2-19-2010

# Biogeography of the American Pika (*Ochotona princeps*) In Oregon and Southern Washington: Illuminating Genetic Relationships Among Disjunct Populations

George Washington Batten III
*Portland State University*

THESIS APPROVAL

The abstract and thesis of George Washington Batten III for the Master of Science in Biology were presented February 19, 2010 and accepted by the thesis committee and the department.

COMMITTEE APPROVALS:

[redacted]

Luis A. Ruedas, Chair

[redacted]

Deborah A. Duffield

[redacted]

Michael T. Murphy

DEPARTMENTAL APPROVAL:

[redacted]

Anna–Louise Reysenbach, Chair
Department of Biology

ABSTRACT

An abstract of the thesis of George Washington Batten III for the Master of Science in Biology presented February 19, 2010.

Title: Biogeography of the American Pika (*Ochotona princeps*) in Oregon and Southern Washington: Illuminating Genetic Relationships Among Disjunct Populations.

The American pika (*Ochotona princeps*) finds moderately warm temperatures (>25°C) lethally stressful, and at the end of the last Ice Age 10,000 years ago was forced to disperse to cooler, "sky island" mountaintops where they are almost exclusively found today. Thirty six subspecies are recognized, all established using morphological characters, and it is uncertain whether these subspecies' designations are corroborated by genetic analyses. This study elucidates three hypotheses regarding populations in Oregon and southern Washington: 1) *O. p. fumosa* constitutes a subspecies distinct form *O. p. brunnescens*, 2) the Columbia River constitutes a barrier to gene flow giving rise to two subspecies rather than the single subspecies *O. p. brunnescens*, and 3) populations in eastern Oregon (*O. p. jewetti* and *O. p. taylori*) are genetically distinct from populations in the Cascade Range (*O. p. brunnescens* and *O. p. fumosa*).

Genetic sequence data from cytochrome *b* and the control region of the mitochondrial genome were analyzed using both genetic distance and Maximum Parsimony, Maximum Likelihood, and Bayesian criteria. *Ochotona princeps fumosa* was not shown to be a monophyletic clade, refuting hypothesis 1. Populations of *O. p. brunnescens* north of the Columbia River were not found to be reciprocally monophyletic with *O. p. brunnescens* south of the Columbia River, refuting hypothesis 2. Eastern populations and western populations were reciprocally monophyletic and exhibited genetic distances ranging from 5.2 – 8.55%. Further support for hypothesis 3 was given by differing alarm calls: single syllable in the west, double syllable in the east.

Genetic analyses did not corroborate current subspecies designations, suggesting further research to determine the genetically most diverse populations. Given the sensitivity of pikas to warming temperatures, this research should be done soon.

# BIOGEOGRAPHY OF THE AMERICAN PIKA *(OCHOTONA PRINCEPS)* IN OREGON AND SOUTHERN WASHINGTON: ILLUMINATING GENETIC RELATIONSHIPS AMONG DISJUNCT POPULATIONS

by

GEORGE WASHINGTON BATTEN III

A thesis submitted in partial fulfillment of the
requirements for the degree of

MASTER OF SCIENCE
in
BIOLOGY

Portland State University
2010

**TABLE OF CONTENTS**

## LIST OF FIGURES

## INTRODUCTION

The end of the Wisconsinian Ice Age ca. 10,000 years ago moved the Pacific Northwest into a period of retreating glaciers, rapid warming, and a change in species composition and abundance. During its glacial maximum 50,000 to 20,000 years ago, nearly half of North America was covered with ice—a virtual biological desert—and the subsequent warming and melting opened large areas of habitat for colonization by both plants and animals (Pielou 1991). The magnitude of change from biological desert to lush ecosystem in a relatively short span of time was tremendous. Still, the transition must have required numerous, successive stages of plant and animal colonization to assemble the contemporary (or pre–Columbian) mixture of species. But what of the plants and animals adapted to life on the cold edge of a continent-sized glacier? Did they have time to adapt to the warmer, wetter climes, or were they forced to follow the retreating glaciers northward in order to maintain habitat suitable for survival and persistence?

One such species is the American pika (*Ochotona princeps*; Lagomorpha: Leporidae). Well adapted to cold weather with its thick fur and high metabolism, *O. princeps* finds even brief exposure to moderately warm temperatures (>25°C) lethally stressful (Smith 1974, Smith 1990), and so must have been forced to either follow the retreating glaciers northward, or head uphill to the cooler mountaintops. Indeed, *O. princeps* is now found almost exclusively at high elevations in Western North America. Find a talus slope near an alpine meadow on many western mountains, and you have found good habitat for *O. princeps*. Severe heat-

intolerance isolates pikas to these high elevations, effectively producing a set of "sky island" populations. How are these "island" populations related to each other? Are mountain passes elevated enough to allow gene flow, and if so, are there geographical features that have created a correlated genetic structure?

The rare exception to high altitude habitat is the Columbia River Gorge splitting the states of Washington and Oregon, where the shade of high, steep cliffs maintains reasonably cool temperatures throughout the year. The Gorge can also be thought of as an island, as temperatures just east or west would prove lethal to pikas during the warmer months of the year. While the cooling effect of the high, steep cliffs seems unique to the Columbia River Gorge, certain mountain passes might have similar topography that allows dispersal and gene flow between two mountains. This would seem reasonable for the fairly well connected mountains of the Cascade Range, but less likely for the mountains of Eastern Oregon, namely the Steens, Strawberries, and Wallowas, which are separated by many miles of high desert. Connectivity in the Cascades suggests some possibilities for gene flow, while isolation in the east suggests little to no gene flow. Hence, fewer clades in the Cascade Range of Oregon as compared to the mountains of the eastern part of the state are expected.

***Ochotona princeps***

While pikas may be confused for rodents, they are members of the Order Lagomorpha along with rabbits and hares. Pikas communicate regularly with high-

pitched calls, while other Lagomorphs are essentially non-vocal as adults (Maser 1998). The Family Ochotonidae is hypothesized to have originated in Asia where there are approximately 28 extant species living both in montane and grassland environments (Niu et al. 2004, but see Smith 1990, and Maser 1998). Ancestors to *O. princeps* dispersed across the Bering Land Bridge in the early Pleistocene, and fossils of *O. princeps* were found in 36 of 46 known Quaternary fossil sites of *Ochotona* in North America (Mead 1987). That most of the latter date to within the last 500,000 years (Mead 1987) indicates a relatively recent dispersal. The Wisconsinian glacial period probably forced the separation and subsequent speciation of *O. princeps* and *O. collaris* from a common ancestor (Guthrie 1973); *O. princeps* was pushed south and *O. collaris* north into the Bering refugium by the expanding glacial ice. *Ochotona princeps* and *O. collaris* are the only extant species of pika in North America. The current range of *O. collaris* is from

Fig. 1 *Ochotona princeps* (http://www.companyofadventurers.com)

southeast Alaska to northwestern Canada.

Although *O. princeps* is found exclusively in talus slopes today, populations from the Quaternary Period also resided in grasslands at lower elevations as much as 100km farther south of its present distribution (Grayson 1987, Mead 1987). With the retreat of Wisconsinian glaciers, heat intolerant *O. princeps* moved upslope to form isolated montane populations by 7,000 years ago (Grayson 1977). Hafner (1994) estimated the minimum permafrost elevation during the height of the Wisconsinian in the southern Rocky Mountains, and showed that current isolated populations were indeed connected at that time. Hafner (1994) also demonstrated that most current populations of pika in the southern Rocky Mountains exist within 20km of estimated altithermal permafrost, meaning that pika populations have dispersed little from their most thermally restricted period 6000 years ago.

*Ochotona princeps* are small, ranging in body length from 162 to 216 mm (Hall 1981), and in mean body mass from 121 to 176 g (Hall 1981; Smith 1978). Their feet and short legs are covered with thick fur except for exposed toe pads. Ears are large, rounded, and suborbicular, with fur on both sides. No tail is visible externally. Pelage ranges from reddish-brown to gray on the dorsal surface, and whitish on the ventral. Ears are normally dark with white margins. The twice annual molt of *Ochotona princeps* corresponds to a summer and warmer winter coat (Smith 1990). Interestingly, males have no scrotum or baculum and females

no vulva. A single "pseudo-cloacal" opening is used for both rectal and urogenital functions (Smith 1990).

*Ochotona princeps* do not hibernate, but spend the winter in or near talus slopes which are typically buried by 2.4 meters or more of snow (Maser 1998). Spaces between talus provide both shelter and tunnels leading to the edge of the talus where pikas dig down to the vegetation for foraging. Once snows have melted enough to expose meadow herbs and forbs, *O. princeps* spend much of the day traveling from talus to meadow to collect vegetation. Returning to the talus, the vegetation, or "hay", is laid in the sun to dry, and eventually haypiles are formed under rocks in the talus slope and used to augment food supplies during the winter. Only some of the hay is consumed and over time its degradation produces nutrient rich soils, thereby allowing for increased plant growth in and around scree slopes (Aho et al. 1998). Haying activity may last from June until November (Maser 1998) and is more frequent in the early morning and later afternoon in order to avoid the midday heat. So frequent are the trips from talus to meadow that trails are often worn in. *Ochotona princeps* are highly territorial, and establish territories as soon as two weeks after weaning (Maser 1998). Both sexes defend territories, and males and females tolerate each other's presence only during their brief mating season. Territorial defense may require a substantial time investment including frequent warning vocalizations and occasional aggressive actions (Smith 1990).

Fig. 2 Pika haypile on Mt. Adams, Washington (approximately 3 ft. wide)

*Ochotona princeps* are highly territorial, and establish territories as soon as Young must establish a territory quickly to have time to collect enough hay for the winter, and the low likelihood of dispersal across non–talus terrain leads to the establishment of territories in the natal range (Smith 1990). Individuals born late in the summer (usually a second litter) often do not have enough time to grow to the size required to establish territories and collect enough hay before winter arrives. Natal philopatry suggests minimal gene flow among populations, particularly for populations on adjacent mountains separated by a low elevation valley.

**Genetic Structure**

Using allozymes, Hafner and Sullivan (1995) suggested the existence of four major genetic units prior to the Wisconsinian Glacial Period, which they

labeled Northern Rocky Mountains, Southern Rocky Mountains, Cascade Range, and Sierra Nevada. While this coarse scale structure may tell us something about *O. princeps* in general, it does not illuminate the finer scale questions I attempt to address. If populations of *O. princeps* were able to homogeneously interbreed across their geographic range as a result of their continuous lower elevation habitat prior to 7,000 years ago, some of their current genetic structure may have arisen subsequent to this time, as populations effectively became isolated on sky islands.

There are some 36 recognized subspecies of *Ochotona princeps*, all of which originally were described on the basis of morphology. But what constitutes a subspecies, and are these 36 subspecies valid? Long and Kittles (2003) identified four major definitions of subspecies: essentialist, taxonomic, population, and lineage. The essentialist concept (Hooton 1926) understands a subspecies as a group of individuals sharing, through common descent, a particular combination of unique characters. The taxonomic subspecies concept (Mayr 1969) defines a subspecies as a group of populations restricted to a subdivision of the species' range and which are phenotypically similar. In the population subspecies concept, Dobzhansky (1970) defines the subspecies as, "genetically distinct Mendelian populations." Templeton's (1998) lineage concept demands both historical continuity and contemporary genetic differentiation to define a subspecies. In other words a subspecies is a distinct evolutionary lineage of a genetically differentiating species that resulted from a consistent historical barrier to gene flow.

Are the four concepts of subspecies arbitrary or do they have an identifying biological basis? If they do have a biological basis, can we unify these concepts? Lewontin (1972) argued that the variation found in human populations was too great to differentiate humans into races or subspecies; however, Edwards (2003) argued that Lewontin was correct only when considering one locus or trait. By using multiple loci or traits, Edwards (2003) showed that the likelihood of being able to differentiate subspecies increases because locus and trait frequencies may generally tend to be correlated within a subspecies; i.e. a subspecies is a correlational structure of traits (Woodley 2010). Woodley maintained that this "correlation structure" idea unifies the four subspecies concepts, but that it does not address the arbitrariness of what is meant by distinctive.

