

National Science Foundation Graduate Research Proposal

Mapping the impacts of Pleistocene glaciation on Arctic-alpine population distribution
in North America using a phylogeographic approach

Nicholas Penzel
February 12, 2019

Department of Organismal Biology and Ecology, Colorado College

KEYWORDS

Phylogeography, Phylogenetics, Arctic-alpine, Pleistocene, Population distribution

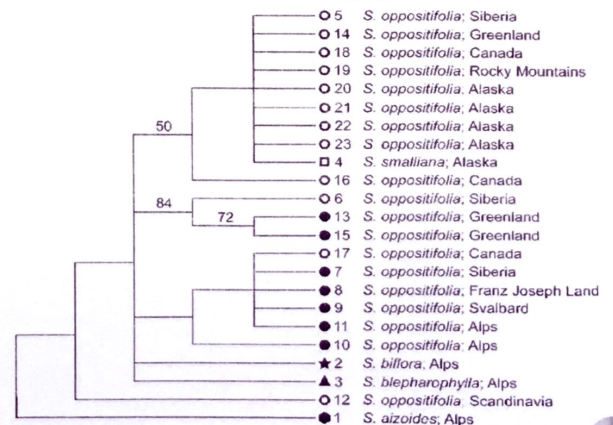
I. Introduction :

The idea that there is a level of interconnectedness between arctic and alpine species has been understood in at least some sense for many years. Darwin himself hypothesized that glaciation during the Pleistocene was responsible for many distribution patterns in the Northern Hemisphere's circumpolar regions. Observing that there were great similarities in alpine species in both the new and old world, Darwin concluded that these species must have derived from northern Arctic regions. Climate change then caused species to move into lower regions during glaciation and were subsequently left in alpine refugia during warming periods. Although our perception of biogeographic patterns in the Arctic, circumarctic, and alpine regions has advanced with modern molecular and genetic testing as well as new fossil finds suggesting refugia of species that survived glaciation in the Arctic (Abbott and Brochman, 2003), some of the fundamental ideas proposed by Darwin still hold true today: Pleistocene glaciation is largely responsible for the distribution of Arctic-alpine species. While biogeographic distribution and phylogenetic relationships have been investigated in relation to Pleistocene glaciation (Brown and Knowles 2012, Holderegger and Abbott 2003), it is a relatively new area of interest and there are many unanswered questions. To investigate how biogeographic relationships and phylogenies are affected, I will focus my study onto three species, all who exhibit characteristics of arctic-alpine taxa: pika, purple mountain saxifrage, and the white-tailed ptarmigan.

The genus of *Ochotona* consists of two distinct North American species of pika. American pika (*O. princeps*) and collared pika (*O. collaris*) are both closely related to *Leporidae* and exist in rocky, mountainous areas. *O. princeps* has an extremely low thermal tolerance and begins to suffer from hyperthermia at 28°C (MacArthur and Wang 1973). Additionally, like some *Leporidae*, pikas do not hibernate. Instead, they rely on subnivean networks and food stores to subsist throughout the long winters. Snow depth is thus very important for pika survival (Morrison et al. 2009). These two factors combined make pika extremely susceptible to climatic changes and also serve to cause significant isolation of populations as they are confined to their sky islands refugia, especially in southern regions like the Rocky Mountains.

The genus *Saxifraga* is a widely distributed Arctic-alpine flower with 400 plus species found throughout the Arctic and alpine regions in both the new and old world. Purple mountain saxifrage (*Saxifraga oppositifolia*) is a common species of *Saxifraga* and has a wide distribution from the Arctic into the Southern Rockies. The phylogeny and phylogeography of *S. oppositifolia* have been well studied compared to many other Arctic plants flowers, but there are some notable gaps in our knowledge that deserve further investigation. Abbott and Comes (2003) suggest that there are two distinct lineages of *S. oppositifolia* populations diverged 5.37-3.76 Ma during the mid-Pliocene. Furthermore, Holderegger and Abbott (2003) have constructed a phylogeny of *S. oppositifolia* as well as several other closely related *Saxifraga* species.

