INVESTIGATING THE GENETIC BASIS OF CLIMATE ADAPTATION IN THE AMERICAN PIKA

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INTRODUCTION

The impacts of climate change on global ecosystems are wide-ranging and pervasive. As changes such as shifts in community structure and altered species phenology become more numerous and severe (Parmesan & Yohe 2003), understanding and managing for climate change is becoming increasingly vital. This research focus is of particular importance in areas of high conservation priority, such as the U.S. National Park System. As evidenced by the current research priorities of the Seattle City Light Wildlife Research Grant Program, high-elevation species are of particular concern to land managers in protected areas, due to alpine species' limited potential for upward elevational shifts. The limited space available upslope for alpine species provides them with three options for climate change response: 1) adapt genetically, behaviorally, or both; 2) disperse, moving upwards in altitude or poleward, likely shifting outside the protective reach of our existing preserves; or 3) perish, becoming locally extinct in the region of interest. A better understanding of species' adaptive potential will provide critical information for managers developing species conservation plans in a new, warmer world, including guiding prioritization of populations/areas for protection.

This study investigates a charismatic and important member of the North Cascades ecosystem: the American pika (*Ochotona princeps*). The American pika is a small lagomorph discontinuously distributed in mountainous areas throughout western North America from central British Columbia and Alberta, south to the Sierra Nevada in California and east to New Mexico, USA. Pikas are restricted to talus slopes in proximity to meadows that provide their food (Smith & Weston 1990). Exhibiting one of the least

nonrandom distributions across mountaintop habitats, average elevation of Great Basin *O. princeps* populations is currently ~582 m higher than during the late Wisconsinan (Grayson 2005). In general, lower elevational limits are constrained by an inability to tolerate high temperatures, while high altitude distribution is enabled by adaptation to hypoxic environments (Beever & Smith 2008). The fragmented nature of their habitats has propelled *O. princeps* to a focal mammalian species for studies of metapopulation dynamics, island biogeography, source-sink dynamics (Peacock & Smith 1997), and extinction risk in the face of climate change (Beever et al. 2011). In fact, American pika are predicted by some to become the first mammalian species to go extinct due to the direct effects of climate change (Smith et al. 2004).

Our research team features PIs with extensive experience researching American pika conservation genetics. Previous research by Dr. Russello's group established three elevational transects in Tweedsmuir South Provincial Park, BC, ranging from sea level to 1500 m, and developed methods for non-invasively sampling these elusive animals. Evidence for limited gene flow and divergent selection were found both longitudinally and altitudinally (Henry & Russello 2013; Henry et al. 2011; Henry et al. 2012a; Henry et al. 2012b).

It is currently unknown, however, if these trends are unique to the range periphery of American pikas or if they exist elsewhere in the species' range, including core sites in the United States. From the perspective of population genetic theory, small, geographically marginal populations exhibit reduced gene flow and increased susceptibility to stochastic processes. These general features of peripheral populations may lead to elevated levels of differentiation and accelerated rates of divergent selection, processes that underlie local adaptation (Mayr 1963; Simpson 1944). The degree to which these predictions hold in American pikas is currently unknown, but critical to understand, as a study of geographic range contraction of 245 species revealed that the vast majority collapsed to the once peripheral parts of their ranges (Channell & Lomolino 2000). This trend is particularly important for Washington and British Columbia, as the most common pattern in the northern hemisphere is for species to collapse to the northern and western edge of their ranges (Channell & Lomolino 2000).

This study was designed to address the WRP research question, "How is climate affecting high-elevation mammal populations such as pikas?" Our primary goal was to use population genomics and next-generation sequencing techniques to investigate neutral and adaptive population divergence of American pikas at different elevations within North Cascades National Park (NOCA). Specifically, we sought to reconstruct genetic patterns to provide information regarding population connectivity and dispersal behavior within and among elevations. We also conducted a preliminary analysis of microclimatic variation across elevationally distributed sites. Combined, these results contribute insights into the role of climatic factors in NOCA in potentially driving local adaptation.

METHODS AND RESULTS

Fieldwork

Site selection and sampling

Sample transects in the North Cascades National Park, Washington were selected on the basis of available habitat, pika presence, and accessibility. After consulting local park staff, inspecting aerial photographs, and scouting potential sites, three transects were identified. Preference was given to south-facing slopes, but one north-facing slope was also included. Transects included: Sourdough Mountain (SD), Thornton Lakes (TL), and Pyramid Peak (PP). Transects were located in the west side of the park in close proximity (< 10 miles) to Newhalem. Four sample sites were located along each transect at approximately 500m, 750m, 1200m, and 1600m (sites 1, 2, 3, and 4 respectively). Each site was given a unique identifier comprised of the two letter transect abbreviation with an alphanumeric code for the site elevation (e.g., TL3T would be the 1200m elevation site along the Thornton Lakes transect).

