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Complex history of isolation and gene flow in hoary, Olympic, and endangered Vancouver Island marmots

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Climate change resulting in a reduction of alpine habitat is believed to pose a considerable risk to alpine-dependent species, including many marmots. Hoary marmots (*Marmota caligata*) range throughout much of the mountainous Pacific Northwest (PNW) and Rocky Mountains while the closely related Olympic and Vancouver Island marmots (*M. olympus* and *M. vancouverensis*, respectively) are restricted to small isolated regions of the PNW. The endemic Vancouver Island marmot is currently classified as Critically Endangered and the Olympic marmot has recently experienced dramatic population declines. Previous phylogenetic studies of PNW marmot species have had limited power as they focused on resolving interspecific relationships, implicitly assumed an absence of gene flow among currently recognized species, included relatively few individuals, and relied heavily or entirely on mitochondrial DNA. We sequenced 2 mitochondrial and 4 nuclear markers from 167 hoary, 4 Vancouver Island, and 5 Olympic marmots in order to investigate phylogenetic relationships and historic gene flow among these species. We recovered 2 monophyletic (and predominantly allopatric) mitochondrial clades of hoary marmots that are not sister groups. Instead, Vancouver Island marmots formed a monophyletic mitochondrial sister clade to 1 of the hoary marmot clades. Nuclear loci did not recover the 2 mitochondrial clades of hoary marmots and suggest that Vancouver Island marmots may have experienced mitochondrial introgression from coastal mainland hoary marmots. Additionally, our nuclear results suggest possible gene flow between hoary and Olympic marmots despite different chromosomal formulas. Rather than resolving what has previously been considered a straightforward 3-taxon phylogenetic question, our findings suggest a complicated history of rapid divergence of the 3 species followed by intermittent and possibly ongoing gene flow between hoary marmots and both Olympic and Vancouver Island marmots. These results therefore have significant implications for the conservation of the latter 2 species, both of which are conservation concerns.

Key words: alpine mammal, hoary marmot, Olympic marmot, Pacific Northwest, phylogenetics, Pleistocene refugia, Vancouver Island marmot

Pleistocene glacial cycles shaped much of the genetic structure of the North American biota (Rand 1948, 1954; Hoffmann 1981; Shafer et al. 2010). During this time, much of Beringia and the southern portion of the Pacific Northwest (PNW) remained ice free and served as separate glacial refugia north and south of the continental ice sheet, respectively (Hultén 1937; Pielou 2008). In the PNW (defined here as including the Rocky Mountains and areas west to the Pacific Ocean from western Montana and Idaho north to Alaska), 1 or more southern refugia likely existed in the Coast/Cascade Mountains of Oregon and Washington and the northern Rocky Mountains of Montana and southern Canada (Fig. 1; Brunsfeld and Sullivan 2005; Shafer et al. 2010). The hoary marmot (*Marmota caligata*) is the only alpine marmot whose current distribution includes regions that served as Pleistocene refugia both north and south of the historic Cordilleran and Laurentide ice sheets as well as areas that were glaciated during the Pleistocene (Steppan et al. 1999). Post-Pleistocene colonization of mammals into glaciated and non-glaciated regions of the PNW generally fall into 1 of 2 categories: southward expansion from a northern refugium or northward expansion from one or more southern refugia (Weksler et al. 2010). The current distribution of hoary marmots (Fig. 1) suggests they were present in 1 or more Pleistocene refugia. To date, the number of hoary marmot specimens included in molecular phylogenetic studies has been limited to 1 or 2 individuals (Kruckenhauser et al. 1999; Steppan et al. 1999; Brandler and Lyapunova 2009; Steppan et al. 2011) and no phylogeographic studies have

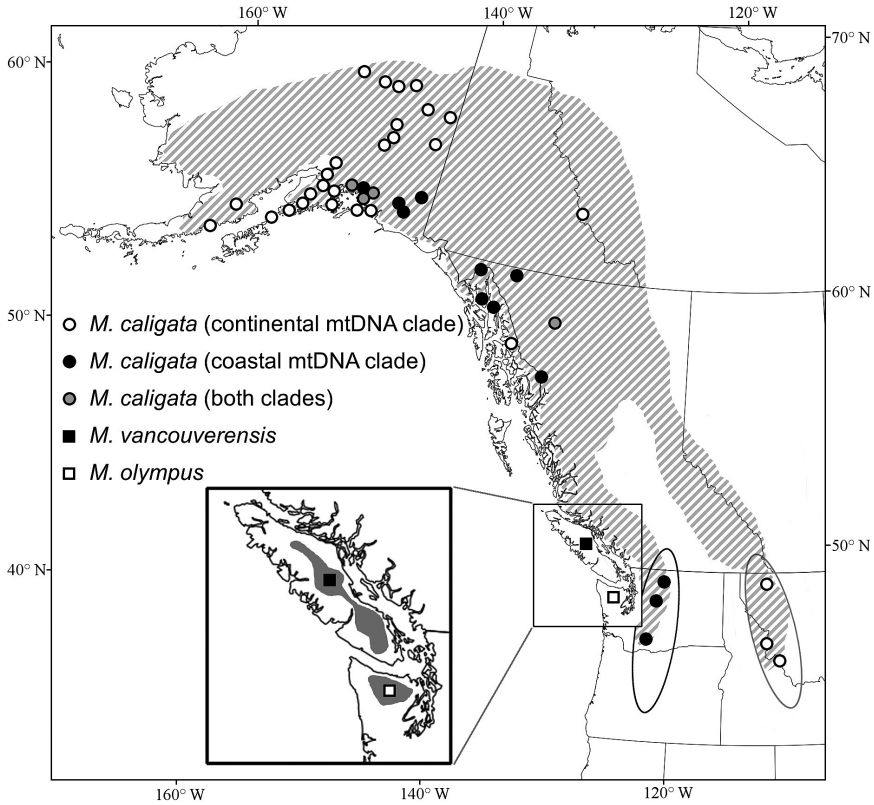

Fig. 1.—Distribution of specimens used in this study. *Marmota caligata* clades are based on mitochondrial DNA results. The hashed region represents the generalized *M. caligata* distribution (modified from Braun et al. 2011). Black and gray oval outlines refer to the predicted Pleistocene refugia of *M. caligata* discussed in the text (based on Shafer et al. 2010) in the Coast/Cascade and northern Rocky mountains, respectively. The distributions of *M. vancouverensis* and *M. olympus* are shown in gray in inset (modified from Aaltonen et al. 2009 and Edelman 2003, respectively). All 7 *M. caligata* specimens from Washington (3 localities) have a signature of nuclear introgression with *M. olympus*.

been published. As a result of these limited sample sizes, the Pleistocene distribution, and mode of post-Pleistocene colonization of hoary marmots remain unknown.

Many species present in the historic southern refugia show a phylogeographic division between the Coast/Cascade and the northern Rocky Mountains (reviewed by Brunsfeld et al. 2001), a pattern supporting a refugia-within-refugia model in the PNW, in which a purported single refugium was actually composed of multiple isolated refugia (Gómez and Lunt 2007; Shafer et al. 2010). Recent research has uncovered reciprocally monophyletic mitochondrial DNA (mtDNA) clades in both the Coast/Cascade and the northern Rocky Mountains in the American pika, *Ochotona princeps* (Galbreath et al. 2009). Pleistocene isolation also likely led to speciation between sooty (*Dendragapus fuliginosus*) and dusky grouse (*D. obscurus*), which today inhabit the Coast/Cascade and the northern Rocky Mountains, respectively (Barrowclough et al. 2004). Furthermore, the Coast/Cascade and the northern Rocky Mountains each served as refugia for a unique assemblage of shrews (*Sorex* spp.—Demboski and Cook 2001; Hope et al. 2014). Thus, if hoary marmots were present in the southern refugia, we expect a phylogeographic division between the Coast/Cascade and northern Rocky Mountain populations (refugia-within-refugia) and relatively deeper phylogenetic divisions among southern populations than among northern populations.

Marmots (*Marmota* spp.) are the largest members of the squirrel family (Sciuridae) and most species are at least moderately social (Barash 1989). There are currently 15 recognized species, 9 of which occur in Eurasia and 6 in North America (Thorington and Hoffmann 2005; Brandler et al. 2008). Two subgenera (*Petromarmota* and *Marmota*) have been recognized based on molecular and phenotypic (pelage) evidence (Steppan et al. 1999). With the exception of the woodchuck (*M. monax*), all marmot species in the PNW belong to the subgenus *Petromarmota*. These include the yellow-bellied (*M. flaviventris*), hoary, Olympic (*M. olympus*), and Vancouver Island (*M. vancouverensis*) marmots (Steppan et al. 1999). *M. caligata*, *M. flaviventris*, and *M. vancouverensis* all have a diploid chromosome number of 42 (Rausch and Rausch 1965, 1971) while *M. monax* and *M. olympus* possess 38 and 40 chromosomes, respectively (Couser et al. 1963; Rausch and Rausch 1965). The most recent molecular phylogeny to include all members of *Petromarmota* recovered yellow-bellied marmots as the basal member of the subgenus, followed by Olympic marmots, with hoary and Vancouver Island marmots sister to one another (Steppan et al. 2011; see below).

