INVESTIGATING THE GENETIC BASIS OF ADAPTATION IN A CLIMATE CHANGE SENSITIVE SPECIES : THE AMERICAN PIKA

by

Philippe O Henry

BSc, University of Lausanne, 2005

MSc, University of Lausanne, 2007

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

The College of Graduate Studies

(Biology)

UNIVERSITY OF BRITISH COLUMBIA

(Okanagan)

December 2011

Abstract

When faced with rapidly changing environmental conditions, wildlife species are left to adapt *in situ*, disperse or disappear. While it is commonly accepted that environmental changes are important drivers in shaping the evolutionary paths of organisms, the functional mechanisms of adaptations to changing environments are not fully understood. With the present thesis, I begin to investigate the relative importance of stochastic (genetic drift) versus deterministic (natural selection) processes in shaping genetic variation in the American pika (*Ochotona princeps*; Richardson, 1828). I begin by describing a novel and noninvasive sampling technique to collect hair from American pikas (Chapter 2). In that chapter, I test different DNA isolation techniques and identify the most promising approach, which was then used to extract DNA from a large number of samples distributed along three elevation gradients in the central Coast Mountains of British Columbia. In Chapter 3, I use a conservation genetic approach and microsatellite data to assess population genetic structure and gene flow among populations. In this chapter I found my populations to be genetically impoverished in comparison to populations from the centre of their range. The BC populations also exhibited limited gene flow, even among geographically proximate sites. Additionally, correlative analyses suggested that warm summer temperature limit pika dispersal across low elevation valley bottoms. In Chapter 4, I use Amplified Fragment Length Polymorphism (AFLP)-based genomic scans to shed light on the genetic basis of adaptation in this system. Based on a set of independent analyses including population genomic and landscape genomic approaches, I revealed that a small proportion (1.5%) of the genome displays evidence of natural selection. These outlier loci were found to differ among each elevation transect. Additionally, some loci that were under selection were also significantly associated with environmental variables such as mean annual precipitation and summer mean

maximum temperature. This highlights the importance of these environmental variables in driving evolutionary adaptation in American pikas. Overall, the work presented here suggests that American pikas from the central Coast Mountains of BC will rely on local adaptations or plasticity *in situ* rather than range shifts to cope with changing climates.

Preface

The following parts of this thesis have been published in or submitted to international peer reviewed journals.

A version of **Chapter 2** has been published in the ***European Journal of Wildlife Research*** and gave rise to a video article published in the ***Journal of Visualized Experiments*** :

- **Henry, P.** & Russello, M.A. (2011) Obtaining high quality DNA from elusive small mammals using low-tech hair snares. ***European Journal of Wildlife Research***, 57, 429-435.

Michael Russello participated in the study design, and provided feedback about sampling strategy, labwork and edited the manuscript. I participated in the study design, undertook field and lab work, analysed the data and drafted the manuscript.

- **Henry, P.,** Henry, A. & Russello, M.A. (2011) A Noninvasive Hair Sampling Technique to Obtain High Quality DNA from Elusive Small Mammals. ***Journal of Visualized Experiments,*** 49. http://www.jove.com/index/details.stp?id=2791, *DOI: 10.3791/2791*

Michael Russello participated in the study design, and provided feedback about sampling strategy, labwork and edited the manuscript. Alison Henry participated in fieldwork and filmed and produced the video article. I participated in the study design, undertook field and lab work, analysed the data and drafted the manuscript.

Chapter 3 was submitted to ***PLoS One*** on the 1st of November 2011 and has been sent out for peer review.

- **Henry, P.**, Sim, Z. & Russello M.A. (Submitted) Genetic evidence for highly restricted dispersal along continuous altitudinal gradients in the climate change-sensitive American pika (*Ochotona princeps*).

Michael Russello participated in the study design, and provided feedback about labwork and analyses and edited the manuscript. Zijian Sim participated in fieldwork, undertook the lab work, and provided comments on a previous version of the manuscript. I participated in the study design, undertook the fieldwork and participated in the labwork, I also analysed the data and drafted the manuscript.

Appendix 1 was submitted to ***Northwest Science*** on the 27th of July 2011 and is currently in revision.

- **Henry, P**., Henry, A., and Russello, M.A. (Submitted) Divergence in microhabitat characteristics in American pikas from low and high elevations.

Michael Russello participated in the study design, and provided feedback about fieldwork and analyses and edited the manuscript. Alison Henry undertook the fieldwork, participated in data analyses and produced a report on which this manuscript is based. I assisted in fieldwork, conducted data analyses, and drafted the manuscript.

The work on which this thesis is based was undertaken following the animal care protocol from the University of British Columbia (# A07-0126) and a sampling permit (# 78470-25) from the Ministry of Environment of the Province of British Columbia.

Table of Contents

List of Tables

List of Figures

Acknowledgements

I would like to extend my sincere gratitude to the members of my PhD committee for their roles in directing, motivating and refining my progress over the course of my studies. My supervisor, Michael Russello was an exceptional mentor, he suported my work both financially and intellectually, and provided assistance in grant proposal writing, and by his example I learned to become a successful professional scientist. Karl Larsen and Karen Hodges both provided interesting insight with regards to sampling protocols. Michael Pidwirny contributed to the environmental component of my work. Mark Rheault was a rigorous source of comments with regards to the direction of my laboratory work.

The members of the Ecological and Conservation Genomics Lab (ECGL) provided a collegial and intellectually stimulating environment that contributed to fostering my academic growth. Anders Gonçalves da Silva has been a friend and role model. Likewise, Stephanie Kirk and Danielle Lalonde offered friendship and valuable insights that improved my work. Brodie Granger, Daniel Rissling, Alison Henry and Adam Goodwin all provided cheerful company while working in the field in the Bella Coola valley, and contributed to designing the noninvasive sampling protocol used in my thesis. Zijian Sim was an instrumental part of the labwork, with his important contribution to microsatellite genotyping. Matt Lemay and Karen Frazer both contributed in discussions on the analysis of population genomic data. Beyond my colleagues from the ECGL, members of the graduate student community at the Universtiy of British Columbia, Okanagan campus, especially Kevin Beiler and Travis Dickson are among the individuals that provided support in the form of their friendships, and many ski trips revolving around passionate discussion of the world, science and the realities of graduate studies.

My research benefited from the contributions of scientists beyond the community at the University of British Columbia. Kurt Galbreath and Mary Peacock both contributed samples that were used to optimize the protocols used throughout my thesis. Mary Peacock and her lab at the University of Nevada, Reno also provided unpublished microsatellite markers that were used in my thesis. Mary Peacock and Chris Ray (University of Colorado, Boulder) also contributed by providing comments on a version of Chapter 2. Tweedsmuir Provincial Park staff are also thanked for their collaboration throughout all field seasons. Katy Hayhurst and Dennis Kuch are thanked for their hospitality in Stuix and for their help in discovering sea level pikas. Will Simpson of the U.S. Fish and Wildlife Service also provided comments that improved the manuscript that is presented in Appendix 1.

My work was funded by a variety of sources, including a NSERC discovery grant (# 341711-07) to Michael Russello and a UBC Okanagan Individual Research Grant (# F08-04551) to Michael Russello and myself. I was also supported in part by University Graduate Fellowships from UBC Okanagan campus as well as a doctoral fellowship from the Swiss National Science Foundation (# PBSKP3_128523).

I am especially thankful to my family for their endless love and support: My parents Pierre-Olivier and Brigitte Henry for inspiring me, from a young age to seek the wilderness, and enjoy the simple things that life has to offer. My siblings Linda, Carine and David Henry were part of my journey all along, and with them I share a childhood that led me to this moment and shaped my personality and ambitions. Lastly, I would like to thank my wife and my daughter, Alison and Shilo Henry for being constant reminders of the essential importance of maintaining a balanced and productive life.

Dedication

I would like to dedicate this thesis to my daughter Shilo : May you grow and learn and one day help influence your generation in building a society that will acquire and maintain a balance with nature.

Chapter 1. Introduction

1.1. Humans as drivers of biodiversity loss

Human societies are dominating ecosystems through their activities: habitat destruction and fragmentation, the alteration of biogeochemical cycles, the introduction of alien organisms and global climate change all adversely affect nature (Pimm and Raven, 2000; Vitousek, 1997). With individual's ecological footprints growing, these trends are on the rise (Dietz *et al.*, 2007; Pimentel and Pimentel, 2003). One of the more dire consequences of these anthropogenic changes is the decline and disappearance of species from their natural habitats. Besides the philosophical reasons that compel humans to care for their living kin (Wilson, 1984), ecosystems services [including air and water purification, pollination, timber, pharmaceuticals, and food] warrant the conservation of the diversity of life on Earth for the sake of human welfare (Costanza *et al.*, 1997; Daily, 1997).

There is now ample evidence that biodiversity has been negatively affected by anthropogenic activities (Venter *et al.*, 2006; Wilcove *et al.*, 1998). Some authors refer to this environmental crisis as "the last extinction" (Myer and Pimm, 2003), as an estimated 25% of known species are currently facing risks of extinction (Schipper *et al.*, 2008), and extinction rates are predicted to rise two to three orders of magnitude above their historical background levels (Novacek and Cleland, 2001). While numerous conservation efforts have been undertaken in an attempt to slow the rate of biodiversity loss, and have sometimes resulted in success stories (Butchart *et al.*, 2006; Rodrigues, 2006), the current trend is still towards a rapid loss of species (Hoffmann *et al.*,

2010). Thus, identifying and prioritizing species or populations for conservation action remains an integral part of effective measures to slow the rate of biodiversity loss (Brooks *et al.*, 2006).

1.2. Documented effects of global climate changes on biodiversity

According to the Intergovernmental Panel on Climate Change (IPCC 2007), the Earth has warmed on average 0.6°C in the last century as a result of increased greenhouse gases concentrations and is predicted to warm an additional 2 - 6°C by the end of the century. On the regional and local scales, patterns appear more complex, with uneven warming occurring from the poles to the equator, and high latitudes warming at faster rates (Walther *et al.*, 2002).

The most obvious impacts of global climate change are shifts in phenology, i.e. the timing of biological phenomena with respect to climatic conditions, such as flowering, breeding and migration. To date the most comprehensive meta-analysis compiling published data from 820 species of amphibians, birds, invertebrates, trees and shrubs and other plants found consistent and significant advance in vernal activities in all taxonomic groups examined, with a mean advance of 2.8 days per decade over the last century (Parmesan, 2007). Amphibians and butterflies responded twice and three times as fast respectively as other groups. Species at higher latitudes also tended to be more affected than species from lower latitudes (Parmesan, 2007). While most studies of phenology in vertebrates have largely focused on birds, some studies have documented change occurring in mammals. For example, a population of red squirrels (*Tamiasciurus hudsonicus*; Erxleben, 1777) from the Yukon advanced its breeding date by 3.7 days per decade over the last 50 years as a result of both plastic and genetic responses to climate warming (Reale *et al.*, 2003). Another study documented a 5-12 day advance in key reproductive parameters over

the past three decades in a wild population of red deer (*Cervus elaphus scoticus*; Linnaeus, 1758) in Scotland (Moyes *et al.*, 2011).

Another major response of species to climate change has been shifts in geographical range. Species of butterflies, birds and alpine plants have shifted their distributions on average 6 km northwards and 6 m upwards per decade over the last century (Parmesan and Yohe, 2003). Species distribution models have shown that shifting geographical ranges will result in species loss ranging from 10% to 50% depending on the species' dispersal abilities and the severity of forecasted climate change (Thomas *et al.*, 2004). While it is thought that most species will shift their geographical ranges in response to global climate change (Parmesan, 2006) rather than adapt *in situ*, factors such as habitat fragmentation will act synergistically to impair species' dispersal to more favorable conditions, thus exacerbating the loss of species.

In addition, species living at high altitudes and species with limited dispersal capacities will most likely be unable to track climate changes due to geographical and physical constraints. In this case, the species will either rely on adaptations to mitigate the adverse effects of global change, or if evolutionary constraints such as low standing genetic variation or mutation rates that are too low to cope with the rates of changes, the species will undergo local extinction (Hewitt and Nichols, 2005). Perhaps the most striking example of local extinction directly correlated with recent trends in climate comes from a small alpine mammal, the American pika (*Ochotona princeps*). Recent research involving long-term monitoring of some populations from the Great Basin, USA have documented an upward range retraction averaging 145 m per decade (Beever *et al.*, 2003; 2011), about 1.5 orders of magnitude larger than previous average estimates of upward movement of organisms in response to climate change (Parmesan and Yohe, 2003). Given the above, *O. princeps* may represent an ideal system in which to study the extent of local

adaptation and the relative importance of stochastic (genetic drift) versus deterministic (natural selection) genetic processes in shaping genetic variation within a climate change-sensitive species.

1.3. Conservation genetics

The field of conservation genetics arose in the early 1980s as a crisis discipline with the central tenet that small isolated populations will inherently suffer genetic erosion due to drift (i.e. the random change in allele frequencies over time, leading to stochastic fixation or loss of alleles) and inbreeding (Frankel and Soulé, 1981). Population genetic theory predicts that increased homozygosity caused by genetic drift and inbreeding will lead to an increased frequency of deleterious alleles, thus reducing the short-term viability of a population. Moreover, the loss of genetic variability is expected to have negative effects on the long-term viability of populations, resulting in a decreased evolutionary adaptive potential (Frankham, 2003).

Major contributions range from tests of the central tenet of conservation genetics, i.e. correlations between population size, genetic diversity, inbreeding and fitness related traits (e.g. Markert *et al.*, 2010; Reed and Frankham, 2003), to more practical conservation issues such as population genetic structure, demographic history and gene flow (e.g. Estes-Zumpf *et al.*, 2010; Henry *et al.*, 2009), species and individual identification (e.g. Haag *et al.*, 2009; Sloane *et al.*, 2000), molecular sexing (e.g. Fontanesi *et al.*, 2008), and *ex situ* conservation (e.g. Goncalves da Silva *et al.*, 2010). Noninvasive genetic sampling (e.g. Henry *et al.*, 2011; Taberlet *et al.*, 1999) has gained popularity among conservation geneticists as a means to obtain samples with a minimal impact on their species of interest. While most conservation genetics studies used a

limited number of genetic markers that were assumed to be neutral, such as microsatellites and mitochondrial DNA, advances in molecular techniques are now enabling researchers to assay a large number of genetic markers that are potentially under selection, opening new horizons for future research in conservation biology. Characterizing adaptive variation in populations of conservation concern will contribute to predictions on the potential effects of forecasted global climate change on sensitive species.

1.4. Conservation genomics

Neutral genetic markers, such as microsatellites, have been widely used to study population level patterns and processes in a myriad of organisms. Such studies have brought about a wealth of knowledge regarding population history and demography, and will continue to yield important insights to inform conservation management strategies. While it has previously been assumed that the amount of neutral genetic variation in a population is a surrogate for its evolutionary potential (Reed and Frankham, 2003), this assumption may be specious since natural selection likely affects only a small number of loci in the genome (Nielsen 2005). It is now possible to assess adaptive genetic diversity in wild populations by employing a population genomics approach (Luikart *et al.*, 2003). Population or conservation genomics entails the characterization of a large set of molecular markers, such as AFLPs (Amplified Fragment Length Polymorphisms) or SNPs (Single Nucleotide Polymorphisms) in a large number of individuals (Luikart *et al.* 2003). The basic premise of this approach is that only a small number of loci in the genome are affected by natural selection (locus-specific effects) as opposed to the genome-wide effects brought about by genetic drift and gene flow (Nielsen, 2005), resulting in a few loci with very

high genetic differentiation indices (termed outlier loci), and many loci with low to intermediate genetic differentiation indices consistent with neutral expectations (Nosil *et al.*, 2009). This idea was initially proposed by Lewontin and Krakauer (1973) and was further refined by Beaumont and Nichols (1996), Vitalis and colleagues (2003), Beaumont and Balding (2004) and Foll and Gaggiotti (2008). This statistical framework has been successfully applied to systems where adaptive genetic variation segregates into two divergent ecotypes (e.g. Campbell and Bernatchez, 2004; Kirk and Russello, Submitted) along altitudinal gradients (e.g. common frog; Bonin *et al.*, 2006) and in populations exposed to environmental pollutants (Williams and Oleksiak, 2008).

1.5. Study species

The American pika (Fig. 1.1) is a small lagomorph, discontinuously distributed in mountainous areas throughout western North America (Fig. 1.2). Pikas are largely restricted to talus slopes and broken rock debris in proximity to meadows that provide their food (Smith and Weston, 1990). *O. princeps* is found at higher elevations in the southern parts of its range, and may occur along a range of elevations (2500 - 3500 m; Grinnell, 1917). Yet, some exceptions have been noted, such as in the Columbia River Gorge, Oregon (Simpson, 2009), on the western slopes of the Cascades, Oregon (Manning and Hagar, 2011), Craters of the Moon National Monument and Preserve, Idaho (Rodhouse et al. 2010) and in Hays Canyon mountain range, northwestern Nevada (Beever *et al.* 2008). Some recent studies suggest that contemporary global warming may have recently led to the extirpation of 28% of pika populations surveyed in the Great Basin, USA (Beever *et al.*, 2003) and contributed to the displacement of the species 145 m upwards in elevation (Beever *et al.*, 2011). Other work suggests that global warming is not acting

in isolation from other anthropogenic factors such as grazing and transportation (Beever *et al.*, 2008b). Yet this may not be the case throughout the range of the pika as the latest study on the topic shows no such declines in populations from Wyoming, Colorado and New Mexico (Erb *et al.,* 2011). An interesting situation exists at the northwestern edge of the *O. princeps* distribution (Coastal Mountains of British Columbia, Canada), where the species occurs along an altitudinal gradient ranging from sea level to 1500 m. Such elevation gradients provide a rare system to study adaptation as specific environmental conditions (e.g. temperature) change rapidly over short distances (e.g. Bonin et al. 2006). In a spatial context, altitudinal gradients can thus be used as a surrogate for the expected temporal changes in selection pressures caused by global climate change (Davis and Shaw, 2001; Davis *et al.*, 2005; Reusch and Wood, 2007). Indeed, average temperatures along an altitudinal gradient at our study location typically show differences of up to six degrees Celsius from top to bottom (Henry *et al.*, Submitted-a; Appendix 1), thus mimicking the differences in temperature predicted by the most pessimistic scenarios of climate change for the end of this century (IPCC, 2007).

Figure 1.1. A photograph of an American pika (*Ochotona princeps*) taken at Heckman's pass at the top of the Bella Coola Valley, British Columbia. © Philippe Henry

1.5.1. Taxonomy and distribution

Pikas (*Ochotona* sp.) are lagomorphs of the Family Ochotonidae. They diverged from other members of their order (Leporidae, i.e. rabbits and hares) 37 million years ago, during the Oligocene (Smith, 2008). The genus *Ochotona* includes 30 extant species, which are mainly

found in the highlands of central Asia (Hoffmann and Smith, 2005; Niu *et al.*, 2004). While no taxonomic consensus has been reached to date, an accepted view is to further split the genus into three groups (Yu *et al.*, 2000): the northern pikas (subgenus *Pika*), the shrub-steppe pikas (subgenus *Ochotona*) and the mountain pikas (subgenus *Conothoa*). In North America, two species of pikas (subgenus *Pika*) inhabit rocky slides and talus slopes in mountainous regions: the Collared pika (*O. collaris*) which is distributed in the mountains of Alaska, the Northwest Territories, the Yukon and the northern tip of BC, and the American pika (*O. princeps*) which is discontinuously distributed in mountainous areas throughout western North America from central BC south to the Sierra Nevada in California and east to New Mexico, USA (Fig. 1.2). A gap of several hundred kilometers separates the ranges of *O. collaris* and *O. princeps*. Both species are monophyletic in comparison to Asian *Ochotona* species, and diverged from the Palearctic representatives of the subgenus *Pika* sometime between 4.7 to 15.7 million years ago (depending on the calibration of Ochotonidae-Leporidae split; Lanier and Olson, 2009).

Due to the discontinuous distribution of *O. princeps*, several subspecies have been described. A recent revision of the specie's taxonomy using evidence from morphology (skull morphometry, fur coloration and body size), molecular evidence (allozymes, mitochondrial DNA and nuclear DNA) as well as dialects of calls has resulted in a taxonomic consensus consisting of five subspecies (Fig.1.2; Hafner and Smith, 2010): *O. p. princeps* (Northern Rocky Mountains), *O. p. fenisex* (Coast Mountains and Cascade Range), *O. p. saxatilis* (Southern Rocky Mountains), *O. p. schisticeps* (Sierra Nevada and Great Basin), and *O. p. uinta* (Uinta Mountains and Wasatch Range of central Utah).

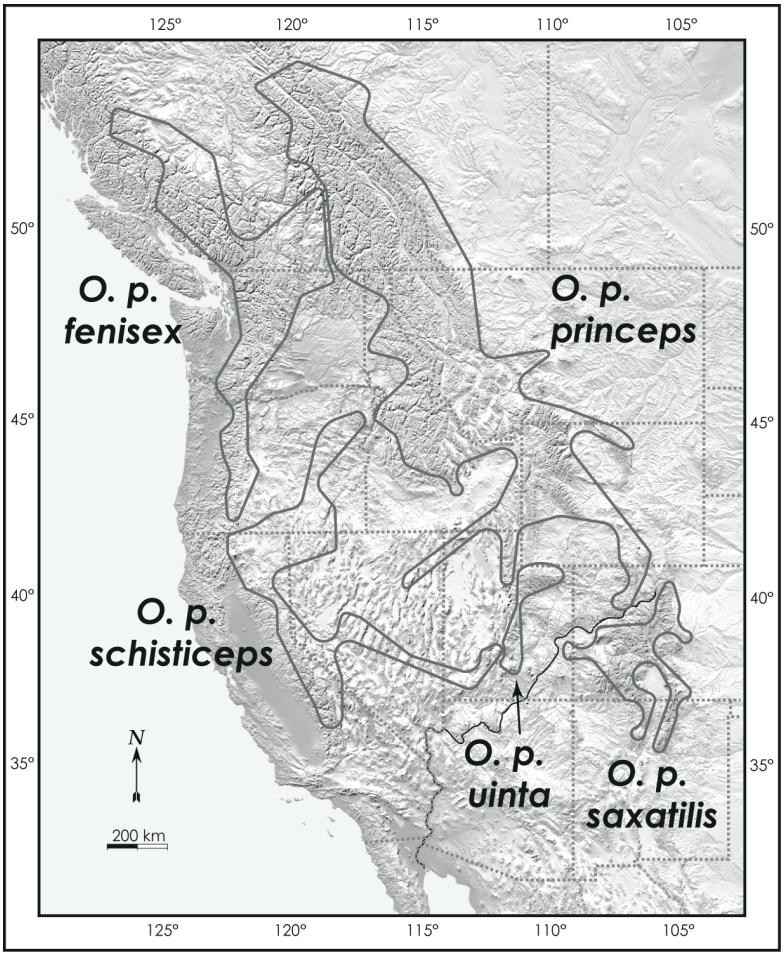


Figure 1.2. Geographic distribution of the five *Ochotona princeps* subspecies in western North America (Hafner and Smith, 2010; Reproduced with permission from David Hafner, University of New Mexico).

1.5.2. Natural History

O. princeps is a small (< 200g), diurnal inhabitant of talus and broken rock slopes in close proximity to meadows that provide their food. The dens of pikas are built in the interstices of the talus slopes that provide protection from predators and thermal stresses (Smith, 1974b). Pikas do not hibernate and their activities include grazing, defending territories and watching for predators. Haying, the active collection of plant material in haypiles for later consumption during

the winter months, becomes the predominant activity in late summer (Mckechnie *et al.*, 1994). Pikas defend individual territories by scent marking, vocalization and aggression (Smith and Ivins, 1986). They preferentially establish territories close to the talus-vegetation interface (Brandt, 1989) as it may limit their exposure to potential predators while feeding. Female pikas exhibit preferential mating with males who have territories close to the talus-vegetation interface and dens with multiple entrances (Brandt, 1983). Territories range from 600 to 3000 m^2, and population density is generally low (4-8 individuals per hectare). Individuals of different sexes tend to establish adjacent home ranges as it increases their chances of finding a mate and may reduce aggression between individuals of the same sex (Brown *et al.*, 1989). A first litter (typically three offspring) is initiated shortly before snow melt, generally in April and gestation lasts 30 days (Golian and Whitworth, 1985). Offspring are typically weaned within a month, after which juveniles disperse and establish their own territories. Females generally initiate a second litter shortly after the first litter is weaned, yet this second litter is rarely successful, unless the first litter is lost (Smith and Ivins, 1983a). Juveniles attain adult size within three months (Golian and Whitworth, 1985). They are often philopatric and tend to settle in the first available territories left vacant by over-winter adult mortality (Smith, 1978). After establishing territories, juveniles start haying and generally reproduce as yearlings the following season. Adult mortality per year is 37-45% and pikas are relatively long-lived, with an average lifespan of five to seven years (Smith and Weston, 1990).

1.5.3. Dispersal abilities

The obligatory habitat of pikas (e.g. talus adjacent to meadows) is scattered in the landscape

like islands in a matrix of inhospitable terrain. Although juveniles tend to settle on the first available territories, dispersal to other patches of habitat will occur when no vacant territories are available within the natal talus slope (Peacock, 1997). Successful dispersal of juveniles will thus depend on traveling across the matrix unharmed to settle in a neighboring or distant patch. Juveniles are faced by two main dangers while dispersing: predators and elevated temperatures. Regarding the latter, even short exposures to temperatures above 28°C has been documented to be fatal (Smith and Weston, 1990). In studies conducted in the Californian Sierra Mountains, Smith (1974a; b) found dispersal distances > 300 m to be rare at low elevations, while individuals at higher elevation or cooler latitude may be able to disperse up to 10 km (Hafner and Sullivan, 1995). Although mortality during dispersal is thought to be high (Smith and Ivins, 1983b), gene-flow has been shown to occur between patches of habitat separated by 2-10 km (Peacock, 1997). However, a recent consensus adopted by pika researchers is to consider the approximate upper limit of dispersal of American pikas to be 3 km (Beever *et al.*, 2008; 2010; C. Ray, personal communication).

1.5.4. Susceptibility to climate change

During the late Pleistocene (c. 30,000 years before present), *O. princeps* displayed a more widespread distribution than that observed today, and could be found both at lower elevations and in more easterly locations in North America (Hafner, 1993). The current distribution of Pikas in mountainous areas of western North America is presumably the direct consequence of warming temperatures after the end of the last Ice age (c. 10,000 BP), that led to an upwards movement of populations of 800 m in elevation (Grayson, 2005). Contemporary global warming

is an important factor driving the decline of pikas in the Great Basin, USA, where 28% of surveyed populations have gone extinct during the 20th century alone (Beever *et al.*, 2003), and this trend has increased in severity in the last decade, as a new study revealed a five-fold increase in the rate of extinction of pikas across the Great Basin (Beever *et al.*, 2011).

While *O. princeps* may become increasingly threatened by increasing temperatures, American pikas are listed as a species of least concern by the International Union for the Conservation of Nature (Beever and Smith, 2008), and a recent status review by the US Fish and Wildlife Services concluded that the species did not warrant listing under the Endangered Species Act (Ray *et al.*, 2010).

1.6. Objectives

I used the continuous distribution of pika populations along altitudinal gradients in the Bella Coola valley as a surrogate for forecasted global climate change. Conservation genetic and genomic approaches were used to shed light on demographic and evolutionary processes occurring in pika populations sampled across altitudinal gradients ranging from sea level to 1500m. Specifically, this was explored in the three following chapters:

- In Chapter 2, I describe a novel noninvasive method to collect pika hair samples. While most conservation genetic studies rely on live trapping to acquire samples from wild species, my approach ensures a minimal impact on the species of interest, thus providing a safer and more ethical way to collect samples from pika populations.

- In Chapter 3, I characterize the amount and distribution of neutral genetic variation present within and between sampled populations. Analyses are directed at identifying the underlying population genetic structure and population demography to inform on the potential of dispersal as a mitigation strategy.