All 12 sites were sampled between late July and early September 2013 using noninvasive hair snares following the approach of Henry and Russello (2011). Sites were surveyed for pika presence to identify the best hair snare locations. Approximately 20 hair snares were set at each sample site. To minimize the likelihood of resampling the same pika, snares were set at least 15m apart. Snares were checked 1-2 nights after deployment and samples were collected and preserved in test tubes with an internal silica

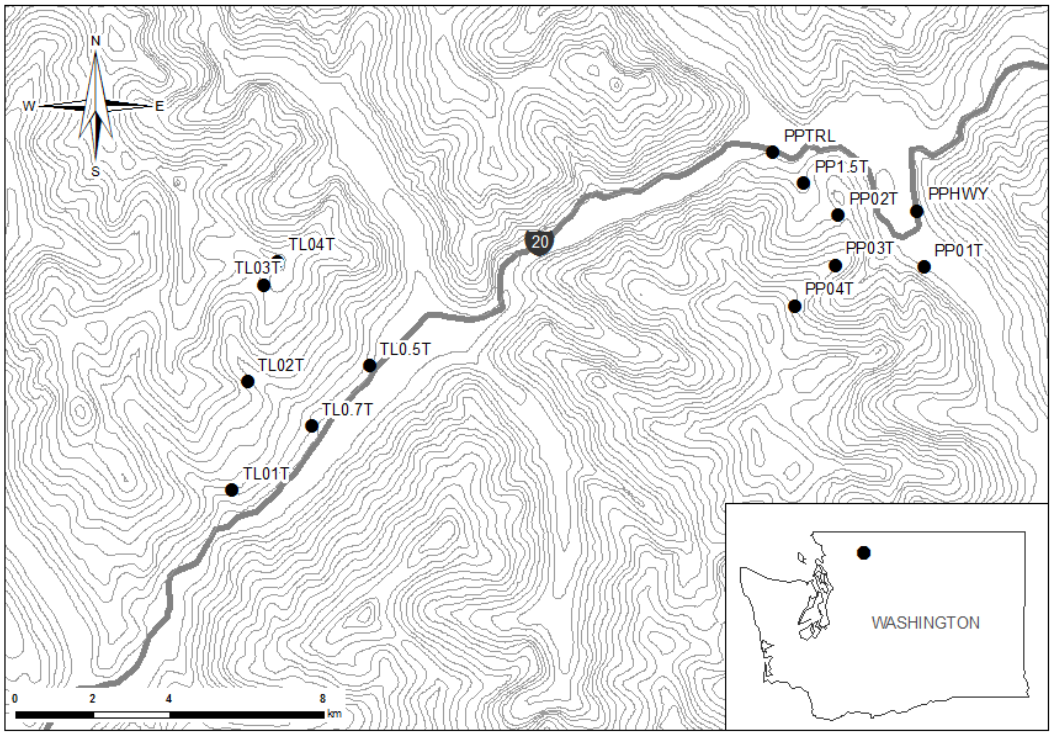


Figure 1. Site map showing the Thornton Lakes (TL) and Pyramid Peak (PP) transects including the supplemental low sites (TL0.5T, TL0.7T, PPTRL, PPHWY, and PP1.5T). desiccant. Each sample was required to have a minimum of 20 hairs to ensure sufficient

genetic material was present. We estimated the number of individuals sampled by considering all samples from the same hair snare a single individual.

Overall, we conservatively estimate 744 person-hours were dedicated to hair sampling during the 2013 sampling period. A total of 234 hair samples were collected, representing an estimated 143 unique individuals. The quantity of hair on each sample varied from the minimum of 20 hairs to hundreds of hairs with the majority of the samples providing enough hair for multiple DNA extractions. An average of 12 individuals were sampled from each sample site, with SD2T and SD3T having notably low sample sizes (n=4 and n=1, respectively). Baited hair snares (peanut butter and spinach) were attempted with little success at these low sample size sites. It is likely that pika density was too low to achieve the minimum sample size at these sites. Replacement locations were not located for these sites, but future efforts could focus on identifying alternate locations for these two sample sites to complete the Sourdough transect.

Table 1. Site name, elevation (m), and initial sample sizes for non-invasively collected pika samples (2013) and live trapped pika (2014). For 2013, the number in parentheses indicates the sample size retained for downstream analyses after quality control.

Site	Elevation	2013 (Hair)	2014 (Tissue)
TL0.5T	150	0	2
TL0.7T	265	0	1
TL01T	490	13 (6)	7
TL02T	780	13(10)	11
TL03T	1390	13 (13)	8
TL04T	1700	12 (9)	4
PPTrl	330	0	1
PPHwy	415	0	1
PP01T	450	12 (8)	5
PP1.5T	615	0	1
PP02T	820	12 (5)	6
PP03T	1330	8 (5)	3
PP04T	1580	13 (11)	9
Total		96 (67)	59

During the summer of 2014, 59 pikas were live-trapped using Tomahawk (Hazelhurst, WI) model 202 collapsible traps from eight sites along two independent elevational transects (TL and PP) and opportunistically around the two low sites (TL01T and PP01T) (Table 1, Figure 1). Two small (3mm) ear hole punches were removed from each pika for a genetic sample. Additionally, a small (20mg) hair sample was taken for a backup genetic sample. To determine the age class of captured animals, cranial diameter was taken using calipers along with weight. Individuals with a weight under 150 grams and cranial diameter under 5 cm were considered juveniles.

Genomic data collection and analysis

DNA Extraction and genotyping-by-sequencing non-invasively collected samples

Our approach for simultaneously discovering single nucleotide polymorphisms (SNPs) and genotyping individuals (NextRAD sequencing) required a minimum of 10ng of DNA to scan approximately 50,000 loci across the genome. Numerous efforts were made to optimize DNA extraction protocols for use with pika hair samples. Our results showed

that the Promega DNA IQ extraction kit was capable of producing sufficient quantity and quality of DNA and required a minimum of 60 hairs per sample. The Thornton Lakes (TL) and Pyramid Peak (PP) transects were selected for preliminary analysis since these transects had the most complete sampling. An average of 12 hair samples from the 2013 field season were selected from each of the 8 main transect sites, for a total of 96 samples. Extractions proceeded with minimum alterations to the manufactures protocol and samples were quantified using a fluorescent real-time PCR method utilizing PicoGreen. The mean starting DNA concentration recovered from the non-invasively collected hair samples was 0.55ng/µl with as little as 1 ng total for some samples.