Hoary marmots are predominantly alpine with an expansive range that spans over 20° of latitude, the greatest of any alpine marmot. The species occurs throughout the PNW from central Idaho, southwest Montana, and southern Washington north to

the Yukon River in Alaska (Gunderson et al. 2009; Braun et al. 2011). While hoary marmots are not a species of conservation concern, the alpine habitat and northern latitudes they inhabit are predicted to be particularly vulnerable to climate change (Krajick 2004; Walther et al. 2005). Within-species variation and taxonomy in hoary marmots is poorly defined and has relied exclusively on qualitative morphological characters.

The Olympic marmot is found only on the Olympic Peninsula in Washington State. Despite its restricted range, *M. olympus* is currently classified as Least Concern by the International Union for Conservation of Nature (IUCN—Linzey 2012), although the State of Washington has considered it a candidate for listing as endangered, threatened, or sensitive since 2008 (Washington Department of Fish and Wildlife 2013). With a small and declining estimated population size (≤ 1,000—Witczuk et al. 2008), increasing population fragmentation (Griffin et al. 2009), and one of the smallest ranges of any North American mammal, the Olympic marmot likely warrants a heightened conservation status.

The Vancouver Island marmot is found only on Vancouver Island, British Columbia, Canada and is classified as Critically Endangered by the IUCN (Nagorsen and Keddie 2000; Nagorsen 2012), Endangered by the Committee on the Status of Endangered Wildlife in Canada (2008), and Endangered under the United States Endangered Species Act. Conservation efforts include ongoing captive breeding and reintroduction programs (Keeley et al. 2011). mtDNA sequence data suggest that Vancouver Island and hoary marmots are closely related (1.2% sequence divergence) and recently (0.4–1.2 million years ago [mya]) diverged from a common ancestor (Steppan et al. 1999, 2011). The genetic similarity and geographic proximity of Vancouver Island and hoary marmots led Steppan et al. (2011:1034) to hypothesize that the hoary marmot "seems likely to be paraphyletic with respect to *M. vancouverensis*." In contrast, geometric morphometric analysis of the skull and mandible clearly separate Vancouver Island marmots from hoary marmots (Cardini et al. 2007, 2009). Clarifying the phylogenetic position of *M. vancouverensis* within a broader geographic sample of *M. caligata* may therefore prove critical to conservation efforts if genetic rescue becomes necessary for the former (Hedrick and Fredrickson 2009).

Previous molecular phylogenetic studies have disagreed over the relationships among hoary, Olympic, and Vancouver Island marmots (Kruckenhauser et al. 1999; Steppan et al. 1999; Herron et al. 2004; Steppan et al. 2011). Steppan et al. (2011) showed that the *M. olympus* sequence reported by Kruckenhauser et al. (1999) was actually *M. vancouverensis*, the likely result of lab contamination. However, all but 1 of these studies relied exclusively on mtDNA. Steppan et al. (2011) attempted to resolve the phylogenetic relationship of PNW marmots using 2 mtDNA markers (1,140 bp of cytochrome *b* and a 2,029-bp region spanning ND3/ND4) and a nuclear exon (RAG1). The results from their nuclear analyses yielded 2 equally supported phylogenies, 1 representing a polytomy composed of *M. caligata*, *M. olympus*, and *M. vancouverensis* and the other supporting Vancouver Island marmots as sister to yellow-bellied marmots (Steppan et al. 2011). Additional nuclear markers are therefore needed to clarify the phylogenetic relationships and history of gene flow between these taxa.

Previous phylogeographic studies of PNW taxa have relied primarily on mtDNA markers (Shafer et al. 2010). Mitochondrial markers are often favored due to their smaller effective population size (leading to faster lineage sorting) relative to nuclear markers, the absence of recombination in the mitochondrial genome, and the ease of acquiring mtDNA sequence data. However, mtDNA can provide a misleading phylogenetic signal due to incomplete lineage sorting and its inheritance as a single linkage group (Funk and Omland 2003). Evidence of hybridization in Asian marmots (Brandler et al. 2010) suggests that mtDNA introgression is possible in the genus and that nuclear and mtDNA markers should therefore be used together to infer phylogenetic relationships among closely related species.

We conducted phylogenetic analyses using 2 mitochondrial and 4 nuclear markers to address 3 questions. First, what is the phylogenetic history of *M. caligata*, and what, if any, intraspecific divisions exist? Second, are the phylogenetic inferences drawn from mitochondrial and nuclear markers concordant and/or compatible in the subgenus *Petromarmota*? Finally, is there evidence of recent or ongoing gene flow among *M. caligata*, *M. olympus*, and *M. vancouverensis*?

Materials and Methods

Specimens.—We generated and analyzed DNA sequence data from 165 marmot specimens housed at the University of Alaska Museum and 13 from other natural history museums. Museum catalog numbers and locality data are provided in Appendix I.

Laboratory protocols.—DNA was extracted from organ or muscle tissue from 167 *M. caligata*, 2 *M. flaviventris*, 5 *M. olympus*, and 4 *M. vancouverensis* specimens using the Gentra PureGene (Qiagen Inc., Valencia, California) DNA extraction kit following the manufacturer's fresh tissue protocol. All PCR reactions were carried out on unquantified 1:10 extraction dilutions using the standard protocols provided with the reagents and/or those outlined in Gunderson et al. (2009).

We amplified and sequenced 2 mtDNA and 4 nuclear loci. The entire mitochondrial cytochrome *b* gene (1,140 bp) was amplified in 2 overlapping segments using 2 flanking universal primers (L41724 and H15915) from Irwin et al. (1991) and 3 *M. caligata*-specific primers (MACA-L4, MACA-R4, and MACA-R7) designed for this study (Supporting Information S1). A 571-bp-segment of the mitochondrial control region was amplified using primers CR-HLF1 and CR-HLR1 (Supporting Information S1). Two nuclear introns were amplified using the eponymous CAT (599 bp) and BGN (715 bp) primers from Lyons et al. (1997). Primers spanning intron 4 of the E3 ubiquitin ligase Cullin 4A (Cul4A) and intron 8 of the lysosomal-associated membrane protein 1 (Lamp1) genes were designed based on GenBank sequences of the house mouse (*Mus musculus*) and the corresponding but as-yet unannotated region of the

draft genome of the 13-lined ground squirrel (*Ictidomys tridecemlineatus*) and are provided in Supporting Information S1. Cul4A primers amplify 362 intronic nucleotides and Lamp1 primers amplify 10 exonic and 490 intronic nucleotides. Because Cul4A and Lamp1 are within < 14 kb of each other in the closely related 13-lined ground squirrel, we treated them as linked. We tested for recombination in the BGN, CAT, and concatenated Cul4A and Lamp1 loci using the program IM*gc*, which identifies the largest nonrecombining block of sequence data and/or individuals that do not exhibit evidence of recombination (Woerner et al. 2007).

PCR reactions were purified using Exo-Sap (Affymetrix, Cleveland, Ohio) and Sanger sequencing reactions were carried out using ABI (Applied Biosystems, Foster City, California) reagents and standard protocols at either the University of Alaska Fairbanks Institute of Arctic Biology's Core Facility (Fairbanks, Alaska) or the High-Throughput Genomics Unit (Seattle, Washington) on ABI 3100 and 3730xl DNA analyzers, respectively. We sequenced in both directions when a single sequencing reaction failed to amplify the entire region of interest and/or when a single reaction did not provide unambiguous results. All sequence data were visualized, assembled, and aligned using Sequencher ver. 5.1 (Gene Codes Corp. 2012). Indels were aligned by eye using homozygous (for a given indel) individuals. Individuals that were heterozygous for indels were identified as those having clean, unambiguous chromatograms along the length of a sequencing reaction until reaching the putative indel sites, after which multiple equally intense overlapping peaks were observed. Information regarding length heterogeneity within an individual was used when inferring the gametic phase and coded as missing data in other analyses. All new sequence data have been deposited to GenBank (accession KJ457348-KJ458415) and nexus files of the aligned sequence data have been included as Supporting Information S2.

The program Phase ver. 2.1.1 was used to infer haplotypes of nuclear loci with multiple heterozygous sites (Stephens et al. 2001; Stephens and Scheet 2005). Only haplotypes inferred with posterior probabilities ≥ 0.95 were included in our analysis using phased data. Input files for Phase were created using the program PhaseIn 1.0 (see Acknowledgments) and Se-Al ver. 2.0a11 (Rambaut 2013). We had a disproportionately large (n = 25) number of *M. caligata* specimens from Sud Island, Alaska. To decrease computation time and bias in our data, we randomly selected 5 specimens from Sud Island, Alaska, to use in the STRUCTURE, *BEAST, and isolation with migration (IM) analyses (below). All trees were rooted with *M. flaviventris*, which has been recovered as the sister species to the focal taxa in previous molecular analyses (Steppan et al. 1999, 2011).

