Gene flow directionality and functional genetic variation among Ontario, Canada *Ursus americanus* populations.

A Thesis Submitted to the Committee on Graduate Studies in Partial Fulfillment of the Requirements for the Degree of Master of Science in the Faculty of Arts and Science

Trent University

Peterborough, Ontario, Canada

Environmental and Life Sciences M.Sc. Graduate Program

September 2022

ABSTRACT

Gene flow directionality and functional genetic variation among Ontario, Canada *Ursus americanus* populations.

Evan Paul Kopsala

Rapidly changing landscapes introduce challenges for wildlife management, particularly for large mammal populations with long generation times and extensive spatial requirements. Understanding how these populations interact with heterogeneous landscapes aids in predicting responses to further environmental change. In this thesis, I profile American black bears using microsatellite loci and pooled whole-genome sequencing. These data characterize gene flow directionality and functional genetic variation to understand patterns of dispersal and local adaptation; processes key to understanding vulnerability to environmental change. I show dispersal is positively density-dependent, male biased, and influenced by food productivity gradients suggestive of source-sink dynamics. Genomic comparison of bears inhabiting different climate and forest zones identified variation in genes related to the cellular response to starvation and cold. My thesis demonstrates source-sink dynamics and local adaption in black bears. Population management must balance dispersal to sustain declining populations against the risk of maladaptation under future scenarios of environmental change.

Keywords: Genomics, American black bear, Gene Flow Directionality, Functional Genetic Variation, Dispersal, Local Adaptation

ACKNOWLEDGEMENTS

In the latter years of my undergraduate degree at Trent University, I developed a passion for wildlife genetics and genomics and am tremendously grateful for the opportunity to have pursued these interests in my graduate studies.

To start, I would like to thank Trent University. Trent has been a home to me since I moved into Gzowski College many years ago. Since then, I am fortunate to have met so many incredible friends, classmates, and teammates, without whom, it is unlikely I would have had the fantastic post-secondary experience that I did.

Science is a collaborative pursuit. I consider myself very lucky to have been part of the Kyle and Northrup Labs these past few years and would like to thank all their members for truly enriching my experience as a graduate student. Your help in the lab and in the world of bioinformatics made this thesis possible. I would also like to thank everyone at the Ontario Ministry of Northern Development, Mines, Natural Resources and Forestry who played a role in the Barbed Wire Hair Trap (BWHT) project who were instrumental in my thesis.

From getting various trucks and ATVs stuck and un-stuck at sampling sites to enjoying a cold drink at The Mad Musher after a long day of field work, I had an incredible time working on the BHWT project.

To Dr. Christina Davy, thank you so much for your thoughtful comments, guidance, time, and for always adding smiley faces throughout your edits to make them seem more encouraging 😊. I am very grateful to have had you as a member of my committee.

Lastly, I would like to thank Dr. Christopher Kyle and Dr. Joseph Northrup. As far as potential graduate students go, you both took a chance on me, for which I will be forever grateful. I only hope you were able to get something out of it yourselves. I cannot thank you enough for the time and effort you have put into guiding me through my research, and I will never forget it.

TABLE OF CONTENTS

LIST OF TABLES

Chapter 2

Chapter 3

LIST OF FIGURES

(positive genetic flux), and light green represents high net emigration (negative genetic flux) from a node. Points represent node centroid locations and are sized according to density (large point = high bear density, small point = low bear density).

Figure 2.4. Harvest rate (proportion of population harvested) calculated at the wildlife management unit (WMU) level. Darker blue WMUs indicate higher harvest rate relative to lighter blue WMUs. Grey WMUs did not have density estimates available, or are protected areas with no harvest, and therefore, we were unable to calculate harvest rate for those WMUs.

Chapter 3

Figure 3.1. Sample locations relative to (a) the two climate comparisons we conducted and (b) the two forest zone comparisons we conducted. Blue represents high snowfall while green represents low snowfall (annual average from 1980-2010). Snowfall is considered the most accurate way to visualize the divide between climate zones located in north-central Ontario (Row et al., 2014). Lines dividing the province latitudinally represent differences in forest zone: north forest = boreal, south forest = GLSL/mixed deciduous. White areas represent unsampled forest regions (deciduous and Hudson Bay lowlands).

Figure 3.2. Frequent poor-food years in eastern Ontario (Atlantic climate zone) appear to drive genetic variation in genes related to the cellular response to starvation, selecting for resilience against food shortages. Colour gradient represents annual precipitation, which is considered the most accurate way to visualize difference in climate zone, within Ontario.

Figure 3.3. In American black bears in eastern Ontario, temperature differences along a latitudinal gradient likely drive genetic variation in genes influencing the cellular response to cold. Colour gradient represents annual average low temperature, where red represents warmer temperatures and blue represents colder temperatures (Range = -7 to +6 degrees Celsius).

CHAPTER 1: GENERAL INTRODUCTION

Landscapes are in constant states of change, yet anthropogenic influences have accelerated these changes owing to climate and land use change (Hetem et al., 2014). This era of rapid environmental change has given rise to new challenges for maintaining healthy wildlife populations (Valladares et al., 2014). These challenges are particularly pertinent for large mammals, which are more vulnerable to environmental change due to long generation times and large space requirements (Hetem et al., 2014). Understanding how wildlife populations move across and have adapted to heterogeneous landscapes can aid in assessing population viability under different scenarios of environmental change. This information can also be used in determining what management efforts may mitigate potential negative consequences of said change (Merilä & Hendry, 2014; Shafer et al., 2014; Stapley et al., 2010).

Dispersal is the process of moving from a natal location to a different breeding location (Baguette et al., 2012) and is a fundamental driver of spatial patterns of species distribution, abundance, and genetic differentiation (Travis et al., 1999). Dispersal governs population interactions through immigration/emigration and influences patterns of population productivity, metapopulation structure, and genetic diversity (Amarasekare, 2006; Andrade-Restrepo et al., 2019; de Bona et al., 2019; Ronce, 2007). Dispersal also influences species' abilities to respond to rapid environmental change such as that caused by climate and land-use change and is an important factor in long-term population viability (Liu et al., 2018; Sheard et al., 2020). Characterizing patterns of dispersal has been used to ensure immigration to small/declining populations, reducing the likelihood of extirpation by buffering against demographic stochasticity (Eriksson et

al., 2014) or increased harvest pressures (Andreasen et al., 2012). Therefore, understanding patterns of dispersal are fundamental to making informed management and conservation decisions (Poethke & Hovestadt, 2001).

Traditional approaches to studying dispersal include mark recapture or radio telemetry. Using these approaches to study wide-ranging, and sometimes elusive, mammals can be logistically difficult due to financial, logistical, and time requirements (Howe et al., 2022; Stokes et al., 2010). Genetic studies provide a viable alternative to these traditional approaches where inferences can be made regarding underlying ecological processes shaping genetic structure (Dyer et al., 2010; W. D. Koenig et al., 1996). For example, using microsatellite data to characterize patterns of relatedness and gene flow allow for inferences on dispersal and population connectivity (Koen et al., 2016; Rayfield et al., 2011; Sawaya et al., 2014), both of which influence population viability (Liu et al., 2018; Sheard et al., 2020).

As with patterns of dispersal, how populations adapt to their environments have profound influences on likelihoods of persistence depending on how environments change (Hohenlohe et al., 2020; Kawecki & Ebert, 2004; Merilä & Hendry, 2014). Understanding these patterns of adaptation across heterogeneous landscapes aids in predicting responses to specific changes in selective pressures (M. E. Feder & Mitchell-olds, 2003; Shafer et al., 2014). Characterizing patterns of adaptation can also aid conservation efforts, where matching genotypes to current or future environmental conditions can optimize restoration and reintroduction efforts (Flanagan et al., 2018).

As the importance of genetic and genomic studies in understanding processes critical for the management and conservation of species are increasingly recognized (Blanchong et al., 2016; Hohenlohe et al., 2020; Morin et al., 2004), it is now possible to sequence whole genomes (Hohenlohe et al., 2020; Morozova & Marra, 2008; Savolainen et al., 2013; Stapley et al., 2010). Whole genome sequencing (WGS) has allowed for identification of genes driving adaptive divergence at both the species (Johnson et al., 2018; Lv et al., 2014; Qiu et al., 2012) and population level (Hamala & Savolainen, 2019; Muir et al., 2014; Zhao et al., 2013). Sufficient sample sizes are required to reliably identify differentiated regions of the genome, particularly in larger populations with high effective population size (N_e) and high recombination rates. Sampling a sufficient number of individuals for whole genome sequencing can be costly and computationally taxing, so alternative methods have been developed (Fuentes-Pardo & Ruzzante, 2017). RAD-seq evaluates genetic variation present around restriction cut sites. While cost-effective, RAD-seq randomly screens a small and dispersed amount of a genome where sequence data only covers ~1-5% of the total genome (K. R. Andrews et al., 2016; Fuentes-Pardo & Ruzzante, 2017). A promising balance of WGS and RAD-seq is pooled whole genome sequencing (pool-seq), which operates by pooling individuals (typically >44; Schlötterer et al., 2014) prior to sequencing. Although individual identity is lost, resulting sequence data from a single pooled sample reflects population level polymorphism patterns, and can be used to identify genes underlying adaptive traits (Ferretti et al., 2013).

Using available genetic tools, such as microsatellites or pool-seq, to further our understanding of dispersal and adaptation across heterogeneous landscapes is ever more important given rapidly changing environments (Hetem et al., 2014; Hohenlohe et al., 2020). Elucidating how factors such as harvest, population density, or landscape variables influence dispersal are key to informed population management, particularly if any of these features are projected to change (Baguette et al., 2011; Ims & Andreassen, 2005; Matthysen, 2005). Further, understanding how animals have adapted to their local environments provides insight into what key traits are under selection and how resilient populations may be if selective pressures change (Merilä & Hendry, 2014; Savolainen et al., 2013).

Clarifying patterns of dispersal and local adaptation are particularly important for large mammal populations that are more likely to experience negative consequences of landscape changes given large space requirements and long generation times; factors inhibiting their ability to adapt at a rate consistent with current climate and land-use change (Hetem et al., 2014; Hohenlohe et al., 2020; Thuiller et al., 2006). Many large mammal species have high dispersal capabilities and may be able to track suitable habitats in response to environmental change, however this is not the case for populations inhabiting fragmented landscapes where dispersal potential is limited (Dixon et al., 2007; Thuiller et al., 2006). Thus, characterizing patterns of dispersal and adaptation in large mammal populations is particularly important given their vulnerability to environmental change.

Study System

My thesis uses microsatellite data and pooled whole-genome sequencing to characterize patterns of gene flow directionality and functional genetic variation in American black bears (*Ursus americanus*) across Ontario, Canada. In doing so, I make inferences on patterns of dispersal and local adaptation. The Ontario black bear population presents an excellent system to assess these patterns in large mammals given their expansive and continuous distribution across heterogeneous landscapes. Further, the Ontario black bear population is largely undisturbed in its northern ranges where patterns of dispersal and adaptation have likely not yet been significantly altered by environmental change present in southern Ontario.

American black bears are an economically and ecologically significant species that inhabits much of North America's forested regions (Lariviere, 2001; Scheick & Mccown, 2014; Figure. 1.1). Black bears are habitat generalists and populate a diverse array of habitats that presumably represent significant differences in habitat quality (Scheick & Mccown, 2014). In southern latitudes, black bears occupy warmer ecoregions where food resources are often more plentiful, relative to northern ecoregions. In most southern ecoregions, black bears hibernate for shorter periods of time, and sometimes forgo hibernation entirely (Hellgren, 1998). Hibernation is a process employed to withstand shortages in food production in colder winter months (Luu et al., 2018). This being the case, bears often hibernate for months at a time in northern ecoregions such as the boreal forest (Hellgren, 1998; Mosnier et al., 2008). Although black bears in southern North America have access to more food annually, they suffer from increased

habitat fragmentation, limiting their dispersal capabilities. This has resulted in reduced genetic diversity in small/declining populations that are now of conservation concern (Coster & Kovach, 2012; Dixon et al., 2007; S. D. Miller, 1990; Whittle, 2009). Conversely, black bear populations at northern latitudes are largely panmictic and demonstrate isolation by distance genetic patterns (Paetkau & Strobeck, 1994; Pelletier et al., 2012).

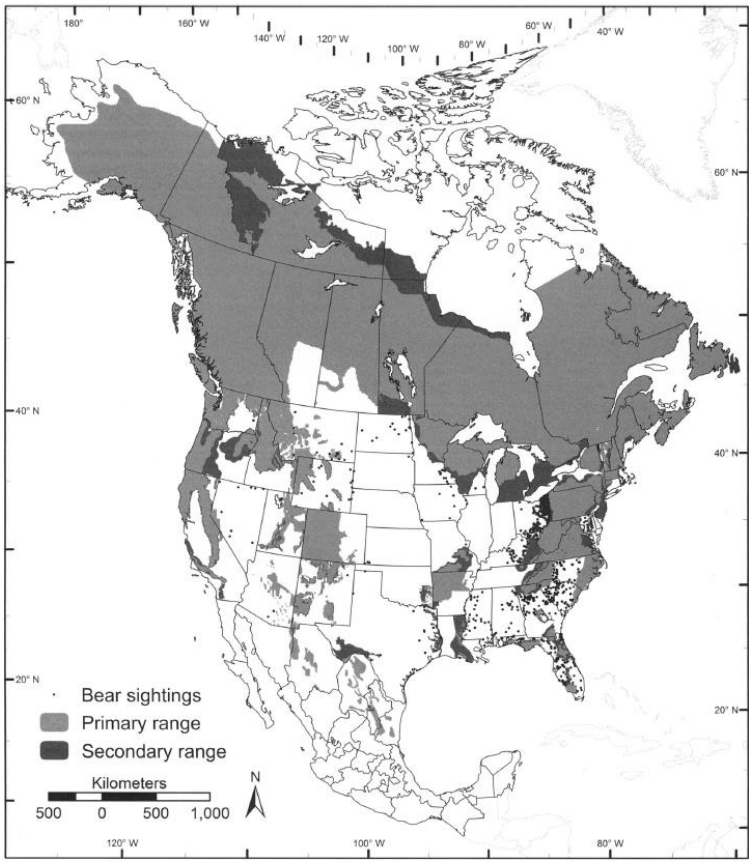


Figure 1.1. Estimated primary and secondary range for American black bears in North America 2009-2012, with sightings reported 2006-2010 outside of range. Only primary range was designated in Mexico (Scheick & Mccown, 2014).