Each sample produced an average of 1.9 million DNA sequence reads. Ten samples yielded less than 100,000 sequencing reads, likely due to the degraded quality and very low quantity of starting DNA. Nineteen additional samples had less than 50% of their sequencing reads mapping to *O. princeps*. Sixteen of these samples had high proportions of sequence reads matching with two small mammals that likely co-occur in the sampling area [*Mus musculus* (n=13) and *Spermophilus* (n=3)], with others matching *Homo sapiens* (n=2) and *Zea mays* (n=1). The above samples (n=29) were removed leaving 67 individuals (Table 2). Additionally, only sequences aligning to the American pika genome were used in subsequent analysis. Consequently, 3,830 SNPs were identified of which 27 deviated from Hardy-Weinberg expectations (HWE) and were eliminated. All downstream analyses were based on genotypic data at 3,803 SNPs.

Polymorphic loci were screened for statistical outliers using the Bayesian simulation method of Beaumont and Balding (2004) as implemented in BAYESCAN 2.1 (Foll and Gaggiotti 2008) and independently run for each transect. This analysis identified 37 loci along the TL transect and 18 unique loci along the PP transect as outliers potentially under natural selection. These loci were segregated into an 'outlier' dataset and subjected to a BLASTN (Altschul et al. 1990) search of all sequences in the NCBI non-redundant database. The remaining loci were grouped into a 'neutral' data set. We tested for genetic structure within and among these two transects in each dataset using a Bayesian model-based clustering method implemented in STRUCTURE 2.3.4 (Pritchard et al. 2000). These results indicated substantial structure between transects, but relatively weak neutral genetic structure within each transect (Figure 2). There was

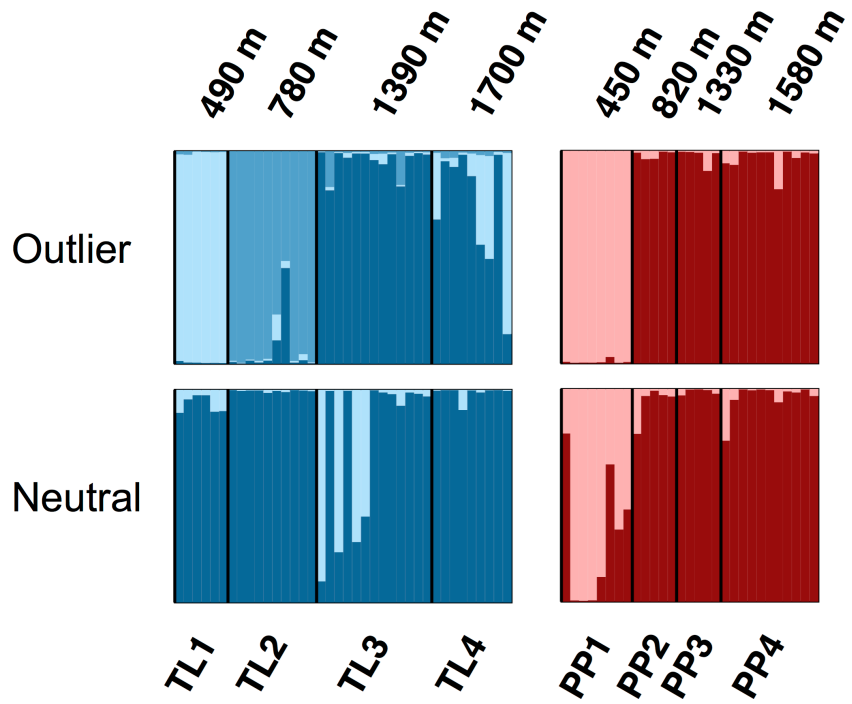


Figure 2. STRUCTURE bar plots depicting the model-based clustering results for Thornton Lake (TL) and Pyramid Peak (PP) sites based on outlier loci (above) and neutral loci (below). Analyses for the TL transect revealed evidence for both $K = 2$ (ΔK = 473.3) and $K = 3$ (ΔK = 314.6; plot shown) based on 37 outlier loci, and $K = 1$ ($K = 2$ plot shown for display purposes) based on 3,748 neutral loci. Analyses for the PP transect revealed evidence for $K = 2$ (ΔK =123.1) based on 18 outlier loci, and $K = 2$ (ΔK = 33.1) based on 3,748 neutral loci.

evidence for a unique genetic unit at the low site of each transect based on outlier loci alone (Figure 2). We estimated conventional genetic diversity metrics from the 'neutral' dataset, including percent of polymorphic loci (P), observed (H_o) and expected (H_s) heterozygosity, gene diversity (N_g), and inbreeding estimates (F_{is}). Interestingly, a linear regression showed a positive correlation between elevation and measures of genomic diversity (Figure 3). The finding of significant genome-wide evidence of heterozygote deficit at low elevation sites in both transects further suggest inbreeding may be leading to the observed patterns (Table 2), a particular concern for PP1T, TL1T and TL2T given their apparent distinctiveness from higher elevation sites.

Overall, we showed that non-invasively collected samples could be used in conservation genomic analysis. These results hold great promise for informing conservation-related studies, substantially increasing the number of markers to allow for

more accurate and precise estimates of population structure and demographic parameters (Primmer 2009), as well as the ability to detect adaptive genetic variation for informing conservation unit delimitation (Funk et al. 2012) and decision frameworks aimed at reducing the long-term impacts of climate change on biodiversity (Hoffmann and Sgrò 2011). These results were recently published in the open-access journal *PeerJ* (Russello et al. 2015). Yet, several characteristics of the data set, including low genomic coverage, cross species contamination, and relatively few loci recovered, limited population-level inferences regarding the American pikas of the North Cascades National Park. Consequently, we decided to live-trap during the 2014 field season to collect tissue samples in order to obtain higher quality and greater quantity DNA which would support broader-scale genomic analysis (sampling described above).