Model selection and phylogenetic analysis.—Maximum likelihood (ML) and Bayesian analyses were conducted using the programs GARLI ver. 2.0 (Zwickl 2006) and MrBayes ver. 3.2 (Ronquist et al. 2012), respectively. For each of these analyses, the best-fit model of nucleotide substitution for each locus was selected using the Akaike Information Criterion (AIC). The AIC values for the ML analysis were calculated using Modeltest ver. 3.7 (Posada and Crandall 1998). MrModeltest ver. 2.3 (Nylander 2004) was used to calculate the AIC values for all Bayesian analyses. Potential problems with parameter estimates have been noted for nucleotide substitution models that include both a proportion of invariable sites (I) and gamma-distributed rates (G—Ren et al. 2005; Yang 2006). To ensure including both parameters did not bias our results, we confirmed results of models with I + G by also analyzing the data with only G. The respective best-fit models of nucleotide substitution for cytochrome *b* and the control region were TrN + I and GTR + I + G for the ML analysis and GTR + I and HKY + I + G for the Bayesian analysis. The ML and Bayesian analyses shared the same best-fit model of nucleotide substitution for the BGN and concatenated Cul4A and Lamp1 loci, HKY and F81 + I, respectively. For the CAT locus, best-fit models were TVM and GTR for the ML and Bayesian analysis, respectively. To meet the assumption of no recombination in the nuclear data, we excluded 1 or both sequences from 1 individual at the CAT locus and 8 individuals and the first 128 bp of the concatenated Cul4A and Lamp1 loci, as determined using IM*gc*.

We conducted individual ML and Bayesian analysis of the BGN, CAT, and concatenated Cul4A and Lamp1 loci. To compare mitochondrial and nuclear phylogenies, we conducted separate ML and Bayesian analysis of both the combined mitochondrial and the combined nuclear loci. To account for variation between loci, we partitioned the data by locus and used the best-fit model of nucleotide substitution for each locus. Partitioning combined data by locus may still allow undue influence of 1 or more loci, but when analyses of individual loci are not in conflict, this method may provide a useful estimation of the overall phylogenetic signal. In all analyses, the Cul4A and Lamp1 loci were concatenated and treated as a single linked partition. We conducted 20 replicates of each GARLI run and checked that there was no significant variation in log likelihood (lnL) values between runs to ensure the program was sufficiently searching tree space. A 1,000-replicate bootstrap analysis was conducted using the program GARLI. The program SumTrees—part of DendroPy ver. 3.12.0 (Sukumaran and Holder 2010)—was used to summarize the output of the GARLI bootstrap analysis. Bayesian analysis consisted of 4 chains run for 2.5×10^7 Markov chain Monte Carlo (MCMC) generations and sampled every 1,000 generations.

Clustering analysis of haplotypes from the phased nuclear data was conducted using STRUCTURE ver. 2.3 (Pritchard et al. 2000). We used an admixture model with correlated allele frequencies and a 10^5 burn-in followed by 5×10^5 MCMC iterations. We assumed the true number of groups (K) was between 1 and 10 and ran 10 iterations for each group size. Results from the multiple runs were analyzed using STRUCTURE HARVESTER (Earl and vonHoldt 2012) and averaged using CLUMPP (Jakobsson and Rosenberg 2007). CLUMPP results were visualized using DISTRUCT (Rosenberg 2003). We determined the number of genetic clusters using both the peak in the mean probability of the data (Pritchard et al. 2000) and the ΔK method of Evanno et al. (2005) in the hierarchical framework presented by Coulon et al. (2008).

We used the Bayesian Evolutionary Analysis by Sampling Trees (BEAST) software package (BEAST ver. 1.7—Drummond et al. 2012) to analyze our phased nuclear data. The graphical user-interface application Bayesian Evolutionary Analysis Utility (BEAUti, ver. 1.5.1, part of the BEAST software package) was used to generate our BEAST XML input file. To estimate the species tree from the multilocus nuclear data, we enabled *BEAST (Heled and Drummond 2010) in BEAST and allowed each major mtDNA clade to be treated as a "species." Because BEAST assumes that discordance among gene trees is the result of incomplete lineage sorting and not hybridization, we ran the *BEAST analysis without hoary marmot specimens from Washington ($n = 7$), all of which shared haplotypes (potentially representing hybridization) with Olympic marmots.

For the *BEAST analysis, we selected an unlinked substitution model for the BGN, CAT, and concatenated Cul4A and Lamp1 loci, a strict molecular clock, a Yule speciation process, the HKY model of nucleotide substitution, and an estimated mutation rate. The *BEAST analysis was conducted in relative time (i.e., without external calibration) and the molecular clock rate was fixed at 1.0. To reduce computation time, we combined the results of 3 MCMC simulations each allowed to run for 10^8 steps sampling every 10^3 steps. We used LogCombiner v1.7.41 (part of the BEAST software package) to combine the log and tree files from the 3 runs using a 10% burn-in. Output files were viewed and summarized using Tracer ver. 1.5 (Rambaut et al. 2009) and TreeAnnotator ver. 1.7.4 (part of the BEAST software package). To ensure our priors were not having unexpected effects on posterior values, we also ran the analysis with empty alignments (created in BEAUti). Phylogenetic analyses were conducted on the University of Alaska Life Sciences Bioinformatics cluster.

An ultrametric tree of *Marmota* species divergence times based on the cytochrome *b* and ND3/ND4 loci was presented in Steppan et al. (2011). To estimate the divergence time of the previously unrepresented *M. caligata* continental mtDNA clade (see below), we reran the BEAST analysis used to create the ultrametric tree of Steppan et al. (2011) including 2 randomly selected *M. caligata* continental mtDNA specimens (GenBank accessions KJ458068 and KJ458094). We followed the methods presented in Steppan et al. (2011), using only the cytochrome *b* data, increasing the run time to 4×10^6 generations, and using the HKY + I + G model of nucleotide sequence evolution. We did not use the sequences of Thomas and Martin (1993) used by Steppan et al. (2011) because they are not on GenBank or otherwise available online. In place of the sequences of Thomas and Martin (1993), we used the following sequences from GenBank: *Callospermophilus lateralis* (AF157887); *C. saturatus* (AF157916); *I. tridecemlineatus* (AF157870); *Sciurus carolinensis* (FJ200744); *Urocitellus columbianus* (AF157882); and *U. richardsonii* (AF157914—Harrison et al. 2003; Barber 2007).

To test for gene flow between marmot species, we fit an IM model to our mtDNA and phased nuclear data using the program IMa2 (Hey 2010). IMa2 uses coalescent-based Bayesian methods to infer effective population sizes, migration rates, and divergence times between populations or closely related species (Nielsen and Wakeley 2001). IMa2 allows for a single analysis of multiple populations/species, but requires a user-specified phylogenetic tree. Because we lacked certainty in the phylogenetic relationship between *M. caligata*, *M. olympus*, and *M. vancouverensis*, we conducted 2 pairwise analyses (*M. caligata* versus *M. olympus* and *M. caligata* versus *M. vancouverensis*).

For the IM analysis, the 2 mtDNA markers were concatenated and treated as a single locus with an inheritance scalar of 0.25. The location of BGN in the marmot genome is unknown, but it is located on the X-chromosome in both *M. musculus* and *Rattus norvegicus* so we treated it as X-linked. For the BGN locus, we excluded specimens of unknown sex ($n = 22$), only included 1 of the 2 identical haplotypes for males, and used an inheritance scalar of 0.75. Cul4A and Lamp1 were similarly concatenated and treated as a single locus with an inheritance scalar of 1. To scale IM model parameters to years, we used a per locus mtDNA mutation rate of $3\%/10^6$ years and a generation time of 4.5 years based on information inferred from *M. flaviventris* (Schwartz et al. 1998). We used the HKY model of nucleotide substitution for the concatenated mtDNA and the infinite sites model for all nuclear loci.

For both IM comparisons, we conducted several preliminary runs to determine optimal prior settings and MCMC chain heating and swap terms. We used update rates, trend plots, and effective sample size values to determine when adequate mixing had been achieved. To ensure we were obtaining consistent results, we performed 2 independent runs of each IM analysis. To reduce computation time, we ran and combined the results of 4 independent MCMC runs for each comparison and used a total of 10^5 saved genealogies for the subsequent L-mode analyses. Each MCMC run had a unique starting seed, 60 heated chains, and a 3×10^6 burn-in. We used the L-mode analysis to compare 5 migration models: (1) migration between species with each species having a migration rate; (2) migration between species with a single migration rate; (3) no migration from species 0 to species 1; (4) no migration from species 1 to species 0; and (5) no migration between species. Results from the L-mode analyses were ranked using AIC following the procedures outlined in Carstens et al. (2009).

Results

Mitochondrial loci.—Both ML and Bayesian analyses of the concatenated cytochrome *b* and the control region produced nearly identical well-supported topologies. *M. caligata* was not recovered as monophyletic; instead, *M. vancouverensis* was strongly supported as the sister clade to 1 of 2 *M. caligata* haplotype clades (Fig. 2). *M. olympus* was recovered as basal to both the *M. caligata* and *M. caligata* + *M. vancouverensis* clades (Fig. 2). There were no appreciable differences between the results of models using I + G and only G.

