PHYLOGEOGRAPHY AND MOLECULAR PHYLOGENETICS OF THE HOARY MARMOT (*MARMOTA CALIGATA*)

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Abstract

In this dissertation I documented the phylogeographic history of the Hoary Marmot (*Marmota caligata*) and its phylogenetic relationships with the Vancouver Island (*M. vancouverensis*) and Olympic (*M. olympus*) marmots. The Hoary Marmot is an iconic alpine mammal that is broadly distributed throughout the Pacific Northwest (PNW) from Washington and central Idaho in the south to Alaska in the north. Vancouver Island and Olympic marmots have much more restricted geographic distributions, occurring only on Vancouver Island (British Columbia, Canada) and the Olympic Peninsula (Washington, USA), respectively. In my first chapter I used mitochondrial DNA (mtDNA) sequence data to document the existence of 2 mtDNA clades in Hoary Marmots. I also used mtDNA and nuclear sequence data to infer historic gene flow from Hoary into Vancouver Island marmots, which resulted in the latter "capturing" the mitochondrial genome of the former. Analyses of nuclear sequence data also suggested the potential for historic gene flow between Hoary Marmots and Olympic Marmots in Washington.

In my second chapter I investigated the origins of Hoary Marmots on Sud Island, Alaska, part of the Maritime National Wildlife Refuge. This island population of marmots was purported to have been introduced by humans and detrimental to nesting seabirds. As a result, the United States Fish and Wildlife Service undertook efforts to eradicate the Hoary Marmot population on Sud Island between 2009-2011. I conducted a literature review of marmot introductions in Alaska and used molecular data to determine the geographic origin of marmots on Sud Island. Through my literature review I found no direct evidence that marmots were introduced to Sud Island or any documentation that they were detrimental to nesting seabirds on this island. Molecular analysis identified the Hoary Marmot population on Sud Island as a distinct genetic

cluster, with divergence time estimates similar to those of a naturally occurring island population, suggesting a natural colonization of Sud Island by Hoary Marmots.

In my third chapter I investigated potential refugia used by Hoary Marmots during the Last Glacial Maximum (LGM) and the potential effects of climate change on the future distribution of suitable habitat. To address these questions I used species distribution models (SDMs) based on all available museum specimens and population genetic summary statistics calculated from mtDNA sequence data. I found the most likely areas of LGM refugia were located south of the glacial margins of the Pleistocene and along the PNW coast. Habitat in the southernmost portion of the Hoary Marmot current geographic distribution was predicted to be the most negatively impacted by future climate change. Additionally, populations from this region were the most genetically diverse, indicating that these populations may be important for conservation of the species as a whole.

In my final chapter I used microsatellite and sequence (mtDNA and nuclear) data to revisit the findings of my first chapter and to test for gene flow between Hoary, Vancouver Island, and Olympic marmots, as well as between the 2 Hoary Marmot mtDNA clades. I also improved the known distribution of the Hoary Marmot mtDNA clades by determining clade membership of 98 museum specimens for which no fresh tissues exist. Analysis of the combined sequence and microsatellite data confirmed previous findings that introgression led to Vancouver Island Marmots capturing the mitochondrial genome of Hoary Marmots. The addition of microsatellite data did not resolve the origin of nuclear alleles shared between Hoary Marmots from Washington and Olympic Marmots. Regarding the 2 Hoary Marmot mtDNA clades, molecular results suggested unidirectional gene flow between the clades and that male-biased dispersal is likely occurring in the species. The additional mtDNA clade membership data from

the 98 museum specimens revealed that British Columbia is predominantly occupied by a single mtDNA clade.

Overall, my research has shown that populations in the southern portion of the Hoary Marmot's geographic distribution are likely to be the most important for conservation and that additional research in this region is needed. I also documented the existence of introgression between Hoary and Vancouver Island marmots, highlighting the importance of using multiple unlinked loci for phylogenetic and phylogeographic analysis. Lastly, my findings call attention to the importance of rigorously verifying primary sources of information before undertaking species eradications.

Table of Contents

List of Figures

Page

List of Tables

Page

List of Appendices

Page

General Introduction

A thorough knowledge of the geographic distribution of biota and genetic diversity is important to both conservation and management. The biogeographic and phylogeographic histories of species in the Pacific Northwest (PNW) of North America have been largely shaped by the glacial cycles during the Pleistocene (reviewed in Shafer et al. 2010a). Climatic oscillations during this epoch likely shifted the geographic distribution of species, resulting in periods of both isolation and contact between populations and species (Brunsfeld et al. 2001; Shafer et al. 2010a). As a result, species in the PNW may harbor genetic structure from historic isolation as well as introgression with currently allopatric species. Documenting and delimiting the phylogeographic structure of PNW species can provide insights into the locations of Pleistocene refugia and hotspots of genetic diversity (Hampe and Petit 2005). The Hoary Marmot (*Marmota caligata*) is broadly distributed throughout the PNW and well suited to make general inferences regarding the phylogeographic history of the region.

Hoary Marmots are house-cat-sized mammals that occur primarily in alpine habitats from southern Washington, central Idaho, and western Montana north and west into Alaska south of the Yukon River (Gunderson et al. 2009; Braun et al. 2011). This broad geographic distribution encompasses several proposed Pleistocene refugia in the PNW (Brunsfeld et al. 2001; Weksler et al. 2010; Shafer et al. 2010a). However, the only previous research that included a large number of Hoary Marmot specimens from throughout their geographic distribution was a taxonomic revision conducted over a century ago (Howell 1915). Subsequent molecular-based research has generally relied on a limited number of specimens that were not representative of the species'

full distribution and often focused on higher-level phylogenetic questions (Kruckenhauser et al. 1999; Steppan et al. 1999; 2011).

Pleistocene refugia in the PNW have been documented both north (i.e., eastern Beringia) and south of the glacial margins of the Cordilleran and Laurentide ice sheets, with post-Pleistocene colonization following a north-to-south or south-to-north pattern (Shafer et al. 2010a; Weksler et al. 2010). Coastal refugia west of the Cordilleran Ice Sheet have also been documented in the PNW (Cook et al. 2006; Shafer et al. 2010b; Chavez et al. 2014). Two predominantly allopatric mitochondrial (mtDNA) clades that diverged during the Pleistocene have been documented in the Hoary Marmot (Kerhoulas et al. 2015; Lanier et al. 2015). The current geographic distribution of the Hoary Marmot and the 2 mtDNA clades suggests any of the aforementioned PNW Pleistocene refugia may have been used by this species.

Three recent papers have addressed the location of the Pleistocene refugia used by Hoary Marmots and each came to a different conclusion: 1) 2 northern refugia (Lanier et al. 2015), 2) 2 southern refugia or a southern refugium and a coastal refugium (Kerhoulas et al. 2015), and 3) both a northern refugium and a coastal refugium (Knowles et al. 2016). The 2 studies to propose a northern refugium used a limited number of specimens that did not include samples from the southern portion of the species' range, which may have influenced their findings.

In addition to molecular-based methods, species-distribution models (SDMs) can be used to infer the biogeographic history of a species by providing predictions of where suitable habitat likely existed across spatial and temporal scales. For example, SDMs can be used to explore the potential distribution of a species during various important times such as glacial maxima (Waltari et al. 2007). Additionally, SDMs can be used to predict the future distribution of

potentially suitable habitat and provide insight as to where conservation efforts and/or increased monitoring may be warranted.

At a broader scale, the phylogenetic relationships among the Hoary, Olympic (*M. olympus*), and Vancouver Island (*M. vancouverensis*) marmots (believed to be each other's closest relatives) are not well resolved (Kerhoulas et al. 2015). The Vancouver Island Marmot is endemic to Vancouver Island, British Columbia, is classified as Critically Endangered by the International Union for the Conservation of Nature (IUCN)(Roach 2017), and is part of an ongoing intensive captive breeding and reintroduction program (Keeley et al. 2011). The Olympic Marmot only occurs on the Olympic Peninsula of western Washington and, despite having a small ($\leq$ 1000 individuals) and possibly declining population (Griffin et al. 2008), is currently classified as Least Concern by the IUCN (Cassola 2017). The molecular phylogenetic relationships among these 3 species has been presented in previous studies that relied predominantly on mtDNA loci (Kruckenhauser et al. 1999; Steppan et al. 1999; 2011). For example, only 1 previous study included nuclear data (a single locus) and these data alone did not resolve a single, well-supported phylogeny (Steppan et al. 2011).

Based primarily on mtDNA data it was hypothesized that Hoary Marmots may be paraphyletic with respect to Vancouver Island Marmots (Steppan et al. 2011). Although mtDNA markers have been widely used to infer phylogenies, they represent a single linkage group and can produce a misleading phylogenetic signal (Funk and Omland 2003). Furthermore, hybridization has been documented in Asian marmot species (Brandler et al. 2010), further emphasizing the need for including nuclear markers when inferring phylogenies. Given the substantial effort that has been put forth to recover the Vancouver Island Marmot and the potential for similar efforts becoming necessary to perpetuate the Olympic Marmot, constructing

a well-resolved phylogeny of these 3 species and documenting potential introgression is clearly warranted to make well-informed management decisions.

Despite being predominantly found in alpine habitats, Hoary Marmots have also been documented at or near sea level (Heller 1910; Braun et al. 2011). The propensity of Hoary Marmots to occur at low elevation coastal sites may have important implications regarding locations of Pleistocene refugia as well as colonization after the Last Glacial Maximum in the PNW. Additionally, the existence of sea level populations likely facilitated the colonization of several islands in Alaska by Hoary Marmots, where they currently exist and/or have been observed over the past century (Heller 1910; MacDonald and Cook 2009). However, the origin of small mammals on Alaskan islands is obfuscated by rampant and poorly documented introductions made for fur farming ca. 1750-1930 (Bailey 1993; Isto 2012). For example, to establish a prey base, fox farmers "…filled barrels with squirrels and other small mammals and released them on islands where there were none" (Peterson 1967:123). Not surprisingly, the introduction of mammals to islands in Alaska has disrupted native biota such as ground-nesting birds, prompting efforts to eradicate introduced species from islands (Ebbert and Byrd 2002).

A Hoary Marmot population on Sud Island, Alaska (part of the Alaska Maritime National Wildlife Refuge) was believed to have been introduced, and the United States Fish and Wildlife Service undertook efforts to eradicate it between 2009 and 2011 (US Department of Interior Fish and Wildlife Service 2010). Sud Island is a small (ca. 110 hectare) island in the Barren Archipelago, situated between the southern tip of the Kenai Peninsula and Kodiak Island. An exploratory molecular analysis suggested the Hoary Marmot population on Sud Island might have been the result of natural colonization, prompting further investigation. An extensive literature review of animal introductions in Alaska produced no substantiated accounts of Hoary

Marmots having been introduced to Sud Island, leaving additional molecular analyses as the only method available to determine the origin of this island population.

The major objectives of this dissertation were to: 1) describe the phylogeographic history of the Hoary Marmot using specimens from throughout the geographic range of the species, multiple molecular markers, and SDMs, 2) infer the phylogenetic relationships between Hoary, Olympic, and Vancouver Island marmots using mtDNA and nuclear loci, 3) document the occurrence and directionality of interspecific gene flow between the Hoary Marmot and both the Olympic and Vancouver Island marmots, and 4) determine the origin of Hoary Marmots occurring on Sud Island.

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Chapter 1 Complex history of isolation and gene flow in Hoary, Olympic, and endangered Vancouver Island marmots[1]

Abstract

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 178 Hoary Marmots as well as multiple specimens of Vancouver Island and Olympic marmots in order to investigate phylogenetic relationships and historic gene flow among members of 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

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[1] KERHOULAS, N. J., A. M. GUNDERSON AND L. E. OLSON. 2015. Journal of Mammalogy 96:810–826.

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.

Introduction

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 1992). 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. 1.1) 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 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 taxa to one another (Steppan et al. 2011).

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, USA (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, USA. Despite its restricted range, *M. olympus* is currently classified as Least Concern by the International Union for the 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 ($\leq$ 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

(COSEWIC, www.cosewic.gc.ca), and Endangered under the U.S. Endangered Species Act. Conservation efforts include ongoing captive breeding and reintroduction programs (Keeley et al. 2011). Mitochondrial DNA 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) 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 (1140 bp of cytochrome *b* and a 2029-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 Table 1.A-1.

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, CA) 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 (Table 1.A-2). A 571-bp-segment of the mitochondrial control region was amplified using primers CR-HLF1 and CR-HLR1 (Table 1.A-2). Two nuclear introns were amplified using the eponymous CAT (599-bp product) and BGN (715-bp product) 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 Table 1.A-2. Cul4A primers amplify 362 intronic nucleotides and Lamp1 primers amplify 10 exonic and 490 intronic nucleotides. Because Cul4A and Lamp1 are within <14kb 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 non-recombining block of sequence data and/or individuals that do not exhibit evidence of recombination (Woerner et al. 2007).

PCR reactions were cleaned using Exo-Sap (Affymetrix, OH) and Sanger sequencing reactions were carried out using ABI (Applied Biosystems, Foster City, CA) 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 5.1 (Genecodes Corp., Ann Arbor, MI). 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).

The program Phase 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 (pp) $\geq$0.95 were included in our analysis using phased data. Input files for phase were created using the program PhaseIn 1.0 (see Acknowledgements) 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 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. 3.7 (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 (Sukumaran and Holder 2010)—part of the DendroPy python library ver. 3.12.0—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 1000 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 1-10 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 (tree.bio.ed.ac.uk/software/tracer/) 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, 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 $4x10^6$ 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. saturates* (AF157916); *Ictidomys tridecemlineatus* (AF157870); *Sciurus carolinensis* (FJ200744); *Urocitellus columbianus* (AF157882); and *U. richardsoni* (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* vs. *M. olympus* and *M. caligata* vs. *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 *Mus 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 (ESS) 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 $3x10^6$ 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 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 one of two *M. caligata* haplotype clades (Fig. 1.2). *M. olympus* was recovered as basal to both the *M. caligata* and *M. caligata* + *M. vancouverensis* clades (Fig. 1.2). There were no appreciable difference 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, 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 were 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 non-recombining 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. 1.3). 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. 1.3). Bayesian and ML analyses 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. 1.4). 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 probability 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. 1.5).

The phylogenetic relationships between *M. caligata*, *M olympus*, and *M vancouverensis* were not well resolved in the *BEAST species tree analyses.

In the ultrametric species tree the 2 *M. caligata* specimens from the continental clade were basal 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* 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 1.1). 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 1.1).

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. 1.1). 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 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 recovered several admixed *M. caligata* clusters, none of which corresponded to the coastal

and/or continental mtDNA clades (Fig. 1.4). 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. 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

Marmota vancouverensis was recovered as the sister lineage to the coastal mtDNA clade of *M. caligata* in analyses of the 2 mitochondrial loci (Fig. 1.2). 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. 1.5). 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. 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 (Grady and Heaton 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 mitochondrial DNA 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. 1.4). *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 (Grady and Heaton 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. 1.2). 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 the 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 ~155km 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 conservation 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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Figures

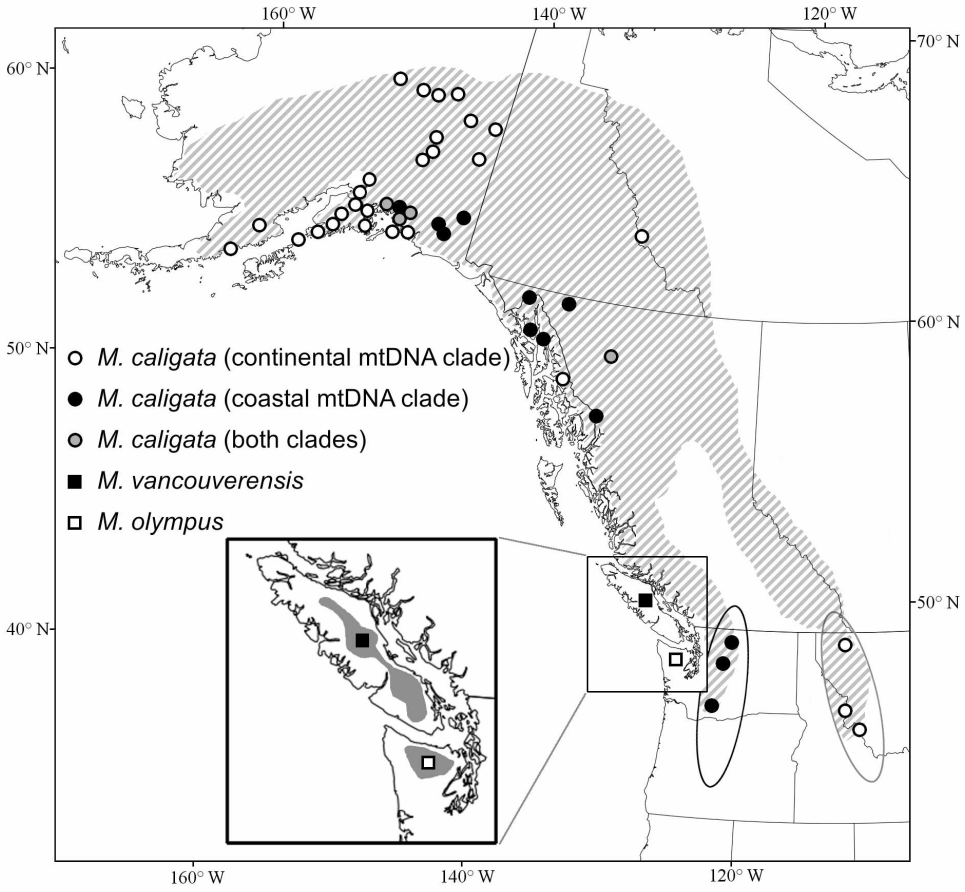

Figure 1.1. Distribution of specimens used in this study. *Marmota caligata* clades are based on mtDNA results. The hashed region represents the generalized *M. caligata* distribution (modified from Braun et al. 2011). Black and gray oval outlines refer to the predicted Pleistocene refugia of *M. caligata* 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*.

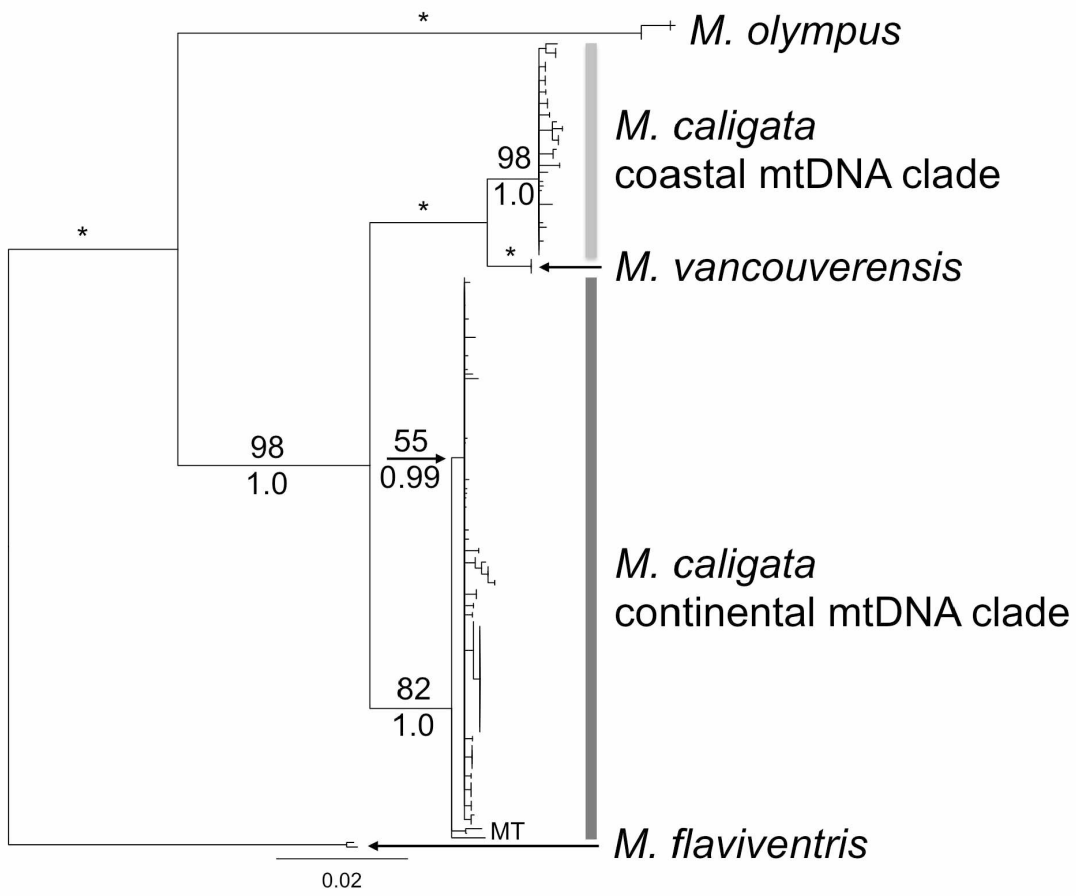

Figure 1.2. Maximum likelihood phylogram of the entire cytochrome *b* gene and 571 bp of the control region for *Marmota caligata*, *M. vancouverensis*, and *M. olympus* rooted with *M. flaviventris*. MT denotes 3 of the 4 *M. caligata* specimens from Montana; the additional specimen was nested within the continental clade. In both the ML 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.

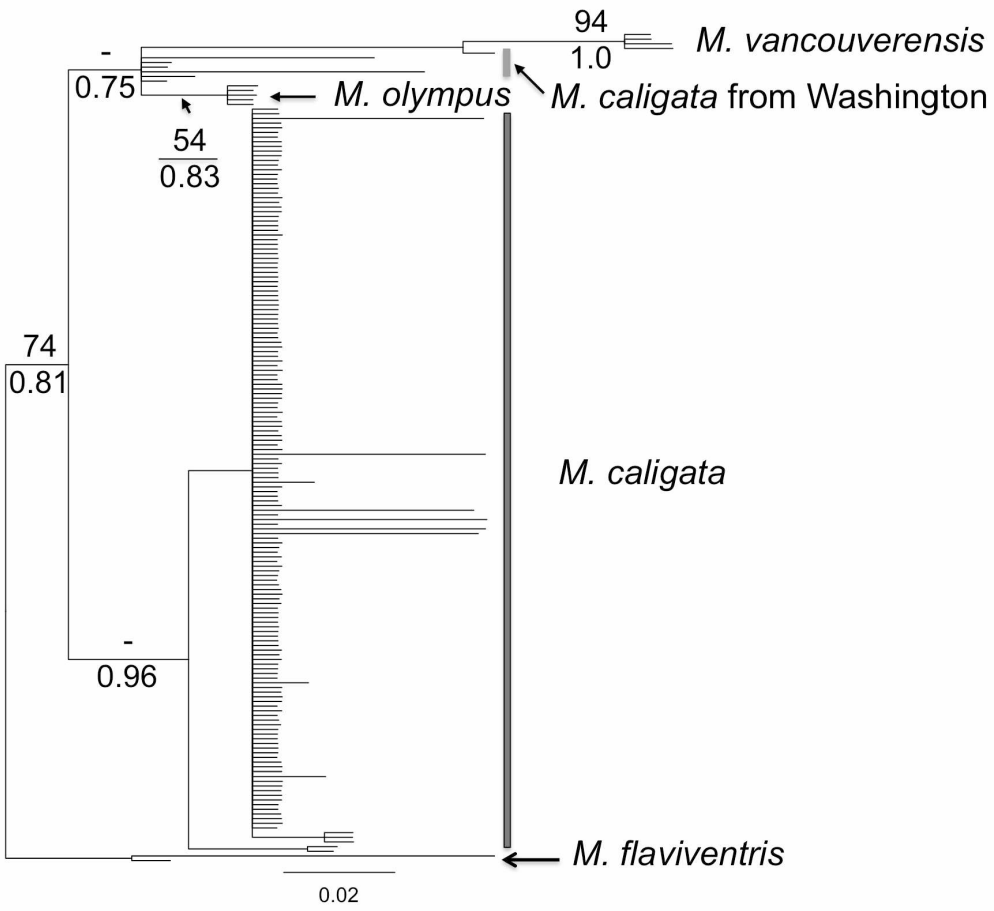

Figure 1.3. Bayesian phylogram of the partitioned BGN, CAT, and concatenated Cul4A and Lamp1 loci for *Marmota caligata*, *M. vancouverensis*, and *M. olympus* rooted with *M. flaviventris*. In both the Bayesian and ML analyses the BGN, CAT, and concatenated Cul4A and Lamp1 loci were analyzed as separate data partitions. A Maximum likelihood 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.

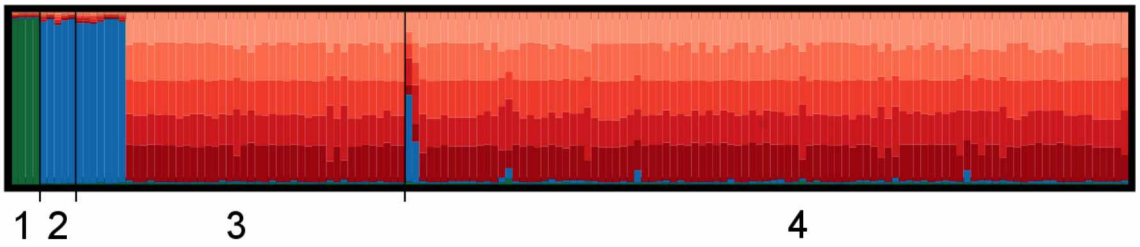

Figure 1.4. Results of a clustering analysis of haplotypes for 4 nuclear loci in *Marmota vancouverensis* (1), *M. olympus* (2), and *M. caligata* coastal (3) and continental (4) mitochondrial DNA (mtDNA) haplotype clades. Each vertical bar represents an individual and color represents relative membership in 1 of the 7 populations discussed in the text. *M. vancouverensis* is very homogenous (green bars), M. olympus and M. caligata specimens from Washington state share membership in a common group (blue bars), and all remaining *M. caligata* specimens belong in part to one of the 5 remaining groups (red bars). *M. caligata* populations do not correspond to the 2 mtDNA clades.