Table 2. Genetic variation within American pika samples sites along the Pyramid Peak (PP) and Thornton Lake (TL) elevational transects in North Cascades National Park. Asterisk indicates a significant reduction in observed genetic diversity (H_o) relative to expectations (H_e) or significant inbreeding (*Fis;* $p < 0.05$).

Site	Elevation	*n*	*P*	H_o	H_e	N_g	F_{is}
PP1	450	8	0.774	0.282*	0.372	0.183	0.260*
PP2	820	5	0.661	0.314*	0.425	0.213	0.295*
PP3	1330	5	0.837	0.403	0.403	0.329	0.001
PP4	1580	11	0.943	0.383	0.359	0.295	-0.071
TL1	490	6	0.777	0.368*	0.400	0.202	0.088*
TL2	780	10	0.839	0.339*	0.362	0.237	0.067*
TL3	1390	13	0.947	0.336	0.349	0.292	0.039*
TL4	1700	9	0.908	0.356	0.364	0.272	0.023*

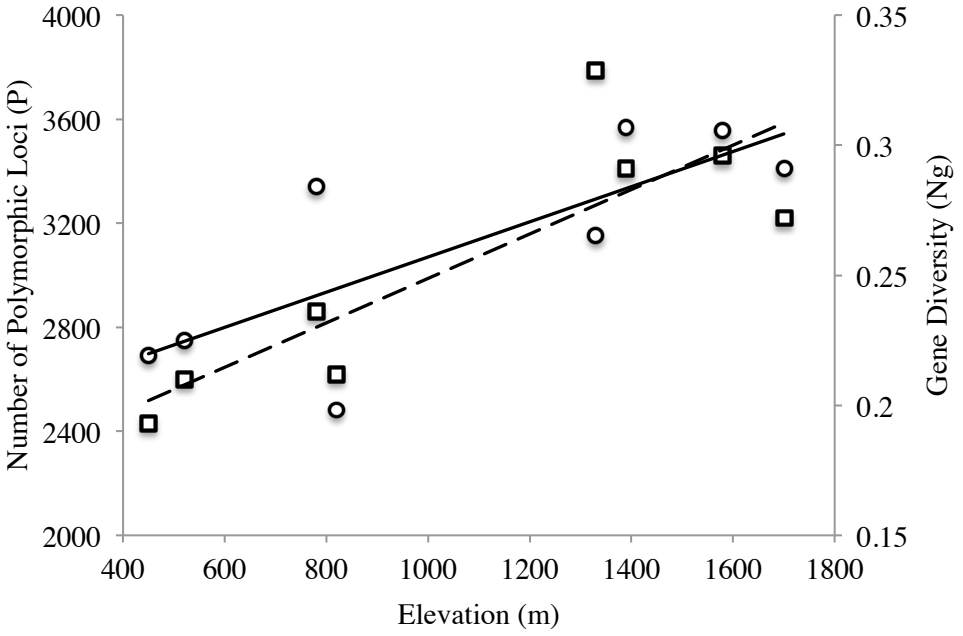


Figure 3. Elevational patterns of genomic diversity within American pika samples in the North Cascade National Park. Solid line shows the correlation between the number of polymorphic loci (circles) with elevation (F=9.232, df=1,6, r^2=0.606 p=0.023). Dashed line shows the correlation between gene diversity (squares) with elevation (F=15.44, df=1,6, r^2=0.720 p=0.008).

DNA extraction, sequencing, and genomic analysis of tissue samples

DNA was extracted from each of the 59 samples obtained via live-trapping during the summer of 2014 (Table 1). Genomic sequencing proceeded using a modified method described by Baird et al (2008) which utilizes restriction-site associated DNA (RAD) sequencing to genotype each sample. Approximately, 500ng of genomic material from each sample was digested with the *SbfI* restriction enzyme and ligated to a unique barcode to facilitate parallel sequencing of pooled samples (i.e., a genomic library). The library was sequenced two independent times on an Illumina HiSeq2000, which produced a total of 5.7 million DNA sequence reads per sample. After dropping ambiguous barcodes, low quality reads, and ambiguous cut sites, 3.9 million reads per sample were retained.

The library was demultiplexed using STACKS v.1.09 (Catchen et al. 2013). Following cleaning, reads were aligned *de novo* with each other to identify putative RAD

Table 3. Pairwise estimates of 'neutral' differentiation between sites (θ) significance was based on 1,000 permutations (* indicates p-value < 0.05). Grey inset indicates pairwise comparisons between transects.

	PP10T	PP02T	PP03T	PP04T	TL01T	TL02T	TL03T
PP10T							
PP02T	0.058*						
PP03T	0.038	0.000					
PP04T	0.044*	0.036*	0.005				
TL01T	0.188*	0.168*	0.170*	0.144*			
TL02T	0.230*	0.217*	0.216*	0.196*	0.113*		
TL03T	0.177*	0.167*	0.147*	0.156*	0.053*	0.048*	
TL04T	0.196*	0.184*	0.166	0.169*	0.088*	0.088*	0.021*

tags. Using the *population* module of STACKS, RAD tags were filtered using a minimum coverage of 10x (each RAD tag was independently sequenced 10 times). Only SNPs present in 70% of the samples and with a minor allele frequency greater than 0.05 were retained, producing 31,077 SNPs. Six samples, three from each transect, were excluded for having greater than 30% missing data and are being sequenced in subsequent genomic libraries. As a final quality control, GENODIVE (Meirmans and Van Tienderen 2004) was used to detect loci out of HWE. Any locus out of HWE in 3 or more sites was excluded, leaving 29,818 SNPs.