Nuclear loci.—There were 43 and 63 specimens heterozygous for length polymorphisms at the Cul4A and Lamp1 loci,

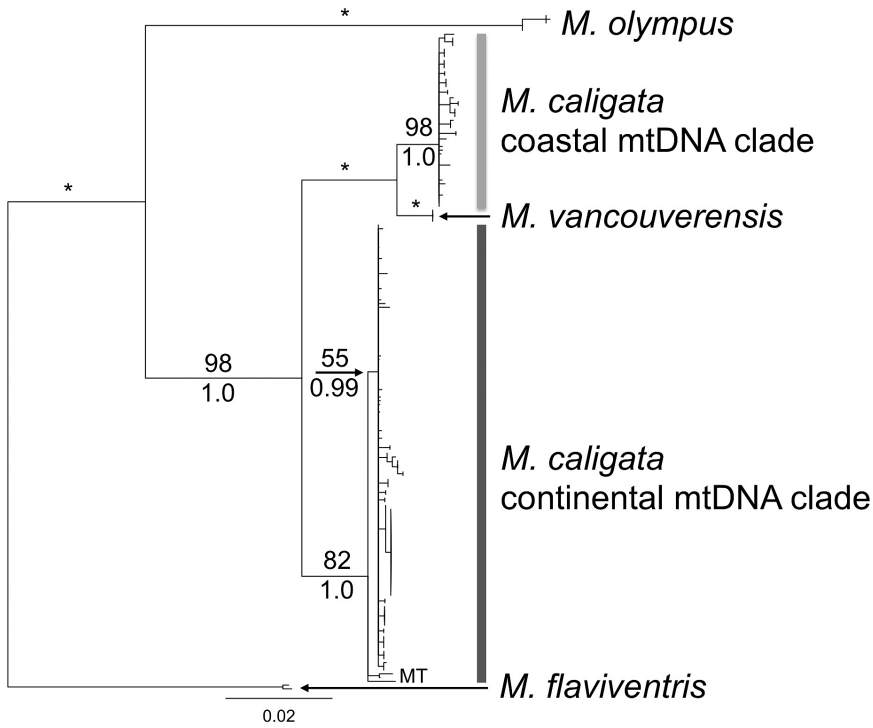

Fig. 2.—Maximum likelihood phylogram of the entire cytochrome *b* gene and 571 bp of the control region for *Marmota caligata*, *M. vancouverensis*, and *M. olympus* rooted with *M. flaviventris*. MT denotes 3 of the 4 *M. caligata* specimens from Montana; the additional specimen was nested within the continental clade. In both the maximum likelihood and Bayesian analyses, the cytochrome *b* and control region data were analyzed as separate data partitions. A Bayesian analysis produced a tree with nearly identical topology. Numbers above the line are the results of a 1,000-replicate bootstrap analysis and numbers below the line are Bayesian posterior probabilities. Asterisks denote 100% bootstrap support and a posterior probability of 1.0.

respectively. Sequencing in both directions resolved heterozygous length polymorphisms for all but 7 specimens, which appeared to be heterozygous for 2 noncontiguous length polymorphisms at the Cul4A locus. For these 7 specimens, we obtained 238 bp of the 363 bp locus. All 363 bp of the Cul4A locus was used in analyses with any unresolved portion of the locus coded as missing data. Among ingroup taxa, there were a total of 8, 11, and 13 variable nucleotide positions at the BGN, CAT, and concatenated Cul4A and Lamp1 loci, respectively. We were able to infer or observe the gametic phase of 178, 178, and 153 individuals for the BGN, CAT, and the concatenated Cul4A and Lamp1 loci, respectively. There were 7, 7, and 6 unique haplotypes for the phased nonrecombining ingroup sequences of the BGN, CAT, and concatenated Cul4A and Lamp1, respectively.

Only a monophyletic *M. vancouverensis* clade nested within *M. caligata* and *M. olympus* was well supported in the majority-rule consensus 1,000-replicate ML bootstrap analysis of the partitioned nuclear data (Fig. 3). Bayesian analysis of the same data recovered 2 well-supported clades, a monophyletic *M. vancouverensis* clade and a clade consisting of all *M. caligata* specimens except those from Washington (Fig. 3). Bayesian and ML analysis of the individual nuclear loci produced few well-resolved clades, all of which were concordant with the concatenated analyses of the nuclear data. Bayesian analysis of the mtDNA and nuclear loci combined and partitioned by locus produced a tree topology not appreciably different from that of the mtDNA alone. The majority-rule 1,000-replicate ML bootstrap analysis of these data produced similar results, with the *M. caligata* + *M. vancouverensis* clade nested within—and not sister to—the other *M. caligata* clade.

We included 147 *M. caligata*, 5 *M. olympus*, and 4 *M. vancouverensis* specimens in the STRUCTURE analysis. The mean likelihood value of the STRUCTURE analysis plateaued at $K = 7$ (Fig. 4). There were 5 groups of *M. caligata*, 1 of *M. olympus* and *M. caligata* from Washington, and 1 of *M. vancouverensis*. Using the ΔK method implemented in STRUCTURE HARVESTER, $K = 2$ was selected as the most probable number of groups. One group was composed of *M. caligata* specimens from Washington, 4 other *M. caligata* specimens, *M. olympus*, and *M. vancouverensis*. The other group included all remaining *M. caligata* specimens. Using the ΔK method on a subsequent STRUCTURE analysis of the group containing the 3 marmot species found $K = 3$ as the most probable number of groups, with each species forming a unique cluster. Additional analysis of the group consisting of only *M. caligata* found the mean likelihood was greatest for $K = 1$, suggesting no additional structure.

The species tree inferred from the phased nuclear loci in *BEAST did not recover a sister relationship between *M. vancouverensis* and the coastal *M. caligata* clade as observed in the mtDNA analysis. Instead, *M. caligata* formed a well-supported monophyletic clade (Fig. 5). The phylogenetic relationships between *M. caligata*, *M. olympus*, and *M. vancouverensis* were not well resolved in the *BEAST species-tree analyses.

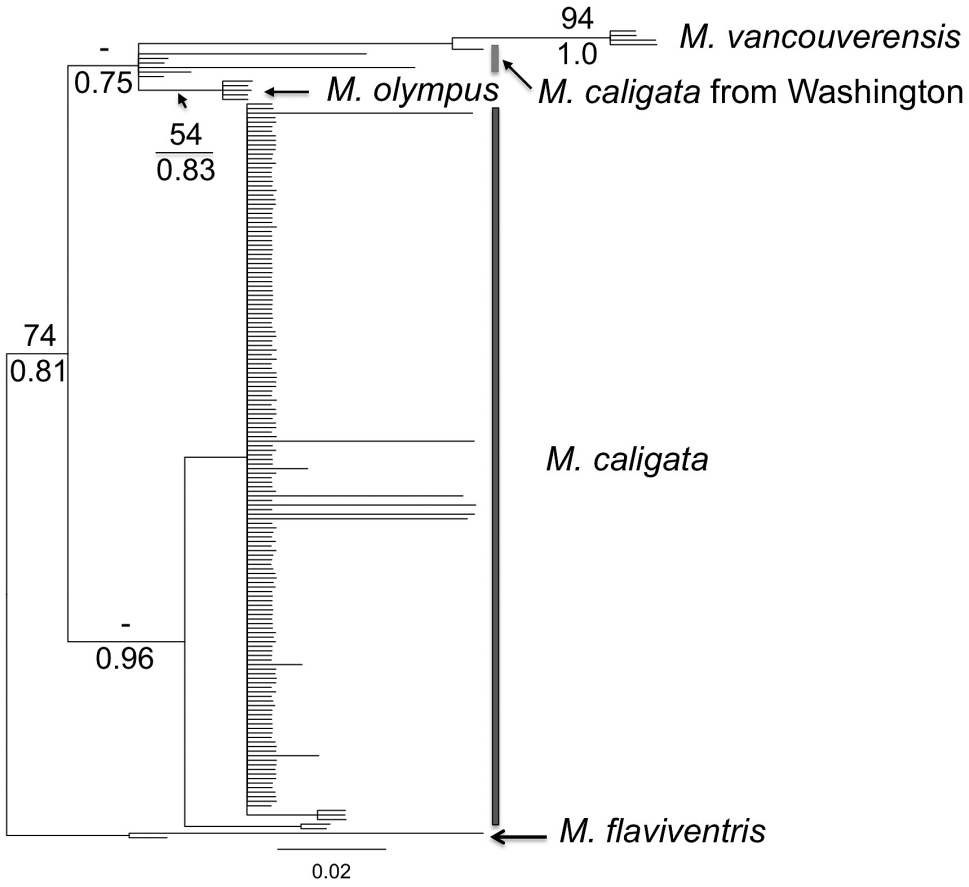

Fig. 3.—Bayesian phylogram of the partitioned BGN, CAT, and concatenated Cul4A and Lamp1 loci for *Marmota caligata*, *M. vancouverensis*, and *M. olympus* rooted with *M. flaviventris*. In both the Bayesian and maximum likelihood (ML) analyses, the BGN, CAT, and concatenated Cul4A and Lamp1 loci were analyzed as separate data partitions. A ML analysis did not recover the sister relationship between *M. caligata* from Washington, *M. olympus*, *M. vancouverensis*, and the remaining *M. caligata* specimens, denoted with dash. Numbers above the line are the results of a 1,000-replicate bootstrap analysis and numbers below the line are Bayesian posterior probabilities.

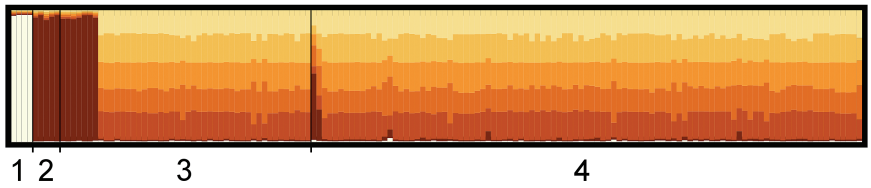

Fig. 4.—Results of a clustering analysis of haplotypes for 4 nuclear loci in *Marmota vancouverensis* (1), *M. olympus* (2), and *M. caligata* coastal (3) and continental (4) mitochondrial DNA (mtDNA) haplotype clades. Each vertical bar represents an individual and color represents relative membership in 1 of the 7 populations discussed in the text. *M. vancouverensis* is very homogenous (lightest bars), *M. olympus* and *M. caligata* specimens from Washington state share membership in a common group (darkest bars), and all remaining *M. caligata* specimens belong in part to one of the 5 remaining groups (intermediate bars). *M. caligata* populations do not correspond to the 2 mtDNA clades.