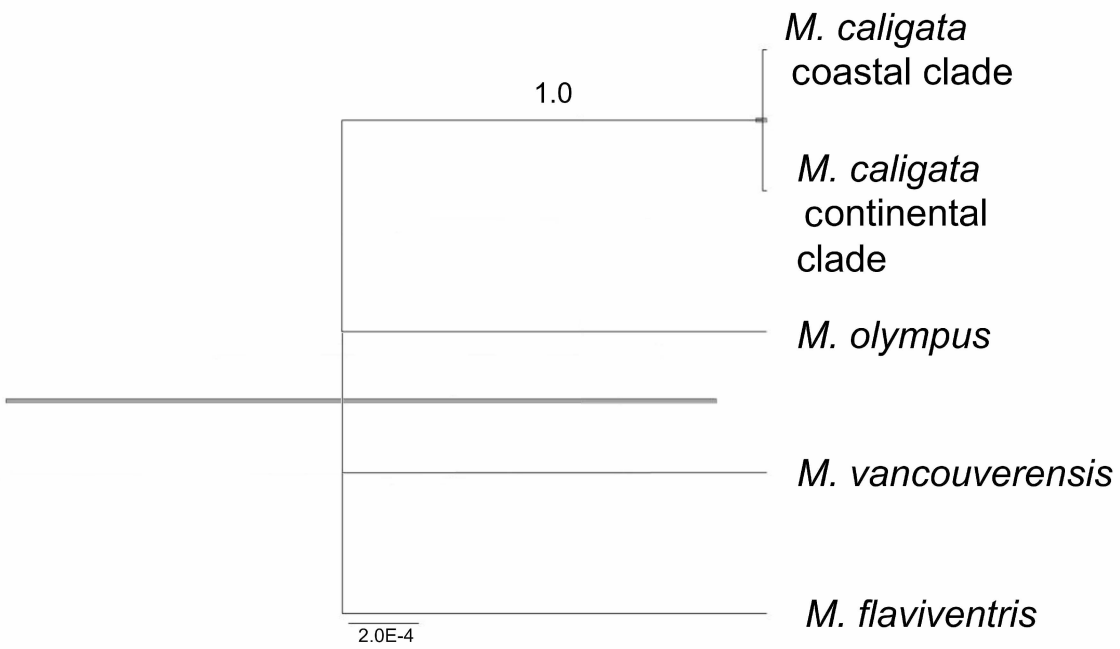

Figure 1.5. Species tree of marmots in the subgenus *Petromarmota* inferred from 4 nuclear loci using the major mtDNA clades as 'species'. Only nodes with a posterior probability > 0.80 are shown. Numbers above the lines are the Bayesian posterior probabilities. Gray bars represent 95% highest probability density of node age in relative time.

Table

Table 1.1. 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 geologies. 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, AIC = Akaike Information Criterion, Δ_i = difference in AIC from best model, ω_i = Akaike weights, and $E_{\min/i}$ = evidence ratio.

Species compared	Model	Migration from Hoary Marmot	Migration to Hoary Marmot	Log(P)	K	AIC	Δ_i	ω_i	$E_{\min/i}$
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 Peninsula 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

Appendices

Table 1.A-1. Species, collection localities, source museums, and catalog numbers of *Marmota* specimens used in this study. Museum abbreviations: Museum of Southwestern Biology, Albuquerque, NM (MSB); Royal Ontario Museum, Toronto, ON (ROM); University of Alaska Museum, Fairbanks, AK (UAM); University of Washington Burke Museum, Seattle, WA (UWBM); and the Yale Peabody Museum of Natural History, New Haven, CT (YPM).

Country	State/Province	Museum	Catalog no.	Latitude	Longitude
Canada	British Columbia	UAM	33803	58.1881	-129.8881
Canada	British Columbia	UAM	35130	58.1881	-129.8881
Canada	British Columbia	UAM	49848	56.1700	-130.0500
Canada	British Columbia	UAM	112310	59.7200	-133.3804
Canada	British Columbia	UAM	112316	58.1895	-129.8937
Canada	British Columbia	UAM	112366	59.7200	-133.3805
Canada	Northwest Territories	MSB	10002070	62.4500	-129.2000
Canada	Northwest Territories	MSB	10002071	62.4500	-129.2000
USA	Alaska	UAM	22914	58.2500	-134.5167
USA	Alaska	UAM	24122	58.2500	-134.5167
USA	Alaska	UAM	30932	57.0833	-132.7333
USA	Alaska	UAM	31724	61.2167	-149.5833
USA	Alaska	UAM	32649	58.2839	-134.5203
USA	Alaska	UAM	35129	56.0339	-130.0433
USA	Alaska	UAM	38302	58.5506	-135.4792
USA	Alaska	UAM	38303	58.5506	-135.4792

Table 1.A-1 cont.

USA	Alaska	UAM	38304	58.5506	-135.4792
USA	Alaska	UAM	48486	58.3042	-134.4083
USA	Alaska	UAM	53836	65.3928	-145.9994
USA	Alaska	UAM	57693	61.0585	-143.3634
USA	Alaska	UAM	58238	64.8110	-143.7790
USA	Alaska	UAM	58239	64.8110	-143.7790
USA	Alaska	UAM	58240	64.8110	-143.7790
USA	Alaska	UAM	58241	64.8110	-143.7790
USA	Alaska	UAM	65635	63.6667	-142.2167
USA	Alaska	UAM	78239	59.6374	-136.1291
USA	Alaska	UAM	78240	59.6374	-136.1291
USA	Alaska	UAM	85858	65.2947	-149.9973
USA	Alaska	UAM	85859	65.2596	-150.0502
USA	Alaska	UAM	86413	60.7709	-148.7506
USA	Alaska	UAM	86414	60.2753	-150.1504
USA	Alaska	UAM	94705	58.7667	-154.9667
USA	Alaska	UAM	98299	60.7819	-149.5456
USA	Alaska	UAM	101845	60.7709	-148.7506
USA	Alaska	UAM	101919	60.2849	-150.1584
USA	Alaska	UAM	102367	61.6124	-142.0313
USA	Alaska	UAM	102368	61.6134	-142.0388
USA	Alaska	UAM	102374	61.6125	-142.0394
USA	Alaska	UAM	102436	60.9763	-143.1291
USA	Alaska	UAM	102474	63.3958	-145.6603
USA	Alaska	UAM	102476	63.3958	-145.6610
USA	Alaska	UAM	103458	63.1285	-146.2803
USA	Alaska	UAM	103473	58.5344	-134.8308

Table 1.A-1 cont.

USA	Alaska	UAM	103474	58.2596	-134.6393
USA	Alaska	UAM	103475	58.3141	-134.6605
USA	Alaska	UAM	103476	60.3559	-146.1937
USA	Alaska	UAM	103477	58.2596	-134.6393
USA	Alaska	UAM	103489	58.8975	-152.2094
USA	Alaska	UAM	103490	58.8975	-152.2094
USA	Alaska	UAM	103491	58.8975	-152.2094
USA	Alaska	UAM	106200	65.4938	-145.3841
USA	Alaska	UAM	106220	65.2084	-148.0575
USA	Alaska	UAM	106221	65.2111	-148.0603
USA	Alaska	UAM	107658	60.5514	-145.3621
USA	Alaska	UAM	111555	65.2116	-148.0608
USA	Alaska	UAM	111557	65.2206	-148.0507
USA	Alaska	UAM	111561	65.2111	-148.0604
USA	Alaska	UAM	111565	65.4854	-145.4000
USA	Alaska	UAM	111626	65.2195	-148.0545
USA	Alaska	UAM	111634	65.2111	-148.0604
USA	Alaska	UAM	111786	58.8799	-152.2055
USA	Alaska	UAM	112286	58.8969	-152.2115
USA	Alaska	UAM	112287	58.8969	-152.2115
USA	Alaska	UAM	112288	58.8969	-152.2115
USA	Alaska	UAM	112289	58.8969	-152.2115
USA	Alaska	UAM	112290	58.8969	-152.2115
USA	Alaska	UAM	112291	58.8969	-152.2115
USA	Alaska	UAM	112292	58.8969	-152.2115
USA	Alaska	UAM	112293	58.8969	-152.2115
USA	Alaska	UAM	112294	58.8969	-152.2115

Table 1.A-1 cont.

USA	Alaska	UAM	112295	58.8969	-152.2115
USA	Alaska	UAM	112296	58.8969	-152.2115
USA	Alaska	UAM	112297	58.8969	-152.2115
USA	Alaska	UAM	112298	58.8969	-152.2115
USA	Alaska	UAM	112299	58.8969	-152.2115
USA	Alaska	UAM	112300	58.8969	-152.2115
USA	Alaska	UAM	112301	58.8969	-152.2115
USA	Alaska	UAM	112302	58.8969	-152.2115
USA	Alaska	UAM	112303	58.8969	-152.2115
USA	Alaska	UAM	112304	58.8969	-152.2115
USA	Alaska	UAM	112305	58.8969	-152.2115
USA	Alaska	UAM	112306	58.8969	-152.2115
USA	Alaska	UAM	112324	59.5097	-151.4527
USA	Alaska	UAM	112325	61.1540	-146.5978
USA	Alaska	UAM	112326	61.1342	-145.7744
USA	Alaska	UAM	112338	58.6245	-134.9362
USA	Alaska	UAM	112342	59.5099	-151.4512
USA	Alaska	UAM	112351	58.6245	-134.9362
USA	Alaska	UAM	112353	65.3902	-146.5982
USA	Alaska	UAM	112354	59.5099	-151.4512
USA	Alaska	UAM	112359	63.0841	-146.3847
USA	Alaska	UAM	112360	60.3461	-146.2685
USA	Alaska	UAM	112364	60.3448	-146.3126
USA	Alaska	UAM	112367	65.1492	-147.0182
USA	Alaska	UAM	112368	65.1492	-147.0182
USA	Alaska	UAM	112369	65.1492	-147.0182
USA	Alaska	UAM	112457	58.4228	-134.4431

Table 1.A-1 cont.

USA	Alaska	UAM	112458	58.4228	-134.4431
USA	Alaska	UAM	112579	59.4278	-151.1522
USA	Alaska	UAM	112580	59.3669	-151.6978
USA	Alaska	UAM	112581	59.4356	-151.1800
USA	Alaska	UAM	112582	59.7913	-150.5125
USA	Alaska	UAM	112583	59.6410	-151.0583
USA	Alaska	UAM	112585	59.4299	-151.1579
USA	Alaska	UAM	112587	65.1492	-147.0182
USA	Alaska	UAM	113733	59.6473	-151.0580
USA	Alaska	UAM	113734	59.6411	-151.0640
USA	Alaska	UAM	113735	59.6410	-151.0583
USA	Alaska	UAM	113736	59.4292	-151.1555
USA	Alaska	UAM	113737	59.4343	-151.1583
USA	Alaska	UAM	113738	59.4338	-151.1636
USA	Alaska	UAM	113739	59.4335	-151.1633
USA	Alaska	UAM	113878	61.1998	-147.4813
USA	Alaska	UAM	113886	60.9262	-146.2006
USA	Alaska	UAM	113889	63.4980	-145.8129
USA	Alaska	UAM	113892	61.7599	-149.3060
USA	Alaska	UAM	113901	61.7606	-149.3110
USA	Alaska	UAM	113902	61.7631	-149.3035
USA	Alaska	UAM	113903	63.5000	-145.8057
USA	Alaska	UAM	113904	61.2010	-147.4751
USA	Alaska	UAM	113905	60.9195	-146.2027
USA	Alaska	UAM	113906	61.2002	-147.4827
USA	Alaska	UAM	113907	65.3675	-146.9370
USA	Alaska	UAM	113925	65.3674	-146.9384

Table 1.A-1 cont.

USA	Alaska	UAM	113930	65.3665	-146.9374
USA	Alaska	UAM	113950	60.9262	-146.2006
USA	Alaska	UAM	113951	61.0548	-147.1226
USA	Alaska	UAM	114143	61.1413	-145.7593
USA	Alaska	UAM	114146	65.4917	-145.3895
USA	Alaska	UAM	114296	61.2018	-147.4709
USA	Alaska	UAM	114298	63.7833	-145.7918
USA	Alaska	UAM	114323	61.2002	-147.4827
USA	Alaska	UAM	114365	60.9278	-146.2128
USA	Alaska	UAM	115699	57.5538	-155.9849
USA	Alaska	UAM	115715	61.1418	-145.7616
USA	Alaska	UAM	115716	61.0548	-147.1226
USA	Alaska	UAM	115718	63.7876	-145.7916
USA	Alaska	UAM	115723	61.1337	-145.7751
USA	Alaska	UAM	115724	61.2016	-147.4731
USA	Alaska	UAM	115797	61.1370	-145.7662
USA	Alaska	UAM	115798	61.1385	-145.7645
USA	Alaska	UAM	115799	61.1333	-145.7773
USA	Alaska	UAM	115800	61.1330	-145.7780
USA	Alaska	UAM	115801	61.1439	-145.7559
USA	Alaska	UAM	115802	61.2017	-147.4716
USA	Alaska	UAM	115803	63.7834	-145.7907
USA	Alaska	UAM	115809	59.4333	-151.1626
USA	Alaska	UAM	117977	64.7920	-141.7312
USA	Alaska	UAM	117978	64.7699	-141.7528
USA	Alaska	UAM	117979	64.7938	-141.7296
USA	Alaska	UAM	117980	64.7924	-141.7288

Table 1.A-1 cont.

USA	Alaska	UAM	117981	64.7809	-141.7227
USA	Alaska	UAM	117982	64.7879	-141.7176
USA	Alaska	UAM	117983	64.7745	-141.7493
USA	Alaska	UAM	117984	64.7723	-141.7542
USA	Alaska	YPB	14820	63.0693	-145.7405
USA	Montana	UAM	112564	45.4223	-113.7225
USA	Montana	UAM	112566	48.5778	-114.4290
USA	Montana	UAM	112575	46.1562	-114.4761
USA	Montana	UAM	112576	48.5747	-114.4256
USA	Washington	UAM	112565	48.5140	-120.6873
USA	Washington	UAM	112570	48.5142	-120.6450
USA	Washington	UAM	112571	47.7331	-121.0717
USA	Washington	UAM	112573	48.5142	-120.6450
USA	Washington	UAM	112574	47.7310	-121.0695
USA	Washington	UAM	112577	47.7331	-121.0717
USA	Washington	UWBM	82114	46.1631	-121.5153
USA	Idaho	UAM	112562	45.3194	-114.5376
USA	Idaho	UAM	112567	45.3246	-114.4368
USA	Washington	UWBM	79553	n/a	n/a
USA	Washington	UWBM	79554	n/a	n/a
USA	Washington	UWBM	79849	n/a	n/a
USA	Washington	UWBM	80739	n/a	n/a
USA	Washington	UWBM	81033	n/a	n/a
Canada	British Columbia	ROM	116794	n/a	n/a
Canada	British Columbia	ROM	116795	n/a	n/a
Canada	British Columbia	ROM	117714	n/a	n/a

Table 1.A-1 cont.

Canada	British Columbia	ROM	117716	n/a	n/a

Table 1.A-2 Primers developed for this study to amplify the cytochrome b gene, part of the mitochondrial control region, and 2 nuclear introns in North American marmots.

Name	Sequence
MACA-L4	5'-GAATCTGAGGCGGATTCTCA-3'
MACA-R4	5'-GAATAAGGAGAAGAACTCCAAGC-3'
MACA-R7	5'-CCACGCCAGGGTAATGTTTA-3'
CR-HLF1	5'-GAGGACAACCCGTTGAACACCC-3'
CR-HLR1	5'-CCCTGAAGTAAGAACCAGATGTC-3'
Lamp1-F	5'-CTCMTACAAGTGCAACACTGAGGA-3'
Lamp1-R	5'-GGTAGGCAATGAGGACGATGAGGA-3'
Lamp1-NK-F	5'-GCGACAGGTTTGGGTCTG-3'
Lamp1-NK-R	5'-CCACAGCTATGGGGATCAG-3'
Cul4A-F	5'-ACCTGCTGGCAGGACCACTGCAGACA-3'
Cul4A-R	5'-CAGGAAGATGCYTCTGAYCATGA-3'
Cul4A-NK-F	5'-GCTGCAGTCTCCTCTCTCCT-3'
Cul4A-NK-R	5'-AGCAATTGTCCTTCCAGAGC-3'

Chapter 2 Ambiguous origins of a genetically discrete and recently eradicated island population of Hoary Marmots[2]

Abstract

Conservation and restoration of endemic island biota has received much attention. Islands in the Gulf of Alaska have been the subjects of intentional introductions and subsequent eradications. Hoary marmots (*Marmota caligata*) were purportedly introduced to Sud Island located off the southwest coast of the Kenai Peninsula, Alaska, in the early 20th century. The decline of a Rhinoceros Auklet (*Cerorhinca monocerata*) nesting colony on Sud Island was attributed to the presence of Hoary Marmots, and in 2010 the US federal government undertook efforts to eradicate the marmots. We found only ambiguous and circumstantial evidence suggesting that marmots were introduced to Sud Island. We also uncovered evidence that North American River Otters (*Lontra canadensis*) were likely responsible for the decline of Rhinoceros Auklets on Sud Island. To determine the origin of Sud Island marmots we analyzed sequence data (mitochondrial and nuclear) and microsatellite loci. Our analyses could not reject the possibility of a recent introduction, but multiple endemic mitochondrial haplotypes were found on Sud Island. STRUCTURE analysis of microsatellite data recovered the Sud Island population as distinct. Our molecular results and the absence of evidence of anthropogenic introduction or a negative impact on nesting seabirds, suggest Sud Island marmots were the result of unassisted overwater colonization, which is consistent with the occurrence of Hoary Marmots on other islands and their common occurrence at sea level throughout much of Alaska. Our findings highlight the

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[2] KERHOULAS, N. J. AND L. E. OLSON. 2015. In preparation Conservation Biology.

challenges in determining the origin of island populations and the need for rigorous scientific review before eradication actions are undertaken.

Introduction

A predominant risk to the loss of biodiversity is the invasion of exotic species (reviewed in Courchamp et al. 2003). The effects of introduced species on biodiversity are often more pronounced on islands (Ebenhard 1988). Historically, many Alaskan islands were stocked with commercially valuable furbearers and small mammals to provide a prey base ca. 1750-1930 (Bailey 1993; Isto 2012). Foxes (*Vulpes vulpes* and *V. lagopus*) were the most widely introduced furbearing mammals on Alaska islands, with populations established on more than 450 islands by the 1930s (Bailey 1993). Many of these insular fox populations were removed by trappers or became naturally extirpated (Paul 2009). To date, conservation efforts have resulted in the removal of introduced foxes from 42 Alaskan islands (Paul 2009).

In contrast, there is little documentation of small mammal introductions to Alaskan islands (Bailey 1993). Fox farmers were noted to have, "…filled barrels with squirrels and other small mammals and released them on islands where there were none" (Peterson 1967:123). A 1950 US Fish and Wildlife memorandum on introductions in Alaska stated that marmots and ground squirrels, "[s]hould probably be considered on islands for marten food where marten are proposed" (Burris 1965:98). The introduction of foxes is believed to have resulted in the extirpation of some native insular populations of small mammals in Alaska (Heller 1910). The natural distribution of many species of small mammals on islands in Alaska remains unclear (e.g., Cook et al. 2010) as a result of the uncertain impacts of fur farming and a lack of biological inventories.

The Barren Islands are a seven-island archipelago in the Alaska Maritime National Wildlife Refuge located ~30 km southwest of the southern tip of the Kenai Peninsula, 50 km east of the Alaska Peninsula, and ~110 km north of Kodiak Island, Alaska (Fig 2.1). Foxes were

stocked on Ushagat Island (the largest of the Barrens) in 1928 (Bailey 1993) and persisted until their removal in 1987 (Paul 2009). Arctic ground squirrels (*Urocitellus parryii*) are present on Ushagat Island and were noted as abundant on the Barren Islands in 1900 (Osgood 1901), before the earliest documented introduction of foxes. A recent molecular study concluded that arctic ground squirrels were "probably indigenous" to Ushagat Island (Cook et al. 2010:1404).

Fur farming may have also taken place on East and West Amatuli Islands (the second- and third-largest Barren Islands, respectively). A newspaper article suggests foxes were introduced to these islands sometime between 1935-1936 ("The fur trail" 1938). To date we have found no additional information regarding fur farming on the Amatuli Islands. The only other Barren Island with evidence of potential fur farming is Sud Island.

At ~110 hectares, Sud Island is the fourth-largest Barren Island. The US Department of Interior Alaska Planning Group (1975:51) noted that, "[a]ctivity ceased on Sud Island in 1939 when a fur farming lease expired" and collapsed cabins on Ushagat and Sud Islands are believed to have been used by fox farmers. This report is the only evidence of fur farming on the island and our efforts to find the lease mentioned in the report were unsuccessful. The paucity of historic documentation of fur farming on the Barren Islands leaves an open question as to the origin of its small mammal fauna.

The first biological inventory of the Barren Islands was conducted between 1974 and 1975 and led to the discovery of a breeding colony of Rhinoceros Auklets (*Cerorhinca monocerata*) on Sud Island in 1975 (Bailey 1976). Rhinoceros auklets are burrow-nesting seabirds, making them difficult to observe at their nesting colonies. Based on breeding burrow surveys, the number of nesting pairs of Rhinoceros Auklets was initially estimated to be 500 (Bailey 1976; Manuwal and Boersma 1977) and later increased to 750 when an additional

nesting area was discovered (Manuwal 1978). Hoary marmots (*Marmota caligata*, Eschscholtz, 1829) received little attention in these reports but were noted to "abound on the island" (Bailey 1976:8).

Rhinoceros auklet colonies on Sud Island were not surveyed again until 1994. The colonies described in the 1970s were revisited and only two shallowly dug, unoccupied nesting burrows were found; former colonies were overgrown and appeared to have had little or no use for the past 10-15 years (D. Roseneau, personal communication). During the 1994 survey, North American River Otters (*Lontra canadensis*; hereafter river otters) were observed and appeared common on the island based on an abundance of scat and well-worn trails (D. Roseneau, personal communication). In 2009 the Rhinoceros Auklet population on Sud Island was estimated to consist of, "…only a few pairs… [with] only about 20 burrows left" (US Department of Interior Fish and Wildlife Service 2010). The belief that Hoary Marmots were introduced and having a deleterious impact on the Rhinoceros Auklets through unspecified habitat alteration was the primary rationale used to argue for the eradication of marmots from Sud Island (US Department of Interior Fish and Wildlife Service 2010).

Hoary marmots are large ground-dwelling sciurids that range from central Idaho, western Montana, and southern Washington north to the Yukon River in Alaska and the Mackenzie Mountains in Yukon Territory (Gunderson et al. 2009; Braun et al. 2011). This species is primarily associated with alpine habitat, but is also found in coastal habitats and on several Alaskan islands (MacDonald and Cook 2009; Braun et al. 2011; pers. obs.). Although they are not known to occur on any islands outside of Alaska, Hoary Marmots are believed to be indigenous to Douglas, Hinchinbrook, Knight, Montague, and Perl Islands (Fig. 2.1; Heller 1910; MacDonald and Cook 2009; University of Alaska Museum [UAM]:Mamm:120619;

UAMObs:Mamm:203). They have also been reported from Hawkins Island, Alaska (Klein 1965), but no contemporary museum specimens or observations are known from this island and the author believes he may have misreported this (D. Klein, personal communication). Based on three specimens at the National Museum of Natural History (Smithsonian), Hoary Marmots may also occur on Elizabeth Island (Fig. 2.1). The reported collection locality of these three specimens (Cape Elizabeth) is on Elizabeth Island, but accounts of other specimens from this locality suggest it may have been misinterpreted as the southern terminus of the Kenai Peninsula and not part of Elizabeth Island (Merriam 1903).

Marmots were widely used by Alaska Natives and zooarcheological remains have been found at sites on Afognak, Hawkins, and Kodiak Islands. Marmot remains are known from multiple archeological sites on Kodiak Island (Hrdlička 1944; Kopperl 2003; M. Etnier unpublished data) and from a single site on both Afognak (M. Etnier unpublished data) and Hawkins Islands (Yarborough and Yarborough 1998). These remains (Table 2.A-1) are presumably Hoary Marmots, as they are the only marmot species in the region. Marmots are not currently known to occur on Afognak, Hawkins, or Kodiak Islands and these remains are believed to be the result of Native peoples transporting marmots, or parts thereof, to the islands.

Generally, introduced species are easily identified because they are outside their known range and/or the introduction was documented. The origins of some island populations are difficult to determine because the species in question occurs naturally on nearby mainland areas and only anecdotal or unpublished records support the introduction (Bailey 1993; Cook et al. 2010). Determining the origin of Hoary Marmots on Sud Island, Alaska, is one such challenging case study. Hoary marmots were presumed introduced and the subject of eradication efforts before the uncertainty regarding their origin was uncovered.

The first Hoary Marmot specimens were collected from Sud Island in 2009 during reconnaissance for the marmot eradication project. The Environmental Assessment (EA) of this project was completed in January 2010 (US Department of Interior Fish and Wildlife Service 2010). A Finding of No Significant Impact was issued for the preferred action of the EA (removal of marmots) by the Alaska Regional Chief of the National Wildlife Refuge System in February 2010 (Logan 2010), and eradication efforts by Wildlife Services (a branch of the U.S. Department of Agriculture's Animal and Plant Health Inspection Service) in collaboration with the United States Fish and Wildlife Service commenced that summer. A total of 189 Sud Island Hoary Marmots were killed between 2009 and 2011 (ADFG) when the eradication was halted. Twenty-five specimens were retained as vouchers and deposited in the Mammal Collection at the University of Alaska Museum (UAM) (Table 2.A-2).

While conducting a phylogenetic analysis of Pacific Northwest marmot species (Kerhoulas et al. 2015) we discovered that all Sud Island marmots had unique mitochondrial haplotypes compared to Hoary Marmots from throughout their known range. This prompted us to investigate the timing and source of the marmot introduction reported in the EA. We were unable to find all of the literature cited in the EA and only circumstantial evidence supporting an introduction. Because of the ambiguity in the literature regarding the purported introduction of marmots and in light of our molecular results, the eradication was halted in 2011. At the time it was believed that at least one and possibly as many as 10 marmots still remained on the island (S. Delahanty, personal communication). The current status of Hoary Marmots on Sud Island is unknown.

We present the results of a thorough literature review regarding the introduction of marmots to Sud Island. Additional mainland Hoary Marmot specimens were collected and

microsatellite data generated to use in conjunction with sequence data reported in Kerhoulas et al. (2015). We compared the genetic structure and relative divergence times of marmots from Sud Island, a native island population (Hinchinbrook), and a 'pseudo island' population to mainland populations in an attempt to determine whether marmots were introduced to Sud Island.

Materials and Methods

We began by reviewing all of the relevant literature available. Because much of the information regarding wildlife introductions in Alaska is contained in government documents and unpublished reports ('gray' literature), we searched for these documents at online repositories in addition to libraries. We inquired about potential historic reports of marmot introductions at the Alaska Department of Fish and Game office in Juneau, Alaska. To investigate military activity in the Barren Islands, we submitted information requests to the National Archives in Seattle, WA and College Park, MD. When possible, we also contacted the authors of the early Sud Island literature and biologists familiar with these efforts.

We also used molecular tools to investigate the origin of marmots on Sud Island. For molecular analyses we used specimens from Sud Island (n = 25), the Kenai Peninsula (n = 22), the nearby Prince William Sound mainland (n = 21), the Alaska Peninsula (n = 5), and Hinchinbrook Island (n = 3) (Fig. 2.1; Table 2.A-2). These specimens were chosen because they are of closest geographic proximity to Sud Island with archived fresh tissue samples, and under both colonization scenarios (anthropogenic or natural) they represent the most likely source population. Specimens from Hinchinbrook Island were included because they represent the nearest native insular population of Hoary Marmots with fresh tissue samples available and provide an opportunity to examine the molecular variation in a presumably native island

population. DNA sequence data, extraction methods, and GenBank accession numbers were presented in, or obtained following, the methods of Kerhoulas et al. (2015).