- In Chapter 4, I use a genomic scan approach to identify genomic regions under selection in the populations studied. Furthermore, I aim to identify the major environmental factors driving adaptive change in this system. This approach represents a stepping-stone towards discovering the genes underlying ecologically important traits in this system.

Chapter 2. Obtaining high quality DNA from elusive small mammals using low-tech hair snares

2.1. Overview

Molecular approaches have become common to ecological and evolutionary studies of wildlife populations. DNA-based tools have contributed significantly to our understanding of wildlife biology, with advances ranging from identifying cryptic species (Gomez *et al.*, 2007; Russello *et al.*, 2005) to discovering genes responsible for adaptation in heterogeneous environments (Nachman, 2005). Traditionally, sampling involved live-trapping and non-destructive collection of tissue or blood from wild animals, followed by DNA extraction and downstream Polymerase Chain Reaction (PCR; Mullis *et al.*, 1986) amplification of informative DNA fragments (mitochondrial DNA or nuclear microsatellite markers; DeYoung and Honeycutt, 2005; Sunnucks, 2000). Concomitant advances in analytical methods and development of user-friendly software (Excoffier and Heckel, 2006) have further led to the expansion and application of DNA-based studies to the fields of ecology, evolution and conservation (DeSalle and Amato, 2004).

Noninvasive genetic sampling (NGS; Taberlet *et al.*, 1999) has gained in popularity as an alternative to traditional live-trapping sampling techniques for several reasons. First, by unobtrusively collecting biological material (e.g., feces, hairs, feathers, saliva and mucus) from wild populations, researchers can study these species without disturbing, handling, or even observing them, thus reducing risks to both animals and researchers. Second, NGS enables biologists to study populations of elusive and rare species, a task that can prove difficult with

more traditional live-trapping approaches (Piggott and Taylor, 2003; and references therein). And third, NGS can potentially increase sample sizes by reducing disturbance to animals, sampling efforts, and costs, thus helping to minimise biases in estimates of population parameters (Banks *et al.*, 2003; Litvaitis *et al.*, 2006). This latter point may prove crucial when dealing with threatened species, since biased estimates of population parameters may result in inappropriate management.

NGS has been applied to identify species (e.g. Rudnick *et al.*, 2007), identify sexes and individuals (e.g. Hedmark *et al.*, 2004; Russello and Amato, 2001; Sastre *et al.*, 2009), estimate population structure and gene flow (e.g. Henry *et al.*, 2009; Regnaut *et al.*, 2006), and monitor populations (e.g. Kendall *et al.*, 2009; Paetkau, 2003; Woods *et al.*, 1999). Despite the advantages of NGS, there are important caveats associated with this approach including poorer quality and quantity of recovered DNA, lower PCR success rates, greater potential for contamination, and enhanced rates of genotyping errors compared to those observed when using traditional tissue or blood samples. While beyond the scope of this chapter, these limitations are well understood and have been addressed by others (e.g. Beja-Pereira *et al.*, 2009; Broquet and Petit, 2004; Piggott *et al.*, 2004; Taberlet *et al.*, 1996; Waits and Paetkau, 2005).

Many innovative approaches for sampling hair have been developed, but these have mainly targeted medium-sized or large mammals (e.g. Amendola-Pimenta *et al.*, 2009; Bremner-Harrison *et al.*, 2006; Kendall and McKelvey, 2008; Mullins *et al.*, In Press; Pauli *et al.*, 2008; Toth, 2008). At present, we are unaware of a noninvasive hair sampling method that specifically targets small mammals. To fill this gap, we describe a novel and inexpensive method to obtain hair from the American pika (*Ochotona princeps*), an elusive and climate sensitive lagomorph from western North America. We evaluated the quality and quantity of DNA obtained from two

different amounts of starting material using four commercially available DNA isolation kits. We further quantified PCR amplification success of resulting DNA extracts across a series of mitochondrial DNA fragments and nuclear microsatellites of varying length. Amplification success was also evaluated for fragments of the *ZFX/ZFY* genes used for molecular sexing.

2.2. Materials and methods

2.2.1. Study species

The American pika (*Ochotona princeps*) is a small lagomorph, discontinuously distributed in mountainous areas throughout western North America from central British Columbia (BC), Canada, south to the Sierra Nevada in California and east to New Mexico, USA. Pikas are restricted to talus slopes and broken rock debris in proximity of meadows that provide their food (Smith and Weston, 1990). Recent extirpations of pika populations in the Great Basin, USA have been attributed, in part, to increasing temperatures (Beever *et al.*, 2003). Pikas are individually territorial and concentrate their daily activities on the collection of plant material stored in hay-piles at the center of their territories. Pikas are elusive animals that emit alarm calls and rapidly retreat underground upon intrusion of their territories by predators. Live-trapping pikas can be resource intensive, with previous studies reporting an effort of over 12 hours/trapped animal (Peacock, 1997). In addition, trapping is stressful for the pikas. Thus, noninvasive genetic sampling has the potential to improve sampling efficiency while minimising stress levels inflicted by live-trapping and handling.

2.2.2. Study site

This study was carried out 3 km southwest of Heckman's Pass at the top of the Bella Coola Valley, British Columbia, Canada (N52°31'01.0'' W125°49'36.7''; altitude 1500 meters above sea level). The area was surveyed on the 24th of June 2009, and 12 putative pika territories were identified over an area of approximately 6 hectares. We targeted pika travel routes by setting up snares (described below) at hay-piles and latrine sites in each of the 12 individuals' territories. Snares were set up on the 24th and 25th of June 2009, and hairs were collected daily from June 25th to the 31st.

2.2.3. Hair snares

Each snare consisted of a scaffold of fishing line wrapped around a boulder approximately 30 cm above the ground. Pieces of clear packing tape (3M, St. Paul, MN, USA) ranging from 10 to 50 cm long were rolled up to provide a 360° sticky surface. The pieces of packing tape were then folded around the fishing line and stuck to each other in a zigzag fashion to cover a maximum of the space from the ground up (Fig. 2.1A). The operation of setting up a hair snare usually takes about 30 minutes per site. Pikas travelling in and out of their hay-piles or latrine sites brush up against the sticky surface of the snares, resulting in hairs being stuck to the tape (Fig. 2.1B). Pikas are known to intrude into neighboring territories, potentially leading to the sampling of multiple individuals in a single hair snare. Yet, when a pika runs through a hair snare, the piece of tape was entirely covered by hair at the impact location. Thus, it is very likely that hair collected from a single impact location belongs to a single individual.

The collected hair samples were removed from the sticky tape using sterile forceps and stored in 2 ml cryogenic tubes (Eppendorf AG, Hamburg Germany) in a liquid nitrogen-filled dry shipper (Thermo Scientific, Waltham, MA, USA) until reaching to the lab. Samples were then transferred to an -80°C freezer until DNA isolation.

Figure 2.1. Photograph of a hair snare used to noninvasively sample pikas in the Bella Coola Valley, BC. A) General configuration, including a fishing line scaffold and rolled up clear packing tape arranged in a zigzag fashion around an individual haypile. B) Successful hair snare containing a large number of plucked hairs at a single impact location on the tape.

2.2.4. DNA extractions and PCR amplifications

In order to assess the optimal amount of starting material to obtain DNA extracts suitable for downstream PCR amplification of nuclear and mitochondrial markers, we used a factorial design of two amounts of hair using 4 extraction methods. We used hair sampled from four individuals and separated the hair from each of the four individuals into two tubes, one containing a single hair and another containing 25 hairs. DNA was then extracted for each sample using one of four commercially available DNA isolation kits: A) DNA IQ™ Tissue and Hair Extraction Kit (Promega, Madison, WI, USA); B) Nucleospin® Tissue kit (Macherey-Nagel, Düren, Germany); C) DNeasy® Blood and Tissue kit (Qiagen, Valencia, CA, USA) and, D) Chelex® 100 resin (Bio-Rad, Hercules, CA, USA). The DNA IQ™ system uses DNA binding paramagnetic resin to isolate a set amount of DNA. We followed the manufacturer's protocol for DNA extraction from hair with DNA being eluted in 100 μl of elution buffer. The Nucleospin® and DNeasy® columns harness the DNA binding capacities of silica-based membranes. We followed the manufacturers' protocol for hair extractions and samples were eluted in 100 μl of elution buffer. Chelex® is an ion exchange resin that acts as a chelating agent to bind polyvalent ions, especially magnesium, thus inactivating nucleases. DNA present in the starting material remains in solution and can be used for PCR. Chelex® extractions were performed according to the forensics grade protocol for hair developed by the National Forensic Science Technology Cen ter (http://static.dna.gov/labmanual/DNA_Lab_Manual.zip). In all cases, water was used as a negative control and 20 mg of liver from four different individuals were used as positive controls and a basis for comparison with hair extractions. DNA was quantified using a NanoDrop® ND-1000 Spectrophotometer (Thermo Scientific, Waltham, MA, USA). DNA quality was assessed by 260 nm and 280 nm absorbance ratio (A260/A280 ratio; Manchester, 1995).

Isolated DNA (1 and 25 hairs as well as a negative and positive control for each extraction method) was used as template for a series of PCR reactions to amplify nuclear and mitochondrial DNA fragments of varying size. Specifically, we designed species-specific primers to amplify mitochondrial cytochrome b fragments of 250 and 800 basepairs (bp; Appendix 3; Supplementary material Table A3.1) and used one primer previously described for the genus *Ochotona* (Yu *et al.,* (2000). Likewise, nuclear DNA amplification was tested at two microsatellite loci previously described for the American pika, Ocp8 (250 bp) and Ocp2 (400 bp) (Peacock *et al.*, 2002). PCRs were performed using a Veriti® thermal cycler (Applied Biosystems, Foster City, CA, USA) in a 25 µL volume containing: 1 - 20 ng DNA, 0.5 µM of each primer, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM dNTPs, 10 µg bovine serum albumin (BSA; New England Biolabs, Ipswich, MA, USA) and 0.5 U AmpliTaq Gold® DNA polymerase (Applied Biosystems Foster City, CA, USA). Cycling parameters for the microsatellite loci were optimised using a touchdown cycling program (10 min at 95°C, 35 cycles at 95°C for 30 s, 30 s annealing, and 45 s at 72°C, followed by a final step at 72°C for 10 min). The annealing temperature decreased by 1°C per cycle from 60 to 55°C until reaching the sixth cycle, at which point the 29 remaining cycles continued at 55°C. Cycling parameters for the mitochondrial fragments were as described above but consisted of 35 cycles with an annealing temperature of 50°C. Each PCR was repeated three times to check for consistency in amplification success. PCR products were then run alongside a 100 bp ladder (New England Biolabs, Ipswich, MA, USA) on a 1.5% agarose gel containing 2.5% SYBR® Safe DNA gel stain (Invitrogen, Carlsbad, CA, USA) and visualised using a RED® personal gel imaging system (Alpha Innotech, San Leandro, CA, USA).

Lastly, molecular sexing was undertaken using the PCR-RFLP of *ZFX/ZFY* loci with *Hinf*I restriction enzyme digestion according to Fontanesi *et al.,* (2008). This approach relies on the observation that the Y chromosome possesses a *Hinf*I restriction site in this region that the X chromosome lacks. Thus females produce 2 co-migrating fragments of 432 bp while males produce fragments of 432 bp, 261 bp and 171 bp. Products of the PCR-RFLP sexing were then run on a 3% agarose gel as described above.

2.3. Results

2.3.1. Hair snare success

We found pika hair in eight of 12 hair snares (67% success) yielding between one and four impact patches ranging from 10 to over 100 hairs (Fig. 2.1B). Each patch was considered to come from a single individual. Additionally, we checked snares daily and sampled collected hairs in order to decrease the probability of collecting hairs from multiple individuals in the same tube. We also found hair in one of the twelve snares that was identified in the field as belonging to a species other than pikas. It was later confirmed in the lab that these hairs yielded a 100% mtDNA sequence similarity (12s rDNA and 16S rDNA (data not shown) to the American Red Squirrel (*Tamiasciurus hudsonicus*), another small mammal that is sympatric with *O. princeps* in BC.

2.3.2. DNA extractions and PCR amplifications

The various DNA extraction techniques yielded widely different levels of DNA purity and quantity (Table 2.1). Mean DNA concentrations (± Standard Error) were 4.47 ng/µl (± 2.24)

across all DNA isolation methods using a single hair, 86.7 ng/µl (± 43.3) for extractions using 25 hairs, and 1307 ng/µl (± 653) for extractions using liver. As a general trend, samples extracted using the DNA IQ™ system had a rather constant concentration of DNA across different quantities of starting material (which was expected given the finite binding capacity of the resin) as opposed to that observed using the other three methods, where positive controls differed by two to three orders of magnitude (Table 2.1). DNA purity was similar for DNA IQ™, Nucleospin® and DNeasy® with mean A260/A280 ratios of 1.48, 1.30 and 1.42 respectively. Chelex®produced a mean 260/280 ratio of 0.89, which is substantially lower than the metric expected for 'pure' DNA (i.e. 260/280 = 1.8).

PCR amplification success varied across both amounts of starting material and molecular marker type and size, but was correlated to the purity and quantity of template DNA. In general the use of multiple hairs in a DNA extraction increased the amplification success. With regard to the choice of DNA isolation method, the best amplification success was obtained with using the DNA IQ™ with 100% amplification success over three consecutive repetitions of PCRs for each marker class used (Table 2.1). This step was followed by the DNA extraction using 25 hairs and the DNeasy® kit with 83.3% of success in amplification of microsatellite loci, 100% of mitochondrial DNA fragments, but without any success at amplifying the sex-linked gene. PCR amplification success was assessed using band presence and intensity on an agarose gel. Although DNA IQ™ yielded greater quantities of amplification products, the other methods used here (except Chelex®) also produced scorable genotypic data when run on a capillary DNA Analyser (Table 2.1; 16.7% of extractions using Nucleospin® kits also produced a genotype on a capillary assay while no apparent bands were detected on the agarose gel).

Table 2.1. DNA concentration, purity and amplification success for each of four DNA isolation methods across two different amounts of hair and a positive control (liver)

DNA Extraction Method	Cost per extraction (€) [a]	Tissue	Amount of Starting Material	Concentration (ng/µl)	Absorbance Ratio (260/280)	Amplification success for Microsatellites (%) [b]	Amplification success for mtDNA (%) [b]	Amplification success for ZFX/ZFY (%) [b]
DNA IQ™ Tissue and	3.4	Hair	1	10.2	1.29	33.3	100	0
Hair Extraction Kit		Hair	25	183	1.63	100	100	100
		Liver	20 mg	184	1.53	100	100	100
Nucleospin® Tissue	1.4	Hair	1	0.5	1.11	0	66.7	0
Kit		Hair	25	4.9	1.07	0	83.3	0
		Liver	20 mg	412	1.71	100	100	100
DNeasy® Blood and	1.7	Hair	1	1.1	0.89	0	50	0
Tissue Kit		Hair	25	1.8	1.38	83.3	100	0
		Liver	20 mg	406	1.98	100	100	100
Chelex®100 Resin	0.2	Hair	1	2.8	0.49	0	33.3	0
		Hair	25	100	0.84	0	33.3	0
		Liver	20 mg	2939	1.35	0	33.3	0

[a] Calculated for a single extraction based on manufacturer's list price

[b] Over three PCR repetitions

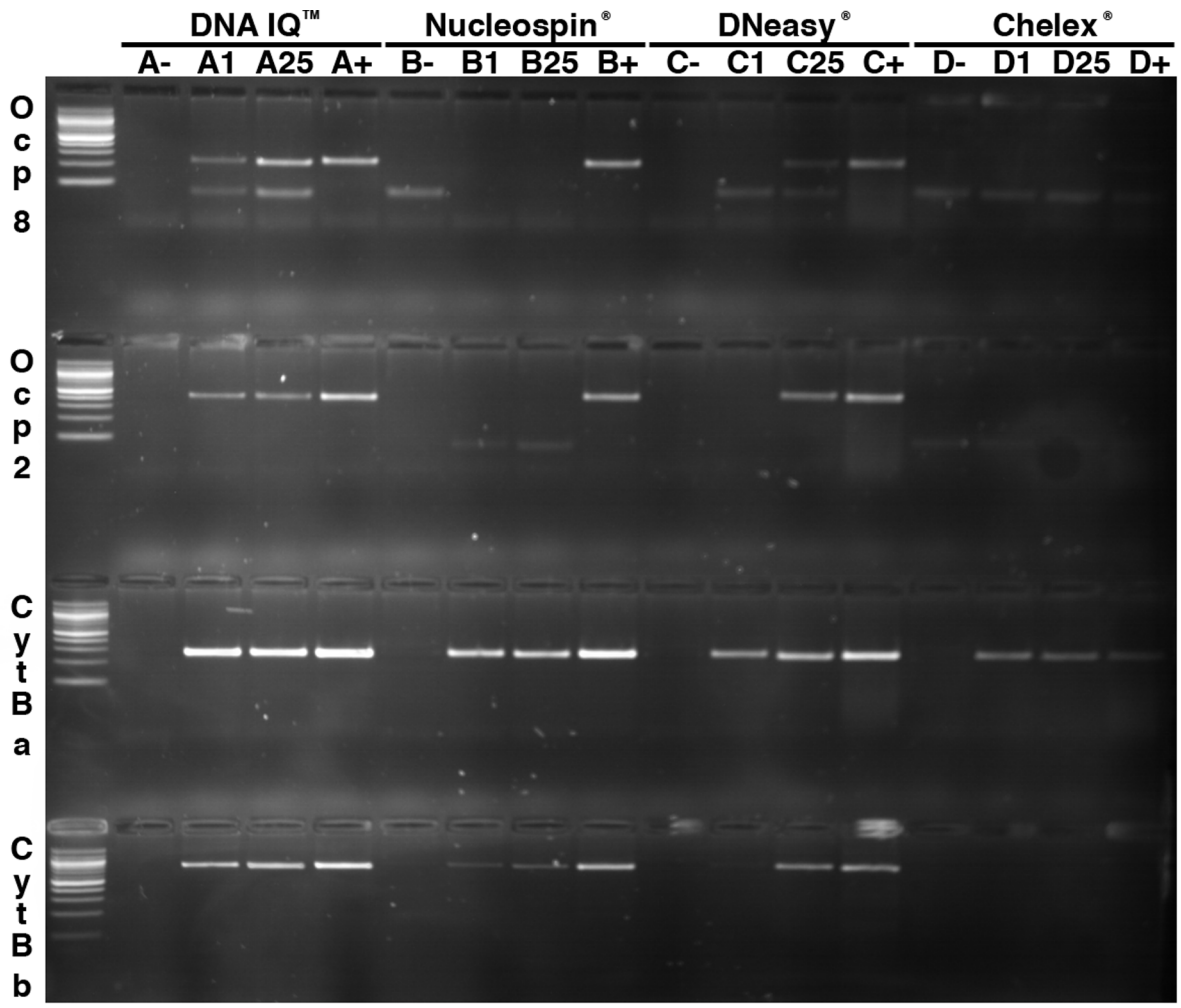


Figure 2.2. A representative gel pattern showing the specific amplifications of two nuclear microsatellite markers [Ocp8 (~250 bp) and Ocp2 (~400 bp); top rows] as well as two mitochondrial Cytochrome b fragments [Cyt B a (~250 bp) and Cyt B b (~800 bp)] for pika DNA extracted using four different commercially available techniques: A) DNA IQ™ Tissue and Hair Extraction Kit (Promega), B) Nucleospin® Tissue kit (Macherey-Nagel), C) DNeasy® Blood and Tissue kit (Qiagen) and D) Chelex® 100 resin (Bio-Rad) and two different quantities of starting material: 1 and 25 hairs as well as a positive control (+; 20 mg of liver) and a negative control (-, water). The first lane is 100 bp ladder (New England Biolabs).

With regard to marker choice and sizes, the two nuclear microsatellites (Ocp 2, Ocp 8) of varying size successfully amplified across both quantities of starting material for the DNA IQ™ (A1, A25) and for 25 hairs using the DNeasy® kit (C25). No microsatellite amplification was observed for the Nucleospin® or Chelex® extractions (Fig. 2.2). Overall, mitochondrial cytochrome b fragments amplified better than nuclear microsatellite loci with 100% amplification success of the smaller 250 bp mitochondrial DNA fragment across all extraction methods and quantities of starting material. Only the Chelex® hair extractions failed to amplify the larger 800 bp mitochondrial DNA fragment (Fig. 2.2). Lastly, only the DNA extracted using 25 hairs as starting material and the DNA IQ™ system produced successful PCR-RFLP of the *ZFX/ZFY* loci to enable sex determination of pikas (Fig. 2.3).

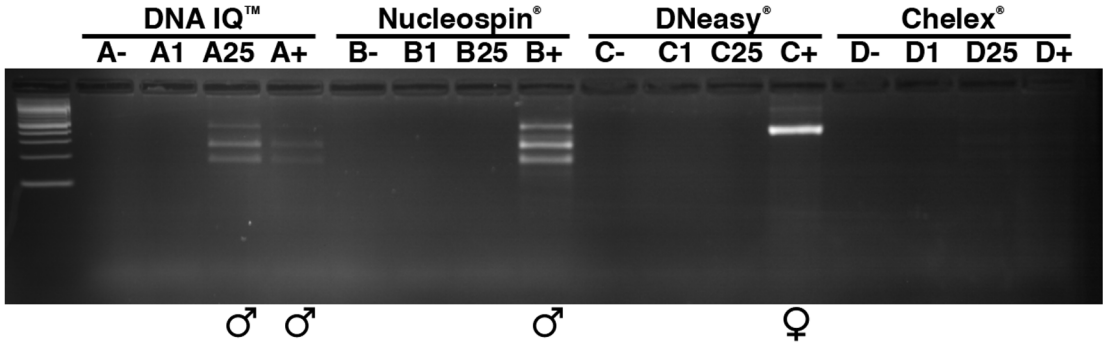


Figure 2.3. A representative gel pattern showing the PCR-RFLP of *ZFX/ZFY* loci with *Hinf*I restriction enzyme following Fontanesi *et al.* (2008) for DNA extracted using four different commercially available techniques: A) DNA IQ™ Tissue and Hair Extraction Kit (Promega), B) Nucleospin® Tissue kit (Macherey-Nagel), C) DNeasy® Blood and Tissue kit (Qiagen) and D) Chelex® 100 resin (Bio-Rad) and two different quantities of starting material: 1 and 25 hairs as well as a positive control (+; 20 mg of liver) and a negative control (-; water). The first lane is 100 bp ladder (New England Biolabs).

2.4. Discussion

2.4.1. Hair snare success

While live-trapping enables non-destructive collection of DNA of high quality and quantity, trapping efficiencies below 50% are commonly reported for small mammals (Connior and Risch, 2009). This capture frequency is likely much lower when dealing with elusive animals such as *O. princeps* (C. Ray, personal observation). Our noninvasive hair snares allowed us to efficiently sample eight pikas over a seven-day period. The four unsuccessful traps possibly reflect vacant territories in the pika population we surveyed. Additional studies comparing both traditional and noninvasive trapping techniques are required to compare trapping efficiencies and to estimate capture probabilities for each method.

2.4.2. DNA extractions and PCR amplifications

Three of the four DNA extraction methods we tested reliably extracted high quality DNA from pika hair (DNA IQ™, Nucleospin®, and DNeasy®). It is not clear why hair samples extracted using Chelex® did not yield successful amplification for the nuclear microsatellite loci, since Chelex® has been classically employed in forensic studies and routinely used to extract DNA from hair collected from wildlife (Mitrovski *et al.*, 2005). Although DNA concentrations were high, it is possible that the Chelex® extractions contained more impurities than the other methods that employed several washing steps, which is consistent with the low 260/280 ratios observed. These impurities may have inhibited the PCR reactions, yet sequential dilutions of the Chelex® extract did not provide better results than the straight extracts (data not shown). Additional optimization of the Chelex® protocol may be required in order to match the success obtained in

other studies using mammalian hair as a starting material. Moreover, pika hairs differ from forensic material such as human hair since the root bulbs are substantially smaller, likely containing only a fraction of the nucleated cells found in human hair (P. Henry, personal observation). Additionally, Chelex® resin may inhibit PCR reactions (Willard *et al.*, 1998).

The length of the amplicons proved to be an important factor in PCR amplification success, with smaller nuclear and mitochondrial fragments being recovered more frequently than larger ones, corroborating similar observations in other species (e.g. Morin *et al.*, 2001). In order to target nuclear markers, researchers should try to remove as much of the hair shaft as possible, as nuclear DNA is found primarily in the root bulb (Beja-Pereira *et al.*, 2009). This operation was not undertaken here since we targeted both nuclear and mitochondrial DNA. Conditions under which hair samples are collected and stored may also have direct implications for DNA amplification success. We collected hair daily to minimise exposure to the elements. Storing samples at -20°C to -80°C and minimising the time between hair collection and DNA extractions may substantially increase amplification success, especially with regards to larger amplicons (Roon *et al.*, 2003).

2.5. Summary

Overall, our results suggest that the novel tape snares described here are effective at obtaining adequate amounts of hair that yield DNA of sufficient quality and quantity for PCR. We anticipate that this approach could provide an effective way to collect samples for downstream population genetic analyses of elusive small mammals. For pikas, this method may also enable sampling of juvenile individuals and estimation of the frequency with which pikas

intrude into neighbouring territories, a task that until now required hours of observation. We found the greatest success in amplifying fragments of both mitochondrial DNA (~800 bp), nuclear microsatellites (~400 bp) and *ZFX/ZFY* loci (432 bp) when using 25 hairs as starting material and the DNA IQ™ system. These results may vary depending upon species and study conditions, but offer a cost-effective [estimated cost of € 2.00 per trap and € 3.4 per DNA extraction (manufacturer's list price at www.promega.com)] and noninvasive approach for collecting population genetic data to inform studies of small mammal ecology, evolution and conservation.

Chapter 3. Genetic evidence for highly restricted dispersal along continuous altitudinal gradients in the climate change-sensitive American pika (*Ochotona princeps*)

3.1. Overview

Anthropogenic activities are having an unprecedented impact on wild populations. Perhaps one of the largest challenges facing wildlife will be coping with climate change (Reusch and Wood, 2007). Altered selection regimes caused by increasing air and water temperatures will operate in the near future (IPCC, 2007), however, some particularly sensitive species have already become extirpated due to contemporary temperature changes (Beever *et al.*, 2011). When faced with rapidly changing environmental conditions, species must adapt, disperse or disappear (Lovejoy and Hannah, 2005). While it has been suggested that most species will shift their geographical ranges rather than adapt *in situ* (Parmesan, 2006), factors such as habitat fragmentation may act synergistically to impair species' dispersal to more favorable conditions (Stockwell *et al.*, 2003). Range shifts may be particularly challenging for species with limited dispersal capacity or those with highly specialized habitat requirements (Sgro *et al.*, 2011). Consequently, there is value in investigating the connectivity of populations of species inhabiting markedly different environments in order to evaluate dispersal as a potential strategy for persistence in the face of predicted climate change.

Species that have successfully established populations along a continuous range of elevations may represent ideal systems in which to study the synergistic effects of genetic drift, inbreeding and natural selection on the extent and distribution of genetic variation as environmental

conditions (e.g. temperature) change rapidly over short distances (Gebremedhin *et al.*, 2009). In a spatial context, altitudinal gradients can thus be used as surrogates for the expected temporal changes in selection pressures caused by climate change (Reusch and Wood, 2007).

The American pika (*Ochotona princeps*) is a small lagomorph discontinuously distributed in mountainous areas throughout western North America from central British Columbia and Alberta, Canada, south to the Sierra Nevada in California and east to New Mexico, USA. Pikas are restricted to talus slopes and broken rock debris in proximity to meadows that provide their food (Smith and Weston, 1990). The fragmented nature of their habitats has propelled *O. princeps* to the position of a model mammalian species for studies of metapopulation dynamics, island biogeography and source-sink dynamics (e.g. Peacock and Smith, 1997a). In recent years, pikas have also gained notoriety as a model system for testing extinction dynamics in the face of climate change (Beever *et al.*, 2011). In that vein, pikas are considered harbingers of global warming, predicted by some to constitute the first mammalian species to go extinct due to the direct effects of climate change (Smith *et al.*, 2004).