All four definitions rely on the distinctiveness of a population; however, disagreement as to what merits "distinctiveness" is considerable (Woodley 2010). Morphology may seem like a good metric, but one gene may influence multiple phenotypic traits (pleiotropy), potentially leading to large morphological differences with slight variation in the gene. The most common example is the variety of body types found in the dog, all of which are grouped into a single subspecies (*Canis lupus familiaris*). With respect to birds, Zink (2004) determined that "97% of continentally distributed avian subspecies lack population genetic structure indicative of a distinct evolutionary unit." This statistic undermines morphological and geographical methods of determining subspecies.

Analysis of molecular data is therefore one method of assessing the taxonomic level of classification (Avise 2000), but has been unevenly applied at the level of subspecies. Heterozygosity (allele frequency) theoretically should be directly proportional to the number of subspecies, but a literature search does not bear this out (Woodley 2010). For example, the observed heterozygosity of chimpanzees (*Pan troglodytes*) is 0.63 – 0.73 (Reinartz et al. 2000, Wise et al. 1997, Gander et al. 1997), that of the wolf (*Canis lupus*) in North America is 0.528 (Garcia-Moreno et al. 1996), and that of the coyote (*Canis latrans*) is 0.583 (Garcia-Moreno et al. 1996) yet there are only 4 recognized subspecies of chimpanzees compared with 37 recognized subspecies of wolf and 19 of coyote. The species with the highest level of heterozygosity has the fewest subspecies while the species with the lowest level of heterozygosity has the highest. Other molecular metrics such as genetic distance have also been applied unevenly (Woodley 2010).

Zink (2004) suggested that Avise's (2000) concept of reciprocal monophyly was a measure that could accurately be applied to determine the validity of recognized subspecies. If a subspecies has evolved independently for $2N_{ef}$ generations, where $2N_{ef}$ is the inbreeding effective size of the female population, the mtDNA gene tree should show a common ancestral sequence not found in other subspecies (Zink 2004); i.e. the subspecies is monophyletic. Thus, any recognized subspecies that is shown to not be monophyletic should not be regarded as a true subspecies.

From a taxonomic perspective, four subspecies of pika are currently recognized in Oregon: *O. p. taylori* extends from southeast and south–central Oregon into northeastern California; *O. p. jewetti* includes northeastern Oregon and the Wallowa, Blue, and Strawberry Mountains; *O. p. fumosa* is restricted to the area immediately adjacent to Mount Jefferson and the Three Sisters; and *O. p. brunnescens*' range extends from southern Oregon along the Cascades into the western Washington Cascades and ends in the Coast Range of British Columbia. All four subspecies belong to Hafner and Sullivan's Cascade Range group of *O. princeps* (Hafner and Sullivan 1995).

The designation of these subspecies is based on morphology and their validity has not been corroborated via genetic means. The geographic isolation of *O. p. taylori* and *O. p. jewetti* (i.e. confinement to the Wallowa/Strawberry Mountains and Steens Mountain, respectively) suggest genetic isolation, hence the validity of subspecific designation. On the other hand, *O. p. brunnescens* and *O. p. fumosa* are widely distributed in apparently contiguous populations throughout the Cascade Range, and as a consequence, may not be sufficiently isolated to have evolved characters suggestive even of subspecific recognition. Populations of *O. p. brunnescens* exist both north and south of *O. p. fumosa*, possibly linked by a narrow corridor to the west of Mt. Jefferson, as proposed by Hall (1981). If pikas dispersed southward, as suggested by Hafner and Sullivan (1995), such a separation into two subspecies is unlikely. Indeed, if *O. p. fumosa*'s designation is legitimate, pikas in the southern range of *O. p. brunnescens* should be more closely related

to *O. p. fumosa* than to northern *O. p. brunnescens*, and/or constitute their own subspecies. Another possibility is *O. p. brunnescens* dispersed from the eastern part of the state in which case *O. p. brunnescens* is more closely related to *O. p. jewetti* or *O. p. taylori.*

Another inconsistency with the current subspecies designations of *Ochotona* is the extensive and narrow north-south distribution of *O. p. brunnescens*. While the Cascades are relatively continuous in Oregon and in Washington, the Columbia River presents a significant obstacle for gene flow between the two states. The catastrophic Missoula Floods, a major vicariant event that coincided with the end of the Wisconsinian in the Pacific Northwest, may have separated *O. princeps* into populations north and south of the river, possibly leading to current genetic structure. At the end of the Wisconsinian, ice dams that formed Lakes Columbia and Missoula repeatedly (as many as 40 times; Benito and O'Connor 2003) formed and burst to produce floods of incredible proportions that extended from Montana to the mouth of the Columbia River. The eastern end of the Columbia River Gorge experienced floodwaters up to 300m above present river levels, while the water that exited the Gorge on the western end was up to 150m above present levels (Alt 2001). Such depths and gradient must certainly have severed all contact between *O. princeps* populations north and south of the Columbia River.

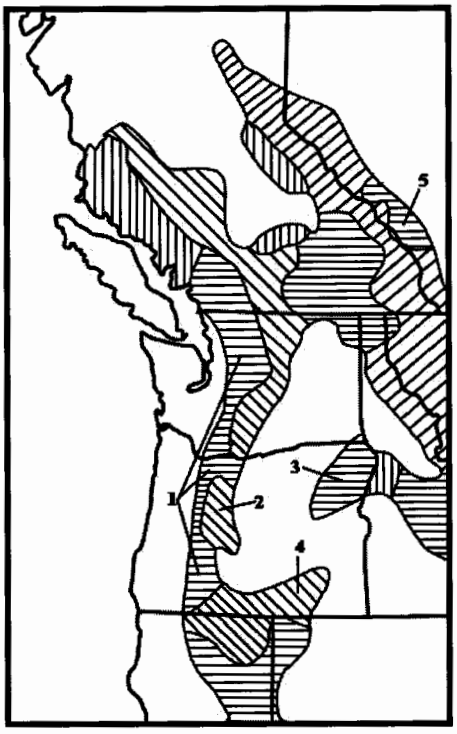


Fig. 3 Subspecies of *Ochotona princeps* in the Pacific Northwest: (1) *O. p. brunnescens*, (2) *O. p. fumosa*, (3) *O. p. jewetti*, (4) *O. p. taylori*, and (5) *O. p. princeps* (Other subspecies are included that are not part of this study.) Adapted from Hall (1981).

For this study, I use the concept of subspecies as reciprocally monophyletic clades to test the validity of the four subspecies of *O. princeps* found in Oregon. By applying phylogenetic analysis of DNA sequence data from the mitochondrial cytochrome *b* and control region loci I test the hypotheses that 1) *O. p. fumosa* constitutes a subspecies distinct from *O. p. brunnescens*, 2) that the Columbia River constitutes a barrier to gene flow giving rise to two subspecies rather than the single subspecies *O. p. brunnescens*, and 3) that populations in eastern Oregon (*O. p. jewetti* and *O. p. taylori*) are genetically distinct from populations in the Cascade Range (*O. p. brunnescens* and *O. p. fumosa*).

## MATERIALS AND METHODS

### Trapping Sites

Trapping localities were determined first at a coarse scale based on the hypotheses to be tested. Testing the validity of the *O. p. fumosa* subspecies designation required samples from *O. p. fumosa* populations, centered on Mt. Jefferson, for comparison with samples from *O. p. brunnescens* populations, found throughout most of the remainder of the Oregon Cascade Range, and *O. p. jewetti* from the Wallowa and Strawberry Mountains. Samples north and south of the Columbia River Gorge were compared to test the hypothesis that *O. p. brunnescens* should be split into two clades. Specimens from the Cascade Range and the eastern ranges (Wallowa, Strawberry, and Steens) were also collected to test the east-west disjunction hypothesis.

After this coarse geographic evaluation, specific trapping sites were determined using museum collection lists, reference sources (Verts and Carraway 1998), contact with local biologists, and personal reconnaissance. *Ochotona princeps* habitat is relatively easy to locate because they are nearly exclusively restricted to talus slopes that are located near meadows, which can often be seen at great distances from high vantage points. Two exceptions are sites in the Columbia River Gorge and the type locality of *O. p. fumosa* on Mt. Jefferson, both of which are in relatively dense coniferous forests.

Fig. 4 Typical *O. princeps* habitat. Strawberry Mountains, Oregon.

Nine sites were chosen for the study including the type locality of *O. p. princeps* in Jasper National Park Alberta, Canada. The sites listed north to south are: **Jasper National Park**, Canada; **Snoqualmie Pass**, Washington; **Mt. Adams**, Washington; **Columbia River Gorge**, Oregon; **Mt. Hood**, Oregon; **Wallowa Mountains**, Oregon; **Mt. Jefferson**, Oregon; **Strawberry Mountains**, Oregon; and **Steens Mountain**, Oregon (see Appendix A).

I was not able to obtain specimens from all locations and therefore used museum specimens from the Portland State University Museum of Vertebrate Biology to augment the data. *Ochotona collaris* was chosen as an outgroup since it is considered the nearest extant relative to *O. princeps*; sequences of *O. collaris*

were obtained courtesy of Link Olson of the University of Alaska Fairbanks Museum of the North.

**Trapping Methods**

Live trapping *O. princeps* is difficult because of their susceptibility to heat stress and the consequent requirement for near constant monitoring of the traps. Moreover, the remoteness of many sites made constant monitoring difficult. Given this, and the fact that animals were to be deposited as specimens in the Portland State University Museum of Vertebrate Biology, I used common, rat-sized snap traps to quickly and humanely euthanize the animals.

Haypiles are constructed during the warm summer months, and several trails that extend from haypile to nearby meadow often are apparent. These trails proved ideal for trap placement with one trap "pointed" up trail, and the other down trail. In some sites, no trails were obvious despite large haypiles; in these cases I placed traps on and around the haypile and in any well-used tunnels formed by the talus around the haypile. All traps were baited with a combination of peanut butter and oats. Trap efficacy varied greatly from site to site, from a low of no captures in 225 trap days (Mt. Jefferson) to a high of 4 in 50 trap days (Strawberry Mountains).

**Call Recording**

While this study used phylogenetic analyses of genetic data to test hypotheses, calls were recorded at five sites (Mt. Adams, Mt. Hood, Mt.

Jefferson, Steens Mountain, and Wallowa Mountains) using a parabolic dish antenna and a digital recorder. Recordings were visualized using *SoundRuler* Acoustic Analysis software (Gridi-Papp 2003 – 2007). *Ochotona princeps* has two calls, a short alarm call, and a longer call produced mainly by males in the breeding season (Broadbooks 1965, Smith 1990). The short alarm call, which is heard much more often than the long call, was recorded at each site. An extensive analysis of calls was not performed, but I noted obvious differences among sites.

**Preservation Method and DNA Extraction**

All specimens were placed in 95% Ethanol and stored in the laboratory at room temperature. Cubes of muscle approximately 0.5cm on edge were removed from the right rear quadriceps of all specimens, and stored individually in 95% Ethanol in Eppendorf tubes. Scalpel blades were thoroughly cleaned with DNA Away prior to tissue extraction for each specimen. Whole genomic DNA was then extracted from the tissue of the freshly collected specimens using a Qiagen DNeasy® tissue kit (Qiagen, Valencia, CA).

The muscle tissue was removed from the Ethanol and incubated overnight at 55°C in a tube containing 180µL of Buffer ATL and 20µL of Proteinase K, following the DNeasy protocol suggested by Qiagen. After incubation, 200µL of Buffer AL was added and the tube incubated 10 minutes at 70°C. 200µL of 95% Ethanol was added and the entire solution was transferred to a mini-column for

elution. Buffers AW1 and AW2 were run through the column before eluting the DNA with Buffer AE. Extracted DNA was stored at –80°C.

DNA was also extracted from the skin and fur of museum specimens. Pieces of skin with fur approximately 0.5cm square were removed from the ventral surface of each specimen. Care was taken to remove pieces along the ventral suture in order to minimize damage to the study skins. Scalpel blades were thoroughly cleaned with DNA Away prior to tissue extraction for each specimen.

Using a Qiagen DNeasy® tissue kit (Qiagen, Valencia, CA), each piece of tissue was incubated overnight at 55°C in a tube containing 450µL of Buffer ATL and 50µL of Proteinase K. 125µL of the resulting supernatant was then mixed with 625µL of Buffer PB and run through a purification column. I then ran 750µL of Buffer PE through the column before eluting the DNA with Buffer EB. This extraction technique worked well on 67% of the samples. Museum samples were taken from specimens collected primarily in the mid-1960s.