Sequence data included two mitochondrial (entire cytochrome-*b* [1140 bp] and partial control region [571 bp]) and four nuclear loci: BGN (715 bp), CAT (599 bp), Cul4A (362 bp), and Lamp1 (500 bp). Mitochondrial loci were concatenated and treated as a single locus in all analyses. Similarly, the Cul4A and Lamp1 loci were concatenated and treated as a single locus because of their proximity in the genome of the closely related 13-lined ground squirrel (*Ictidomys tridecemlineatus*) (see Kerhoulas et al. 2015). The nuclear loci used here are primarily intronic with relatively limited variation in Hoary Marmots (Kerhoulas et al. 2015). Despite their modest variation, they are the most polymorphic known for Hoary Marmots to date. Sequence data were only obtained from one (UAM:Mamm:94705) of the five Alaska Peninsula specimens from which microsatellite data were generated.

We screened 15 microsatellite loci designed for marmots or closely related species. Microsatellite loci were amplified using a forward primer with an m13(-21) tail and one of four dye-labeled m13(-21) primers in a nested PCR reaction as outlined in (Schuelke 2000). Because the aforementioned method of florescent labeling the PCR product precludes multiplexing, we simulated multiplex PCR reactions for fragment analysis. The simulated reactions were composed of 1.5μl PCR product from each of three independent PCR reactions of different loci for the same individual and 5.5μl Hi-Di Formamide (Applied Biosystems, Foster City, CA). To avoid ambiguity no loci in the simulated multiplex reactions shared the same dye.

Fragment analysis of microsatellite loci was conducted at the DNA Analysis Facility on Science Hill at Yale University using their in-house Liz-500 (Applied Biosystems, Foster City, California) size standard. Alleles were scored using GeneMarker ver. 2.6.0 (SoftGenetics, State

College, PA). The program MICROSATELLITE ANALYSER (MSA) ver. 4.05 (Dieringer and Schlötterer 2003) was used to reformat data. Deviations from Hardy-Weinberg equilibrium and linkage disequilibrium of the microsatellite loci were tested using the web interface of GENEPOP (Raymond and Rousset 1995; Rousset 2008). For the GENEPOP analyses we assumed four populations: (1) Sud Island, (2) the mainland surrounding Prince William Sound and the Kenai Peninsula, (3) Hinchinbrook Island, and (4) the Alaska Peninsula (Fig. 2.1; Table 2.A-2).

We used STRUCTURE ver. 2.3 (Pritchard et al. 2000) to determine if marmots from Sud and Hinchinbrook Islands each formed unique genetic clusters distinct from mainland populations. The STRUCTURE analyses included all genetic data and followed the methods presented in Kerhoulas et al. (2015). Because our nuclear data were phased we used a diploid model and treated the absent second copy of the concatenated mitochondrial loci as missing data. We implemented an admixture model assuming correlated allele frequencies, with a 10^5 burn-in, and 10^6 subsequent Markov-Chain Monte Carlo (MCMC) iterations. We assumed a maximum of 10 populations (K) and ran 10 iterations of the program for each assumed population size. The number of genetic clusters was determined using both the peak in the mean probability of the data (Pritchard et al. 2000) and the ΔK method (Evanno et al. 2005). Multiple STRUCTURE runs were analyzed, averaged, and visualized using the programs STRUCTURE HARVERSTER (Earl and vonHoldt 2012), CLUMPP (Jakobsson and Rosenberg 2007), and DISTRUCT (Rosenberg 2003) respectively.

The program IMa2 (Hey 2010) was used to conduct an isolation-with-migration (IM) analysis. We used IM to estimate the relative divergence times of marmots on Sud Island, Hinchinbrook Island, and a 'pseudo island' from the mainland population. The pseudo island

population was composed of nine specimens from a single locality on the Kenai Peninsula (Table 2.A-2). Because Sud Island was believed to have been glaciated during the Last Glacial Maximum (LGM) (Kaufman and Manley 2004), the isolation of Hoary Marmots on the island (regardless of origin) was likely post-LGM. With such a recent divergence time, estimating molecular rates that account for the time-dependent rates of molecular evolution is extremely difficult (Ho 2005; Ho et al. 2011). To circumvent this issue we compared the estimated 95% highest posterior density interval (HPD) of the divergence times (not scaled to demographic units) of the insular (including the pseudo island) populations from the mainland population. We compared the 95% HPDs of divergence times to determine if there was a significant difference (i.e. no overlap) between the estimated divergence times of marmots from Sud Island to a native insular population (Hinchinbrook Island) and a population that presumably has not diverged (pseudo island).

The IM analyses used to infer relative divergence times included all directly sequenced loci (6) following the methods used in Kerhoulas et al. (2015) and the 10 successfully amplified microsatellite loci. For all IM analyses we assumed no migration between populations. Each IM run used a unique starting seed, 120 heated chains, a $3x10^6$ burn-in, and was allowed to run for 10^7 steps. Update rates, trend plots, and effective sample size (ESS) values were used to determine if adequate mixing had been attained. For all IM analyses we performed two independent runs to ensure that the results obtained were consistent.

Results

Literature Review

Our literature review found two conflicting accounts of the timing and origin of marmot introduction(s) to Sud Island, both authored by the same person. The earliest published account

states marmots were probably introduced by the military (Bailey 1976). The navy installed an automatic weather station on Sud Island ~20 February 1945 (US Navy 1945). Removal of the weather station was planned for May or June 1949 (US Navy 1949), but we found no documentation of the completed removal. In addition to the weather station, remnants of "wooden barracks" have been noted on Sud Island (US Department of Interior Fish and Wildlife Service 2010). We were unable to locate any military documentation regarding these barracks. The more recent (and seemingly more accepted) account (Bailey 1993) states that marmots were introduced to Sud Island in ~1930. It appears that, "an island near Shuyak" (Fig. 2.1) as reported by Elkins and Nelson (1954:15) was interpreted to be Sud Island in the more recent account (Bailey 1993), but no explanation or mention of this assumption was provided.

The relevant section in Elkins and Nelson (Elkins and Nelson 1954:15) states that, "[s]ome 13 marmot[s] from the Juneau area were placed on Prince of Wales Island [Fig. 2.1] in 1930 and 1931... The 1935 report gives no reason for this transplant, but states that '—conditions on the island are suitable for these rodents—'. Marmot[s] were placed on an island near Shuyak in 1930 and existed there as late as 1948, according to Meyer."

It is unclear if the personal communication with Meyer is referencing marmots being placed on an island near Shuyak, marmots persisting on the island until as late as 1948, or both. The name Marcus W. Meyer is given in a preceding section of Elkins and Nelson (1954) and we believe this is the Meyer referred to in the marmot section. According to his obituary (Anderson 2014), Marcus W. Meyer was in the Kodiak area between 1944-49, but not during the 1930s. Additionally, Sud Island was named in 1908 (Orth 1967:2785), well before the purported introduction, so if the "island near Shuyak" was in fact Sud Island (as interpreted by Bailey [1993]), it is odd that it was not referred to by name. Furthermore, we found a report that stated

marmots were introduced to Shuyak (Island) and not "an island near" in 1930 (Burris 1965). These inconsistencies highlight the ambiguous and unreliable nature of the accounts claiming marmots were introduced to Sud Island.

In addition to the documents mentioned above, we note the likely existence of a potentially relevant 1935 Alaska Game Commission report that we were not able to locate. Elkins and Nelson (1954) quote a 1935 report, regarding suitable environmental conditions for marmots on Prince of Wales Island. The citation provided for this 1935 report directs the reader to "Alaska Game Commission — Annual reports 1925 to 1952." Similarly, Burris and McKnight (1973) also cite a 1935 report regarding the introduction of marmots to Prince of Wales Island. The Alaska Game Commission produced various annual reports and to date we have been unable to find a 1935 report that contains the text quoted in Elkins and Nelson (1954) or the marmot-specific information presented in Burris and McKnight (1973).

Molecular

Two mtDNA haplotypes unique to Sud Island were recovered that differ from mainland haplotypes at either one or two positions. SNPs were found in both the cytochrome-*b* gene and the partial control region. None of the sequenced nuclear loci had SNPs unique to Sud Island.

We amplified 15 microsatellite loci and included 10 in our analysis (GS14, GS25: Stevens et al. 1997; SS-Bilb18: Goossens et al. 1998; MS53, MS56, MS6: Hanslik and Kruckenhauser 2000; MA018, MA066: Da Silva et al. 2003; 2h6b, 3b1: Kyle et al. 2004). Five microsatellite loci (SS-Bilb25: Goossens et al. 1998; ST10 Hanslik and Kruckenhauser 2000; MA091, MA001: Da Silva et al. 2003; 2g2: Kyle et al. 2004) were omitted because they contained a large amount of unusable data (i.e., multiple peaks) or amplified products that varied greatly in size from previously published work on the focal or closely related species. Two loci

(MA066 and MS6) were monomorphic. A deficiency in heterozygotes was observed in the Prince William Sound and the Kenai Peninsula population at the SS-Bibl18 ($p = 0.023$) and MS56 ($p = 0.038$) loci and in the Sud Island population at the 2h6b ($p = 0.021$) locus. We detected no significant linkage disequilibrium among the loci using a Bonferroni correction. There were no microsatellite alleles unique to Sud Island. Hinchinbrook Island specimens all shared a single unique microsatellite allele at the MA018 locus.

The STRUCTURE analysis recovered $K = 4$ as the most likely number of clusters using the peak in the mean probability of the data. Individuals from Sud and Hinchinbrook Islands each formed distinct clusters (Fig. 2.2). Using the ΔK method $K = 4$ also appears to be the most likely number of clusters, but $K = 2$ also received some support (Fig. 2.A-1). In the $K = 2$ model individuals from Sud and Hinchinbrook Islands formed one cluster with all mainland individuals forming the other.

The two independent IM runs for each "island" group were identical or differed only slightly in their estimates of divergence time from the mainland population (Table 2.1). The 95% HPD estimate of divergence time for marmots from Sud Island, Hinchinbrook Island, and the pseudo island from the mainland population overlapped. The pseudo island population was the only one to have a 95% HPD estimate of divergence time that included zero. The 95% HPD of divergence time for Sud and Hinchinbrook Island populations were similar and generally greater than that of the pseudo island population (Table 2.1).

Discussion

Our literature review failed to uncover any original documentation of marmots having been introduced to Sud Island. We found two accounts of purported marmot introductions to Sud Island, both authored by the same person at different times (Bailey 1976; 1993). The earliest

account suggests the military transported marmots to Sud Island. The Navy installed an automatic weather station on Sud Island in February 1945 (US Navy 1945), but it is exceedingly unlikely marmots could have been transported at this time as they would have been in hibernation (Braun et al. 2011). Furthermore, Hoary Marmots do not occur on Kodiak Island (the site of the naval base), so transporting marmots would involve an additional stop to collect marmots before landing on Sud Island. Additionally, we found no military documentation regarding the "wooden barracks" reported in the EA (US Department of Interior Fish and Wildlife Service 2010). Overall, we found no evidence supporting this account, but we did not personally review the military documents housed at the National Archives. The more recent account relies on interpreting "an island near Shuyak" to be Sud Island and is based on personal communication at best.

Regarding the personal communication cited as evidence in the more recent account, we find it peculiar that the source (Meyer) visited Sud Island but neglected to note its name, the name of any other islands in the archipelago, or that the archipelago is known as the Barren Islands, all of which are much closer than Shuyak Island. This suggests the personal communication with Meyer regarding a marmot introduction to "an island near Shuyak" as cited in Elkins and Nelson (1954) may have been in reference to the persistence of the marmots until 1948 and not their introduction. If Meyer was the source regarding marmots being introduced to "an island near Shuyak," then his account was likely secondhand.

Further complicating this issue, Burris (1965) states an unknown number of marmots from an unknown locality were introduced to Shuyak in 1930 (and not an island near). In a subsequent publication with the same title and coauthored by Burris, there is no mention of a marmot introduction to Shuyak Island (Burris and McKnight 1973). We found no evidence that

marmots were ever present on Shuyak Island. The claim of marmots being introduced to Shuyak Island made in Burris (1965) may have been the result of him misreading and not citing Elkins and Nelson (1954). Regardless, this further highlights the inconsistencies present in the gray literature regarding the history of marmot introductions.

We found no evidence of state or federal agency participation in the introduction of marmots, aside from the failed attempt on Prince of Wales Island (Paul 2009), which is located over 1,100 km SE of the Barren Islands. The introduction of marmots and ground squirrels for marten food on islands was proposed, but ranked, "doubtful proposals under consideration, require further investigation" (Burris 1965:97). We were unable to find the 1935 Alaska Game Commission report quoted in Elkins and Nelson (1954) and cited in Burris and McKnight (1973) that appears to contain specific details (e.g. collection locality, date of release, sex of individuals) of the marmot introduction on Prince of Wales Island and may also contain additional information regarding marmot introductions in general.

The origins of island populations of arctic ground squirrels in Alaska were also found to be incomplete and largely anecdotal (Cook et al. 2010). Molecular data suggest arctic ground squirrels on Ushagat Island (<2 km from Sud Island) were "probably indigenous" (Cook et al. 2010:1404). The arctic ground squirrels on Ushagat were reported as introduced in Clark (2010), but no supporting literature was cited and attempts by us to contact the author were unsuccessful. If the genetic signal found in arctic ground squirrels on Ushagat Island is the result of natural colonization or even a prehistoric introduction it is possible that the marmots on Sud Island share a similar history.

Arctic ground squirrels occur on several islands in the region and are commonly confused with marmots. Similarities between the Alaska Native as well as Russian words for marmots and

ground squirrels have also led to confusion. The epitome of this is Marmot Island (~44 km from Shuyak Island), which lacks marmots and was instead misnamed for its resident arctic ground squirrels (reviewed in Orth 1967:623). Because of the confusion between marmots and arctic ground squirrels it is possible that Meyer (or his source) was actually referring to arctic ground squirrels and not marmots having been introduced to "an island near Shuyak." If this was the case, Dark Island, Alaska (named in 1849 Orth 1967:257) may have been the "Island near Shuyak." Dark Island is <5 km from Shuyak Island and has a population of arctic ground squirrels believed to have been introduced (US Department of Interior Fish and Wildlife Service 1988).

Our STRUCTURE analysis, including all genetic data, identified marmots from Sud and Hinchinbrook Islands as forming unique clusters (Fig. 2.2). The estimated divergence time of marmots on Sud and Hinchinbrook Islands from the mainland were similar, but overlapping with the estimated divergence time of our pseudo island population. Similarities in divergence time estimates among these populations are likely the result of our data lacking the information needed to resolve such recent events. Additional loci with more variation are needed to compare the divergence time of the Sud Island marmot population with mainland and naturally occurring island populations.

If Hoary Marmots colonized Sud Island naturally, it would be the most remote island known to have been colonized in this manner by the species. The nearest mainland Hoary Marmots occur on the Kenai Peninsula. The shortest overwater distance between the Kenai Peninsula and Sud Island is ~38 km. The recently discovered population of Hoary Marmots on Perl Island ~36 km from Sud Island is the closest known population. Hoary marmots on Montague Island appear to have crossed the widest water barrier. Using current sea levels and

assuming marmots colonized from the nearest known population (Hinchinbrook Island) the minimum overwater distance to reach Montague Island is ~11.5 km.

This region was likely glaciated during the LGM (Kaufman and Manley 2004), suggesting colonization of these islands took place post-LGM. Lower sea levels of the late Pleistocene and early Holocene (Mix et al. 2001) may have facilitated island colonization if it took place shortly after the LGM.

According to the two introduction accounts, Hoary Marmots were transported to Sud Island ~1930 or 1945 (Bailey 1976; 1993; US Department of Interior Fish and Wildlife Service 2010). If one of these scenarios is correct then the largest Rhinoceros Auklet population ever documented on the island occurred either 31 or ~46 years after Hoary Marmots were introduced and had presumably become well established (Manuwal and Boersma 1977). This timing suggests that an additional factor(s) and not marmots (introduced or native) were responsible for the decline of Rhinoceros Auklets. Because Sud Island was never documented without marmots it is difficult to determine the effects of their presence. Furthermore, it is clear that even if marmots were introduced, seabird populations appeared to be unaffected for three or four decades.

We uncovered evidence suggesting river otters may have been responsible for the near-extirpation of Rhinoceros Auklets from Sud Island after 1979. River otters were considered the most important seabird predator on East Amatuli Island (a Barren Island <11 km from Sud Island, Fig. 2.1) between 1976-79 and were not known to occur on Sud Island until 1979 (Manuwal 1980). Additionally, river otters are believed responsible for the extirpation of a Rhinoceros Auklet colony on Seabird Rocks, British Columbia (Carter et al. 2012). The existence of a large Rhinoceros Auklet colony that only declined after the arrival of river otters,

combined with evidence that river otters decimated a similar a Rhinoceros Auklet population, suggests they are likely responsible for the drastic decline of Rhinoceros Auklets on Sud Island. Additionally, in 1976, a large portion of the Rhinoceros Auklet colony site on Sud Island was observed to be eroding away (Manuwal and Boersma 1977) and the impact this may have had on the colony is unclear.

The purpose of the eradication of Hoary Marmots from Sud Island as given in the EA was to "restore native ecosystems on these islands" (US Department of Interior Fish and Wildlife Service 2010, p. 7). As we have shown, Hoary Marmots may well have been part of Sud Island's "native" ecosystem, and no unambiguous evidence to the contrary has been uncovered or adduced. The EA later (p. 10) claims that "introduced Hoary Marmots cause problems for native species on small islands," yet no other introduced island population of Hoary Marmots is known. The EA goes on (p. 10) to say that "[o]n Sud Island, after being introduced, [marmots] became overabundant and competed with native seabirds for nest sites" and that "chronic grazing by marmots" (p. 25) was evident. However, no evidence of direct competition was noted.

Conservation and management often require making difficult decisions with limited information, especially in a state as large, rugged, and remote as Alaska. However, when the information used to assess irreversible actions such as eradications is ambiguous, it is paramount that the uncertainties are clearly acknowledged. We found only circumstantial evidence that marmots may have been introduced to somewhere near Sud Island. Furthermore, we discovered that river otters and not marmots might have been responsible for the decline of Rhinoceros Auklets on Sud Island. The molecular markers we used lacked the resolution to definitively determine the origin of marmots on Sud Island but did recover the population as genetically differentiated from all other sampled populations, a finding not consistent with a recent

introduction. This work serves as a reminder to conduct careful synthesis from primary sources, especially when the consequences are the irreversible loss of biodiversity.

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Figures

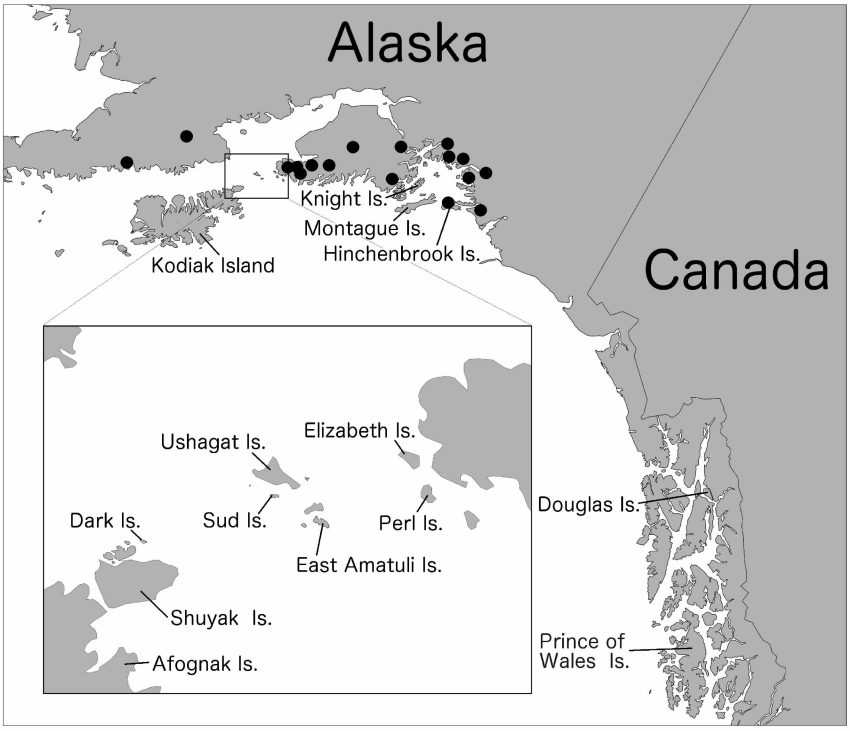

Figure 2.1. Map of the islands discussed in the text and the collection localities (black circles) of Hoary Marmot (*Marmota caligata*) specimens used in this study. No circle was placed for the Hoary Marmot specimens collected on Sud Island, Alaska.

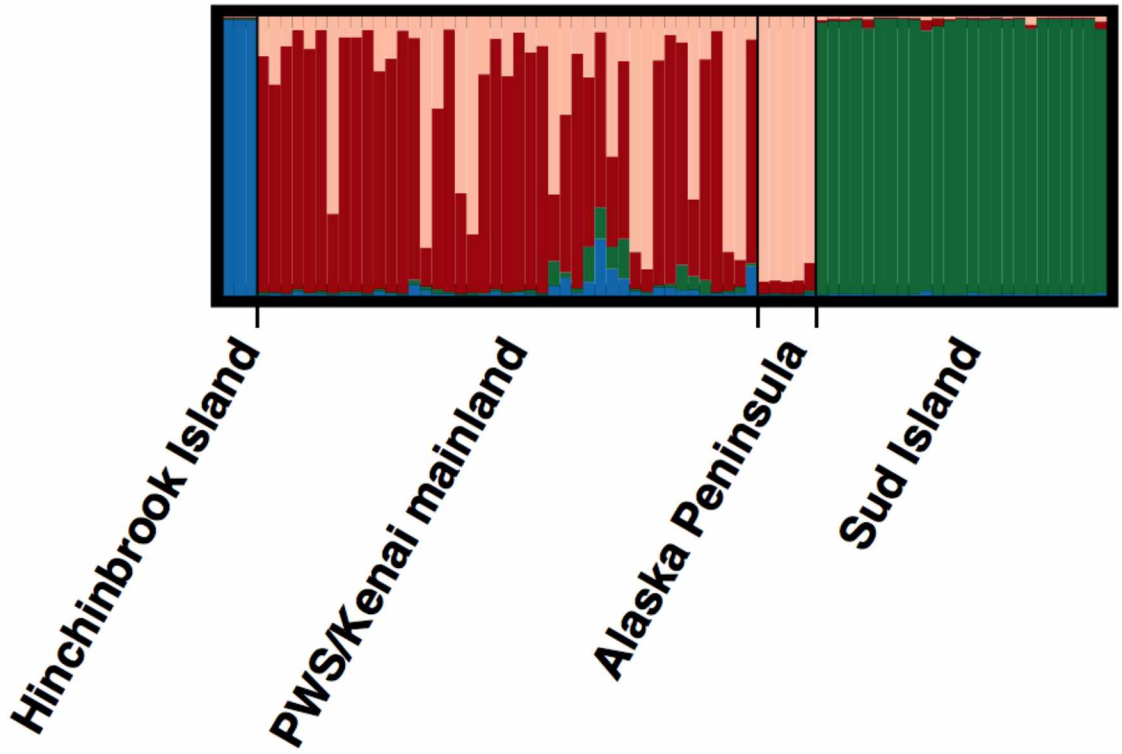

Figure 2.2. Results of a STRUCTURE analysis of *Marmota caligata* haplotypes for two mtDNA and four sequenced nuclear loci and 10 microsatellite loci. Each individual specimen is represented by a vertical bar. Bar color represents relative membership to the four genetic clusters. PWS and Kenai refer to Prince William Sound and the Kenai Peninsula, respectively.

Table

Table 2.1. Results of the 95% highest posterior density interval (HPD) of divergence time (not scaled to demographic units) of insular populations *Marmota caligata* from the mainland population, estimated using the program IMa2. Two independent runs were conducted for each comparison.

	Sud Island	Hinchinbrook Island	Pseudo island
run 1	0.00275 - 0.04375	0.00225 - 0.05075	0 - 0.01725
run 2	0.00225 - 0.04225	0.00275 - 0.05175	0 - 0.01725

Appendices

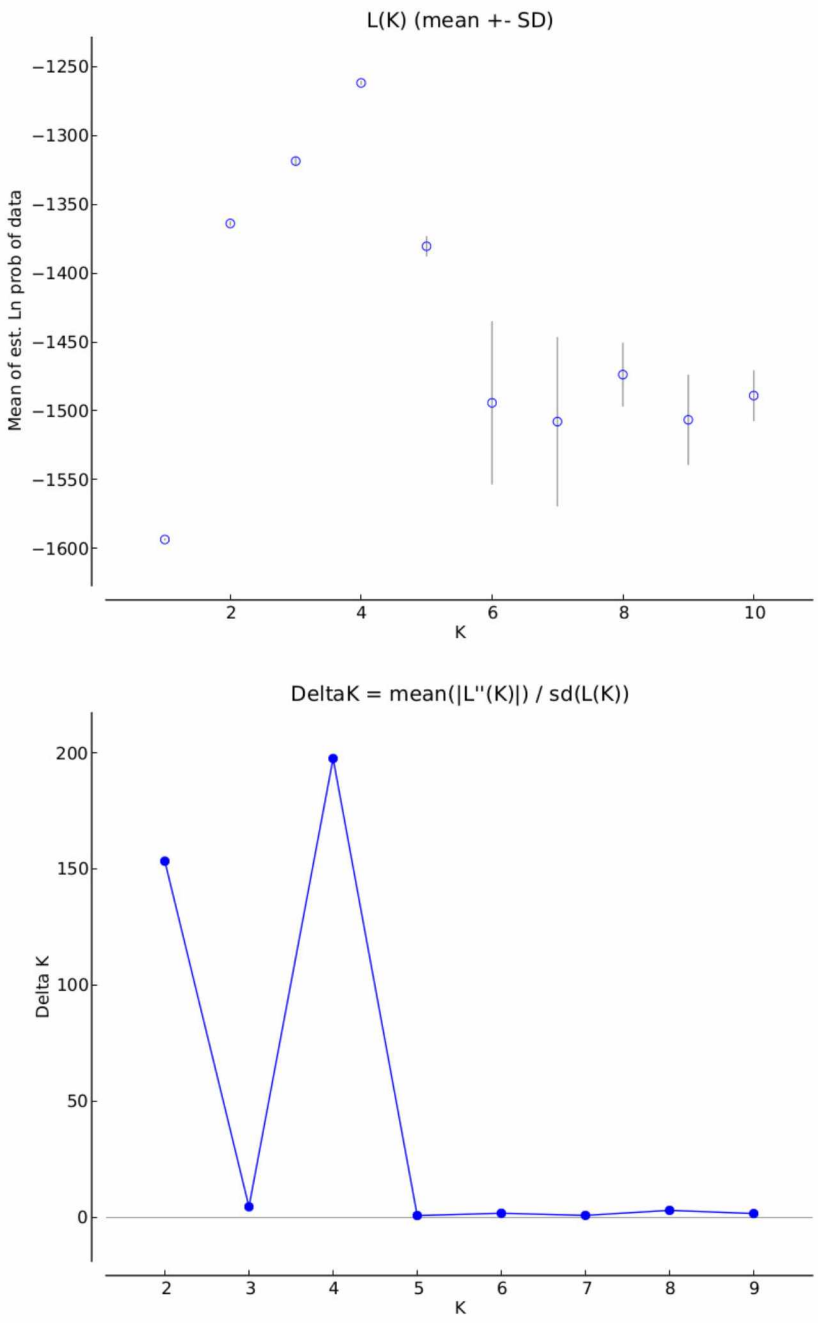

Figure 2.A-1. Results of STRUCTURE HARVERSTER showing the peak in mean probability (top) and ΔK (bottom).