Putative loci under natural selection were identified using an outlier detection method implemented in BAYSCAN 2.1. This analysis was conducted independently for each transect identifying 57 loci along the TL transect and 31 unique loci along the PP transect as statistical outliers (q-value < 0.20). GENEPOP V4.2 (Raymond and Rousset 1995; Rousset 2008) was used to verify these loci were in linkage equilibrium along their consecutive transects. We segregated loci into two datasets for downstream analyses including: 1) all loci identified as an outlier ('outlier dataset'); and 2) all loci not identified as an outlier ('neutral dataset')

Genetic structure within the 'neutral' and 'outlier' datasets was inferred using a Bayesian model-based clustering method implemented in STRUCTURE 2.3.4. Overall, the genetic structure was similar to the non-invasively caught samples. The neutral genetic dataset resolved little genetic structure along each transect with the main genetic division between the two transects (Figure 4). Yet, pairwise estimates of θ, a measure of genetic

Table 4. Analysis of molecular variation showing hieratical organization of genomic variation within the 'neutral' dataset. All groupings were significant ($p < 0.001$).

Source of Variation	Percent of variation
Within Individuals	82.8%
Among Transects	10.9%
Among Populations	4.9%
Among Individuals	1.4%

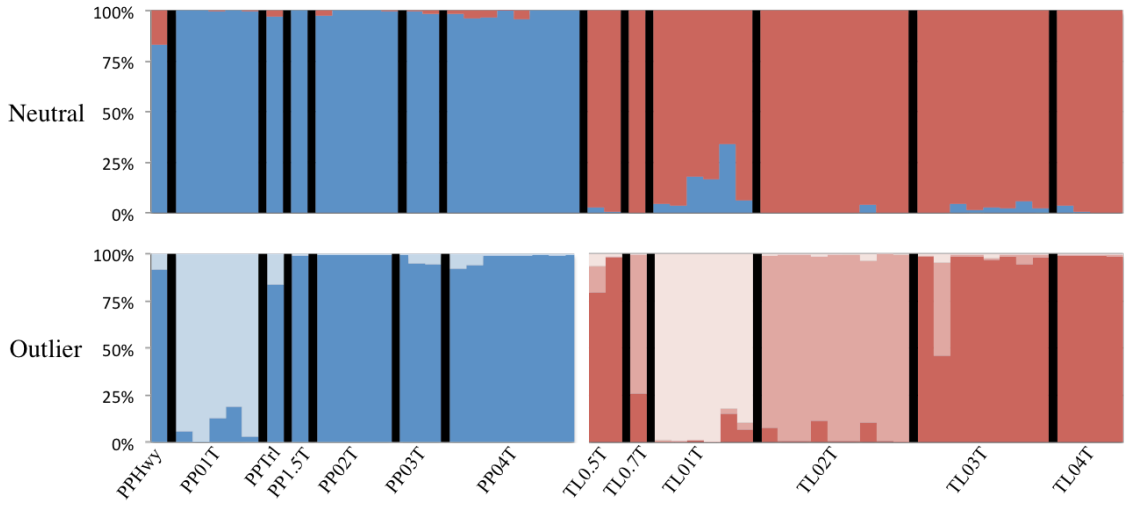


Figure 4. Genetic structure plots showing 'neutral' genetic structure (top; n=29,464 loci) and 'outlier' structure for the PP transect (bottom left; n=31 loci) and TL transect (bottom right; n=57 loci). All samples (n=53) were used in 'neutral' genetic structure and resolved a high degree of support for two genetic units (ΔK = 2,094). Genetic structure was analyzed independently for the PP transect (n = 23) and TL transect (n = 30). Both the PP and TL transects showed a high degree of support (ΔK = 402, 1061, respectively) for 2 and 3 genetic units, respectively.

divergence, revealed evidence for significant structure between most pairwise site comparisons within and among transects (Table 3). An analysis of molecular variance confirmed that the majority of the genomic variation in the 'neutral' dataset was explained by the differentiation between transects (Table 4). The 'outlier' dataset showed the low sites at each transect comprised a unique genetic unit, with supplementary sites (PPHwy, PPTrl, PP1.5T, TL0.5T, TL0.7T) showing affinity to mid- and high-elevation

sites. Given the small sample sizes of the supplementary sites, it is difficult to interpret the recovered patterns; greater sampling effort at these sites would be required to make more definitive inferences.

Microclimate analysis

Microclimate variation was assessed between sites by deploying two ambient and two talus temperatures sensors (DS1921G Thermochron i-Button, Maxim Integrated Products, Sunnyvale, CA) at each of the eight main transect sites. All sensors were deployed in weather-proof housing; ambient sensors were placed 1.5m above the talus in neighboring trees while talus sensors were deployed 0.8m below the talus surface in a central region of the sites talus. Temperatures were taken every four hours from August 24, 2014 to July 23, 2015. Temperature readings were averaged between the two ambient and two talus sensors at each site and used to generate relative microclimate measurements (Table 5).