Recent studies have examined American pika phylogeography in the context of climate change since the late Pleistocene (Galbreath *et al.*, 2010). Additionally, DNA fingerprinting has been applied to shed light on mating behaviour and dispersal in this species (Peacock and Smith, 1997a; b). Given the above, *O. princeps* may represent an ideal system in which to study the relative importance of stochastic (genetic drift) versus deterministic (natural selection) genetic processes in shaping genetic variation within a climate change-sensitive species.

In the present study, I begin to address these questions by sampling *O. princeps* populations found along three elevation gradients in the central Coast Mountains of British Columbia,

Canada, ranging from sea level to greater than 1500 m. Specific objectives include: 1) investigating the extent and distribution of genetic variation within and among populations; 2) quantifying gene flow among populations along continuous, altitudinally distributed transects as well as disjunctly among transects; 3) reconstructing population demographic histories; and 4) evaluating the degree to which patterns of genetic variation correlate with observed environmental variation.

3.2. Materials and methods

3.2.1. Study Site

This study was carried out in the Bella Coola Valley, BC (Fig. 3.1). This area was initially chosen because historical records show the presence of *O. princeps* from sea level to tree line and the presence of an extensive network of roads connecting the bottom of the valley to higher elevations. American pikas were sampled from August 2008 to September 2010 at 10 sites along three elevation gradients ranging from sea level to 1500 m using recently developed noninvasive hair snares (Fig. 3.1) (Henry *et al.*, 2011; Henry and Russello, 2011). Pikas at site *A1* were extirpated in the late summer of 2009 due to a forest fire that burned through the area, but the site was recolonized as of summer 2011 (M.A. Russello, personal communication). Samples were collected in sites *B*, *C*, *D* and *E* in 2008, 2009 and 2010, from site *A1* in 2008 and 2009, and from sites *A2, F, G, H and I* in 2010. A total of 288 geo-referenced individual hair snares were set up across these ten sites (Fig. 3.1) and samples were brought back to the laboratory for subsequent DNA extraction and PCR amplification.

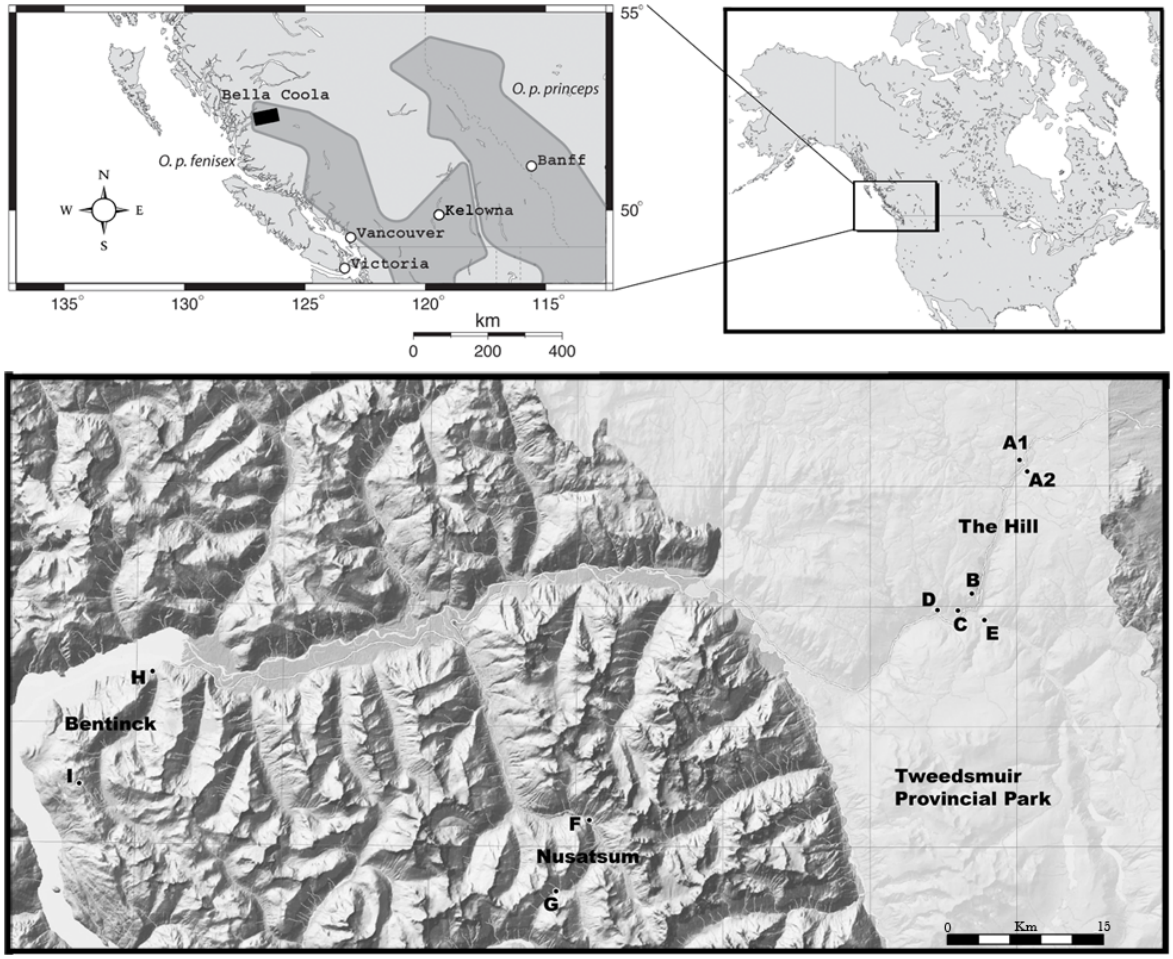


Figure 3.1. Map of the study area in the Bella Coola Valley, British Columbia, Canada, with the ten sampling sites located along three altitudinal gradients: the Hill, Nusatsum and Bentinck from east to west.

3.2.2. DNA Isolation, PCR Amplification and Genotyping

I previously determined that DNA extracted from 15-20 hairs per sample yielded DNA of sufficient quality and quantity for downstream assays (Henry and Russello, 2011). Given these findings, I isolated DNA from 206 individual samples with sufficient starting material. DNA was

extracted using the DNA IQ™ Tissue and Hair Extraction Kit (Promega, Madison, WI, USA) and a modified version of the manufacturer's protocol as demonstrated in Henry *et al.* (2011).

Isolated DNA was used as template for PCR amplification of 28 nuclear microsatellite loci previously described for the American pika (Appendix 3; Supporting material Table A3.2; Peacock *et al.*, 2002; Peacock *et al.*, unpublished). Initially I used a representative sample of 16 individuals distributed throughout our study area to assess amplification success and polymorphism of all microsatellite loci (Appendix 3; Supplementary material Table A3.2). Based on results from the pilot analysis, ten polymorphic loci were retained and screened on the remainder of the sampled individuals (see Results). All PCRs were performed using a Veriti® thermal cycler (Applied Biosystems, Foster City, CA, USA) in a 12.5 µL volume containing: 1 - 20 ng DNA, 0.5 µM of labelled M13, 0.5 µM reverse primer and 0.05 µM forward primer, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM dNTPs, 10 µg bovine serum albumin (BSA; New England Biolabs, Ipswich, MA, USA) and 0.5 U AmpliTaq Gold® DNA polymerase (Applied Biosystems). All forward primers were 5'-tailed with an M13 sequence [5'-TCCCAGTCACGA-CGT -3'] to facilitate automated genotyping. Specifically, the M13-tailed forward primer was used in combination with an M13 primer of the same sequence 5'-labeled with one of four fluorescent dyes (6-FAM, VIC, NED, PET; Applied Biosystems; Appendix 3; Supplementary material Table A3.2), effectively incorporating the fluorescent label into the resulting PCR amplicon (Schuelke, 2000).

Cycling parameters were optimised using a touchdown cycling program (10 min at 95°C, 40 cycles at 95°C for 30 s, 30 s annealing, and 45 s at 72°C, followed by a final step at 72°C for 10 min; Appendix 3; Supplementary material Table A3.2). The annealing temperature decreased by 1°C per cycle from 60 to 55°C until reaching the sixth cycle, at which point the 34 remaining

cycles continued at 55°C. Two of the markers produced stuttering using this touch down protocol and thus were amplified using a program that consisted of the same PCR cycling conditions except the annealing temperature was kept constant at 50°C for 40 cycles (Appendix 3; Supplementary material Table A3.2). PCR products were multiloaded and run on an ABI 3130XL genetic analyser (Applied Biosystems) with GeneScan™ 500 LIZ® size standard and genotypes were called using GENEMAPPER 4.0 (Applied Biosystems). Two people (P. Henry and Z. Sim) called alleles independently and incongruent calls were repeated from the PCR stage.

3.2.3. Quality Control and Genetic Variation

Since our sampling strategy involved the use of a non-conventional DNA source, I applied a multi-tube approach in which each PCR was repeated at least twice for heterozygote genotypes and at least three times in order to confirm homozygotes. Genotyping errors were quantified and consensus genotypes were obtained using PEDANT 1.0 (Johnson and Haydon, 2007). Additional tests for stuttering and allelic dropout were undertaken using MICROCHECKER 2.2.3 (Van Oosterhout *et al.*, 2004). Incidence of repeated genotypes were identified using GENALEX 6.4.1 (Peakall and Smouse, 2006) and duplicated multilocus genotypes were removed. Deviations from Hardy-Weinberg equilibrium (HWE) as well as linkage disequilibrium (LD) within sampled sites were assessed using ARLEQUIN 3.5.1.2 (Excoffier and Lischer, 2010) with the following settings: 1,000,000 steps in Markov Chain Monte Carlo (MCMC), and 100,000 dememorisation steps. Significance of deviations from HWE and LD were determined after sequential Bonferroni correction for multiple comparisons.

Within population genetic variation was quantified using observed and expected heterozygosity (H_O, H_E) and the number of alleles (N_A) calculated in ARLEQUIN 3.5.1.2 (Excoffier and Lischer, 2010). In addition, I used a measure of allelic richness (A_R) based on a rarefaction index accounting for differences in sample sizes as implemented in FSTAT 2.94 (Goudet, 1995). The inbreeding coefficient (F_{IS}) was also calculated for each site and its deviation from zero was assessed using 100,000 permutations between loci using FSTAT 2.94 (Goudet, 1995).

3.2.4. Population Genetic Structure

Genotypic differentiation between pikas in different study sites was tested using a log-likelihood G-test not assuming *HWE* within samples using FSTAT 2.94 (Goudet, 1995) and based on 100,000 permutations of alleles between sites. In order to shed additional light on underlying population genetic structure, I used discriminant analysis of principle components (DAPC; Jombart *et al.*, 2010). This model-free approach extracts information from genetic data by transforming the genotypes into uncorrelated components using principal components analysis (PCA). A discriminant analysis is then applied to a number of principal components retained by the user in order to maximize the among-population variation and minimize the variation within predefined groups. The fact that these methods lack underlying assumptions such as *HWE* and LD make them applicable to a wide range of situations where such assumptions are not met, as is often the case with small populations. I ran these analyses using the R package ADEGENET 1.3-1 (Jombart, 2008), retaining 30 principal components representing 92% of the total genetic information and using each site as *a priori* populations.

I estimated rates and direction of recent migration events between each sample site using the Bayesian method implemented in BAYESASS+ 1.3 (Wilson and Rannala, 2003) with the following parameters: 3,000,000 iterations with sampling every 2000 iterations, 999,999 burnin and delta values of 0.15. This method makes use of gametic disequilibrium information in a Bayesian inferential framework to estimate recent (last two generations) migration rates from one population into another. This approach has one major assumption, namely that the loci used are in linkage equilibrium, but it does not require that populations are in HWE.

To test for associations between local environmental conditions and observed population genetic structure, I applied a hierarchical Bayesian method that estimates local *FST* values and relates them to environmental variables of interest as implemented in GESTE 2 (Foll and Gaggiotti, 2006). Our framework included the comparison of genetic differentiation (*FST*) with population geographical isolation (Foll and Gaggiotti, 2006), elevation and five other uncorrelated environmental variables (mean annual temperature, mean annual precipitation, precipitation as snow, mean maximum summer temperature and mean minimum winter temperature) calculated for each site using CLIMATEBC 3.1 (Wang *et al.*, 2006). This software downscales and interpolates PRISM 1961-1990 monthly normal data (2.5 x 2.5 arcmin) into 100 m x 100 m resolution and outputs a number of measured and derived variables. Initially, I targeted the 39 annual and seasonal environmental variables available through CLIMATEBC. In order to remove redundant information from this large number of variables, I performed a principal component analysis (PCA) and calculated correlation coefficients between each pair of variables using the R packages ADE4TKGUI (Thioulouse and Dray, 2007) and RCMDR (Fox, 2005) respectively. Variables were considered as redundant if they produced a correlation coefficient higher than 0.9, in which case the variables that made least biological relevance (e.g.

derived variables or variables that *a priori* do not affect the species) were removed from further analyses.

Generalized linear models were run using all seven factors and resulting in a total of 2^7 (128) models. The method evaluates the posterior probabilities of each factor and their combinations in shaping the observed population genetic structure using reversible jump MCMC. For example, the model that compares only genetic differentiation and geographic isolation can be viewed as a null model for isolation by distance, while other models can incorporate more complex scenarios. I followed the approach of Gaggiotti *et al.* (2009) in that I first ran GESTE using all seven factors and then performed a second round of analyses using the three factors with the highest cumulative posterior probabilities from the first round of analyses.

3.2.5. Demographic history

Genetic signatures of demographic contraction were assessed within each site and each transect using three different approaches: 1) the heterozygote excess test and 2) the mode-shift test, both implemented in the software package BOTTLENECK 1.2.02 (Piry *et al.*, 1999), as well as 3) the *M*-ratio test using M_P_VAL.EXE and CRITICAL_M.EXE (Garza and Williamson, 2001). For the heterozygote excess test, significance was assessed using 100,000 iterations with the Wilcoxon sign-rank test and under a Two Phase Model (TPM) consisting of 10% multi-state change and a variance among multiple steps of 12 as recommended by Piry *et al.* (1999). For the *M*-ratio test, I used a TPM mutation model with 10% multi-state change, assuming a marker mutation rate μ of 5 x 10^{-4} and a pre-bottleneck N_e ranging from 500 to 12,500 resulting in a value of θ ($4 N_e \mu$) ranging from 1 to 25 and 3.5 bases steps for multi-step mutations. I used both

approaches since the heterozygosity excess and mode shift test have been suggested to reflect recent demographic contractions, while *M*-ratio reflects historical contractions (Williamson-Natesan, 2005).

I tested for signatures of population expansion using two separate tests implemented in the EXCEL macro KGTESTS (Bilgin, 2007). The within-locus *k* test assesses the distribution of allele lengths against the expectation of unimodality in expanding populations (Reich *et al.*, 1999), the significance of which is based on a simulated one-tailed binomial distribution. The second test is the interlocus *g* test, which is based on the expectation that the variance in the widths of the distribution of allele lengths will be low in expanding populations, as determined by the 5% cutoff value from simulations (Table 1 in Reich *et al.*, 1999).

3.3. Results

3.3.1. Quality Control and Genetic Variation

Of the 28 microsatellite loci screened, six markers failed to amplify and 12 markers were monomorphic (Appendix 3; Supplementary material Table A3.2). Ten markers were polymorphic and used to genotype the 206 hair samples. The overall dataset contained 2.8% missing data, ranging from 0 % (*Ocp* 13) to 7.7 % (*Ocp* 12).

The multi-locus probability of identity for this suite of ten microsatellite loci was 8.3×10^{-6}. Of the 206 hair samples genotyped, 38 pairs harbored identical multi-locus genotypes. I removed one sample from each pair, resulting in a final dataset containing 168 individuals. The mean number of individuals sampled per site was 16.2, ranging from 5 (*H*) to 32 (*D*) (Table 3.1).

After correcting for multiple tests, eight out of ten microsatellite loci were found to deviate from Hardy-Weinberg expectations (*HWE*) in at least one of the ten sampled sites (Appendix 3; Supplementary material Table A3.3). *Ocp 15* showed the highest evidence for violation of this assumption, deviating from *HWE* in five of ten sites. The other loci that deviated from *HWE* did so at only one or two sites (Appendix 3; Supplementary material Table A3.3). Since I found no systematic deviation of *HWE* in all sites, all loci were retained for further analyses, but for tests that assumed loci were in *HWE*, I repeated the analyses without the loci that violated this assumption.

Linkage disequilibrium (*LD*) occurred between four pairs of loci, yet this significant linkage was never observed at all sites (Appendix 3; Supplementary material Table A3.4). The most frequent pair of loci exhibiting deviation from linkage equilibrium (*Ocp12*/*Ocp23*) occurred in five of 10 sites (*C, D, E, F, G*; Appendix 3; Supplementary material Table A3.4). The other three instances of *LD* were specific to only one (*D*: *Ocp2/Ocp11*; *E*: *Ocp15/Ocp22*) or two sites (*A, D*: *Ocp2/Ocp6*; Appendix 3; Supplementary material Table A3.4). As above, when the underlying assumptions of the tests required that loci be at linkage equilibrium, one locus from each linked pair was omitted from these calculations. Furthermore, no evidence for stuttering was detected and allelic dropout and false allele rates were estimated based on repeated genotypes to be as low as 0.002 and 0.0015 respectively, resulting in a total error rate of 0.0035.

Our samples harboured 73 alleles at ten microsatellite loci, ranging from four to 11 alleles per locus. The three sites with the lowest sample sizes contained monomorphic loci: *A2* (*Ocp12* and *Ocp23*), *H* (*Ocp22* and *Ocp 7*) and *I* (*Ocp12*, *Ocp22*, *Ocp23* and *Ocp7*) while all other sites were polymorphic at all ten loci (Appendix 3; Supplementary material Table A3.5). Within population genetic variation was moderate with a mean observed heterozygosity of 0.47 (SD =

0.1), ranging from 0.63 in site *B* to 0.28 in site *I* (Table 3.1). A similar pattern was revealed for allelic richness with a mean A_R of 2.95 (SD = 0.66) with values ranging from 3.7 in site *D* to 1.7 in site *I*. Within-site inbreeding coefficients (F_{IS}) were always positive and differed significantly from zero in all instances except one (site *B*; Table 3.1).

3.3.2. Population Genetic Structure

Log-likelihood tests of population differentiation between sites were all significant, except in the pairwise comparisons that involved the sites with the smallest sample sizes (*A2*-*H*, *A2*-*I*, *H*-*I*). While average F_{ST} values for most sites were centred around 0.20 (0.17 – 0.26), the high elevation site from the Bentinck transect (*I*) displayed an unusually high F_{ST} value of 0.36 (Table 3.2).

The DAPC analysis grouped the sites into clusters corresponding to the three elevation transects (Hill, Nusatsum and Bentinck; Fig. 3.2). The Hill samples were separated from the Bentinck sites by the first axis of the DAPC and from the Nusatsum samples by the second axis, while the Nusatsum samples were separated from the Bentinck samples by the first axis only (Fig. 3.2). While the samples from the Hill and Nusatsum transects overlap with samples within their respective transects, the two sites from the Bentinck transects were completely separated.

Estimates of recent migration rates (*m*) yielded results that were consistent with observed patterns of population differentiation, in that mean migration rates were very low in all cases, (0.001 to 0.07; Table 3.3; Appendix 3; Supplementary material Table A3.6).

Table 3.1. Site-specific information including site names, transect, geographical location, elevation, sample size *(N)*, observed (*HO*) and expected (*HE*) heterozygosites, number of alleles (*NA*), allelic richness (*AR*), and within-site inbreeding coefficient (*FIS*).

Site	Transect	Latitude	Longitude	Elevation (m)	N	Ho	He	Na	Ar	Fis
A1	Hill	N52˚ 18' 36"	W125˚ 29' 47"	1433	15	0.51 (0.23)	0.58 (0.19)	3.2 (1.0)	2.8 (0.84)	0.10
A2	Hill	N52˚ 18' 26"	W125˚ 29' 34"	1338	6	0.40 (0.31)	0.61 (0.13)	3.1 (1.3)	2.6 (1.29)	0.38
B	Hill	N52˚ 15' 9"	W125˚ 31' 39"	793	17	0.63 (0.17)	0.67 (0.11)	4.4 (1.7)	3.5 (0.82)	*0.06*
C	Hill	N52˚ 14' 56"	W125˚ 32' 14"	362	26	0.52 (0.15)	0.68 (0.09)	4.6 (1.2)	3.5 (0.64)	0.23
D	Hill	N52˚ 14' 49"	W125˚ 33' 15"	301	32	0.56 (0.14)	0.69 (0.08)	5.4 (1.4)	3.7 (0.70)	0.20
E	Hill	N52˚ 14' 39"	W125˚ 31' 14"	329	21	0.55 (0.18)	0.60 (0.09)	5.0 (1.2)	3.2 (0.42)	0.08
F	Nusatsum	N52˚ 9' 37"	W126˚ 11' 29"	707	10	0.38 (0.19)	0.56 (0.14)	3.2 (0.6)	2.9 (0.48)	0.34
G	Nusatsum	N52˚ 7' 46"	W126˚ 13' 4"	1058	30	0.46 (0.16)	0.64 (0.14)	4.3 (1.1)	3.3 (0.63)	0.28
H	Bentinck	N52˚ 13' 21"	W126˚ 29' 22"	2	5	0.45 (0.18)	0.59 (0.12)	2.6 (0.7)	2.3 (0.95)	0.26
I	Bentinck	N52˚ 10' 22"	W126˚ 32' 5"	1282	6	0.28 (0.19)	0.36 (0.17)	2.3 (0.5)	1.7 (0.73)	0.25

These values are orders of magnitude lower than the suggested migration rate at which two populations are considered to exchange sufficient migrants to influence each other's dynamics (m = 0.1; Hastings, 1993).

Table 3.2. Pairwise population differentiation indices.

	A1	A2	B	C	D	E	F	G	H	I
A1	-	*	*	*	*	*	*	*	*	*
A2	0.20	-	*	*	*	*	*	*	NS	NS
B	0.14	0.22	-	*	*	*	*	*	*	*
C	0.14	0.16	0.11	-	*	*	*	*	*	*
D	0.15	0.22	0.11	0.05	-	*	*	*	*	*
E	0.19	0.19	0.17	0.15	0.14	-	*	*	*	*
F	0.25	0.21	0.14	0.16	0.20	0.23	-	*	*	*
G	0.17	0.20	0.12	0.18	0.16	0.19	0.10	-		*
H	0.26	0.29	0.22	0.22	0.25	0.29	0.25	0.19	-	NS
I	0.40	0.43	0.35	0.35	0.34	0.32	0.38	0.30	0.38	-

FST values are represented below the diagonal. Asterisks above the diagonal represent a significant differentiation based on log-likelihood G-tests (Goudet *et al.*, 1996).

Bayesian model testing using all seven factors identified mean maximum summer temperature as the variable with the highest cumulative probability (*Pr* = 0.5) in explaining the observed population genetic structure, followed by geographic isolation (*Pr* = 0.497) and mean minimum winter temperature (*Pr* = 0.493). The model with the highest probability (*Pr* = 0.01) contained all three variables listed above. The second round of MCMC containing only these three factors identified that the model with the highest posterior probability contained a constant term (a_0 = -2.13) and mean maximum summer temperature only (*Pr* = 0.134). This latter model resulted in a positive regression coefficient (a_1 = 0.205), suggesting that genetic differentiation

increases with increasing temperature. Yet, a high proportion of the variance remains unexplained by this model as illustrated by a relatively large σ^2 value (1.13).

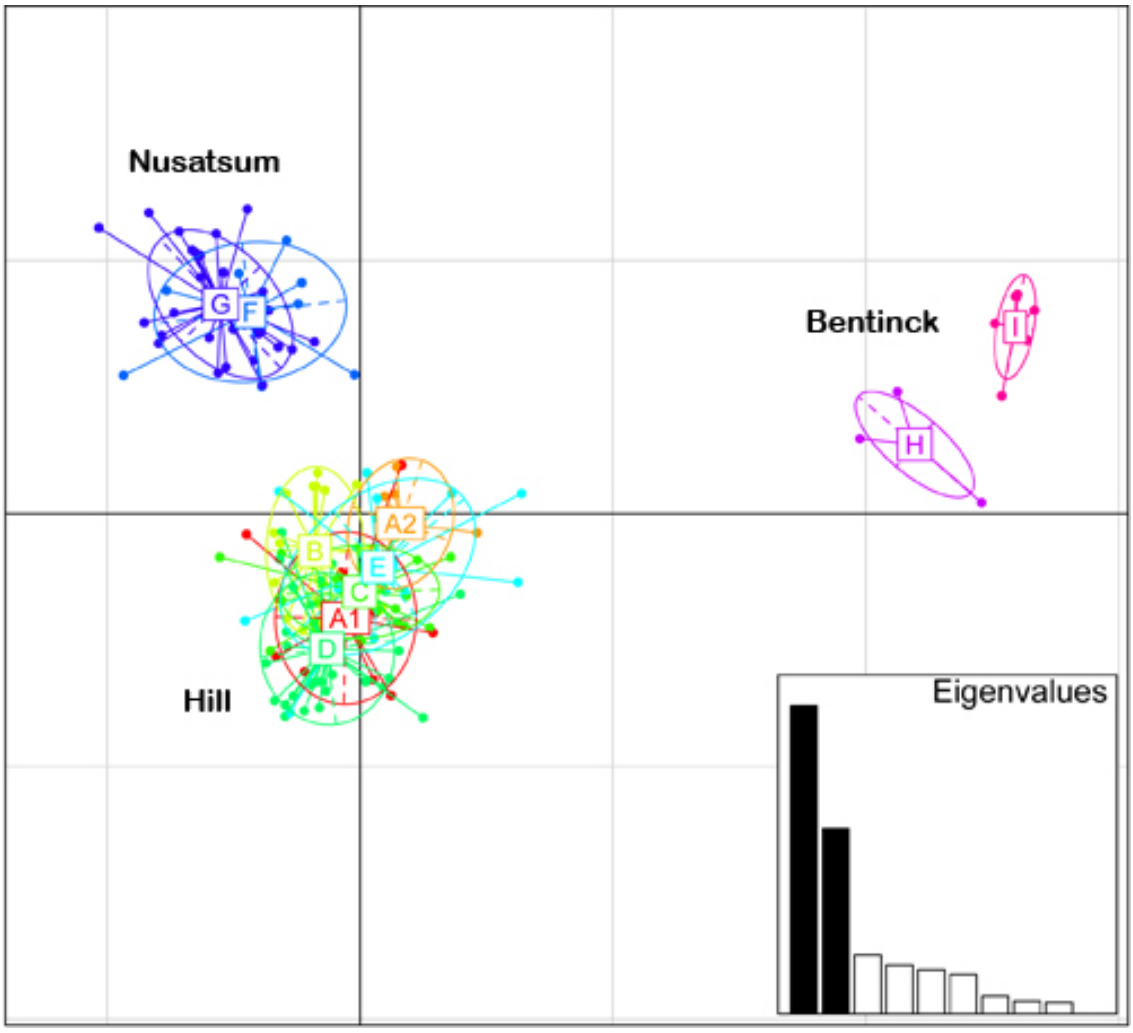


Figure 3.2. Scatter plot from the Discriminant Analysis of Principal Components (DAPC; Jombart *et al.*, 2010) showing the regional cohesion of sample sites into clusters that represent the three elevation gradients sampled.

3.3.3. Demographic history

Signatures of recent demographic contraction were evidenced in half the sites as they displayed a significant excess heterozygosity relative to the number of alleles expected at mutation-drift equilibrium (Appendix 3; Supplementary material Table A3.7). Four of these five sites (*A1*, *C*, *G* and *H*) also displayed shifted allele frequency distributions, another indication of recent demographic contraction. These results were not affected when loci that deviated from *HI* expectations were excluded from the analyses.