In the ultrametric species tree, the 2 *M. caligata* specimens from the continental clade were sister to the clade composed of *M. caligata* specimens from the coastal mtDNA clade and *M. vancouverensis*. For the *M. caligata* and *M. caligata* + *M. vancouverensis* mtDNA clades, the inferred divergence time and 95% highest posterior density intervals (HPD) were 1.22 mya (HPD: 0.76–1.84 mya). The coastal *M. caligata* and *M. vancouverensis* mtDNA clades diverged 0.73 mya (HPD: 0.42–1.15). *M. olympus* diverged from *M. caligata* and *M. vancouverensis* 2.58 mya (HPD: 1.76–3.59). Relative to Steppan et al. (2011), all phylogenetic relationships were concordant with negligible differences between divergence times and HPDs. The rate of molecular evolution has been shown to be time dependent for recent divergence times (Ho 2005; Ho et al. 2011) and we currently lack a reliable calibration to estimate the rate curve of this time dependency. Given this and the calibration points used, we acknowledge that actual divergence events in *M. caligata* and *M. caligata* + *M. vancouverensis* and the coastal *M. caligata* clade and *M. vancouverensis* are likely even more recent than our estimates suggest.

We did not use divergence time (t) estimates from our IM analyses. Estimates of t were unimodal, but the upper tail did not converge at 0 before reaching the user-defined upper limit of ~9 mya. Independent IM runs of identical data did not differ

with respect to the ranking of models in the L-mode analysis. A model of unidirectional forward migration from *M. caligata* to *M. vancouverensis* was the best supported by the L-mode analysis of IMa2 (Table 1; Supporting Information S3). For *M. caligata* and *M. olympus*, a model of bidirectional migration with a single rate was the best supported, although support for this model was similar to support for a model with no migration and a model of bidirectional migration with 2 rates (Table 1; Supporting Information S3).

Discussion

Hoary marmot.—The expansive distribution of *M. caligata* in the PNW makes it well suited to investigate Pleistocene vicariance and the 2-clade pattern observed in several species in the region. The *M. caligata* and *M. caligata* + *M. vancouverensis* mtDNA clades appear to have diverged during the mid-Pleistocene at the latest, in the northern Rocky and the Coast/Cascade Mountains, respectively. This general pattern of unique assemblages in the Coast/Cascade (coastal clade) and/or the northern Rocky Mountains (continental clade) has been observed in other PNW-distributed taxa and attributed to Pleistocene isolation in these species (Shafer et al. 2010). The regions of proposed Pleistocene refugia in the Coast/Cascade and the northern Rocky Mountains each currently contain a unique *M. caligata* mtDNA clade. These 2 haplotype clades are sympatric where mountains link the Coast/Cascade and the northern Rocky Mountains near Dease Lake, British Columbia, further supporting Pleistocene isolation in 2 refugia south of the Cordilleran and Laurentide ice sheets and a northward expansion following glacial retreat (Fig. 1). The 2 mtDNA clades are syntopic near Valdez, Alaska, where representatives of both have been collected from the same social group. Previous studies (Steppan et al. 1999, 2011) did not recover the 2 *M. caligata* mtDNA clades because they only included specimens from the coastal mtDNA clade. Additionally, the collection locality of specimen AF 2384 (UAM 22914, GenBank AF143920) used in these studies was misreported as "USA, Alaska, vic. Fairbanks"; we have determined that this specimen is actually from Juneau, in coastal Southeast Alaska, and has a cytochrome *b* sequence identical to another specimen from this area.

The coastal and continental haplotype clades recovered in the mtDNA analysis were not recovered in the analysis of our nuclear data. The STRUCTURE analysis of the nuclear loci

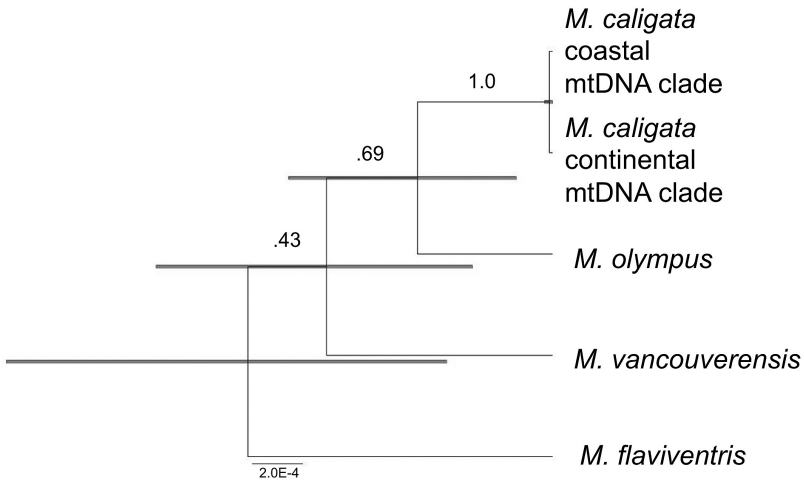

Fig. 5.—Species tree of marmots in the subgenus *Petromarmota* inferred from 4 nuclear loci using the major mitochondrial DNA clades as "species." Numbers above the lines are the Bayesian posterior probabilities. Gray lines represent 95% highest probability density of node age in relative time.

Table 1.—Results of 2 pairwise IMa2 L-mode analyses with ranked nested models of migration for 3 species of *Marmota*. Each pairwise comparison is based on 10^5 saved genealogies. Values in brackets were fixed as per the assumptions of the model. All migration is in the forward direction. Values presented are: K = number of model parameters, Δ_i = difference in AIC from best model, ω_i = Akaike weights, and $E_{min/i}$ = evidence ratio. AIC = Akaike Information Criterion.

Species compared	Model	Migration from hoary marmot	Migration to hoary marmot	Log(P)	K	AIC	Δi	ωi	$E_{min/}$i
Hoary and Vancouver Island marmots	Migration unidirectional	0.4611	(0.000)	0.2542	4	7.492	0.000	0.555	1.000
	Migration bidirectional (2 rates)	0.4612	0.000	0.2542	5	9.492	2.000	0.204	2.718
	No migration	(0.000)	(0.000)	−2.243	3	10.486	2.994	0.124	4.469
	Migration bidirectional (1 rate)	0.0389	(0.039)	−1.694	4	11.388	3.896	0.079	7.016
	Migration unidirectional	(0.000)	0.000	−2.424	4	12.848	5.356	0.038	14.559
Hoary and Olympic marmots	Migration bidirectional (1 rate)	0.213	(0.213)	−3.523	4	15.046	0.000	0.256	1.000
	No migration	(0.000)	(0.000)	−4.564	3	15.128	0.082	0.246	1.042
	Migration bidirectional (2 rates)	0.740	0.115	−2.625	5	15.250	0.204	0.231	1.107
	Migration, unidirectional	1.4171	(0.000)	−3.897	4	15.794	0.748	0.176	1.454
	Migration, unidirectional	(0.000)	0.000	−4.564	4	17.128	2.082	0.090	2.832

recovered several admixed *M. caligata* clusters, none of which corresponded to the coastal and/or continental mtDNA clades (Fig. 4). Additionally, both the ML and Bayesian analysis of the nuclear data did not recover multiple *M. caligata* clades. There are several possible explanations for the lack of concordance among nuclear and mitochondrial loci. Given the strong association of the mtDNA clades with regions that served as Pleistocene refugia for other taxa, the most likely of these explanations is incomplete lineage sorting of the nuclear markers (see below). However, failure to infer the species tree from the signal in the nuclear data as well as a misleading mtDNA signal resulting from sex-biased dispersal could also explain the lack of concordance (Funk and Omland 2003).

The 4-fold larger effective population size of nuclear loci and the stochasticity of mtDNA coalescence can require a much longer period of isolation for nuclear loci to reflect monophyly observed in mtDNA (Hudson and Turelli 2003). Since the 2 *M. caligata* mtDNA clades are likely the result of vicariance in the mid-Pleistocene at the latest, it seems similarly likely there was insufficient time to allow the sorting of nuclear loci to reflect this isolation. Both *M. olympus* and *M. vancouverensis* are believed to have arisen during the Pleistocene (Steppan et al. 2011) and are morphologically distinct (Cardini et al. 2009). However, despite the predominant use of morphology to describe as many as 9 subspecies of *M. caligata* (reviewed in Braun et al. 2011), no morphological features congruent with the 2 mtDNA clades have been identified, further suggesting the 2 mtDNA clades are the result of recent isolation.

As in previous studies of North American and European marmots (Rassmann et al. 1994; Steppan et al. 2011), we found limited variation at nuclear loci. As a result, we cannot rule out failure to detect the species tree from the signal in the nuclear data. Unlike previous studies, we targeted introns with the expectation that they would provide more phylogenetic signal. Among the ingroup taxa, the nuclear loci we analyzed had 32 variable nucleotide positions; the only other study to include nuclear sequence data used a single nuclear exon variable at only 2 positions with respect to ingroup taxa (Steppan et al. 2011). Additional studies incorporating more (and more variable) loci are needed to assess the nuclear signal in this species complex.