Table 2.A-1. Zooarcheological remains referred to in the text. Burke Museum = Burke Museum of Natural History and Culture, University of Washington, Seattle.

Museum	Catalog number
National Museum of Natural History	USNM 256721
Burke Museum Archaeology	49-KOD-363, Kopperl ID: 5.010
Burke Museum Archaeology	49-KOD-363, Kopperl ID: 5.090
Burke Museum Archaeology	49-AFG-016, Etnier ID:AM34.7078.002

Table 2.A-2. Collection localities, University of Alaska Museum Mammal Collection catalog numbers, and coordinates (WGS84) of *Marmota caligata* specimens used in this study. Specimens with bold location were used as the pseudo island population in the IM analysis.

Location	Catalog no.	Latitude	Longitude	Error (m)
Alaska Peninsula	94705	58.7667	-154.9667	3219
Alaska Peninsula	117504	57.6364	-155.7204	300
Alaska Peninsula	117501	57.6344	-155.7428	100
Alaska Peninsula	117492	57.6405	-155.7717	100
Alaska Peninsula	117489	57.6364	-155.7204	300
Hinchinbrook Island	103476	60.3559	-146.1937	100
Hinchinbrook Island	112360	60.3461	-146.2685	3
Hinchinbrook Island	112364	60.3448	-146.3126	3
Kenai Peninsula	101845	60.7709	-148.7506	1000
Kenai Peninsula	101919	60.2849	-150.1584	10
Kenai Peninsula	112324	59.5097	-151.4527	10
Kenai Peninsula	112326	61.1342	145.7744	3
Kenai Peninsula	112354	59.5099	-151.4512	10
Kenai Peninsula	112579	59.4278	-151.1522	3
Kenai Peninsula	112580	59.3669	-151.6978	8
Kenai Peninsula	112581	59.4356	-151.18	3
Kenai Peninsula	112582	59.7913	-150.5125	9
Kenai Peninsula	112583	59.641	-151.0583	3
Kenai Peninsula	112585	59.4299	-151.1579	3
Kenai Peninsula	112587	65.1492	-147.0182	3
Kenai Peninsula	113733	59.6473	-151.058	3
Kenai Peninsula	113734	59.6411	-151.064	3
Kenai Peninsula	113735	59.641	-151.0583	3

Table 2.A-2 cont.

Kenai Peninsula	113736	59.4292	-151.1555	3
Kenai Peninsula	113737	59.4343	-151.1583	3
Kenai Peninsula	113738	59.4338	-151.1636	7
Kenai Peninsula	113739	59.4335	-151.1633	12
Kenai Peninsula	113885	60.9262	-146.2006	91
Kenai Peninsula	113886	60.9262	-146.2006	274
Kenai Peninsula	115809	59.4333	-151.1626	7
Prince William Sound	107658	60.5514	-145.3621	5
Prince William Sound	112325	61.154	-146.5978	3
Prince William Sound	112342	59.5099	-151.4512	10
Prince William Sound	113878	61.1998	-147.4813	5
Prince William Sound	113904	61.201	-147.4751	10
Prince William Sound	113905	60.9195	-146.2027	80
Prince William Sound	113906	61.2002	-147.4827	5
Prince William Sound	113950	60.92624	-146.20058	3
Prince William Sound	113951	61.0548	-147.1226	5
Prince William Sound	114143	61.1413	-145.7593	4
Prince William Sound	114296	61.2018	-147.4709	10
Prince William Sound	114365	60.9278	-146.2128	30
Prince William Sound	115715	61.1418	-145.7616	5
Prince William Sound	115716	61.0548	-147.1226	5
Prince William Sound	115723	61.1337	-145.7751	10
Prince William Sound	115724	61.2016	-147.4731	10
Prince William Sound	115798	61.1385	-145.7645	10
Prince William Sound	115799	61.1333	-145.7773	10
Prince William Sound	115800	61.133	-145.778	10
Prince William Sound	115801	61.1439	-145.7559	152
Prince William Sound	115802	61.2017	-147.4716	10
Sud Island	103489	58.8975	-152.2094	1000
Sud Island	103490	58.8975	-152.2094	1000
Sud Island	103491	58.8975	-152.2094	1000
Sud Island	111786	58.8799	-152.2055	1000
Sud Island	112286	58.8969	-152.2115	1000
Sud Island	112287	58.8969	-152.2115	1000
Sud Island	112288	58.8969	-152.2115	1000
Sud Island	112289	58.8969	-152.2115	1000
Sud Island	112290	58.8969	-152.2115	1000
Sud Island	112291	58.8969	-152.2115	1000
Sud Island	112292	58.8969	-152.2115	1000
Sud Island	112293	58.8969	-152.2115	1000
Sud Island	112294	58.8969	-152.2115	1000

Table 2.A-2 cont.

Sud Island	112295	58.8969	-152.2115	1000
Sud Island	112296	58.8969	-152.2115	1000
Sud Island	112297	58.8969	-152.2115	1000
Sud Island	112298	58.8969	-152.2115	1000
Sud Island	112299	58.8969	-152.2115	1000
Sud Island	112300	58.8969	-152.2115	1000
Sud Island	112301	58.8969	-152.2115	1000
Sud Island	112302	58.8969	-152.2115	1000
Sud Island	112303	58.8969	-152.2115	1000
Sud Island	112304	58.8969	-152.2115	1000
Sud Island	112305	58.8969	-152.2115	1000
Sud Island	112306	58.8969	-152.2115	1000

Chapter 3 Phylogeography, distributional limits, and future outlooks for Hoary Marmots (*Marmota caligata*)[3]

Abstract

The phylogeographic history of a species can provide insights into populations and regions that are the most important for the conservation of genetic diversity. We used species distribution maps and molecular data to infer the most important areas of future conservation for the Hoary Marmot (*Marmota caligata* (Eschscholtz 1829)) and to resolve conflicting hypotheses regarding the Pleistocene refugia used by the species. We georeferenced all available Hoary Marmot museum specimens and created species distribution maps for past, present, and future climatic scenarios. Several previous molecular studies relied on a limited number of samples that did not include the southern distribution of the species, so we revisited those results using specimens from throughout the species' distribution. Our results suggest that Hoary Marmots likely existed south and/or west of the glacial margins of Last Glacial Maximum and found little support for a proposed northern refugium. These findings suggest the southernmost (and possibly coastal) populations of Hoary Marmots are the most genetically diverse and that additional research in these areas would be the most useful to inform conservation efforts and future management.

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[3] KERHOULAS, N. J., H.C. LANIER AND L. E. OLSON. 2015. In preparation for the Canadian Journal of Zoology.

Introduction

Terrestrial vertebrate populations are experiencing extremely high rates of localized extirpation (Ceballos et al. 2017). These declines are likely the result of a number of factors including climate change, which may disproportionately impact alpine species. Because of their distribution at or near mountain tops, alpine-dependent species have limited potential to mitigate climate change with upslope migration (Krajick 2004). Thus, identifying the current distribution and phylogeographic history of a species may be particularly important for conservation and management as it allows us to determine which areas are disproportionately important to maintaining genetic diversity as climate shifts (Hampe and Petit 2005). One species that deserves more attention is the Hoary Marmot (*Marmota caligata* (Eschscholtz 1829), a predominantly alpine species found in the Pacific Northwest (PNW) of North America.

Hoary Marmots are large, ground-dwelling squirrels distributed throughout the PNW, from southern Washington, central Idaho, and southwestern Montana north to Alaska south of the Yukon River (Gunderson et al. 2009; Braun et al. 2011). As with many alpine species, a fine-scale distribution with respect to specific mountain ranges and internal range margins is poorly known, in part because many of the areas they inhabit are difficult to survey effectively. Their broad distribution includes three general regions of documented Pleistocene refugia: eastern Beringia, south of the glacial margins of the LGM ice sheets, and along the PNW coast. Isolation during the Pleistocene appears to have resulted in two reciprocally monophyletic mitochondrial DNA (mtDNA) lineages in Hoary Marmots (Kerhoulas et al. 2015; Lanier et al. 2015). These clades are generally distributed from the Rocky Mountains in southwestern Montana north to interior Alaska (continental clade) and in the Cascade and Coastal Mountains of the PNW from southern Washington to Prince William Sound, Alaska (coastal clade) (Fig. 3.1). Despite the

well-documented presence of this historical separation, recent studies have provided conflicting hypotheses regarding the location of the refugia that resulted in this pattern.

Glacial cycles of the Pleistocene were a dominant force in shaping the recent phylogeographic history of the PNW (Pielou 1992; Weksler et al. 2010; Shafer et al. 2011), and thus for partitioning much of the genetic diversity that we see in today's landscapes. Glacial refugia in the region are believed to have been predominantly north (in eastern Beringia, unglaciated Alaska and northwestern Canada, hereafter Beringia) and south of the Cordilleran and Laurentide ice sheets (reviewed in Shafer et al. 2010a). Common patterns of post-Last Glacial Maximum (LGM) range expansion from these refugia is a northward migration from a southern refugium and southward migration from a northern refugium (Pielou 1992; Weksler et al. 2010; Shafer et al. 2011). There is also evidence of coastal PNW Pleistocene refugia, i.e., west of Pleistocene glaciation (Ogilvie 1997; Shafer et al. 2010b; Chavez et al. 2014). The Haida Gwaii archipelago (Fig. 3.1, formerly known as the Queen Charlotte Islands) off the coast of central British Columbia and the Alexander Archipelago in southeastern Alaska (Fig. 3.1) are believed to have served as a Pleistocene refugium for several species of plants and animals (Carrara et al. 2007; reviewed in Shafer et al. 2010a). For example, a coastal refugium in Southeast Alaska has been proposed for the North American Mountain Goat (*Oreamnos americanus*) (Shafer et al. 2010b). Lastly, portions of Vancouver Island, British Columbia (Fig. 3.1), also appear to have served as a Pleistocene refugium (Ogilvie 1997; Chavez et al. 2014). Distinguishing the refugial origins and historical population sizes of extant populations can be important for identifying areas of unique genetic diversity within the species.

Conflicting hypotheses exist regarding the locations of the LGM refugia used by the two Hoary Marmot mtDNA clades and the subsequent mode of postglacial colonization. Some

research suggests the mtDNA clades persisted within southern and/or coastal refugia (Kerhoulas et al. 2015). In contrast, using a comparative approach across multiple species, Lanier et al. (2015) suggested these clades might have used Beringia refugia. Further support for northern and coastal Pleistocene refugia was reported in Knowles et al. (2016). However, neither study reporting evidence of northern refugia in Hoary Marmots included samples from the southern portion of the Hoary Marmot's range (~ an additional 10° latitude), which may have impacted previous findings regarding the potential of a southern refugium.

Species distribution models (SDMs) offer an opportunity to infer the potential current and historic distributions of a species during various important times, such as glacial maxima (Waltari et al. 2007), as well as to predict the future distribution of suitable habitat for a species under likely climatic scenarios. In order to identify areas of potential Pleistocene refugia and those of particular conservation importance, we created SDMs using all possible museum specimens and combined these with population genetic summary statistics calculated using existing molecular data from specimens collected throughout the distribution of the species. Specifically, we ask: 1) Where do SDMs predict suitable Hoary Marmot habitat existed during the LGM? 2) Do these predictions concur with most likely regions of Pleistocene refugia for each mtDNA clade and how do these results compare to those of previous research? 3) Based on these results, which populations of Hoary Marmots are likely to contain the greatest genetic diversity? 4) How will Hoary Marmot habitat be impacted by future climate scenarios?

Materials and methods

Specimen records and georeferencing

We compiled a comprehensive database of *M. caligata* specimens from all North American museums searchable online and/or through visits by one of us to individual collections

to circumscribe an accurate distributional map. All specimen records were vetted for ambiguities and/or irregularities and clarified to the extent possible with museum staff. We georeferenced or verified previously assigned locality data for all specimens in our database following the BioGeomancer protocol (Chapman and Wieczorek 2010). Collector-assigned locality data were noted and not altered. The extent of common but often difficult-to-delimit terms were calculated as follows: "mountain/mount"—centered on the peak extending to the base of the peak or most distant saddle when in a mountain range, "head of river/creek"—centered on the point of the highest mapped segment of the waterway (determined via the highest resolution topology map available) extending to summit of nearest peak, and "lake"—centered on the lake extending to summit of adjacent mountains. Because Hoary Marmots do not commonly occur in populated places, when the specific locality was a populated place we arbitrarily added 10 km to the extent of the locality. Uncertainty was determined using the Georeferencing Calculator (Wieczorek and Wieczorek 2015) assuming exact coordinate precision and 10 meters of measurement error.

Species distribution modeling

We estimated the distribution of suitable *M. caligata* habitat using SDMs implemented in the program MAXENT 3.3 (Phillips et al. 2006), using specimens collected since 1950 with an uncertainty $\leq$ 5 km combined with 19 bioclimatic layers (WorldClim.org). In order to capture population movements over time, SDMs were created for the last interglacial, the LGM, the mid-Holocene, current, and future conditions based on two greenhouse-gas concentration trajectories (representative concentration pathway [RCP]): year 2050 with a RCP of 2.6, year 2050 with a RCP of 8.5, year 2070 with a RCP of 2.6, and year 2070 with a RCP of 8.5. A resolution of 2.5 arc-minutes was used for all bioclimatic layers except for the last interglacial, where 30 arc-second data were used, as they were the only available layer. We used bioclimatic layers

constructed using the Community Climate System Model (CCSM4) for past and future times and cropped these layers to an appropriate size using SDMTOOLBOX 1.1c (Brown 2014).

Occurrence data were rarefied to minimize overfitting due to biased sampling (i.e., greater sampling from more accessible areas [Boria et al. 2014]). Background sampling bias files were created to ensure pseudo-absence points did not include highly unsuitable regions (Anderson and Raza 2010; Barbet-Massin et al. 2012). All background sampling bias files also accounted for latitudinal bias (Brown 2014). We also used SDMTOOLBOX to rarefy the occurrence data used to create the background sampling bias files. We rarefied the occurrence data at 10, 25, and 50 km and created background sampling bias files using the entire map, 100-km, and 300-km radial buffers of the occurrence data. We spatially jackknifed and conducted independent tests of model parameters using SDMTOOLBOX and the methods presented in Knowles et al. (2016). All nine combinations of sampling bias files and rarefied occurrence data were run to assess the effects of these treatments on the resulting SDMs. We enabled clamping in MAXENT to deal with out-of-range variables (i.e., those outside the range sampled in our training data) in future and past predictive models. We visually inspected MESS maps (Elith et al. 2010) to determine if clamping was influencing the predictions at localities of interest.

Molecular

We constructed phylogenetic trees, calculated population genetic summary statistics, and created Bayesian skyline plots using the mitochondrial sequence data available on GenBank to infer phylogeographic history as well as recent demographic changes that may be of conservation importance (Kerhoulas et al. 2015). These included 1737 bp of mtDNA: 1140 bp of cytochrome *b* and 597 bp of control region, concatenated and treated as a single locus.

Mitochondrial sequence data represented 147 *M. caligata* specimens: 101 specimens from the continental mtDNA clade and 46 specimens from the coastal mtDNA clade.

Phylogenetic trees were created using both maximum likelihood (ML) and Bayesian methods, implemented in the programs GARLI 2.0 (Zwickl 2006) and MRBAYES 3.2 (Ronquist et al. 2012), respectively. Because the cytochrome *b* and control region represent protein-coding and non-protein-coding regions, respectively, they were treated as separate data partitions for tree construction. The best-fit model of nucleotide substitution for each partition was selected using the programs MODELTEST 3.7 (Posada and Crandall 1998) and MRMODELTEST 2.3 (Nylander 2004) under the Akaike Information Criterion (AIC). The TIM+I and K81uf+I+G models and the GTR+I and HKY+I+G models were used for the cytochrome *b* and control regions in the ML and Bayesian analyses, respectively. Support for ML trees was determined using a 1,000-replicate bootstrap analysis. Trees were rooted with the Olympic Marmot (*M. olympus*). Because Vancouver Island Marmots (*M vancouverensis*) appear to have recently captured the mitochondria of *M. caligata* (Kerhoulas et al. 2015), they were not included in our analyses. Program settings, runtimes, and tree summarization follow Kerhoulas et al. (2015).

To investigate demographic changes (specifically, population expansions and contractions), we calculated Tajima's *D*, Fu's *Fs*, and R_2 using DNASP 5.10.01 (Librado and Rozas 2009) for the noncoding mtDNA control region. Both Tajima's *D* and Fu's *Fs* show negative values for recent population expansion and positive values for population bottlenecks (Tajima 1989; Fu 1997). The R_2 statistic is reported to be more sensitive to demographic change when small sample sizes are used, with low values indicating recent population events (Ramos-Onsins and Rozas 2002). We calculated summary statistics for each of the two mtDNA clades and subsets of each clade. Using the ice sheet margins presented in Dyke et al. (2002), clades

were split into 7 subgroups to explore the demographic history of proposed LGM refugia and to provide a direct comparison to previous research that only included specimens from northern populations (Lanier et al. 2015). The continental mtDNA clade was split into four subgroups (all northern, northern from proposed Beringia refugia, northern from non-refugia, and southern) and the coastal clade was split into three subgroups (northern, central, and southern) (Fig. 3-A1). Statistical significance of these summary statistics was calculated by running 10,000 coalescent simulations in DNASP.

Bayesian skyline plots were constructed using all mtDNA data and the Bayesian Evolutionary Analysis by Sampling Trees (BEAST 1.74) software package (Drummond et al. 2012). We used BEAUTI (part of the BEAST software package) to construct BEAST input files. Data were treated as a single partition using the HKY+I+G model of nucleotide substitution, based on the results of MRMODELTEST 2.3 (Nylander 2004) and the AIC. For BEAST analyses, we selected a strict molecular clock with the rate fixed at 1.0, set the number of groups to 10, and ran Markov-chain Monte Carlo (MCMC) chains for 5×10^8 generations, sampling every 5×10^3 iterations. We conducted three independent runs for each BEAST analysis to test for convergence of the MCMC chains. Results were summarized, viewed, and plotted using TRACER 1.5 (Rambaut et al. 2009).

Results

Georeferenceing museum specimens

We identified 1213 *M. caligata* museum specimen records in 34 natural history museums (Table 3.A-1), of which we were able to verify or assign coordinates with uncertainty ≤5 km for 600 specimens and ≤25 km for 975 specimens. Locality data at or below the level of state or province was assigned to 1175 specimens. The known distribution of Hoary Marmots was

mapped using specimens from all collection years that had an uncertainty ≤25 km (Fig. 3.2). The two following specimens represent unique (and possibility dubious) collection localities and were omitted from our analyses. Specimen MCZ 4870 is the only known Hoary Marmot potentially from a locality in Alaska north of the Yukon River. The specific locality of this specimen, "Fort Yukon," does not appear to be suitable habitat for marmots and both the specimen and original collector notes are missing. Fort Yukon was and is an active center of trade for furs brought in from throughout eastern Interior Alaska (and Canada, in historic times), and it is likely this specimen was collected elsewhere. Additionally, MCZ specimen 11605 is noted as being collected in Yellowstone Park, MT. We have visually confirmed that this specimen is a Hoary Marmot, but have no other information to determine the collection locality. Hoary Marmots are not known from Yellowstone Park and the nearest verified specimen was collected >200 km away.

Species Distribution Modeling

Species distribution models were created using 125, 96, and 74 occurrence localities for the 10, 25, and 50 km rarefaction treatments, respectively. The SDMs were largely concordant across the various rarefaction and background sampling treatments (Fig. 3.3, Fig. 3. A-2). Mid-Holocene SDM models indicate suitability was greater in the Coast/Cascade Mountains and reduced in interior Alaska and the high-elevation southern Rocky Mountains during this time relative to the present. Areas of suitable habitat during the LGM were more variable among treatments, but regions in the Coast/Cascade and Rocky Mountains south of the LGM glacial margin were identified as highly suitable in all models. The coastal margin of the Kenai and Alaska Peninsula were also identified as suitable in all models. Regions of interior Alaska were identified as suitable, but the limits varied among models. The Wrangell-Saint Elias area and the

northern portions of Haida Gwaii also appear to have been suitable during the LGM. The latitudinal distribution of suitable habitat during the last interglacial was similar to the current distribution, but generally reduced in expanse and largely absent from interior Alaska. All future scenarios predict a reduction in suitable habitat, with the greatest reduction at the higher RCPs. The distribution of future habitat is similar to that of the last interglacial and largely absent from regions recovered as suitable during the LGM.

Molecular

Both ML and Bayesian analyses produced phylogenetic trees with a sister relationship between the coastal and continental clades (Fig. 3.4). Clades were well supported in the Bayesian analysis, but the continental clade received low bootstrap support in the ML analysis. Bayesian analysis recovered the three of the four specimens from historically sub-Laurentide localities (i.e., Montana) as sister to the remainder of the continental clade including the additional sub-Laurentide specimen (Fig. 3.4). The best ML tree recovered a similar relationship regarding the sub-Laurentide specimens, but this relationship received low bootstrap support and was not recovered in the majority rule consensus tree of the bootstrap analysis.

A signal of recent population expansion was found in the continental clade for all three population genetic summary statistics and in the coastal clade for Fu's *Fs* (Table 3.1). Significant negative values of Fu's *Fs* were also recovered for two of the continental clade subgroups: all northern specimens and northern specimens from regions likely to have been glaciated (i.e. non-refugia) during the LGM (Table 3.1). No significant results were found for any of the coastal clade subgroups. Both the coastal and continental clades had similar levels of diversity. The southernmost continental subgroup had the highest level of genetic diversity, which was more than double that of any other clade or subgroup (Table 3.1).

Bayesian skyline plots show evidence of a recent population increase in the continental clade and little to no change in the coastal clade (Fig. 3.5). Both clades have similar maximum effective population sizes and steep population declines at recent timescales. The continental clade had an older (~3x in relative terms) coalescence time than the coastal clade.

Discussion

Our results suggest the largest region of suitable habitat during the LGM was south of the glacial margins of the Pleistocene (Fig. 3.3). Locations of potential coastal habitat were also recovered for this time period in Southeast Alaska and near present day Haida Gwaii, British Columbia (Fig. 3.3). Our models indicated little evidence of suitable habitat in regions of Beringia previously proposed as Hoary Marmot refugia during the LGM. A southern refugium is also supported by Hoary Marmot fossils that predate the LGM from two localities in southern Alberta (Harington 2011) where SDM models of the last interglacial (~120-140 kya) predict suitable habitat. We did not verify the identification of these fossils, but they were found near areas currently inhabited by Hoary Marmots. Although fossil evidence (even if positively confirmed as Hoary Marmots) does not preclude extirpation and subsequent recolonization, it does provide circumstantial evidence of Hoary Marmots near the sub-Laurentide region of proposed LGM refugium during the last interglacial period.

Similar to the findings of our SDMs a recent study using fossils and climatic habitat modeling found that LGM habitat suitable for Hoary Marmots likely existed in Beringia and south of the glacial margins, with the majority of the suitable habitat in the Great Plains and Midwest (Polly et al. 2015). While these results are generally in agreement with our hypothesis of a southern refugium, that likely harbors the greatest genetic diversity, several fossil specimens included in their study were not confidently identified as *M. caligata* (Polly and Head 2004).

More importantly, the climatic habitat model created by Polly et al. (2015) may be biased by their use of an outdated distribution map of *M. caligata* that included the entire known range of the allopatric Alaska Marmot (*M. broweri*), whose range extends far north of the Hoary Marmot's (Gunderson et al. 2009) and this may account for the potential Beringian refugium found in their study. Regardless, the fossils used in Polly et al. (2015) (if confirmed to be Hoary Marmot) suggest a far more expansive LGM refugium than has been previously proposed and underscores the need for sampling from the extreme southern distribution of the species in Idaho and Montana. Additional study of marmot fossils, including the use of ancient DNA, may be warranted to confirm the identification of these fossil specimens and the extent of the Hoary Marmot's historic distribution.

Our revised distribution of Hoary Marmots (Fig. 3.2) is generally consistent with recently published maps (e.g. Braun et al. 2011), but it is the first to incorporate an exhaustive review of voucher specimens housed in North American museums and standardized georeferencing methods. Most range maps of Hoary Marmots suggest their absence from coastal areas of British Columbia east of Haida Gwaii. Although we found relatively few museum specimens from this region, our SDMs suggest ample areas of suitable habitat. It is therefore likely that the purported absence of the species from much of western British Columbia is merely a reflection of inadequate sampling due to the region's remoteness and ruggedness.

In contrast to previous findings, our molecular analyses suggest that both coastal and continental Hoary Marmot mtDNA clades likely underwent some level of recent population expansion (Fig. 3.5, Table 3.1). Summary statistics yielded evidence of population expansions in both clades—not an unexpected result—as both currently inhabit regions that were glaciated during the LGM. Bayesian skyline plots also support a recent expansion in the continental clade,

but not in the coastal clade. The lack of a recent expansion signal in the Bayesian skyline plot of the coastal clade may be a result of our sample failing to capture the actual genetic signal or the result of limited genetic diversity in the clade. Both coastal and continental clade Bayesian skyline plots yield evidence of a very recent population decline. This result may represent a population decline since the mid-Holocene, as our SDM predicts highly suitable habitat to have existed during this time (Fig. 3.3), but is likely the result of limited sampling of highly structured populations (Heller et al. 2013).

Our population genetic summary statistics suggest that limited sampling of such a widely distributed species (Fig. 3.2) might produce misleading results with regard to historic demographic changes. Since the persistence of Hoary Marmots in Beringian refugia (Lanier et al. 2015) was based on a limited number of specimens, all from Alaska and northwestern British Columbia, we believe the results supporting this hypothesis may be an artifact of limited sampling (14 specimens with four haplotypes from the continental clade and 12 specimens with five haplotypes from the continental clade). While our study includes a greater number of specimens than previous research, we note additional sampling from throughout the distribution of Hoary Marmots is likely to further refine our understanding of the phylogeographic history of the species.

A "simultaneous" divergence of Hoary Marmot mtDNA clades with those of four other alpine species, that also likely diverged in Beringia given their current distribution and fossil record, was also presented as evidence of a Beringian refugium for Hoary Marmots (Lanier et al. 2015). As noted by the authors, a simultaneous divergence does not preclude these species from having diverged at approximately the same time in other locations. Furthermore, the temporal boundaries of what may be considered simultaneous were not defined, so species diverging in

different localities as a response to shared climatic shifts in the region may ultimately be responsible for this finding.

Continental clade

Our SDM suggest the Hoary Marmot continental mtDNA clade likely persisted south of the glacial margins of the LGM. Supporting this hypothesis, population genetic summary statistics showed evidence of a recent population expansion of the northern populations of this clade, but no such signal in the southernmost specimens. These results strongly suggest a south-to-north post-Pleistocene expansion in this clade (Table 3.1). Successive bottlenecking in the leading-edge populations during range expansion may have led to a reduction in the genetic diversity, as observed in the continental clade (Hewitt, 1996). The high level of genetic diversity observed in the southern specimens of the continental clade (> 2x that of any other subgroup) is also consistent with a southern refugium, rather than recent colonization from a northern refugium. Additionally, southern specimens of this clade were also recovered as sister to, and nested within, the remainder of the continental clade in our Bayesian tree analyses (Fig. 3.4). Despite the limited number of southern samples, our findings strongly support a sub-Laurentide origin of the continental clade.