Black bears in Ontario mirror continental-scale patterns, where southern regions of the province are more fragmented relative to the largely undisturbed northern ranges

of boreal forest (Pelletier et al., 2017; Scheick & Mccown, 2014). In these northern ranges, genetic structure likely represents patterns of dispersal and adaptation that have not yet undergone significant transformation due to environmental change. Across this range, Ontario's black bear population spans heterogeneous landscapes along longitudinal and latitudinal gradients and is subject to different anthropogenic influence and harvest pressures.

Boreal forest spans the majority of northern Ontario and represents the largest continuous habitat occupied by American black bears (Mosnier et al., 2008). To the south is the Great Lakes-St. Lawrence forest (GLSL; mixed deciduous; Rowe, 1972). As productivity typically decreases along latitudinal gradients (Hawkins et al., 2003), the boreal forest represents a less productive environment for black bears where winters are longer and colder, and predominant tree species in the boreal forest, such as pine, spruce, and birch do not provide the fatty mast used by bears in southern ecoregions (Bonan et al., 1992; Mosnier et al., 2008; Rogers, 1987). Conversely, the GLSL forest represents a more productive habitat for black bears where hard mast species such as beech and oak, coupled with greater berry production, provide more food options (Howe et al., 2012; Obbard & Howe, 2014; Rogers, 1987). Given the known importance of calorie intake prior to winter dormancy (Hellgren, 1998; Toien et al., 2011), bear movement is often governed by food availability (Obbard et al., 2014, 2017). Thus, the differences between Ontario's forest zones represent significant differences in habitat quality for numerous herbivores and omnivores (Boonstra et al., 2014) including black bears (Howe et al., 2013).

In addition to environmental differences along latitudinal gradients, Ontario has two different climate zones that differ longitudinally. The Atlantic climate zone influences weather patterns in eastern Ontario and the continental climate zone influences weather patterns in western Ontario (Hurrell, 1996; Hurrell et al., 2003). Weather conditions that differ between these climate zones include wind speeds, heat and moisture, precipitation, number of storms, their trajectories, and their intensities. Thus, differences in climate zone can have a significant influence on vegetation productivity. Similar to differences between forest zone, vegetation differences between climate zone can have profound influence on habitat quality for black bears. Further, black bears in Ontario are subject to spring and fall hunting seasons, with harvest pressures that vary spatially and are largely a function of human population density (McLaren et al., 2009; Obbard et al., 2014). An exception is made for the fragmented Bruce Peninsula population (Obbard, Coady, et al., 2010; Pelletier et al., 2017).

This thesis seeks to better our understanding of how heterogeneous landscapes influences patterns of dispersal and adaptation in a large mammal population, and how these processes may influence resilience to contemporary and future environmental change. The Ontario black bear population is an excellent system to assess these patterns given its climatic and ecoregion variation, coupled with spatially varying harvest pressures and population densities, across a large area with no major barriers to dispersal. Variation in these landscape and demographic factors likely have significant influence on habitat quality and subsequent selective pressures. In my first data chapter

(chapter 2), I characterize gene flow directionality, to which I fit spatial models to test hypotheses regarding drivers of putative dispersal patterns. I predict that dispersal patterns will be best explained by differences in food productivity and population densities. In my second data chapter (chapter 3), I use pooled whole-genome sequencing to characterize genetic variation between forest zones, and between climate zones. In doing so, I seek to identify differentiated genes that are functionally relevant to environmental differences and make inferences on underlying adaptive mechanisms driving these patterns. I predict that differentiated genes will be functionally relevant to differences in food productivity between forest and climate zones. For example, genes influencing resilience to lower calorie intake.

Chapters two and three are currently formatted for submission to academic journals. As such, certain repetition exists given I have written chapters two and three to be independent and ready for peer-review publication.

CHAPTER 2: BROAD-SCALE GENETIC MONITORING REVEALS DENSITY DEPENDENT DISPERSAL IN A LARGE CARNIVORE

ABSTRACT

Dispersal strategies govern population interactions, subsequently influencing population productivity and diversity. Thus, characterizing patterns and drivers of dispersal are fundamental to our understanding of animal ecology and ultimately informing species conservation and management strategies. In this study, we used microsatellite data from 3941 individual black bears (*Ursus americanus*) occupying 73 spatially distinct sampling areas across a large heterogeneous landscape to characterize dispersal via gene flow directionality. We fit spatial models to quantified gene flow to test hypotheses regarding drivers of putative dispersal patterns.

Specifically, we tested the relative influence of food productivity gradients, bear density, and harvest. Additionally, we evaluated differences in gene flow patterns within and between sexes as a means of assessing sex-biased dispersal. We found evidence of positive density-dependent male-biased dispersal; a pattern documented in other systems, but evidence for which was generally weak. Results also showed evidence of a relationship between dispersal and broad food productivity gradients. Specifically, male bears displayed preferential dispersal towards mixed deciduous forests with higher food productivity relative to less productive boreal forests. Given the dense sampling scheme across a continuous population, occupying a large heterogeneous landscape, these results provide key insight to dispersal patterns of a wide-ranging mammals and underlying drivers. We postulate that observed dispersal patterns are the result of differential human-caused mortality, and thus suggest population sinks exist in highly productive but heavily harvested areas. We interpret these data to suggest intensive

harvesting can have substantial impacts on large carnivore populations that can be masked by natural dispersal patterns.

INTRODUCTION

Dispersal is the process of moving from a natal location to a different breeding location and is an important driver of spatial patterns of species distribution, abundance, genetic diversity, as well as overall population productivity (Amarasekare, 2006; Travis et al., 1999). Therefore, quantifying dispersal, and underlying drivers, is key to understanding contemporary patterns of distribution, metapopulation structure, genetic variation, and local adaptation (Andrade-Restrepo et al., 2019; De Bona et al., 2019; Koen et al., 2016; Ronce, 2007). Additionally, dispersal is an important trait determining the ability for species to adapt to rapid environmental change, such as climate and land-use change (Sheard et al., 2020). Therefore, understanding patterns of dispersal can be used to predict responses to environmental or demographic stressors and adjust management practices accordingly (Poethke & Hovestadt, 2002).

Factors driving dispersal vary across species, populations, and individuals (Clobert et al., 2009; Sheard et al., 2020) where density of conspecifics, both in natal and destination sites, is expected to be an important predictor of dispersal. Conspecific density links directly to intraspecific competition for resources (e.g., food, mates, territories), which in turn influence individual decisions to disperse. Density-dependent dispersal has been documented in a variety of taxa including insects (R. G. Harrison, 1980), fish (Yeakel et al., 2018), birds (Doncaster, 2000), and mammals (Matthysen,

2005). Positive density-dependent dispersal, moving from a high population density to low (Yeakel et al., 2018), is associated with resource availability, kin competition (Clobert et al., 2009), and inbreeding avoidance (Lawson Handley & Perrin, 2007). Positive density-dependent dispersal is the most widespread hypothesis pertaining to density-dependent dispersal, particularly in mammals (Matthysen, 2005). Conversely, negative density-dependent dispersal, moving from low population density to high (Travis & Dytham, 1999), can be beneficial when it achieves lower male biased sex ratios leading to greater reproductive success for dispersing males (Ims & Andreassen, 2005). Population density can also be an indicator of habitat quality that promotes negative density-dependent dispersal (Baguette et al., 2011).

Density-dependent dispersal has been thoroughly studied in invertebrates, yet studies of large, wide-ranging mammals are notably less common given logistical difficulties associated with geographic study scales relevant to dispersal for these species (Loe et al., 2009; Matthysen, 2005). Large mammals, particularly carnivores, are often highly susceptible to land-use change and anthropogenic influence because of their need for large areas (Pillay et al., 2011). Dispersal is fundamental for stabilizing population fluctuations in heterogeneous landscapes (Sheard et al., 2020), where climate and land-use change continue to increase heterogeneity beyond normal bounds (Grimm et al., 2008). Further, many large mammal populations are subject to high rates of harvest and other forms of human-caused mortality. Dispersal directionality (i.e., immigration and emigration) is a fundamental component of population growth rates for which information is needed to ensure harvest-induced mortality rates are

sustainable (A. K. Harrison, 2011). When properly quantified and accounted for in a harvested system, dispersal can maintain neighboring populations experiencing fluctuations. However, if not taken into account, harvest has the potential to deplete source populations that are sustaining adjacent sink populations through density-dependent dispersal (Gaggiotti, 1996). Thus, understanding source-sink dynamics is of key importance when managing harvested large mammal populations (Labonte et al., 1998; Robinson et al., 2008).

Past research on density-dependent dispersal across mammalian species have shown somewhat equivocal results. Aars and Ims (2000) report negative density-dependent dispersal in root voles (*Microtus oeconomus*), where males dispersing from high to low density patches experience a greater male-biased sex ratio, and thus, lower reproductive success than those that disperse from low to high density patches (Aars & Ims, 2000). Conversely, positive density-dependent dispersal has been observed in meerkats (*Suricata suricatta*), potentially owing to an increased cost of kin competition that exceeds the cost of cooperation at high densities (Maag et al., 2018). Given the importance of dispersal strategies in understanding species ecology and evolution (Amarasekare, 2006), characterizing patterns of density-dependent dispersal is of key interest.

American black bears (*Ursus americanus*) are large, generalist mammals that occupy forested regions across much of North America. Genetic differentiation among black bear populations is often attributed to the presence of physiogeographic barriers (e.g., anthropogenic development, water bodies, mountain ranges) that discourage,

although may not entirely preclude, dispersal and subsequent gene flow (Dixon et al., 2007; Peacock et al., 2007; Pelletier et al., 2012; Puckett et al., 2015). Genetic differentiation in black bears is also associated with isolation due to habitat loss and fragmentation (Dixon et al., 2007; Pelletier et al., 2017). In northern portions of the species' range, genetic structure is largely consistent with isolation by distance (IBD; Brown et al., 2009; Pelletier et al., 2012; Puckett et al., 2015), as expected in systems that lack prominent barriers to dispersal. These contrasting patterns of genetic structure reflect underlying dispersal patterns driving gene flow in black bears (Lawson Handley & Perrin, 2007).

Previous work suggests black bear dispersal can be driven by food productivity and availability (Karelus et al., 2019), inbreeding avoidance (Rogers, 1987), and harvest (Moore et al., 2014), all of which relate to the relative density of populations. Powell et al. (1996) examined population dynamics of black bears in Northern California, USA, relative to harvest near a protected sanctuary. They found increased bear density within the sanctuary resulted in net emigration leading to harvest outside the sanctuary where population density was lower and resources more readily available. Source-sink patterns such as these can sustain populations subject to harvest pressure (A. K. Harrison, 2011; Powell et al., 1996; Robinson et al., 2008). Similar patterns are observed in Algonquin Provincial Park (protected area) in Ontario, Canada, where bears are seven times more likely to die outside the park, where they frequently make forays for food and are legally harvested (Obbard et al., 2017). As black bears are an intensively managed and harvested species across much of their range (Hristienko & McDonald, 2007),

elucidating factors influencing dispersal at broad scales have important conservation and management implications.

Black bear dispersal and density dependence studies have yielded inconsistent results. Schenk *et al.* (1998) suggested no evidence of female philopatry in a high-density population in a protected region of Ontario, in contrast to the male-biased dispersal reported in other populations of black bears (Costello, Creel, Kalinowski, Vu, & Quigley, 2008; Obbard et al., 2017; Rogers, 1987). In another study by Costello et al. (2008), positive density-dependent dispersal was observed in New Mexico black bears. Conversely, negative density dependent dispersal is documented in a different black bear population (Roy et al., 2012). These studies have limitations, however, such as sampling on small spatial scales given black bears can disperse hundreds of kilometers (Moore et al., 2014). Further, differences in population densities across sampled regions were minor in some cases (Costello et al., 2008; Roy et al., 2012). Overall, these inconsistences make results difficult to apply broadly and have implications for the need for larger-scale studies relevant to black bear ecology and metapopulation patterns.

Genetic techniques and network theory have been applied to characterize density-dependent and sex-biased dispersal patterns in large mammals (Draheim et al., 2016; Herrero et al., 2021; VanderWaal et al., 2014) based on genetic structure and patterns of gene flow directionality (Peery et al., 2008). Calculating gene flow directionality offers an advantage over traditional methods, such as mark-recapture or radio telemetry, that are expensive, labor intensive, and may lead to biased assessments of dispersal due to small sample size and limited geographic and temporal scope (W. D.