Table 3.3. Mean estimates of the distribution of recent migration rates (*m*) calculated using BAYESASS+ (Wilson and Rannala, 2003), given as the proportion of migrant individuals per population per generations.

	A1	A2	B	C	D	E	F	G	H	I
A1	**0.971**	0.074	0.004	0.004	0.005	0.011	0.005	0.001	0.009	0.004
A2	0.003	**0.710**	0.003	0.003	0.003	0.004	0.006	0.001	0.008	0.005
B	0.003	0.015	**0.965**	0.047	0.005	0.008	0.006	0.002	0.011	0.005
C	0.003	0.043	0.004	**0.899**	0.015	0.017	0.006	0.001	0.008	0.004
D	0.003	0.015	0.005	0.017	**0.946**	0.006	0.005	0.002	0.008	0.004
E	0.004	0.017	0.004	0.003	0.013	**0.914**	0.005	0.001	0.008	0.005
F	0.003	0.021	0.004	0.011	0.003	0.007	**0.933**	0.002	0.009	0.005
G	0.004	0.046	0.004	0.007	0.004	0.009	0.015	**0.986**	0.009	0.005
H	0.003	0.016	0.004	0.005	0.003	0.010	0.006	0.002	**0.918**	0.005
I	0.003	0.043	0.004	0.004	0.002	0.016	0.011	0.001	0.014	**0.959**

Columns represent the incoming migration rates and rows represent the outgoing migration rates. Bold values represent the proportion of non-migrant individuals in a population

Conversely, the *M*-ratios were always larger than the critical *M* value simulated for a stable population with the same number of individuals and loci used here, suggesting historically stable populations. The outcome of the *M*-ratio tests remained unchanged when θ was varied from 1 to

25. Likewise, results from the *k* and *g* tests yielded no evidence for population expansion in any of the ten sites, also suggesting that these populations have had historically stable population sizes (Appendix 3; Supplementary material Table A3.7).

3.4. Discussion

3.4.1. Quality Control and Genetic Variation

In the present study, I used a noninvasive sampling approach that enabled us to effectively collect population-level samples of the American pika. Following appropriate quality control, our final microsatellite dataset presented a 0.35% error rate corresponding to 0.2% allele dropout (seven false homozygotes out of 3360 alleles) and 0.15% of false alleles (five false heterozygotes out of 3360 allelles), values that are towards the low end of reported genotyping errors for high quality starting material such as plucked hair (Bonin *et al.*, 2004).

Given the extent of quality control used in our study, the observed deviations from *HWE* at several loci in some of our sites are most likely biologically meaningful, associated with pika life history rather than by genotyping errors (Morin *et al.*, 2009). Indeed, *O. princeps* populations are usually small (at most 10 individuals per ha; Merideth, 2002) and exhibit non-random mating (Peacock and Smith, 1997b). Additionally, inbreeding coefficients were significantly above zero in nine out of ten of our study sites, indicating that the hypothesis of random mating should be rejected. These results are in contrast with those observed in *O. princeps* populations from the centre of their range, where Merideth (2002) did not find evidence of inbreeding at six sites in Nevada. Other life history characteristics that may have contributed to high within-site inbreeding coefficients and deviations from *HWE* are the potential for overlapping generations, post-glacial

colonization, limited dispersal capacities (see below) and metapopulation structure. Regarding the latter, I directly observed the impacts of environmental stochasticity over the course of this study, as illustrated by the likely extirpation of one of the high elevation sites on the Hill transect (*A1*) due to a 2010 forest fire and subsequent re-emergence or recolonization in 2011. Further research would be required, however, in order to definitively determine whether pikas observed at *A1* in 2011 were the result of re-emergence or recolonization from a neighbouring source population that was not sampled in the current study. Overall, these results suggest that the Bella Coola pikas may be distributed in meta-population structure, as previously evidenced in other parts of their range (Peacock and Smith, 1997a).

The amount of genetic variation found in the *O. princeps* samples from the Bella Coola Valley was lower, on average, than that previously reported in other studies of *Ochotona* spp. that employed an overlapping subset of the microsatellite markers used here (Merideth, 2002; Peacock *et al.*, 2002; Yin *et al.*, 2009; Zgurski *et al.*, 2009). Although these studies only incorporated four and three out of the ten loci I used [(Ocp2, Ocp 6, Ocp 7 and Ocp 9 in Merideth, 2002; Peacock *et al.*, 2002; Zgurski *et al.*, 2009) and (Ocp2, Ocp 7 and Ocp 9 in Yin *et al.*, 2009)], meaningful comparisons are still possible. After removing the non-overlapping loci and the three sites for which I had fewer than 10 samples (*A2*, *H* and *I*), mean observed heterozygosity was 0.50 (SD = 0.19). This value is substantially lower than those reported in previous studies of *O. princeps* from Montana, California and Nevada [0.65, SD = 0.14 (Peacock *et al.*, 2002); 0.64, SD = 0.15 (Merideth, 2002)]. The levels of observed heterozygosity reported here are also lower than those found in previous studies of congenerics, including 0.64 (SD = 0.19) from Yukon *O. collaris* (Zgurski *et al.*, 2009), and 0.72 (SD = 0.25) from *O. curzoniae* sampled from the Qinghai-Xizang plateau, China (Yin *et al.*, 2009). Additionally, the allelic

richness I observed (A_R = 3.3; SD = 0.33) was also lower than that found in *O. princeps* populations in Nevada, USA [A_R = 4.8; SD = 0.99; (Merideth, 2002)].

The low level of genetic variation recovered in our samples may be explained by one or a combination of the following: 1) relatively small population sizes; 2) measurable levels of inbreeding; and 3) location of the Bella Coola Valley at the northwestern tip of the American pika distribution. Based on this latter consideration, lower levels of genetic variation observed in Bella Coola Valley *O. princeps* populations may be a result of a more recent post-glacial expansion from southerly refugia compared to core populations in the USA (Galbreath *et al.*, 2009). In practical terms, these observed low levels of genetic variability may represent an impediment to the evolutionary potential of our study populations (Sgro *et al.*, 2011).

3.4.2. Population Genetic Structure, lack of dispersal and the effect of increasing temperatures

The marked population genetic structure revealed in this system is not surprising given the life history characteristics of pikas (e.g. limited dispersal abilities, high mortality of dispersing individuals). All sites displayed significant differentiation except for the pairwise comparisons of the three sites that contained the fewest samples (*A2*, *H*, *I*; n < 10). As the F_{ST} values for these sites were still very high, and in the case of site *I*, the highest observed in our samples, it is quite likely that the lack of significance for these comparisons is an artefact of small sample sizes. Consequently, each sampled site likely represents an independent unit characterized by a unique allele frequency distribution. While it has been suggested that pikas are able to disperse to neighbouring talus patches located up to three km apart (Merideth, 2002; Peacock, 1997), I found no evidence for such a pattern here, even in cases where the geographical distances between sites

was less than three km (*A1 – A2*: 0.6 km, *B – C* : 1.3 km, *C – D* : 2 km, *D – E* : 2 km). Estimates of migration rates corroborated this finding, failing to yield any evidence of recent gene flow between any of the study sites, even those with close geographical distances (*A1 – A2*: 0.6 km).

Although low levels of gene flow were generally detected between sites, I did uncover regional cohesion at the level of the transect (Fig. 3.2). These results suggest that all sites within a transect have a common origin, with some historical exchange of migrants. On a broader scale, the low elevation of the Bella Coola Valley floor (sea level to 300m) may constitute a major barrier to gene flow between transects over the distances studied here (35 to 70 km).

Along the same line, I identified that the mean maximum summer temperature is the one predictor that best explains the underlying population genetic structure, exhibiting the highest posterior probability of any alternative model including the null model for geographical isolation (classically used to study isolation by distance). This result suggests there may be an association between elevated summer temperatures and lack of migration among the sampled transects.

Previous studies have shown that pikas are at high risk of hyperthermia if exposed to ambient temperatures above 27˚C and behaviourally thermoregulate by seeking shelter in their rocky habitat where temperatures remain cooler (Macarthur and Wang, 1974). Dispersal thus represents a major source of mortality due, in part, to the inability for thermoregulation during attempted migrations, which may differ at varying elevations. Indeed, I found that temperatures differed significantly along one of our elevation gradients (the Hill; Fig. 3.1), varying up to six degree Celsius from low to high elevation sites (Henry *et al.*, Submitted-a; Appendix 1). Moreover, this work quantified the insulation properties of talus habitat, finding that below talus temperatures were significantly lower than above talus temperatures throughout the afternoon,

and were significantly warmer than above talus temperatures in the morning and night (Henry *et al.*, Submitted-a; Appendix 1).

Previous studies have shown that increasing rates of environmental change over the past decade are correlated with pika population extirpation in the southern part of their range (Beever *et al.*, 2011). Results from the present study suggest that warmer temperatures may not only increase the rate of population extirpation, but may also reduce the dispersal occurring between sites. Thus, limited thermal tolerance and restricted dispersal capacity may interact synergistically with future climate warming to inhibit recolonization of extirpated patches and reduce survival of resident animals, leading local populations into an extinction vortex. Yet, the association between pika population genetic structure and mean maximum summer temperature revealed here should be taken with caution as alternative biotic or abiotic factors or historical factors not considered may also explain these patterns.

3.4.3. Demographic history

Tests of demographic contraction yielded no evidence of historical bottlenecks occurring in any of the ten sites as illustrated by high *M*-ratios. On the other hand, tests for recent population bottlenecks displayed evidence of population contraction in half the sampled sites, found at different elevations. A similar pattern was described by Merideth (2002), who found that two of six populations displayed genetic signatures of population decline, but failed to detect evidence of historical contractions. This pattern is congruent with a high turnover of populations and colonization of empty sites by a few migrants. Lastly, our analyses did not reveal any evidence for population expansion, which may contradict the hypothesis that recent post-glacial

colonization of the Bella Coola Valley contributed to lower levels of observed population genetic variation than previously reported for *O. princeps*. Yet, a combination of recent population contraction, with population turnover and inbreeding may be responsible for the lack of expansion signal in our dataset, as has been suggested by Galbreath *et al.,* (2009). Based on mitochondrial DNA sequence data collected from *O. princeps* in the northern part of their distribution, Galbreath and colleagues (2009) concluded that the magnitude of population growth exhibited in post-glacial lineages, such as the pikas from the Bella Coola Valley, might have remained under a given threshold to leave a genetic signature of expansion.

3.5. Summary

The present study revealed low levels of neutral genetic variation in *O. princeps* populations from the northern edge of their distribution. While the implications of such impoverished gene pools on the persistence of *O. princeps* are unknown, low levels of genetic variation may increase risks of extinction (Spielman *et al.*, 2004). Additionally, the lack of evidence of migration between local populations sampled along continual altitudinal gradients suggest that in the face of climate change, *O. princeps* will have to rely on local adaptations or phenotypic plasticity in order to survive that magnitude of environmental change. The latter remains to be investigated, including the use of non-neutral molecular markers in order to shed light on the evolutionary potential of *O. princeps* and the importance of local adaptation versus plasticity.

Chapter 4. Adaptive population divergence and associated environmental correlates detected in the climate change-sensitive American pika (*Ochotona princeps*)

4.1. Overview

A significant challenge facing wildlife species will be coping with contemporary climate change (Reusch and Wood, 2007). When challenged with environmental stresses, species have three options: disperse, adapt or go extinct (Hewitt and Nichols, 2005). Altered selection regimes caused by increasing air and water temperatures will operate in the near future (IPCC, 2007) and while it has been suggested that most species will shift their geographical ranges rather than adapt *in situ* (Parmesan, 2006), factors such as habitat fragmentation may act synergistically to impair species' dispersal to more favorable conditions (Stockwell *et al.*, 2003). Such a strategy may be particularly challenging for species with limited dispersal capacity or those with highly restricted habitat requirements (Sgro *et al.*, 2011), thus local adaptation may represent the only option for continued persistence.

Species that have successfully established populations along environmental gradients may represent ideal systems in which to study the effects of natural selection on the extent and distribution of genetic variation, as environmental conditions (e.g. temperature, precipitation) change rapidly over short distances (Bonin *et al.*, 2006; Gebremedhin *et al.*, 2009). In a spatial context, environmental gradients may thus be used as surrogates for the expected temporal changes in selection pressures generated by climate change (Davis and Shaw, 2001; Davis *et al.*, 2005; Reusch and Wood, 2007).

Investigating the genetic basis of species' adaptations to their environments continues to be an important focus in evolutionary biology (Nielsen, 2005). Besides an essential contribution to a more profound understanding of species' evolutionary histories, elucidating the genetic basis underlying ecologically important traits can yield other fundamental knowledge regarding the biology of the species of interest. For examples, quantifying: 1) the number of genes and alleles involved in adaptations (Orr and Coyne, 1992); 2) the degree to which adaptations differ in their genetic basis among populations (e.g. Nachman, 2005); and 3) the extent to which such information may guide conservation strategies for the long-term persistence of species (Hoffmann and Parsons, 1997), especially with regards to defining conservation units in a changing environment (Bonin *et al.*, 2007a; Sgro *et al.*, 2011).

The identification of genes underlying ecologically important traits has traditionally been limited to well-studied or model organisms using a targeted gene approach within systems where individual adaptive traits segregate into contrasting [e.g. the detection of the genetic basis of fur coloration in mammals (Hoekstra, 2006)]. Additionally, associations between phenotypes and genotypes have been characterized using quantitative trait loci (QTL). Yet, QTL studies are restricted to well-studied taxa that can be experimentally manipulated and crossed (Stinchcombe and Hoekstra, 2008). Moving beyond model systems, the development of population genomic approaches (Luikart *et al.*, 2003) have enabled the investigation of the genetic basis of adaptation in natural populations of non-model organisms. By screening large numbers of loci distributed throughout the genome, researchers are able to tease apart neutral (genome-wide) and adaptive (locus-specific or "outlier") effects. This approach has been applied at multiple scales for investigating the genetic basis of adaptation, from continuous distributions along gradients of altitude (Bonin *et al.*, 2006) and temperature (Jump *et al.*, 2006), to phenotypically discrete

ecotypes (Campbell and Bernatchez, 2004; Nosil *et al.*, 2008) and subspecies (Nunes *et al.*, 2011).

The American pika (*Ochotona princeps*) is a small lagomorph discontinuously distributed in mountainous areas throughout western North America from central British Columbia and Alberta, Canada, south to the Sierra Nevada in California and east to New Mexico, USA. Pikas are restricted to talus slopes and broken rock debris in proximity of meadows that provide their food (Smith and Weston, 1990). The fragmented nature of their habitats has propelled *O. princeps* to the position of a model mammalian species for studies of metapopulation dynamics, island biogeography and source-sink dynamics (Clinchy *et al.*, 2002; Moilanen *et al.*, 1998; Peacock and Smith, 1997b; Smith, 1974a). In recent years, pikas have also gained notoriety as a model system for testing extinction dynamics in the face of climate change (Beever *et al.*, 2003; 2010; 2011). In that vein, pikas are considered harbingers of global warming, predicted by some to constitute the first mammalian species to go extinct due to the direct effects of climate change (Smith *et al.*, 2004).

In the present study, I used population and landscape genomic approaches to investigate the genetic basis of adaptation in populations of *O. princeps* found along three elevation gradients in the central Coast Mountains of British Columbia, Canada, ranging from sea level to 1500 m. Specifically I addressed the following questions: 1) what proportion of the American pika genome is under positive selection in this system? 2) What are the main environmental variables associated with adaptive population divergence in this species? 3) Are different adaptive gene variants detected along independent elevation and longitudinal gradients?

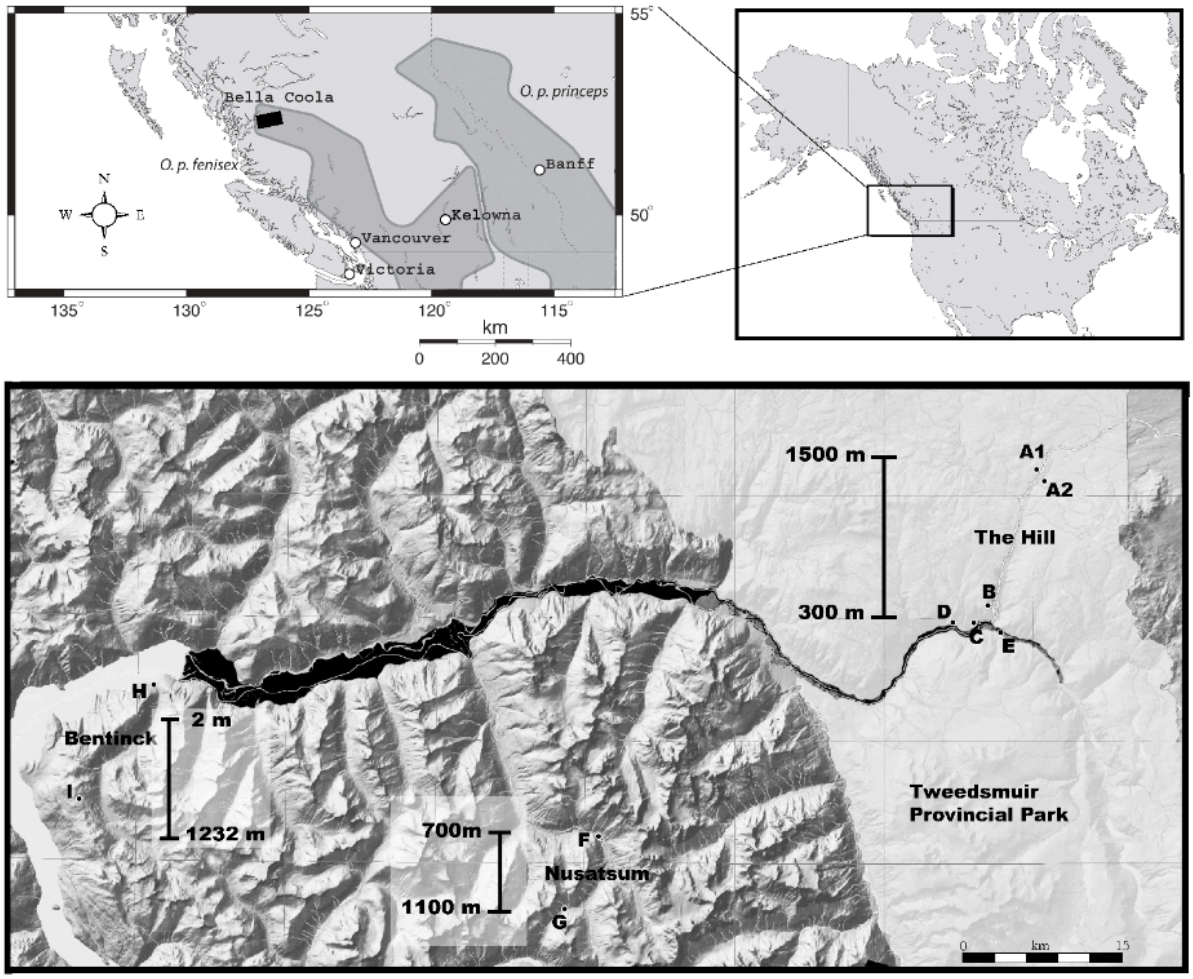


Figure 4.1. Map of the study area in the Bella Coola Valley, British Columbia, Canada, with the ten sampling sites located along three elevation gradients: the Hill, Nusatsum and Bentinck from east to west. For each elevation gradient, the extent of altitudinal differences is described. The valley bottom is indicated as the dark grey area at the centre of the map.

4.2. Materials and methods

4.2.1. Study Site

This study was carried out in the Bella Coola Valley, British Columbia, Canada (Fig. 4.1). This area was chosen for several reasons, including historical records of the occurrence of

O. princeps from sea level to tree line and the presence of an extensive network of roads connecting the bottom of the valley to higher elevations. The valley runs from east to west and thus provides a longitudinal gradient from the interior to the coast, with marked differences in precipitation. American pikas were sampled from August 2008 to September 2010 at 10 sites along three elevation gradients (Table 4.1; Hill, Nusatsum and Bentinck gradients) using recently developed noninvasive hair snares (Fig. 4.1) (Henry *et al.*, 2011; Henry and Russello, 2011). Altitudes at which pikas are found in the Bella Coola Valley range from sea level to over 1500 m, and such elevation transects represent strong temperature gradients, with differences of up to six degrees Celsius from the bottom to the top of the Hill over a distance of only 16 kilometres (Fig. 4.1; Henry *et al.*, Submitted-a; Appendix 1).

Table 4.1. Site-specific information including site names, transect, geographical location, elevation and sample size *(N*).

Site	Transect	Latitude	Longitude	Elevation (m)	N
A1	Hill	N52˚ 18' 36"	W125˚ 29' 47"	1433	15
A2	Hill	N52˚ 18' 26"	W125˚ 29' 34"	1338	6
B	Hill	N52˚ 15' 9"	W125˚ 31' 39"	793	17
C	Hill	N52˚ 14' 56"	W125˚ 32' 14"	362	26
D	Hill	N52˚ 14' 49"	W125˚ 33' 15"	301	32
E	Hill	N52˚ 14' 39"	W125˚ 31' 14"	329	21
F	Nusatsum	N52˚ 9' 37"	W126˚ 11' 29"	707	10
G	Nusatsum	N52˚ 7' 46"	W126˚ 13' 4"	1058	30
H	Bentinck	N52˚ 13' 21"	W126˚ 29' 22"	2	5
I	Bentinck	N52˚ 10' 22"	W126˚ 32' 5"	1282	6

4.2.2. DNA isolation, AFLP genotyping and band scoring

I previously determined that DNA extracted from 15-20 hairs per sample yielded DNA of sufficient quality and quantity for downstream assays (Henry and Russello, 2011). Given these findings, I used DNA isolated from a total of 168 samples previously shown to represent unique multilocus genotypes based on 10 microsatellite markers (Henry *et al.*, Submitted-b; Chapter 3).

I used Amplified Fragment Length Polymorphism (AFLP; Vos *et al.*, 1995)-based genomic scans to screen large numbers of molecular markers in our samples. AFLPs produce a large number of markers at a relatively low cost and are popular in studies of non-model organisms, as they do not require prior sequence information (Bonin *et al.*, 2007b; Meudt and Clarke, 2007). Additionally, AFLPs are distributed throughout the genome and thus represent an ideal marker choice for genomic scans (Bensch and Akesson, 2005). About 50 ng of isolated DNA was digested sequentially using *Taq*I and *Eco*RI restriction endonucleases (New England Biolabs, Ipswitch, MA, USA) following the protocol outlined in Bonin *et al.* (2005) with the following modifications: double stranded adapters (Table 4.2) were prepared fresh each time the procedure was repeated and ligation took place at 16°C overnight in order to maximize the efficiency of the reaction (Papa *et al.*, 2005). Following ligation, the reaction volume was diluted four instead of 10 times. Pre-selective and selective amplifications were performed using a Veriti® thermal cycler (Applied Biosystems, Foster City, CA, USA) in a 25µL volume (Bonin *et al.*, 2005). Initially, I used a representative sample of 16 individuals distributed throughout our study area and across elevation gradients to assess amplification success and polymorphism of all possible selective primer combinations (Table 4.2). Based on results from the pilot analysis, twenty primer combinations that produced a large number of bands with high repeatability were retained and screened on the remainder of the sample. In order to quantify error rates and check

repeatability of our protocol, 42 individuals (25% of total sample) were randomly selected as duplicates.

Cycling parameters for pre-selective and selective amplification were carried out according to Bonin *et al.* (2005) with the exception that I used KAPA *Taq* (KAPA Biosystems, Cape Town, South Africa) for the pre-selective amplification. Each *Eco*RI primer was fluorescently labelled (Table 4.2) to enable analysis on a capillary system. PCR products were multiloaded and run on an ABI 3130XL genetic analyser (Applied Biosystems, Foster City, CA, USA) with GeneScan™ 600 LIZ® size standard.

AFLP profiles were called using a semi-automated approach in GENEMAPPER 4.0 (Applied Biosystems, Foster City, CA, USA). First, I allowed GENEMAPPER to automatically generate bins of 1 base pair (bp) width between 50 bp and 600 bp. Then I manually checked all bins and removed overlapping bins. Fragments with relative fluorescent unit (rfu) less than 50 were discarded as this threshold was used to represent instrument noise (Keyghobadi *et al.*, 2009) and the peak heights were left as un-normalized. The output from GENEMAPPER (fragment size and peak heights) was then imported into SCANAFLP 1.3 (Herrmann *et al.*, 2010) for further processing. Marker selection proceeded by first discarding fragments with the following characteristics: 1) peaks lower than 200 rfu; 2) heights lower than 10% of the mean height of the maximum height frequency class; and/or 3) a coefficient of variation higher than one. In addition, markers that differed by more than one fragment among replicates were also discarded. The resulting binary matrix was handled with AFLPDAT (Ehrich, 2006) to produce files formatted for further analyses.

4.2.3. *Environmental data*

Climatic data were calculated based on geographical location and elevation for each site using CLIMATEBC 3.1 (Wang *et al.*, 2006). This software downscales and interpolates PRISM (Daly *et al.*, 2002) 1961-1990 monthly normal data (2.5 x 2.5 arcmin) into 100 m x 100 m resolution and outputs a number of measured and derived variables. Initially, I targeted the 39 annual and seasonal environmental variables available through CLIMATEBC. In order to remove redundant information from this large number of variables, I performed a principal component analysis (PCA) and calculated correlation coefficients between each pair of variables using the R packages ADE4TKGUI (Thioulouse and Dray, 2007) and RCMDR (Fox, 2005), respectively. Variables were considered redundant if they produced a correlation coefficient higher than 0.8 (Manel *et al.*, 2010), in which case the variables that were deemed less biologically-relevant (e.g. derived variables or variables that *a priori* do not affect the species) were removed from further analyses. I thus retained altitude (ALT), mean annual precipitation (MAP), mean annual temperature (MAT), precipitation as snow (PAS), summer mean maximum temperature (T_{MAX}) and winter mean minimum temperature (T_{MIN}) as the explanatory variables in tests of associations with allele frequency data.

Table 4.2. AFLP adapters, primers and primer combinations used.