While our results are in general agreement with a southern refugium we did not recover evidence of a recent population expansion in specimens from areas of proposed Beringian refugia (Table 3.1). Given the elevated level of genetic diversity recovered in the southernmost specimens relative to those from unglaciated Beringia, we hypothesize that the absence of a signal of recent population expansion from this region is likely a consequence of early post-glacial colonization. The earliest known fossils identified as Hoary Marmot (not verified by us) from Beringia are from an archeological site in Interior Alaska dated between 9,300-10,500 BP

(Yesner 2001), suggesting Hoary Marmots may have already been well established near what is now the northern margin of their distribution by this time as they were apparently being used as a resource by indigenous peoples. An early post-LGM colonization of northern latitudes (Heintzman et al. 2016) would have provided more than 2,000 generations (Schwartz et al. 1998) with little subsequent demographic change, which may explain this result and the inferred historic stability documented in Hoary Marmots from interior Alaska in a recent analysis of RADseq data (Knowles et al. 2016). Furthermore, the results of Knowles et al. (2016) may have also been influenced by sampling bias (DeGiorgio and Rosenberg 2013) as the current known range of Hoary Marmots extends some 2,000 km southeast of their sampling. Overall, our results suggest the greatest genetic diversity and potential for conservation in this clade likely exists in the southernmost populations.

Coastal clade

Based on the distribution of suitable habitat during the LGM, the most likely Pleistocene refugium for this clade was the Cascade Mountains south of the current Hoary Marmot range (Fig. 3.3). However, we did not recover elevated levels of genetic diversity in marmots from the Cascade Mountains in Washington, as might be expected if the region had served as a Pleistocene refugium (Hewitt, 1996). Low genetic diversity does not preclude the region serving as a refugium, since it may be a result of historic bottlenecking of the species during the glacial cycles of the Pleistocene. Additionally evidence of potential introgression with Olympic Marmots in the region (Kerhoulas et al. 2015) suggests that, while predicted as suitable, the Cascade Mountains may have instead been occupied by Olympic Marmots.

In addition to the Cascade Mountains, SDMs also identified areas of potential LGM refugia from coastal British Columbia and Southeast Alaska (Fig. 3.3). Further supporting

potential refugia in this area, fossil evidence places Hoary Marmots in Southeast Alaska (Prince of Wales Island, Fig. 3.1) during the last glacial period (>44,500- 23,560 BP) (Grady and Heaton 2003). These fossils and the predicted suitability of habitat in this region during the last interglacial (Fig. 3.3) indicate the potential of a long history of Hoary Marmots in Southeast Alaska. The location of predicted LGM habitat in Southeast Alaska is congruent with an area believed to have served as an LGM refugium for North American Mountain Goats (Shafer et al. 2010a). However, in contrast to the findings in Mountain Goats (Shafer et al. 2010a), we did not recover increased levels of genetic diversity consistent with the region serving as a LGM refugium in Hoary Marmots. While our SDM also suggests Haida Gwaii—long considered a Pleistocene refugium for several taxa (reviewed in Shafer et al. 2010a)—contained suitable Hoary Marmot habitat during the Pleistocene to date, no marmot remains have been recovered from the archipelago, nor do marmots occur there today. British Columbia in general is represented by a limited number of Hoary Marmot specimens with archived tissue samples, limiting genetic-based inference.

The current distribution of the coastal clade in areas glaciated during the LGM and our molecular results both indicate a recent population expansion (Table 3.1). In contrast, our Bayesian skyline plot suggests negligible demographic change (Fig. 3.5). This discrepancy may be the result of population bottlenecks (Hewitt 1996), limited sampling, and/or failing to sample the region(s) of Pleistocene refuge. Exploratory analysis (results not shown) of a similar number of continental specimens from northern locations most likely glaciated during the LGM (i.e. recently colonized) produced a Bayesian skyline plot nearly identical to that of the coastal clade, suggesting we may have failed to sample populations from near the LGM refugium. In addition, the similar levels of genetic variation and lack of a population expansion signal observed in the

three coastal subpopulations may also be a result of a limited sampling of the species across its range and/or limited sample size.

Although the phylogeographic history of the coastal clade remains largely unresolved, the potential survival of this clade in Beringia during the LGM (Lanier et al., 2015) is unlikely for a number of reasons. First, the coastal clade does not occur in parts of Beringia believed to have been unglaciatated during the LGM. This does not preclude LGM survival of the coastal clade in a northern refugium followed by a complete displacement by the continental clade, but persistence in a southern and/or coastal refugium is more parsimonious. Second, we recovered no evidence of demographic expansion in any of the three subpopulations tested, which suggests that the absence of an expansion signal may be a result of limited sampling and/or limited genetic diversity rather than demographic stability. Finally, our SDM of the LGM recovered highly suitable habitat south of the glacial margins and in areas of the PNW coast. Overall the phylogeographic history of the coastal clade is enigmatic and additional sampling (especially in western British Columbia) is needed to elucidate areas of historic refugia and how genetic diversity can best be conserved.

Future Outlook

Species distribution models, fossils, and molecular data are congruent with the persistence of Hoary Marmots south of the glacial margins of the LGM. The increased level of genetic diversity documented in the southernmost continental clade specimens suggests this area is likely the most important for the long-term conservation of genetic diversity in this clade. Museum records support the presence of Hoary Marmots in many localities that may have served as refugia during the LGM, e.g., southwestern Alberta, southeastern British Columbia, Idaho, and Montana (Fig. 3.2); but to date there are only four museum specimens from this region with

archived tissue samples (necessary for molecular analyses), highlighting the desperate need for addition research in this region.

The region of greatest genetic diversity in the coastal mtDNA clade was not resolved. Fossil evidence (Grady and Heaton 2003), genetic diversity estimates (Table 3.1), and SDMs (Fig. 3.3) are compatible with survival in a coastal refugium. However, SDMs indicate a large region south of the glacial margins of the LGM was highly suitable for the species (Fig. 3.3), but genetic diversity was lowest in specimens from this region (Table 1). Additional sampling of this clade from throughout its distribution is needed to determine where the greatest genetic diversity and regions of conservation priority exist.

Our SDMs of future Hoary Marmot habitat suitability suggests a general decrease in availability across all latitudes. All future scenarios predict habitat in the northern Rocky Mountains will become subdivided and increasingly isolated. Given the high levels of genetic diversity observed in this region, additional studies of diversity and gene flow may be useful to future conservation and management decisions. Although reduced, large portions of suitable habitat are predicted to remain in much of the northern portion of the Hoary Marmot's range. This is likely because at higher latitudes Hoary Marmots occur at lower elevations, increasing the potential for upslope movement in northern regions, relative to more southern populations. Additionally, the Brooks Range appears to provide suitable habitat in future scenarios. However, the Alaska Marmot is currently found in the Brooks Range (Gunderson et al. 2009), so it is uncertain if migration to this region will be possible and/or desirable as it may displace an endemic species.

In addition to the importance of southern populations and the need for additional sampling we also note that over the course of a decade of field surveys and our review of

museum specimens, we confirmed the presence of Hoary Marmots in non-alpine habitats at sea level along coastal areas of Southeast, Southcentral, and Western Alaska. The existence of sea-level populations of Hoary Marmots has been documented for some time (e.g. Heller 1910), but most studies and reviews omit or incorrectly delimit this peculiarity (e.g., Braun et al. [2011]). Given that Hoary Marmots are widely considered to be predominantly alpine (Howell 1915, Braun et al. 2011) and particularly sensitive to climate change as a result (Krajick 2004), and given the long-term yet muddled history of the species along the PNW coast, additional investigation of sea level populations seems warranted both for biogeographic resolution and conservation prioritization.

Finally, our SDMs and molecular results are consistent with Hoary Marmots persisting in southern and/or coastal refugia during the LGM. To date, the southernmost Rocky Mountain populations appear to be the most diverse genetically, but additional sampling (especially in British Columbia) and analysis of the nuclear genome is needed to confirm this finding. Predictions of future habitat all show the greatest reduction in the southern portion of the species distribution, highlighting the need for additional study in this region.

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Figures

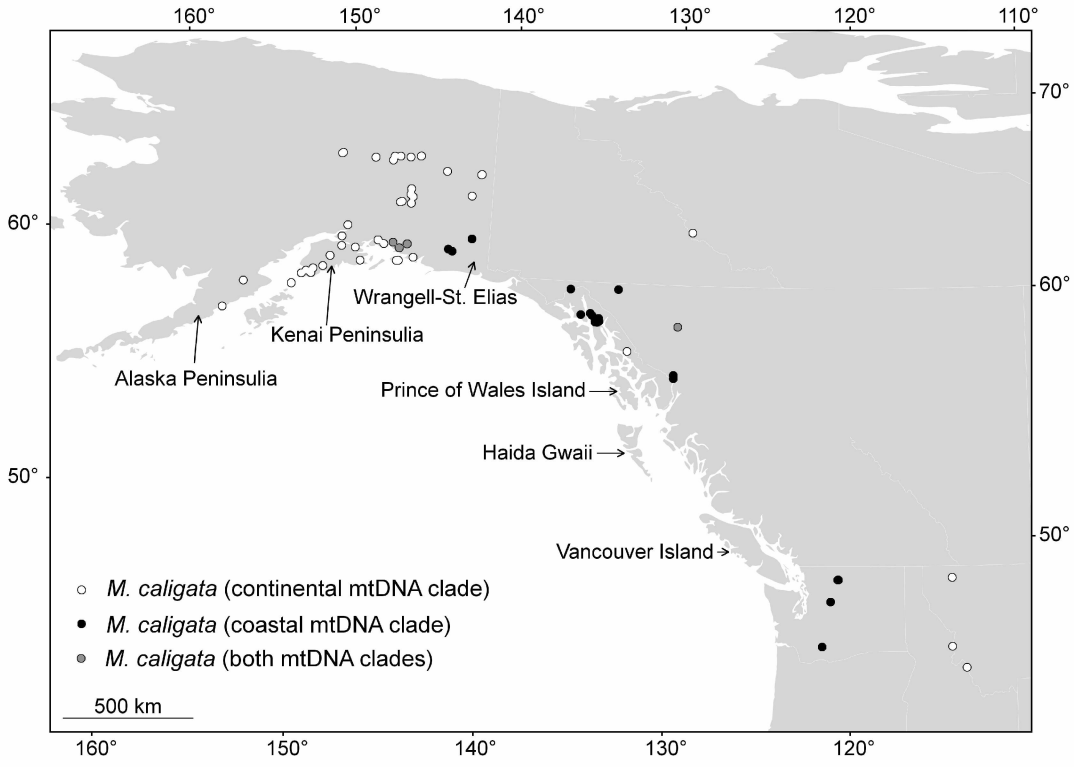

Figure 3.1. Map of the Pacific Northwest (Albers equal-area conic projection) with the distribution and mtDNA clade assignment of the Hoary Marmot (*Marmota caligata*) specimens used in this study. The Alexander Archipelago includes islands along the southeastern coast of Alaska, including Prince of Wales Island.

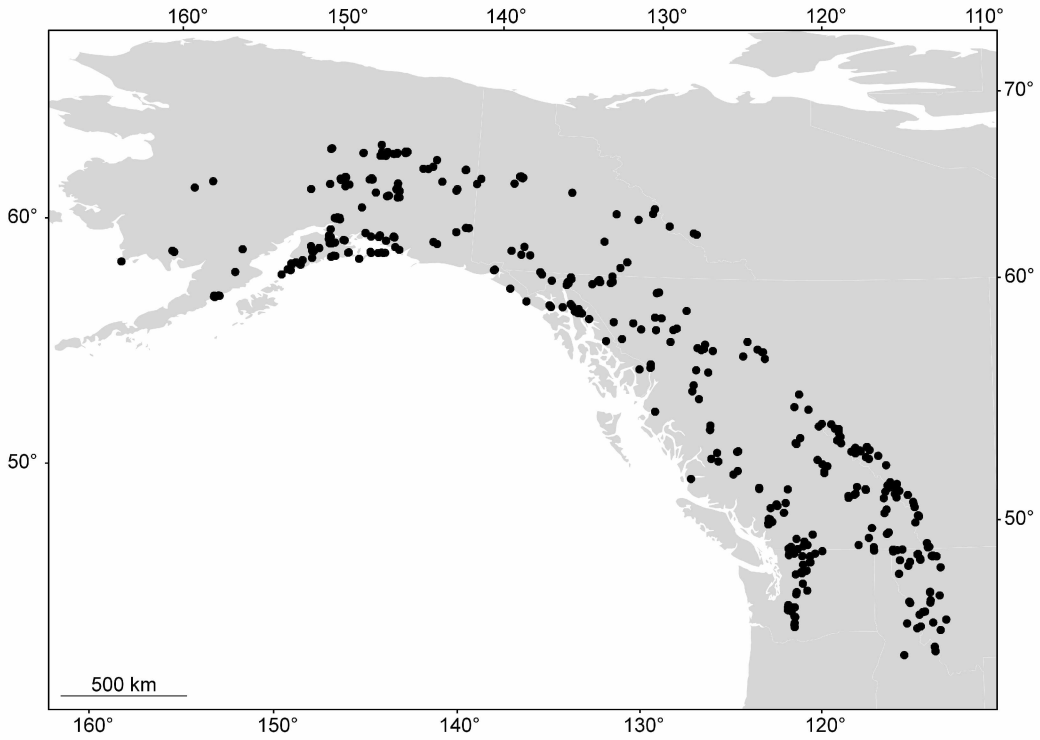

Figure 3.2. Map of the Pacific Northwest (Albers equal-area conic projection) including all georeferenced *Marmota caligata* museum specimens with an uncertainty ≤25 km (n = 975). Specimens are represented by black dots, which are ~25 km in diameter.

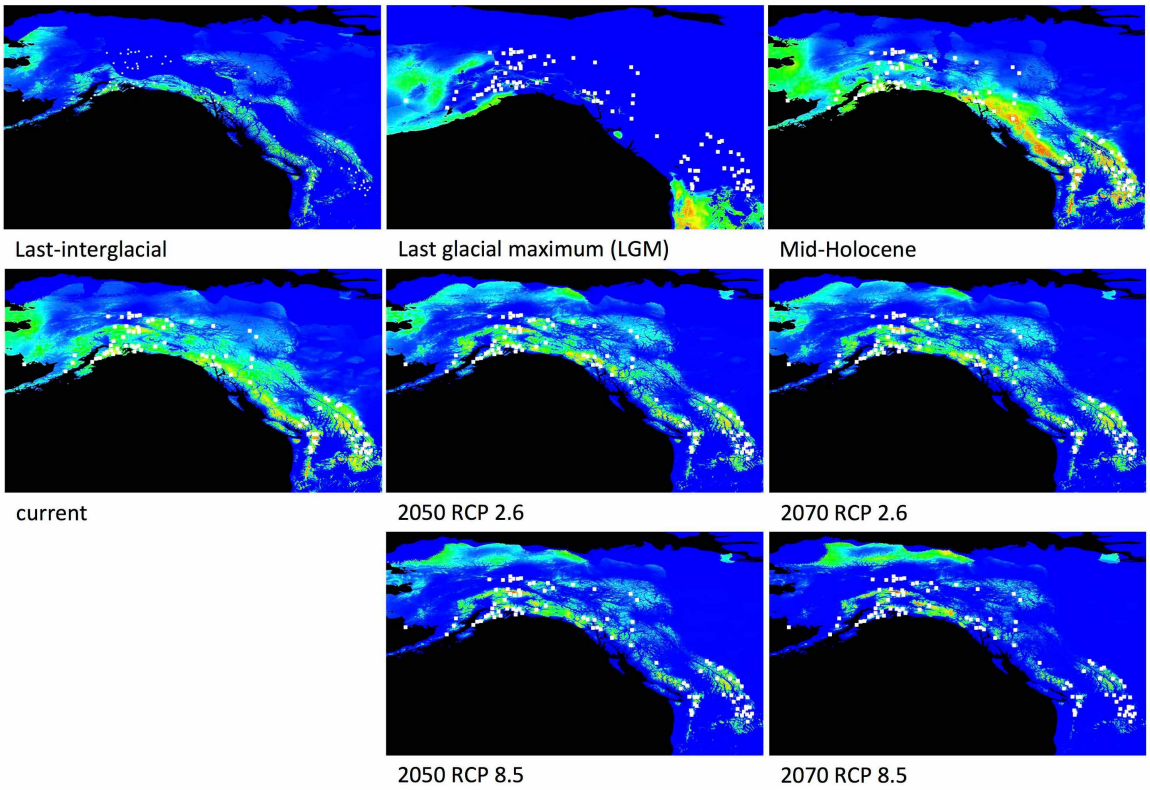

Figure 3.3. Species distribution models for *Marmota caligata* across time. Model was constructed using occurrence points rarified at 25 km and radial buffer of 300 km around occurrence points to construct bias files.

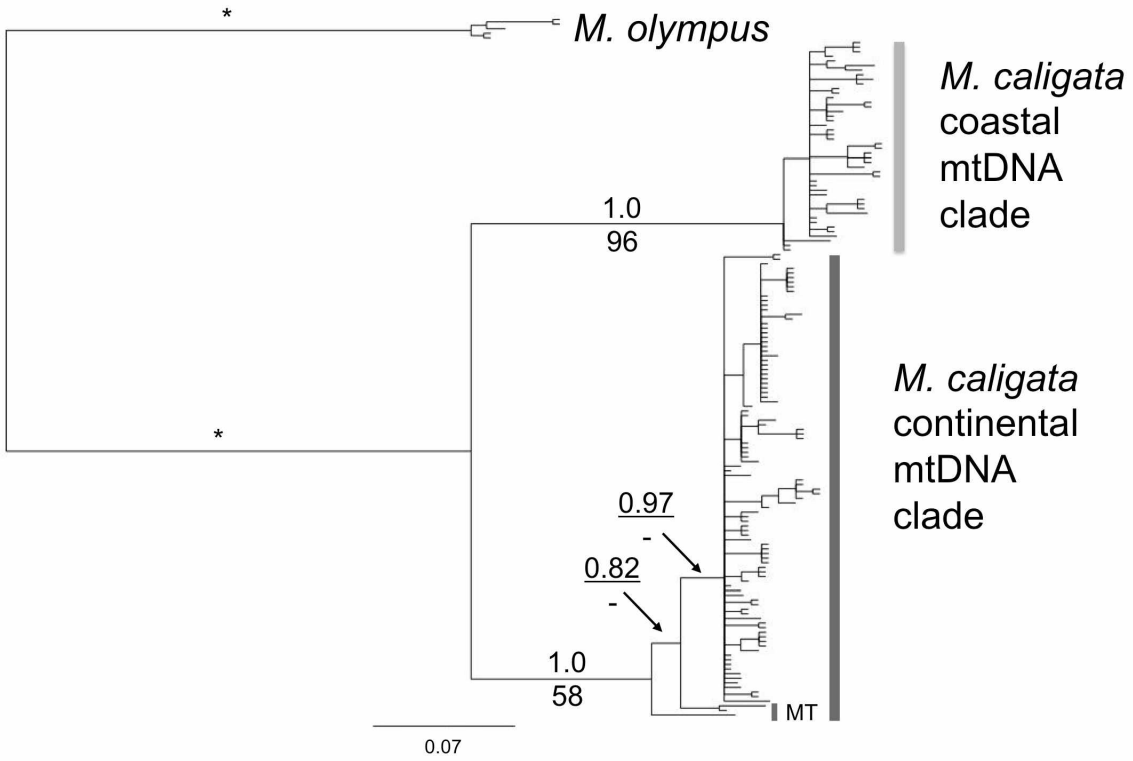

Figure 3.4. Bayesian phylogram of concatenated and partitioned cytochrome *b* and partial control region mtDNA sequence data for the *Marmota caligata* specimens used in this study. Numbers above the line represent posterior probabilities and numbers below the line are the results of a 1000-replicate bootstrap analysis. An asterisk denotes a posterior probability of 1 and a bootstrap score of 100%. A dash denotes a bootstrap value < 50. MT denotes three of the four specimens from Montana; the remaining specimen was nested within the continental clade.

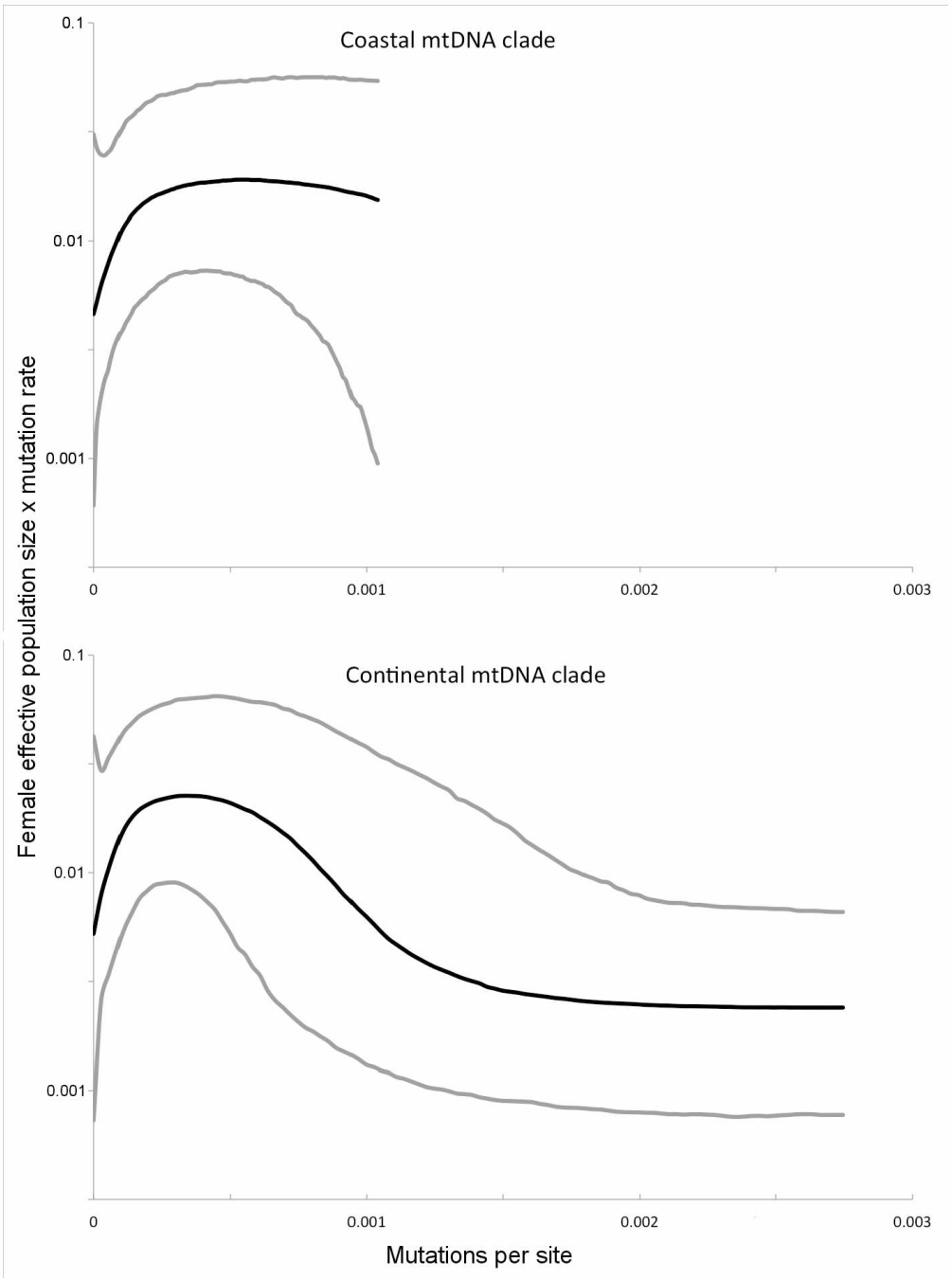

Figure 3.5. Bayesian skyline analysis of the two major Marmota caligata mtDNA clades showing the change in effective female population size across relative time (mutation per site). The black line represents the median value and the gray lines represent the 95% highest posterior density interval.

Table

Table 3.1. Population genetics summary statistics of major Hoary Marmot (*Marmota caligata*) mtDNA clades and subgroups. Summary statistics were calculated using 597 bp of the mitochondrial control region. Significant results are shown in bold. Abbreviations are: *n* = number of samples, *h* = number of haplotypes, θπ = Theta based the number of pairwise differences, S = number of segregating sites, *Fs* = Fu's *Fs*, R_2 = R_2 test, Taj *D* = Tajima's *D*. Three continental clade specimens from interior British Columbia were not included in a subgroup.

Marmota caligata subgroup	*n*	*h*	S	θπ	*Fs*	R_2	Taj *D*
Continental clade all	101	40	39	0.00680	**-28.6034**	**0.0513**	**-1.4361**
Continental clade northern (all)	94	34	27	0.00625	**-21.4247**	0.0681	0.8905
Continental clade northern (unglaciated)	35	8	10	0.00293	-1.4715	0.0849	0.8585
Continental clade northern (glaciated)	59	28	25	0.00672	**-16.5210**	0.0784	-0.8170
Continental clade southern	4	4	12	0.0134	0.0687	0.1563	-0.2259
Coastal clade all	46	24	27	0.00649	**-13.6903**	0.0681	-1.2318
Coastal clade north	18	10	15	0.00719	-1.8010	0.1373	-0.0582
Coastal clade central	21	10	15	0.00590	-1.9320	0.1104	-0.5645
Coastal clade south	7	4	5	0.00271	-0.5380	0.0905	-1.0238

Appendices

Figure 3.A-1. Link to Berkeley Mapper file of the geographical locations of specimens assigned to each subgroup for the population genetic summary statistic analysis. https://goo.gl/EympU3

Figure 3.A-2. Results of historic, current, and future species distribution models for Hoary Marmots (*Marmota caligata*). Models were created with occurrence points rarified at 10, 25, and 50km and using radial bias files using buffer of 100 and 300km from occurrence points and the entire map. File is available for download at: https://goo.gl/3Ajx1A

Table 3.A-1. List of all known *Marmota caligata* specimens. We verified or determined collection locality and uncertainty for all specimens. File is available for download at: https://goo.gl/6342d3

Chapter 4 Patterns of historic and contemporary gene flow in Hoary, Vancouver Island, and Olympic marmots based on multiple classes of molecular data[4]

Abstract

Gene flow appears to have occurred among alpine-dependent marmot species in the Pacific Northwest. We combined data from 9 microsatellite loci with existing sequence data to confirm historic gene flow between the critically endangered Vancouver Island Marmot (*Marmota vancouverensis*) and the Hoary Marmot (*M. caligata*) and determined the origin of nuclear alleles shared between the Olympic Marmot (*M. olympus*) and nearby populations of Hoary Marmots. We also used these data to investigate intraspecific gene flow between 2 reciprocally monophyletic mitochondrial clades of Hoary Marmots. Additionally, to improve the known geographic distribution of Hoary Marmot mitochondrial clades, we extracted and amplified DNA from skin samples of 98 museum specimens without archived fresh tissue to determine clade membership. We created species distribution models to determine if bioclimatic variables could explain the current geographic distribution of the two Hoary Marmot mitochondrial clades. Our findings suggest there was historic gene flow between Hoary and Vancouver Island marmots. We failed to resolve the origin of the nuclear alleles shared between Olympic Marmots and nearby populations of Hoary marmots. We also documented unidirectional gene flow between the 2 Hoary Marmot mitochondrial clades. Bioclimatic variables did not appear to influence the current geographic distribution of these clades. We observed a discord between the geographic distributions of the 2 mitochondrial clades and the structuring of nuclear loci, suggesting male-biased gene flow in Hoary Marmots. Finally, our

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[4] KERHOULAS, N. J. AND L. E. OLSON. 2015. Submitted to the Journal of Mammalogy.

results suggest that Hoary Marmots in Washington may represent a population of potential conservation concern and highlights the need for additional sampling from Washington and southern British Columbia.