Male-biased dispersal could have resulted in nuclear gene flow with limited to no mitochondrial gene flow. Sex-biased dispersal favoring males has been documented in *M. flaviventris* (Downhower and Armitage 1981). However, there are no empirical data to suggest that males are better dispersers (i.e., can cross barriers females cannot), only that males likely disperse more often (Kyle et al. 2007). It is unlikely that reduced female dispersal could lead to sufficient isolation necessary to produce the 2 mtDNA clades given 1) the limited amount of gene flow needed to prevent genetic divergence (Wright 1931) and 2) the apparent dispersal ability of *M. caligata* as evidenced by their expansive range, much of which has only become available after the last glacial maximum (LGM).

Vancouver Island marmot.—M. vancouverensis was recovered as the sister lineage to the coastal mtDNA clade of *M. caligata* in analyses of the 2 mitochondrial loci (Fig. 2). Previous mtDNA-based research also recovered a sister relationship and limited sequence divergence between *M. vancouverensis* and *M. caligata*, leading to the suggestion that *M. vancouverensis* may be a recently diverged member (or "allospecies" sensu Steppan et al. 1999) of the *M. caligata* superspecies. However, the nuclear loci used in this study do not support this (Figs. 3–5).

Several lines of evidence suggest that *M. vancouverensis* is a distinct lineage based on nuclear loci. The Bayesian clustering analysis implemented in STRUCTURE recovered *M. vancouverensis* as a unique cluster that did not group with members of the coastal *M. caligata* mtDNA clade. Also, the *BEAST species-tree analysis did not recover a sister relationship between *M. vancouverensis* and the coastal *M. caligata* mtDNA clade (Fig. 5). Both the ML and Bayesian analysis of nuclear loci failed to recover a well-supported *M. olympus* clade (a well-accepted species with a unique chromosomal formula) while recovering *M. vancouverensis* as a well-supported monophyletic assemblage. These findings are congruent with previous geometric morphometric analyses of the cranium and mandible, which found *M. vancouverensis* to be the most morphologically distinct member of the subgenus *Petromarmota* (Cardini et al. 2003; Cardini and O'Higgins 2004; Cardini et al. 2007, 2009).

Forward migration of *M. caligata* to *M. vancouverensis* was the best-supported model of our IM analysis (Table 1; Supporting Information S3). This is consistent with the persistence of *M. vancouverensis* in a refugium on or near Vancouver Island (giving rise to the Vancouver Island marmot's distinctive morphology and unique nuclear alleles) and subsequent introgression of *M. caligata* mtDNA into *M. vancouverensis*. If introgression is responsible for the discordance between the mtDNA and nuclear loci then the mtDNA divergence represents the timing of that introgression event, (~ 0.73 mya at the latest). Marmot fossils from coastal localities that predate the LGM are known from both Prince of Wales Island in Southeast Alaska and Vancouver Island (Heaton and Grady 2003; Ward et al. 2003). Further analysis of these fossils including ancient DNA analysis may provide insight into the rate of time-dependent molecular evolution in *Petromarmota*, the possible existence of a more expansive coastal Pleistocene refugium, and the origin of *M. vancouverensis*.

Recent evidence suggests that codistributed tree squirrels in the genus *Tamiasciurus* likely persisted in a glacial refugium on Vancouver Island (Chavez et al. 2014). *T. douglasii* and *T. hudsonicus* are parapatric and known to hybridize in northern Washington and southern British Columbia (Chavez et al. 2011). The nuclear and mtDNA of *Tamiasciurus* on Vancouver Island are most closely related to *T. douglasii* and *T. hudsonicus*, respectively, suggesting introgression and subsequent divergence (~ 40 kya) in this insular population as well (Chavez et al. 2014).

Introgression and subsequent fixation of *M. caligata* mtDNA in the small *M. vancouverensis* population could explain the nestedness of the latter within the former in phylogenetic analyses of mtDNA, the unique nuclear haplotypes of

M. vancouverensis found in this study, and the morphological distinctiveness found in previous studies (Cardini et al. 2009; Nagorsen and Cardini 2009). However, our analyses did not include samples from the region of British Columbia immediately adjacent to Vancouver Island.

Rapid change as a result of a small founding population has been suggested as an explanation of the morphological distinctiveness observed in *M. vancouverensis* (Nagorsen and Cardini 2009). If a small founding population was responsible for the observed molecular and morphological patterns, we might expect to find a similar pattern in the nearby and closely related *M. olympus*. However, in *M. olympus*, we see the inverse pattern: less morphological distinctiveness (Cardini et al. 2009), greater mtDNA sequence divergence (Steppan et al. 1999), a unique karyotype (Hoffmann and Nadler 1968), and nuclear haplotypes shared with *M. caligata* populations from Washington (Fig. 4). *M. vancouverensis* appears more distinct than *M. olympus*, a well-accepted species, suggesting that *M. vancouverensis* likely evolved in isolation and recently experienced introgression leading to complete mitochondrial capture (Good et al. 2008) of *M. caligata* mtDNA.

Olympic marmot.—At the species level, our mtDNA results are in agreement with the findings of Steppan et al. (1999, 2011) and congruent with their suggestion that the *M. olympus* sequence of Kruckenhauser et al. (1999) was the result of contamination. In contrast, all *M. caligata* specimens from Washington (n = 7) shared at least 1 nuclear allele with *M. olympus*, despite their mtDNA divergence and different chromosomal formulas, suggesting incomplete lineage sorting and/or recent gene flow. The prospect of gene flow between *M. olympus* and *M. caligata* is perplexing as they have been shown to have 40 and 42 chromosomes, respectively (Rausch and Rausch 1965; Hoffmann and Nadler 1968; Rausch and Rausch 1971).

Hybridization before chromosomal differences became fixed and/or incomplete lineage sorting are the most plausible explanations for the haplotypes shared between *M. caligata* and *M. olympus*. Haplotypes are shared between *M. olympus* and all *M. caligata* specimens from the proposed Pleistocene refugium in the Coast/Cascade Mountains. The geographic proximity of the shared haplotypes suggests they resulted from introgression rather than lineage sorting. Results of the IM analysis with respect to migration between *M. olympus* and *M. caligata* were inconclusive, failing to rule out gene flow as an explanation of the shared nuclear haplotypes. The estimated mtDNA divergence of *M. olympus* and *M. caligata* is 2.6 mya (Steppan et al. 2011) and likely reflects the true divergence time of the species. The Pleistocene distribution of *M. olympus* is not well understood, but it has been proposed that *M. olympus* was formerly distributed over a larger region of the PNW than is currently occupied (Steppan et al. 2011). If true, gene flow from a relictual (and now extirpated or assimilated) population of *M. olympus* from the Cascades to *M. caligata* could also explain the shared haplotypes and why they have so far only been recovered in Washington.

Biogeography.—The Pleistocene range of *M. caligata* is poorly known, limiting inference into the mid-Pleistocene vicariance that presumably led to the *M. caligata* and *M. caligata* + *M. vancouverensis* mtDNA clades. The earliest known fossils of *M. caligata* have been radiocarbon dated to ~ 35 kya during the Wisconsin Glaciation and are from the Rocky Mountains in southern Alberta and coastal Southeast Alaska (Heaton and Grady 2003; Harington 2011). These fossils suggest that *M. caligata* survived the Pleistocene south (and potentially west) of the Cordilleran and Laurentide ice sheets. Additionally, 3 of the 4 *M. caligata* specimens from Montana form a mitochondrial haplotype clade sister to all other members of the *M. caligata* continental clade (Fig. 2). The early divergence of specimens from Montana and lack of any similar phylogenetic structure for specimens from interior Alaska (where a northern refugium would have been) further suggests that the *M. caligata* continental clade persisted in a southern refugium.

We recovered no additional phylogenetic structure in the coastal *M. caligata* mtDNA clade. This lack of structure may be the result of incomplete sampling and/or repeated colonization and extirpation throughout the glacial cycles of the Pleistocene (Hewitt 1996). Fossil evidence from Southeast Alaska suggests a potential coastal refugium for *M. caligata*. We cannot rule out a coastal refugium, but given the evidence of gene flow between *M. caligata* and both *M. olympus* and *M. vancouverensis* as well as the current distribution of these species, it appears likely *M. caligata* occupied the Coast/Cascade Mountains during Pleistocene.

Marmot fossils that predate the LGM (potentially *M. vancouverensis*) and *M. vancouverensis* fossils from the Holocene have been recovered on Vancouver Island (Nagorsen et al. 1996; Ward et al. 2003). The earliest-known marmot fossils from Vancouver Island are from Port Eliza cave (Ward et al. 2003; Al-Suwaidi et al. 2006), ~ 55 km southeast of the Brooks Peninsula, a proposed Pleistocene refugium on Vancouver Island (Ogilvie 1997). To date, there is no evidence of the Brooks Peninsula serving as a Pleistocene refugium for mammals. However, it does share several plant species associated with Haida Gwaii (Queen Charlotte Islands) and the Alexander Archipelago (Ogilvie 1997), part of an area believed to have served as a cryptic coastal refugium in the Pleistocene (reviewed by Shafer et al. 2010).