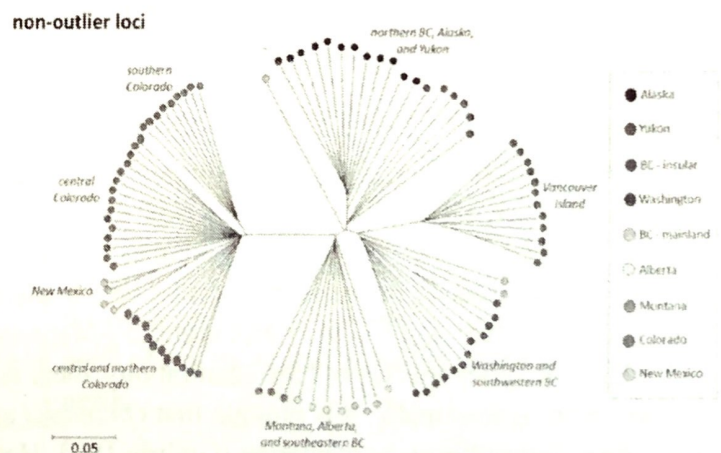
Fig 1. A fifty percent majority rule phylogeny tree created by Holderegger and Abbott (2003) using ITS DNA analysis with bootstrap support values. It is important to note that there is only one sample from the U.S. which was collected in Washington. Additionally, the resolution is too poor to allow many intraspecies comparisons. Using new SNP techniques like ddRADseq can provide higher resolution



However, in their work Holderegger and Abbott only sampled *S. Saxifraga* from the United States in Washington, and didn't include the furthest southern reaches of the population which extend through the Colorado Rockies into New Mexico. Being so far south, these populations may have had significant historical isolation and warrant further investigation.

White-tailed ptarmigan (*Lagopus leucura*) is an alpine bird that spends almost all of its life in the alpine. *L. leucura* can be found farther south than any other species of ptarmigan and have a range from central Alaska to New Mexico (Martin et al. 2015). However, due to the species necessity for an alpine environment, especially in more southerly populations, the species is fragmented throughout the American Rockies (Martin et al. 2015). Much of the analysis of *L. leucura* has been focused on the conservation of the species because of habitat threats related to climate change. There are five subspecies of *L. leucura* described, but Langin et al. (2018) found that there are additional genetic clusters. For instance, in Colorado, there appear to be distinct groups in Southern Colorado ranges like the San Juans, and Northern Colorado populations. Additionally, Langin et al. (2018) found that it may not make sense to combine Montana populations with other more southerly populations. This work demonstrates that there are detectable genetic variation and distinct populations between the various "sky islands" that *L. leucura* calls home.

Fig 2. A Neighbor-joining tree constructed by Langin et al. (2018) using non-outlier SNPs. Colors correspond to the geographic location of an individual. Note the clearly distinguished groups that make *L. leucura* such a good candidate for my study.



While research has been conducted on how the Arctic-alpine species are currently distributed and how glaciation has affected these populations, large holes still exist in our understanding of how historic glaciation during the Pleistocene has led to the modern phylogeographic distributions that we see today. For *S. oppositifolia* much of the focus has been on circumpolar distributions and arctic refugium during glaciation, but the farthest most reach of *S. oppositifolia* in the Southern Rockies is less understood. The North American *Ochotona* have been well studied, specifically *O. princeps*, however, there is still much to learn by making both inter and intraspecific comparisons, something that has rarely been done. *L. leucura* have had genetic population mapping done, but the phylogeny of the subspecies of *L. leucura* still requires investigation. In the case of these three study species, I plan to apply a phylogeographic and phylogenetic approach to determine how Pleistocene glaciation led to speciation and isolated populations. While the methods of doing this for each species will differ, by using the same approach of looking at population and species divergence along a latitudinal gradient I will attempt to draw general conclusions about species distribution and evolution in respect to climatic changes in North America.

will be to use
al. (2017),
model
Be

II. Hypothesis:

Although Darwin was probably wrong in assuming that Arctic species migrated south during glacial maximums and where left at high elevation refugium—the modern hypothesis is that alpine species moved north during warming and eventually ended up in the arctic—his fundamental hypothesis is still understood to be true. The ultimate goal of my researching is looking for phylogenetic patterns that have been shaped by the pattern of glaciation that Darwin described in *Origin of Species*. While each species will have its own unique history and distribution, *O. princeps/collaris*, *S. oppositifolia*, and *L. leucura* all share similarities in that they are Arctic-alpine taxon, have large ranges along a latitudinal gradient and have genetically distinct populations. I believe that these similarities may result in conclusions that can be drawn across species about how glacial maximums during the Pleistocene affected the ancestors of our current Arctic-alpine species.