Adapters and primers used	Primer code	Fluorescent label	No. Of selective bases	Adapter and primer sequences or primer combinations
*Eco*RI-adapter				5′-CTCGTAGACTGCGTACC-3′ (top strand)
				3′-CATCTGACGCATGGTTAA-5′(bottom strand)
*Eco*RI-primers	E01		+ 1 (A)	5′-GACTGCGTACCAATTCA-3′
	E31	FAM	+ 3 (AAA)	5′-GACTGCGTACCAATTCAAA-3′
	E32	NED	+ 3 (AAC)	5′-GACTGCGTACCAATTCAAC-3′
	E33	VIC	+ 3 (AAG)	5′-GACTGCGTACCAATTCAAG-3′
	E34	PET	+ 3 (AAT)	5′-GACTGCGTACCAATTCAAT-3′
	E38	FAM	+ 3 (ACT)	5′-GACTGCGTACCAATTCACT-3′
	E43	NED	+ 3 (ATA)	5′-GACTGCGTACCAATTCATA-3′
	E44	VIC	+ 3 (ATC)	5′-GACTGCGTACCAATTCATC-3′
	E46	PET	+ 3 (ATT)	5′-GACTGCGTACCAATTCATT-3′
*Taq*I-adapter				5′-CGGTCAGGACTCAT-3′ (top strand)
				3′-CAGTCCTGAGTAGCAG-5′(bottom strand)
TaqI-primers	T01		+ 1 (A)	3′-AAGCCAGTCCTGAGTAG-5′
	T32		+ 3 (AAC)	3′-CAAAGCCAGTCCTGAGTAG-5′
	T35		+ 3 (ACA)	3′-ACAAGCCAGTCCTGAGTAG-5′
	T37		+ 3 (ACG)	3′-GCAAGCCAGTCCTGAGTAG-5′
	T38		+ 3 (ACT)	3′-TCAAGCCAGTCCTGAGTAG-5′
	T39		+ 3 (AGA)	3′-AGAAGCCAGTCCTGAGTAG-5′
	T43		+ 3 (ATA)	3′-ATAAGCCAGTCCTGAGTAG-5′
	T44		+ 3 (ATC)	3′-CTAAGCCAGTCCTGAGTAG-5′
	T45		+ 3 (ATG)	3′-GTAAGCCAGTCCTGAGTAG-5′
Primer combinations				E31/T37 (1); E31/T39 (2); E31/T43 (3); E32/T35 (4);
				E33/T32 (5); E33/T37 (6); E33/T39 (7); E38/T32 (8);
				E38/T37 (9); E43/T35 (10); E43/T37 (11); E43/T44 (12);
				E44/T38 (13); E44/T44 (14); E46/T38 (15); E34/T44 (16);
				E46/T45 (17); E34/T45 (18); E34/T38 (19); E43/T43 (20)

4.2.4. Detection of outlier loci

I used a set of alternative methods to identify loci potentially subject to selection in our system: 1) two frequentist *FST* outlier detection methods relying on different simulation frameworks; 2) a Bayesian *FST* outlier detection method; and 3) a spatial analysis method incorporating multiple linear regression models to correlate AFLP band frequencies with environmental variables. I chose to use the multiple methods available as each approach has its own assumptions and algorithm. I applied the frequentist method implemented in MCHEZA (Antao and Beaumont, 2011), which is based on the algorithm of FDIST (Beaumont and Nichols, 1996a). This software estimates allele frequencies using the Bayesian method of Zhivotovsky (1999) and calculates *FST* indices (Weir and Cockerham, 1984) between predefined populations (ten sample sites). Coalescent simulations are performed under a finite island model to generate an *FST* null distribution. Loci with unusually high or low *FST* values contingent on their allele frequency are considered outliers, and thus potentially under selection. I performed these analyses using 1,000,000 iterations and the most stringent setting including a 0.995 confidence interval (CI) and 1% false discovery rate (FDR) to guard against false positives. All other parameters used default values with $\theta = 0.1$, β-a and β-b = 0.25 and a critical frequency of 0.99. Additionally, I used the neutral mean F_{ST} and force mean F_{ST} options. This undertakes an initial run and removes potential outliers in order to compute an unbiased neutral F_{ST}. The second frequentist method, also derived from FDIST (Beaumont and Nichols, 1996a) and implemented in ARLEQUIN 3.5 (Excoffier and Lischer, 2010), applies a hierarchical island model (Excoffier *et al.*, 2009) that takes underlying population genetic structure into account in calculating F_{ST}. This method has been shown to substantially reduce false positive rates in hierarchically structured populations (Excoffier *et al.*, 2009), such as that observed in our system (Henry *et al.*, Submitted-

b; Chapter 3). I thus grouped populations belonging to the same elevation transect as suggested in this previous study and used a 99% CI to identify outliers. I applied 1,000,000 iterations and simulated 100 demes per group for 10 groups with minimum and maximum expected heterozygosities bounded between 0 and 1, and a minimum DAF = 0 under a pairwise difference model.

The second general approach I used for outlier detection was the Bayesian method implemented in BAYESCAN 2.01 (Foll and Gaggiotti, 2008). This approach directly estimates the probability that each locus is subject to selection by teasing apart population-specific and locus-specific components of F_{ST} coefficients using a logistic regression. The posterior probability of a given locus being under selection is assessed by defining two alternative models, one including the locus-specific effect and the other excluding it. Departure from neutrality is assumed when the locus-specific component is necessary to explain the observed pattern of diversity using a reversible jump Markov chain Monte Carlo (MCMC) algorithm which takes into account all loci at once, thus resolving the issue of multiple testing of a large number of loci. I ran chains of 1,000,000 iterations with a thinning of 10, resulting in 100,000 iterations considered following a burnin of 50,000. As above, all other parameters were kept as default, including 20 pilot runs of 5,000 iterations in length, prior odds for the neutral model of 10, and a uniform distribution of F_{IS} between 0 and 1. As above, I opted for the most stringent settings, implementing a 1% false discover rate in order to identify outlier loci.

The third independent approach I used for outlier detection was the spatial analysis method, SAM (Joost *et al.*, 2008), that computes multiple univariate logistic regression models to test for associations between the frequency of AFLP bands and data from selected environmental variables. To ensure the robustness of the method, likelihood ratio (*G*) and Wald statistical tests

are implemented to assess the significance of coefficients calculated by the logistic regression function. A model is considered significant only if the null hypothesis is rejected by both tests, after Bonferroni correction for multiple testing. For both tests, the null hypothesis is that the model with the examined variable does not explain the observed distribution better than a model with a constant only (Joost *et al.*, 2007). I used a significance threshold corresponding to a 99% CI after Bonferroni correction. After identifying the loci that showed outlier behavior or significant association with the selected environmental variables using the approaches described above, I performed linear regression analysis using R (R Development Core Team, 2011). For each linear regression, the residuals were extracted and the assumption of normality was verified by plotting the distribution of residuals and corresponding Normal Quantile Plots. Homoscedasticity was verified by look at each residual plots and using a Levene test for homogeneity of variances. Given the normality and homoscedasticity of residuals, I calculated the adjusted R^2 values (R^2_{adj}) for each regression model separately and for each environmental variable. The R^2_{adj} values were used as they provide an unbiased estimate of the explanatory power of each alternative model (Ohtani, 2000). All above analyses were repeated four times, once at a regional scale using all the sampled populations representing a longitudinal gradient from the interior to the coast, and once across each of the three elevation gradients separately. In the latter cases, the analyses using hierarchical population structure implemented in ARLEQUIN 3.5 (Excoffier and Lischer, 2010) were omitted since it is not applicable to within-transect comparisons.

4.3. Results

4.3.1. Detection of outlier loci across a longitudinal gradient

A total of 1509 AFLP loci ranging in size from 50 to 473 base pairs produced using 20 selective primer combinations were reliably scored in our sample (Appendix 3; Supplementary material Table A3.8). Each primer combination yielded on average 75 bands, with a mean error rate of 4.5% ranging from 3% to 6.4%.

Using a combination of four different analytical methods, I detected 21 outlier loci across all sampling sites along this longitudinal gradient (1.4% of the genomic scan; Table 4.3). The first approach, MCHEZA identified 13 loci with F_{ST} values significantly greater than that expected under a neutral model, indicative of positive or directional selection, nine of which were also identified by at least one other approach (Table 4.3). ARLEQUIN identified 11 loci under selection, nine of which were shared with MCHEZA. The Bayesian approach of BAYESCAN was the most conservative of all, and detected five outliers, two of which were also identified by both ARLEQUIN and MCHEZA. Lastly the SAM identified six loci potentially associated with at least one of the six environmental variables under study (MAP, PAS and T_{MAX}), two of which were also identified as outliers by BAYESCAN.

One outlier (E43T37_215) was identified by all methods and was found to be associated with mean annual precipitation (MAP), yet this outlier did not produce a significant R^2_{adj}. After performing linear regression analysis on each of the 21 outliers with each of the six environmental variables separately, only a single locus (E31T37_104) showed a negative, strong and significant association with an environmental variable, in this case MAP (Figure 4.2; R^2_{adj} = 0.84, F-test, F= 47.27, DF= 8, p = 0.0001).

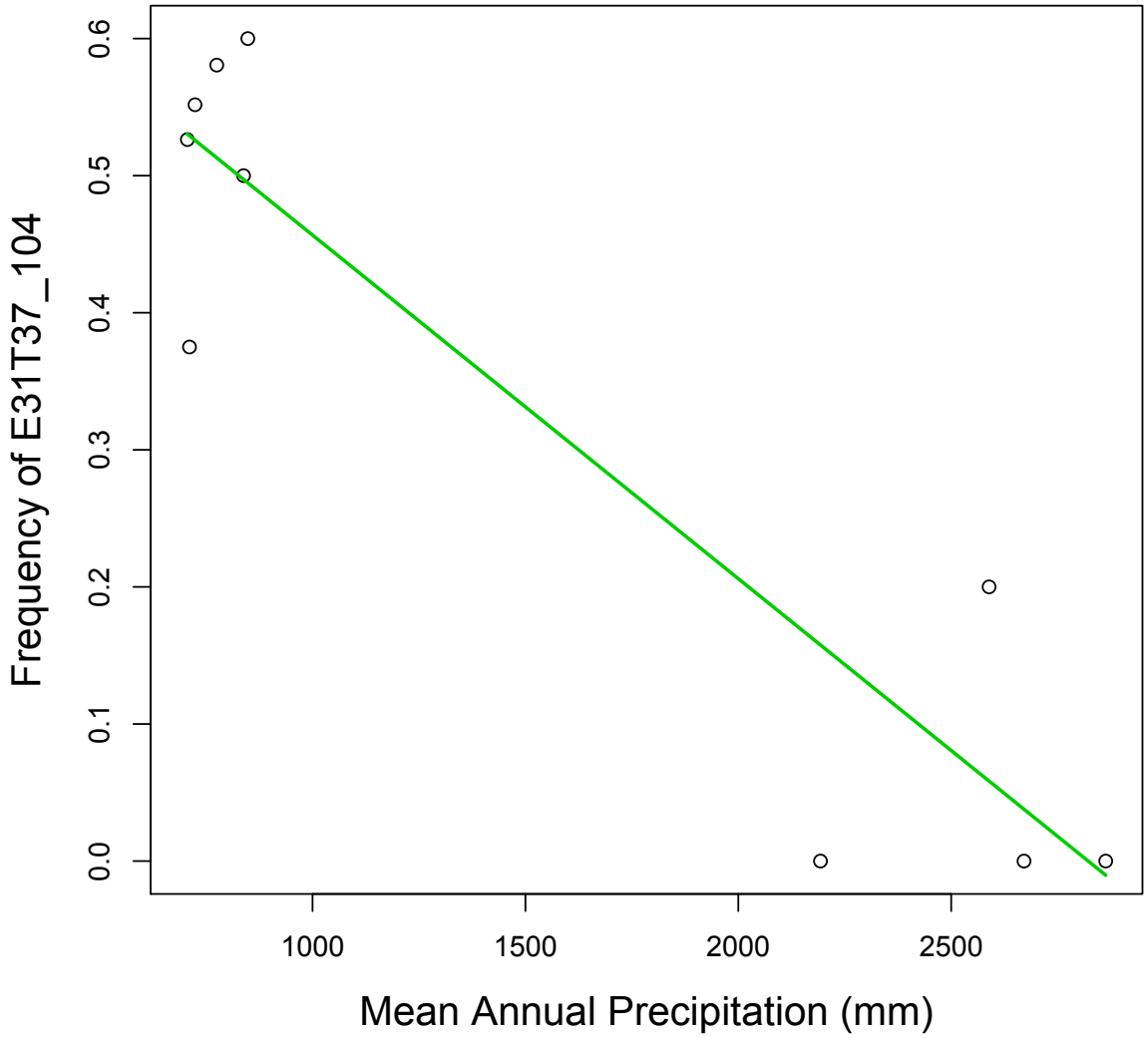


Figure 4.2. Linear regression of the frequency of E31T37_104 against mean annual precipitation, depicting a significant negative relationship (R^2_{adj} = 0.84, F-test, F = 47.27, DF = 8, p = 0.0001) across the longitudinal gradient from coast to interior. Points indicate sampling locations.

Table 4.3. List of outlier loci detected by four methods across our entire sample. For MCHEZA an "x" indicates that the locus was detected as an outlier at the 99.5% significance level and using a 1% FDR. In ARLEQUIN, an "x" indicates significance at the 99% CI, and using BAYESCAN, an "x" indicates a posterior probability above 0.99 and a 1% FDR. For the SAM, the environmental variables significantly correlated at a 99% CI and after Bonferroni corrections are indicated. MAP- mean annual precipitation, PAS- precipitation as snow and Tmax- summer mean maximum temperature. The bold values indicate a significant linear regression between AFLP band frequency and a given environmental variable.

Outlier	Mcheza	Arlequin	Bayescan	SAM	R2adj
E31T37_100		x			0.654
E31T37_104				**MAP**, PAS	**0.977**
E31T39_108	x	x			0.768
E31T39_53				MAP, PAS	0.785
E31T39_56	x	x			0.826
E31T39_84			x	MAP, PAS	0.59
E31T39_88	x	x			0.701
E31T43_104	x				0.88
E31T43_82	x	x			0.738
E33T39_54	x	x			0.949
E33T39_56	x	x	x		0.782
E33T39_86			*x*		0.782
E33T39_91			x	Tmax	0.566
E38T32_71	x				0.534
E38T32_91		x			0.876
E38T37_155	x				0.619
E43T35_63	x				0.818
E43T37_194	x	x			0.764
E43T37_213	x	x			0.868
E43T37_215	x	x	x	MAP	0.898
E46T45_76				Tmax	0.646

4.3.2. Detection of outlier loci across elevation gradients

The above analyses were repeated separately for each transect in order to assess if there was evidence for adaptive population divergence by elevation. In the Hill transect, which consisted of three low elevation (C, D, E), a mid elevation (B) and two high elevation (A1, A2) populations, a total of 20 outliers were detected (1.3% of the genomic scan; Table 4.4). A large number of these outliers (n=16) were detected by MCHEZA, nine of which also showed a significant association with at least one environmental variable based on the SAM (MAP, MAT, PAS, T_{MAX}). Similar to the regional scale analysis, BAYESCAN was the most conservative approach and identified a single outlier (E38T37_260) that was also identified by all other approaches. After regression analysis, two loci showed significant and strong association with an environmental variable: E38T32_136 showed a negative and significant correlation with MAP (Figure 4.3a; R^2_{adj} = 0.82, F-test, F= 24.6, DF= 5, p = 0.008) and a positive correlation with T_{MAX} (Figure 4.3b; R^2_{adj} = 0.81, F-test, F= 23, DF= 5, p = 0.009). Additionally, E43T43_80 showed a negative relationship with T_{MAX} (Figure 4.4; R^2_{adj} = 0.79, F-test, F= 20.28, DF= 5, p = 0.01).

Table 4.4. List of outlier loci detected by three methods across the elevation gradient "the Hill". For MCHEZA an "x" indicates that the locus was detected as an outlier at the 99.5% significance level and using a 1% FDR. In BAYESCAN, an "x" indicates a posterior probability above 0.99 and a 1% FDR. For the SAM, the environmental variables significantly correlated at a 99% CI and after Bonferroni corrections are indicated. MAP- mean annual precipitation, MAT- mean annual temperature, PAS- precipitation as snow and Tmax- summer mean maximum temperature. The bold values indicate a significant linear regression between AFLP band frequency and a given environmental variable.

Outlier	Mcheza	Bayescan	SAM	R2adj
E31T43_104	x			-0.6
E31T43_169	x		MAT,PAS,Tmax	0.99
E32T35_68	x			-0.91
E33T37_107	x			0.79
E33T39_77	x			-0.08
E33T39_91	x			0.07
E34T45_103	x			-3.55
E38T32_136			**MAP, Tmax**	**0.98**
E38T37_260	x	x	MAT, Tmax	0.99
E38T37_60			MAT,PAS,Tmax	0.8
E38T37_80	x		MAP	0.98
E43T35_99	x			-0.6
E43T43_80	**x**		**Tmax**	**0.98**
E43T44_104	x			-0.01
E44T44_111	x			0.24
E44T44_81	x			0.66
E44T44_97			MAP	0.88
E46T38_51	x			-0.5
E46T45_76			MAT,PAS,Tmax	0.69
E46T45_145	x			-0.1

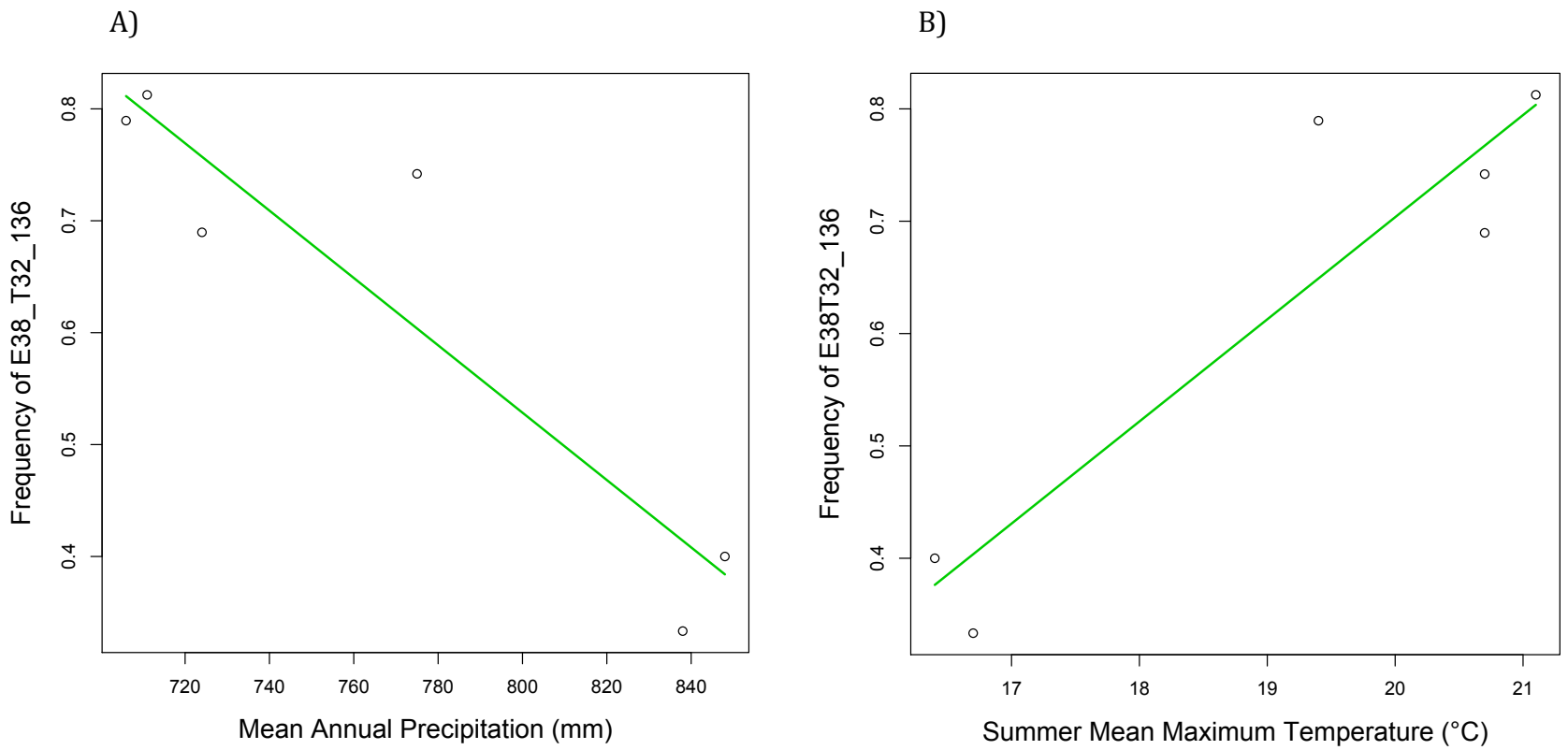


Figure 4.3. Linear regression of the frequency of E38T32_136 against: A) mean annual precipitation, depicting a negative and significant correlation (R^2_{adj} = 0.82, F-test, F = 24.6, DF = 5, p = 0.008) and B) summer mean maximum temperature, depicting a positive correlation (R^2_{adj} = 0.81, F-test, F = 23, DF = 5, p = 0.009) across the elevation transect at the Hill ranging from 300m to 1500m. Points indicate sampling locations.

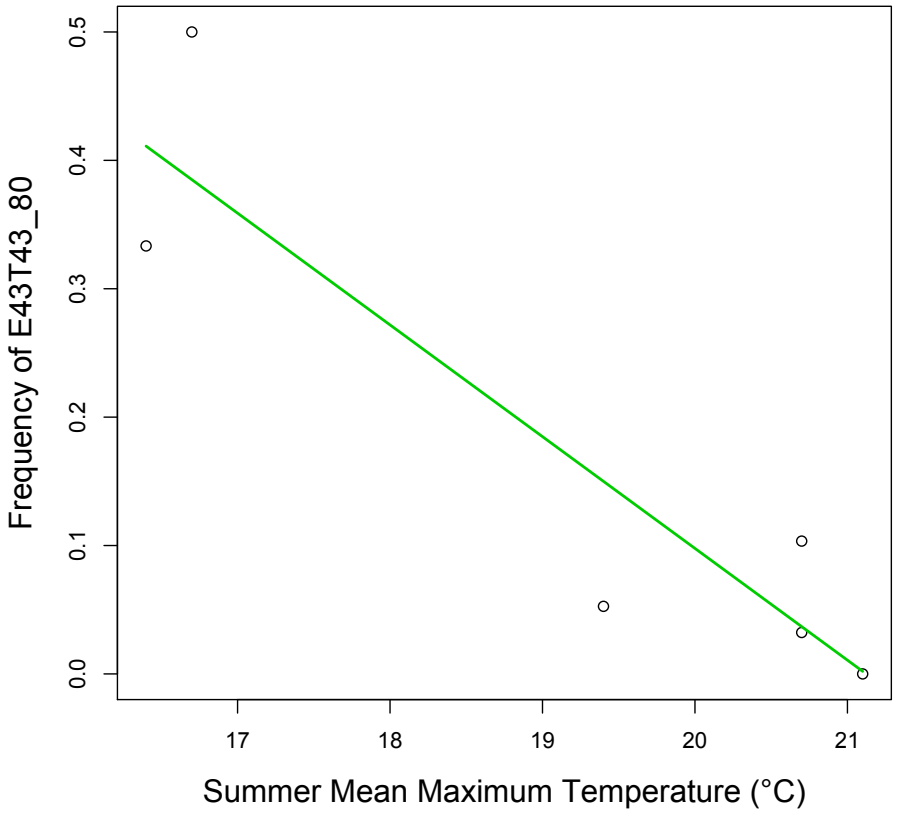


Figure 4.4. Linear regression of the frequency of E43T43_80 against summer mean maximum temperature, showing a significant negative relationship (R^2_{adj} = 0.79, F-test, F = 20.28, DF = 5, p = 0.01) across the elevation transect at the Hill ranging from 300m to 1500m. Points indicate sampling locations.

Since both the Nusatsum and Bentinck transects consisted of only two populations, no linear regression or SAM were undertaken. In Nusatsum, MCHEZA identified 19 outliers (1.2% of genomic scan), one of which was also identified using BAYESCAN (E44T38_115; Table 4.5). In the Bentinck transect only three outliers (0.3% of genomic scan) were detected by MCHEZA, one of which was also identified by BAYESCAN (Table 4.6). Interestingly, I found no overlap in outliers among elevation transects as each gradient was characterized by its own set of outliers.

Table 4.5. List of outlier loci detected by two methods across the elevation gradient "Nusatsum". For MCHEZA an "x" indicates that the locus was detected as an outlier at the 99.5% significance level and using a 1% FDR. In BAYESCAN, an "x" indicates a posterior probability above 0.99 and a 1% FDR.

Outlier	Mcheza	Bayescan
E33T32_68	x	
E33T32_71	x	
E33T37_73	x	
E33T37_99	x	
E33T39_106	x	
E34T38_103	x	
E34T45_159	x	
E38T32_112	x	
E38T32_76	x	
E38T32_88	x	
E38T37_105	x	
E38T37_92	x	
E43T35_136	x	
E44T38_104	x	
E44T38_108	x	
E44T38_115	x	x
E46T38_125	x	
E46T38_70	x	

Table 4.6. List of outlier loci detected by two methods across the elevation gradient "Bentinck". For MCHEZA an "x" indicates that the locus was detected as an outlier at the 99.5% significance level and using a 1% FDR. In BAYESCAN, an "x" indicates a posterior probability above 0.99 and a 1% FDR.

Outlier	Mcheza	Bayescan
E43T43_64	x	
E46T38_101	x	
E46T38_65	x	x

4.4. Discussion

While climate change is regarded as a potential threat to humanity and other species, relatively little knowledge on the genetic basis underlying biotic responses to environmental changes is available. This information will likely become increasingly important for the implementation of future conservation strategies that will explicitly take environmental changes into consideration. Population genomic approaches offer innovative analytical tools that may be applied in that regard, by screening the genomes of non-model organisms and assessing the genetic changes incurred by varying environmental conditions. In this study I used an AFLP-based genomic scan to screen for loci potentially under selection in *O. princeps* populations experiencing contrasting environmental conditions. Our AFLP-based genomic scan produced >1500 loci that were analysed with three independent outlier detection methods with their most stringent thresholds to reveal several candidate loci under divergent selection across a longitudinal and three elevation gradients. I also identified significant association of several loci with environmental variables across these gradients.

4.4.1. Detection of outlier loci across a longitudinal gradient

At the regional scale, I identified 1.4% of the loci analyzed as displaying evidence for positive selection or significant association with an environmental variable. This percentage falls below the reported values for AFLP-based genomic scans using DFDIST (5 - 10%; Nosil *et al.*, 2009). Several reasons may be responsible for the low percentage of outliers detected in our study. First, our study was conducted within a subspecies of American pika (*O. princeps fenisex*) and at a finer scale in comparison to previous work. Indeed, other studies have used populations

that were separated by several hundred kilometers (e.g. Nunes *et al.*, 2011), or at both continental and regional scales (e.g. Manel *et al.*, 2010). The only comparable study to our knowledge is that of Bonin *et al* (2006), which focused on common frog populations sampled along an altitudinal gradient and recovered a similar percentage of outliers as those reported here. Second, I chose to use the most stringent significance levels permitted by the outlier detection analyses to minimize our false discovery rate, incorporating corrections for multiple testing where applicable. With these precautions in mind, the low level of outliers detected here may not represent an unusually low level of loci under selection, but rather, previous studies at similar scales may have contained a higher percentage of false positives (Hermisson, 2009; Perez-Figueroa *et al.*, 2010).

In that vein, I used the algorithm implemented in ARLEQUIN, which takes hierarchical population structure into account in the detection of outlier loci. This approach performed similarly to MCHEZA, which does not account for population structure and implements a finite island model. The two approaches were mostly consistent with one another, with two loci being identified by the latter, but not the former. These two loci may thus represent false positives caused by the use of an over simplistic model of the underlying population genetic structure present in this system.

As previously reported (Perez-Figuera *et al.* 2010; Narum & Hess, 2011), BAYESCAN was the most conservative of all approaches used, detecting five loci under positive selection across the longitudinal sampling, four of which were also detected by at least one other approach. Perez-Figueroa e*t al.* (2010) suggested that a true outlier locus is one that is identified by multiple, independent methods. Using that criterion, I may have identified four true outliers and one false positive (see Fig. 3 in; Perez-Figueroa *et al.*, 2010). The use of coincident outliers detected by multiple, independent methods may provide a conservative approach for detecting true outliers to

be targeted for further analyses, such as linking candidate loci with genomic regions or gene sequences in order to understand the functional basis of adaptation (Storz and Wheat, 2010).

The spatial analysis method identified loci that were significantly correlated with several environmental variables. Four out of six loci were correlated with mean annual precipitation, which may be considered the main driver of population differentiation across this longitudinal gradient ranging from the wet coast to the dry interior. Targeting these loci as candidates for further analyses may shed light on the genomic region or identity of the gene responsible for adaptations to different environmental conditions in this species. For example, locus E31T43_104 displayed a negative association with mean annual precipitation (Fig. 4.2), and was entirely absent from populations experiencing high amounts of precipitation. Identifying the functional gene represented by or linked to this locus would enable us to move from correlation to causation.