Koenig et al., 1996). Leveraging genetic techniques to characterize patterns of gene flow directionality allows for study of dispersal at spatial scales relevant to true dispersal capabilities of large, wide-ranging, and sometimes elusive, mammals (Howe et al., 2022; Stokes et al., 2010).

In this study, we employ a genetic network-based approach, similar to that of Draheim *et al.* (2016), to assess patterns of density-dependent and sex-biased dispersal in American black bears over a large area in Ontario, Canada (>1 000 000 km^2). Ontario black bears occupy a range that reflects continental patterns: a large continuous population across its northern range limit, with smaller fragmented populations to the south. The largest continuous black bear distribution in Ontario consists of a heterogeneous landscape, spanning multiple forest and climate zones, as well as variable exposure to anthropogenic influence (Obbard et al., 2017). Given the large extent, high variation in density (Howe et al., 2013), and landscape heterogeneity of the Ontario black bear population, it serves as an ideal system to study dispersal and its underlying drivers. Consistent with findings in other mammalian systems (Costello et al., 2008; Fattebert et al., 2015; Maag et al., 2018), we hypothesize that dispersal is largely driven by variation in resources. Further, we hypothesize the observed variation in resource availability is influenced by harvest-mediated bear density. We examine drivers of dispersal in black bears using genetic data that spans nearly their entire range across the province. We predicted that food productivity and density gradients would best explain patterns of black bear dispersal with bears dispersing from areas of lower productivity and higher density to those of higher productivity and lower density.

MATERIALS AND METHODS

Sample Collection

Genetic samples were collected as a part of the Ontario Ministry of Northern Development, Mines, Natural Resources, and Forestry's (NDMNRF) ongoing black bear monitoring program that monitors black bear populations across the province using non-invasive genetic sampling (Howe et al., 2013, 2022). Samples were collected from arrays of approximately 40 baited barbed-wire corrals (Woods et al., 1999), spaced roughly 1.5 km apart along secondary roads, over 5 weekly sampling occasions in spring and early summer. Individual sampling arrays were separated by a minimum of 25km. Although black bear range can vary from five to 250km^2 (Tri, 2013), it was unlikely that male bears would be detected at arrays greater than 25km apart during our sampling period in spring and early summer. During this time, black bear range is typically smaller (e.g., <25km; Humm et al., 2017; Noyce & Garshelis, 2011) before it expands in the fall when bears undergo seasonal food forays (Humm et al., 2017, Noyce & Garshelis, 2011; Obbard et al., 2017; supported by genotypes that found < 0.1% of detected across arrays). Here, we used samples collected from 73 arrays sampled in 2017, 2018, and 2019 (Figure 2.1) for a total of 2952 unique trap locations. Traps were constructed with a single strand of barbed wire strung around a series of trees at 50cm above the ground creating a corral (Howe et al., 2013). Wire height was considered sufficient to exclude sampling bears < 2 years of age that are mostly shorter than this height (NDMNRF unpublished data). A tree central within the corral was used to hang 3 cans of sardines to serve as bait; traps were rebaited weekly during sample collection. Black bears step

over or crawl under barbed wire, snagging hair that is subsequently used for DNA extraction and individual identification.

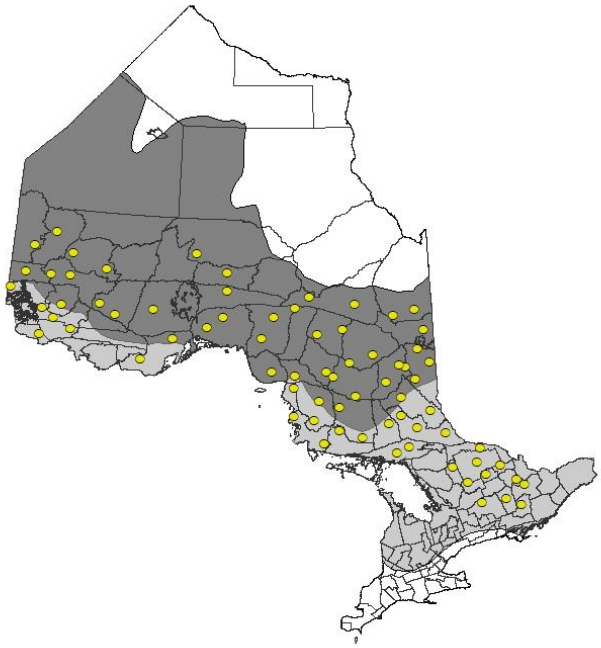


Figure 2.1. Trap array node centroids (trap 20 of each array), main forest regions and wildlife management unit (WMU) in Ontario, Canada. Dark grey represents boreal forest, while light grey represents Great Lakes St. Lawrence (GLSL; mixed deciduous) forest (Rowe, 1972). Lines dividing the province into different sections depict WMUs, which are the primary unit for making harvest management decisions.

DNA Extraction and Microsatellite Amplification

A minimum of 5 (average 20) hairs per sample were used for DNA extraction. DNA extraction followed previous, noninvasive, black bear DNA sampling projects (Howe et al., 2022; Pelletier et al., 2017; see Appendix A, S1). Samples were genotyped at 15 microsatellite loci and amelogenin locus (for sex determination) previously used by

Pelletier *et al.* (2017) and Howe *et* al. (2022) with modifications to optimize genotyping. Two microlitres of stock DNA was used to amplify all loci using the Qiagen Multiplex PCR Kit in two 12µL multiplex reactions. Cycling conditions were as follows: 95°C 15min; 30-32 cycles of 94°C for 30s, T_A for 90s, 72°C for 60s; and a final extension of 60°C for 45min. Reaction-specific cycling conditions are included in Appendix A, Table S1.

Individual Identification

PCR products were size-separated on an ABI 3730 with Genescan 500 LIZ (Applied Biosystems). Scoring was performed using GeneMarker v.2.6.4 (SoftGenetics). Individual identification of bears from the genotypes was conducted separately for each trapline. To avoid artificial creation of individual genotypes, when inconsistencies between closely related genotypes could not be confirmed as scoring error, dropout, or pullup, they were reamplified. If reamplification did not remedy inconsistencies, or clearly demonstrate an individual genotype, samples were discarded (see Appendix A, S2 for details). Individual genotypes were given a unique ID, after which all traplines were combined to analyse all individuals in allelematch. Bears found on more than one trapline ($n<5$) were assigned the first ID to ensure individuals were not counted multiple times.

Broad Scale Genetic Clustering

We first assessed sex-biased dispersal by analyzing separate datasets containing all sampled individuals, only males, and only females. We assessed broad scale genetic

structure using STRUCTURE v2.3.4 (Pritchard et al., 2000) and CLUMPAK (Kopelman et al., 2015). STRUCTURE is a Bayesian genetic clustering program used to determine the model (number of genetic clusters; K) that has the highest likelihood, based on provided genotype data (Pritchard et al., 2000). STRUCTURE v2.3.4 implements an aspatial method that considers admixture – one genotype existing in multiple genetic clusters. STRUCTURE then calculates membership proportions (*q*) of each individual genotype to each inferred cluster (Falush et al., 2003).

We ran STRUCTURE using StrAuto – a python utility that combines STRUCTURE analysis with ΔK analysis, outlined by Evanno *et al*. (2005), and visualization of results using STRUCTURE HARVESTER that allows for multiple Markov chain Monte Carlo (MCMC) runs in parallel (Chhatre & Emerson, 2017). We ran 10 iterations of STRUCTURE at each of k_{max} (k being a possible value of K) = 1-10 (100 total runs), with a burn in of 200 000 iterations, followed by 500 000 sampled MCMC iterations. We then used the 'Best K' feature of CLUMPAK to estimate the most likely model (number of genetic clusters; K) as described by Evanno *et al*. (2005). CLUMPAK uses ln(Pr(X|K)) values in order to identify k for which Pr(K=k) is highest. In instances where K=2 (all datasets), we performed post-hoc hierarchical STRUCTURE and ΔK analyses to assess if additional genetic structure existed within clusters identified in K=2 models (Janes et al., 2017). After the most likely number of genetic clusters (K) was determined, we proceeded with STRUCTURE results of that K value using the main CLUMPAK pipeline to visualize population structure using DISTRUCT (Rosenberg, 2004). STRUCTURE plots were organized by geographic location (southeast to northwest). We then visualized

STRUCTURE results spatially in ArcMap 10.7.1 using the interpolation tool (IDW). Data used for interpolation were population assignment values (northwest genetic cluster) for respective nodes.

Characterizing and modelling dispersal patterns

To infer dispersal direction, we calculated genetic flux – a metric developed by Draheim *et al*. (2016) to measure gene flow into, or out of, a particular area; in our case, arrays of traps. Genetic flux refers to gene flow directionality calculated using the method described by Draheim *et al*. (2016). This metric utilizes a combination of population genetic data and network theory. Networks are structures comprised of nodes and edges. Nodes are individual elements of the network (in this case single arrays), while edges represent the relationship between nodes (Minor & Urban, 2008). The concept of using networks has been applied in various studies to demonstrate population connectivity (Dyer et al., 2010; Koen et al., 2012; Rayfield et al., 2011). In these approaches, nodes are represented by mean pairwise genetic relatedness of individuals within a node and edges are represented by mean pairwise relatedness between nodes. Although this approach is useful to assess population connectivity, it is limited in that does not provide directionality to observed gene flow. To address this, an extension of this approach was developed by Draheim *et al*. (2016) to calculate genetic flux, which provides information on the directionality of gene flow between nodes.

We followed the general approach of Draheim et al. (2016) and defined each array of traps as a node. We selected a trap near the middle of the array (trap 20) as the

node centroid (Figure 2.1). We measured the net flux into, and out of, each of these nodes. Net flux was calculated using the formula:

$$F_{net} = F_{in\ i} - F_{out\ i} \quad (1)$$

Where $F_{in\ i}$ represents the flux into node *i* and $F_{out\ i}$ represents flux out of node *i*

$$F_{in\ i} = \Sigma f_{ji} \quad (2.1)$$

$$F_{out\ i} = \Sigma f_{ij} \quad (2.2)$$

Where Σf_{ij} represents flux from node *i* to node *j* and Σf_{ji} represents flux from node *j* to node *i*

$$f_{ij} = q_i \times p_{ij} \quad (3.1)$$

$$f_{ji} = q_j \times p_{ij} \quad (3.2)$$

Where p_{ij} represents dispersal probability between node *i* and node *j*, and q_i represents the quality metric for node *i*. Mean within-node relatedness was used as the quality metric for a given node. To calculate q_i, we used the maximum likelihood estimator within the program ML-Relate (Kalinowski et al., 2006) to calculate pairwise relatedness within each node. The mean pairwise relatedness value served as a quality metric for its respective node. Probability of dispersal was calculated as

$$p_{ij} = \exp(k \times d_{ij}) \quad (5)$$

where d_{ij} is the genetic distance (a metric assessing genetic differentiation) between node *i* and node *j*, calculated in R using gstudio (Dyer, 2012) and *k* is a distance decay coefficient that determines the steepness of the decline in the probability of dispersing between two nodes as a function of distance and is calculated as

$$k = \log(0.05/d) \quad (6)$$

Where d is equal to maximum dispersal distance. Maximum dispersal distance in black bears is typically greater in northern ranges, and smaller in areas of high food productivity (Moyer et al., 2007). Moore *et* al. (2014) determined that black bears on the Michigan peninsula, a similar habitat to Ontario, had a maximum dispersal distance of 187.2km for females, and 251.2km for males. Based on this information, we calculated the decay coefficient (k) using four different maximum dispersal distances (d; 150km, 200km, 250km, and 300km) and carried each resulting value of *k* through genetic flux calculations to assess influences on results. After net genetic flux was calculated for each node, these values were used as data points to visualize patterns of dispersal in ArcMap 10.7.1 using the interpolation tool.

We next sought to assess drivers of gene flow by fitting statistical models to calculated genetic flux values, treating each node's net flux as a data point in a linear regression. We fit a multivariate linear regression model with the covariates of bear density, forest zone, and harvest density to each of the three datasets (all individuals, male individuals, female individuals). Density estimates were calculated by the NDMNRF using a spatially explicit capture recapture (SECR) approach similar to that described in (Howe et al., 2013, 2022). Forest zone (Figure 2.1) was split into three separate covariates: Great Lakes St. Lawrence (GLSL; mixed deciduous), Boreal Buffer (coniferous), and GLSL Buffer, where buffer regions refer to the area bordering another forest (e.g., GLSL buffer is the area of GLSL forest adjacent to boreal forest). In this analysis, boreal forest was used as a reference class. Forest zone and black bear harvest density covariates were measured at the WMU level (Figure 2.1), while bear density was

measured at individual nodes. Harvest density was calculated as a function of total harvest and total land area, per wildlife management unit (WMU; Figure 2.1), where the total number of bears harvested was divided by the total land area in each WMU. We assumed that harvest density directly influences bear density, which is what we predicted drives dispersal patterns rather than bear density itself. Therefore, we replicated the model fitting without harvest density as a covariate. After fitting basic linear regressions, we used the residuals to calculate Moran's I to determine if spatial autocorrelation existed between nodes. Spatial autocorrelation ($p<0.05$) was observed for all datasets; Therefore, we refit linear regression models with spatially correlated errors using the package spaMM (Rousset, 2017) in the R statistical software. Following fitting, we recalculated Moran's I to ensure no residual spatial autocorrelation. We calculated 95% confidence intervals around estimated covariate coefficients and inferred significant effects when the confidence interval did not include zero.