**Selection of Primers and PCR**

Mitochondrial DNA (mtDNA) was used as the source of genetic data because recombination is not an issue, thus leading to a geographic sorting of lineages (Avise et al. 1984), and higher resolution is obtained compared with a similar length of nuclear DNA (Slade et al. 1994). Cytochrome *b* (cyt *b*) is a common mitochondrial gene used for inter- and intraspecific phylogenetic studies, but typically when studying divergences occurring around 50,000 years ago.

The hypotheses of this study rest mostly on events that took place within the last 10,000 years. Cyt *b* was thus used to detect divergence events that occurred prior to the end of the Wisconsinian. DNA from the mitochondrial control region is non-coding and known to evolve at a more rapid rate than cyt *b* (Stoneking et al. 1991). Consequently, I used the control region to address the hypotheses of this study. While several control region primers obtained in the literature (Slade et al. 1994; Waltari et al. 2004) were tried, I had success only with Formozov et al.'s (2006), which were subsequently used in the analysis (see Appendix C).

All primers were obtained from Integrated DNA Technologies (San Diego, CA), reconstituted with TE buffer (Promega, Madison, WI), and diluted with $H_2O$ to 10nmol. Master mixes were created for each primer pair using the basic formula: 1μL of primer A, 1μL of primer B, and 20μL of $H_2O$. 22μL of the master mix was combined with 3μL of DNA and cycled in a Minicycler. By raising and lowering the temperature of the reactants to specific temperatures for specific lengths of time, each desired sequence was copied multiple times utilizing a polymerase chain reaction. Program GWBCYTb was used to set the temperatures and durations for cytochrome *b* primers and program GWBCR000 was used for control region primers (see Appendix D). Programs were obtained from Zachary Harlow. Quality of PCR product was determined by visualizing DNA on Invitrogen® (Carlsbad, CA) agarose E-gels. After clearing the gel for 2 minutes at 70 volts, 5μL of PCR product and 9μL of $H_2O$ were added to each well. 14μL of

$H_2O$ were added to empty wells to prevent drying and distortion of the gel. Single bands indicated that the primers successfully selected a unique piece of the genome; lanes that exhibited a single banding were selected for subsequent sequencing.

**Sequencing Methods**

Before sequencing, PCR product was cleaned using the Qiaquick PCR Purification Kit (Qiagen, Valencia, CA) to ensure that all PCR residues were removed. 125μL of Buffer PB was added to the PCR product, placed in a column, and centrifuged for 60 seconds at 8000 rpm. The flowthrough was discarded, and 750μL of Buffer PE was added to the column and spun for 60 seconds. Flowthrough was discarded and the column was spun an additional 60 seconds. The column was placed in a 1.5mL Eppendorf tube and the DNA eluted by adding 40μL of Buffer EB and spinning for 60 seconds.

Master mixes were created for each primer using the basic recipe: 2.5μL of primer, 1μL of 5X buffer, 2μL of Big Dye, and 2μL of $H_2O$. For the sequencing reaction, primers were diluted to 2.5nmol. 7.5μL of master mix was added to 2.5μL of PCR product and cycled in a thermal cycler using program GWBCYTb for cytochrome *b* and GWBCR000 for the control region. Reaction product was plated and sent to the Oregon State University Center for Gene Research and Biotechnology for sequencing on an Applied Biosystems, Inc. ABI 3100 automated

sequencer. Bases not read by the sequencer were manually determined using SEQ-ED software (Nilsson and Gunnar 1984) to visualize the chromatograms.

**Phylogenetic Analyses**

MacClade v. 4.06 (Maddison and Maddison 2000) and ModelTest v. 3.7 (Posada and Crandall 1998) were used to manually align the sequences and test for the optimal model of molecular evolution respectively. ModelTest evaluates 56 models of evolution based on Akaike information criterion (AIC – used for control region) or hierarchical likelihood ratio tests (hLRTs – used for cytochrome *b*). Posada and Buckley (2004) show there are some advantages to using AIC, but that hLRTs also yield interpretable and valid results.

Maximum parsimony (MP), maximum likelihood (ML), and metropolis coupled Markov chain Monte Carlo Bayesian inference (MCMCMC) were used to estimate the phylogenetic relationship among populations. MP and ML were implemented using the software PAUP* v. 4.0b10 (Swofford 2002) while MrBayes v.3.1.2 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) was used for the Bayesian analysis.

**Cytochrome *b***

A heuristic MP search was run using the tree-bisection-reconnection (TBR) algorithm with gaps treated as missing. Branch support was evaluated using nonparametric bootstrap analysis with 1000 replicates (Felsenstein 1985). A

heuristic ML search was run using TBR and the best-fit model (TrN+G) as determined by ModelTest 3.7 (Posada and Crandall 1998). Branch support was evaluated using nonparametric bootstrap analysis with 500 replicates (Felsenstein 1985). ML and MP analyses were implemented in PAUP* (Swofford 2002).

A Bayesian search using Metropolis-coupled Markov chain Monte Carlo sampling was implemented using MrBayes (Huelsenbeck and Ronquist 2001). One cold and eight warm chains were run at a metropolis temperature of 0.02 and a swap frequency of 10. The analysis was run for 20,000,000 generations, sampled every 500 generations, and a consensus tree constructed from the last 10,000 samples. All three trees were rooted using the sequence of *O. collaris* obtained from Genbank. Two other Genbank sequences of *O. princeps* from Idaho were included in the analysis. A genetic distance matrix was created using PAUP* (Swofford 2002).

**Control Region**

MP, ML, and Bayesian analyses were implemented using the same protocol as for cyt *b* except that I used a different best-fit model (HKY+I+G) and ran the Bayesian analysis for 100,000,000 generations. The two Genbank sequences of *O. princeps* used in the cyt *b* analysis were not used in the control region analysis. Trees were rooted using *Ochotona collaris* sequences obtained courtesy of Link Olson.

## RESULTS

Newly caught specimens accounted for 57% of the 28 specimens used for my analyses. A complete list of specimens, and whether they were newly caught or of museum origin, is found in Appendix B.

Individual calls were recorded at 5 sites: Mt. Hood (4 calls), Mt. Adams (4), Mt. Jefferson (4), Steens Mountain (1), and the Wallowa Mountains (2). In addition to the recordings, at least 4 individual pikas were heard at the Strawberry Mountains and the above sites, except for Steens Mountain where 2 were heard. Recordings of alarm calls show calls in the Cascade Range were distinct from calls in the eastern mountains. Specifically, western *O. princeps* had a single syllable alarm call, where eastern *O. princeps* had a double syllable alarm call (Fig. 5).

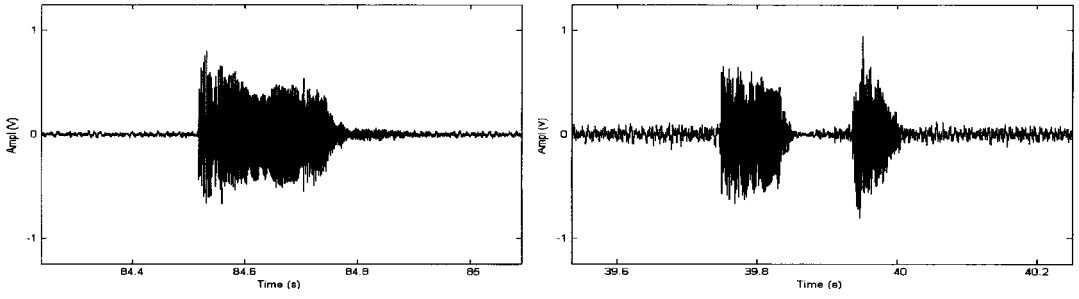


Fig. 5 (left) Oscillogram of pika call from Mt. Adams showing single syllable; (right) Oscillogram of pika call from Wallowa Mts. showing double syllable.

This simple distinction groups specimens from Mt. Adams, Mt. Hood, and Mt. Jefferson into one clade, and those from the Wallowa Mountains and Steens Mountain into a separate clade (Fig. 6). While not recorded, single syllable alarm

calls were heard at the Mt. Adams, Snoqualmie Pass, and Multnomah Falls sites, and double alarm calls were heard at the Strawberry Mountains site.

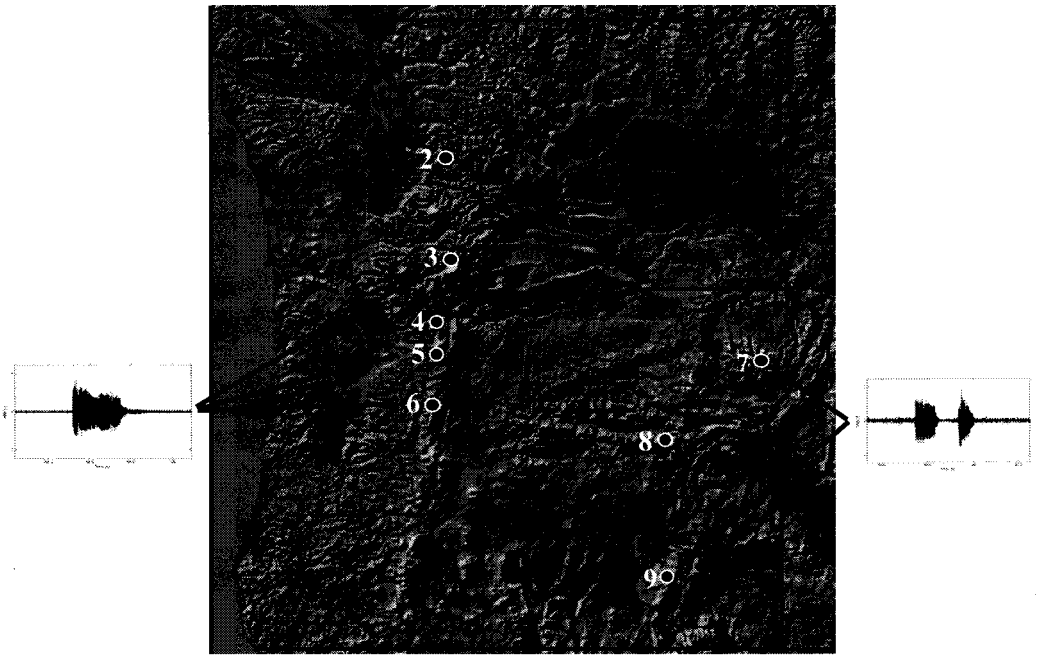


Fig. 6 Recording location of single syllable calls in the west and double syllable calls in the east. Single calls were heard at all western sites, and double calls were heard at all eastern sites (See Appendix A for site names.)

DNA was successfully extracted from all newly caught specimens and most of the museum specimens from the 1960's. Specimens collected from the 1950's all were too degraded to yield sufficient DNA for extraction. The cyt *b* gene of an initial set of specimens (Jasper, Strawberry Mountains, Wallowa Mountains, Snoqualmie Pass, Mt. Adams, Mt. Hood, and two Idaho specimens) was successfully sequenced, but the phylogenetic analysis, while useful, had too limited a resolution to thoroughly address two of the hypotheses of this study. Thus,

attention was focused on the more rapidly evolving control region. While several primers were used to sequence the control region, the only ones to work were those developed by Formozov et al. (2006).

The three analytical algorithms for cyt *b* yielded similar tree topologies (Figs. 7–9), with ML providing the best resolution. Two major clades with good bootstrap support (84 to 100%) are apparent: a western and eastern group. The Jasper and Idaho specimens form a clade which is sister to specimens from the Wallowa Mountains (*O. p. jewetti*), Strawberry Mountains (*O. p. jewetti*), and Steens Mountain (*O. p. taylori*). Mt. Hood (*O. p. brunnescens*) is shown as a sister clade to Mt. Adams (*O. p. brunnescens*) and Snoqualmie Pass (*O. p. brunnescens*), a relationship not recovered by MP and Bayesian analyses.

The three analyses for the control region (Figs. 10–12) yielded similar topologies except that the Maximum Likelihood analysis placed P5 Mt. Adams in a sister relationship to all other western specimens, while Maximum Parsimony grouped the Oregon and Washington specimens in one clade distinct from Jasper. ML and Bayesian analyses indicate three clades: eastern, western, and Jasper. Though considered two subspecies, the control region sequence data showed no resolution between the Wallowa Mountains and Steens Mountain. The Linn County specimens, representing *O. p. fumosa*, are found within a clade comprised of *O. p. brunnescens* specimens (Multnomah Falls, Government Camp, and Mt. Hood) on all three phylogenetic trees.