I hypothesize that by creating a time-calibrated phylogeny within species, I will see population divergence happening along a latitudinal cline. Phylogenetic approaches have concluded that southern alpine regions were important habitats for arctic-alpine species (Ikeda et al. 2008). Additionally, it has been shown that species may have migrated from these sky islands northwards after the last glacial maximum (Li et al., 2014, Weber 2003). As such, I expect to see a pattern arise where the southernmost reaches of a population become separated the earliest with populations becoming successively isolated later and later the farther north you sample.

III. Research Plan:

The methods for creating time-calibrated phylogenies will differ slightly for each species depending on what data is available and what research has already been done. For instance, Langin et al (2018) have already established a number of genetically isolated populations of *L. leucura* using both traditional genetic microsatellites and single nucleotide polymorphisms (SNP). Pikas are understood to have high levels of intraspecific genetic variation as a result of a fractured distribution, but relative to other species of hares and rabbits, there is little interspecific variation (Lanier and Olson 2009). Additionally, it is thought that *O. collaris* and *O. princeps* underwent allopatric speciation during Pliocene glaciation (Guthrie 1973, Harington 1978).

For each species, I will begin my analysis by mapping current distributions of the species in ArcMap 10.6. This will allow me to establish a basic range as well as a data set for comparison. Arctic-alpine endemic species are often poor dispersers and cannot survive above their thermal maximum or below their altitudinal range. Because of this, I can use the existing rough distributions as well as physical range limitations to establish geographically isolated populations. This is a rough method at best because there is so much variation on physical limitations of range across such a large latitudinal gradient. As such, this initial GIS analysis will serve as a preliminary tool. Once I have established populations, I will sample sufficient genetic material from what I believe to be isolated populations. Because of the massive area being studied and the remoteness of the species being studied, I may have to simplify areas. For instance, while Langin et al (2018) established isolated populations of *L. leucura* in Colorado, considering that this study focuses on more broad themes, it may make sense to combine these populations.

After these data are collected, I will conduct a STRUCTURE analysis of populations to determine distinct groups, while there have been noted issues with STRUCTURE, I believe that the intraspecific isolation of arctic-alpine species could result in statistically provable separate populations. The case of *Ochotona* is slightly different because there are two different species so I will conduct a STRUCTURE analysis for both species individually. The final step of my research

will be to use a Bayesian molecular dating model to identify divergence within species. Bromhan et al. (2017) have noted that there are varying degrees of accuracy among the different Bayesian models and that the newest model may not be the best fit. Thus, it may be necessary to tailor the Bayesian model used for each individual species. Hopefully, the combination of the GIS analysis, the STRUCTURE analysis, and the Bayesian molecular clock model will provide insight into the biogeography each species as well as allowing for broader across species conclusions to be made.

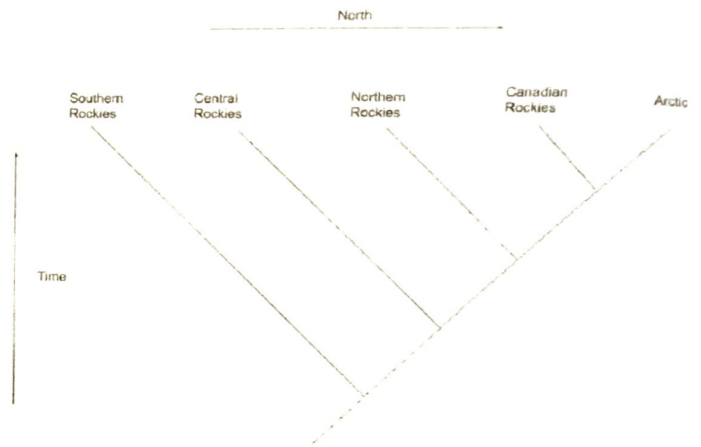
The actual method of genetic testing will be different for each species. Holderegger and Abbott (2003) used intergenic spacers of chloroplast (cp) DNA and internal transcribed spacers (ITS) of ribosomal DNA to map phylogenies of *S. oppositifolia*. They found that ITS phylogeny did not have a high enough resolution to detect intraspecific variations. While cpDNA was more accurate, it still may not have the resolution I require. Adding more sequencing regions may provide enough information for my purposes, but if this fails I may use more modern SNP techniques like ddRADSeq to have the desired resolution. For *L. leucura*, Langin et al. (2018) had good results with their use of 14,866 SNPs and I will use their procedure as a guide. Lanier and Olson (2009) had data for inferring the divergence or pika in North America using mtDNA, but more recent studies like Lanier et al. (2015) have been using RADseq to determine 4156 SNPs with good success and this will probably be more accurate than older mtDNA techniques. I would like to base my research out of Colorado State University because it's biology department is well know for work in alpine ecosystems and its location in the Central Rockies is ideal. Dr. Dan Sloan, Dr. Mark Simmons, and Dr. Jennifer Neuwald all have research that focuses on evolution and would be compatible with my research. Additionally, there may be opportunities for working with University of Anchorage for the parts of my study that would take place in the far north.