As a post-hoc analysis, we repeated the above-mentioned model fitting procedure using harvest rate instead of harvest density. Harvest rate is a function of population size rather than land area. We used bear density estimates to estimate total population size for each WMU. In the case that there were multiple arrays in a single WMU, we averaged density estimates obtained from each array. We combined this with WMU-level harvest data (average number of bears harvested annually between 1998-2018) to calculate harvest rate as the proportion of bears harvested in each WMU.

RESULTS

Broad Scale Genetic Clustering

ΔK analyses found the two-cluster model was most likely based on available data for all three datasets (Appendix A, Figure S1). We averaged results from all (n=10) iterations for K=2 in the main CLUMPAK pipeline. STRUCTURE depicted two genetic clusters (Figure 2.2) with partial admixture as locations moved from southeast (dark brown) to northwest (light yellow) - a consistent finding in all three datasets. STRUCUTRE maps were similar between all three datasets (male and female STRUCTURE maps found in Appendix A, Figure S3). Similar to the underlying heat map, assignment probabilities show a gradient as nodes move from southeast to northwest Ontario. This is consistent with past findings of Pelletier *et al.* (2012) who documents an isolation by distance pattern in the Ontario black bear population.

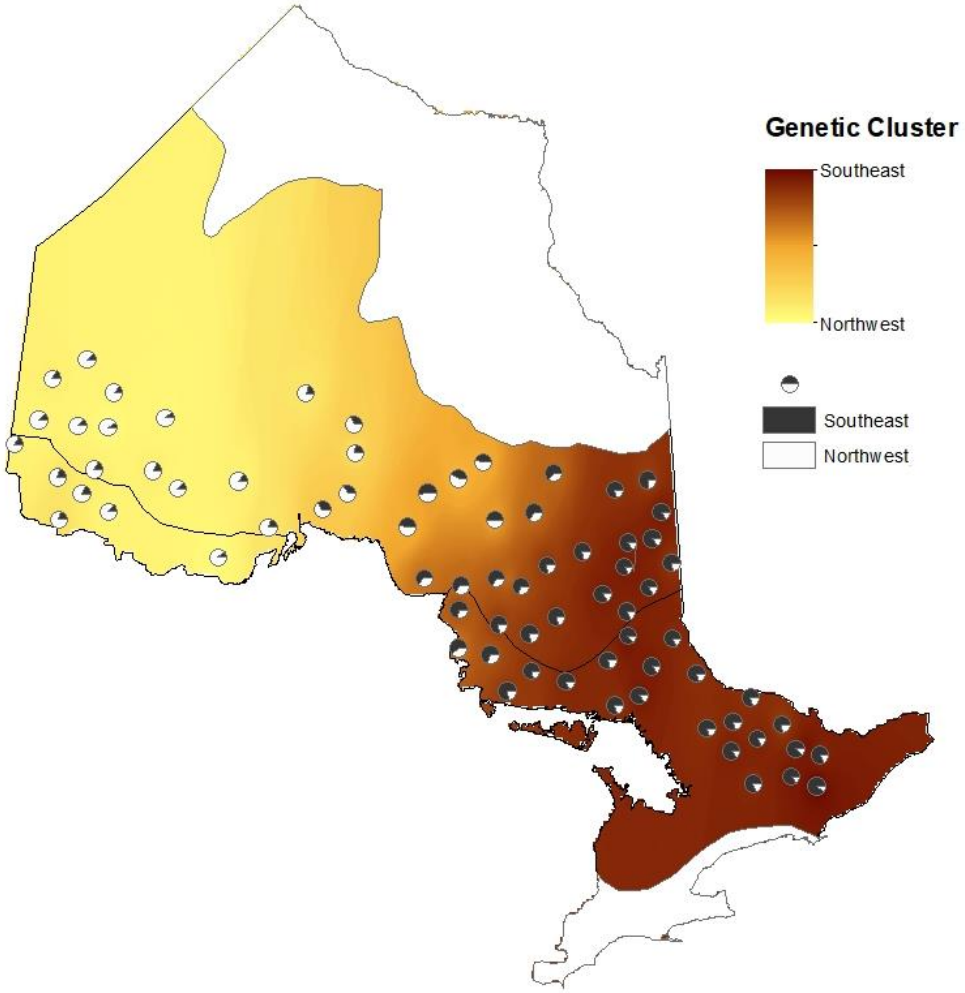


Figure 2.2. Spatial visualization of STRUCTURE results (K = 2) for 3941 American black bears (*Ursus americanus),* estimated using ArcMap 10.7.1's interpolation tool (IDW). Dark brown represents strong population assignment probability to the southeast genetic cluster, while light yellow represents strong population assignment probability to the northwest genetic cluster. Each node centroid is represented by a pie chart with black representing population assignment probability to the southeast cluster, and white representing population assignment probability to the northwest genetic cluster, for that particular node.

Hierarchical ΔK analyses suggest the western cluster is further divided into 3 sub-clusters for each of the three datasets. The eastern cluster was divided into two sub-clusters for the datasets using all and male bears and five sub-clusters for the dataset using female bears (Appendix A, Figure S4). Although more sub-clustering may be present, among these analyses, individual assignment probabilities were seldom greater than 0.5 when K was greater than 2 (Appendix A, Figure S5-S7).

Characterizing and modelling dispersal patterns

Across the four values of maximum dispersal distance (d; 150, 200, 250, 300), there was little difference in the final genetic flux values and patterns (Appendix A, Table S2). Based on these findings, we selected 200km as the maximum dispersal distance, reflecting observed dispersal distances and life history characteristics (Tri, 2013), and proceeded with a subsequent *k*-value of -0.0149. We visualized patterns of dispersal in the form of a heat map informed by net genetic flux values at individual nodes (Figure 2.3). The northwestern region of the province appeared to have the most emigration, while there was strong immigration into a large swath of the central part of the province (Figure 2.3). This pattern was consistent for all three datasets (dispersal map generated using all individuals can be found in Appendix A, Figure S8). A notable difference was observed in the male dispersal map (Figure 2.3a), where immigration was more prominent in the GLSL (mixed deciduous) forest than in the Boreal. In contrast, the dispersal map for females (Figure 2.3b) displayed increased immigration in

higher-density nodes in northwest Ontario, and emigration from a couple of nodes in central Ontario.

a)

b)

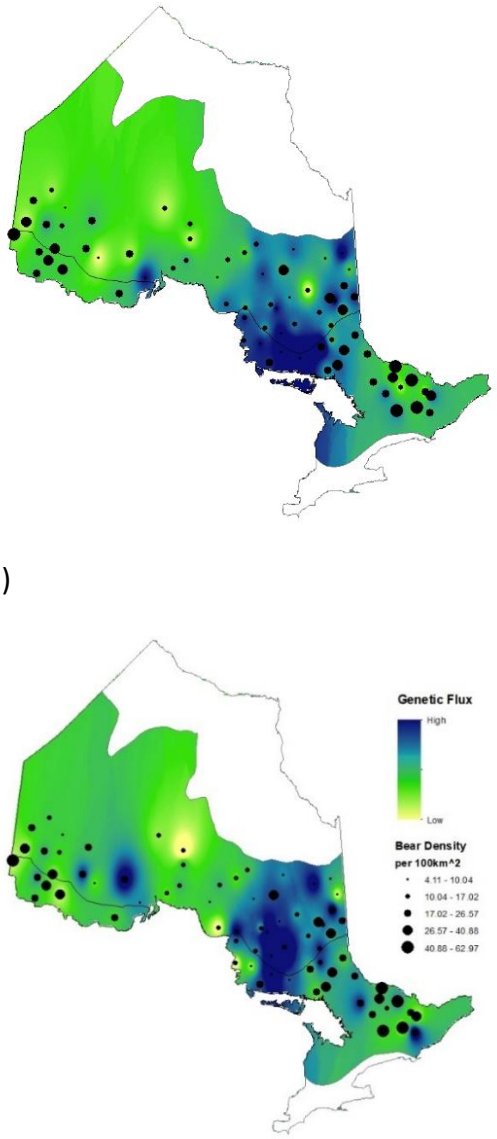


Figure 2.3. Dispersal maps, informed by net genetic flux values, constructed using the interpolation tool (IDW) in ArcMap 10.7.1 for a) male black bears (*Ursus americanus*; n=2250) and b) female black bears (n=1691). Dark blue represents high net immigration (positive genetic flux), and light green represents high net emigration (negative genetic

flux) from a node. Points represent node centroid locations and are sized according to density (large point = high bear density, small point = low bear density).

There was no significant residual spatial autocorrelation in the model with spatially correlated errors. No significant relationships were identified between genetic flux and either harvest density or harvest rate in any of the three datasets. Additionally, there were no significant relationships between genetic flux and any covariates in data from females only or data from both sexes combined. However, we identified significant relationships between genetic flux and both demographic and landscape covariates in data from males. Bear density, GLSL, and GLSL buffer all had significant relationships with genetic flux; a consistent finding in models run with and without harvest covariates. As density increased, there was greater net flux out of a node, with higher flux into low density nodes (coefficient = -0.103, 95% CIs = -0.205 - -0.005). There was also greater net flux into nodes in the GLSL and GLSL buffer relative to the boreal. One of the two forest covariates, GLSL buffer, demonstrated a stronger relationship than GLSL (coefficients = 0.54 and 0.28, 95% CIs = 0.238-0.864 and 0.101-0.560, respectively).

Table 2.1. Results of spatial models including harvest rate covariate. Columns represent dataset used (all bears, male bears, and female bears, respectively). Covariates are bear density (per 100km^2), Harvest Rate, Boreal buffer, GLSL, and GLSL buffer. Table values are coefficients, with standard errors in brackets. Significant results are bolded with an asterisk. Results for spatial models using the covariate harvest density, as well as no harvest covariates, found in Appendix A.

	All bears	Male Bears	Female Bears
Intercept	-0.088 (0.07)	-0.305 (0.108)	-0.076 (0.121)
Density	-0.063 (0.035)	**-0.108 (0.05)***	-0.110 (0.071)
Harvest Rate	0.006 (0.029)	-0.019 (0.041)	0.084 (0.060)
Boreal Buffer	0.087 (0.087)	0.217 (0.123)	0.060 (0.174)
GLSL	0.045 (0.099)	**0.295 (0.139)***	-0.344 (0.194)
GLSL buffer	0.124 (0.103)	**0.544 (0.144)***	-0.151 (0.195)

DISCUSSION

Quantifying patterns and drivers of dispersal are critical to understanding ecological and evolutionary patterns at both historical and contemporary scales. These data are also key to informed management and conservation decisions aimed at sustaining populations that are subject to increasing anthropogenetic impacts from harvest, habitat loss, and climate change. In this study, we were able to sample over spatial extents that capture both maximum dispersal distances and provide meaningful variation in underlying factors that may influence animal movement and directionality of movement. Matching our predictions, we found that dispersal in male black bears was related to density and productivity gradients, where bears moved from areas of

high density and low food productivity to areas of lower density and higher productivity. Importantly, we did not see similar patterns in female bears, or when combining data from males and females, supporting past research that black bear dispersal is male-biased (Costello et al., 2008; Obbard et al., 2017; Rogers, 1987). These patterns were apparent despite limited population structure at large geographic scales, as evidenced by STRUCTURE results. These findings advance our understanding of dispersal in large mammals, suggesting the presence of source-sink dynamics in a harvested system.

Density

Our finding that males dispersed away from areas of high density towards those with fewer conspecifics has important implications for understanding population dynamics, particularly for harvested species. Density-dependent feedback between vital rates (births, deaths, immigration, and emigration) is an integral part of sustainable harvest (A. K. Harrison, 2011), especially in systems exhibiting source-sink dynamics (Draheim et al., 2016). For example, in metapopulations with sources and sinks linked by dispersal, overharvest of the source can have profound influence on both populations. Typical wildlife management approaches often involve measuring population densities and growth rates, but do not monitoring metapopulations and interactions of constituent populations (A. K. Harrison, 2011; Novaro et al., 2005). Our gene flow directionality results suggest the existence of potential source and sink populations. In harvested systems where sink populations are subject to increased harvest pressure reducing population density, populations can remain stable at low densities through

support of a source population (Gaggiotti, 1996). This pattern has been documented in black bears in select regions of Ontario (Obbard et al., 2017) as well as other large mammals (Robinson et al., 2008). However, some studies on black bear dispersal addressing source-sink dynamics have produced contrasting results. Some suggest increased harvest pressures can create population sinks (Gantchoff et al., 2020; S. Miller & Tutterrow, 1999; Obbard et al., 2017), while other suggest increased harvest is indicative of high bear density suggesting a source (Draheim et al., 2016). These contrasting results could be due to a lack of broad systematic sampling and demographic data (Draheim et al., 2016; Gantchoff et al., 2020; S. Miller & Tutterrow, 1999; Obbard et al., 2017). Our results provide unique and relevant information that remedy these inconsistencies and are useful for managing large, harvested mammals. For species that can disperse over distances much greater than the size of management units, careful consideration of possible source-sink dynamics is needed for effective management and conservation at appropriate spatial scales. Establishing designated source areas to sustain adjacent sub-populations can be a more effective means of sustainable harvest relative to regulations based solely on demographic rates (A. K. Harrison, 2011; Novaro et al., 2005).

Harvest

We suspect that much of the pattern between flux and bear density is driven by harvest, despite no significant relationship between flux and harvest. This is likely due to the coarse resolution for harvest metrics relative to bear density and genetic flux.