The following figures show all phylogenetic trees produced in this study. Unless otherwise noted all specimens are *Ochotona princeps*, and subspecies correspond with the following geographical names: *O. p. brunnescens* (Snoqualmie Pass, Mt. Adams, Multnomah Falls, Mt. Hood, and Government Camp); *O. p. fumosa* (Linn County); *O. p. jewetti* (Strawberry and Wallowa Mountains); and *O. p. taylori* (Steens Mountain).

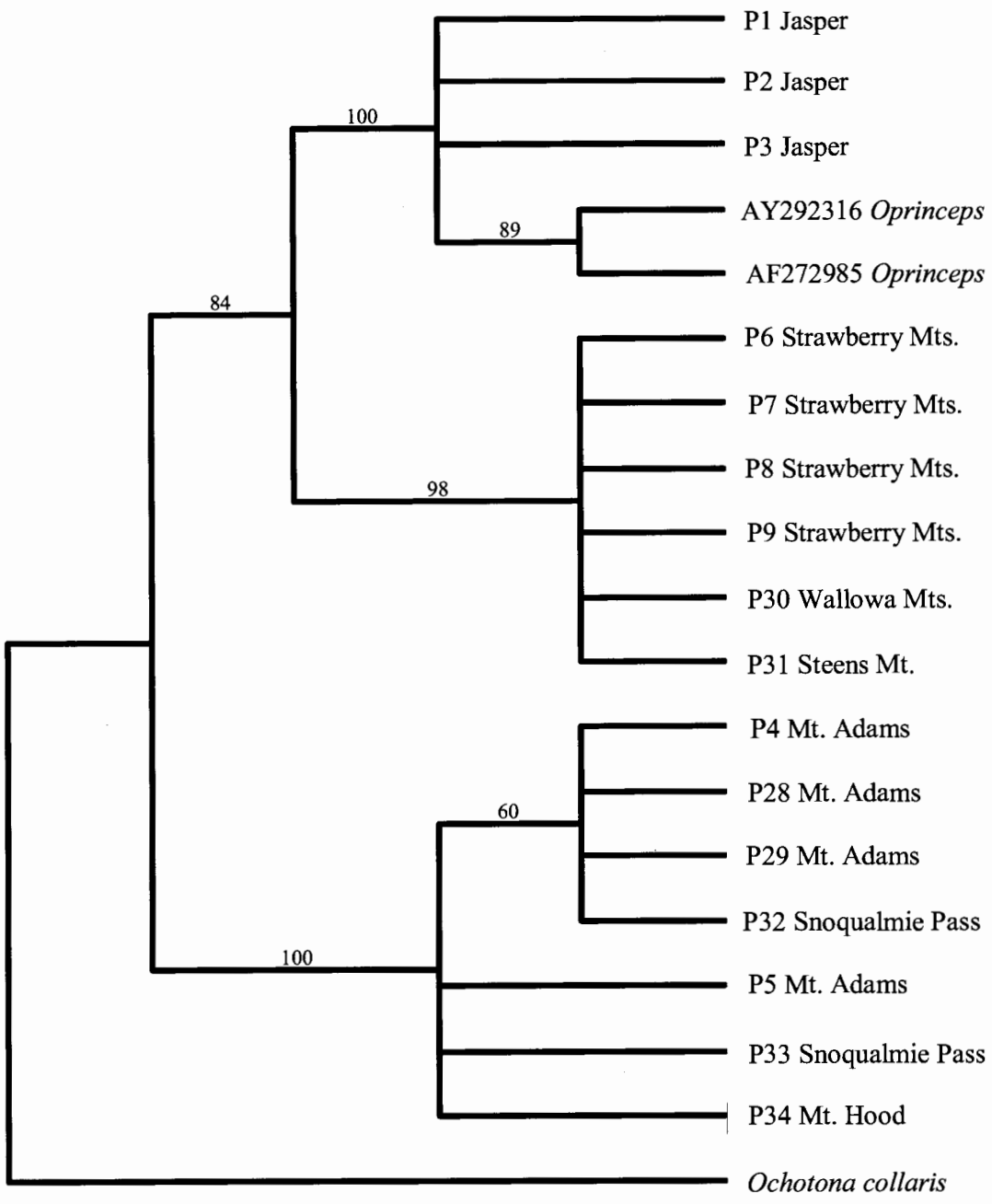


Figure 7. Phylogenetic tree derived using the Maximum Parsimony criterion from the sequence data for cytochrome *b*. Values above the lines indicate percent bootstrap support (500 replicates), and the tree is a 50% majority consensus tree.

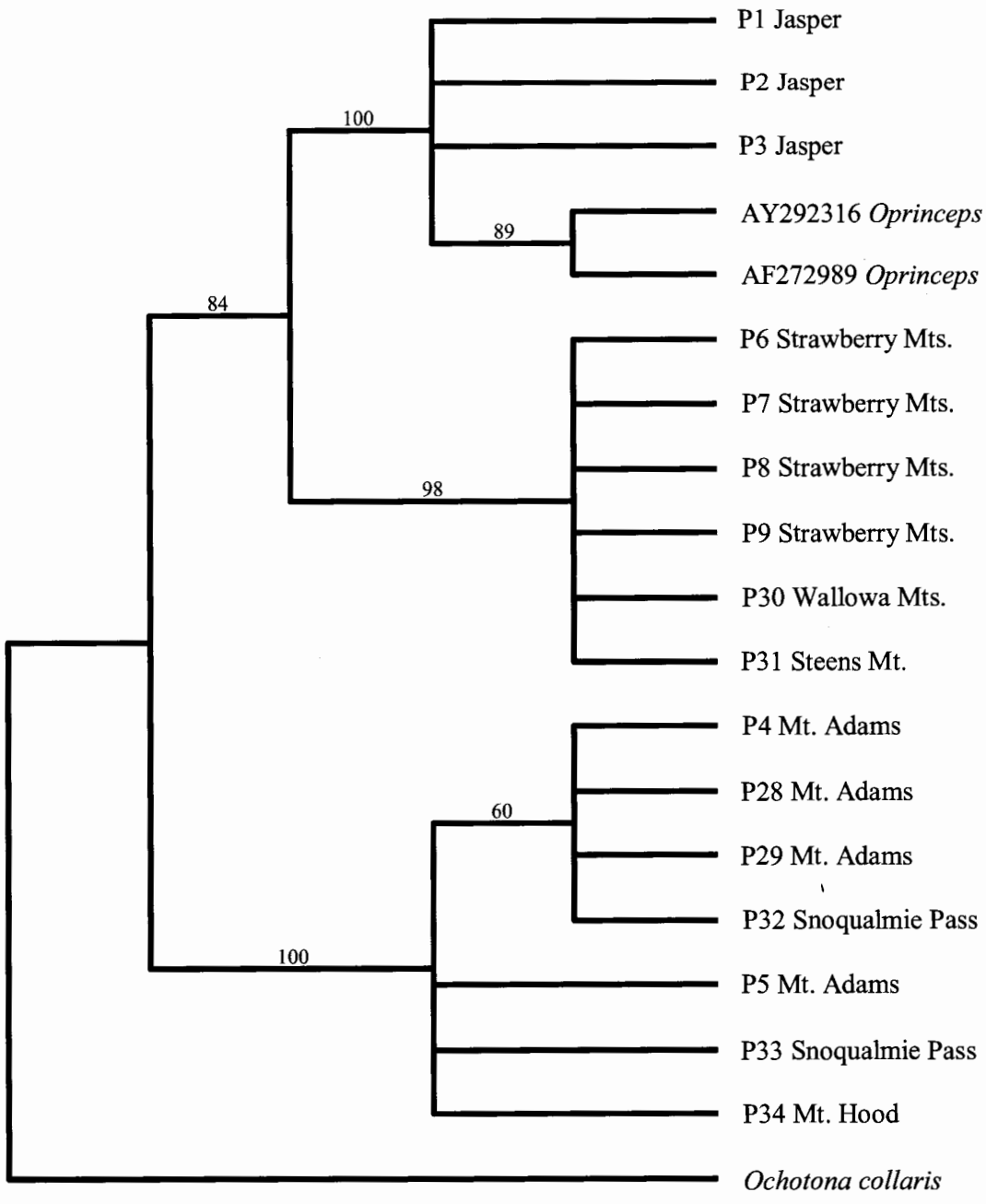


Figure 8. Phylogenetic tree derived using the Bayesian criterion from the sequence data for the cytochrome *b*. The tree is a 50% majority consensus tree.

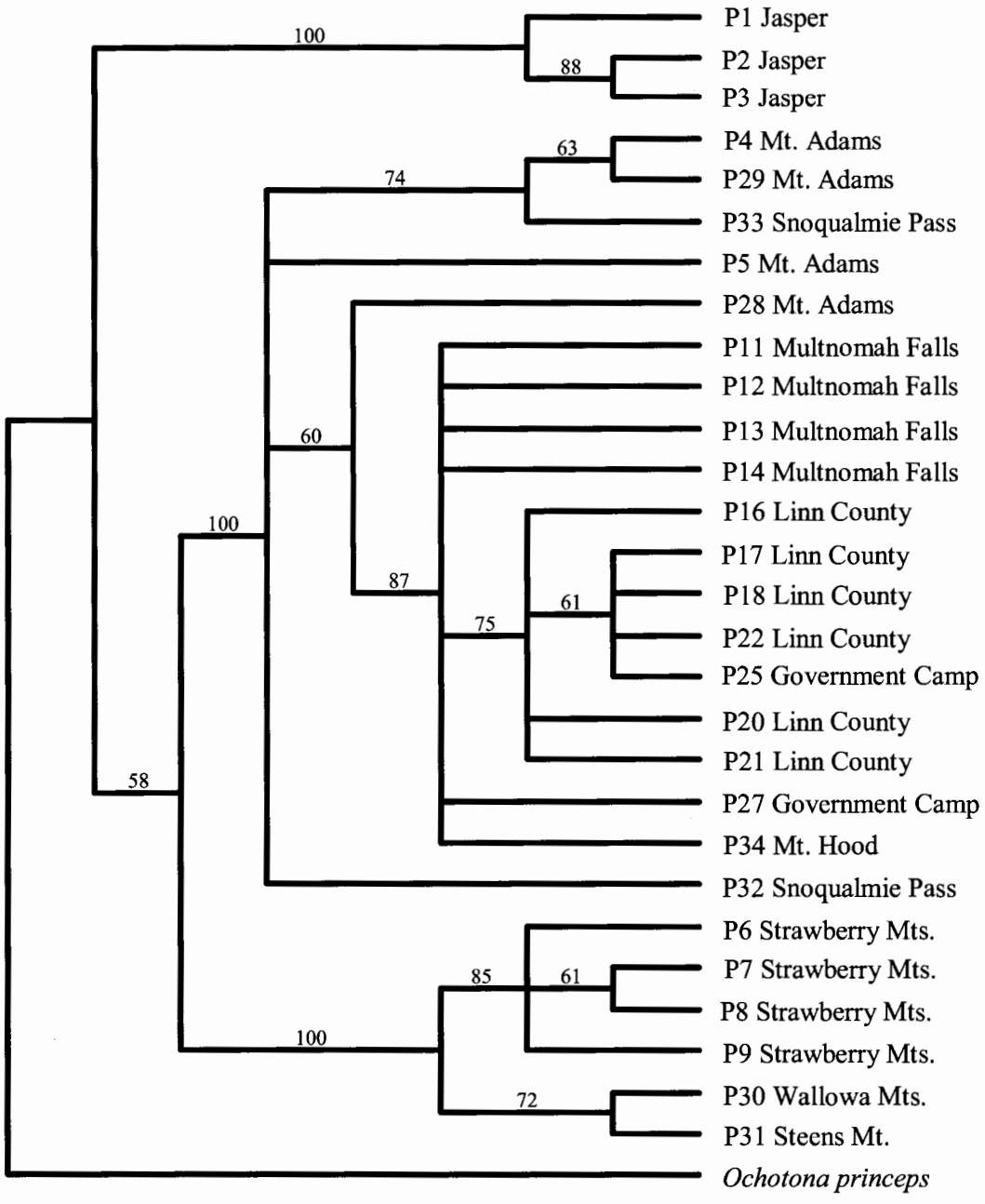


Figure 9. Phylogenetic tree derived using the MaximumParsimony criterion from the sequence data for the control region. Values above the lines indicate percent bootstrap support (500 replicates), and the tree is a 50% majority consensus tree.