In general, transects showed a linear decrease of 5.1°C decrease in temperatures with an increase of 1,000 m elevation. This is close to the global average of 6°C per 1,000 m (Briggs et al. 1997). Interestingly, while mean ambient temperatures were comparable between these two sites, talus temperatures were significantly lower at the two PP low sites then the TL low sites, possibly owing to the northern aspect of these sites. Importantly, talus temperatures never exceeded 28°C, a temperature thought to be a thermal threshold for pika (Beever et al. 2010). However, ambient temperatures routinely exceeded 28°C at low sites (TL01T and PP01T) in both transects but not at the high sites (TL04T and PP04T; Figures 5 and 6). These preliminary analyses indicate that significant microclimate variations is represented across the sampled transects; the obtained data will be useful in future environment-genotype association studies. Additionally, this direct measurement of microclimate variation will facilitate the assessment of long-term climate variation using downscaled long-term climate data and general circulate models as employed by the ClimateWNA model (Wang et al. 2012).

Table 5. Summary of temperature sensor data. At each site, two sensors were placed below the talus surface approximately 0.8m deep and two sensors were placed above the surface at a height of approximately 1.5m. Sensors recorded temperature every 4 hours; one logger in each location (sub-talus and ambient) recorded data from August 24, 2014 through July 23, 2015. The other logger in each pair recorded data from September 8, 2014 through August 15, 2015. This configuration allowed for continuous data collection from August 24, 2014 through August 15, 2015. Data in the overlapping period (September 8, 2014 through July 23, 2015) was averaged for use in calculating the summary metrics below. All temperatures shown in Celsius.

		Ambient					Sub-Talus				
Site	Elevation	Daily Minima < -10° C	Daily Maxima >28° C	Mean Annual Temp	Mean Summer (Jun-Aug) Temp	Mean Winter (Dec-Feb) Temp	Daily Minima < -10° C	Daily Maxima >28° C	Mean Annual Temperature	Mean Summer (Jun-Aug) Temp	Mean Winter (Dec-Feb) Temp
PP01T	450	0	29	10.03	19.76	2.76	0	0	7.57	16	0.79
PP02T	820	3	22	8.82	18.62	1.65	0	0	6.12	13.74	0.25
PP03T	1330	6	3	6.14	15.39	-0.14	0	0	4.48	13.24	-1.18
PP04T	1580	5	0	4.58	13.8	-0.84	0	0	3.86	11.83	0.02
TL01T	490	0	16	10.58	19.25	4.19	0	0	9.96	18.13	3.93
TL02T	780	3	22	8.95	17.93	2.64	0	0	7.33	15.16	1.57
TL03T	1390	4	1	5.83	14.06	0.32	0	0	3.69	10.46	-0.45
TL04T	1700	8	0	4.21	12.46	-1.14	0	0	3.67	11.17	-0.14

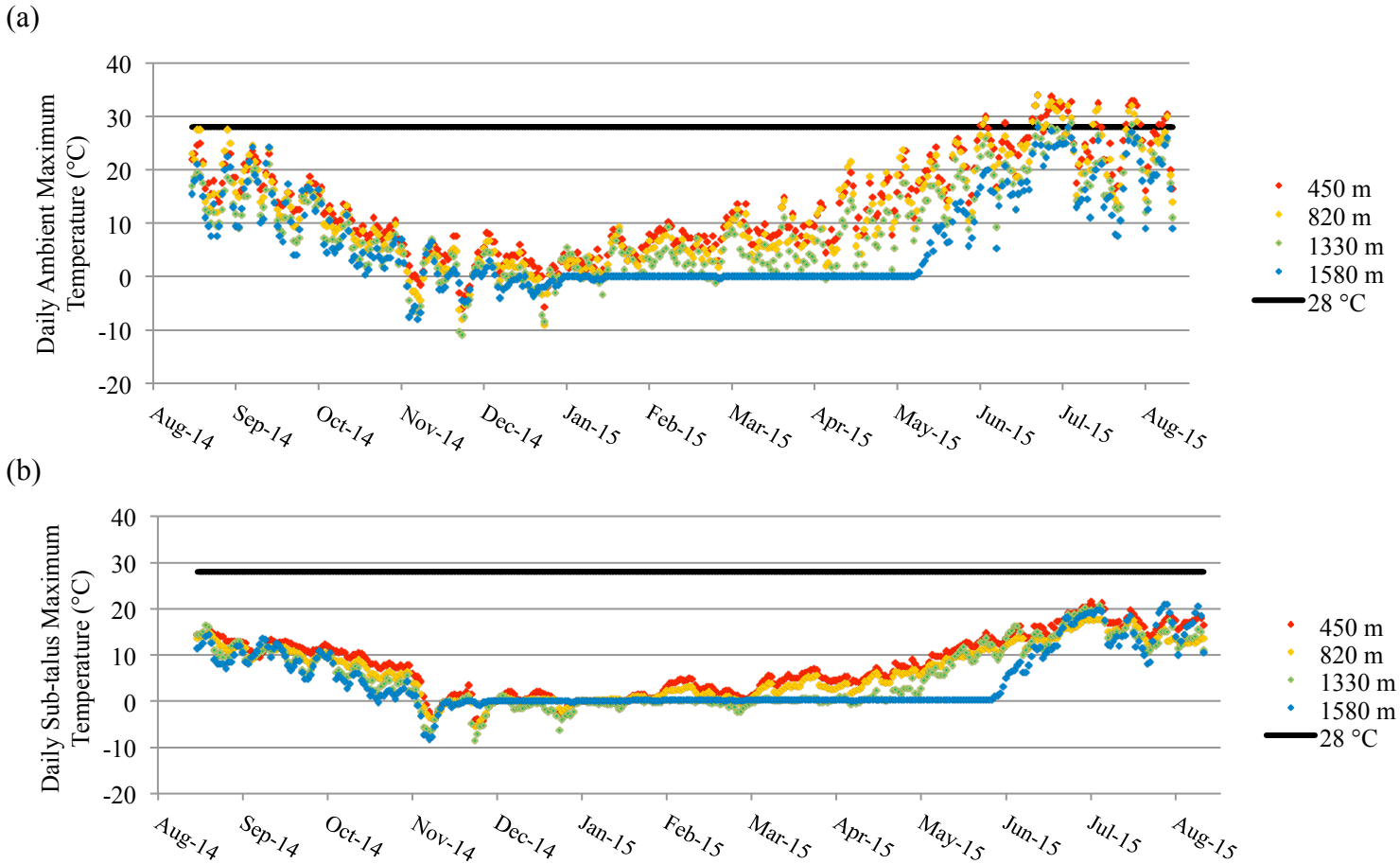
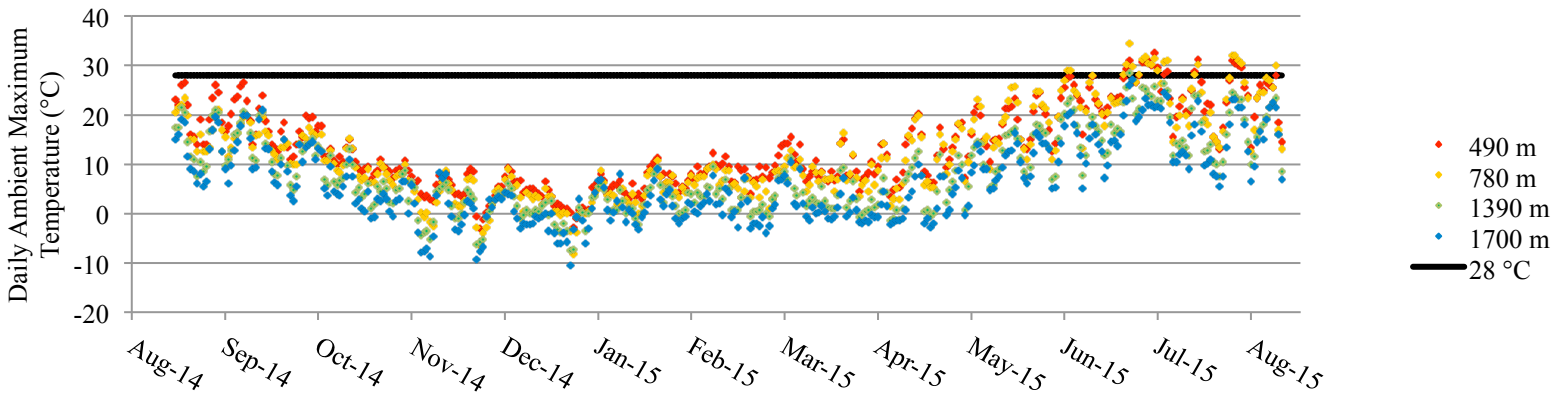


