWBM 76393	GQ903645	Skin	41
L	WA	Cascades	46.62	−121.24	UWBM 26617	GQ903646	Skin	35
M	WA	Cascades	46.39	−121.94	UWBM 55978	GQ903647	Skin	42
M	WA	Cascades	46.40	−121.61	UWBM 74006e	GQ903648	Liver	39
M	WA	Cascades	46.00	−121.99	UWBM 75886	GQ903649	Liver	42
M	WA	Cascades	46.00	−121.99	UWBM 75887	GQ903650	Liver	42
M	WA	Cascades	46.00	−121.99	UWBM 75888	GQ903651	Liver	45
M	WA	Cascades	46.00	−121.99	UWBM 75889	GQ903652	Liver	42
M	WA	Cascades	46.00	−121.99	UWBM 75890	GQ903653	Liver	42
N	WA	Olympic Mts.	47.91	−123.73	UWBM 79821	GQ903654	Liver	44
N	WA	Olympic Mts.	47.91	−123.72	UWBM 79828	GQ903655	Liver	44
N	WA	Olympic Mts.	47.91	−123.72	UWBM 79832g	GQ903656	Liver	45
N	WA	Olympic Mts.	47.91	−123.72	UWBM 79833	GQ903657	Liver	46
N	WA	Olympic Mts.	47.91	−123.72	UWBM 79835	GQ903658	Liver	45
N	WA	Olympic Mts.	47.91	−123.73	UWBM 79844	GQ903659	Liver	45
O	OR	Cascades	45.19	−121.70	PSM 1903	GQ903661	Skin	48
O	OR	Cascades	45.19	−121.70	PSM 1904	GQ903662	Skin	49
O	OR	Cascades	45.19	−121.70	PSM 1905	GQ903663	Skin	48
P	OR	Cascades	43.15	−122.11	PSM 1902i	GQ903660	Skin	47
P	OR	Cascades	44.13	−122.11	UWBM 58465	GQ903665	Skin	51
P	OR	Cascades	44.13	−122.11	UWBM 58466	GQ903666	Skin	52
P	OR	Cascades	44.22	−122.16	UWBM 58468	GQ903667	Skin	52
P	OR	Cascades	44.40	−121.86	UWBM 61187h	GQ903668	Skin	53
P	OR	Cascades	44.44	−121.77	UWBM 75417	GQ903664	Liver	50
Q	OR	Cascades	43.62	−121.78	UWBM 80840	GQ903669	Liver	54
Q	OR	Cascades	43.63	−121.78	UWBM 80841	GQ903670	Liver	55
Q	OR	Cascades	43.62	−121.79	UWBM 80842	GQ903671	Liver	56
Q	OR	Cascades	43.60	−121.80	UWBM 80843	GQ903672	Liver	54
Q	OR	Cascades	43.61	−121.80	UWBM 80844	GQ903673	Liver	56
Q	OR	Cascades	43.61	−121.80	UWBM 80845	GQ903674	Liver	54
R	CA	Sierra Nevada	37.83	−119.50	MVZ 207658	GQ903675	Liver	57
R	CA	Sierra Nevada	38.06	−119.34	MVZ 216651j	GQ903676	Liver	58

[a] Letters a–j indicate individuals used for divergence dating as shown in Fig. 3.