Research Expense	Cost (USD)
Field Expenses (Travel and food ect.)	5,000
Additional Materials (GPS, sampling material)	2,000
Genome sequencing and genetic testing	7,500
Yearly Salary	30,000
Total	44,500

IV. Anticipated Results:

I hypothesized that there would be a temporal gradient of population divergence stretching from south to north along a species' range. If the theory that many Arctic species migrated from circumpolar alpine habitats is true, I believe it would lead to phylogenies that reflect southern populations being isolated first with subsequent northerly populations being established as glacial maximums receded. There could be many factors that could cause this hypothesis to not be accepted though. For instance, past climate fluctuations that happened after the glacial maximum could have allowed for more gene flow then we see in today's climate. Populations could have separated and recombined many times. Additionally, Abbott (2010) has pointed out that in *S. oppositifolia*, as well as many arctic plants, there is very little morphological difference in extant species as well as the fossil record. Instead, the molecular data point to speciation from polyploidy in many species. With pika, there doesn't appear to be strong divergent selection being applied, even though populations have little gene flow (Brown 2012). With both pika and saxifrage, it might be that most differences are a result of genetic drift instead of natural selection. This means that the distinctions in populations might not be as clear as I hope, and finding genetic evidence to suggest populations diverging could not be possible even though populations are clearly geographically isolated from each other.

Fig. 3 A possible phylogeny that my data could support. The geographic regions are greatly simplified. Investigating the phylogeography on a smaller scale could reveal additional distribution patterns not seen through a broader lens. This could result in a phylogeny with many more branches for each region



It is also worth noting that while my ultimate goal in studying three very different organisms is to be able to try and draw large scale conclusions about arctic-alpine distributions, it is very possible that I will not be able to find any distinguishable patterns across species. However, the research I propose serves to fill important gaps in the current knowledge of each species individually. The data I collect for each species can stand independently from the other parts of my research and can function to provide interesting and new evolutionary information about the species.

V. Broader Impacts:

Arctic and alpine species are very susceptible to climate change because of their specialized adaptations for living in an extreme climate. Climate change will likely increase maximum summer temperature, decrease snowpack, and shift seasons, all of which would devastate Arctic and alpine ecosystems. If we wish to conserve biodiversity in these regions, we are going to have to have an advanced understanding of how these ecosystems evolved.

Unfortunately, climate change will probably impact these ecosystems regardless. My proposed study would contribute to the scientific community, and overall conservation, by looking at how Arctic-alpine species responded to substantial climate change in the past. With this knowledge, we may be able to understand what to expect in coming years. The information from my proposed research would also provide valuable data on species' ability to adapt to their climates. Understanding population distribution will be important as Arctic-alpine habitats shrink. With my data, we will be able to help stimulate gene flow between groups and maintain genetic diversity, thereby giving Arctic-alpine species the best chance for adaptation to a new climate. Even though my study focuses on population level evolution, its finding could be used for many different fields, like evolutionary biology, conservation biology, biogeography, and population genetics.

People love the flora and fauna found in the mountains and the far north. These regions are some of the most barren and extreme places on earth, but they harbor amazing biodiversity. Polar bears rambling across sea ice and an unbelievably delicate flower hunkered down against a ridge on a mountain slope have captivated people for centuries. Moreover, much of the Inuit culture is built on the animals that inhabit the Arctic. Because these ecosystems will be so hard hit by climate change, we must try and save these species in order to save unique and wonderful cultures, and for the sake of everyone who has been captured by the stunning beauty of the tundra. I hope that by better understanding the evolution that shaped these amazing places, my research can contribute to society by allowing these places to exist for many more generations.