NDMNRF collects harvest data at the WMU scale, and some WMUs cover large areas. There were up to 4 trap arrays within a WMU, and these arrays/nodes often showed substantial differences in density (Howe et al., 2022). Harvest analyses assigned each of these nodes the same harvest density even though harvest pressure varied spatially within WMUs. Although we hoped calculating harvest rate would ameliorate this potential limitation, bear density was still averaged across all trap arrays within a WMU to estimate population size. Further, if density patterns are the result of long-term depletion of local populations, contemporary harvest rates are unlikely to be a meaningful measure as they could differ significantly from past rates. Thus, using density as the independent variable is likely a more robust assessment because it compares local genetic patterns to local densities.

Interestingly, however, the area displaying the highest immigration also has one of the highest harvest rates in the province (Figure 2.4), where harvest rate in this area (0.32) is nearly double the next highest (0.18). This is likely unsustainable for black bears absent a continual influx of dispersing individuals, based on past studies of harvest rate and its influence on populations growth rate (Hristienko & McDonald, 2007; Loosen et al., 2019; S. D. Miller, 1990). The only area in this study system exhibiting a higher harvest rate (0.5) was a small WMU immediately south of Algonquin Provincial Park, a large, protected area with a high bear density (27.6/100km^2). This further suggests active harvest-driven source-sink dynamics, as black bears move from the park to this lower density area (13.9/100km^2) on food forays in the late summer/early fall, where they are highly vulnerable to harvest (Obbard et al. 2017). Harvest-driven source-sink

dynamics have been suggested in several other wide-ranging mammals such as mountain lions (Andreasen et al., 2012; Robinson et al., 2008), elephants (Jeke et al., 2019), and a variety of ungulates (Naranjo & Bodmer, 2007). Some of these systems demonstrate unsustainable rates of harvest (Naranjo & Bodmer, 2007). In contrast, other systems establish designated source regions that sustain sink populations subject to increased harvest pressure through a continual influx of dispersing individuals (Robinson et al., 2008). Thus, understanding source-sink dynamics can serve to improve population management strategies, particularly in harvested systems.

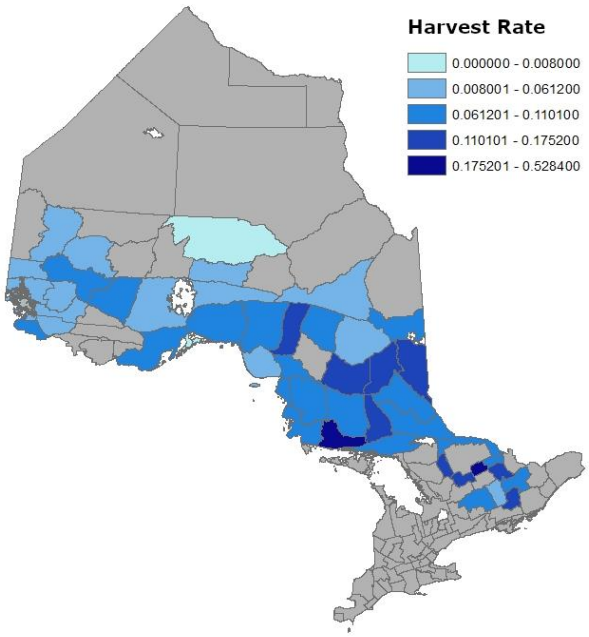


Figure 2.4. Harvest rate (proportion of population harvested) calculated at the wildlife management unit (WMU) level. Darker blue WMUs indicate higher harvest rate relative to lighter blue WMUs. Grey WMUs did not have density estimates available, or are protected areas with no harvest, and therefore, we were unable to calculate harvest rate for those WMUs.

Forest Type

Both dispersal maps (Figure 2.3) show increased immigration into the low-density nodes located in central Ontario. However, male bears (Figure 2.3a) appear to show higher immigration to the nodes located in the GLSL forest as opposed to the Boreal forest. These findings likely reflect the strong influence of known productivity gradients between forest zones where black bear movement has been related to food productivity and availability (Karelus et al., 2019). Prominent tree species in the Boreal forest include pine, black spruce, and white birch (Bonan et al., 1992). In contrast, the GLSL forest (mixed deciduous) provides a greater diversity of soft mast-producing trees and shrubs. The GLSL forest also contains a higher proportion of hard mast species such as oak and beech that produce high-calorie nuts preferred by black bears in fall months prior to hibernation (Rowe, 1972). Late summer/early fall is also a time when the range of male black bears extends as they search for food (Obbard, Coady, et al., 2010). Bear density itself was much higher, on average, in the GLSL (Howe et al. 2022), a pattern previously identified in Ontario (Howe et al., 2013). Higher bear density in the GLSL forest further supports that these areas are more productive for black bears than the boreal forest. Importantly, our models account for the effect of density and still show this strong effect of productivity. Together with density results, these findings suggest that dispersing male bears are seeking areas with fewer competitors and high forage availability. This directly corresponds with the known behaviour and social hierarchy of black bears, where large, older males can dominate breeding opportunities, and where individuals need to obtain sufficient calories prior to denning (Kolenosky, 1990).

Male-biased dispersal

Significant relationships were only identified in data from males, suggesting male-biased dispersal consistent with most past findings in black bears (Costello et al., 2008; Moore et al., 2014; Pelletier et al., 2017, 2012; Rogers, 1987; but see Schenk et al. 1998), and large mammals in general (Greenwood 1980). In wildlife populations exhibiting strong male-biased dispersal, STRUCTURE plots using male individuals typically display increased admixture compared to STRUCTURE plots using female individuals (Herrero et al., 2021) reflecting increased gene flow of dispersing males. Our STRUCTURE results differ from this pattern but remain consistent with previous work (Pelletier et al., 2012), identifying two broad genetic clusters that can be described as southeast and northwest. At a broad scale, this pattern is indicative of clinal isolation by distance. Although we did not identify notable differences in our STRUCTURE results between males and females, multivariate models that incorporate landscape and demographic variables, similar to the ones we use in our genetic flux modelling, perform significantly better at explaining population structure compared to IBD models (Storfer et al., 2010). In contrast to prior studies, we used a larger dataset of systematically collected samples from a much larger study area. (Costello et al., 2008; Moore et al., 2014; Obbard et al., 2017; Pelletier et al., 2012; Rogers, 1987).

Conclusions

Accurately identifying source and sink populations can be difficult as it requires sampling of all populations within a metapopulation. The structured and dense black

bear sampling scheme across Ontario, made possible by a large-scale monitoring program, allowed for sampling over a broad geographic extent while still capturing fine-scale variation. Overall, results of our study suggest male-biased positive density-dependent dispersal that is also influenced by broad food productivity gradients. We speculate that this type of dispersal is further driven by harvest pressure, maintaining low bear density in highly productive regions of the province. These results are highly relevant to black bears in other areas, as well as other large mammal species, as similar patterns to the ones documented herein likely exist for species with high dispersal capabilities across strong gradients in harvest and forage productivity. This description characterizes most large carnivore species as well as other large mammals. Thus, our findings suggest these dynamics are likely more prevalent than currently understood. Our results can be used in tandem with demographic and environmental data to make informed conservation and management decisions in large, harvested, mammals, now and in the future. Future studies might measure variation in harvest pressure at finer scales to further our understanding of the implications of harvest on source-sink dynamics.

REFERENCES

Aars, J., & Ims, R. A. (2000). Population Dynamic and Genetic Consequences of Spatial Density-Dependent Dispersal in Patchy Populations. *The American Naturalist*, *155*(252–265).

Amarasekare, P. (2006). Productivity, dispersal and the coexistence of intraguild predators and prey. *Journal of Theoretical Biology*, *243*(1), 121–133.

Andrade-Restrepo, M., Champagnat, N., & Ferriere, R. (2019). Local adaptation, dispersal evolution, and the spatial eco-evolutionary dynamics of invasion. *Ecology Letters*, *22*(5), 767–777.

Andreasen, A. M., Stewart, K. M., Longland, W. S., Beckmann, J. P., & Forister, M. L. (2012). Identification of source-sink dynamics in mountain lions of the Great Basin. *Molecular Ecology*, *21*(23), 5689–5701. https://doi.org/10.1111/j.1365-294X.2012.05740.x

Baguette, M., Benton, T. G., & Bullock, J. M. (2012). *Dispersal ecology and evolution*. Oxford University Press.

Baguette, M., Clobert, J., & Schtickzelle, N. (2011). Metapopulation dynamics of the bog fritillary butterfly: experimental changes in habitat quality induced negative density-dependent dispersal. *Ecography*, *34*(1), 2011.

Bonan, G. B., Pollard, D., & Thompson, S. L. (1992). Effects of boreal forest vegetation on global climate. *Nature*, *359*(6397), 716.

Brown, S. K., Hull, J. M., Updike, D. R., Fain, S. R., & Ernest, H. B. (2009). Black Bear Population Genetics in California: Signatures of Population Structure, Competitive Release, and Historical Translocation. *Journal of Mammalogy*, *90*(5), 1066–1074.

Chhatre, V. E., & Emerson, K. J. (2017). StrAuto: automation and parallelization of STRUCTURE analysis. *BMC Bioinformatics*, *18*(1), 1–5.

Clobert, J., Le Galliard, J. F., Cote, J., Meylan, S., & Massot, M. (2009). Informed dispersal, heterogeneity in animal dispersal syndromes and the dynamics of spatially structured populations. *Ecology Letters*, *12*(3), 197–209. https://doi.org/10.1111/j.1461-0248.2008.01267.x

Costello, C. M., Creel, S. R., Kalinowski, S. T., Vu, N. v., & Quigley, H. B. (2008). Sex-biased natal dispersal and inbreeding avoidance in American black bears as revealed by spatial genetic analysis. *Molecular Ecology*, *17*(21), 4713–4723.

De Bona, S., Bruneaux, M., Lee, A. E. G., Reznick, D. N., Bentzen, P., & Lopez-Sepulcre, A. (2019). Spatio-temporal dynamics of density-dependent dispersal during a population colonisation. *Ecology Letters*, *22*(4), 634–644.

Dixon, J. D., Oli, M. K., Wooten, M. C., Eason, T. H., McCown, J. W., & Cunningham, M. W. (2007). Genetic consequences of habitat fragmentation and loss: The case of the Florida black bear (Ursus americanus floridanus). *Conservation Genetics*, *8*(2), 455–464. https://doi.org/10.1007/s10592-006-9184-z

Doncaster, C. P. (2000). Extension of ideal free resource use to breeding populations and metapopulations. *Oikos*, *89*(1), 24–36. https://doi.org/10.1034/j.1600-0706.2000.890103.x

Draheim, H. M., Moore, J. A., Etter, D., Winterstein, S. R., & Scribner, K. T. (2016). Detecting black bear source–sink dynamics using individual-based genetic graphs. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1835). https://doi.org/10.1098/rspb.2016.1002

Dyer, R. J. (2012). *The gstudio Package*.

Dyer, R. J., Nason, J. D., & Garrick, R. C. (2010). Landscape modelling of gene flow: Improved power using conditional genetic distance derived from the topology of population networks. *Molecular Ecology*, *19*(17), 3746–3759. https://doi.org/10.1111/j.1365-294X.2010.04748.x

Falush, D., Stephens, M., & Pritchard, J. K. (2003). Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics*, *164*(4), 1567–1587.

Fattebert, J., Balme, G., Dickerson, T., Slotow, R., & Hunter, L. (2015). Density-Dependent Natal Dispersal Patterns in a Leopard Population Recovering from Over-Harvest. *PLoS ONE*, *10*(4).

Gaggiotti, O. E. (1996). Population genetic models of source – sink metapopulations. *Theoretical Population Biology*, *50*(2), 178–208.

Gaillard, J. M., Hewison, A. J. M., Kjellander, P., Pettorelli, N., Bonenfant, C., Van Moorter, B., Liberg, O., Andren, H., Van Laere, G., Klein, F., Angibault, J. M., Coulon, A., & Vanpe, C. (2008). Population density and sex do not influence fine-scale natal dispersal in roe deer. *Proceedings of the Royal Society B: Biological Sciences*, *275*(1646), 2025–2030.

Gantchoff, M. G., Hill, J. E., Kellner, K. F., Fowler, N. L., Petroelje, T. R., Conlee, L., Beyer, D. E., & Belant, J. L. (2020). Mortality of a large wide-ranging mammal largely caused by anthropogenic activities. *Scientific Reports*, *10*(1), 1–11. https://doi.org/10.1038/s41598-020-65290-9

Greenwood, P. J. (1980). Matingsystems, philopatry and dispersal in birds and mammals. *Animal Behaviour*, *28*(4), 1140–1162.

Grimm, N. B., Foster, D., Groffman, P., Grove, J. M., Hopkinson, C. S., Nadelhoffer, K. J., Pataki, D. E., & Peters, D. P. C. (2008). The changing landscape: ecosystem responses to urbanization and pollution across climatic and societal gradients. *Frontiers in Ecology and the Environment*, *6*(5), 264–272.

Hanski, I. (1998). Metapopulation dynamics. *Nature*, *396*(6706), 41–49. https://doi.org/10.1038/23876

Harman, R. R., Goddard II, J., Shivaji, R., & Cronin, J. T. (2020). Frequency of Occurrence and Population-Dynamic Consequences of Different Forms of Density- Dependent Emigration. *The American Naturalist*, *195*(5), 851–867.

Harrison, A. K. (2011). *Dispersal and compensatory population dynamics in a harvested mammal*.

Harrison, R. G. (1980). Dispersal polymorphism in insects. *Annual Review of Ecology and Systematics*, *11*(1), 95–118.