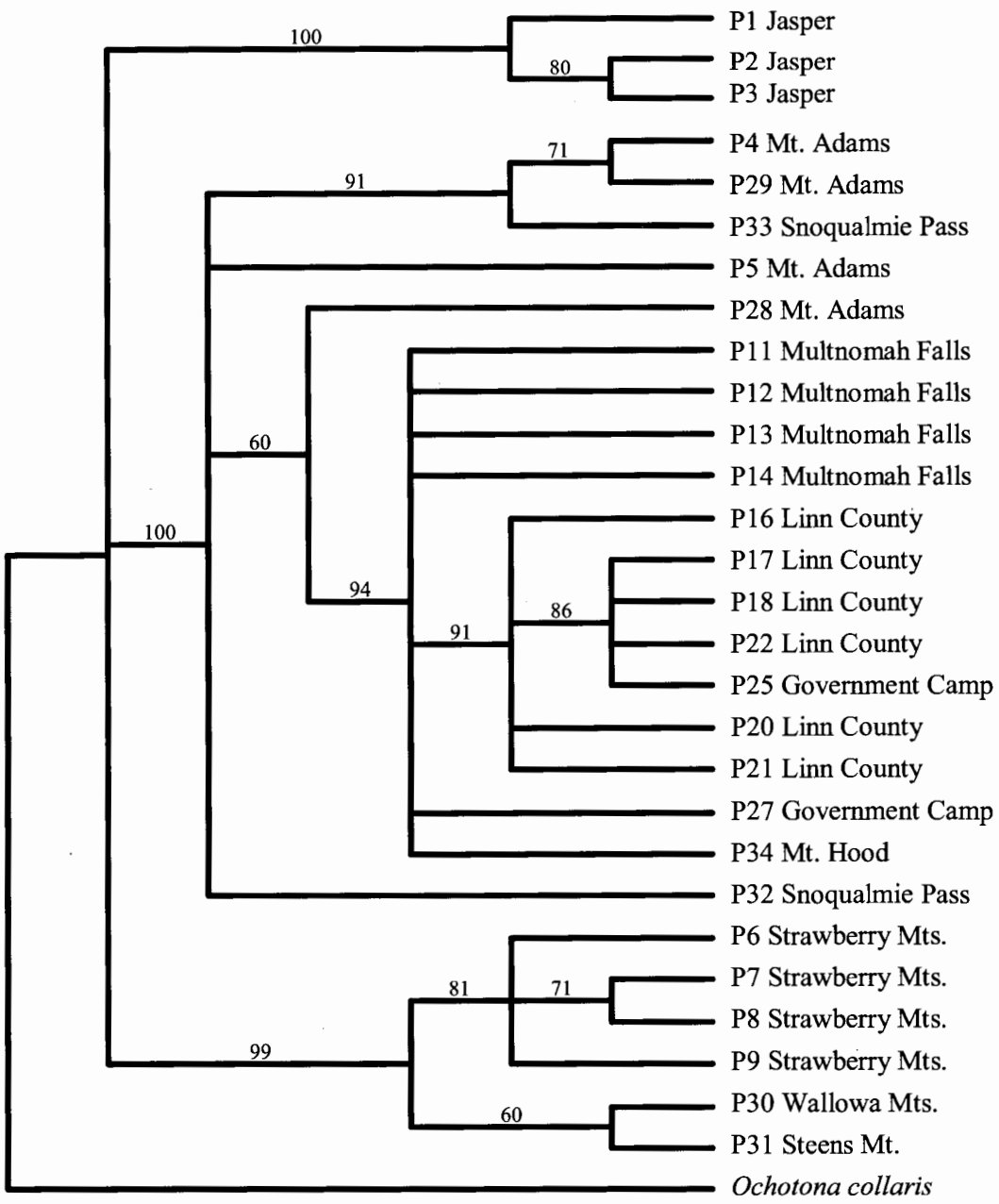


Figure 10. Phylogenetic tree derived using the Bayesian criterion from the sequence data for the control region. The tree is a 50% majority consensus tree.

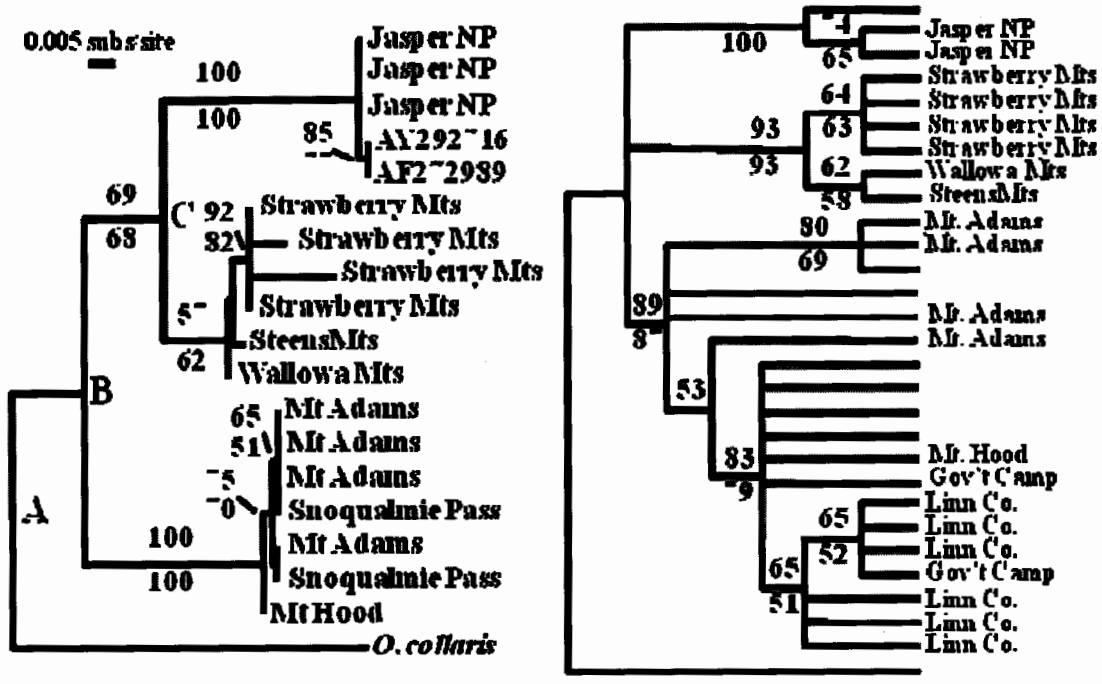


Fig. 11. Phylogenetic tree derived using the Maximum Likelihood (ML) criterion from the sequence data for cytochrome *b* (left) and ML consensus tree for the control region (right). Values above the lines indicate percent bootstrap support (500 replicates); below are percent jackknife support values (500 replicates). In the cytochrome *b* tree, letters indicate Tamura–Nei genetic distances at the major nodes: node A (*O. collaris v. O. princeps*) averages 9.577%; B (*O. princeps*, Cascades *v.* Rockies), 6.790%; C (*O. princeps*, Eastern *v.* Western Rockies), 5.135%.

## DISCUSSION

Knowledge of population genetic relationships among island populations is required to determine whether one population constitutes a novel species. Such a determination is not limited only to true islands: rather, the same logic applies to ecological islands of unique habitat formed by topography or other abiotic factors. This is precisely the case in *Ochotona princeps*. Comparing a map of subspecies in Oregon and southern Washington with a map of topography raises questions. Why does *O. p. fumosa* disrupt the range of *O. p. brunnescens* which otherwise extends from Canada to southern Oregon? Why is the distribution of *O. p. brunnescens* not disrupted by the Columbia River? While topography would suggest a separation of subspecies in eastern and western Oregon, given that the ranges may be continuous in northern Washington and Canada, will such separations withstand genetic scrutiny? The phylogenetic analyses in this study have, to some degree, illuminated these questions.

### Hypothesis 1: *O. p. fumosa* is a subspecies

*Ochotona princeps* individuals could have dispersed into the current range of *O. p. fumosa* by one of three routes: from the north, from the south, or from the east. According to Hall (1981), there is a thin band of *O. p. brunnescens* which skirts around the western edge of *O. p. fumosa* connecting northern Oregon populations of *O. p. brunnescens* with southern ones. The Cascade Range forms a

continuous chain of mountains and high mountain passes; no geographical feature corresponding to this hypothesized habitat band is known to exist or otherwise be discernable as distinct from the habitat that comprises the range of *O. p. fumosa*; thus it would seem that this band does not exist. If the pikas' dispersal routes went from north to south, any separation between *O. p. fumosa* and *O. p. brunnescens*, if it happened, would have occurred as a result of sympatric speciation after *O. p. brunnescens* extended its range to their present day southern terminus. Unless there is north-south gene flow via the thin putative western habitat band, southern populations of *O. p. brunnescens* should be more closely related to *O. p. fumosa* than to northern populations of *O. p. brunnescens* because their most recent ancestors would have come from habitat immediately to the north. It would be very unlikely that northern and southern populations of *O. p. brunnescens* would experience identical evolutionary trajectories with *O. p. fumosa* located geographically in between.

The same logic applies in the case of the reverse possibility: that dispersing pikas moved from south to north. If *O. p. fumosa* is a separate subspecies, *O. p. brunnescens* populations in the north should be more closely related to *O. p. fumosa* than to *O. p. brunnescens* in southern Oregon for reasons stated above. While no specimens from southern Oregon were used in this study, if *O. p. fumosa* is within a clade that includes *O. p. brunnescens* from the north, the direction of dispersal is irrelevant: *O. p. fumosa* is not a separate subspecies since it is not monophyletic.

The third option is that *O. princeps* dispersed to the current range of *O. p. fumosa* from the eastern part of the state during a colder period, when habitat would have been continuous and appropriate. This can be tested genetically: a closer relationship between *O. p. fumosa* and *O. p. taylori* or *O. p. jewetti* than with *O. p. brunnescens* would support this hypothesis.

All phylogenetic analyses of the control region produced trees showing that the clade containing all specimens of *O. p. fumosa* also contain a specimen from Government Camp. This lack of reciprocal monophyly falsifies the hypothesis that *O. p. fumosa* constitutes a taxonomically distinct subspecies. In addition, the specimens examined also fell within a larger clade comprised of all specimens in the western part of Oregon, i.e. *O. p. fumosa* is more closely related to *O. p. brunnescens* than it is to *O. p. jewetti* or *O. p. taylori*. This closer relationship with *O. p. brunnescens* refutes the east-to-west dispersal hypothesis.

**Hypothesis 2: *O. p. brunnescens* forms distinct clades north and south of the Columbia**

The Columbia River and the Columbia River Gorge form a large physical and ecological barrier between Washington and Oregon, which is largely impassable for small terrestrial mammals. During the colder periods of the Wisconsinian, the Columbia River was reduced to very low flows, and passage across the river was likely. However, any route across was violently removed by the catastrophic Missoula Floods, the waters being some 120m higher in present

day Portland, Oregon. This series of floods, beginning 12,000 years ago (Alt 2001), marked the beginning of 12,000 years of separation between populations of *O. p. brunnescens* north and south of the river. Because the mtDNA control region evolves at a very fast rate (useful at microevolutionary scales of just a few thousand years (Ward et al. 1993, McMillan and Palumbi 1997)), 12,000 years should be enough time for these populations to have developed separate evolutionary trajectories.

While the cyt *b* data corroborate this hypothesis, it is with only one specimen south of the river and with poor (<50%) bootstrap support. The control region analyses do not support this hypothesis since the thirteen southern specimens rest within a clade (93% bootstrap support) including Snoqualmie Pass and Mt. Adams. Either 12,000 years was not enough time to create genetic separation or sufficient gene flow occurs to prevent separation. Possible routes for gene flow across the river are the multiple landslides that occurred near present day Cascade Locks, Oregon. The Bonneville Slide, in particular, is estimated to have occurred between 1060 to 1760 CE (Reynolds 2001), and there is evidence that multiple slides occurred in the area. At its maximum, the slide formed a dam 3.5 miles long, providing ample physical linkage between *O. princeps* populations north and south of the Columbia River.

**Hypothesis 3: East – West clades in Oregon**

According to Hoffmann and Smith (2005), all four subspecies of *O. princeps* in Oregon are part of the Cascade Group as defined by Hafner and Sullivan (1995), but is there an east – west split within that grouping? In Oregon and southern Washington, the Cascade Range is separated from the eastern mountains by an expanse of high desert that is completely inhospitable to *O. princeps*. It is possible that gene flow between the Cascade Range and the eastern mountains occurred during the Wisconsinian; however, the distances are large, suggesting that dispersal in these highly philopatric animals would have been unlikely. The results of the phylogenetic analyses indicate that reciprocally monophyletic eastern and western clades have strong bootstrap support for both cyt *b* (67 – 100%) and control region (85 – 94%) data sets with a correspondingly large (5.2 – 7.9%) genetic distance. Likewise, the alarm call data suggest a strong east – west split, providing additional credence to hypothesis 3.