Introduction

Several species of marmots from North America's Pacific Northwest (PNW) are dependent on or associated with alpine habitats, which are believed to be especially vulnerable to climate change (Krajick 2004). As a result, these marmot species may face greater negative impacts of climate change relative to species found at lower elevation. The Hoary Marmot (*Marmota caligata*) is the most broadly distributed of these species, occurring from southern Washington, central Idaho, and southern Montana north into Alaska and neighboring Yukon and Northwest Territories of Canada (Gunderson et al. 2009; Braun et al. 2011). The southernmost populations may harbor the highest levels of genetic diversity (Kerhoulas et al. 2015) but, as these populations already occur near mountaintops, they have limited potential to move up in elevation to mitigate changing climatic conditions. The Vancouver Island Marmot (*M. vancouverensis*) is endemic to Vancouver Island, British Columbia, and is classified as Critically Endangered by the International Union for Conservation of Nature (IUCN 2017). The Olympic Marmot (*M. olympus*) only occurs on the Olympic Peninsula in Washington, and while not currently considered a conservation risk (IUCN 2017), the species' small (≤1000 individuals) population (Witczuk et al. 2008) suggests it may warrant a heightened threat status and focused conservation efforts.

Molecular data support Vancouver Island, Olympic, and Hoary marmots as a monophyletic assemblage (Steppan et al. 2011), although phylogenetic relationships among these species are not well resolved (Kerhoulas et al. 2015). Glacial cycles of the Pleistocene have played a major role in shaping the phylogeographic patterns of the PNW (reviewed in Shafer et al. 2010) and molecular dating is consistent with a Pleistocene divergence of these 3 species (Steppan et al. 2011; Kerhoulas et al. 2015). It is likely that the glacial and interglacial cycles of

the Pleistocene provided multiple periods of isolation and secondary contact between these currently allopatric marmot species.

Isolation during the Pleistocene appears to have resulted in 2 reciprocally monophyletic mitochondrial (mtDNA) clades in Hoary Marmots (Kerhoulas et al. 2015; Lanier et al. 2015). A coastal clade has been documented in the Cascade and Coastal mountains of the PNW from southern Washington north to near Valdez, Alaska, and a continental clade that ranges from the Rocky Mountains in southwestern Montana and central Idaho north and west into interior, southwestern, and southcentral Alaska (Kerhoulas et al. 2015). The clades are known to be syntopic in the Interior Mountains of northern British Columbia near Dease Lake as well as in the vicinity of Valdez, Alaska (Fig. 4.1). Further investigation of these mtDNA clades would be interesting as similar mammalian clades from this general region have been representative of species level divergences, both with and without gene flow (Chavez et al. 2011; Arbogast et al. 2017). While previous analysis of nuclear loci suggests genetic admixture of the coastal and continental mtDNA clades in Hoary Marmots (Kerhoulas et al. 2015), patterns of gene flow and directionality remain unclear.

The phylogenetic relationship between Hoary and Vancouver Island marmots is not resolved. Phylogenetic analysis of mtDNA recovers Vancouver Island Marmots as sister to the Hoary Marmot coastal mtDNA clade, which together form a clade sister to the Hoary Marmot continental mtDNA clade. Nuclear loci and morphological data support the Vancouver Island Marmot as distinct from the Hoary Marmot (Cardini et al. 2009; Nagorsen and Cardini 2009; Kerhoulas et al. 2015). The discord between mtDNA and the nuclear and morphological data appears to be the result of Vancouver Island Marmots capturing the mitochondrial genome of the Hoary Marmot coastal clade during the Pleistocene (Kerhoulas et al. 2015). Vancouver Island

Marmots have been the subject of a substantial conservation effort that includes an ongoing captive breeding and reintroduction program (Keeley et al. 2011). Understanding the phylogenetic relationships and history of gene flow between Vancouver Island and Hoary marmots may prove critical to conservation, particularly if genetic rescue (Hedrick and Fredrickson 2009) of the former becomes necessary.

The phylogenetic position of Olympic Marmots relative to Vancouver Island and Hoary marmots is likewise unresolved. Mitochondrial data place Olympic Marmots as sister to Vancouver Island and Hoary marmots (Steppan et al. 2011; Kerhoulas et al. 2015). In contrast, several nuclear alleles are shared exclusively between Olympic and Hoary marmots from nearby populations in Washington (Kerhoulas et al. 2015). It remains unclear if these shared alleles are the result of incomplete lineage sorting and/or gene flow. If gene flow is responsible for the nuclear alleles shared between these species it may indicate that chromosomal rearrangements do not necessarily inhibit genetic exchange as Olympic and Hoary marmots have 40 and 42 chromosomes, respectively, the apparent result of a Robertsonian translocation (Rausch and Rausch 1965; Hoffmann and Nadler 1968; Rausch and Rausch 1971) . We note that the Olympic Marmot chromosomal number appears to be based on a single male specimen (Rausch and Rausch 1965; 1971) and may warrant further analysis.

Olympic Marmots have the second-smallest range and population of all North American marmot species (the smallest belonging to Vancouver Island Marmots). At the state level Olympic Marmots have been a candidate species for listing as Endangered, Threatened, or Sensitive since 2008 (Washington Department of Fish and Wildlife 2013; 2017), but currently lack any protective conservation status. Their small population size and range, as well as recent population declines (Witczuk et al. 2008), strongly suggests Olympic Marmots will soon be a

conservation concern. Determining the origin of nuclear alleles shared between Olympic and Hoary marmots may be useful for future conservation plans. If Olympic Marmot alleles are present in Hoary Marmots from Washington, these populations could potentially aid in the conservation of Olympic Marmot genetic diversity and/or be useful for genetic rescue.

We combine microsatellite data with existing molecular sequence data to: 1) determine if gene flow is occurring or has recently occurred between the 2 mtDNA clades of Hoary Marmots and, if so, its directionality, 2) confirm previous findings that Vancouver Island Marmots captured Hoary Marmot mtDNA, and 3) determine the origin of nuclear alleles shared between Olympic and Hoary marmots. We also clarify the known distribution of the 2 Hoary Marmot mtDNA clades by extracting and amplifying DNA from historic museum specimens collected at locations not represented by archived fresh tissue. Finally, we create species distribution models (SDMs) for each Hoary Marmot mtDNA clade to test if bioclimatic variables could accurately predict current clade distributions.

Materials and Methods

Molecular

We amplified 15 microsatellite loci previously developed for use in marmots and closely related species: 2h6b, 3b1, 2g2 (Kyle et al. 2004), GS14, GS25 (Stevens et al. 1997), MA018, MA066, MA001, MA091 (Da Silva et al. 2003), MS53, MS56, MS6, ST10 (Hanslik and Kruckenhauser 2000), SS-Bilb18, SS-Bilb25 (Goossens et al. 1998). Amplification of microsatellite loci followed the method of Schuelke (2000). Because this method does not allow for multiplex reactions, they were simulated for analyses by combining 1.5 μl from each of 3 PCR reactions of independent loci for the same individual (each locus using a different dye) and combined with 5.5 μl Hi-Di Formamide (Applied Biosystems, Foster City, CA) for fragment

analysis. Microsatellite loci were analyzed at the DNA Analysis Facility on Science Hill at Yale University using a Liz-500 size standard (Applied Biosystems, Foster City, California). We scored alleles using GENEMARKER ver. 2.6.0 (SoftGenetics, State College, PA) and reformatted microsatellite data using MICROSATELLITE ANALYSER (MSA) ver. 4.05 (Dieringer and Schlötterer 2003). Microsatellite loci were checked for linkage disequilibrium and deviations from Hardy-Weinberg equilibrium using GENEPOP ver. 1.2 (Raymond and Rousset 1995; Rousset 2008), treating each of the genetic clusters identified in our STRUCTURE analysis as a distinct population.

To test for gene flow we conducted an isolation with migration (IM) analysis using the program IMa2 ver. 8.27.12 (Hey 2010). These analyses included 9 microsatellite loci combined with existing sequence data from 2 mitochondrial loci (cytochrome *b*, 1140 bp and control region, 597 bp) and 4 nuclear loci (primarily intronic, 2049 bp total) (see Kerhoulas et al. 2015). IM analyses followed the methods of Kerhoulas et al. (2015), with microsatellite data converted to integers based on the number of repeats (inferred from allele length) and analyzed using a stepwise mutation model.

Because the phylogenetic relationship among the 3 species remains uncertain, we conducted pairwise IM analyses between Hoary Marmots and both Vancouver Island and Olympic marmots. We also performed IM analysis between the 2 Hoary Marmot mtDNA clades. When testing for intraspecific gene flow in Hoary Marmots, we excluded specimens from Washington (where nuclear alleles are shared with Olympic Marmots) to avoid potentially confounding effects of introgression. For each IM analysis, we determined optimal prior settings based on several preliminary runs. Final Markov-chain Monte Carlo (MCMC) runs used a unique starting seed, a 3×10^6 burn-in, and 10^7 steps with a total of 10^5 saved genealogies. We

checked effective sample size values, trend plots, and update rates to optimize prior settings and determine when adequate runtimes had been reached. We conducted 2 independent runs of each final IM analysis to ensure that the results obtained were consistent. We compared 5 migration models using the IM L-mode analysis: (1) no migration; (2) unidirectional migration from group 0 to group 1; (3) unidirectional migration from group 1 to group 0; (4) bidirectional migration with a single rate; and (5) bidirectional migration with 2 rates.

To test for introgression and additional support for the mtDNA clades a clustering analysis of all nuclear data (9 microsatellite and 4 nuclear loci) was conducted using STRUCTURE ver. 2.3.4 (Pritchard et al. 2000). For this analysis sequence data were assigned integers based on haplotype. We used the sampling location for each individual (coded as an integer) as prior information using the LOCPRIOR model (Hubisz et al. 2009) to assist with clustering. We conducted several STRUCTURE runs using the hierarchical method outlined in (Coulon et al. 2008). We used an admixture model with correlated allele frequencies and assumed the true number of groups (K) was between 1 and 15 for the initial run and 1 and 10 for subsequent hierarchical method analyses. For each value of K we performed 10 independent runs, with a 10^5 burn-in followed by 5×10^5 MCMC iterations. Automation and parallelization of the STRUCTURE analysis was conducted using StrAuto ver. 1.0 (Chhatre and Emerson 2017). Results were analyzed, averaged, and visualized using STRUCTURE HARVESTER ver. 0.6.94 (Earl and vonHoldt 2012), CLUMPP ver. 1.1.2 (Jakobsson and Rosenberg 2007), and DISTRUCT ver. 3.2.57 (Rosenberg 2003), respectively. We used both the peak in the mean probability (Pritchard et al. 2000) and the ΔK method (Evanno et al. 2005) to determine the number of genetic clusters.

To better delimit the distribution of Hoary Marmot mtDNA clades, we extracted DNA from skin snips or adventitious tissue removed from skeletal material from 138 museum specimens that were not associated with archived fresh tissue (Table 4.A-2). DNA extraction was conducted in the University of Alaska Museum's ancient DNA extraction facility using the Promega DNA IQ system (Promega Corp. Madison, WI) following the manufacturer's protocol. We used one universal primer and Hoary Marmot-specific primers to amplify and sequence overlapping segments of the mitochondrial cytochrome-*b* gene (Table 4.A-1). PCR thermal-cycling parameters follow those presented in Kerhoulas and Arbogast (2010), but used an annealing temperature of 48° for 1.5 minutes. Once a successful PCR was obtained, clade assignment was determined via Sanger sequencing and/or the use of a restriction enzyme.

To classify specimens to an mtDNA clade using a restriction enzyme, we first amplified a 222 bp segment of the cytochrome-*b* gene using primers MACA-L4 and MACA-R4 (Table 4.A-1). We then added 3.75 µl of NE Buffer 3 and 0.35 µl of restriction enzyme Bs1I (New England BioLabs, Ipswich, MA) to 25 µl of each successful PCR reaction (confirmed via gel electrophoresis) and incubated at 55 °C for 70 minutes, followed by 80 °C for 20 minutes, and then held at 4 °C. Gel electrophoresis was used to visualize the size and number of fragments produced. Members of the coastal clade lacked a binding site for this restriction enzyme, producing a single (222 bp) band, while members of the continental clade had a single binding site and produced bands at 91 and 131 bp. Selection of this restriction site was based on sequence data from 167 Hoary Marmot specimens with known clade membership (Kerhoulas et al. 2015).

Georeferencing specimen records

For the Hoary Marmot museum specimens, we georeferenced and/or verified previously assigned spatial data following the BioGeomancer protocol (Chapman and Wieczorek 2010).

Commonly used ambiguous terms were dealt with as follows using the highest resolution United States Geological Survey (USGS) topology maps available: "mountain/mount" = centered on peak extending to mountain base; "head of river/creek" = centered on highest terminal point of waterway extending to summit of nearest peak; "lake" = centered on lake extending to summit of adjacent mountains. Uncertainty was determined using the Georeferencing Calculator ver. 20151221 (Wieczorek and Wieczorek 2015).

Species distribution modeling.— We modeled the distribution of suitable habitat for both the coastal and continental clades using SDMs created using the program MAXENT ver. 3.3 (Phillips et al. 2006) to explore whether the current geographic distributions of the two Hoary Marmot mtDNA clades could be attributed to specific bioclimatic factors and whether these factors differed between haplotype clades (suggesting some degree of ecological divergence). We used all specimens assigned to a clade from the current and previous studies and georeferenced with an uncertainty $\leq$ 10 km with the exceptions of two specimens: USNM 241748 and MVZ 964 (justified in results). Occurrence data were spatially rarefied at 25 km to reduce sampling bias (Boria et al. 2014). SDMs used bioclimatic data for current conditions with a 5 arc-minute resolution obtained from WorldClim.org (Hijmans et al. 2005). A bias file was used to limit background sampling to a 300-km radial buffer of occurrence points and to correct for latitudinal background selection bias (Anderson and Raza 2010; Barbet-Massin et al. 2012; Brown 2014). For all SDMs, we spatially jackknifed models, splitting the landscape into 3 regions and testing all model feature classes with 12 rate parameters evenly spaced between 0.25 and 3, with five replicates run for each combination. SDMtoolbox (Brown 2014) was used to crop bioclimatic layers, rarefy occurrence data, create bias files, and set up the MAXENT analyses.

Results

Nine of the 15 microsatellite loci screened produced useable results. The 6 omitted loci (2g2, MA066, MA001, MA091, ST10, SS-Bibl25) had regions of ambiguous peaks and/or produced a product greatly different in size than previously observed in marmots or closely related species. We found no deficiency in heterozygotes or significant linkage disequilibrium among loci using a Bonferroni corrected alpha value that accounted for multiple tests (i.e. $\alpha = 0.05/8 = 0.00625$).

The direction of gene flow inferred by ranking migration models in the L-mode analyses of IMa2 was consistent among independent replicates. For Hoary Marmot clades, unidirectional gene flow from the coastal to the continental clade was the best-ranked model and supported by Akaike weights and evidence ratios (Burnham et al. 2010) (Table 4.1). In one replicate, a unidirectional migration model with 1 additional parameter (i.e. a bidirectional migration model with 2 rates, one of which being zero) also received moderate support. Unidirectional gene flow from Hoary to Vancouver Island marmots was the best-ranked model and was also supported by Akaike weights and evidence ratios (Table 4.1). As above, a unidirectional model with an additional parameter was also supported. No gene flow between Hoary and Olympic marmots was the best model, but all models received similar levels of support (Table 4.1).

The mean likelihood of the STRUCTURE analysis peaked at $K = 8$. Well-defined clusters were recovered for Vancouver Island Marmots, Olympic Marmots, and Hoary Marmots from Montana, Washington, Douglas Island, and Hinchinbrook Island (Fig. 4.2). Hoary Marmots from the region of syntopy near Dease Lake were slightly admixed, but generally belonged to a single cluster, with all remaining Hoary Marmots comprising the remaining cluster (Fig. 4.2). Using the ΔK method, $K = 2$ was the most probable number of genetic clusters. Under this

scenario, one group contained Vancouver Island, Olympic, and Hoary marmots from Washington and Montana and the other group was composed of all remaining Hoary Marmot specimens. For both of these groups subsequent analyses recovered $K = 4$ as the most likely number of genetic clusters using both the peak in the mean probability and the ΔK methods. The resulting 8 clusters (Fig. 4.A-2) were nearly identical to those found using the peak in the mean likelihood (Fig. 4.2). Notable differences were that in this analysis Hoary Marmots from the region of syntopy near Dease Lake formed a unique cluster and those from near Juneau were nearly equally admixed between 3 clusters (Fig. 4.A-2).

We determined mtDNA haplotype clade membership for 98 of 138 Hoary Marmot museum specimens that lacked archived fresh tissue (Table 4.A-2). Of the museum specimens assigned to a mtDNA clade, Sanger sequencing and restriction enzymes were used to classify 17 and 81 specimens, respectively. For all but 2 of these specimens (USNM 241748 and MVZ 964), the geographic distribution of clades was compatible with the distribution of 167 specimens previously assigned in Kerhoulas et al. (2015). Restriction enzyme products indicated that both of these specimens belonged to the coastal clade. Specimen USNM 241748 is from Interior Alaska, a region otherwise occupied exclusively by the continental clade, and thus likely represents contamination and/or erroneous locality data. Specimen MVZ 964 was collected on Hinchinbrook Island, AK, where only continental clade specimens have been previously documented (Kerhoulas et al. 2015). The assignment of this specimen to the coastal clade is ambiguous for a number of reasons. Coastal clade specimens have been collected from the mainland within 65 km of Hinchinbrook Island, but previous molecular analyses of specimens with archived fresh tissue placed Hinchinbrook Island Hoary Marmots in the continental clade (Kerhoulas et al. 2015). The specimen in question was collected from a different region of the

island more than 100 years before those assigned to the continental clade were collected; the island therefore could possibly be home to both haplotype clades and represent multiple separate colonization events. In any case, we ultimately excluded these two questionable specimens from further analyses and confidently assigned 46 and 50 specimens to the coastal and continental clades, respectively (Table 4.A-2, Fig. 4.1, Fig. 4.A-3).

Combining specimens assigned to a clade by this study with the results of Kerhoulas et al. (2015) yielded a total of 223 specimens with an uncertainty $\leq$ 10 km available for SDMs. Of these, 71 and 152 belonged to the coastal and continental clades, respectively. After spatial rarefication, a total of 28 coastal and 48 continental specimens were retained. Habitat from throughout the known distribution of the coastal clade was recovered as suitable for both clades (Fig. 4.A-2). Suitable habitat outside the known distribution of the coastal clade was predicted in portions of the Rocky Mountains from central and southern Alberta and eastern British Columbia, the southernmost portion of the Kuskokwim Mountains in Alaska, and the Kenai and Alaska Peninsulas (Fig. 4.A-2) Portions of Southeast Alaska and coastal British Columbia were not recovered as suitable for the coastal clade (Fig. 4.A-2). Habitat from throughout the entire known distribution of Hoary Marmots (i.e. both mtDNA clades) was recovered as suitable for the continental clade (Fig. 4.A-2).

Discussion

Results of our IM and STRUCTURE analyses support gene flow between the coastal and continental mtDNA clades of Hoary Marmots. Our IM analysis suggests ongoing unidirectional gene flow from the coastal to the continental Hoary Marmot mtDNA clade is the most likely (Table 4.1). However, the STRUCTURE analysis of nuclear loci alone did not recover population clusters congruent with the coastal and continental mtDNA clades (Fig. 4.2). An

additional STRUCTURE analysis of the Hoary Marmot using only microsatellite loci (not shown) produced similar results.

Given the relatively fast sorting time of microsatellite loci, the lack of congruence between mtDNA clades and nuclear loci may reflect sex-biased gene flow. Male Hoary Marmots have been shown to disperse from the natal colony at a higher rate than females (Kyle et al. 2007), as is the case for most mammal species (Greenwood 1980). Male-biased dispersal has also been documented in the closely related Yellow-bellied Marmot (*Marmota flaviventris*) (Downhower and Armitage 1981). Male-biased dispersal does not necessarily preclude females from contributing the same (or more) to gene flow between colonies, if females have a greater probability of assimilation and/or reproductive success once reaching an existing colony. However, our results of limited sympatry between the mtDNA clades and shared population structure in the nuclear data are consistent with male-biased gene flow.

The greatest genetic diversity in the continental clade appears to occur at the southern extent of its distribution (Kerhoulas et al. 2015), suggesting that this region served as a Pleistocene refugium (Hewitt 1996) and that a northward post-Pleistocene expansion was most likely. Under this scenario marmots presumably expanded north after the Last Glacial Maximum (LGM) along the ice-free corridor present on the eastern slope of the Rocky Mountains that opened ~13,400 BP (Heintzman et al. 2016). The limited mtDNA variation present in the coastal clade provides scant insight into the phylogeographic history of this group. However, the increased structure of the nuclear data observed in Hoary Marmot specimens from Washington (coastal mtDNA clade) suggests the existence of a southern refugium and a rapid northward expansion in this clade as well. Alternatively, the limited genetic variation observed in the coastal clade might be the result of a population bottleneck during Pleistocene isolation within a

coastal refugium (Kerhoulas et al. 2015; Knowles et al. 2016). A post-LGM expansion of this clade may have occurred via the alpine regions of the Coast and Cascade mountains and/or along a coastal corridor(s), as the species is known to occur at and near sea level (Braun et al. 2011). Although a northern refugium has also been suggested (Lanier et al. 2015; Knowles et al. 2016), previous tests of this hypothesis did not include specimens from the southern half of the species' distribution.

The Interior Mountains of northern British Columbia connect the Coastal and Rocky mountains, suggesting they would likely be the first place the 2 Hoary Marmot clades would have experienced secondary contact, assuming post-Pleistocene expansion from southern and/or coastal refugia. The prevalence of the coastal clade in this region (Fig. 4.1, Fig. 4.A-3) may suggest its dominance and/or that a nearby coastal refugium in northern British Columbia and/or Southeast Alaska facilitated early colonization. Members of both haplotype clades currently occur in this region and SDMs recovered much of the area currently occupied by the coastal clade as slightly more suitable for the continental clade (Fig. 4.A-2). Our STRUCTURE analyses of nuclear data found specimens from this region formed a unique cluster that was slightly admixed (Fig. 4.A-2). This clustering of the nuclear loci and evidence of ongoing unidirectional gene flow would suggest that dispersal might be limited in this region. Unfortunately, all specimens with archived fresh tissue from this region are from a single locality, limiting our inference and highlighting the need for additional sampling in northern British Columbia.

In the other region of Hoary Marmot haplotype clade sympatry near Valdez, Alaska, both clades were found in similar numbers in syntopy (Fig. 4.A-3). In this region specimens from both mtDNA clades were assigned to the same genetic cluster based on nuclear data (Fig. 4.2, Fig. 4.A-4), suggesting ongoing gene flow between the clades, as predicted by our IM results (Table

1). In contrast, specimens from the region of mtDNA clade syntopy in the Interior Mountains of British Columbia exhibited some population structure based on nuclear data (Fig. 4.2), suggesting the potential for different patterns or degrees of gene flow at each area of secondary contact.

Populations of Hoary Marmots from islands that were colonized post-LGM provide insight into the history of colonization and the ability of our data to resolve recent isolation. Hoary Marmots from Montague (no recent specimens) and Hinchinbrook islands, Alaska, belong to the continental clade (Fig. 4.1, Fig.4.A-3), suggesting this haplotype clade was likely the first to reach this region. Additionally, marmots from Douglas Island, Alaska (coastal clade) and Hinchinbrook Island each formed unique or nearly unique genetic clusters in the STRUCRURE analyses (Fig. 4.2, Fig. 4.A-4). Since both of these islands were likely overrun with ice during the LGM (Kaufman and Manley 2004) and thus colonized post-LGM, the presence of distinct genetic clusters suggests that the markers used were sufficient to detected recent population isolation.

The known distribution of the Hoary Marmot's mtDNA clades was greatly improved by the addition of 98 museum specimens (Fig. 4.1). As noted above, the coastal clade appears to dominate northern British Columbia, but additional sampling from the northwestern portion of the province may recover additional continental specimens. We note that nearly half of the specimens sampled from northern British Columbia were collected over a century ago and thus may not be entirely representative of the current distribution of the haplotype clades. Despite the expansive distribution of Hoary Marmots in western Canada, museum specimens with fresh tissue samples are extremely limited: 10 from British Columbia (representing 3 localities) and 2

from the Northwest Territory (from a single locality). This study highlights the need for additional sampling and tissue archiving for Hoary Marmots in Canada.

Our results clearly support previous findings that the Vancouver Island Marmot recently captured the mitochondrial genome of the Hoary Marmot (Kerhoulas et al. 2015). Our IM analysis recovered unidirectional gene flow from Hoary to Vancouver Island Marmots as the best model and our STRUCTURE analysis of nuclear loci found that Vancouver Island Marmots formed a unique genetic cluster. This, combined with the morphological distinctiveness of Vancouver Island Marmots (Cardini et al. 2007; 2009), suggests genetic exchange with Hoary Marmots was limited, thereby preserving the majority of the unique nuclear diversity of Vancouver Island Marmots. Contemporary gene flow between these species is likely inhibited by geographic isolation, but reproductive incompatibility remains untested. Overall our results corroborate the hypothesis of historic gene flow with Hoary Marmots leading to mitochondrial capture in this Critically Endangered species.

Our study was unable to resolve the origin of nuclear alleles shared between Olympic and Hoary marmots from Washington. The best-ranked IM model was "no migration," which suggests incomplete lineage sorting, but all models received similar levels of support so introgression could not be ruled out (Table 4.1). In contrast to previous research (Kerhoulas et al. 2015), our STRUCTURE analysis recovered Olympic Marmots as a unique group and not part of a group that included Hoary Marmots from Washington (Fig. 4.2). Regardless of origin, the genetic diversity recovered in Hoary Marmots from Washington should be considered an important conservation priority. This diversity represents Olympic Marmot genes that have introgressed into Hoary Marmots and/or the most genetically diverse region of the Hoary Marmot coastal clade. If the shared alleles originated from Olympic Marmots, this provides an

additional path to conserving Olympic Marmot genetic diversity outside of the Olympic Peninsula as well as a potential source population for genetic rescue. If this diversity is the result of incomplete lineage sorting, it likely represents the increased genetic diversity often documented at the rear edge of expanding populations and an important diversity hotspot for the Hoary Marmot coastal clade that may warrant consideration as a conservation priority (Hampe and Petit 2005).