Herrero, A., Klütsch, C. F. C., Holmala, K., Maduna, S. N., Kopatz, A., Eiken, H. G., & Hagen, S. B. (2021). Genetic analysis indicates spatial-dependent patterns of sex-biased dispersal in Eurasian lynx in Finland. *PLoS ONE*, *16*(2 February 2021), 1–22. https://doi.org/10.1371/journal.pone.0246833

Howe, E. J., Obbard, M. E., & Kyle, C. J. (2013). Combining data from 43 standardized surveys to estimate densities of female American black bears by spatially explicit capture–recapture. *Population Ecology*, *55*, 595–607. https://doi.org/10.1007/s10144-013-0389-y

Howe, E. J., Potter, D., Beauclerc, K. B., Jackson, K. E., & Northrup, J. M. (2022). Estimating animal abundance at multiple scales by spatially explicit capture-recapture. *Ecological Applications*.

Hristienko, H., & McDonald, J. E. (2007). Going into the 21st century: a perspective on trends and controversies in the management of the American black bear. *Ursus*, *18*(1), 72–88.

Humm, J. M., McCown, W. J., Scheick, B. K., & Clark, J. D. (2017). Spatiall Explicit Population Estimates for Black Bears Based on Cluster Sampling. *The Journal of Wildlife Management*, *81*(7), 1187–1201.

Ims, R. A., & Andreassen, H. P. (2005). Density-dependent dispersal and spatial population dynamics. *Proceedings of the Royal Society B: Biological Sciences*, *272*, 913–918.

Janes, J. K., Miller, J. M., Dupuis, J. R., Malenfant, R. M., Gorrell, J. C., Cullingham, C. I., & Andrew, R. L. (2017). The K = 2 conundrum. *Molecular Ecology*, *26*(14), 3594–3602.

Jeke, A., Chanyandura, A., Muposhi, V. K., Madhlamoto, D., & Gandiwa, E. (2019). Trophy Hunting and Possible Source-Sink Dynamics in Protected Areas: Insights from Trophy Size and Offtake Patterns in Southeast Zimbabwe. *International Journal of Zoology*, *2019*. https://doi.org/10.1155/2019/1313927

Kalinowski, S. T., Wagner, A. P., & Taper, M. L. (2006). ML-RELATE: a computer program for maximum likelihood estimation of relatedness and realtionship. *Molecular Ecology Notes*, *6*(2), 576–579.

Karelus, D. L., McCown, J. W., Scheick, B. K., van de kerk, M., Bolker, B. M., & Oli, M. K. (2019). Incorporating movement patterns to discernhabitat selection: black bears as a case study. *Wildlife Research*, *46*(1), 76–88.

Koen, E. L., Bowman, J., Garroway, C. J., Mills, S. C., & Wilson, P. J. (2012). Landscape resistance and American marten gene flow. *Landscape Ecology*, *27*(1), 29–43. https://doi.org/10.1007/s10980-011-9675-2

Koen, E. L., Bowman, J., & Wilson, P. J. (2016). Node-based measures of connectivity in genetic networks. *Molecular Ecology Resources*, *16*, 69–79.

Koenig, W. D., van Vuren, D., & Hooge, P. N. (1996). Detectability, philopatry, and the distribution of dispersal distances in vertebrates. *Trends in Ecology & Evolution*, *11*(12), 514–517.

Kolenosky, G. B. (1990). Reproductive Biology of Black Bears in East-Central Ontario. *Ursus*, *8*, 385–392.

Kopelman, N. M., Mayzel, J., Jakobsson, M., Rosenberg, N. A., & Mayrose, I. (2015). CLUMPAK: a program for identifying clustering modes and packaging population structure inferences across K. *Molecular Ecology Resources*, *15*(5), 1179–1191. https://doi.org/10.1111/1755-0998.12387

Labonte, J., Oullet, J.-P., Courtois, R., & Belisle, F. (1998). Moose Dispersal and Its Role in the Maintenance of Harvested Populations. *The Journal of Wildlife Managment*, *62*(1), 225–235.

Lawson Handley, L. J., & Perrin, N. (2007). Advances in our understanding of mammalian sex-biased dispersal. *Molecular Ecology*, *16*(8), 1559–1578. https://doi.org/10.1111/j.1365-294X.2006.03152.x

Loe, L. E., Mysterund, A., Veiberg, V., & Langvatn, R. (2009). Negative density-dependent emigration of males in an increasing red deer population. *Proceedings of the Royal Society B: Biological Sciences*, *276*, 2581–2587.

Loosen, A. E., Morehouse, A. T., & Boyce, M. S. (2019). Land tenure shapes black bear density and abundance on a multi-use landscape. *Ecology and Evolution*, *9*(1), 73–89. https://doi.org/10.1002/ece3.4617

Maag, N., Cozzi, G., Clutton-Brock, T., & Ozgul, A. (2018). Density-dependent dispersal strategies in a cooperative breeder. *Ecology*, *99*(9), 1932–1941. https://doi.org/10.1002/ecy.2433

Marjamaki, P. H., Contasti, A. L., Coulson, T. N., & McLoughlin, P. D. (2013). Local density and group size interacts with age and sex to determine direction and rate of social dispersal in a polygynous mammal. *Ecology and Evolution*, *3*(9), 3073–3082.

Matthysen, E. (2005). Density-dependant dispersal in birds and mammals. *Ecography*, *28*, 403–416.

Miller, S. D. (1990). Population Managment of Bears in North America. *Ursus*, *8*, 357–373.

Miller, S., & Tutterrow, V. Leigh. (1999). Characteristics of nonsport mortalities to brown and black bears and human injuries from bears in Alaska. *Ursus*, *11*, 239–252.

Minor, E. S., & Urban, D. L. (2008). A graph-theory framework for evaluating landscape connectivity and conservation planning. *Conservation Biology*, *22*(2), 297–307. https://doi.org/10.1111/j.1523-1739.2007.00871.x

Moore, J. A., Draheim, H. M., Etter, D., Winterstein, S., & Scribner, K. T. (2014). Application of large-scale parentage analysis for investigating natal dispersal in highly vagile vertebrates: A case study of American black bears (Ursus americanus). *PLoS ONE*, *9*(3). https://doi.org/10.1371/journal.pone.0091168

Moyer, M. A., McCown, J. W., & Oli, M. K. (2007). Factors Influencing Home-Range Size of Female Florida Black Bears. *Journal of Mammalogy*, *88*(2), 468–476.

Naranjo, E. J., & Bodmer, R. E. (2007). Source-sink systems and conservation of hunted ungulates in the Lacandon Forest, Mexico. *Biological Conservation*, *138*(3–4), 412–420. https://doi.org/10.1016/j.biocon.2007.05.010

Nathan, R. (2001). The challenges of studying dispersal. *Trends in Ecology and Evolution*, *16*(9), 481–483.

Novaro, A. J., Funes, M. C., & Walker, R. S. (2005). An empirical test of source-sink dynamics induced by hunting. *Journal of Applied Ecology*, *42*(5), 910–920.

Noyce, K. V., & Garshelis, D. L. (2011). Seasonal migrations of black bears (Ursus americanus): Causes and consequences. *Behavioral Ecology and Sociobiology*, *65*(4), 823–835. https://doi.org/10.1007/s00265-010-1086-x

Obbard, M. E., Coady, M. B., Pond, B. A., Schaefer, J. A., & Burrows, F. G. (2010). A distance-based analysis of habitat selection by American black bears (Ursus americanus) on the Bruce Peninsula, Ontario, Canada. *Canadian Journal of Zoology*, *88*, 1063–1076. https://doi.org/10.1139/Z10-072

Obbard, M. E., Newton, E. J., Potter, D., Orton, A., Patterson, B. R., & Steinberg, B. D. (2017). Big enough for bears? American black bears at heightened risk of mortality during seasonal forays outside Algonquin Provincial Park, Ontario. *Ursus*, *28*(2), 182–194. https://doi.org/10.2192/ursu-d-16-00021.1

Peacock, E., Peacock, M. M., & Titus, K. (2007). Black bears in Southeast Alaska: the fate of two ancient lineages in the face of contemporary movement. *Journal of Zoology*, *271*(4), 445–454.

Peery, Z. M., Beissinger, S. R., House, R. F., Berube, M., Hall, L. A., Sellas, A., & Palsboll, P. J. (2008). Characterizing source – sink dynamics with genetic parentage assignments. *Ecology*, *89*(10), 2746–2759.

Pelletier, A., Obbard, M. E., Harnden, M., McConnell, S., Howe, E. J., Burrows, F. G., White, B. N., & Kyle, C. J. (2017). Determining causes of genetic isolation in a large carnivore (Ursus americanus) population to direct contemporary conservation measures. *PLoS ONE*, *12*(2), 1–23. https://doi.org/10.1371/journal.pone.0172319

Pelletier, A., Obbard, M. E., Mills, E. J., Burrows, F. G., White, B. N., & Kyle, C. J. (2012). Delineating genetic groupings in continuously distributed species across largely homogeneous landscapes: a study of American black bears (Ursus americanus) in Ontario, Canada. *Canadian Journal of Zoology*, *90*, 999–1014. https://doi.org/10.1139/Z2012-068

Pillay, R., Johnsingh, A. J. T., Raghunath, R., & Madhusudan, M. D. (2011). Patterns of spatiotemporal change in large mammal distribution and abundance in the southern Western Ghats, India. *Biological Conservation*, *144*(5), 1567–1576.

Poethke, H. J., & Hovestadt, T. (2002). Evolution of density- and patch-size-dependent dispersal rates. *Proceedings of the Royal Society B: Biological Sciences*, *269*(1491), 637–645. https://doi.org/10.1098/rspb.2001.1936

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*(2), 945–959.

Puckett, E. E., Etter, P. D., Johnson, E. A., & Eggert, L. S. (2015). Phylogeographic analyses of American black bears (Ursus americanus) suggest four glacial refugia and complex patterns of postglacial admixture. *Molecular Biology and Evolution*, *32*(9), 2338–2350. https://doi.org/10.1093/molbev/msv114

Rayfield, B., Fortin, M.-J., & Fall, A. (2011). Connectivity for conservation: a framework to classify network measures. *Ecology*, *92*(4), 847–858.

Robinson, H. S., Wielgus, R. B., Cooley, H. S., & Cooley, S. W. (2008a). Sink populations in carnivore management: Cougar demography and immigration in a hunted population. *Ecological Applications*, *18*(4), 1028–1037. https://doi.org/10.1890/07-0352.1

Rogers, L. L. (1987). Factors influencing dispersal in the black bear. *Mammalian Dispersal Patterns: The Effects of Social Structure on Population Genetics*, 75–84.

Ronce, O. (2007). How does it feel to be like a rolling stone? Ten questions about dispersal evolution. *Annual Review of Ecology, Evolution, and Systematics*, *38*, 231–253. https://doi.org/10.1146/annurev.ecolsys.38.091206.095611

Rosenberg, N. A. (2004). DISTRUCT: a program for the graphical display of population structure. *Molecular Ecology Notes*, *4*(1), 137–138.

Rousset, F. (2017). *An introduction to the spaMM package for mixed models.*

Roy, J., Yannic, G., Côté, S. D., & Bernatchez, L. (2012). Negative density-dependent dispersal in the American black bear (Ursus americanus) revealed by noninvasive sampling and genotyping. *Ecology and Evolution*, *2*(3), 525–537. https://doi.org/10.1002/ece3.207

Schenk, A., Obbard, M. E., & Kovacs, K. M. (1998). Genetic relatedness and home-range overlap among female black bears (Ursus americanus) in Northern Ontario, Canada. *Canadian Journal of Zoology*, *76*(8), 1511–1519.

Sheard, C., Neate-Clegg, M. H. C., Alioravainen, N., Jones, S. E. I., Vincent, C., MacGregor, H. E. A., Bregman, T. P., Claramunt, S., & Tobias, J. A. (2020). Ecological drivers of global gradients in avian dispersal inferred from wing morphology. *Nature Communications*, *11*(1). https://doi.org/10.1038/s41467-020-16313-6

Storfer, A., Murphy, M. A., Spear, S. F., Holderegger, R., & Waits, L. P. (2010). Landscape genetics: Where are we now? *Molecular Ecology*, *19*(17), 3496–3514. https://doi.org/10.1111/j.1365-294X.2010.04691.x

Travis, J. M. J., & Dytham, C. (1999). Habitat persistence, habitat availability and the evolution of dispersal.pdf. *Proceedings of the Royal Society of London. B: Biological Sciences*, *266*(1420), 723–728.

Travis, J. M. J., Murrell, D. J., & Dytham, C. (1999). The evolution of density dependent dispersal. *Proceedings of the Royal Society of London. B: Biological Sciences*, *266*(1432), 1837–1842.

Tri, A. (2013). *Temporal, Spatial, and Environmental Influences on the Demographics and Har aphics and Harvest Vulnerability of American Black Bears ability of American Black Bears (Ursus americanus) in Urban Habitats in New Jersey, Pennsylvania and West Virginia*. West Virginia University.

VanderWaal, K. L., Atwill, E. R., Isbell, L. A., & McCowan, B. (2014). Linking social and pathogen transmission networks using microbial genetics in giraffe (Giraffa camelopardalis). *Journal of Animal Ecology*, *83*(2), 406–414. https://doi.org/10.1111/1365-2656.12137

Woods, J. G., Paetkau, D., Lewis, D., McLellan, B. N., Proctor, M., & Strobeck, C. (1999). Genetic Tagging of Free-Ranging Black and Brown Bears. *Wildlife Society Bulletin*, *27*(3), 616–627.