Hafner and Sullivan (1995) proposed that two dispersal routes existed during the initial colonization from the north: one down the Cascade Range and into the Sierras and another down the Rocky Mountains. They suggested that the Wallowa and Strawberry Mountains were populated by individuals that dispersed from the Cascade Range while the Steens Mountain population originated from the Sierra Group. However, the cyt *b* data show a closer relationship between the eastern mountains and Jasper than the eastern mountains and the Cascades. Indeed

the eastern mountains form a sister clade to Jasper, which suggests they were established by dispersal down the Rocky Mountains. It is also possible that the Sierra Group was originally established by dispersal from the Rocky Mountains and that the Steens, Strawberry, and Wallowa populations were established from later dispersal from the Sierra Group.

**Taxonomic Conclusions**

Current taxonomy of *Ochotona princeps* does not fully reflect my analysis of sequence data from mitochondrial cyt *b* and the control region. Specifically, *O. p. fumosa* is not a separate subspecies, but is subsumed by *O. p. brunnescens*. Additionally, cyt *b* data show no resolution between the Strawberry Mountains (*O. p. jewetti*), Steens Mountain (*O. p. taylori*), and the Wallowa Mountains (*O. p. jewetti*), while the control region data show that pikas from the Strawberry Mountains are a sister clade to one comprised of pikas from the Steens and the Wallowa Mountains. Either all three localities should be combined into a single subspecies or, the geographical boundaries should be redrawn to group the Wallowas and Steens together. A larger data set from the Wallowa Mountains and Steens Mountain should clarify these relationships. Finally, this study shows strong support for the existence of two large clades: the Cascade Range and the eastern Oregon mountains. These clades have genetic distances as large 8.55% (P29Mt. Adams and P8Strawberry Mts.) which is greater than the genetic distance

between *O. collaris* and Steens Mt. (8.47%) suggesting that these clades represent separate species.

**Climate Change and Population Extirpation**

Collins (2009) noted that many individual global change drivers (e.g. increased nitrogen levels) decrease biodiversity and lead ultimately to less resilient ecosystems. An argument can thus be made that ecological research should prioritize conservation of biodiversity. Because temperatures are warming faster than the capacity for animal species to adapt to temperature change (Loarie et al. 2009), this is a particularly pressing problem for North American pikas, which occur at low population densities in highly restricted montane habitats. Since *O. princeps* are isolated on mountains, as temperatures warm they can neither move upward (because the shape of the mountain results in smaller and smaller area), nor do they have the ability to disperse north. Beever et al. (2003) first raised the concern that some *O. princeps* populations in the Great Basin had become extirpated and showed evidence to support the idea that warmer temperatures were at least partially to blame. Since then, *O. princeps* have been labeled a "canary in the global warming coal mine", and, at least in the Great Basin, is considered on the brink of extinction (Grayson 2005). In an effort to avoid this fate, the Center for Biological Diversity filed petitions in 2007 with both the State of California and the U.S. Fish and Wildlife Service (USFWS) to list *O. princeps* as an endangered species. The USFWS, in early 2010, ruled against listing *O. princeps* as an

endangered species. Had they been listed, *O. princeps* would have been the first species listed under the Endangered Species Act as a direct result of global warming.

The importance of identifying biologically valid subspecies becomes apparent in conservation efforts. Limited time and resources mean that we must focus on saving populations that represent the greatest amount of biodiversity, which implies a need to make determinations as to biologically valid subspecies. A population that is not reciprocally monophyletic may have adapted phenotypically in ways important to that population's survival (Zink et al. 2000). It can therefore be argued that phenotypic variability could be considered a reasonable measure for determining upon which populations to focus conservation efforts on. Zink (2004) pointed out, however, that phenotypic variability occurs relatively quickly compared to the evolution of reciprocally monophyletic clades, and that emphasis should thus more appropriately be placed on the genetic structure of the population. It follows that in order to successfully effect conservation of threatened populations of *O. princeps*, a more comprehensive knowledge is required of the genetic structure of this species. Studies such as the present should be undertaken to evaluate the status of all purported subspecies. Results of this and other research can then inform those tasked with allocating conservation resources. For example, efforts should be placed on preserving representative populations in both the Cascade Range and the mountains of eastern Oregon – the two large clades supported by this study.

## LITERATURE CITED


Aho, Ken, Nancy Huntly, John Moen, and Tarja Oksanen. 1998. Pikas (*Ochotona princeps*: Lagomorpha) as allogenic engineers in an alpine ecosystem. *Oecologia.* 114: 405–409.

Alt, David. 2001. Glacial Lake Missoula and its humongous floods. Mountain Press Publishing Company. Missoula, Montana. 197 pp.

Avise, J. C., J.E. Neigl, and J. Arnold 1984. Demographic influences on mitochondrial DNA lineage survivorship in animal populations. *Journal of Molecular Evolution.* 20:99–105.

Avise, J. C. 2000. Phylogeography. Harvard University Press. Cambridge, Massachusetts. 447pp.

Beever, Erik A., Peter F. Brussard, and Joel Berger. 2003. Patterns of apparent extirpation among isolated populations of pikas (*Ochotona princeps*) in the Great Basin. *Journal of Mammalogy.* 84(1): 37–54.

Benito, G. and J. E. O'Connor. 2003. Number and size of last–glacial Missoula Floods in the Columbia River valley between the Pasco Basin, Washington and Portland, Oregon. *Geological Society of America Bulletin.* 115(5):624–638.

Broadbooks, Harold E. 1965. Ecology and distribution of the pikas of Washington and Alaska. *American Midland Naturalist.* 73(2):299–335.

Collins, Scott L. 2009. Biodiversity under global change. *Science.* 326:1353–1354.

Dobzhansky, T. 1970. Genetics of the evolutionary process. Columbia University Press. New York, New York.

Edwards, A. W. F. 2003. Human genetic diversity: Lewontin's fallacy. *Bioessays.* 25:798–801.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution.* 39:783–791.

Formozov, Nikolai, T. V. Grigoreva, and V. L. Surin. 2006. Molecular systematics of pikas of the subgenus Pika (Ochotona; Lagomorpha).

*Zoologicheskii Zhurnal.* 85(12); 1465–1473. (Primer MDLO incorrect in article. Correct bases are:CCG CCA TCA GCA CCC AAA GC per personal communication with Formozov.)

Gonder, M. K., J. F. Oates, T. R. Disotell, M. R. J. Forstner, J. C. Morales, and D. J. Melnick. 1997. A new west African chimpanzee subspecies?. *Nature.* 388(6640):337–337.

Garcia–Moreno, J., M. Matocq, M. Roy, E. Geffen, and R. K. Wayne. Relationships and genetic purity of the endangered Mexican wolf based on analysis of microsatellite loci. *Conservation Biology.* 10:376–389.

Grayson, Donald K. 1977. On the Holocene history of some northern Great Basin Lagomorphs. *Journal of Mammalogy* 58(4): 507–513.

Grayson, Donald K. 1987. The biogeographic history of mammals in the Great Basin: observations on the last 20,000 years. *Journal of Mammalogy* 68(2): 359–357.

Grayson, Donald K. 2005. A history of Great Basin pikas. *Journal of Biogeography* 32: 2103–2111.

Gridi-Papp, M (ed.). 2003–2007. SoundRuler: Acoustic Analysis for Research and Teaching. http://soundruler.sourceforge.net.

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

Hafner, David J. 1994. Pikas and permafrost: post-Wisconsin historical zoogeography of *Ochotona* in the southern Rocky mountains, U.S.A. *Arctic and Alpine Research.* 26(4):375–382.

Hafner, David J. and Robert M. Sullivan. 1995. Historical and ecological biogeography of nearctic pikas (Lagomorpha: Ochotonidae). *Journal of Mammalogy.* 76(2): 302–321.

Hall, E. Raymond. 1981. The mammals of North America, 2nd ed. John Wiley and Sons. New York. 1:1–606 +90.

Hoffmann, R. S. and A. T. Smith. 2005. Order Lagomorpha. PP. 185–211 *in*: Wilson, D. E. and D. M. Reeder (eds.), Mammals Species of the World; a taxonomic and geographic reference, 3rd ed. Baltimore, Maryland, Johns Hopkins University Press, v. 1, xxxv+743 pp.

Hooton, E. A. 1926. Methods of racial analysis. *Science*. 63:75–81.
Huelsenbeck, JP and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*. 17(8):754–755.

Lewontin, R. C. 1972. The apportionment of human diversity. *Evolutionary Biology*. 6:381–397.

Loarie, Scott R., Philip B. Duffy, Healy Hamilton, Gregory P. Asner, Christopher B. Field, and David D. Ackerly. 2009. The velocity of climate change. *Nature*. 462(24):1052–1055.

Long, J. C. and R. A. Kittles. 2003. Human genetic diversity and the nonexistence of biological races. *Human Biology*. 75:449–471.

Maddison, W. P. and D. R. Maddison. 1992. MacClade 4: analysis of phylogeny and Character evolution, version 4.0. Sinauer Associates, Inc. Sunderland, Massachusetts.

Maser, Chris. 1998. Mammals of the Pacific Northwest from the Coast to the High Cascades. Oregon State University Press, Corvallis, Oregon. 406 pp.

Mayr, E. 1969. Principles of Systematic Zoology. McGraw-Hill. New York, New York.

McMillan, W. O. and S. R. Palumbi. 1997. Rapid rate of control region evolution in Pacific butterflyfishes (*Chaetodontidae*). *Journal of Molecular evolution*. 45:473–484.

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

Nilsson, Mats T. and Gunnar O. Klein. 1984. SEQ-ED: and interactive computer program for editing, analysis and storage of long DNA sequences. *Bioinformatics*. 1(1):29-34.

Niu, Yedong, Fuwen Wei, Ming Li, Xiaming Liu, and Zuojian Feng. 2004. Phylogeny of pikas (Lagomorpha, *Ochotona*) inferred from mitochondrial cytochrome *b* sequences. *Folia Zool.* 53(2): 141–155.

Pielou, E. C. 1991. After the ice age: the return of life to glaciated North America. University of Chicago Press, Chicago, Illinois. 366pp.

Posada, D. and K. A. Crandall. 1998. Modeltest: testing the model of DNA substitution. *Bioinformatics* 14(9): 817–818.

Posada, David and Thomas R. Buckley. 2004. Model selection and model averaging in phylogenetics: advantages of Akaike information criterion and Bayesian approaches over likelihood ratio tests. *Systematic Biology*. 53(5):793–793.

Reinartz, G. E., J. D. Karron, R. B. Phillips, and J. L. Weber. Patterns of microsatellite polymorphism in the range-restricted bonobo (*Pan paniscus*): considerations for interspecific comparison with chimpanzees (*Pan troglodytes*). *Molecular Ecology*. 9:315–328.

Reynolds, Nathaniel D. 2001. Dating the Bonneville landslide with lichenometry. *Washington Geology*. 29(3/4):11–16.

Ronquist, F. and JP Huelsenbeck. 2003. MrBayes 3; Bayesian phylogenetic inference under mixed models. *Bioinformatics*. 19(12):1572–1574.

Slade, Robert W., Craig Moritz, and Anita Heideman. 1994. Multiple nuclear-gene Phylogenies: application to pinnepeds and comparison with a mitochondrial DNA gene phylogeny. *Mol. Bio. Evol.* 11(3):341–356.

Smith, Andrew T. 1974. The distribution and dispersal of pikas: influences of behavior and climate. *Ecology*. 55(6): 1368–1376.

Smith, Andrew T. 1978. Comparative demography of pikas (*Ochotona*): effect of patial and temporal age-specific mortality. *Ecology*. 59:133–139.

Smith, Andrew T. 1990. *Ochotona princeps*. *Mammalian Species*. 352: 1-8.

Stoneking, M., D. Hedgecock, R. G. Higuchi, L. Vigilant, and H. A. Erlich. 1991. Population variation of human mtDNA control region sequences detected by enzymatic amplification and sequence–specific oligonucleotide probes. *American Journal of Human Genetics*. 48:370–382.