We have shown that ongoing unidirectional genetic exchange from the coastal to the continental mtDNA clade is likely occurring in Hoary Marmots. Evidence of gene flow, the discord between the geographic distribution of the mtDNA clades, as well as the structuring of the nuclear data suggests male-biased gene flow. The SDMs show that bioclimatic factors do not appear to be influencing the distribution of the Hoary Marmot mtDNA clades and that habitat suitability is similar for both clades at regions of syntopy. Clade assignment of museum specimens without archived tissue samples found that most of northwestern British Columbia was occupied by the coastal clade of Hoary Marmots. This finding suggests the coastal mtDNA clade may have been the first to colonize northwestern British Columbia or that it has displaced the continental clade in this region. Our results further support Vancouver Island Marmots as a distinct species that likely captured the mitochondria of Hoary Marmots. Finally, the origin of nuclear alleles shared between Olympic and Hoary Marmots from Washington remains enigmatic. Regardless of origin, the genetic diversity documented in Hoary Marmots from Washington may represent a region of conservation priority and we recommend additional sampling in southern British Columbia to document the extent of this diversity hotspot.

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Figures

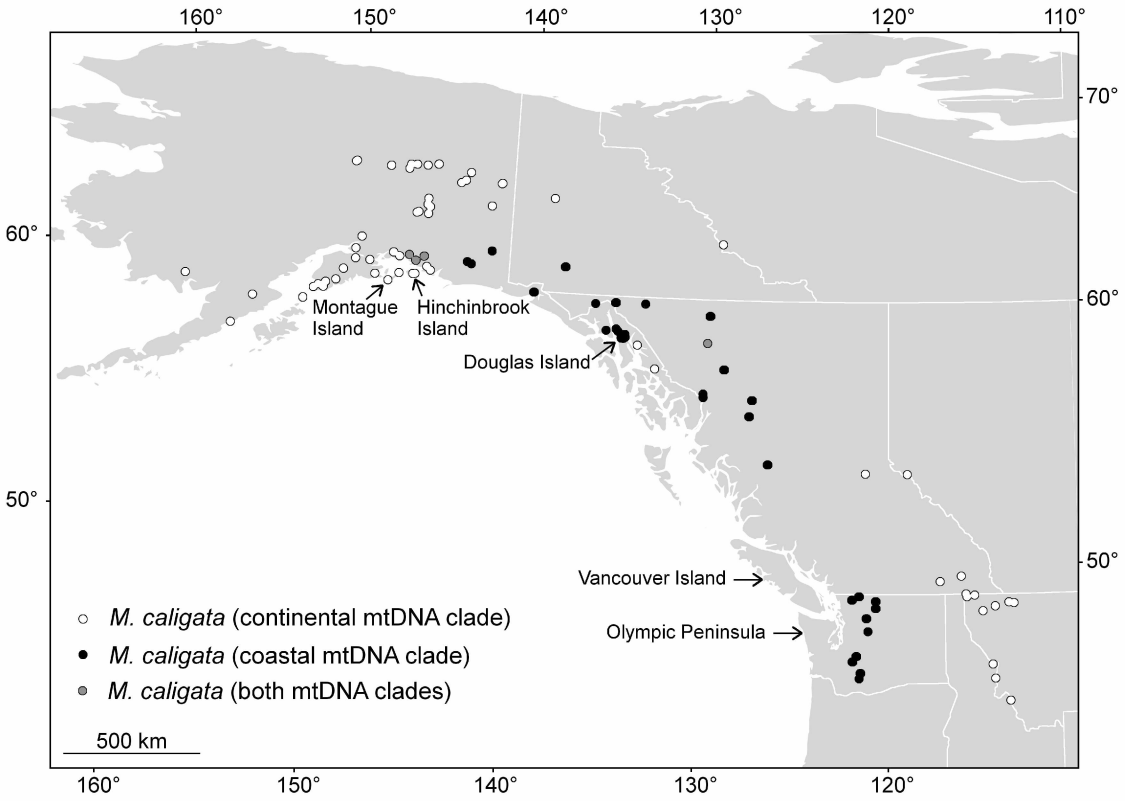

Figure 4.1. Distribution of *Marmota caligata* specimens and mitochondrial (mtDNA) clade membership. Only specimens with a coordinate uncertainty ≤ 25 km (~ the size of the dots used) are shown. For an interactive map of all specimens with mtDNA clade information see Fig. 4.A-3.

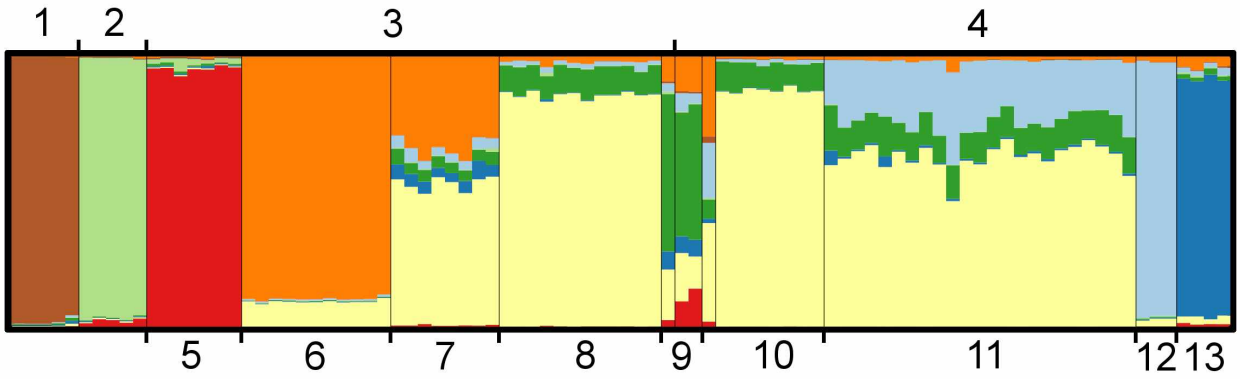

Figure 4.2. Results of a STRUCTURE analysis (K = 8) of 4 nuclear and 9 microsatellite loci for Vancouver Island, Olympic, and Hoary Marmots. Numbers 1-4 correspond to: (1) Vancouver Island Marmots, (2) Olympic Marmots, (3) Hoary marmots from the coastal mtDNA clade, and (4) Hoary Marmots from the continental mtDNA clade. Numbers 5-13 correspond to Hoary Marmots from the following localities: (5) Washington State, (6) Douglas Island, AK, (7) near Juneau AK, (8) Prince William Sound (coastal mtDNA clade), (9) British Columbia (area surrounding where clades are syntopic), (10) Prince William Sound (continental mtDNA clade), (11) Kenai Peninsula, (12) Hinchinbrook Island, AK, and (13) Montana.

Table

Table 4.1. Ranking of nested models of migration from an IMa2 L-mode analysis. Migration rates are presented in the forward direction and values in brackets were fixed to meet the assumptions of the model. The values presented are: K = number of model parameters, AIC = Akaike Information Criterion, Δ_i = difference in AIC from best model, ω_i = Akaike weights, and $E_{min}/_i$ = evidence ratio.

Species/clades compared	Model	Forward migration	Forward migration	log(*P*)	K	AIC	Δ*i*	ω*i*	$E_{min/i}$
Hoary Marmot clades	Migration unidirectional (coastal to continental)	[0.000]	13.081	-6.025	4.000	20.050	0.000	0.831	1.000
	Migration bidirectional (2 rates)	3.059	15.695	-7.241	5.000	24.482	4.432	0.091	9.171
	Migration bidirectional (1 rate)	22.886	[22.886]	-8.380	4.000	24.760	4.710	0.079	10.538
	Migration unidirectional (continental to coastal)	18.781	[0.000]	>100	4.000	>100	>100	0.000	>100
	No migration	[0.000]	[0.000]	>100	3.000	>100	>100	0.000	>100
Hoary and Vancouver Island marmots	Migration unidirectional (*M. caligata* to *M. vancouverensis*)	0.754	[0.000]	2.218	4.000	3.564	0.000	0.583	1.000
	Migration bidirectional (2 rates)	0.754	0.000	2.218	5.000	5.564	2.000	0.214	2.718
	No migration	[0.000]	[0.000]	-0.390	3.000	6.781	3.217	0.117	4.995
	Migration bidirectional (1 rate)	0.000	[0.000]	-0.390	4.000	8.781	5.217	0.043	13.577
	Migration unidirectional (*M. vancouverensis to M. caligata*)	[0.000]	0.000	-0.390	4.000	8.781	5.217	0.043	13.577
Hoary and Olympic marmots	No migration	[0.000]	[0.000]	-1.079	3.000	8.158	0.000	0.341	1.000
	Migration bidirectional (1 rate)	0.2410	[0.2405]	-0.335	4.000	8.669	0.511	0.264	1.291
	Migration unidirectional (*M. olympus to M. caligata*)	[0.000]	0.2120	-0.868	4.000	9.735	1.577	0.155	2.200
	Migration unidirectional (*M. caligata to M. olympus*)	0.1880	[0.000]	-1.017	4.000	10.034	1.876	0.134	2.555
	Migration bidirectional (2 rates)	0.369	0.214	-0.246	5.000	10.492	2.334	0.106	3.212

Appendices

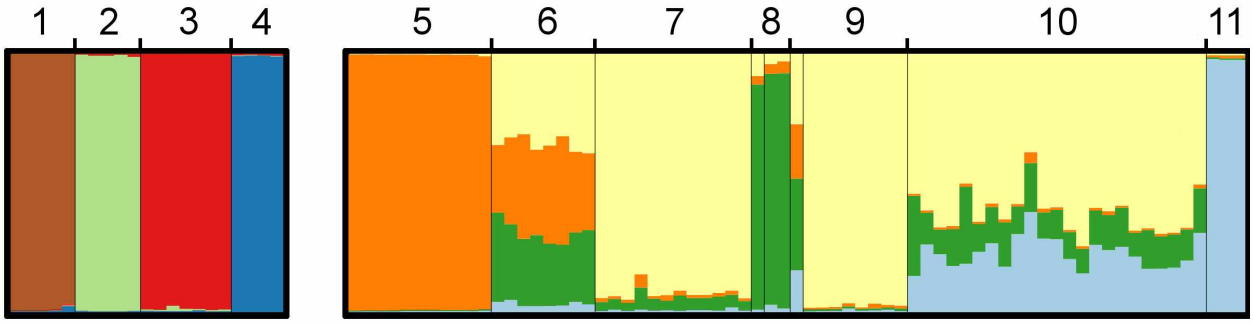

Figure 4.A-1. Results of subsequent STRUCTURE analyses of 2 major groups identified in the initial hierarchical STRUCTURE analysis of Vancouver Island, Olympic, and Hoary Marmots using 4 nuclear and 9 microsatellite loci. For each group the most likely number of clusters was K = 4 using both the ΔK method and the peak in the mean likelihood. One group consisted of: (1) Vancouver Island Marmots, (2) Olympic Marmots, (3) Hoary marmots from Washington, (4) Hoary Marmots from Montana and the other group consisted of Hoary Marmots from: (5) Douglas Island, AK, (6) near Juneau AK, (7) Prince William Sound (coastal mitochondrial clade), (8) British Columbia (area surrounding where clades are syntopic), (9) Prince William Sound (continental mitochondrial clade), (10) Kenai Peninsula, and (11) Hinchinbrook Island, AK.

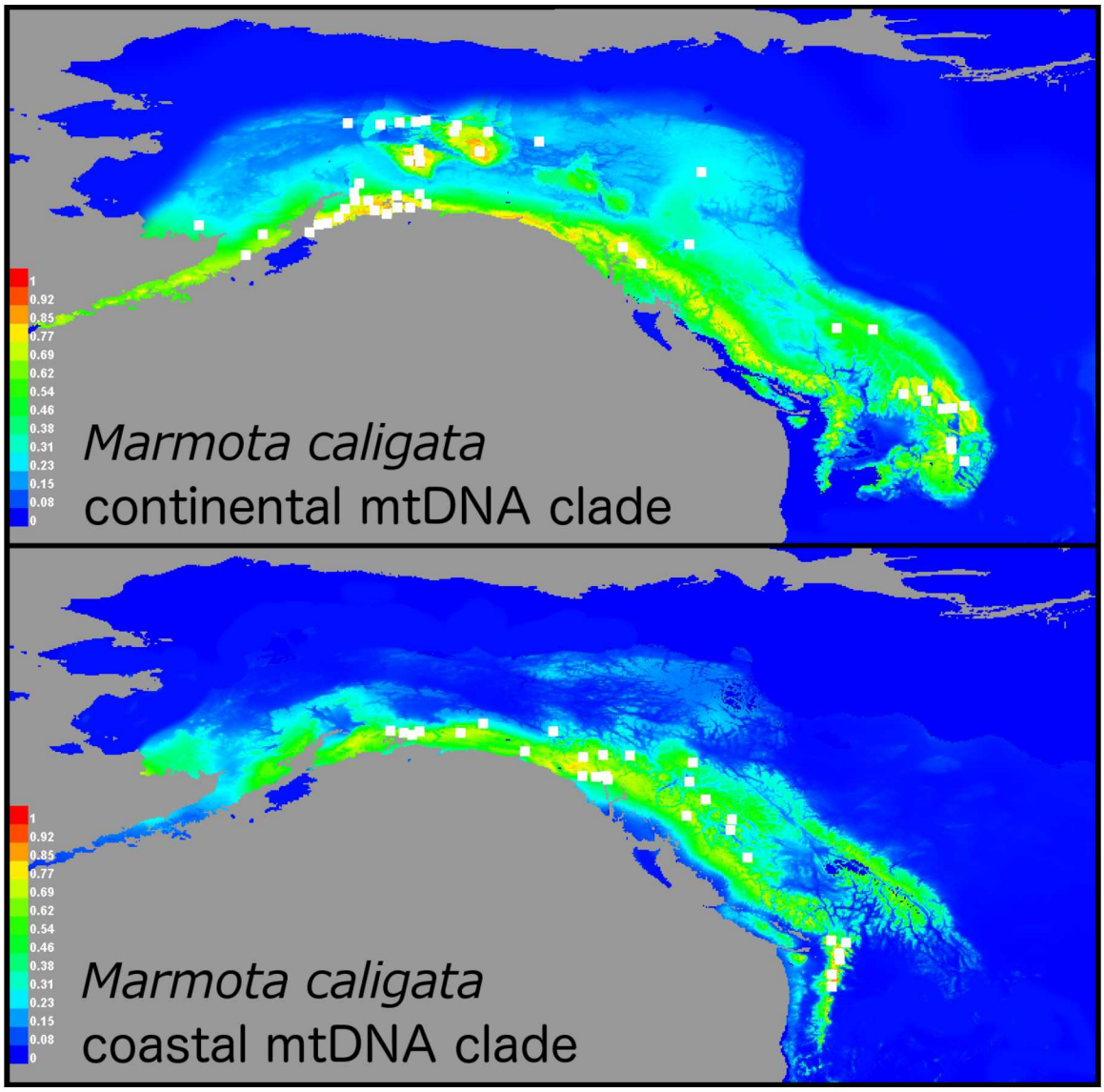

Figure 4.A-2. Results of species distribution models for the 2 Hoary Marmot mitochondrial clades. White squares represent the locality of specimens used to create the map. Colors represent Hoary marmot habitat suitability from low (blue) to high (red). Habitat predicted as suitable from well outside the know distribution of the species has been removed for clarity.

Figure 4.A-3. Link to Berkeley Mapper file that depicts the geographical locations and mtDNA clade membership for Hoary Marmot specimens used in this study. https://goo.gl/Pd00Dq

Figure 4.A-4. Link to Berkeley Mapper file that depicts the geographical locations and major group membership of the STRUCTRURE analysis of all nuclear loci. https://goo.gl/k9KZat

Table 4.A-1. *Marmota* specimens used in this study. Museum abbreviations are as follows: MCZ = Museum of Comparative Zoology, Cambridge, Massachusetts; MSB = Museum of Southwestern Biology, Albuquerque, New Mexico; MVZ = Museum of Vertebrate Zoology, Berkeley, California; ROM = Royal Ontario Museum, Toronto, Ontario; UAM = University of Alaska Museum, Fairbanks, Alaska; USNM = National Museum of Natural History, Washington D.C.; UWBM = University of Washington Burke Museum, Seattle, Washington; YPM = Yale Peabody Museum of Natural History, New Haven, Connecticut. Mitochondrial clade membership for *M. caligata* specimens previously determined using Sanger sequencing of fresh tissue are denoted by plain text. Clade assignments for museum specimens with no archived tissue samples were made using restricting enzymes (bold) or Sanger sequencing (bold italic). Specimens used in STRUCTURE and IM analyses are denoted with an asterisk after the species name.

Species	Country	State or province	Museum	Catalog number	Latitude	Longitude	Uncertainty (m)	Year collected	Clade
M. caligata	United States	Alaska	UAM	22914	58.2866	-134.5598	7300	1992	coastal
M. caligata	United States	Alaska	UAM	24122	58.2866	-134.5598	7300	1992	coastal
M. caligata	United States	Alaska	UAM	32649	58.2508	-134.4705	14000	1995	coastal
M. caligata	United States	Alaska	UAM	35129	56.0339	-130.0433	1450	1995	coastal
*M. caligata**	Canada	British Columbia	UAM	35130	58.1881	-129.8881	500	1995	coastal

Table 4.A-1 cont.

M. caligata	United States	Alaska	UAM	38302	58.4713	-135.4012	1274	1996	coastal
M. caligata	United States	Alaska	UAM	38303	58.4713	-135.4012	1274	1996	coastal
M. caligata	United States	Alaska	UAM	38304	58.4713	-135.4012	1274	1996	coastal
*M. caligata**	United States	Alaska	UAM	48486	58.2543	-134.3117	500	1997	coastal
M. caligata	Canada	British Columbia	UAM	49848	56.1700	-130.0500	2880	1997	coastal
M. caligata	United States	Alaska	UAM	57693	61.0585	-143.3634	100	2001	coastal
M. caligata	United States	Alaska	UAM	78239	59.6374	-136.1291	600	2002	coastal
M. caligata	United States	Alaska	UAM	78240	59.6374	-136.1291	600	2002	coastal
*M. caligata**	United States	Washington	UWBM	82114	46.1631	-121.5153	10	2011	coastal
M. caligata	United States	Alaska	UAM	102367	61.6124	-142.0313	11	2008	coastal
M. caligata	United States	Alaska	UAM	102368	61.6134	-142.0388	8	2008	coastal
M. caligata	United States	Alaska	UAM	102374	61.6125	-142.0394	8	2008	coastal
M. caligata	United States	Alaska	UAM	102436	60.9763	-143.1291	13	2008	coastal
*M. caligata**	United States	Alaska	UAM	103473	58.5344	-134.8308	15	2009	coastal
*M. caligata**	United States	Alaska	UAM	103474	58.3111	-134.6669	383	2009	coastal
*M. caligata**	United States	Alaska	UAM	103477	58.3111	-134.6669	383	2009	coastal
M. caligata	Canada	British Columbia	UAM	112310	59.7200	-133.3804	10	2011	coastal
*M. caligata**	United States	Alaska	UAM	112325	61.1540	-146.5978	3	2011	coastal
*M. caligata**	United States	Alaska	UAM	112338	58.6245	-134.9362	3	2011	coastal
*M. caligata**	United States	Alaska	UAM	112351	58.6245	-134.9362	3	2011	coastal
M. caligata	Canada	British Columbia	UAM	112366	59.7200	-133.3805	10	2011	coastal
*M. caligata**	United States	Alaska	UAM	112457	58.4231	-134.4429	50	2010	coastal
*M. caligata**	United States	Alaska	UAM	112458	58.4231	-134.4429	50	2010	coastal
*M. caligata**	United States	Washington	UAM	112565	48.5140	-120.6873	4	2010	coastal
*M. caligata**	United States	Washington	UAM	112570	48.5142	-120.6450	10	2010	coastal
*M. caligata**	United States	Washington	UAM	112571	47.7331	-121.0717	10	2010	coastal
*M. caligata**	United States	Washington	UAM	112573	48.5142	-120.6450	10	2010	coastal
*M. caligata**	United States	Washington	UAM	112574	47.7310	-121.0695	10	2010	coastal
*M. caligata**	United States	Washington	UAM	112577	47.7331	-121.0717	10	2010	coastal
*M. caligata**	United States	Alaska	UAM	113878	61.1998	-147.4813	5	2012	coastal
*M. caligata**	United States	Alaska	UAM	113904	61.2010	-147.4751	10	2012	coastal
*M. caligata**	United States	Alaska	UAM	113905	60.9195	-146.2027	80	2012	coastal
*M. caligata**	United States	Alaska	UAM	113906	61.2002	-147.4827	5	2012	coastal
*M. caligata**	United States	Alaska	UAM	113950	60.9262	-146.2006	3	2012	coastal
*M. caligata**	United States	Alaska	UAM	114143	61.1413	-145.7593	4	2012	coastal
*M. caligata**	United States	Alaska	UAM	114365	60.9278	-146.2128	30	2012	coastal
*M. caligata**	United States	Alaska	UAM	115715	61.1418	-145.7616	5	2012	coastal
M. caligata	United States	Alaska	UAM	115797	61.1370	-145.7662	15	2012	coastal
*M. caligata**	United States	Alaska	UAM	115798	61.1385	-145.7645	10	2012	coastal
*M. caligata**	United States	Alaska	UAM	115799	61.1333	-145.7773	10	2012	coastal
*M. caligata**	United States	Alaska	UAM	115801	61.1439	-145.7559	152	2012	coastal
M. caligata	United States	Alaska	MVZ	403	58.4510	-136.0892	8219	1907	**coastal**

Table 4.A-1 cont.

M. caligata	United States	Alaska	MVZ	418	58.4510	-136.0892	8219	1907	**coastal**
M. caligata	United States	Alaska	MVZ	420	58.4510	-136.0892	8219	1907	**coastal**
M. caligata	United States	Alaska	MVZ	964	60.3516	-146.4059	19038	1908	**coastal**
M. caligata	United States	Alaska	MVZ	994	59.9811	-139.5701	9369	1906	**coastal**
M. caligata	United States	Alaska	MVZ	8360	55.9383	-130.7015	23949	1909	**coastal**
M. caligata	United States	Alaska	MVZ	8361	55.9383	-130.7015	23949	1909	**coastal**
M. caligata	United States	Washington	UWBM	13561	48.1120	-121.1153	7326	1950	**coastal**
M. caligata	Canada	British Columbia	USNM	19156	55.3217	-126.6224	40000	n/a	**coastal**
M. caligata	United States	Washington	UWBM	31095	48.8333	-121.5626	2149	1977	**coastal**
M. caligata	United States	Washington	UWBM	31096	48.8333	-121.5626	2149	1977	**coastal**
M. caligata	United States	Washington	UWBM	32616	46.8721	-121.5156	231	1975	**coastal**
M. caligata	Canada	British Columbia	MVZ	32763	55.3448	-127.4986	7103	1921	**coastal**
M. caligata	Canada	British Columbia	MVZ	34276	59.7580	-134.9627	3192	1924	**coastal**
M. caligata	Canada	Yukon Territory	MCZ	34503	61.1357	-137.8861	5580	1936	**coastal**
M. caligata	Canada	Yukon Territory	MCZ	34504	61.1357	-137.8861	5580	1936	**coastal**
M. caligata	Canada	Yukon Territory	MCZ	34505	61.1357	-137.8861	5580	1936	**coastal**
M. caligata	United States	Washington	UWBM	35529	48.3936	-120.1379	40781	1987	**coastal**
M. caligata	United States	Alaska	USNM	35622	59.7587	-139.8693	25987	1882	**coastal**
M. caligata	United States	Washington	UWBM	37535	48.7210	-120.6699	260	1990	**coastal**
M. caligata	Canada	British Columbia	UWBM	42161	54.7808	-127.1697	11556	1965	**coastal**
M. caligata	United States	Washington	UWBM	42170	48.7788	-121.8624	3552	1951	**coastal**
M. caligata	Canada	British Columbia	USNM	53594	54.6091	-124.7142	35985	1893	**coastal**
M. caligata	Canada	British Columbia	MVZ	114118	53.7214	-126.4718	7389	1950	**coastal**
M. caligata	United States	Washington	USNM	141948	46.8529	-121.7604	11868	1905	**coastal**
M. caligata	Canada	British Columbia	USNM	170683	57.2089	-128.8367	25025	1910	**coastal**
M. caligata	Canada	British Columbia	USNM	170727	57.5300	-129.4410	49523	1910	**coastal**
M. caligata	Canada	British Columbia	USNM	170739	57.0714	-126.8901	13244	1910	**coastal**
M. caligata	Canada	British Columbia	USNM	202788	55.9793	-127.3760	5434	1913	**coastal**
M. caligata	Canada	British Columbia	USNM	202791	56.8353	-127.1002	11071	1913	**coastal**
M. caligata	Canada	British Columbia	USNM	210674	59.3170	-129.7047	6134	1914	**coastal**
M. caligata	Canada	British Columbia	USNM	210675	59.3170	-129.7047	6134	1914	**coastal**
M. caligata	Canada	British Columbia	USNM	226148	59.2801	-129.8398	10184	1916	**coastal**
M. caligata	United States	Washington	USNM	226713	46.3051	-121.5178	2000	1917	**coastal**
M. caligata	United States	Alaska	USNM	235255	58.5225	-136.1824	3632	1920	**coastal**
M. caligata	United States	Alaska	USNM	235257	58.5225	-136.1824	3632	1920	**coastal**
M. caligata	Canada	British Columbia	USNM	235432	57.1493	-128.9298	8050	1920	**coastal**
M. caligata	United States	Washington	USNM	236593	46.7230	-121.6926	9395	1921	**coastal**
M. caligata	United States	Alaska	USNM	241748	64.0684	-143.1177	3377	1921	**coastal**
M. caligata	Canada	British Columbia	MCZ	BANGS-1750	49.0000	-121.8154	14394	1891	**coastal**
M. caligata	Canada	British Columbia	MCZ	BANGS-1919	49.3828	-121.4386	11204	1894	**coastal**
M. caligata	Canada	British Columbia	MCZ	BANGS-6842	49.0014	-121.7782	27011	1895	**coastal**
M. caligata	Canada	British Columbia	MCZ	BANGS-6843	49.0014	-121.7782	27011	1895	**coastal**

Table 4.A-1 cont.