4.4.2. Detection of outlier loci across elevation gradients

While differences in sampling design for each transect has precluded the use of all the analyses described above, our sample still enabled us to make meaningful comparisons with regards to differences in the genetic basis of adaptation across multiple elevation gradients. As a general trend, each elevation gradient was characterized by its own set of outlier loci. The populations from the Hill displayed the largest amount of outliers with 1.3% of the genome identified as outlier loci by at least one approach, followed by Nusatsum (1.2%) and Bentinck (0.2%). This discrepancy between proportion of outliers may be due to sample size differences, and is correlated with sample size as the Bentinck populations had the lowest sample size. As in

the above analysis including all the populations, MCHEZA identified the bulk of the outlier loci (see results), with BAYESCAN identifying a single and different outlier locus per elevation transect.

Within the Hill transect, six outliers were found to be correlated with mean annual temperature, mean annual precipitation, precipitation as snow, and summer mean maximum temperature. When analyzed separately, two loci (E43T43_80 and E38T32_136) showed significant and strong correlation with summer mean maximum temperature and mean annual precipitation, respectively. Interestingly, one of these (E43T43_80) displayed a negative relationship with summer mean maximum temperature, representing a promising candidate for further studying a potential association with adaptation to cold tolerance. In contrast, E38T32_136 showed the opposite trend, potentially representing a variant associated with adaptation to warmer environmental conditions. In a conservation context, these loci could be screened in other populations and their relative frequencies may be used to calculate a population adaptive index, which may help prioritize populations for conservation action with regards to anticipated climate changes (Bonin *et al.*, 2007a; Sgro *et al.*, 2011).

Although different outliers were detected across different elevation gradients, it is unknown to what extent these different loci overlap with respect to genomic location and putative function. AFLP-based genomic scans are a repeatable and cost effective way to screen the genome of non-model organisms, and represent a feasible way to identify regions under selection. Yet, the fact that AFLP markers are anonymous precludes the identification of the genes responsible for the observed adaptive divergence. Additional work is necessary to isolate, clone and sequence these fragments with the aim to identify candidate genes associated with local adaptation. Thus the present genomic scan represents a first broad attempt at characterizing adaptive population

divergence in *O. princeps*, and follow up studies that will target these candidate loci for sequencing as well as additional studies incorporating the use of novel high throughput sequencing technologies (e.g. Ekblom and Galindo, 2011; Helyar *et al.*, 2011) will likely yield additional knowledge, especially in terms of the chromosomal location and biochemical pathways involved in local adaptation in this system.

4.5. Summary

The present study represents one of the first parallel uses of population and landscape genomic approaches to detect candidate loci under positive selection in a wildlife species potentially threatened by climate change. I illustrate the complementary nature of both approaches in identifying candidates responsible for local adaptation. Because these approaches are prone to false positives, I opted to use the most stringent thresholds available in each method. Strong candidates retained for further analyses were those detected by multiple approaches. Based on this rationale, an unusually small proportion of outlier loci were identified compared to previous published studies. Two main environmental variables, mean annual precipitation and summer mean maximum temperatures were identified as main forces associated with adaptive divergence in this system. The use of the candidate loci that demonstrated strong correlation with these environmental variables may represent an additional tool to inform conservation action for this climate-change sensitive species.

Chapter 5. Conclusion

The present dissertation represents one of the first applications of conservation genetics, conservation genomics and landscape genomics in a climate change-sensitive mammal. Additionally, it is also one of the first studies of American pikas at their Northwestern range margin. In Chapter 2, I described a sampling technique that is minimally invasive and represents a first for this species, since the majority of the work to date has involved live trapping. Within the scope of a conservation project, it is becoming increasingly important, given the ethical guidelines of animal care committees, to use the technique that is most appropriate for the species under investigation and the purpose of the study. Given that live trapping may produce high stress situations and potentially the death of the sampled animal, developing a noninvasive methodology was key to undertaking the work presented here. I anticipate that future conservation genetic studies using pikas or other small mammals as a case species will benefit from using the sampling approach described here. In that vein, collaborating on the production of an open access video article that describes the sampling strategy and DNA extraction protocol (which has gathered almost 8'000 views since April 2011) will greatly enhance the impact of this research on the scientific community.

In Chapter 3, I used neutral microsatellite markers to shed light on the population genetic structure and dispersal in American pikas. This work enabled me to begin to answer the following question: are pikas most likely to disperse to more favorable conditions, or adapt *in situ*? I detected low levels of genetic variation in my sampled populations compared to that previously reported in more southerly latitudes. I found evidence for limited gene flow, even in geographically proximate populations, suggesting local adaptation as the most likely option

available to American pikas as environments change. Of particular note, I used a recently developed analytical tool called Discriminant Analysis of Principal Components (DAPC) to shed light on patterns of population structure. This approach is free from the underlying assumptions of classical population genetics, specifically the Hardy-Weinberg Equilibrium (*HWE*). While most approaches investigating population structure have thus far assumed *HWE* within samples, this assumption is often not realistic in natural populations, especially in species characterized by small population sizes. Thus, the work presented in Chapter 3 may stimulate others scientists that work with endangered taxa or peripheral populations to use similar methodologies. This will likely bring about more accurate knowledge of these species' population genetic structures, resulting in more realistic management plans. Although knowledge on the imperilment of American pika has previously been demonstrated in the light of continued climate change, no work had yet identified limited dispersal as a potential threat to this species. Consequently, the information gathered in Chapter 3 may have practical conservation implications. In particular, the lack of gene flow across elevation gradients may require that assisted migration of pre-adapted genotypes become necessary for American pika to survive in the face of climate change.

In Chapter 4, I used a genomic scan approach to detect loci under selection in pika populations inhabiting steep environmental gradients. This space-for-time approach in which elevation gradients are used as possible surrogates of forecasted climate changes has, to my knowledge, never been undertaken in small mammals. The strengths of this Chapter resides in the use of multiple elevation transects to shed light on the independent evolutionary trajectories of populations from distinct populations in the study area, and the combined use of multiple analytical approaches (population and landscape genomics) to detect loci under positive selection. This approach represents a rigorous and novel tool to limit the amount of false positives detected in the data, a phenomenon known to limit the informativeness of genomic

scans. Indeed, the use of F_{ST}–based outlier detection techniques coupled with regression analyses of AFLP band frequency with selected environmental variables enabled the identification of: i) loci that are potentially under positive selection and ii) the environmental factors possibly driving selection in this system. As these approaches are mainly correlative in nature, other biotic or abiotic covariates may also have contributed to the observed patterns, further experimental studies will be necessary to identify causative agents. Yet, this information will be useful in future studies of pika conservation genomics as it will serve as a stepping stone to build upon a research program that aims to identify the functional basis of adaptation in this system (i.e. gene and pathways responsible for adaptations to novel conditions). Other directions may include complementary experimental studies to test alternative hypotheses of pika persistence, such as reciprocal transplant experiments between elevations, followed by a monitoring of allele frequencies over generations.

On a practical note with regards to loci under selection, additional work is still required to bridge the gap between outlier behavior and functional genes. This work is currently being undertaken and will involve the isolation, cloning and sequencing of the most promising candidate loci for selection identified in Chapter 4. Other future directions based on the work presented here will include the use of next-generation transcriptome sequencing to provide additional genomic information based on genes of functional significance found in populations distributed along these elevation gradients. This technique will lead to the discovery of Single Nucleotide Polymorphism (SNP) markers, which are rapidly replacing microsatellites and AFLPs as the markers of choice in conservation genomics. This novel approach is currently being used in American pikas from the Bella Coola valley to develop a large number of SNPs that will be screened in populations range-wide using a similar analytical framework. This approach will also inform assessments of the relative strength and weaknesses of AFLP and SNP-based genomic

scans with respect to identifying loci under selection and shedding light on population genetic structure.

Overall, the work presented here represents a snapshot of the neutral and adaptive genetic composition of American pika populations from the central Coast Mountains of BC. Additional studies conducted at larger scales, or at similar scales at different locations in their range, will shed light on whether the patterns observed here can be extended to pika populations in other parts of their range. While still in its early days, conservation genomics will likely find a place in the management of sensitive taxa in order to provide evolutionary insight into conservation practices at increasingly finer scales. The example of the American pika provided here presents a valuable case study for conservation practice in a changing world.

References

Amendola-Pimenta M, Garcia-Feria L, Serio-Silva JC, and Rico-Gray V (2009) Noninvasive collection of fresh hairs from free-ranging howler monkeys for DNA extraction. *American Journal of Primatology* **71**, 359-363.

Antao T, and Beaumont MA (2011) MCHEZA: a workbench to detect selection using dominant markers. *Bioinformatics* **27**, 1717-1718.

Banks SC, Hoyle SD, Horsup A, Sunnucks P, and Taylor AC (2003) Demographic monitoring of an entire species (the northern hairy-nosed wombat, *Lasiorhinus krefftii*) by genetic analysis of non-invasively collected material. *Animal Conservation* **6**, 101-107.

Beaumont MA, and Balding DJ (2004) Identifying adaptive genetic divergence among populations from genome scans. *Molecular Ecology* **13**, 969-980.

Beaumont MA, and Nichols RA (1996) Evaluating loci for use in the genetic analysis of population structure. *Proceedings of the Royal Society London B* **263**, 1619-1625.

Beever EA, Brussard PE, and Berger J (2003) Patterns of apparent extirpation among isolated populations of pikas (*Ochotona princeps*) in the Great Basin. *Journal of Mammalogy* **84**, 37-54.

Beever EA, Ray C, Mote PW, and Wilkening JL (2010) Testing alternative models of climate-mediated extirpations. *Ecological Applications* **20**, 164-178.

Beever EA, Ray C, Wilkening JL, Brussard PE, and Mote PW (2011) Contemporary climate change alters the pace and drivers of extinction. *Global Change Biology* **17**, 2054-2070.

Beever EA, and Smith AT (2008) *Ochotona princeps.* In: *2008 IUCN Red List of Threatened Species*.

Beever EA, Wilkening JL, McIvor DE, Weber SS, and Brussard PE (2008) American pikas (*Ochotona princeps*) in northwestern Nevada: A newly discovered population at a low-elevation site. *Western North American Naturalist* **68**, 8-14.

Beja-Pereira A, Oliveira R, Alves PC, Schwartz MK, and Luikart G (2009) Advancing ecological understandings through technological transformations in noninvasive genetics. *Molecular Ecology Resources* **9**, 1279-1301.

Bensch S, and Akesson M (2005) Ten years of AFLP in ecology and evolution: why so few animals? *Molecular Ecology* **14**, 2899-2914.

Bienengreber M, Oczan C, Terzic A (2002) Stable transfection of UCP1 confers resistance to hypoxia/reoxygenation in a heart-derived cell line. **35**, 861-865

Bilgin R (2007) Kgtests: a simple Excel Macro program to detect signatures of population expansion using microsatellites. *Molecular Ecology Notes* **7**, 416-417.

Bonin A, Bellemain E, Eidesen PB*, et al.* (2004) How to track and assess genotyping errors in population genetics studies. *Molecular Ecology* **13**, 3261-3273.

Bonin A, Pompanon F, and Taberlet P (2005) Use of amplified fragment length polymorphism (AFLP) markers in surveys of vertebrate diversity. *Molecular Evolution: Producing the Biochemical Data, Part B* **395**, 145-161.

Bonin A, Taberlet P, Miaud C, and Pompanon F (2006) Explorative genome scan to detect candidate loci for adaptation along a gradient of altitude in the common frog (*Rana temporaria*). *Molecular Biology and Evolution* **23**, 773-783.

Bonin A, Nicole F, Pompanon F, Miaud C, and Taberlet P (2007a) Population adaptive index: a new method to help measure intraspecific genetic diversity and prioritize populations for conservation. *Conservation Biology* **21**, 697-708.

Bonin A, Ehrich D, and Manel S (2007b) Statistical analysis of amplified fragment length polymorphism data: a toolbox for molecular ecologists and evolutionists. *Molecular Ecology* **16**, 3737-3758.

Brandt CA (1983) Den location by and reproductive success of pikas, a resource defence mating system. *American Zoologist* **23**, 932-932.

Brandt CA (1989) Mate choice and reproductive success of pikas. *Animal Behaviour* **37**, 118-132.

Bremner-Harrison S, Harrison SWR, Cypher BL*, et al.* (2006) Development of a single-sampling noninvasive hair snare. *Wildlife Society Bulletin* **34**, 456-461.

Brooks TM, Mittermeier RA, da Fonseca GAB*, et al.* (2006) Global biodiversity conservation priorities. *Science* **313**, 58-61.

Broquet T, and Petit E (2004) Quantifying genotyping errors in noninvasive population genetics. *Molecular Ecology* **13**, 3601-3608.

Brown RN, Southwick CH, and Golian SC (1989) Male-female spacing, territorial replacement, and the mating system of pikas (*Ochotona princeps*). *Journal of Mammalogy* **70**, 622-627.

Butchart SHM, Stattersfield AJ, and Collar NJ (2006) How many bird extinctions have we prevented? *Oryx* **40**, 266-278.

Campbell D, and Bernatchez L (2004) Genomic scan using AFLP markers as a means to assess the role of directional selection in the divergence of sympatric whitefish ecotypes. *Molecular Biology and Evolution* **21**, 1164-1164.

Clinchy M, Haydon DT, and Smith AT (2002) Pattern does not equal process: What does patch occupancy really tell us about metapopulation dynamics? *American Naturalist* **159**, 351-362.

Connior MB, and Risch TS (2009) Live trap for pocket gophers. *Southwestern Naturalist* **54**, 100-103.

Costanza R, dArge R, deGroot R*, et al.* (1997) The value of the world's ecosystem services and natural capital. *Nature* **387**, 253-260.

Daily GC (1997) Nature's services: societal dependence on natural ecosystems. Island Press, Washington, DC.

Daly C, Gibson WP, Taylor GH, Johnson GL, and Pasteris P (2002) A knowledge-based approach to the statistical mapping of climate. *Climate Research* **22**, 99-113.

Davis MB, and Shaw RG (2001) Range shifts and adaptive responses to Quaternary climate change. *Science* **292**, 673-679.

Davis MB, Shaw RG, and Etterson JR (2005) Evolutionary responses to changing climate. *Ecology* **86**, 1704-1714.

DeSalle R, and Amato G (2004) The expansion of conservation genetics. *Nature Reviews Genetics* **5**, 702-712.

DeYoung RW, and Honeycutt RL (2005) The molecular toolbox: Genetic techniques in wildlife ecology and management. *Journal of Wildlife Management* **69**, 1362-1384.

Dietz T, Rosa EA, and York R (2007) Driving the human ecological footprint. *Frontiers in Ecology and the Environment* **5**, 13-18.

Echtay, K. S, Murpy, M. P., Smith, R. A., Talbot, D. A. and Brand, M D. (2002) Superoxide activates mitochondrial uncoupling protein 2 from the matrix side Studies using targeted antioxidants. *Journal of Biochemistry* **277**, 47129-47135

Ehrich D (2006) AFLPDAT: a collection of R functions for convenient handling of AFLP data. *Molecular Ecology Notes* **6**, 603-604.

Ekblom R, and Galindo J (2011) Applications of next generation sequencing in molecular ecology of non-model organisms. *Heredity* **107**, 1-15.

Enerback, C, Martinsson, T and Inerot, A (1997) Evidence that HLA-Cw6 determines early onset of psoriasos, obtained using sequence-specific primer (PCR-SSP). *Acta Dermato-Veneologica* **77**, 273-276

Erb, LP, Ray, C, and Guralnick R (2011) On the generality of a climate-mediated shift in the distribution of the American pika (*Ochotona princeps*). Ecology **92**, 1730-1735.

Estes-Zumpf WA, Rachlow JL, Waits LP, and Warheit KI (2010) Dispersal, gene flow, and population genetic structure in the pygmy rabbit (*Brachylagus idohoensis*). *Journal of Mammalogy* **91**, 208-219.

Excoffier L, and Heckel G (2006) Computer programs for population genetics data analysis: a survival guide. *Nature Reviews Genetics* **7**, 745-758.

Excoffier L, Hofer T, and Foll M (2009) Detecting loci under selection in a hierarchically structured population. *Heredity* **103**, 285-298.

Excoffier L, and Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* **10**, 564-567.

Foll M, and Gaggiotti O (2006) Identifying the environmental factors that determine the genetic structure of Populations. *Genetics* **174**, 875-891.

Foll M, and Gaggiotti O (2008) A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: a Bayesian perspective. *Genetics* **180**, 977-993.

Fontanesi L, Tazzoli M, Pecchioli E*, et al.* (2008) Sexing European rabbits (*Oryctolagus cuniculus*), European brown hares (*Lepus europaeus*) and mountain hares (*Lepus timidus*) with ZFX and ZFY loci. *Molecular Ecology Resources* **8**, 1294-1296.

Fox J (2005) The R commander: A basic-statistics graphical user interface to R. *Journal of Statistical Software* **14**.

Frankel OH, and Soulé ME (1981) *Conservation and evolution* Cambridge University Press, Cambridge, UK.

Frankham R (2003) Genetics and conservation biology. *Comptes Rendus Biologies* **326**, S22-S29.

Gaggiotti OE, Bekkevold D, Jorgensen HBH*, et al.* (2009) Disentangling the effects of evolutionary, demographic, and environmental factors influencing genetic structure of natural populations: atlantic herring as a case study. *Evolution* **63**, 2939-2951.

Galbreath KE, Hafner DJ, and Zamudio KR (2009) When cold is better: climate-driven elevation shifts yield complex patterns of diversification and demography in an alpine specialist (American Pika, *Ochotona princeps*). *Evolution* **63**, 2848-2863.

Galbreath KE, Hafner DJ, Zamudio KR, and Agnew K (2010) Isolation and introgression in the Intermountain West: contrasting gene genealogies reveal the complex biogeographic history of the American pika (*Ochotona princeps*). *Journal of Biogeography* **37**, 344-362.

Garza JC, and Williamson EG (2001) Detection of reduction in population size using data from microsatellite loci. *Molecular Ecology* **10**, 305-318.

Gebremedhin B, Ficetola GF, Naderi S*, et al.* (2009) Frontiers in identifying conservation units: from neutral markers to adaptive genetic variation. *Animal Conservation* **12**, 107-109.

Golian SC, and Whitworth MR (1985) Growth of Pikas (*Ochotona princeps*) in Colorado. *Journal of Mammalogy* **66**, 367-371.

Gomez A, Wright PJ, Lunt DH*, et al.* (2007) Mating trials validate the use of DNA barcoding to reveal cryptic speciation of a marine bryozoan taxon. *Proceedings of the Royal Society B-Biological Sciences* **274**, 199-207.

Goncalves da Silva A, Lalonde DR, Quse V, Shoemaker A, and Russello MA (2010) Genetic Approaches Refine Ex Situ Lowland Tapir (*Tapirus terrestris*) Conservation. *Journal of Heredity* **101**, 581-590.

Goudet J (1995) FSTAT version 1.2: a computer program to calculate F-statistics. *Journal of Heredity* **86**, 485-486.

Goudet J, Raymond M, de Meeûs T, Rousset F (1996) Testing differentiation in diploid populations. *Genetics* **144**, 1933-1940.

Grayson DK (2005) A brief history of Great Basin pikas. *Journal of Biogeography* **32**, 2103-2111.

Grinnell J (1917) Field tests of theories concerning distributional control. *American Naturalist* **51**, 115-128.

Haag T, Santos AS, De Angelo C*, et al.* (2009) Development and testing of an optimized method for DNA-based identification of jaguar (*Panthera onca*) and puma (*Puma concolor*) faecal samples for use in ecological and genetic studies. *Genetica* **136**, 505-512.

Hafner DJ (1993) North-American Pika (*Ochotona princeps*) as a Late Quaternary Biogeographic Indicator Species. *Quaternary Research* **39**, 373-380.

Hafner DJ, and Smith AT (2010) Revision of the subspecies of the American pika, *Ochotona princeps* (Lagomorpha: Ochotonidae). *Journal of Mammalogy* **91**, 401-417.

Hafner DJ, and Sullivan RM (1995) Historical and ecological biogeography of Nearctic pikas (Lagomorpha, Ochotonidae). *Journal of Mammalogy* **76**, 302-321.

Hardison, R (1998) Hemoglobins from bacteria to man: Evolution of different patterns of gene expression. *Experimental Biology* **201**, 1099-1117

Hastings A (1993) Complex Interactions between Dispersal and Dynamics - Lessons from Coupled Logistic Equations. *Ecology* **74**, 1362-1372.

Hedmark E, Flagstad O, Segerstrom P*, et al.* (2004) DNA-based individual and sex identification from wolverine (*Gulo gulo*) faeces and urine. *Conservation Genetics* **5**, 405-410.

Helyar SJ, Hemmer-Hansen J, Bekkevold D*, et al.* (2011) Application of SNPs for population genetics of nonmodel organisms: new opportunities and challenges. *Molecular Ecology Resources* **11**, 123-136.

Henry P, Henry A, and Russello MA (Submitted-a) Significant variation in American pika habitat characteristics along an elevation gradient at their northern range margin. *Northwest Science*.

Henry P, Sim Z, and Russello MA (Submitted-b) Genetic evidence for highly restricted dispersal along continuous altitudinal gradients in the climate-change sensitive American pika (*Ochotona princeps*). *PLoS One*.

Henry P, and Russello MA (2011) Obtaining high quality DNA from elusive small mammals using low-tech hair snares. *European Journal of Wildlife Research* **57**, 429 - 435.

Henry P, Henry A, and Russello MA (2011) A Noninvasive Hair Sampling Technique to Obtain High Quality DNA from Elusive Small Mammals. *JoVE* **49**.

Henry P, Miquelle D, Sugimoto T*, et al.* (2009) In situ population structure and ex situ representation of the endangered Amur tiger. *Molecular Ecology* **18**, 3173-3184.

Hermisson J (2009) Who believes in whole-genome scans for selection? *Heredity* **103**, 283-284.

Herrmann D, Poncet BN, Manel S*, et al.* (2010) Selection criteria for scoring amplified fragment length polymorphisms (AFLPs) positively affect the reliability of population genetic parameter estimates. *Genome* **53**, 302-310.

Hewitt GM, and Nichols RA (2005) Genetic and Evolutionary Impacts of Climate Change. In: *Climate Change and Biodiversity* (eds. Lovejoy TE, andHannah L), pp. 176-192. Yale University Press, New Haven, CT.

Hoekstra HE (2006) Genetics, development and evolution of adaptive pigmentation in vertebrates. *Heredity* **97**, 222-234.

Hoffmann AA, and Parsons PA (1997) *Extreme Environmental Change and Evolution* Cambridge University Press, Cambridge, UK.

Hoffmann M, Hilton-Taylor C, Angulo A*, et al.* (2010) The Impact of Conservation on the Status of the World's Vertebrates. *Science* **330**, 1503-1509.

Hoffmann RS, and Smith AT (2005) Order Lagomorpha. In: *Mammal Species of the World* (eds. Wilson DE, andReeder DM), pp. 185-211. The Johns Hopkins University Press, Baltimore.

IPCC (2007) IPCC Fourth Assessment Report: Climate Change 2007. Cambridge University Press, Cambridge, UK.

Johnson PCD, and Haydon DT (2007) Maximum-likelihood estimation of allelic dropout and false allele error rates from Microsatellite genotypes in the absence of reference data. *Genetics* **175**, 827-842.

Jombart T (2008) ADEGENET: a R package for the multivariate analysis of genetic markers. *Bioinformatics* **24**, 1403-1405.

Jombart T, Devillard S, and Balloux F (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genetics* **11**, 94.

Joost S, Bonin A, Bruford MW*, et al.* (2007) A spatial analysis method (SAM) to detect candidate loci for selection: towards a landscape genomics approach to adaptation. *Molecular Ecology* **16**, 3955-3969.

Joost S, Kalbermatten M, and Bonin A (2008) Spatial analysis method (SAM): a software tool combining molecular and environmental data to identify candidate loci for selection. *Molecular Ecology Resources* **8**, 957-960.

Jump AS, Hunt JM, Martinez-Izquierdo JA, and Penuelas J (2006) Natural selection and climate change: temperature-linked spatial and temporal trends in gene frequency in *Fagus sylvatica*. *Molecular Ecology* **15**, 3469-3480.

Kendall KC, and McKelvey KS (2008) Hair collection. In: *In: Noninvasive Survey Methods for North American Carnivores* (eds. Long RA, MacKay P, Ray JC, andZielinski WJ), pp. 135-176. Island Press, Washington, DC.

Kendall KC, Stetz JB, Boulanger J*, et al.* (2009) Demography and Genetic Structure of a Recovering Grizzly Bear Population. *Journal of Wildlife Management* **73**, 3-17.

Keyghobadi N, Crawford LA, and Maxwell SA (2009) Successful analysis of AFLPs from non-lethally sampled wing tissues in butterflies. *Conservation Genetics* **10**, 2021-2024.

Kirk SL, and Russello MA (Submitted) Detection of outlier loci and their utility for fisheries management. *Evolutionary Applications*.

Kitao, N., Yahata, T., Matsumoto, T., Okamatsu-Oguru, Y., Omachi, A., Kimura, K. and Saito, M. (2007) Molecular cloning and tissue distribution of uncoupling protein 1 (UCP1) in plateau pika (*Ochotona dauurica*). *Biochemistry* **69**, 1065-1068

Lanier HC, and Olson LE (2009) Inferring divergence times within pikas (*Ochotona* spp.) using mtDNA and relaxed molecular dating techniques. *Molecular Phylogenetics and Evolution* **53**, 1-12.

Lewontin RC, and Krakauer J (1973) Distribution of Gene Frequency as a Test of Theory of Selective Neutrality of Polymorphisms. *Genetics* **74**, 175-195.

Litvaitis JA, Tash JP, Litvaitis MK*, et al.* (2006) A range-wide survey to determine the current distribution of New England cottontails. *Wildlife Society Bulletin* **34**, 1190-1197.

Lovejoy TE, and Hannah LJ (2005) *Climate change and biodiversity* Yale University Press, New Haven, CT.

Luikart G, England PR, Tallmon D, Jordan S, and Taberlet P (2003) The power and promise of population genomics: from genotyping to genome typing. *Nature Reviews Genetics* **4**, 981-994.

Macarthur RA, and Wang LCH (1974) Behavioral thermoregulation in pika *Ochotona princeps* - field study using radiotelemetry. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* **52**, 353-358.

Manchester KL (1995) Value of a(260)/a(280) Ratios for measurement of purity of nucleic acids. *Biotechniques* **19**, 208-210.

Manel S, Poncet BN, Legendre P, Gugerli F, and Holderegger R (2010) Common factors drive adaptive genetic variation at different spatial scales in *Arabis alpina*. *Molecular Ecology* **19**, 3824-3835.

Markert JA, Champlin DM, Gutjahr-Gobell R*, et al.* (2010) Population genetic diversity and fitness in multiple environments. *BMC Evolutionary Biology* **10**, 98.

Mckechnie AM, Smith AT, and Peacock MM (1994) Kleptoparasitism in pikas (*Ochotona princeps*) - theft of hay. *Journal of Mammalogy* **75**, 488-491.

Merideth SJ (2002) *The Impact of Habitat Spatila Structure on Pika (Ochotona princeps) Dispersal Dynamics*, MSc Thesis, University of Nevada Reno.

Meudt HM, and Clarke AC (2007) Almost forgotten or latest practice? AFLP applications, analyses and advances. *Trends in Plant Science* **12**, 106-117.

Millar, CI, and Westfall, RD (2010) Distribution and climatic relationships of the American pika (*Ochotona princeps*) in the Sierra Nevada and western Great Basin, U.S.A.; periglacial landforms as refugia in warming climates. *Artic, Antarctic, and Alpine Research,* **42**, 76-88.

Mitrovski P, Heinze DA, Guthridge K, and Weeks AR (2005) Isolation and characterization of microsatellite loci from the Australian endemic mountain pygmy-possum, *Burramys parvus* Broom. *Molecular Ecology Notes* **5**, 395-397.

Moilanen A, Smith AT, and Hanski I (1998) Long-term dynamics in a metapopulation of the American pika. *American Naturalist* **152**, 530-542.