Molecular evidence suggests *M. vancouverensis* diverged from *M. caligata* before the LGM, suggesting the pre-LGM marmot fossils from Vancouver Island are likely those of *M. vancouverensis*. If not, then marmots colonized Vancouver Island multiple times, potentially from a coastal refugium. If marmots colonized Vancouver Island post-LGM it was likely ~ 12 kya, when fossil evidence suggests a reduction (or absence) of the marine barrier between Vancouver Island and the mainlined (Nagorsen and Keddie 2000; Wilson et al. 2009). Additional research is needed to determine if *M. vancouverensis* survived the Pleistocene on Vancouver Island.

To date, no Pleistocene-era marmot fossils have been found in the Cascade or Olympic Mountains and the location of the

Pleistocene refugium presumably occupied by *M. olympus* is enigmatic. The 2 most likely (and not mutually exclusive) refugial areas are nunataks that existed on the partially glaciated Olympic Peninsula and/or the nearby Cascade Mountains (Steppan et al. 2011). Currently, the closest population of hoary marmots to *M. olympus* is ~ 155 km away in the Cascade Mountains. Based on mtDNA, *M. olympus* appears to have diverged from *M. caligata* and *M. vancouverensis* in the early Pleistocene (Steppan et al. 2011; this study). However, given the ambiguity regarding the origin of the nuclear haplotypes shared between *M. olympus* and *M. caligata*, the reliability of the mtDNA divergence time is in question. Further investigations into the origin and distribution of the nuclear haplotypes shared between *M. olympus* and *M. caligata* are needed to clarify the Pleistocene range of these 2 species.

Our findings highlight the importance of rigorous phylogenetic analysis in conservation and the need for further research. We found that *M. caligata* likely experienced isolation in the Coast/Cascade and northern Rocky mountains during the Pleistocene and this isolation gave rise to 2 *M. caligata* mtDNA clades. We were unable to detect a signal of this Pleistocene isolation in the nuclear data, likely the result of incomplete lineage sorting. *M. vancouverensis* is a genetically (and morphologically) distinct species that appears to have recently "captured" the mitochondrial genome of *M. caligata*. We were unable to confidently resolve phylogenetic relationships among *M. caligata*, *M. olympus*, and *M. vancouverensis*. Our mtDNA results were consistent with those of Steppan et al. (1999, 2011) and recovered *M. olympus* as basal to both *M. caligata* and *M. vancouverensis*. In the mtDNA analyses, *M. caligata* was paraphyletic with respect to *M. vancouverensis*. Species-tree analysis of the nuclear loci supported a monophyletic *M. caligata*, but did not confidently resolve the phylogenetic placement of *M. olympus* and *M. vancouverensis*, and warrants further investigation.

Additional *M. caligata* specimens from mainland British Columbia near Vancouver Island are critical to determining if the unique nuclear haplotypes found in *M. vancouverensis* are restricted to Vancouver Island and where the most genetically similar populations of *M. caligata* are located should genetic rescue of *M. vancouverensis* become necessary. Similarly, additional sampling of *M. caligata* from Washington and British Columbia is needed to determine the genetic variation shared between *M. caligata* and *M. olympus*. Determining the spatial and genomic extent of this shared variation may be useful for genetic rescue (if viable hybridization is possible) and to guide management decisions that maximize the preservation of genetic diversity. Given the endangered status of *M. vancouverensis* and the decline in *M. olympus* numbers, further research including additional specimens and markers is paramount to preserving marmot biodiversity in the PNW.

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Supporting Information

The Supporting Information documents are linked to this manuscript and are available at Journal of Mammalogy online (jmammal.oxfordjournals.org). The materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supporting data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Supporting Information S1.—Primers developed for this study to amplify the cytochrome *b* gene, part of the mitochondrial control region, and two nuclear introns in North American marmots.

Supporting Information S2.—Nexus files of aligned sequence data used in this study.

Supporting Information S3.—Posterior distributions for the migration rate (*M*) and migration scaled to reflect the number of effective immigrants (2 *Nm*) per generation for *Marmota caligata*, *M. olympus*, and *M. vancouverensis* using the methods of (Peters et al. 2012). Migration and immigration in the figures is presented in the forward direction.

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Appendix I

Species, collection localities, source museums, and catalog numbers of *Marmota* specimens used in this study. Museum abbreviations: MSB = Museum of Southwestern Biology, Albuquerque, New Mexico; ROM = Royal Ontario Museum, Toronto, Ontario; UAM = University of Alaska Museum, Fairbanks, Alaska; UWBM = University of Washington Burke Museum, Seattle, Washington; YPM = Yale Peabody Museum of Natural History, New Haven, Connecticut. n/a = not available.

Species	Country	State or province	Museum	Catalog number	Latitude	Longitude
M. caligata	Canada	British Columbia	UAM	33803	58.1881	−129.8881
M. caligata	Canada	British Columbia	UAM	35130	58.1881	−129.8881
M. caligata	Canada	British Columbia	UAM	49848	56.1700	−130.0500
M. caligata	Canada	British Columbia	UAM	112310	59.7200	−133.3804
M. caligata	Canada	British Columbia	UAM	112316	58.1895	−129.8937
M. caligata	Canada	British Columbia	UAM	112366	59.7200	−133.3805
M. caligata	Canada	Northwest Territories	MSB	265467	62.4500	−129.2000
M. caligata	Canada	Northwest Territories	MSB	267586	62.4500	−129.2000
M. caligata	United States	Alaska	UAM	22914	58.2500	−134.5167
M. caligata	United States	Alaska	UAM	24122	58.2500	−134.5167
M. caligata	United States	Alaska	UAM	30932	57.0833	−132.7333
M. caligata	United States	Alaska	UAM	31724	61.2167	−149.5833
M. caligata	United States	Alaska	UAM	32649	58.2839	−134.5203
M. caligata	United States	Alaska	UAM	35129	56.0339	−130.0433
M. caligata	United States	Alaska	UAM	38302	58.5506	−135.4792
M. caligata	United States	Alaska	UAM	38303	58.5506	−135.4792
M. caligata	United States	Alaska	UAM	38304	58.5506	−135.4792
M. caligata	United States	Alaska	UAM	48486	58.3042	−134.4083
M. caligata	United States	Alaska	UAM	53836	65.3928	−145.9994
M. caligata	United States	Alaska	UAM	57693	61.0585	−143.3634
M. caligata	United States	Alaska	UAM	58238	64.8110	−143.7790
M. caligata	United States	Alaska	UAM	58239	64.8110	−143.7790
M. caligata	United States	Alaska	UAM	58240	64.8110	−143.7790
M. caligata	United States	Alaska	UAM	58241	64.8110	−143.7790
M. caligata	United States	Alaska	UAM	65635	63.6667	−142.2167
M. caligata	United States	Alaska	UAM	78239	59.6374	−136.1291
M. caligata	United States	Alaska	UAM	78240	59.6374	−136.1291
M. caligata	United States	Alaska	UAM	85858	65.2947	−149.9973
M. caligata	United States	Alaska	UAM	85859	65.2596	−150.0502
M. caligata	United States	Alaska	UAM	86413	60.7709	−148.7506
M. caligata	United States	Alaska	UAM	86414	60.2753	−150.1504
M. caligata	United States	Alaska	UAM	94705	58.7667	−154.9667
M. caligata	United States	Alaska	UAM	98299	60.7819	−149.5456
M. caligata	United States	Alaska	UAM	101845	60.7709	−148.7506
M. caligata	United States	Alaska	UAM	101919	60.2849	−150.1584
M. caligata	United States	Alaska	UAM	102367	61.6124	−142.0313
M. caligata	United States	Alaska	UAM	102368	61.6134	−142.0388
M. caligata	United States	Alaska	UAM	102374	61.6125	−142.0394
M. caligata	United States	Alaska	UAM	102436	60.9763	−143.1291
M. caligata	United States	Alaska	UAM	102474	63.3958	−145.6603
M. caligata	United States	Alaska	UAM	102476	63.3958	−145.6610
M. caligata	United States	Alaska	UAM	103458	63.1285	−146.2803
M. caligata	United States	Alaska	UAM	103473	58.5344	−134.8308
M. caligata	United States	Alaska	UAM	103474	58.2596	−134.6393
M. caligata	United States	Alaska	UAM	113885	60.2006	−148.4004
M. caligata	United States	Alaska	UAM	103476	60.3559	−146.1937
M. caligata	United States	Alaska	UAM	103477	58.2596	−134.6393
M. caligata	United States	Alaska	UAM	103489	58.8975	−152.2094
M. caligata	United States	Alaska	UAM	103490	58.8975	−152.2094
M. caligata	United States	Alaska	UAM	103491	58.8975	−152.2094
M. caligata	United States	Alaska	UAM	106200	65.4938	−145.3841
M. caligata	United States	Alaska	UAM	106220	65.2084	−148.0575
M. caligata	United States	Alaska	UAM	106211	65.2111	−148.0603
M. caligata	United States	Alaska	UAM	107658	60.5514	−145.3621
M. caligata	United States	Alaska	UAM	111555	65.2116	−148.0608
M. caligata	United States	Alaska	UAM	111557	65.2206	−148.0507
M. caligata	United States	Alaska	UAM	111561	65.2111	−148.0604