Works Cited

- Abbott, R. J., & Brochmann, C. (2003). History and evolution of the arctic flora: In the footsteps of Eric Hulten. *Molecular Ecology*, 12(2), 299-313.
- Abbott, R. J. (2008). History, evolution and future of arctic and alpine flora: Overview. *Plant Ecology & Diversity*, 1(2), 129-133.
- Bromham, L., Duchêne, S., Hua, X., Ritchie, A. M., Duchêne, D. A., & Ho, S. Y. (2017). Bayesian molecular dating: Opening up the black box. *Biological Reviews*, 93(2), 1165-1191.
- Brown, J. L., & Knowles, L. L. (2012). Spatially explicit models of dynamic histories: Examination of the genetic consequences of Pleistocene glaciation and recent climate change on the American Pika. *Molecular Ecology*, 21(15), 3757-3775.
- Fedy, B. C., Martin, K., Ritland, C., & Young, J. (2008). Genetic and ecological data provide incongruent interpretations of population structure and dispersal in naturally subdivided populations of white-tailed ptarmigan (*Lagopus leucura*). *Molecular Ecology*, 17(8), 1905-1917.
- Galbreath, K. E., & Hoberg, E. P. (2011). Return to Beringia: Parasites reveal cryptic biogeographic history of North American pikas. *Proceedings of the Royal Society B: Biological Sciences*, 279(1727), 371-378.
- Guthrie, R. D. (1973). Mummified Pika (*Ochotona*) Carcass and Dung Pellets from Pleistocene Deposits in Interior Alaska. *Journal of Mammalogy*, 54(4), 970-971.
- Harington, C. (2011). Pleistocene vertebrates of the Yukon Territory. *Quaternary Science Reviews*, 30(17-18), 2341-2354.
- Holderegger, R., & Abbott, R. J. (2003). Phylogeography of the Arctic-Alpine *Saxifraga oppositifolia* (Saxifragaceae) and some related taxa based on cpDNA and ITS sequence variation. *American Journal of Botany*, 90(6), 931-936.
- Ikeda, H., Senni, K., Fujii, N., & Setoguchi, H. (2008). Survival and genetic divergence of an arctic-alpine plant, *Diapensia lapponica* subsp. *obovata* (Fr. Schm.) Hultén (Diapensiaceae), in the high mountains of central Japan during climatic oscillations. *Plant Systematics and Evolution*, 272(1-4), 197-210.
- Langin, K. M., Aldridge, C. L., Fike, J. A., Cornman, R. S., Martin, K., Wann, G. T., . . . Oyler-McCance, S. J. (2018). Characterizing range-wide divergence in an alpine-endemic bird: A comparison of genetic and genomic approaches. *Conservation Genetics*, 19(6), 1471-1485.
- Lanier, H. C., & Olson, L. E. (2009). Inferring divergence times within pikas (*Ochotona* spp.) using mtDNA and relaxed molecular dating techniques. *Molecular Phylogenetics and Evolution*, 53(1), 1-12.
- Lanier, H. C., & Olson, L. E. (2012). Deep barriers, shallow divergences: Reduced phylogeographical structure in the collared pika (Mammalia: Lagomorpha: *Ochotona collaris*). *Journal of Biogeography*, 40(3), 466-478.
- Lanier, H. C., Massatti, R., He, Q., Olson, L. E., & Knowles, L. L. (2015). Colonization from divergent ancestors: Glaciation signatures on contemporary patterns of genomic variation in Collared Pikas (*Ochotona collaris*). *Molecular Ecology*, 24(14), 3688-3705.

- Li, G., Kim, C., Zha, H., Zhou, Z., Nie, Z., & Sun, H. (2014). Molecular phylogeny and biogeography of the arctic-alpine genus *Lagotis* (Plantaginaceae). *Taxon*, 63(1), 103-115. doi:10.12705/631.47
- Macarthur, R. A., & Wang, L. C. (1973). Physiology of thermoregulation in the pika, *Ochotona princeps*. *Canadian Journal of Zoology*, 51(1), 11-16.
- Weber, W. A. (2003). The Middle Asian Element in the Southern Rocky Mountain Flora of the western United States: A critical biogeographical review. *Journal of Biogeography*, 30(5), 649-685.