Figure 5. Daily ambient (a) and sub-talus (b) maximum temperatures along the Pyramid Peak elevational transect. 28 degrees Celsius is a common threshold used to assess acute heat stress in *Ochotona princeps* (e.g. Beever *et al.* 2010). No sub-talus maxima were above this threshold at any of the four sites sampled, despite many such ambient values (see Table 1). This indicates a talus buffering effect, providing the potential for behavioral thermoregulation at hotter, low elevation sites.

(a)



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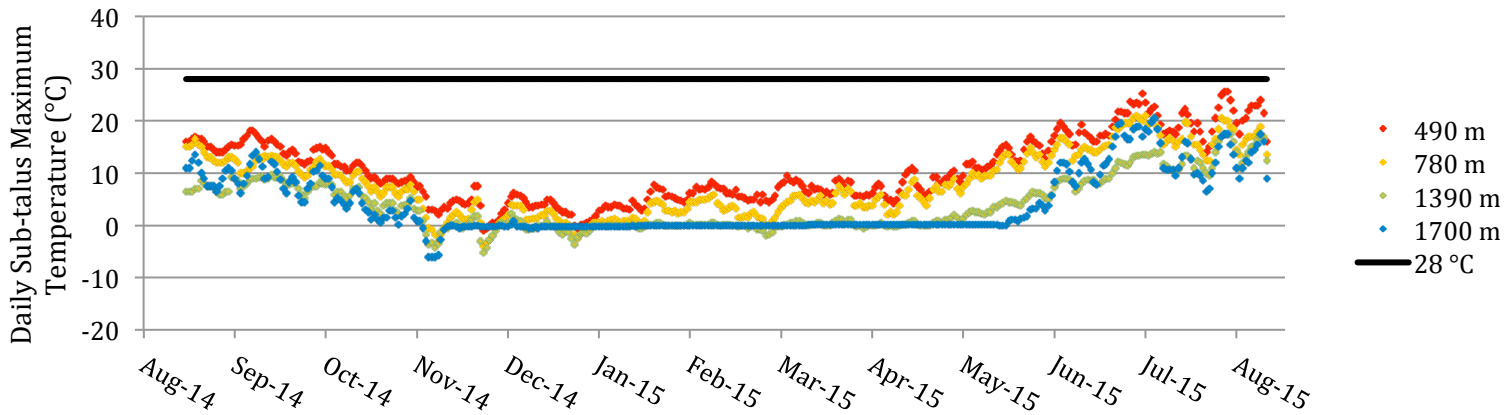

Figure 6. Daily ambient (a) and sub-talus (b) maximum temperatures along the Thornton Lakes elevational transect. 28 degrees Celsius is a common threshold used to assess acute heat stress in *Ochotona princeps* (e.g. Beever *et al.* 2010). No sub-talus maxima were above this threshold at any of the four sites sampled, despite many such ambient values (see Table 1). This indicates a talus buffering effect, providing the potential for behavioral thermoregulation at hotter, low elevation sites.