Morin PA, Chambers KE, Boesch C, and Vigilant L (2001) Quantitative polymerase chain reaction analysis of DNA from noninvasive samples for accurate microsatellite genotyping of wild chimpanzees (*Pan troglodytes verus*). *Molecular Ecology* **10**, 1835-1844.

Morin PA, Leduc RG, Archer FI*, et al.* (2009) Significant deviations from Hardy-Weinberg equilibrium caused by low levels of microsatellite genotyping errors. *Molecular Ecology Resources* **9**, 498-504.

Moyes K, Nussey DH, Clements MN*, et al.* (2011) Advancing breeding phenology in response to environmental change in a wild red deer population. *Global Change Biology* **17**, 2455 - 2469.

Moyer-Horner, L (2010) Effects of temperature on American pika activity. Presentation at the 1st North American pika conference, Teton Schools of Science, Jackson, WY, 25-27 March 2010.

Mullins J, Statham MJ, Roche T, Turner PD, and O'Reilly C (In Press) Remotely plucked hair genotyping: a reliable and non-invasive method for censusing pine marten (*Martes martes*, L. 1758) populations. *European Journal of Wildlife Research*.

Mullis K, Faloona F, Scharf S*, et al.* (1986) Specific enzymatic amplification of DNA in vitro: The Polymerase Chain Reaction. *Cold Spring Harbor Symposium on Quantitative Biology* **51**, 263.

Myer N, and Pimm S (2003) The Last Extinction? *Foreign Policy* **135**.

Nachman MW (2005) The genetic basis of adaptation: lessons from concealing coloration in pocket mice. *Genetica* **123**, 125-136.

Nielsen R (2005) Molecular signatures of natural selection. *Annual Review of Genetics* **39**, 197-218.

Niu YD, Wei FW, Li M, Liu XM, and Feng ZJ (2004) Phylogeny of pikas (Lagomorpha, Ochotona) inferred from mitochondrial cytochrome b sequences. *Folia Zoologica* **53**, 141-155.

Nosil P, Egan SP, and Funk DJ (2008) Heterogeneous genomic differentiation between walking-stick ecotypes: "Isolation by adaptation" and multiple roles for divergent selection. *Evolution* **62**, 316-336.

Nosil P, Funk DJ, and Ortiz-Barrientos D (2009) Divergent selection and heterogeneous genomic divergence. *Molecular Ecology* **18**, 375-402.

Novacek MJ, and Cleland EE (2001) The current biodiversity extinction event: Scenarios for mitigation and recovery. *Proceedings of the National Academy of Sciences of the United States of America* **98**, 5466-5470.

Nunes VL, Beaumont MA, Butlin RK, and Paulo OS (2011) Multiple approaches to detect outliers in a genome scan for selection in ocellated lizards (*Lacerta lepida*) along an environmental gradient. *Molecular Ecology* **20**, 193-205.

Ohtani K (2000) Bootstrapping R^2 and adjusted R^2 in regression analysis. *Economic Modelling* **17**, 473-483.

Orr HA, and Coyne JA (1992) The Genetics of adaptation - a reassessment. *American Naturalist* **140**, 725-742.

Paetkau D (2003) An empirical exploration of data quality in DNA-based population inventories. *Molecular Ecology* **12**, 1375-1387.

Palmeiri F (1994) Mitochondrial carrier proteins. *FEBS Letters* **346**, 48-54

Papa R, Troggio M, Ajmone-Marsan P, and Marzano FN (2005) An improved protocol for the production of AFLP (TM) markers in complex genomes by means of capillary electrophoresis. *Journal of Animal Breeding and Genetics* **122**, 62-68.

Parmesan C (2006) Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology Evolution and Systematics* **37**, 637-669.

Parmesan C (2007) Influences of species, latitudes and methodologies on estimates of phenological response to global warming. *Global Change Biology* **13**, 1860-1872.

Parmesan C, and Yohe G (2003) A globally coherent fingerprint of climate change impacts across natural systems. *Nature* **421**, 37-42.

Pauli JN, Hamilton MB, Crain EB, and Buskirk SW (2008) A single-sampling hair trap for mesocarnivores. *Journal of Wildlife Management* **72**, 1650-1652.

Peacock MM (1997) Determining natal dispersal patterns in a population of North American pikas (Ochotona princeps) using direct mark-resight and indirect genetic methods. *Behavioral Ecology* **8**, 340-350.

Peacock MM, Kirchoff VS, and Merideth SJ (2002) Identification and characterization of nine polymorphic microsatellite loci in the North American pika, *Ochotona princeps*. *Molecular Ecology Notes* **2**, 360-362.

Peacock MM, and Smith AT (1997a) The effect of habitat fragmentation on dispersal patterns, mating behavior, and genetic variation in a pika (*Ochotona princeps*) metapopulation. *Oecologia* **112**, 524-533.

Peacock MM, and Smith AT (1997b) Nonrandom mating in pikas *Ochotona princeps*: evidence for inbreeding between individuals of intermediate relatedness. *Molecular Ecology* **6**, 801-811.

Peakall R, and Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* **6**, 288-295.

Perez-Figueroa A, Garcia-Pereira MJ, Saura M, Rolan-Alvarez E, and Caballero A (2010) Comparing three different methods to detect selective loci using dominant markers. *Journal of Evolutionary Biology* **23**, 2267-2276.

Piggott MP, Bellemain E, Taberlet P, and Taylor AC (2004) A multiplex pre-amplification method that significantly improves microsatellite amplification and error rates for faecal DNA in limiting conditions. *Conservation Genetics* **5**, 417-420.

Piggott MP, and Taylor AC (2003) Remote collection of animal DNA and its applications in conservation management and understanding the population biology of rare and cryptic species. *Wildlife Research* **30**, 1-13.

Pimentel D, and Pimentel M (2003) World population, food, natural resources, and survival. *World Futures* **59**, 145-167.

Pimm SL, and Raven P (2000) Biodiversity - Extinction by numbers. *Nature* **403**, 843-845.

Piry S, Luikart G, and Cornuet JM (1999) BOTTLENECK: A computer program for detecting recent reductions in the effective population size using allele frequency data. *Journal of Heredity* **90**, 502-503.

R Development Core Team (2011) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Ray AJ, Barsugli JJ, Wolter K, and EIsheid J (2010) Rapid-response climate assessment to support the FWS status review of the American Pika (ed. Laboratory NESR), Boulder, CO.

Réale D, McAdam AG, Boutin S, and Berteaux D (2003) Genetic and plastic responses of a northern mammal to climate change. *Proceedings of the Royal Society of London Series B-Biological Sciences* **270**, 591-596.

Reed DH, and Frankham R (2003) Correlation between fitness and genetic diversity. *Conservation Biology* **17**, 230-237.

Regnaut S, Lucas FS, and Fumagalli L (2006) DNA degradation in avian faecal samples and feasibility of non-invasive genetic studies of threatened capercaillie populations. *Conservation Genetics* **7**, 449-453.

Reich DE, Feldman MW, and Goldstein DB (1999) Statistical properties of two tests that use multilocus data sets to detect population expansions. *Molecular Biology and Evolution* **16**, 453-466.

Reusch TBH, and Wood TE (2007) Molecular ecology of global change. *Molecular Ecology* **16**, 3973-3992.

Rodhouse, T.J., Beever, E.A., Garrett, L.K., Irvine, K.M., Jeffress, M.R., Munts, M., and Ray, C. (2010) Distribution of American pikas in a low-elevation lava landscape: conservation implications from the range periphery. *Journal of Mammalogy* **91**, 1287-1299.

Rodrigues ASL (2006) Are global conservation efforts successful? *Science* **313**, 1051-1052.

Roon DA, Waits LP, and Kendall KC (2003) A quantitative evaluation of two methods for preserving hair samples. *Molecular Ecology Notes* **3**, 163-166.

Rudnick JA, Katzner TE, Bragin EA, and DeWoody JA (2007) Species identification of birds through genetic analysis of naturally shed feathers. *Molecular Ecology Notes* **7**, 757-762.

Russello MA, and Amato G (2001) Application of a noninvasive, PCR-Based test for sex identification in an endangered parrot, *Amazona guildingii*. *Zoo Biology* **20**, 41-45.

Russello MA, Glaberman S, Gibbs JP*, et al.* (2005) A cryptic taxon of Galapagos tortoise in conservation peril. *Biology Letters* **1**, 287-290.

Sastre N, Francino O, Lampreave G*, et al.* (2009) Sex identification of wolf (*Canis lupus*) using non-invasive samples. *Conservation Genetics* **10**, 555-558.

Schipper J, Chanson JS, Chiozza F*, et al.* (2008) The status of the world's land and marine mammals: Diversity, threat, and knowledge. *Science* **322**, 225-230.

Schuelke M (2000) An economic method for the fluorescent labeling of PCR fragments. *Nature Biotechnology* **18**, 233-234.

Sgro CM, Lowe AJ, and Hoffmann AA (2011) Building evolutionary resilience for conserving biodiversity under climate change. *Evolutionary Applications* **4**, 326-337.

Simpson WG (2009) American pikas inhabit low-elevation sites outside the specie's previously described bioclimatic envelope. *Western North American Naturalist* **69**, 243-250.

Sloane MA, Sunnucks P, Alpers D, Beheregaray LB, and Taylor AC (2000) Highly reliable genetic identification of individual northern hairy-nosed wombats from single remotely collected hairs: a feasible censusing method. *Molecular Ecology* **9**, 1233-1240.

Smith AT (1974a) Distribution and Dispersal of Pikas - Consequences of Insular Population Structure. *Ecology* **55**, 1112-1119.

Smith AT (1974b) Distribution and Dispersal of Pikas - Influences of Behavior and Climate. *Ecology* **55**, 1368-1376.

Smith AT (1978) Comparative Demography of Pikas (Ochotona) - Effect of Spatial and Temporal Age-Specific Mortality. *Ecology* **59**, 133-139.

Smith AT (2008) The World of Pikas. In: *Lagomorph Biology: Evolution, Ecology, and Conservation* (eds. Alves PC, Ferrand N, andHackländer K), pp. 89-102. Springer-Verlag, Berlin.

Smith AT, and Ivins BL (1983a) Reproductive Tactics of Pikas - Why Have 2 Litters. *Canadian Journal of Zoology-Revue Canadienne De Zoologie* **61**, 1551-1559.

Smith AT, and Ivins BL (1983b) Colonization in a Pika Population - Dispersal Vs Philopatry. *Behavioral Ecology and Sociobiology* **13**, 37-47.

Smith AT, and Ivins BL (1986) Territorial Intrusions by Pikas (*Ochotona princeps*) as a Function of Occupant Activity. *Animal Behaviour* **34**, 392-397.

Smith AT, Weidong L, and Hik DS (2004) Pikas as Harbingers of Global Warming. *Species* **41**, 4-5.

Smith AT, and Weston ML (1990) Ochotona princeps. *Mammalian Species* **352**, 1-8.

Spielman D, Brook BW, and Frankham R (2004) Most species are not driven to extinction before genetic factors impact them. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 15261-15264.

Stinchcombe JR, and Hoekstra HE (2008) Combining population genomics and quantitative genetics: finding the genes underlying ecologically important traits. *Heredity* **100**, 158-170.

Stockwell CA, Hendry AP, and Kinnison MT (2003) Contemporary evolution meets conservation biology. *Trends in Ecology & Evolution* **18**, 94-101.

Storz JF, and Wheat CW (2010) Integrating evolutionary and functional approaches to infer adaptation at specific loci. *Evolution* **64**, 2489-2509.

Sunnucks P (2000) Efficient genetic markers for population biology. *Trends in Ecology & Evolution* **15**, 199-203.

Taberlet P, Griffin S, Goossens B*, et al.* (1996) Reliable genotyping of samples with very low DNA quantities using PCR. *Nucleic Acids Research* **24**, 3189-3194.

Taberlet P, Waits LP, and Luikart G (1999) Noninvasive genetic sampling: look before you leap. *Trends in Ecology & Evolution* **14**, 323-327.

Thioulouse J, and Dray S (2007) Interactive multivariate data analysis in R with the ade4 and ade4TkGUI packages. *Journal of Statistical Software* **22**, 1-14.

Thomas CD, Cameron A, Green RE*, et al.* (2004) Extinction risk from climate change. *Nature* **427**, 145-148.

Toth M (2008) A new noninvasive method for detecting mammals from birds' nests. *Journal of Wildlife Management* **72**, 1237-1240.

Van Oosterhout C, Hutchinson WF, Wills DPM, and Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* **4**, 535-538.

Venter O, Brodeur NN, Nemiroff L*, et al.* (2006) Threats to endangered species in Canada. *Bioscience* **56**, 903-910.

Vitalis R, Dawson K, Boursot P, and Belkhir K (2003) DetSel 1.0: A computer program to detect markers responding to selection. *Journal of Heredity* **94**, 429-431.

Vitousek PM (1997) Human domination of Earth's ecosystems (vol 277, pg 494, 1997). *Science* **278**, 21-21.

Vos P, Hogers R, Bleeker M*, et al.* (1995) AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Research* **23**, 4407-4414.

Waits LP, and Paetkau D (2005) Noninvasive genetic sampling tools for wildlife biologists: A review of applications and recommendations for accurate data collection. *Journal of Wildlife Management* **69**, 1419-1433.

Walther GR, Post E, Convey P*, et al.* (2002) Ecological responses to recent climate change. *Nature* **416**, 389-395.

Wang T, Haman A, Spittlehouse D, and Aitken SN (2006) Development of scale-free climate data for western Canada for use in resource management. *International Journal of Climatology* **26**, 383-397.

Weir BS, and Cockerham CC (1984) Estimating F-Statistics for the Analysis of Population-Structure. *Evolution* **38**, 1358-1370.

Wilcove DS, Rothstein D, Dubow J, Phillips A, and Losos E (1998) Quantifying threats to imperiled species in the United States. *Bioscience* **48**, 607-615.

Willard JM, Lee DA, and Holland MM (1998) Recovery of DNA for PCR amplification from blood and forensic samples using chelating resin. In: *In: Forensic DNA Profiling Protocols* (eds. Lincoln PJ, andThomson J), pp. 9-18. Humana Press, Totowa, NJ.

Williams LM, and Oleksiak MF (2008) Signatures of selection in natural populations adapted to chronic pollution. *Bmc Evolutionary Biology* **8**, -.

Williamson-Natesan EG (2005) Comparison of methods for detecting bottlenecks from microsatellite loci. *Conservation Genetics* **6**, 551-562.

Wilson EO (1984) Biophila. Harward University Press.

Wilson GA, and Rannala B (2003) Bayesian inference of recent migration rates using multilocus genotypes. *Genetics* **163**, 1177-1191.

Woods JG, Paetkau D, Lewis D*, et al.* (1999) Genetic tagging of free-ranging black and brown bears. *Wildlife Society Bulletin* **27**, 616-627.

Yang Y, Cao Y, Jin G, Bai Z, Ma L, Yun H and Ge, R (2007) Molecular cloning and characterization of hemoglobin α and β chains from plateau pika (*Ochotona curoniae*) living at high altitude. *Gene* **403**, 118-123

Yin BF, Yang SM, Wei WH, and Zhang YM (2009) Male reproductive success in plateau pikas (*Ochotona curzoniae*): A microsatellite analysis. *Mammalian Biology* **74**, 344-350.

Yu N, Zheng CL, Zhang YP, and Li WH (2000) Molecular systematics of pikas (genus Ochotona) inferred from mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution* **16**, 85-95.

Zgurski JM, Davis CS, and Hik DS (2009) Isolation and characterization of microsatellite loci for the collared pika (*Ochotona collaris*) and their cross-amplification in five other Ochotona species. *Molecular Ecology Resources* **9**, 867-871.

Zhivotovsky LA (1999) Estimating population structure in diploids with multilocus dominant DNA markers. *Molecular Ecology* **8**, 907-913.

Appendices

Appendix 1. Significant variation in American pika habitat characteristics along an elevation gradient at their northern range margin

Temperature is known as an important environmental variable in shaping the distribution of species. The American pika has recently been identified as a climate change-sensitive species with the extirpation of a number of populations at the southern end of its range. While their limited tolerance to warm temperatures has previously been documented, some evidence now suggests that pikas are able to live outside of cool habitats with the discovery of low elevation pika populations. Here I seek to characterize the temperatures experienced by pikas living along an elevation gradient at the northern end of their distribution. Additionally I recorded temperatures both above and below the talus at one of our sites and tested the relationship between pika activity and temperature. Temperatures differed significantly along the elevation gradient with up to six degree Celsius differences from low to high elevation sites. Below talus temperatures were significantly lower than above talus temperatures at noon and during the afternoon and were significantly warmer than above temperatures in the morning and night, meaning that talus has insulator properties that guard against extreme temperatures. Lastly a significant negative relationship was observed between temperature and pika activity in the afternoon only. Here I show that although at the northern end of pika distribution, ambient temperatures often exceeded the threshold for acute heat stress in pikas, and suggest that behavioral thermoregulation or other adaptations may enable pikas to inhabit low elevation habitat that was previously thought as inhospitable to pikas.

The American pika (*Ochotona princeps*) is a small lagomorph discontinually distributed in mountainous regions of North America. This species is typically described as an obligate inhabitant of talus slopes found in cool moist microclimates of alpine or sub-alpine environments (Smith and Weston, 1990). The main reason for this environmental constraint is thought to be the sensitivity of the species to elevated ambient temperatures (Beever *et al.*, 2008a; Smith, 1974b) as well as acute temperatures (Beever et al. 2010). Interestingly, recent studies have demonstrated that American pikas can survive outside this bioclimatic envelope, as low-elevation populations of the species have been documented in the Columbia River Gorge, Oregon (Simpson, 2009), on the western slopes of the Cascades, Oregon (Manning and Hagar, 2011), Craters of the Moon National Monument and Preserve, Idaho (Rodhouse et al. 2010) and in Hays Canyon mountain range, northwestern Nevada (Beever et al. 2008).

Pikas found in the Bella Coola Valley in British Columbia's Central Coast Mountains (*O. p. fenisex*) also deviate from long-understood habitat restrictions. For example, in 1938, Hamilton Laing and Charles Guiget collected American pikas near sea level at Tweedsmuir Lodge in Stuie, BC (Canadian Museum of Nature Database, 2007). Recent studies in this region have further documented pikas along an altitudinal gradient, ranging from sea level to 1500 m (Henry and Russello, 2011). In order to better characterize American pika micro-habitat at their range margins (both latitudinally and altitudinally), I measured temperature both above and below the talus at three sites at varying elevations (300-1500m) in the Bella Coola Valley, BC (Fig. A1.1). Observational data were also collected to investigate pika activity relative to ambient temperature. In the present note, I aim to characterize the environmental conditions experienced by pikas living along an elevation gradient at the northwestern edge of their distribution and inform on the importance of talus in mitigating extreme temperatures.

Temperature loggers (HOBO® Pro V2, Onset Computer Corporation Bourne, MA) were placed in three sites with known presence of American pikas along an altitudinal gradient: one low elevation population (N52˚ 14' 56", W125˚ 32' 14", 362 m), one mid elevation population (N52˚ 15' 9", W125˚ 31' 39", 793 m), and one high elevation population (N52˚ 18' 36", W125˚ 29' 47", 1433 m). At each site one temperature logger was placed in shaded areas with similar aspect and orientation, 150 cm above the ground (following Simpson 2009), and at the mid elevation site an additional temperature logger was placed 100 cm below the surface of the talus (following Millar and Westfall 2010). Temperatures were then recorded every hour between July 6th and August 23rd 2010.

In order to shed light on the extent of behavioral thermoregulation, observational data was taken at each site. Each site was surveyed once a week and observations were taken at three different times that day: in the morning (6-9am), midday (12-2pm) and at dusk (5-7pm). Observations entailed the recording of sightings and vocalizations (both short and long calls) made by active pikas. An observational unit was defined as the number of different sightings or long call emitted above ground within one hour of the observation. Multiple calls emitted by the same animal were thus counted as one observation and vocalization emitted from under the rock was not counted as an incidence of activity. The exact time of each observation was recorded and was later assigned a temperature value.

The temperatures recorded at the three elevations were plotted in Figure A1.2. I used an Analysis of Variance (ANOVA) and associated Tukey-Kramer HSD test to determine if temperatures were significantly different between sites along the elevation gradient in our study area. I then used a Student's T-test to determine whether there was a statistically significant difference between temperatures recorded above and below the talus at the mid elevation site

(Fig. A1.3). Lastly, I tested the relationship between pika activity and temperature using linear regression analysis. An ANOVA was then undertaken to test for the effects of elevation and time of day when the observations were recorded on the significance of the regression.

Temperature loggers recorded data every hour during summer 2010 and resulted in a total of 1408 individual recordings at each site. A significant difference was found between the mean temperature at high elevation (14.3°C, SD = 2.9°C) compared to both mid (17.6°C, SD = 5.6°C) and low elevations (20.3°C, SD = 5.4°C) [ANOVA, $F=462$, $P<0.001$; Fig A1.3]. The same results were obtained when this analysis was repeated by times of day (data not shown). When comparing the difference in temperature above and below the talus at the mid elevations, results differed depending on the time of day. Below talus temperatures were typically warmer than above talus temperatures during the morning (T-test, $T = 4.2$, $p < 0.001$, Fig. A1.5a) and at night (T-test, $T = 13$, $p < 0.001$, Fig. A1.5e). Significantly colder temperatures were found under the talus slope at noon (T-test, $T = 2$, $p = 0.04$, Fig. A1.5b) and during the afternoon (T-test, $T = 4.2$, $p < 0.001$, Fig. A1.5c). Above and below talus temperatures were not significantly different in the evening (T-test, $T = 1.8$, $p = 0.07$, Fig. 5d). Overall, the talus acts as an insulator and buffers temperature fluctuations, resulting in less extreme temperatures under the talus slope (see Fig. A1.3). In addition, acute heat stress temperatures (25.5°C and above; Smith, 1974) were only rarely reached under the talus (3 observations), whereas above talus temperatures reached temperatures above this threshold 31, 30 and 15 days over the summer in low, mid and high elevation sites.

Pikas at high elevations (n=88) showed higher amounts of activity when compared to both mid (n=50) and low elevation pikas (n=48). Overall, a non-significant negative correlation was found between pika activity and temperature when all sites and all times of day were taken into

consideration (Linear regression; $r^2 = 0.05$, ANOVA; $F = 2.4$, $p = 0.12$, Fig. A1.6). When this analysis was repeated by time of day, the regression using the observation taken in the afternoon (coincident with warmer temperatures) yielded a significant negative relationship between temperature and activity (Linear regression; $r^2 = 0.19$, ANOVA; $F = 6.3$, $p = 0.02$), consistent with the long-held belief that pikas are less active in warmer weather. The significant relationship between pika activity and temperature no longer holds, however, when the analyses are repeated for each elevation separately, as well as for other times of the day. This may be caused by the fact that the sample size was too low to attain the given significance level, or that some other factor, such as local adaptation (genetic or behavioral), may be confounding these analyses. Besides the differences illustrated above, low and mid elevation sites (below 800m) did not harbor detectable haypiles as opposed to the high elevation site. This observation was also made by Simpson (2009) in low elevation populations in Oregon, and he suggests that the lack of snow pack at these sites may have selected against the haying behavior due to the ability to forage year -round.

Even in areas where low elevation dwelling pikas likely experience the mildest summer temperatures found throughout their geographic range, there is evidence that low elevation pikas still may need to use talus for behavioral thermoregulation. Overall, I demonstrated a correlation between pika activity and cool ambient temperatures in the Bella Coola Valley, B.C., consistent with previous studies conducted in more southern parts of its range (Moyer-Horner, 2010). As cooler temperatures are associated with higher elevations, the presence of pikas at low elevations would require specific adaptations to higher temperatures, increased phenotypic plasticity, and/or behavioral thermoregulation. Regarding the latter, I have shown that temperatures within the talus remain significantly cooler despite increasing ambient temperatures, potentially providing a critical refuge enabling their persistence at lower elevations.

Figures

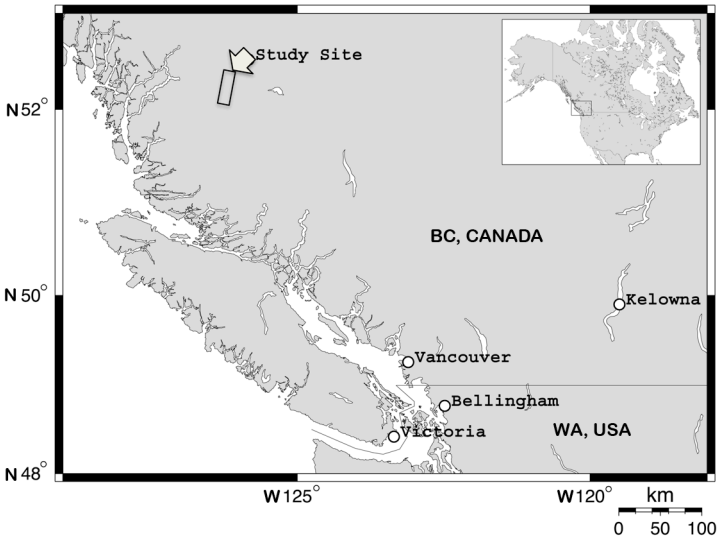


Figure A1.1. Map showing the geographic location of the study site in the Bella Coola Valley, British Columbia, Canada.

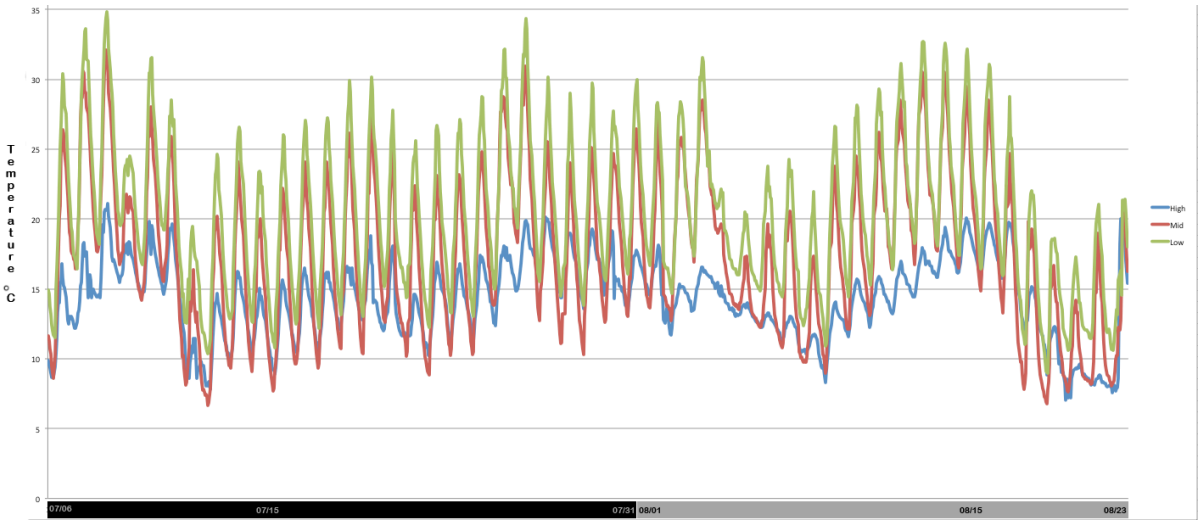


Figure A1.2. Graph depicting the observed temperatures above the talus at low, mid and high elevations along an elevation gradient in the Bella Coola Valley. Temperatures were recorded from July 6th 2010 to August 23rd 2010.