Yeakel, J. D., Gilbert, J. P., Gross, T., Westley, P. A. H., & Moore, J. W. (2018). Eco-evolutionary dynamics, density- dependent dispersal and collective behaviour: implications for salmon metapopulation robustness. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *373*(1746), 20170018.

APPENDIX A

S1. DNA Extraction and Microsatellite Amplification

A minimum of 5 (average 20) hairs were used for DNA extraction using in 300µL of lysis buffer (2M urea, 0.1M NaCl, 0.25% n-lauroylsarcosine, 5mM CDTA, 0.05 M Tris HCl pH 8) and 20µL of Proteinase K (20mg/mL; Bishop Canada). Samples were incubated for 2 hours at 56°C and stored at 4°C until extraction. Samples were extracted using a Promega Wizard® SV96 Genomic Purification System. Samples were heated to 56°C for 1 hour, 300µL of Wizard® SV Lysis Buffer added, and mixed for 1 minute at 56°C. Lysate was transferred to a Promega silica binding plate and centrifuged at max speed (3214g) for 2 minutes. The binding plate was placed on a new collection plate, 750µL Column Wash Solution A (Promega) was added to each well, and plates were centrifuged at max for 2 minutes. This wash process was repeated 2 times, for a total of 3 washes. A final 5-minute centrifugation at max speed was used to fully dry the silica membrane. For elution, 80µL of prewarmed (65°C) $TE_{0.1}$ was added to each well and plates were centrifuged at max speed. After elution, samples were incubated at 56°C for 2 minutes to evaporate residual ethanol.

All extracted samples were genotyped at 15 microsatellite loci and the amelogenin locus for sex determination. Amplified loci are those used by Pelletier *et al*. (2012) and Obbard *et al.* (2010), with modifications to optimize genotyping. Two microlitres stock DNA was used to amplify all loci using the Qiagen Multiplex PCR Kit in two, 12µL multiplex reactions. Cycling conditions were as follows: 95°C 15min; 30-32

cycles of 94°C for 30s, T_A for 90s, 72°C for 60s; and a final extension of 60°C for 45min. Reaction specific cycling conditions are included in Appendix A, Table S1.

S2. Individual Identification

PCR products were size separated on an ABI 3730 with Genescan 500 LIZ (Applied Biosystems). Scoring was performed using GeneMarker v.2.6.4 (SoftGenetics). Individual identification of bears from the genotypes was conducted separately for each trapline. As trap arrays were separated by distances greater than the typical male black bear's home range, it is unlikely that bears would be sampled at multiple trap arrays at this time of year. As an initial quality control step, samples with more than two mixed loci (resulting from more than one individual leaving samples at the trap) and more than 14 missing alleles were considered poor quality and were removed from analyses. Remaining samples were grouped into individual genotypes using allelematch in R (Galpern et al., 2012). Given the non-invasive nature of these samples, allelic dropout was expected for some loci that could lead to mismatches between samples of the same individual. Therefore, according to the zero second minimum algorithm in allelematch, samples with up to 11 allelic mismatches were grouped.

To remedy inconsistencies, such as grouped genotypes with allelic mismatches, we first reviewed grouped genotypes' genemarker project to confirm scoring accuracy. Most inconsistencies were due to scoring error and were corrected. In the case that inconsistencies were scored correctly, we analyzed electropherograms to assess pullup or allelic dropout. If inconsistencies could not be confirmed as scoring error, pullup, or

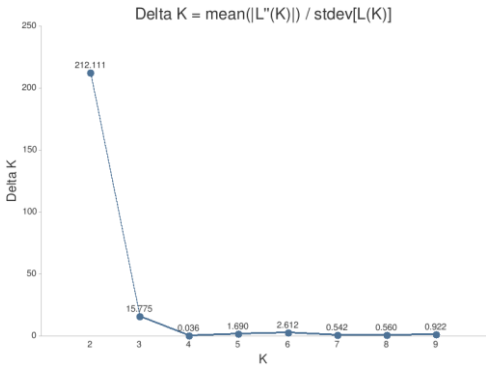
dropout, samples were re-amplified. If reamplification did not clarify genotypic inconsistencies, the sample was discarded.

In the case that an individual was identified from only one sample, more stringent inclusion criteria needed to be met. For the unique genotype to be considered a valid individual, we required at least 10 amplified loci, no mixed loci, and at least 5 loci with peak heights > 2000RFU. While initial inclusion criteria were liberal (ex. allowing up to 14 missing alleles), the final dataset used for analysis consisted 76% of profiles with full genotypes, 23% of profiles with >90% of alleles present, and 1% of profiles with <90% of alleles present. Most profiles missing <10% of the genotype were due to the G10P locus not amplifying. Thus, missing data was often consistent between profiles. After genotypes were either rectified or discarded, individual genotypes were given a unique ID, all traplines were combined to analyse all individuals in allelematch. Individual bears detected at more than one trapline were assigned to the fist assigned ID to ensure individuals were not counted multiple times.

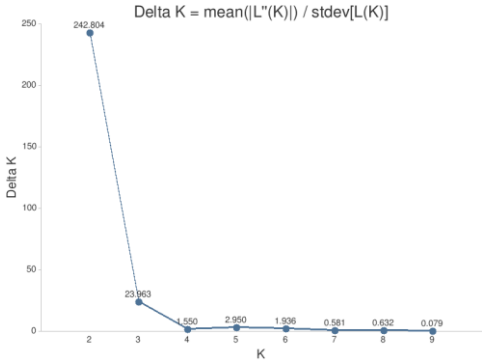
Table S1. Multiplex conditions for 16 loci using Qiagen Multiplex PCR Kit to genotype black bear samples for individual identification.

Locus	Fluorescent Label	Final concentration (μM)	Reference
Multiplex 1: Mastermix final concentration = 0.8X; T_A = 57°C; 30 cycles			
G10C	FAM	0.2	Paetkeau et al. 1995
G10B	FAM	0.2	Paetkau & Strobeck 1994
G10H	FAM	0.2	Paetkau et al. 1998
G10L	VIC	0.2	Paetkau & Strobeck 1994
G1A	NED	0.22	Paetkau & Strobeck 1994
G1D	PET	0.25	Paetkau & Strobeck 1994
MU05	NED	0.22	Taberlet et al. 1997
AMX/Y	VIC	0.05	Ennis & Gallagher 1994
Multiplex 2: Mastermix final concentration = 1X; T_A = 51°C; 32 cycles			
G10X	PET	0.01	Paetkeau et al 1995
G10J	FAM	0.01	Paetkau et al. 1998
G10M	VIC	0.25	Paetkeau et al. 1995
G10P	NED	0.08	Paetkeau et al. 1995
G10U	FAM	0.07	Paetkau et al. 1998
MSUT6	VIC	0.1	Kitahara et al. 2000
MU50	NED	0.1	Taberlet et al. 1997
MU59	NED	0.2	Taberlet et al. 1997

a)



b)



c)

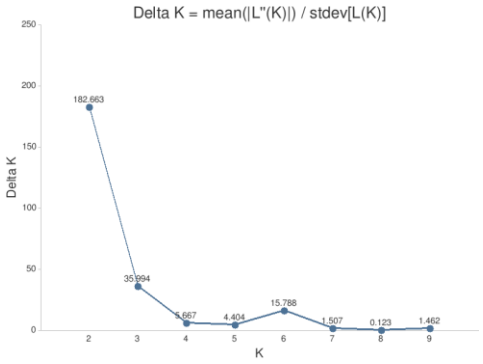


Figure S1. Results of ΔK analysis (Evanno et al., 2005) for each of the three datasets: a) all individuals, b) male individuals, c) female individuals. For all three analyses, two is the optimal number of genetic clusters.

a)

b)

c)

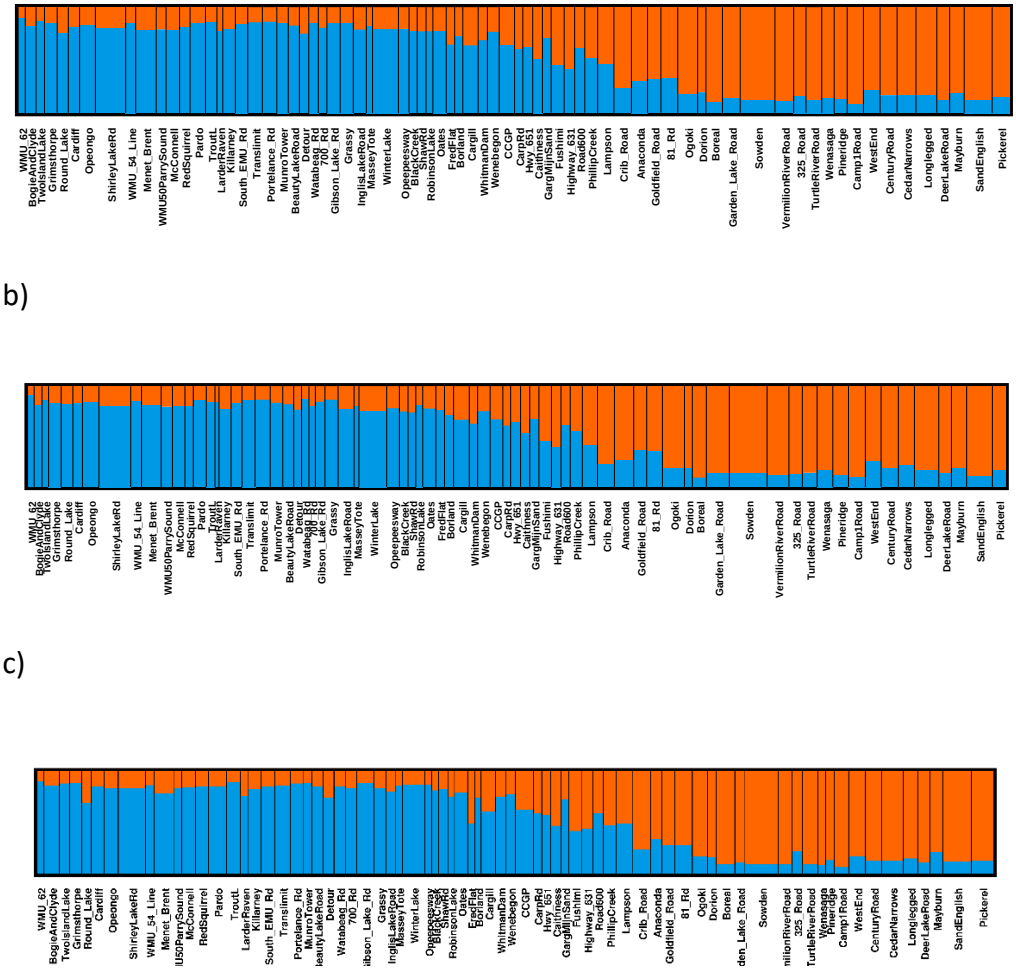


Figure S2. STRUCTURE results visualized using the main pipeline of CLUMPAK for each of the three datasets: a) all individuals, b) male individuals, c) female individuals. Names along the x-axis are the names of individual traplines and are organized by geographic location (southeast to northwest). Orange = southeast genetic cluster, blue = northwest genetic cluster.

a)

b)

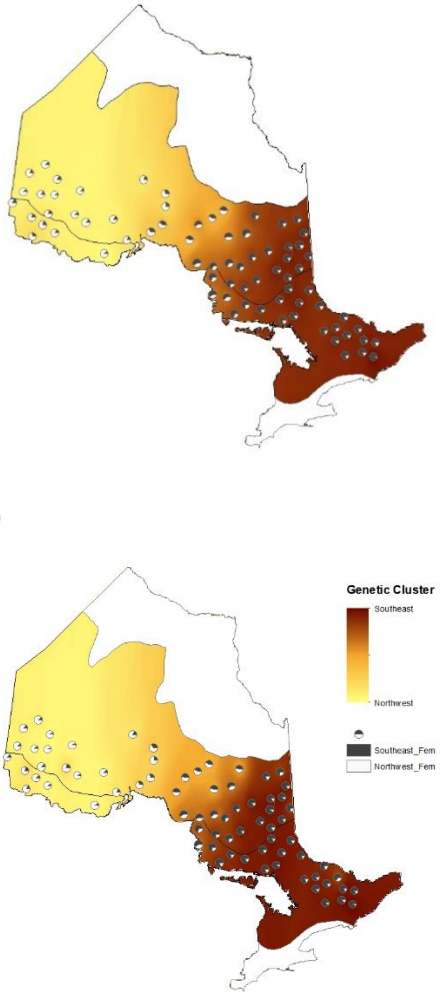
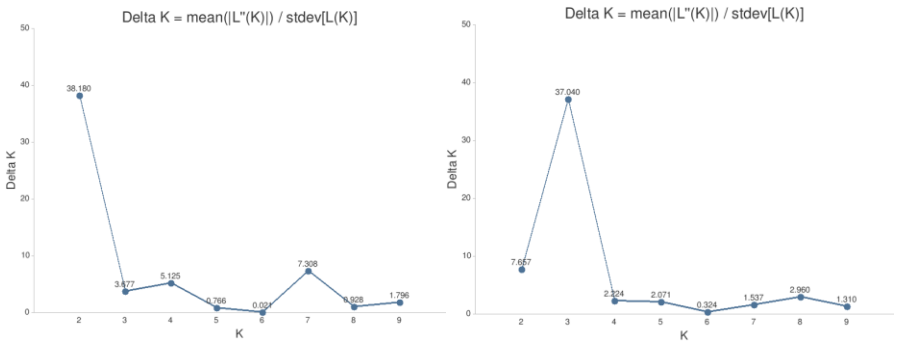
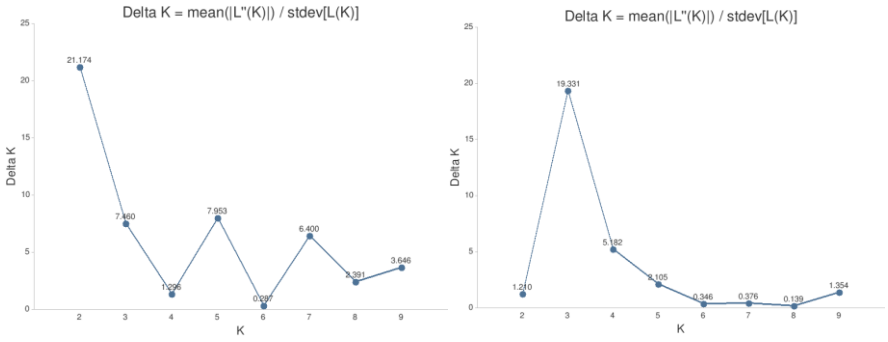