CONCLUSIONS

- Genome-wide genotypic data generated from non-invasively collected hair (n= 67 individuals @ 3,803 SNPs) and ear tissue (n= 59 individuals @ 29,818 SNPs) revealed significant neutral population structure among sampled elevational transects and evidence of highly restricted dispersal among sites within transects. These results suggest that pikas may be limited in their ability to disperse in response to changing environments and that assisted migration may be warranted as a management strategy.
- The detection of outlier loci among sites within elevational transects provides some evidence for signatures of natural selection that could potentially underlie local adaptations, however, the lack of parallel patterns across both transects limits our ability to confidently infer an underlying genetic mechanism.
- Significant microclimate variation exists among sampling sites along elevational gradients, and temperatures exceeding expected upper thermal limits for pikas were present above the talus surface during the study period. These results highlight the importance of talus in buffering against rapidly changing environmental conditions.

SCIENCE COMMUNICATION AND OUTREACH

The funding allocated to public outreach via ScienceLIVE has resulted in two lesson plans using actual data from the project, as well as four videos. All of these materials, along with profiles of and blogs from the research team, can be found free of charge on the ScienceLIVE website (www.science-live.org). A brief description of each of these resources and how they can be accessed is provided below.

1. **Overall Pika Video:** https://vimeo.com/122452931
   This video provides an overview of American pikas, their physiology, ecology, and the current threats to and research on the species.
2. **North Cascades Ecological Genetics Overview:** https://vimeo.com/122543923
   This video provides an overview of the goals of this research project.
3. **Pika Body Size and Adaptation:** https://vimeo.com/122472847

This brief video is meant to accompany the pika body size lesson (listed as #5 below), providing a basic overview of natural selection as it relates to pikas in the North Cascades.

4. **Pika Genetic Data:** https://vimeo.com/122472232
   This brief video explains why and how genetic data were acquired for the lessons.
5. **Lesson on Natural Selection and Body Size of Pikas:** http://science-live.org/teachers/pikabodysize.html

   This multi-part lesson focuses on natural selection of body size in pika populations. Part 1 focuses on the importance of body size in animal thermoregulation, using geometry to assess heat loss in different size pikas. Part 2 is an exercise in natural selection, in which students simulate several generations of pikas and how the population's average body size changes depending on the environment they are in. Part 3 allows students to analyze and graph real pika body size data and interpret their results.

   This very popular lesson can be adapted to elementary through early undergraduate classrooms. It was presented at a Biological Sciences Initiative workshop, "Natural Selection in a Changing World: Using real data to explore adaptation in native bees and pikas," on March 21, 2015 to 20 middle school and high school teachers. It was also presented at the Colorado Biology Teachers Association Spring Symposium on April 11, 2015 to approximately 30 teachers. This lesson was also shared at the North American Pika Consortium Conference in Golden, CO, on April 17, 2015. This was an internationally-attended conference with approximately 40 pika scientists and educators. Since these outreach efforts, this lesson has been visited online 210 times by users from 11 US states. We can confirm use of this lesson in classrooms ranging from 5th grade to undergraduate general biology classes.
6. **Lesson on Pika Population Genetics:** http://science-live.org/teachers/pikagenetics.html

   The goal of this lesson is to answer the question: Are pikas living at low elevations genetically different than those at high elevations? Students investigate this question in two ways, both commonly used by modern population geneticists.

First, students investigate general gene flow in these populations by looking at the proportion of individuals that are heterozygous in a population. Second, they will look at single nucleotide polymorphism (SNP) data to identify DNA regions (loci) that may be undergoing natural selection and will use their creativity to guess possible functions of these genes. Finally, students will develop methods for testing their gene's function and consider the ability of low elevation pikas to survive in the future.

This lesson is for a more advanced audience, targeting advanced placement high school or undergraduate classrooms. This lesson was also presented in the March 2015 Biological Sciences Initiative workshop and shared at the April 2015 pika conference. Exact classroom use of this lesson has been difficult to track due to website limitations, but we are aware of 84 visitors from 9 states to the webpage associated with this lesson.

7. **Other Web Materials Featuring North Cascades Research**

All of the materials above, as well as other information on the pika research team, are regularly accessed by the public on the ScienceLIVE website, which logged approximately 11,000 visits from the public in the last year.

- The overall pika video can be accessed on the pika home page of ScienceLIVE: http://science-live.org/pika.html
- The overview of pika genetics research in the North Cascades can be found under "The Science": http://science-live.org/pikas/science.html
- Features on the North Cascades researchers are found under "The Team": http://science-live.org/pikas/team.html
- Blog entries from the 2013 and 2014 research teams can be found at: http://www.science-live.org/pikas/follow/category/north-cascades-national-park/

ACKNOWLEDGEMENTS

Regina Rochefort and Roger Christophersen provided extensive knowledge of local pika populations and assistance in planning fieldwork. Erik Beever provided the technical details of deploying temperature sensors and experimental design. Bryson Sjodin, Ashley Boksteyn, and Amber Peter all assisted with field and lab work. Holly Buhler and Shane Schoolman assisted with fieldwork and GIS analysis. Kelsey Robson, Aidan Beers and Andrew Veale assisted with fieldwork. Peter Erb created the videos and web content associated with the outreach projects. Cheryl Blair contributed extensive technical expertise and training for live trapping pika. Karl Larsen provided the traps for the 2014 field season. Paul Etter and Eric Johnson provided technical expertise in genotyping-by-sequencing the non-invasive hair samples.

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