Swofford, D. L. 2002. *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods) Version 4*. Sinauer Associates, Sunderland, Massachusetts.

Templeton, A. R. 1998. Human races: a genetic and evolutionary perspective. *Am. Anthropol.* 100:632–650.

Verts, B. J. and Leslie N. Carraway. Land mammals of Oregon. University of California Press. Berkeley, California. 800 pp.

Ward, R. H., A. Redd, D. Valencia, B. Frazier, and S. Pääbo. 1993. Genetic and linguistic differentiation in the Americas. *Proceedings of the National Academy of Science USA*. 90:10663–10667.

Wise, C., M. Sraml, D. Rubinsztein, and S. Easteal. Comparative nuclear and mitochondrial genome diversity in humans and chimpanzees. *Molecular Biology and Evolution*. 14:707–716.

Woodley, Michael A. 2010. Is *Homo sapiens* polytypic? Human taxonomic diversity and its implications. *Medical Hypotheses*. 74:195–201.

Waltari, Eric, John R. Demboski, David R. Klein, and Joseph A. Cook. 2004. A molecular perspective on the historical biogeography of the northern high latitudes. *Journal of Mammalogy*. 85(4):591–600.

Zink, R. M., G. F. Barrowclough, J. L. Atwood, and R. C. Blackwell–Rago. 2000. Genetics, taxonomy, and the conservation of the threatened California gnatcatcher. *Conservation Biology*. 14:1394–1405.

Zink, Robert M. 2004. The role of subspecies in obscuring avian biological diversity and misleading conservation policy. *Proceedings of the Royal Society of London, Series B—Biological Sciences*. 271:561–564.

## APPENDIX A

### Trapping Sites

| Site Name | Latitude | Longitude | Elevation |
|---|---|---|---|
| 1. Jasper National Park | 52°41'16.96"N | 118°2'17.29"W | 2018 m (6600 ft) |
| 2. Snoqualmie Pass | 47°26'19.65"N | 121°25'14.57"W | 932 m (3050 ft) |
| 3. Mount Adams | 46°16'10.84"N | 121°35'13.97"W | 1409 m (4607 ft) |
| 4. Columbia R. Gorge | 45°34'44.57" | 122°06'23.87"W | 80 m (261 ft) |
| 5. Mount Hood | 45°17'21.86"N | 121°47'31.36"W | 1497 m (4895 ft) |
| 6. Mount Jefferson | 44°40'42.6"N | 121°50'58.1"W | ~1682 m (5500 ft) |
| 7. Wallowa Mts. | 45°13'31.64"N | 117°15'57.14"W | 2483 m (8123 ft) |
| 8. Strawberry Mts. | 44°17'34.63"N | 118°40'47.61"W | 2292 m (7498 ft) |
| 9. Steens Mountain | 42°43'4.57"N | 118°34'31.58"W | 2446 m (8000 ft) |

## APPENDIX B

### Specimen List

Specimen numbers in bold indicate samples obtained via trapping. All others are museum samples. In addition to those listed below, two specimens were obtained from GenBank for the cyt *b* analysis (AY292716 and AF272989) and one *Ochotona collaris* specimen was used as the outgroup (obtained from Link Olsen, University of Alaska, Fairbanks) in both the cyt *b* and control region analyses.

| No. | Date Trapped | Location |
|---|---|---|
| **P1** | 8/17/2005 | Jasper, Canada |
| **P2** | 8/18/2005 | Jasper, Canada |
| **P3** | 8/19/2005 | Jasper, Canada |
| **P4** | 10/4/2005 | Mount Adams, WA |
| **P5** | 10/4/2005 | Mount Adams, WA |
| **P28** | 8/19/2006 | Mount Adams, WA |
| **P29** | 8/19/2006 | Mount Adams, WA |
| **P6** | 10/11/2005 | Strawberry Mountains, OR |
| **P7** | 10/11/2005 | Strawberry Mountains, OR |
| **P8** | 10/11/2005 | Strawberry Mountains, OR |
| **P9** | 10/11/2005 | Strawberry Mountains, OR |
| P11 | 7/12/1967 | 0.5 mile east of Multnomah Falls |
| P12 | 7/13/1967 | 0.5 mile east of Multnomah Falls |
| P13 | 7/1/1967 | 0.5 mile east of Multnomah Falls |
| P14 | 7/1/1967 | 0.5 mile east of Multnomah Falls |
| P16 | 8/16/1966 | 32.5 miles east of Sweet Home, Linn Co., OR |
| P17 | 7/12/1967 | 32.5 miles east of Sweet Home, Linn Co., OR |
| P18 | 9/11/1968 | 32.5 miles east of Sweet Home, Linn Co., OR |
| P20 | 9/11/1968 | 32.5 miles east of Sweet Home, Linn Co., OR |
| P21 | 9/11/1968 | 32.5 miles east of Sweet Home, Linn Co., OR |
| P22 | 9/11/1968 | 32.5 miles east of Sweet Home, Linn Co., OR |
| P25 | 7/28/1966 | 2.25 miles east, 1.5 miles north of Government Camp, OR |
| P27 | 7/28/1966 | 2.25 miles east, 1.5 miles north of Government Camp, OR |
| **P30** | 8/30/2006 | Ice Lake, Wallowa Mountains, OR |
| **P31** | 9/5/2006 | Kiger Gorge, Steens Mountain, OR |
| **P32** | 9/13/2006 | Snoqualmie Pass, WA |
| **P33** | 9/13/2006 | Snoqualmie Pass, WA |
| **P34** | 9/20/2006 | Tom, Dick, and Harry Mountain, Mount Hood, OR |

## APPENDIX C

### Primers

Control Region
L159260CH: 5' – ATT ACC CTG GTC TTG TAA ACC – 3' *
MTOCH1R: 5' – GTA CAT CGA GGT GCT CGT CT –3' *

Cytochrome *b*
L14724: 5' – CGA AGC TTG ATA TGA AAA ACC ATC GTT G – 3' #
H15915: 5' – AGG AAT TCC ATT TTT GGT TTA CAA GAC – 3' #

* Obtained from Formozov et al. (2006)

# Obtained from Lori Patrick (lpatrick@pdx.edu)

## APPENDIX D

### PCR Cycler Programs

Cytochrome *b*

*GWBCYTb*

1. 94° for 3 min
2. 94° for 1 min
3. 95° for 1 min
4. 72° for 1.5 min
5. 39 times to 2°
6. 72° for 5 min
7. 4° forever
8. END

Control Region

*GWBCR000*

1. 94° for 5 min
2. 94° for 1 min
3. 62° for 1 min
4. 72° for 3 min
5. 40 times to 2°
6. 72° for 5 min
7. Hold at 4°
8. END

# APPENDIX E

## Cytochrome *b* Genetic Distances

| | P1Jasper | P2Jasper | P3Jasper | P4Adams | P5Adams | P28Adams |
|---|---|---|---|---|---|---|
| P1Jasper | | | | | | |
| P2Jasper | 0.00087854 | | | | | |
| P3Jasper | 0.00087854 | 0 | | | | |
| P4Adams | 0.06709102 | 0.06604198 | 0.06604198 | | | |
| P5Adams | 0.0651191 | 0.06510516 | 0.06510516 | 0.0019704 | | |
| P28Adams | 0.06698813 | 0.06594279 | 0.06594279 | 0 | 0.00197213 | |
| P29Adams | 0.06783549 | 0.06678071 | 0.06678071 | 0 | 0.00199717 | 0 |
| P6Strawberry | 0.04796451 | 0.04697582 | 0.04697582 | 0.06291671 | 0.06292833 | 0.06284134 |
| P7Strawberry | 0.05527252 | 0.05524412 | 0.05524412 | 0.07232881 | 0.07235973 | 0.07237682 |
| P8Strawberry | 0.06064359 | 0.06062598 | 0.06062598 | 0.08405468 | 0.08151861 | 0.08392904 |
| P9Strawberry | 0.04646923 | 0.04644265 | 0.04644265 | 0.06877327 | 0.06619392 | 0.06882057 |
| P30Wallowa | 0.0477178 | 0.04671773 | 0.04671773 | 0.0596164 | 0.06139077 | 0.05966093 |
| P31Steens | 0.04905043 | 0.04805481 | 0.04805481 | 0.05888593 | 0.06057046 | 0.05893425 |
| P32Snoqualmie | 0.06708869 | 0.06604335 | 0.06604335 | 0 | 0.00197395 | 0 |
| P33Snowqualmie | 0.06685378 | 0.06581211 | 0.06581211 | 0.00175793 | 0 | 0.00175793 |
| P34Hood | 0.06779002 | 0.06674835 | 0.06674835 | 0.00264174 | 0.0029398 | 0.00264174 |
| AY292716Oprinceps | 0.00264372 | 0.00175977 | 0.00175977 | 0.06814374 | 0.06746134 | 0.06803714 |
| AF272989Oprinceps | 0.00264372 | 0.00175977 | 0.00175977 | 0.06814374 | 0.06746134 | 0.06803714 |
| Ocollaris | 0.10087962 | 0.10201617 | 0.10201617 | 0.09654311 | 0.09451839 | 0.09638594 |

| | P29Adams | P6Strawberry | P7Strawberry | P8Strawberry | P9Strawberry |
|---|---|---|---|---|---|
| P1Jasper | | | | | |
| P2Jasper | | | | | |
| P3Jasper | | | | | |
| P4Adams | | | | | |
| P5Adams | | | | | |
| P28Adams | | | | | |
| P29Adams | | | | | |
| P6Strawberry | 0.06365016 | | | | |
| P7Strawberry | 0.07259469 | 0.00706895 | | | |
| P8Strawberry | 0.08545333 | 0.01618928 | 0.02571911 | | |
| P9Strawberry | 0.06947018 | 0 | 0.00826045 | 0 | |
| P30Wallowa | 0.05986181 | 0.00445477 | 0.01013184 | 0.0198873 | 0.00337606 |
| P31Steens | 0.05960476 | 0.00619351 | 0.01119578 | 0.0216915 | 0.005644 |
| P32Snoqualmie | 0 | 0.06299049 | 0.07246524 | 0.08399527 | 0.0689136 |
| P33Snowqualmie | 0.00177945 | 0.06085317 | 0.07004433 | 0.08141734 | 0.06628992 |
| P34Hood | 0.00267554 | 0.05991567 | 0.06891922 | 0.08147619 | 0.06629471 |
| AY292716Oprinceps | 0.06889397 | 0.04895653 | 0.05755688 | 0.06306875 | 0.04889997 |
| AF272989Oprinceps | 0.06889397 | 0.04895653 | 0.05755688 | 0.06306875 | 0.04889997 |
| Ocollaris | 0.09765176 | 0.08635103 | 0.09244056 | 0.10692304 | 0.09218144 |

## APPENDIX E (continued)

| | P31Steens | P32Snoqualmie | P33Snowqualmie | P34Hood |
|---|---|---|---|---|
| P1Jasper | | | | |
| P2Jasper | | | | |
| P3Jasper | | | | |
| P4Adams | | | | |
| P5Adams | | | | |
| P28Adams | | | | |
| P29Adams | | | | |
| P6Strawberry | | | | |
| P7Strawberry | | | | |
| P8Strawberry | | | | |
| P9Strawberry | | | | |
| P30Wallowa | | | | |
| P31Steens | | | | |
| P32Snoqualmie | 0.05905969 | | | |
| P33Snowqualmie | 0.05695529 | 0.00177026 | | |
| P34Hood | 0.05602256 | 0.00266635 | 0.00263915 | |
| AY292716Oprinceps | 0.05004941 | 0.0681377 | 0.0678991 | 0.06883534 |
| AF272989Oprinceps | 0.05004941 | 0.0681377 | 0.0678991 | 0.06883534 |
| Ocollaris | 0.08473752 | 0.09649139 | 0.09654219 | 0.09427095 |

| | AF272989Oprinceps | Ocollaris |
|---|---|---|
| P1Jasper | | |
| P2Jasper | | |
| P3Jasper | | |
| P4Adams | | |
| P5Adams | | |
| P28Adams | | |
| P29Adams | | |
| P6Strawberry | | |
| P7Strawberry | | |
| P8Strawberry | | |
| P9Strawberry | | |
| P30Wallowa | | |
| P31Steens | | |
| P32Snoqualmie | | |
| P33Snowqualmie | | |
| P34Hood | | |
| AY292716Oprinceps | | |
| AF272989Oprinceps | | |
| Ocollaris | 0.09974783 | |