M. caligata	Canada	British Columbia	MCZ	BANGS-6844	49.0014	-121.7782	27011	1895	**coastal**
M. caligata	United States	Alaska	MVZ	965	61.0703	-146.6685	2876	1908	***coastal***
M. caligata	Canada	British Columbia	MVZ	52111	52.3715	-126.0438	37635	1932	***coastal***
M. caligata	Canada	British Columbia	MVZ	53805	50.1148	-122.9581	11261	1927	***coastal***
M. caligata	Canada	British Columbia	MVZ	114117	53.7214	-126.4718	7389	1950	***coastal***
M. caligata	United States	Alaska	YPM	14820	63.0693	-145.7405	10	2010	continental
*M. caligata**	United States	Alaska	UAM	30932	57.0833	-132.7333	3352	1993	continental
M. caligata	United States	Alaska	UAM	31724	61.2167	-149.5833	3112	1994	continental
*M. caligata**	Canada	British Columbia	UAM	33803	58.1881	-129.8881	500	1995	continental
M. caligata	United States	Alaska	UAM	53836	65.3928	-145.9994	10	2000	continental
M. caligata	United States	Alaska	UAM	58238	64.8075	-143.7460	680	2001	continental
M. caligata	United States	Alaska	UAM	58239	64.8075	-143.7460	680	2001	continental
M. caligata	United States	Alaska	UAM	58240	64.8075	-143.7460	680	2001	continental
M. caligata	United States	Alaska	UAM	58241	64.8075	-143.7460	680	2001	continental
M. caligata	United States	Alaska	UAM	65635	63.6667	-142.2167	6437	2002	continental
M. caligata	United States	Alaska	UAM	85858	65.2947	-149.9973	5	2006	continental
M. caligata	United States	Alaska	UAM	85859	65.2596	-150.0502	5	2006	continental
M. caligata	United States	Alaska	UAM	86413	60.7709	-148.7506	25	2006	continental
M. caligata	United States	Alaska	UAM	86414	60.2753	-150.1504	9	2006	continental
M. caligata	United States	Alaska	UAM	94705	58.7667	-154.9667	3219	2004	continental
M. caligata	United States	Alaska	UAM	98299	60.7819	-149.5456	32	2005	continental
*M. caligata**	United States	Alaska	UAM	101845	60.7709	-148.7506	1000	2006	continental
*M. caligata**	United States	Alaska	UAM	101919	60.2849	-150.1584	10	2006	continental
M. caligata	United States	Alaska	UAM	102474	63.3958	-145.6603	15	2008	continental
M. caligata	United States	Alaska	UAM	102476	63.3958	-145.6610	6	2008	continental
M. caligata	United States	Alaska	UAM	103458	63.1285	-146.2803	8	2009	continental
*M. caligata**	United States	Alaska	UAM	103476	60.3559	-146.1937	100	2011	continental
M. caligata	United States	Alaska	UAM	103489	58.8975	-152.2094	1000	2009	continental
M. caligata	United States	Alaska	UAM	103490	58.8975	-152.2094	1000	2009	continental
M. caligata	United States	Alaska	UAM	103491	58.8975	-152.2094	1000	2009	continental
M. caligata	United States	Alaska	UAM	106200	65.4938	-145.3841	6	2010	continental
M. caligata	United States	Alaska	UAM	106211	65.2111	-148.0603	4	2010	continental
M. caligata	United States	Alaska	UAM	106220	65.2084	-148.0575	3	2010	continental
*M. caligata**	United States	Alaska	UAM	107658	60.5514	-145.3621	5	2009	continental
M. caligata	United States	Alaska	UAM	111555	65.2116	-148.0608	915	2011	continental
M. caligata	United States	Alaska	UAM	111557	65.2206	-148.0507	936	2011	continental
M. caligata	United States	Alaska	UAM	111561	65.2111	-148.0604	915	2011	continental
M. caligata	United States	Alaska	UAM	111565	65.4854	-145.4000	100	2011	continental
M. caligata	United States	Alaska	UAM	111626	65.2195	-148.0545	924	2011	continental
M. caligata	United States	Alaska	UAM	111634	65.2111	-148.0604	915	2011	continental
M. caligata	United States	Alaska	UAM	111786	58.8974	-152.2117	1000	2010	continental
M. caligata	United States	Alaska	UAM	112286	58.8974	-152.2117	1000	2010	continental

Table 4.A-1 cont.

M. caligata	United States	Alaska	UAM	112287	58.8974	-152.2117	1000	2010	continental
M. caligata	United States	Alaska	UAM	112288	58.8974	-152.2117	1000	2010	continental
M. caligata	United States	Alaska	UAM	112289	58.8974	-152.2117	1000	2010	continental
M. caligata	United States	Alaska	UAM	112290	58.8974	-152.2117	1000	2010	continental
M. caligata	United States	Alaska	UAM	112291	58.8974	-152.2117	1000	2010	continental
M. caligata	United States	Alaska	UAM	112292	58.8974	-152.2117	1000	2010	continental
M. caligata	United States	Alaska	UAM	112293	58.8974	-152.2117	1000	2010	continental
M. caligata	United States	Alaska	UAM	112294	58.8974	-152.2117	1000	2010	continental
M. caligata	United States	Alaska	UAM	112295	58.8974	-152.2117	1000	2010	continental
M. caligata	United States	Alaska	UAM	112296	58.8974	-152.2117	1000	2010	continental
M. caligata	United States	Alaska	UAM	112297	58.8974	-152.2117	1000	2010	continental
M. caligata	United States	Alaska	UAM	112298	58.8974	-152.2117	1000	2010	continental
M. caligata	United States	Alaska	UAM	112299	58.8974	-152.2117	1000	2010	continental
M. caligata	United States	Alaska	UAM	112300	58.8974	-152.2117	1000	2010	continental
M. caligata	United States	Alaska	UAM	112301	58.8974	-152.2117	1000	2010	continental
M. caligata	United States	Alaska	UAM	112302	58.8974	-152.2117	1000	2010	continental
M. caligata	United States	Alaska	UAM	112303	58.8974	-152.2117	1000	2010	continental
M. caligata	United States	Alaska	UAM	112304	58.8974	-152.2117	1000	2010	continental
M. caligata	United States	Alaska	UAM	112305	58.8974	-152.2117	1000	2010	continental
M. caligata	United States	Alaska	UAM	112306	58.8974	-152.2117	1000	2010	continental
*M. caligata**	Canada	British Columbia	UAM	112316	58.1895	-129.8937	10	2011	continental
*M. caligata**	United States	Alaska	UAM	112324	59.5097	-151.4527	1000	2011	continental
*M. caligata**	United States	Alaska	UAM	112326	61.1342	-145.7744	3	2011	continental
*M. caligata**	United States	Alaska	UAM	112342	59.5099	-151.4512	1000	2011	continental
M. caligata	United States	Alaska	UAM	112353	65.3902	-146.5982	400	2011	continental
*M. caligata**	United States	Alaska	UAM	112354	59.5099	-151.4512	1000	2011	continental
M. caligata	United States	Alaska	UAM	112359	63.0853	-146.3888	7	2006	continental
*M. caligata**	United States	Alaska	UAM	112360	60.3461	-146.2685	3	2011	continental
*M. caligata**	United States	Alaska	UAM	112364	60.3448	-146.3126	3	2011	continental
M. caligata	United States	Alaska	UAM	112367	65.1492	-147.0182	137	2011	continental
M. caligata	United States	Alaska	UAM	112368	65.1492	-147.0182	137	2011	continental
M. caligata	United States	Alaska	UAM	112369	65.1492	-147.0182	137	2011	continental
*M. caligata**	United States	Montana	UAM	112564	45.4223	-113.7225	4	2010	continental
*M. caligata**	United States	Montana	UAM	112566	48.5778	-114.4290	3	2010	continental
*M. caligata**	United States	Montana	UAM	112575	46.1562	-114.4761	3	2010	continental
*M. caligata**	United States	Montana	UAM	112576	48.5747	-114.4256	3	2010	continental
*M. caligata**	United States	Alaska	UAM	112579	59.4278	-151.1522	3	2011	continental
*M. caligata**	United States	Alaska	UAM	112580	59.3669	-151.6978	8	2011	continental
*M. caligata**	United States	Alaska	UAM	112581	59.4356	-151.1800	3	2011	continental
*M. caligata**	United States	Alaska	UAM	112582	59.7913	-150.5125	9	2011	continental
*M. caligata**	United States	Alaska	UAM	112583	59.6410	-151.0583	3	2011	continental
*M. caligata**	United States	Alaska	UAM	112585	59.4299	-151.1579	3	2011	continental

Table 4.A-1 cont.

*M. caligata**	United States	Alaska	UAM	112587	59.4343	-151.1687	3	2011	continental
*M. caligata**	United States	Alaska	UAM	113733	59.6473	-151.0580	3	2011	continental
*M. caligata**	United States	Alaska	UAM	113734	59.6411	-151.0640	3	2011	continental
*M. caligata**	United States	Alaska	UAM	113735	59.6410	-151.0583	3	2011	continental
*M. caligata**	United States	Alaska	UAM	113736	59.4292	-151.1555	3	2011	continental
*M. caligata**	United States	Alaska	UAM	113737	59.4343	-151.1583	3	2011	continental
*M. caligata**	United States	Alaska	UAM	113738	59.4338	-151.1636	7	2011	continental
*M. caligata**	United States	Alaska	UAM	113739	59.4335	-151.1633	12	2011	continental
*M. caligata**	United States	Alaska	UAM	113885	60.2006	-148.4004	91	2012	continental
*M. caligata**	United States	Alaska	UAM	113886	60.2180	-148.3640	274	2012	continental
M. caligata	United States	Alaska	UAM	113889	63.4980	-145.8129	3	2012	continental
M. caligata	United States	Alaska	UAM	113892	61.7599	-149.3060	4	2012	continental
M. caligata	United States	Alaska	UAM	113901	61.7606	-149.3110	3	2012	continental
M. caligata	United States	Alaska	UAM	113902	61.7631	-149.3035	6	2012	continental
M. caligata	United States	Alaska	UAM	113903	63.5000	-145.8057	3	2012	continental
M. caligata	United States	Alaska	UAM	113907	65.3675	-146.9370	4	2012	continental
M. caligata	United States	Alaska	UAM	113925	65.3674	-146.9384	4	2012	continental
M. caligata	United States	Alaska	UAM	113930	65.3665	-146.9374	4	2012	continental
*M. caligata**	United States	Alaska	UAM	113951	61.0548	-147.1226	5	2012	continental
M. caligata	United States	Alaska	UAM	114146	65.4917	-145.3895	61	2012	continental
*M. caligata**	United States	Alaska	UAM	114296	61.2018	-147.4709	10	2012	continental
M. caligata	United States	Alaska	UAM	114298	63.7833	-145.7918	10	2012	continental
M. caligata	United States	Alaska	UAM	114323	61.2002	-147.4827	10	2012	continental
M. caligata	United States	Alaska	UAM	115699	57.5538	-155.9849	6	2011	continental
*M. caligata**	United States	Alaska	UAM	115716	61.0548	-147.1226	5	2012	continental
M. caligata	United States	Alaska	UAM	115718	63.7876	-145.7916	15	2012	continental
*M. caligata**	United States	Alaska	UAM	115723	61.1337	-145.7751	10	2012	continental
*M. caligata**	United States	Alaska	UAM	115724	61.2016	-147.4731	10	2012	continental
*M. caligata**	United States	Alaska	UAM	115800	61.1330	-145.7780	10	2012	continental
*M. caligata**	United States	Alaska	UAM	115802	61.2017	-147.4716	10	2012	continental
M. caligata	United States	Alaska	UAM	115803	63.7834	-145.7907	13	2012	continental
*M. caligata**	United States	Alaska	UAM	115809	59.4333	-151.1626	7	2011	continental
M. caligata	United States	Alaska	UAM	117977	64.7920	-141.7312	3	2011	continental
M. caligata	United States	Alaska	UAM	117978	64.7699	-141.7528	3	2011	continental
M. caligata	United States	Alaska	UAM	117979	64.7938	-141.7296	4	2011	continental
M. caligata	United States	Alaska	UAM	117980	64.7924	-141.7288	3	2011	continental
M. caligata	United States	Alaska	UAM	117981	64.7809	-141.7227	5	2011	continental
M. caligata	United States	Alaska	UAM	117982	64.7879	-141.7176	5	2011	continental
M. caligata	United States	Alaska	UAM	117983	64.7745	-141.7493	3	2011	continental
M. caligata	United States	Alaska	UAM	117984	64.7723	-141.7542	3	2011	continental
M. caligata	Canada	Northwest Territories	MSB	265467	62.4500	-129.2000	305	2005	continental
M. caligata	Canada	Northwest Territories	MSB	267586	62.4500	-129.2000	305	2005	continental

Table 4.A-1 cont.

M. caligata	United States	Alaska	MVZ	968	60.6656	-145.6207	4802	1908	**continental**
M. caligata	United States	Alaska	MVZ	969	59.9674	-147.7062	5378	1908	**continental**
M. caligata	Canada	Northwest Territories	MCZ	1559	60.2394	-123.4644	-	-	**continental**
M. caligata	Canada	Northwest Territories	MCZ	4868	60.2394	-123.4644	-	-	**continental**
M. caligata	United States	Alaska	MVZ	8359	58.0047	-133.7950	6905	1909	**continental**
M. caligata	United States	Montana	MCZ	11605	45.0034	-110.7949	-	1907	**continental**
M. caligata	Canada	British Columbia	MVZ	31004	57.2007	-131.7981	11095	1919	**continental**
M. caligata	Canada	British Columbia	MVZ	31005	57.2007	-131.7981	11095	1919	**continental**
M. caligata	United States	Alaska	MVZ	37527	63.7539	-149.2904	20853	1926	**continental**
M. caligata	United States	Alaska	MVZ	37528	63.7539	-149.2904	20853	1926	**continental**
M. caligata	Canada	Yukon Territory	UWBM	39668	64.8826	-138.2874	77279	1964	**continental**
M. caligata	United States	Alaska	UWBM	39790	61.8240	-149.2428	2000	1950	**continental**
M. caligata	Canada	British Columbia	MVZ	40182	53.2663	-121.2498	5856	1928	**continental**
M. caligata	Canada	British Columbia	MVZ	40183	53.2663	-121.2498	5856	1928	**continental**
M. caligata	Canada	British Columbia	MVZ	42502	53.2663	-121.2498	5856	1929	**continental**
M. caligata	Canada	British Columbia	MVZ	42503	53.2663	-121.2498	5856	1929	**continental**
M. caligata	Canada	British Columbia	MVZ	43825	53.2663	-121.2498	5856	1929	**continental**
M. caligata	Canada	British Columbia	USNM	66696	49.4124	-117.3288	3641	1894	**continental**
M. caligata	Canada	British Columbia	USNM	66697	49.4124	-117.3288	3641	1894	**continental**
M. caligata	Canada	British Columbia	USNM	67073	51.2615	-117.4934	10093	1894	**continental**
M. caligata	United States	Montana	USNM	72222	48.6905	-113.5065	7804	1895	**continental**
M. caligata	United States	Montana	USNM	72223	48.6905	-113.5065	7804	1895	**continental**
M. caligata	Canada	Alberta	USNM	81913	52.6598	-118.0631	19077	1896	**continental**
M. caligata	United States	Montana	MVZ	95153	48.6966	-113.7183	2000	1938	**continental**
M. caligata	United States	Alaska	USNM	128082	59.1556	-151.8847	18642	1903	**continental**
M. caligata	United States	Alaska	USNM	128084	59.1556	-151.8847	18642	1903	**continental**
M. caligata	United States	Alaska	USNM	131437	57.6110	-156.0426	11489	1903	**continental**
M. caligata	United States	Alaska	USNM	131535	64.6756	-144.0247	4009	1903	**continental**
M. caligata	United States	Montana	MVZ	134483	48.5051	-115.0140	4557	1966	**continental**
M. caligata	United States	Montana	MVZ	134484	48.9691	-115.9605	3925	1966	**continental**
M. caligata	United States	Montana	MVZ	134485	48.8975	-115.9508	4259	1966	**continental**
M. caligata	United States	Montana	MVZ	134486	48.9461	-115.7390	6203	1966	**continental**
M. caligata	United States	Montana	MVZ	134487	48.9461	-115.7390	6203	1966	**continental**
M. caligata	United States	Alaska	USNM	271701	63.6200	-146.7177	65000	1941	**continental**
M. caligata	United States	Alaska	USNM	512803	65.1879	-143.5431	9920	1975	**continental**
M. caligata	Canada	British Columbia	USNM	551541	49.6111	-116.1938	3454	1924	**continental**
M. caligata	Canada	Alberta	MCZ	BANGS-8611	51.1819	-115.5645	11037	1898	**continental**
M. caligata	United States	Alaska	MVZ	960	59.9674	-147.7062	5378	1908	***continental***
M. caligata	United States	Alaska	MVZ	961	59.9674	-147.7062	5378	1908	***continental***
M. caligata	United States	Alaska	MVZ	962	59.9674	-147.7062	5378	1908	***continental***
M. caligata	United States	Alaska	MVZ	963	59.9674	-147.7062	5378	1908	***continental***
M. caligata	Canada	Northwest Territories	USNM	8728	65.9196	-132.9346	200000	1864	***continental***

Table 4.A-1 cont.

M. caligata	United States	Alaska	USNM	13650	59.3092	-158.7220	8354	1882	***continental***
M. caligata	Canada	Alberta	USNM	76233	52.8767	-118.0631	10383	1895	***continental***
M. caligata	Canada	Yukon Territory	USNM	135161	64.2441	-138.7213	2992	1904	***continental***
M. caligata	United States	Alaska	USNM	137321	60.3389	-147.0736	3960	1905	***continental***
M. caligata	United States	Idaho	USNM	169241	46.6146	-114.7297	119022	1910	***continental***
M. caligata	United States	Idaho	USNM	169242	46.5802	-114.5364	2148	1910	***continental***
M. caligata	Canada	Alberta	USNM	174502	53.1739	-119.1123	3221	1911	***continental***
M. caligata	United States	Alaska	USNM	301301	57.5683	-156.0378	10203	1954	***continental***
*M. caligata**	United States	Alaska	UAM	120630	58.2569	-134.5061	10	2013	-
*M. caligata**	United States	Washington	UWBM	79553	-	-	-	2005	-
*M. caligata**	United States	Washington	UWBM	79554	-	-	-	2003	-
*M. caligata**	United States	Washington	UWBM	79849	-	-	-	2006	-
*M. caligata**	United States	Washington	UWBM	80739	-	-	-	2005	-
*M. caligata**	United States	Washington	UWBM	81033	-	-	-	2008	-
*M. caligata**	Canada	British Columbia	ROM	116794	-	-	-	-	-
*M. caligata**	Canada	British Columbia	ROM	116795	-	-	-	-	-
*M. caligata**	Canada	British Columbia	ROM	117714	-	-	-	-	-
*M. caligata**	Canada	British Columbia	ROM	117715	-	-	-	-	-
*M. caligata**	Canada	British Columbia	ROM	117716	-	-	-	-	-
*M. olympus**	United States	Alaska	UAM	103475	58.3141	-134.6605	15	2009	-
*M. olympus**	United States	Alaska	UAM	117755	58.2569	-134.5061	15	2013	-
*M. olympus**	United States	Alaska	UAM	117756	58.2861	-134.3259	15	2013	-
*M. olympus**	United States	Alaska	UAM	118821	58.2441	-134.4195	10	2013	-
*M. olympus**	United States	Alaska	UAM	119038	58.2441	-134.4195	10	2013	-
*M. vancouverensis**	United States	Alaska	UAM	119131	58.2569	-134.5061	15	2013	-
*M. vancouverensis**	United States	Alaska	UAM	119132	58.2569	-134.5061	15	2013	-
*M. vancouverensis**	United States	Alaska	UAM	119877	58.2936	-134.3717	15	2013	-
*M. vancouverensis**	United States	Alaska	UAM	120456	58.2569	-134.5061	5	2013	-
*M. vancouverensis**	United States	Alaska	UAM	120629	58.2441	-134.4195	10	2013	-

Table 4.A-2. Primers used to amplify and sequence short, overlapping segments of the Cytochrome-*b* gene. L14724 was designed by Irwin et al. (1991) and all other primers were designed by the authors specifically for *Marmota caligata*.

Name	Sequence
L14724	5'-CGAAGCTTGATATGAAAAACCATCGTTG-3'
MACA-R1	5'-CGACAGATGTGGGTGACTGA-3'
MACA-L2	5'-TCATCCAAATCTTTACCGGATT-3'
MACA-R2	5'-GCTATTACTGCAAATAAGAG-3'
MACA-L3	5'-CTATGGCTCATATACCTATTTTG-3'
MACA-R3	5'-AAGGGAGGACAAAGTGGAATGC-3'
MACA-L4	5'-GAATCTGAGGCGGATTCTCA-3'
MACA-R4	5'-GAATAAGGAGAAGAACTCCAAGC-3'
MACA-L5	5'-GATCCCCTTTCACCCGTACT-3'
MACA-R5	5'-GGGCTAAAACGCCTCCTAGT-3'
MACA-L6	5'-TTCCTATTTGCCTACGCTATCC-3'
MACA-R6	5'-CCTCCGATTCAGGTCAGTGT-3'
MACA-L7	5'-AATCCGACCATTAAGCCAAT-3'
MACA-R7	5'-CCACGCCAGGGTAATGTTTA-3'

General Conclusion

The Hoary Marmot (*Marmota caligata*) is one of the most broadly distributed alpine mammals in the Pacific Northwest (PNW) of North America. Given its expansive range, biogeographic and phylogeographic patterns observed in the Hoary Marmot may be indicative of the general patterns of the region. In this dissertation I conducted the first molecular phylogeographic analyses of the Hoary Marmot to include specimens from throughout its geographic range and multiple molecular markers. I also inferred the molecular phylogenetic relationships of Hoary, Olympic, and Vancouver Island marmots. Lastly, I conducted a thorough literature review and molecular analysis to determine the origin of the Hoary Marmot population on Sud Island, Alaska, which was believed to have been introduced and was the subject a recent eradication effort.

In my initial phylogeographic analysis of the Hoary Marmot I documented 2 reciprocally monophyletic mitochondrial (mtDNA) clades whose general distribution are: the Coast and Cascade mountains of Washington and British Columbia north to near Valdez, Alaska (coastal clade), and the Rocky Mountains from central Idaho and southwestern Montana north including the majority of Alaska (continental clade) (Kerhoulas et al. 2015). These clades were not supported by the nuclear DNA sequence data, suggesting either incomplete lineage sorting and/or gene flow between the clades. In a subsequent analysis using microsatellite data in combination with nuclear sequence data, I determined that gene flow from the coastal to the continental clade was occurring and likely responsible for the phylogeographic discord observed between the mtDNA and nuclear data.

Analysis of nuclear data (sequence and microsatellite) found the southernmost (and island) populations of the Hoary Marmot to be the most genetically structured, with the

remaining populations largely homogenous. This suggests Pleistocene refugia for the Hoary Marmot likely existed at or near the southern portion of the species' current range. The limited genetic structure of nuclear data among the remaining populations combined with little documented sympatry between the mtDNA clades strongly suggests male-biased dispersal. To improve our knowledge of the geographic distribution of the mtDNA clades I determined clade membership of 98 museum specimens that lacked archived fresh tissue and identified the coastal clade as dominant in northern British Columbia, a biogeographically important region where the relative representation of the mtDNA clades was previously based on 4 specimens from a single collection locality. I then used the known geographic distribution of the mtDNA clades to create species distribution models (SDMs) for each clade and to infer that bioclimatic factors were likely not responsible for the current distribution of these clades.

To determine where potentially suitable habitat existed during the Last Glacial Maximum (LGM) and how the distribution of habitat will likely be affected by future climate change, I georeferenced all known Hoary Marmot museum specimens and used these data to construct SDMs. These models predict Hoary Marmot LGM refugia likely existed south and potentially west of the Cordilleran and Laurentide glacial margins. Under future climatic scenarios the southernmost Hoary Marmot habitat is predicted to be the most negatively impacted. Using population genetic summary statistics I documented the greatest genetic diversity in the southernmost populations of the continental clade, further supporting a southern refugium and highlighting the potential loss of genetic diversity if the future habitat fragmentation predicted by the SDMs renders this region inhospitable.

Overall the geographic distribution of the mtDNA clades, the genetic structure of the nuclear data, SDMs, and population genetic summary statistics suggest the 2 mtDNA clades

were likely isolated in Pleistocene refugia in the northern Rocky Mountains (Idaho and/or western Montana) and the Cascade Mountains (Washington and/or Oregon) and/or along the PNW coast and not in a northern refugium as previously hypothesized (Lanier et al. 2015; Knowles et al. 2016). These results also suggest the southernmost populations of the continental clade are both the most genetically diverse and the most likely to be negatively impacted by future climatic changes.

Phylogenetic analyses suggests that introgression resulted in Vancouver Island Marmots "capturing" the mitochondrial genome (Good et al. 2008) of the Hoary Marmot. Analyses of mtDNA found Vancouver Island Marmots to be sister to the coastal clade of Hoary Marmots. In contrast, analysis of nuclear loci recovered Vancouver Island Marmots as a distinct genetic cluster and not sister to the Hoary Marmot coastal mtDNA clade. I conducted an isolation-with-migration analysis (Hey 2010) and found gene flow from Hoary Marmots to Vancouver Island Marmots to be the most likely, suggesting that the discord between the mtDNA and nuclear data is the result of historic gene flow between the 2 species.

Olympic Marmots may also have experienced introgression with Hoary Marmots. Nuclear haplotypes were shared between Olympic Marmots and Hoary Marmots collected from nearby populations in Washington. Although the geographic distribution of these shared alleles strongly suggests historic gene flow between these 2 species, molecular analysis produced ambiguous results. However, a population structure analysis of nuclear sequence and microsatellite data recovered Olympic Marmots as a distinct cluster (i.e., not clustered or admixed with Washington Hoary Marmots), indicating recent introgression is unlikely.

Regarding the Hoary Marmot population on Sud Island, my molecular results were largely ambiguous but do suggest natural colonization of the island. Two gray-literature accounts

reported anthropogenic introductions of Hoary Marmots to Sud Island (Bailey 1976; 1993), but these accounts were inconsistent, reliant on secondhand (at best) sources, and ultimately could not be substantiated. I found marmots from Sud and Hinchinbrook (colonized naturally) islands each formed a unique genetic cluster distinct from mainland populations, consistent with a natural colonization scenario for Sud Island. The estimated divergence times of both island populations were also similar, further suggesting a natural colonization of Sud Island, but these divergence times could not be confidently distinguished from that of an unsampled mainland population. I also found evidence in the literature suggesting the decline of nesting seabirds on Sud Island (a justification for marmot eradication) was likely the result of North American River Otters (*Lontra canadensis*) naturally reaching the island and not the presence of marmots (Manuwal 1980; Carter et al. 2012). The unexpected findings that Sud Island Hoary Marmots (a) were likely a naturally occurring population and (b) did not need to be eradicated as a problematic invasive species highlight the importance of rigorous scientific review before eradication efforts are undertaken.

Overall I investigated the phylogeographic history of the Hoary Marmot and the phylogenetic relationships of Hoary, Olympic, and Vancouver Island marmots using a variety of molecular data and SDMs. I documented and delimited the presence of 2 Hoary Marmot mtDNA clades (coastal and continental) that were the result of vicariance during the Pleistocene. Furthermore, I found that ongoing and likely male-biased gene flow is occurring between the mtDNA clades. Adding an important contribution to the field, my SDMs and population genetic summary statistics identified southern and/or coastal regions as the most likely LGM refugia for Hoary Marmots. From a conservation perspective, the increased fragmentation of the southern portion of the Hoary Marmot distribution, predicted by SDMs of future climatic conditions,

combined with the greater relative genetic diversity documented in this region, highlight the need for further study and likely additional conservation in this region. Using mtDNA and nuclear data, I found that Vancouver Island Marmots likely captured the Hoary Marmot mitochondria via hybridization during the Pleistocene. I also documented likely hybridization between Hoary and Olympic marmots based on shared nuclear alleles, although I was unable to confidently resolve the origin of the shared alleles. Finally, emphasizing the need for careful review of management decisions, I found no evidence that Hoary Marmots were introduced to Sud Island, a location where this possibly naturally occurring species was likely intentionally extirpated due to its assumed introduced status. My hope is that the work presented in this dissertation will provide a broad and thorough knowledge base to serve researchers of PNW species long into the future.

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