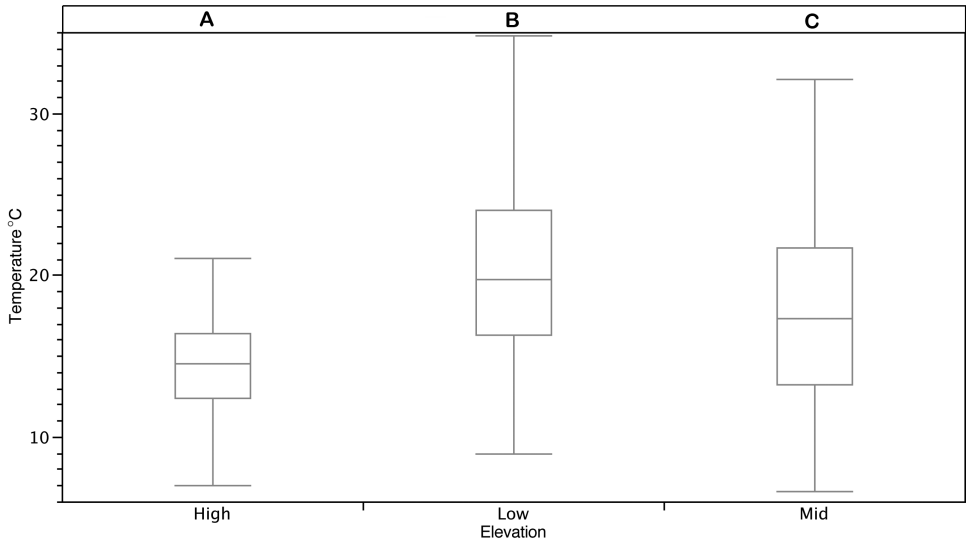


Figure A1.3. Box plot depicting the difference in mean temperature between high, mid and low elevations. Different letters (A, B, C) indicate that the mean temperatures at each elevation is significantly different from all others based on an ANOVA and Tukey's HSD test.

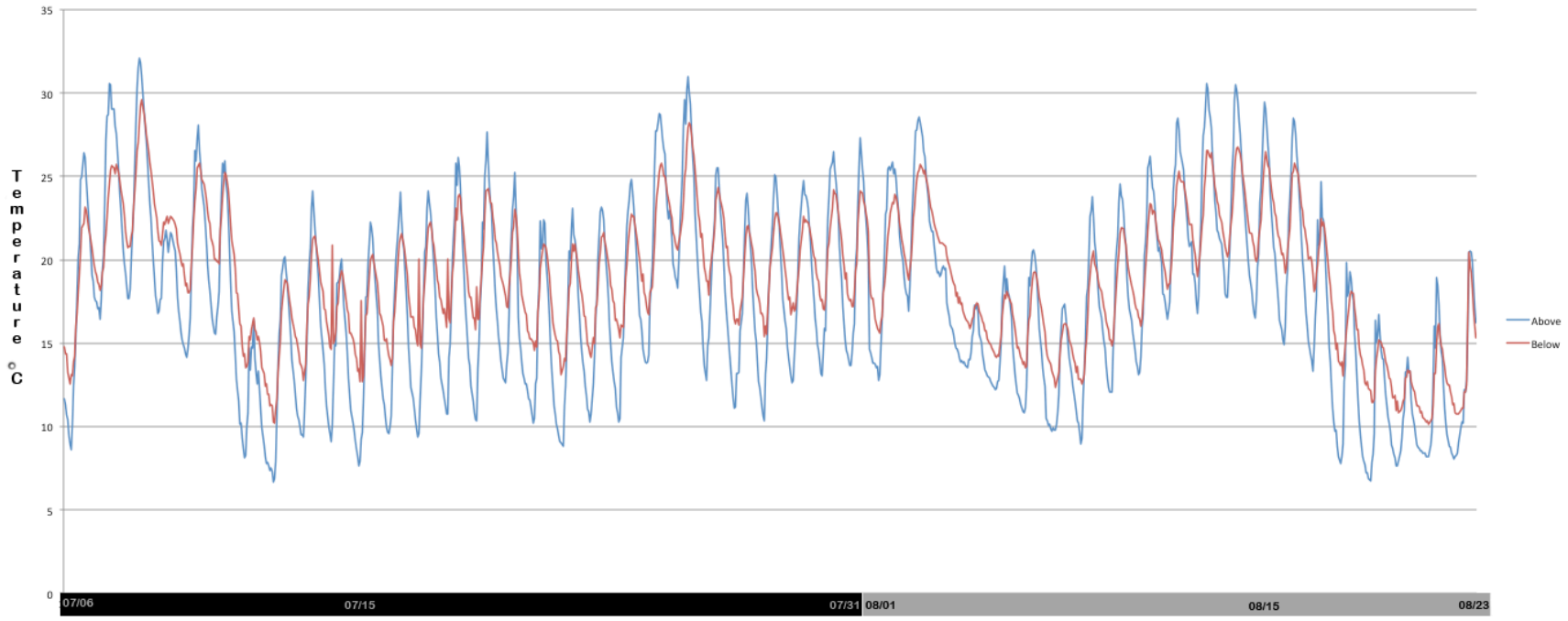


Figure A1.4. Graph depicting the observed temperatures above and below the talus at the mid elevation site in the Bella Coola Valley. Temperatures were recorded from July 6th 2010 to August 23rd 2010. Note that above talus temperatures are always more extreme than below talus temperatures.

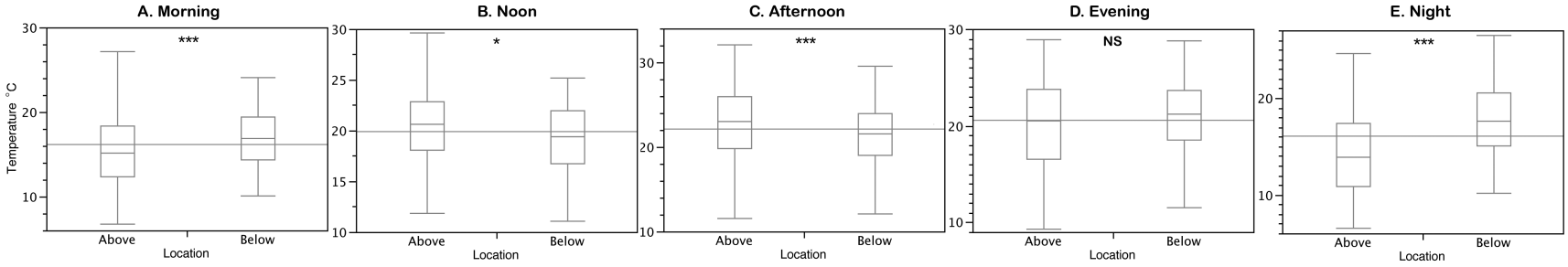
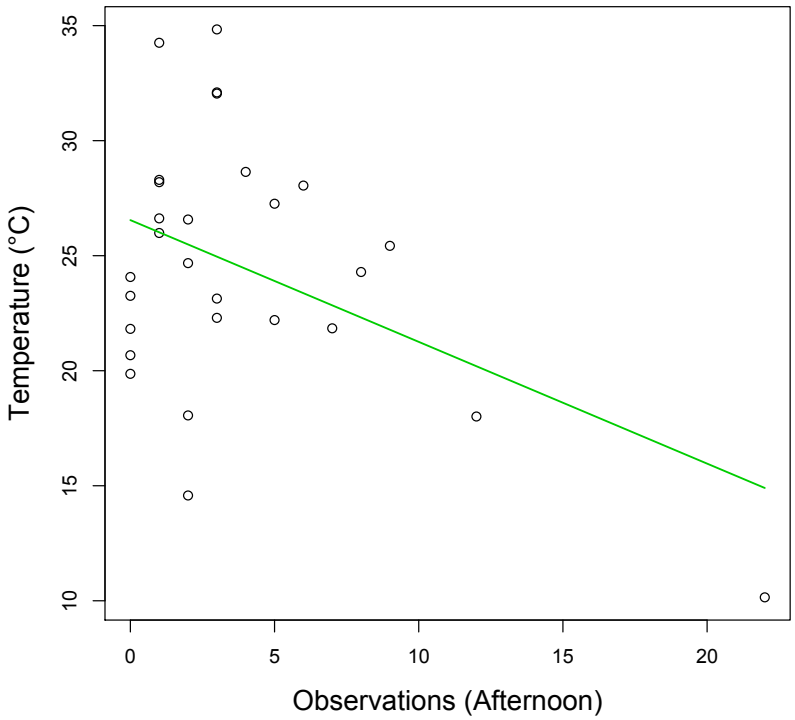


Figure A1.5. Box plots depicting the differences between below and above talus temperatures at different time of day. A) Morning, B) Noon, C) Afternoon, D) Evening and E) Night. * indicates significance at $p < 0.05$, *** indicates significance at $p < 0.001$ and NS indicate a non-significant difference.

A)



B)

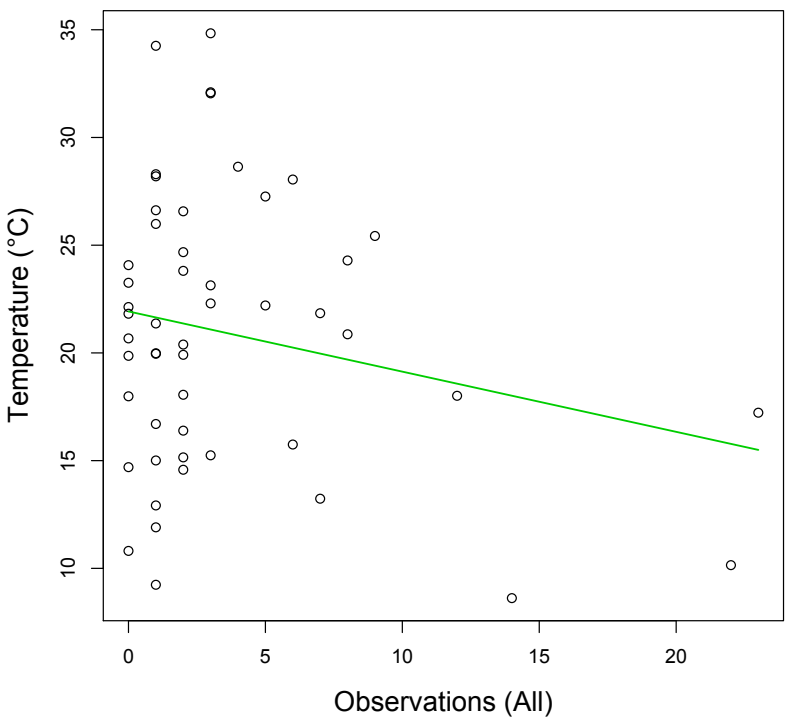


Figure A1.6. Scatter plot depicting the overall decrease in activity of the American Pika with increasing temperature. A) All observations: this relationship is not significant (Linear regression; $r^2 = 0.05$, ANOVA, $F = 2.4$, $p = 0.12$), B) Afternoon observations: a significant negative relationship between temperature and activity is observed (Linear regression; $r^2 = 0.19$, ANOVA; $F = 6.3$, $p = 0.02$).

Appendix 2. An investigation of targeted genes potentially under selection in American pikas

I initially used a candidate gene approach and targeted genes that had been identified as potentially under selection in species closely related to the American pika: Uncoupling protein 1 (UCP 1) and Hemoglobin (Hb) genes. These genes were amplified in a subset of individuals from 5 *O. princeps* populations found in the Bella Coola valley, including 1 high elevation (N = 12), 1 mid elevation, (N = 7) and 3 low elevation, Low1, Low2 and Low3, (N = 13, N = 7, N=7 respectively).

Hemoglobin (Hb) is a metalloprotein tetramer consisting of 2 α-globins and 2 β-globins polypeptides. It is a key component of oxygen storage and regulation in a wide range of living organisms, from yeasts to mammals (Hardison, 1998). The possession of Hb with high oxygen affinity is known to help adaptation of animals to high altitude environments (Yang et al., 2007). Uncoupling proteins (UCPs) are members of the mitochondrial transporter family that dissipate the mitochondrial proton gradient as heat (Palmieri, 1994). UCP 1 is found exclusively in brown adipose tissue (BAT), which help regulate non-shivering thermogenesis in small rodents during cold exposure (Enerback et al. 1997). In addition, UCP 1 plays a role in the protection against reactive oxygen species, whose levels increase drastically during environmental stress, and hypoxic states (Bienengraeber et al. 2002; Echtay et al. 2002).

I used primers PIKAHbA-F, PIKAHbA-R, PIKAHbB-F, PIKAHbB-R, designed for the plateau pika (*Ochotona curoniae*) as described in Yang et al. (2007) for the core region sequence. For the UCP 1, primers UCP1_1F, UCP1_5R, UCP1_8F and UCP1_11R as described in Kitao et al. (2007) were used. PCR reagents used for adaptive genes experiments are as follows: 1.0ul

(1ng/ul) template DNA, 1.0ul (1uM) forward primer, 1.0ul (10uM) reverse primer, 2.5ul 10x buffer (supplied with TAQ gold), 2.5ul (2nM) dNTP, 1.0ul (1mg/ul) BSA, 0.2ul (5U/ul) ApliTaq Gold DNA polymerase and 15.8 ul of ddH_2O for a reaction volume of 25ul. Cycling parameters were as follows: initial denaturation at 95°C for 10 min, followed by 40 cycles denaturation at 95°C for 30sec, annealing at 50°C for 30 sec and extension at 72°C for 45sec. Final extension was conducted at 72°C for 7mins followed by rapid cooling to 4°C.

PCR products were then purified using ExoSAP-IT (GE Healthcare, Pittsburg, PA, USA) and sequenced on an ABI 3130XL genetic analyser (Applied Biosystems, Foster City, CA, USA). Visualization of the amplified product of Hb and UCP 1 was performed using Sequencher v4.10.1 (Gene Codes Corporation, Ann Harbour, MI, USA). A BLAST search was then performed on the sequences obtained (Table A2.1).

Table A2.1. Top BLAST hits for amplified gene sequences

Fragment	Top BLAST Hit
PIKAHbA	Sacalopus aquaticus isolate Nashville 08 hemiglobin subunit alpha 2, mRNA
PIKAHbB	*Sacalopus aquaticus* isolate Nashville 08 hemiglobin subunit delta, mRNA
UCP1_1F/5R	*Clostridium sacchrolyticum* WM1 complete genome
UCP1_8F/11R	*Ochotona dauurica* UCP 1 mRNA

As illustrated above, this targeted gene approach yielded limited success, with only a single fragment of UCP1 being assigned to the species of interest. While other successful PCR products belonged to other taxa. In any case a general pattern was that PCR success for these fragments was low, possibly caused by the lack of suitability of published markers for American pika. This

avenue was thus abandoned early on and the study was focused on using an AFLP-based genomic scan.

Appendix 3. Supplementary material

Table A3.1. Information regarding mitochondrial Cytochrome B fragments amplified, including fragment names, primer names and sequences as well as overall length of fragment amplified.

Fragment name	Primer names	Primer sequences (5' - 3')	Length (bp)
Cyt B a	OpCytB1F	GAAAAACCACCGTTGTAGTTCA	250
	1R_H14994 *	AGGTAGCGGATAATTCAGCC	
Cyt B b	OpCytB1F	GAAAAACCACCGTTGTAGTTCA	800
	OpCYTB3R	CCTAGGAGGTCTGGGGAAAA	

* Yu *et al.,* (2000)

Table A3.2. Information on the microsatellite loci tested in the present study including locus name, Genbank numbers, length (for successful amplification) and PCR program (for loci retained in the present study). Loci with a single fragment length were monomorphic in our samples

Locus	Genbank ♯	Length	Program
Ocp2	AF487493	413-447	TD
Ocp6	AF487497	350-370	TD
Ocp7	AF487498	310-320	TD
Ocp9	AF487500	225-245	TD
Ocp11	GQ461706	290-318	TD
Ocp13	GQ461708	248-272	TD
Ocp15	GQ461710	161-197	TD
Ocp22	GQ461717	220-242	TD
Ocp12	GQ461707	281-313	50
Ocp23	GQ461723	282-294	50
Ocp1	*AF487492*	*292*	-
Ocp3	*AF487494*	*286*	-
Ocp8	*AF487499*	*248*	-
Ocp10	*GQ461705*	*198*	-
Ocp16	*GQ461711*	*289*	-
Ocp17	*GQ461712*	*281*	-
Ocp19	*GQ461714*	*226*	-
Ocp20	*GQ461715*	*242*	-
Ocp25	*GQ461719*	*228*	-
Ocp26	*GQ461720*	*283*	-
Ocp27	*GQ461721*	*226*	-
Ocp28	*GQ461722*	*162*	-
Ocp4	*AF487495*	*Failed*	-
Ocp5	*AF487496*	*Failed*	-
Ocp14	*GQ461709*	*Failed*	-
Ocp18	*GQ461713*	*Failed*	-
Ocp21	*GQ461716*	*Failed*	-
Ocp24	*GQ461718*	*Failed*	-

Table A3.3. Tests of HWE within each site for each loci used. Black boxes indicate significant deviation from HWE per locus per site after corrections for multiple comparisons.

	A1	A2	B	C	D	E	F	G	H	I
Ocp11				■		■				
Ocp12										
Ocp13			■							
Ocp15	■			■	■		■	■		
Ocp2					■					
Ocp22				■		■				
Ocp23										
Ocp6		■								
Ocp7				■				■		
Ocp9			■							

Table A3.4. Test of linkage disequilibrium for each pair of loci calculated within sites. Combined P-values are given below the diagonal, and sites at which the two loci were linked are given above the diagonal.

	Ocp11	**Ocp12**	**Ocp13**	**Ocp15**	**Ocp2**	**Ocp22**	**Ocp23**	**Ocp6**	**Ocp7**
Ocp11	-	-	-	-	**D**	-	-	-	-
Ocp12	0.18850	-	-	-	-	-	**C,D,E,F,G**	-	-
Ocp13	0.07200	0.02850	-	-	-	-	-	-	-
Ocp15	0.04420	0.67400	0.03730	-	-	**E**	-	-	-
Ocp2	**0.00170**	0.60430	0.49390	0.13210	-	-	-	**A,D**	-
Ocp22	0.18800	0.20070	0.07970	**0.00360**	0.18380	-	-	-	-
Ocp23	0.00800	**0.00000**	0.08230	0.86670	0.02050	0.32630	-	-	-
Ocp6	0.05320	0.62570	0.15800	0.47810	**0.00040**	0.17140	0.15410	-	-
Ocp7	0.20720	0.64130	0.02680	0.82270	0.45570	0.90380	0.62630	0.05750	-
Ocp9	0.00500	0.26440	0.05330	0.26610	0.42540	0.17420	0.44840	0.06020	0.20620

Table A3.5. Observed (*HO*) and expected (*HE*) heterozygosities for each loci and each site.

	A1		**A2**		**B**		**C**		**D**		**E**		**F**		**G**		**H**		**I**	
	Ho	He	Ho	He	Ho	He	Ho	He	Ho	He	Ho	He	Ho	He	Ho	He	Ho	He	Ho	He
Ocp11	0.50	0.54	0.33	0.55	0.65	0.70	0.36	0.73	0.60	0.66	0.43	0.57	0.38	0.53	0.41	0.70	0.20	0.64	0.00	0.30
Ocp12	0.11	0.66	Monomorphic		0.80	0.84	0.50	0.70	0.07	0.62	0.27	0.66	0.14	0.56	0.43	0.70	0.00	0.54	Monomorphic	
Ocp13	0.80	0.74	1.00	0.85	0.88	0.67	0.84	0.77	0.81	0.78	0.86	0.60	0.80	0.78	0.54	0.70	0.75	0.54	0.50	0.46
Ocp15	0.21	0.73	0.33	0.75	0.53	0.80	0.32	0.61	0.37	0.75	0.62	0.62	0.20	0.72	0.30	0.80	0.40	0.71	0.17	0.17
Ocp2	0.67	0.69	0.67	0.48	0.42	0.60	0.48	0.61	0.53	0.75	0.45	0.60	0.40	0.56	0.48	0.71	0.50	0.68	0.33	0.33
Ocp22	0.64	0.49	0.00	0.67	0.71	0.77	0.50	0.80	0.73	0.73	0.67	0.67	0.50	0.50	0.10	0.43	Monomorphic		Monomorphic	
Ocp23	0.42	0.52	Monomorphic		0.67	0.53	0.50	0.51	0.29	0.52	0.60	0.64	0.22	0.39	0.68	0.68	0.40	0.53	Monomorphic	
Ocp6	0.83	0.52	0.00	0.67	0.56	0.56	0.57	0.72	0.45	0.68	0.20	0.35	0.71	0.67	0.39	0.65	0.50	0.50	0.40	0.62
Ocp7	0.47	0.62	0.33	0.53	0.35	0.60	0.36	0.66	0.63	0.71	0.58	0.68	0.40	0.64	0.23	0.37	Monomorphic		Monomorphic	
Ocp9	0.07	0.07	0.17	0.53	0.76	0.76	0.62	0.70	0.56	0.71	0.71	0.62	0.40	0.70	0.62	0.73	0.60	0.78	0.50	0.53

Table A3.6. Mean estimates of the distribution of recent migration rates (*m*) calculated using BAYESASS+ (Wilson and Rannala, 2003), given as the proportion of migrant individuals per population per generations.

	A1		A2		B		C		D		E		F		G		H		I	
A1	**0.971**		0.074		0.004		0.004		0.005		0.011		0.005		0.001		0.009		0.004	
	0.909	*0.999*	*0.004*	*0.194*	*1.7E-12*	*0.030*	*4.0E-08*	*0.026*	*5.8E-09*	*0.030*	*5.6E-09*	*0.065*	*1.4E-11*	*0.038*	*5.6E-14*	*0.011*	*3.1E-12*	*0.072*	*3.6E-14*	*0.038*
A2	0.003		**0.710**		0.003		0.003		0.003		0.004		0.006		0.001		0.008		0.005	
	7.3E-13	*0.021*	*0.668*	*0.808*	*3.0E-13*	*0.022*	*1.9E-08*	*0.021*	*3.3E-10*	*0.022*	*8.2E-09*	*0.027*	*2.2E-11*	*0.044*	*7.4E-16*	*0.011*	*2.2E-12*	*0.063*	*6.1E-15*	*0.046*
B	0.003		0.015		**0.965**		0.047		0.005		0.008		0.006		0.002		0.011		0.005	
	2.0E-13	*0.026*	*3.3E-06*	*0.079*	*0.883*	*0.999*	*4.1E-04*	*0.123*	*1.2E-08*	*1.2E-08*	*1.4E-08*	*0.048*	*4.7E-12*	*0.047*	*8.6E-14*	*0.016*	*6.3E-13*	*0.088*	*2.0E-14*	*0.039*
C	0.003		0.043		0.004		**0.899**		0.015		0.017		0.006		0.001		0.008		0.004	
	3.9E-13	*0.023*	*1.2E-04*	*0.147*	*1.7E-13*	*0.030*	*0.797*	*0.979*	*2.5E-08*	*0.062*	*3.4E-07*	*0.063*	*4.4E-12*	*0.045*	*2.4E-14*	*0.012*	*1.3E-11*	*0.065*	*1.9E-13*	*0.036*
D	0.003		0.015		0.005		0.017		**0.946**		0.006		0.005		0.002		0.008		0.004	
	3.2E-12	*0.022*	*1.2E-05*	*0.079*	*3.8E-11*	*0.038*	*3.7E-07*	*0.070*	*0.868*	*0.995*	*7.1E-09*	*0.039*	*5.2E-12*	*0.041*	*3.4E-14*	*0.014*	*3.1E-13*	*0.058*	*2.0E-13*	*0.037*
E	0.004		0.017		0.004		0.003		0.013		**0.914**		0.005		0.001		0.008		0.005	
	1.3E-12	*0.027*	*8.3E-06*	*0.091*	*1.2E-12*	*0.028*	*1.2E-08*	*0.022*	*5.2E-08*	*0.062*	*0.836*	*0.979*	*1.4E-11*	*0.044*	*5.7E-15*	*0.015*	*2.6E-14*	*0.060*	*3.6E-14*	*0.039*
F	0.003		0.021		0.004		0.011		0.003		0.007		**0.933**		0.002		0.009		0.005	
	3.1E-14	*0.025*	*8.6E-06*	*0.099*	*4.1E-11*	*0.034*	*1.1E-07*	*0.061*	*4.9E-10*	*0.022*	*2.0E-08*	*0.041*	*0.810*	*0.998*	*1.4E-13*	*0.015*	*9.3E-12*	*0.072*	*3.2E-14*	*0.047*
G	0.004		0.046		0.004		0.007		0.004		0.009		0.015		**0.986**		0.009		0.005	
	2.6E-13	*0.030*	*9.4E-05*	*0.154*	*2.0E-12*	*0.033*	*1.4E-08*	*0.038*	*5.2E-09*	*0.028*	*1.3E-07*	*0.052*	*1.1E-11*	*0.102*	*0.952*	*1.000*	*8.2E-13*	*0.070*	*3.7E-14*	*0.050*
H	0.003		0.016		0.004		0.005		0.003		0.010		0.006		0.002		**0.918**		0.005	
	1.8E-13	*0.027*	*7.0E-06*	*0.078*	*1.6E-12*	*0.032*	*2.5E-08*	*0.035*	*4.2E-10*	*0.021*	*1.7E-08*	*0.056*	*1.2E-11*	*0.046*	*8.4E-15*	*0.012*	*0.728*	*0.998*	*3.2E-13*	*0.041*
I	0.003		0.043		0.004		0.004		0.002		0.016		0.011		0.001		0.014		**0.959**	
	3.7E-13	*0.031*	*2.2E-04*	*0.137*	*4.1E-13*	*0.029*	*1.4E-08*	*0.025*	*7.6E-10*	*0.015*	*4.2E-07*	*0.068*	*7.6E-12*	*0.072*	*1.6E-13*	*0.013*	*4.9E-13*	*0.106*	*0.875*	*0.998*

Values in italic are the lower and upper bounds of the 95% confidence interval for each mean value. Columns represent the incoming migration rates and rows represent the outgoing migration rates. Bold values represent the proportion of non-migrant individuals in a population

Table A3.7. Results of the tests of demographic history for each site.

	A1	A2	B	C	D	E	F	G	H	I
Het excess	**0.02**	0.06	**0.04**	**0.01**	0.19	0.99	0.29	**0.01**	**0.01**	0.95
Mode Shift	**Shifted**	L- Shaped	L- Shaped	**Shifted**	L- Shaped	L- Shaped	**Shifted**	**Shifted**	**Shifted**	L- Shaped
M-ratio	0.79	0.90	0.84	0.84	0.85	0.86	0.93	0.97	0.96	0.87
Critical M	0.77	0.76	0.77	0.77	0.78	0.78	0.77	0.77	0.75	0.76
negative k	4	3	4	7	4	6	5	4	3	8
k (p-value)	0.72	0.93	0.80	0.15	0.80	0.34	0.59	0.80	0.93	0.04
g	1.04	1.23	1.22	1.01	0.67	1.29	0.49	1.10	2.27	0.79

Population contraction was assessed using three tests (Heterozygosity excess, mode shift and *M*-ratio). Results represent the p-value for the heterozygosity excess, and the shape of the allele frequency distribution for the mode shift test. M-ratios and critical M values are presented where a M-ratio smaller than the critical M for that population indicates historical demographic contraction. Values in bold indicate evidence for demographic contraction. Test of population expansion were based of *k* and *g* statistics: I present the number of loci with a negative k value out of ten along with the p-value of the test. g-values are presented, where any value below (0.23 - 0.17; Table 1 in Reich *et al* 1999) is indicative of population expansion.

Table A3.8. Number of AFLP bands and error rates for each primer combinations used in this study.

Primer combination	No bands	Error rate (%)
E31T37	60	3.6
E32T35	84	4.4
E33T32	79	3.7
E34T44	86	5.5
E31T39	39	4.1
E33T37	116	4.1
E43T35	77	4.7
E46T45	40	3.7
E31T43	48	3.0
E33T39	86	5.0
E34T45	79	5.3
E43T37	39	3.6
E34T38	72	4.8
E38T32	102	4.5
E43T44	78	3.9
E44T38	112	5.2
E38T37	90	4.0
E43T43	52	6.4
E44T44	95	5.3
E46T38	75	4.3
MEAN	**75**	**4.5**
SD	23	0.8