# APPENDIX F

## Control Region Genetic Distances

| | P1Jasper | P2Jasper | P3Jasper | P4MtAdams | P5MtAdams | P28MtAdams |
|---|---|---|---|---|---|---|
| P1Jasper | | | | | | |
| P2Jasper | 0.00838563 | | | | | |
| P3Jasper | 0.00676415 | 0.00137058 | | | | |
| P4MtAdams | 0.07103238 | 0.07450382 | 0.07086776 | | | |
| P5MtAdams | 0.06451419 | 0.06794572 | 0.06440295 | 0.00837329 | | |
| P28MtAdams | 0.07243443 | 0.07282298 | 0.06910882 | 0.00978343 | 0.00978334 | |
| P29MtAdams | 0.07103238 | 0.07450382 | 0.07086776 | 0 | 0.00837329 | 0.00978343 |
| P6Strawberry | 0.06573934 | 0.06566629 | 0.06256751 | 0.05565663 | 0.05719663 | 0.05716101 |
| P7Strawberry | 0.06543968 | 0.06416164 | 0.06229179 | 0.05688848 | 0.0584255 | 0.05533589 |
| P8Strawberry | 0.06370466 | 0.06259947 | 0.06058396 | 0.05516335 | 0.05672175 | 0.05669338 |
| P9Strawberry | 0.06223967 | 0.06263278 | 0.0591126 | 0.05367349 | 0.05522463 | 0.05518926 |
| P11MultnomahFalls | 0.07001141 | 0.06937977 | 0.06668339 | 0.01569686 | 0.01276244 | 0.01133056 |
| P12MultnomahFalls | 0.07048191 | 0.06941495 | 0.06710347 | 0.01291317 | 0.01283885 | 0.01137887 |
| P13MultnomahFalls | 0.07000857 | 0.06935892 | 0.06667188 | 0.01279117 | 0.0127537 | 0.01132147 |
| P14MultnomahFalls | 0.06977104 | 0.06927478 | 0.06642652 | 0.01284614 | 0.01275272 | 0.01128121 |
| P16LinnCounty | 0.07520537 | 0.07421619 | 0.07180496 | 0.01433862 | 0.01139868 | 0.00995638 |
| P17LinnCounty | 0.07925374 | 0.07921194 | 0.075662 | 0.01725466 | 0.01403902 | 0.01258903 |
| P18LinnCounty | 0.07220477 | 0.07253698 | 0.06889639 | 0.01552296 | 0.01265164 | 0.01122253 |
| P20LinnCounty | 0.07591472 | 0.07859927 | 0.075899 | 0.01652878 | 0.01344923 | 0.01204684 |
| P21LinnCounty | 0.07807056 | 0.07711667 | 0.07453912 | 0.01501172 | 0.01195627 | 0.0105587 |
| P22LinnCounty | 0.07319789 | 0.07258449 | 0.06984926 | 0.01572493 | 0.01279106 | 0.01135923 |
| P25GovernmentCamp | 0.07200965 | 0.07099298 | 0.06863751 | 0.01725714 | 0.01429228 | 0.01284098 |
| P27GovernmentCamp | 0.07070335 | 0.07093718 | 0.06738937 | 0.0112376 | 0.01120922 | 0.00978437 |
| P30WallowaMountains | 0.07150683 | 0.07374758 | 0.06984238 | 0.05977788 | 0.05978888 | 0.056681 |
| P31SteensMt | 0.07125214 | 0.06882683 | 0.06655268 | 0.0552163 | 0.05522738 | 0.05826337 |
| P32SnoqualmiePass | 0.06620888 | 0.06960645 | 0.066077 | 0.00696491 | 0.00416984 | 0.01120282 |
| P33SnoqualmiePass | 0.07207634 | 0.07450479 | 0.07191332 | 0.00280468 | 0.00844393 | 0.01276038 |
| P34MtHood | 0.06999093 | 0.06936724 | 0.06666012 | 0.01284614 | 0.01277995 | 0.01133498 |
| Ocollaris | 0.08751918 | 0.09463038 | 0.09281842 | 0.07420174 | 0.07070637 | 0.07594029 |

## APPENDIX F (continued)

| | P6Strawberry | P7Strawberry | P8Strawberry | P9Strawberry | P11MultFalls |
|---|---|---|---|---|---|
| P1Jasper | | | | | |
| P2Jasper | | | | | |
| P3Jasper | | | | | |
| P4MtAdams | | | | | |
| P5MtAdams | | | | | |
| P28MtAdams | | | | | |
| P29MtAdams | | | | | |
| P6Strawberry | | | | | |
| P7Strawberry | 0.00412806 | | | | |
| P8Strawberry | 0.0027497 | 0.00135697 | | | |
| P9Strawberry | 0.00136911 | 0.00271522 | 0.00135643 | | |
| P11MultnomahFalls | 0.05754875 | 0.05759474 | 0.05605966 | 0.05606923 | |
| P12MultnomahFalls | 0.05634168 | 0.05638748 | 0.0548601 | 0.05486769 | 0.00280871 |
| P13MultnomahFalls | 0.05443045 | 0.05441954 | 0.05288843 | 0.05289375 | 0.00280813 |
| P14MultnomahFalls | 0.05567966 | 0.05711882 | 0.05561816 | 0.05412436 | 0.00280389 |
| P16LinnCounty | 0.05782956 | 0.05787634 | 0.05634037 | 0.05634994 | 0.0042365 |
| P17LinnCounty | 0.06383144 | 0.06547083 | 0.06367164 | 0.06202894 | 0.00614439 |
| P18LinnCounty | 0.05881569 | 0.06002268 | 0.0583148 | 0.05682121 | 0.00559259 |
| P20LinnCounty | 0.06155092 | 0.06160801 | 0.05989143 | 0.05990195 | 0.00592938 |
| P21LinnCounty | 0.06013757 | 0.06019241 | 0.05846792 | 0.05847571 | 0.0044186 |
| P22LinnCounty | 0.05914497 | 0.05918597 | 0.05765677 | 0.05766848 | 0.00562815 |
| P25GovernmentCamp | 0.05791296 | 0.0579605 | 0.05642368 | 0.05643336 | 0.00708222 |
| P27GovernmentCamp | 0.05417371 | 0.05539716 | 0.05370815 | 0.05222199 | 0.00421045 |
| P30WallowaMountains | 0.01816999 | 0.01947347 | 0.01799332 | 0.01659647 | 0.06382394 |
| P31SteensMt | 0.01811736 | 0.01660452 | 0.01514114 | 0.01652797 | 0.06080109 |
| P32SnoqualmiePass | 0.05883975 | 0.06005967 | 0.05832505 | 0.0568262 | 0.01423005 |
| P33SnoqualmiePass | 0.06071101 | 0.06072856 | 0.05915799 | 0.05914846 | 0.01576729 |
| P34MtHood | 0.05591989 | 0.0559063 | 0.05438189 | 0.0543892 | 0.00280672 |
| Ocollaris | 0.08703567 | 0.08522853 | 0.08705284 | 0.0870524 | 0.08124156 |

## APPENDIX F (continued)

| | P13MultFalls | P14MultFalls | P16LinnCounty | P18LinnCounty | P20LinnCounty |
|---|---|---|---|---|---|
| P1Jasper | | | | | |
| P2Jasper | | | | | |
| P3Jasper | | | | | |
| P4MtAdams | | | | | |
| P5MtAdams | | | | | |
| P28MtAdams | | | | | |
| P29MtAdams | | | | | |
| P6Strawberry | | | | | |
| P7Strawberry | | | | | |
| P8Strawberry | | | | | |
| P9Strawberry | | | | | |
| P11MultnomahFalls | | | | | |
| P12MultnomahFalls | | | | | |
| P13MultnomahFalls | | | | | |
| P14MultnomahFalls | 0 | | | | |
| P16LinnCounty | 0.00423646 | 0.00422046 | | | |
| P17LinnCounty | 0.006179 | 0.0061373 | 0.00154341 | | |
| P18LinnCounty | 0.00559754 | 0.00556298 | 0.00139505 | | |
| P20LinnCounty | 0.00596413 | 0.00595642 | 0.00151174 | 0.00299161 | |
| P21LinnCounty | 0.00443303 | 0.00441731 | 0 | 0.00148329 | 0.00151104 |
| P22LinnCounty | 0.00562678 | 0.00559898 | 0.00140764 | 0 | 0.00299825 |
| P25GovernmentCamp | 0.00708486 | 0.00705957 | 0.00281746 | 0.00139505 | 0.00445776 |
| P27GovernmentCamp | 0.00138823 | 0.00138632 | 0.00282768 | 0.00415636 | 0.00450685 |
| P30WallowaMountains | 0.06217612 | 0.06194433 | 0.06413389 | 0.06447786 | 0.06980663 |
| P31SteensMt | 0.05916471 | 0.06041889 | 0.06108775 | 0.06297862 | 0.06664849 |
| P32SnoqualmiePass | 0.01133064 | 0.01136591 | 0.01287084 | 0.01408581 | 0.01496997 |
| P33SnoqualmiePass | 0.01286366 | 0.01277423 | 0.01433785 | 0.01569629 | 0.01655659 |
| P34MtHood | 0 | 0 | 0.00423117 | 0.00558979 | 0.00596197 |
| Ocollaris | 0.08124949 | 0.08094247 | 0.08307355 | 0.08484648 | 0.08905894 |

## APPENDIX F (continued)

| | P21LinnCounty | P22LinnCounty | P25GovCamp | P30WallowaMts | P31SteensMt |
|---|---|---|---|---|---|
| P1Jasper | | | | | |
| P2Jasper | | | | | |
| P3Jasper | | | | | |
| P4MtAdams | | | | | |
| P5MtAdams | | | | | |
| P28MtAdams | | | | | |
| P29MtAdams | | | | | |
| P6Strawberry | | | | | |
| P7Strawberry | | | | | |
| P8Strawberry | | | | | |
| P9Strawberry | | | | | |
| P11MultnomahFalls | | | | | |
| P12MultnomahFalls | | | | | |
| P13MultnomahFalls | | | | | |
| P14MultnomahFalls | | | | | |
| P16LinnCounty | | | | | |
| P17LinnCounty | | | | | |
| P18LinnCounty | | | | | |
| P20LinnCounty | | | | | |
| P21LinnCounty | | | | | |
| P22LinnCounty | 0.00149669 | | | | |
| P25GovernmentCamp | 0.00295718 | 0.00140764 | | | |
| P27GovernmentCamp | 0.00297882 | 0.00422516 | 0.00566097 | | |
| P30WallowaMountains | 0.06842057 | 0.06542613 | 0.06421293 | | |
| P31SteensMt | 0.06527399 | 0.06239897 | 0.06115917 | 0.01656028 | |
| P32SnoqualmiePass | 0.01345714 | 0.01426529 | 0.01578284 | 0.06296896 | 0.05836143 |
| P33SnoqualmiePass | 0.01502581 | 0.01575866 | 0.01727783 | 0.06394441 | 0.05775839 |
| P34MtHood | 0.00442816 | 0.0056175 | 0.00708622 | 0.06224477 | 0.05923649 |
| Ocollaris | 0.08754521 | 0.08490901 | 0.08672906 | 0.08712437 | 0.08364108 |

## APPENDIX F (continued)

| | P33SnoqualmiePass | P34MtHood | Ocollaris |
|---|---|---|---|
| P1Jasper | | | |
| P2Jasper | | | |
| P3Jasper | | | |
| P4MtAdams | | | |
| P5MtAdams | | | |
| P28MtAdams | | | |
| P29MtAdams | | | |
| P6Strawberry | | | |
| P7Strawberry | | | |
| P8Strawberry | | | |
| P9Strawberry | | | |
| P11MultnomahFalls | | | |
| P12MultnomahFalls | | | |
| P13MultnomahFalls | | | |
| P14MultnomahFalls | | | |
| P16LinnCounty | | | |
| P17LinnCounty | | | |
| P18LinnCounty | | | |
| P20LinnCounty | | | |
| P21LinnCounty | | | |
| P22LinnCounty | | | |
| P25GovernmentCamp | | | |
| P27GovernmentCamp | | | |
| P30WallowaMountains | | | |
| P31SteensMt | | | |
| P32SnoqualmiePass | | | |
| P33SnoqualmiePass | | | |
| P34MtHood | 0.01281903 | | |
| Ocollaris | 0.07563926 | 0.08101243 | |