Appendix I *Continued*

Species	Country	State or province	Museum	Catalog number	Latitude	Longitude
M. caligata	United States	Alaska	UAM	111565	65.4854	−145.4000
M. caligata	United States	Alaska	UAM	111626	65.2195	−148.0545
M. caligata	United States	Alaska	UAM	111634	65.2111	−148.0604
M. caligata	United States	Alaska	UAM	111786	58.8799	−152.2055
M. caligata	United States	Alaska	UAM	112286	58.8969	−152.2115
M. caligata	United States	Alaska	UAM	112287	58.8969	−152.2115
M. caligata	United States	Alaska	UAM	112288	58.8969	−152.2115
M. caligata	United States	Alaska	UAM	112289	58.8969	−152.2115
M. caligata	United States	Alaska	UAM	112290	58.8969	−152.2115
M. caligata	United States	Alaska	UAM	112291	58.8969	−152.2115
M. caligata	United States	Alaska	UAM	112292	58.8969	−152.2115
M. caligata	United States	Alaska	UAM	112293	58.8969	−152.2115
M. caligata	United States	Alaska	UAM	112294	58.8969	−152.2115
M. caligata	United States	Alaska	UAM	112295	58.8969	−152.2115
M. caligata	United States	Alaska	UAM	112296	58.8969	−152.2115
M. caligata	United States	Alaska	UAM	112297	58.8969	−152.2115
M. caligata	United States	Alaska	UAM	112298	58.8969	−152.2115
M. caligata	United States	Alaska	UAM	112299	58.8969	−152.2115
M. caligata	United States	Alaska	UAM	112300	58.8969	−152.2115
M. caligata	United States	Alaska	UAM	112301	58.8969	−152.2115
M. caligata	United States	Alaska	UAM	112302	58.8969	−152.2115
M. caligata	United States	Alaska	UAM	112303	58.8969	−152.2115
M. caligata	United States	Alaska	UAM	112304	58.8969	−152.2115
M. caligata	United States	Alaska	UAM	112305	58.8969	−152.2115
M. caligata	United States	Alaska	UAM	112306	58.8969	−152.2115
M. caligata	United States	Alaska	UAM	112324	59.5097	−151.4527
M. caligata	United States	Alaska	UAM	112325	61.1540	−146.5978
M. caligata	United States	Alaska	UAM	112326	61.1342	−145.7744
M. caligata	United States	Alaska	UAM	112338	58.6245	−134.9362
M. caligata	United States	Alaska	UAM	112342	59.5099	−151.4512
M. caligata	United States	Alaska	UAM	112351	58.6245	−134.9362
M. caligata	United States	Alaska	UAM	112353	65.3902	−146.5982
M. caligata	United States	Alaska	UAM	112354	59.5099	−151.4512
M. caligata	United States	Alaska	UAM	112359	63.0841	−146.3847
M. caligata	United States	Alaska	UAM	112360	60.3461	−146.2685
M. caligata	United States	Alaska	UAM	112364	60.3448	−146.3126
M. caligata	United States	Alaska	UAM	112367	65.1492	−147.0182
M. caligata	United States	Alaska	UAM	112368	65.1492	−147.0182
M. caligata	United States	Alaska	UAM	112369	65.1492	−147.0182
M. caligata	United States	Alaska	UAM	112457	58.4228	−134.4431
M. caligata	United States	Alaska	UAM	112458	58.4228	−134.4431
M. caligata	United States	Alaska	UAM	112579	59.4278	−151.1522
M. caligata	United States	Alaska	UAM	112580	59.3669	−151.6978
M. caligata	United States	Alaska	UAM	112581	59.4356	−151.1800
M. caligata	United States	Alaska	UAM	112582	59.7913	−150.5125
M. caligata	United States	Alaska	UAM	112583	59.6410	−151.0583
M. caligata	United States	Alaska	UAM	112585	59.4299	−151.1579
M. caligata	United States	Alaska	UAM	112587	65.1492	−147.0182
M. caligata	United States	Alaska	UAM	113733	59.6473	−151.0580
M. caligata	United States	Alaska	UAM	113734	59.6411	−151.0640
M. caligata	United States	Alaska	UAM	113735	59.6410	−151.0583
M. caligata	United States	Alaska	UAM	113736	59.4292	−151.1555
M. caligata	United States	Alaska	UAM	113737	59.4343	−151.1583
M. caligata	United States	Alaska	UAM	113738	59.4338	−151.1636
M. caligata	United States	Alaska	UAM	113739	59.4335	−151.1633
M. caligata	United States	Alaska	UAM	113878	61.1998	−147.4813
M. caligata	United States	Alaska	UAM	113886	60.9262	−146.2006
M. caligata	United States	Alaska	UAM	113889	63.4980	−145.8129
M. caligata	United States	Alaska	UAM	113892	61.7599	−149.3060
M. caligata	United States	Alaska	UAM	113901	61.7606	−149.3110
M. caligata	United States	Alaska	UAM	113902	61.7631	−149.3035
M. caligata	United States	Alaska	UAM	113903	63.5000	−145.8057
M. caligata	United States	Alaska	UAM	113904	61.2010	−147.4751
M. caligata	United States	Alaska	UAM	113905	60.9195	−146.2027

Appendix I *Continued*

Species	Country	State or province	Museum	Catalog number	Latitude	Longitude
M. caligata	United States	Alaska	UAM	113906	61.2002	−147.4827
M. caligata	United States	Alaska	UAM	113907	65.3675	−146.9370
M. caligata	United States	Alaska	UAM	113925	65.3674	−146.9384
M. caligata	United States	Alaska	UAM	113930	65.3665	−146.9374
M. caligata	United States	Alaska	UAM	113950	60.9262	−146.2006
M. caligata	United States	Alaska	UAM	113951	61.0548	−147.1226
M. caligata	United States	Alaska	UAM	114143	61.1413	−145.7593
M. caligata	United States	Alaska	UAM	114146	65.4917	−145.3895
M. caligata	United States	Alaska	UAM	114296	61.2018	−147.4709
M. caligata	United States	Alaska	UAM	114298	63.7833	−145.7918
M. caligata	United States	Alaska	UAM	114323	61.2002	−147.4827
M. caligata	United States	Alaska	UAM	114365	60.9278	−146.2128
M. caligata	United States	Alaska	UAM	115699	57.5538	−155.9849
M. caligata	United States	Alaska	UAM	115715	61.1418	−145.7616
M. caligata	United States	Alaska	UAM	115716	61.0548	−147.1226
M. caligata	United States	Alaska	UAM	115718	63.7876	−145.7916
M. caligata	United States	Alaska	UAM	115723	61.1337	−145.7751
M. caligata	United States	Alaska	UAM	115724	61.2016	−147.4731
M. caligata	United States	Alaska	UAM	115797	61.1370	−145.7662
M. caligata	United States	Alaska	UAM	115798	61.1385	−145.7645
M. caligata	United States	Alaska	UAM	115799	61.1333	−145.7773
M. caligata	United States	Alaska	UAM	115800	61.1330	−145.7780
M. caligata	United States	Alaska	UAM	115801	61.1439	−145.7559
M. caligata	United States	Alaska	UAM	115802	61.2017	−147.4716
M. caligata	United States	Alaska	UAM	115803	63.7834	−145.7907
M. caligata	United States	Alaska	UAM	115809	59.4333	−151.1626
M. caligata	United States	Alaska	UAM	117977	64.7920	−141.7312
M. caligata	United States	Alaska	UAM	117978	64.7699	−141.7528
M. caligata	United States	Alaska	UAM	117979	64.7938	−141.7296
M. caligata	United States	Alaska	UAM	117980	64.7924	−141.7288
M. caligata	United States	Alaska	UAM	117981	64.7809	−141.7227
M. caligata	United States	Alaska	UAM	117982	64.7879	−141.7176
M. caligata	United States	Alaska	UAM	117983	64.7745	−141.7493
M. caligata	United States	Alaska	UAM	117984	64.7723	−141.7542
M. caligata	United States	Alaska	YPB	14820	63.0693	−145.7405
M. caligata	United States	Montana	UAM	112564	45.4223	−113.7225
M. caligata	United States	Montana	UAM	112566	48.5778	−114.4290
M. caligata	United States	Montana	UAM	112575	46.1562	−114.4761
M. caligata	United States	Montana	UAM	112576	48.5747	−114.4256
M. caligata	United States	Washington	UAM	112565	48.5140	−120.6873
M. caligata	United States	Washington	UAM	112570	48.5142	−120.6450
M. caligata	United States	Washington	UAM	112571	47.7331	−121.0717
M. caligata	United States	Washington	UAM	112573	48.5142	−120.6450
M. caligata	United States	Washington	UAM	112574	47.7310	−121.0695
M. caligata	United States	Washington	UAM	112577	47.7331	−121.0717
M. caligata	United States	Washington	UWBM	82114	46.1631	−121.5153
M. flaviventris	United States	Idaho	UAM	112562	45.3194	−114.5376
M. flaviventris	United States	Idaho	UAM	112567	45.3246	−114.4368
M. olympus	United States	Washington	UWBM	79553	n/a	n/a
M. olympus	United States	Washington	UWBM	79554	n/a	n/a
M. olympus	United States	Washington	UWBM	79849	n/a	n/a
M. olympus	United States	Washington	UWBM	80739	n/a	n/a
M. olympus	United States	Washington	UWBM	81033	n/a	n/a
M. vancouverensis	Canada	British Columbia	ROM	116794	n/a	n/a
M. vancouverensis	Canada	British Columbia	ROM	116795	n/a	n/a
M. vancouverensis	Canada	British Columbia	ROM	117714	n/a	n/a
M. vancouverensis	Canada	British Columbia	ROM	117716	n/a	n/a