Figure S3. Spatial visualization of STRUCTURE results using ArcMap 10.7.1's interpolation tool. a) represents results generated using all individuals, and b) female individuals. Green represents strong population assignment probability to the southeast genetic cluster, while red represents strong population assignment probability to the northwest genetic cluster. Each node centroid is represented by a pie chart with black representing population assignment probability to the southeast cluster, and white representing population assignment probability to the northwest genetic cluster, for that particular node.

a)



b)



c)

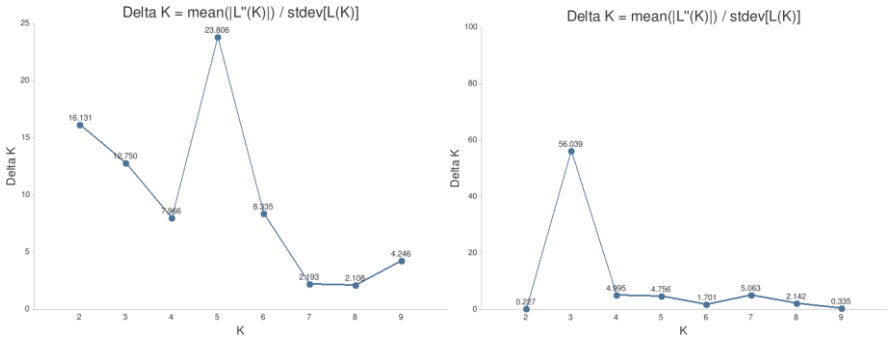
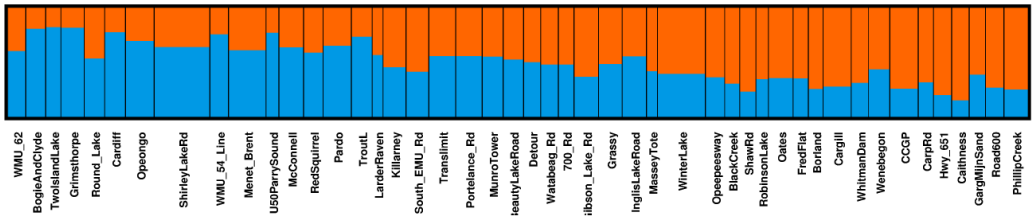


Figure S4. Results of hierarchical ΔK analysis (Evanno et al., 2005; Janes et al., 2017) for each of the three datasets: a) all individuals, b) male individuals, c) female individuals. Figures on the left represent analysis results using data from eastern genetic clusters, and figures on the right represent analysis results using data from western genetic clusters.

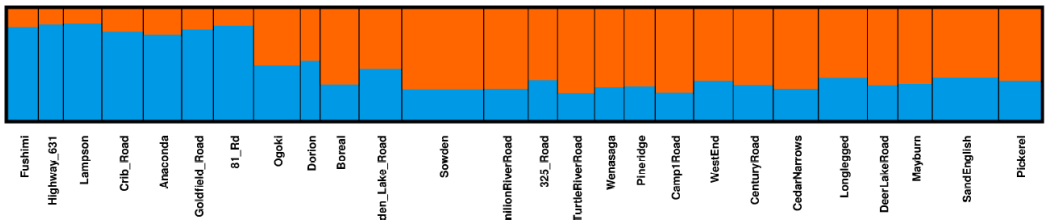
a)

K=2



b)

K=2



K=3

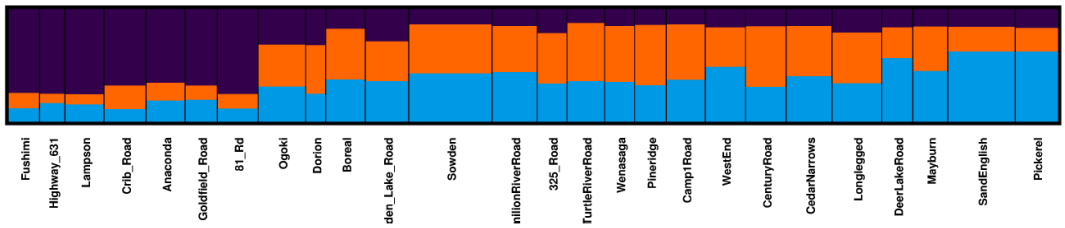
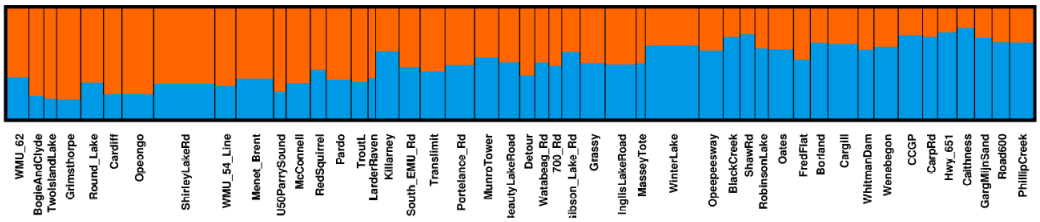


Figure S5. Hierarchical STRUCTURE results using all black bears within the (a) eastern genetic cluster and (b) western genetic cluster. Plots are organized from east to west. STRUCTURE results for K=2 and K=3 are included for western Ontario. Our ΔK analysis suggested the three-cluster model is most likely for western Ontario.

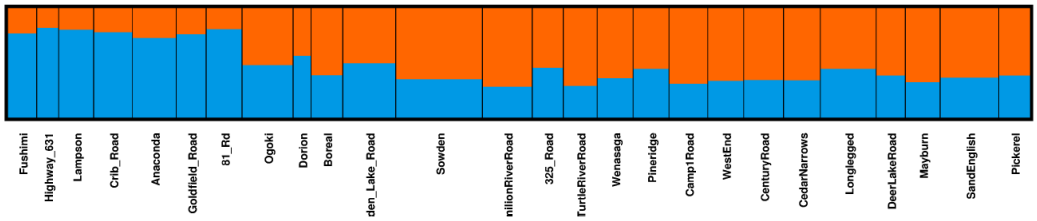
a)

K=2



b)

K=2



K=3

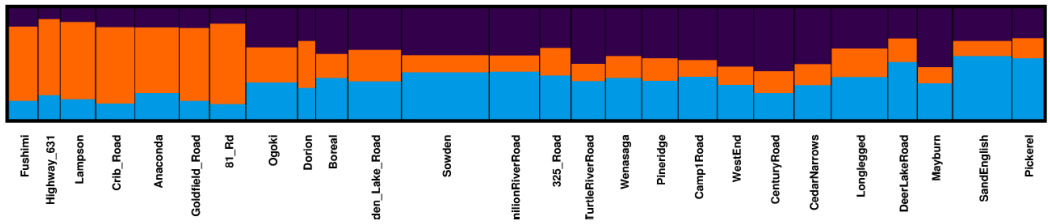
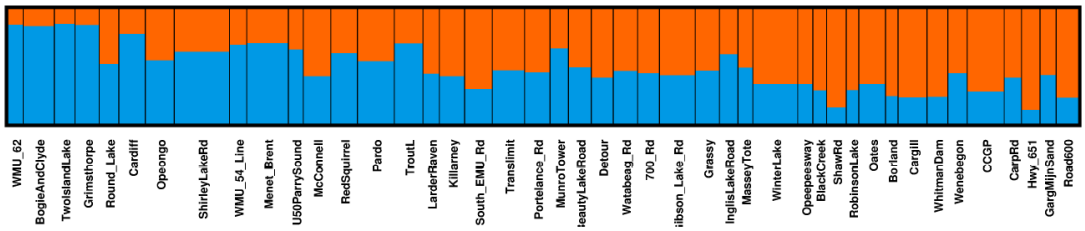


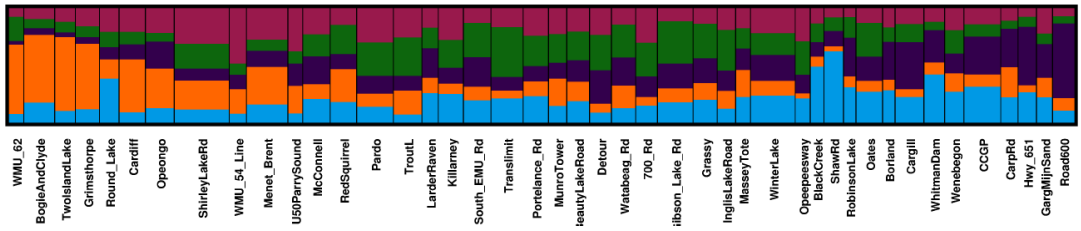
Figure S6. Hierarchical STRUCTURE results using male black bears within the (a) eastern genetic cluster and (b) western genetic cluster. Plots are organized from east to west. STRUCTURE results for K=2 and K=3 are included for western Ontario. Our ΔK analysis suggested the three-cluster model is most likely for western Ontario.

a)

K=2

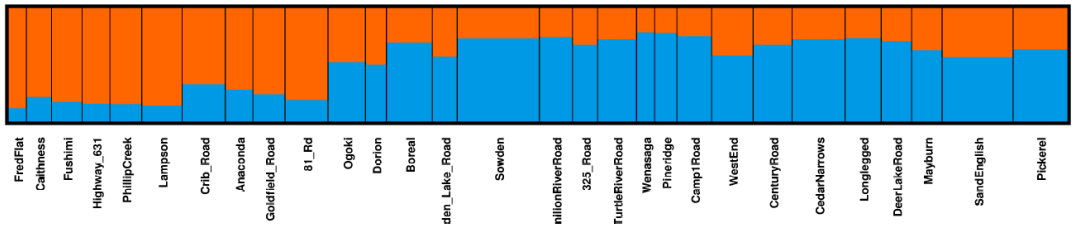


K=5



b)

K=2



K=3

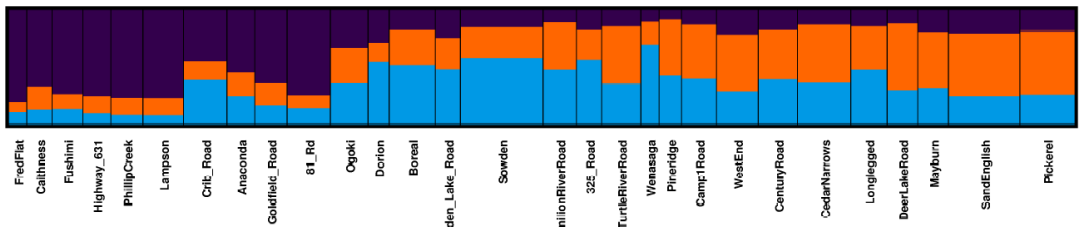


Figure S7. Hierarchical STRUCTURE results using only female black bears within the (a) eastern genetic cluster and (b) western genetic cluster. Plots are organized from east to

west. STRUCTURE results for K=2 and K=5 are included for eastern Ontario. STRUCTURE results for K=2 and K=3 are included for western Ontario. Our ΔK analysis suggested the five cluster model is most likely for eastern Ontario, and the three-cluster model is most likely for western Ontario.

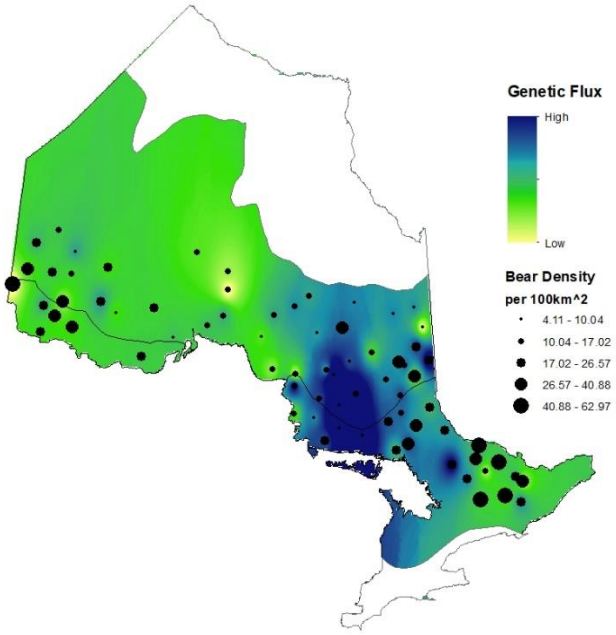


Figure S8. Dispersal map, informed by net genetic flux values, constructed using the interpolation tool in ArcMap 10.7.1 for the dataset containing all individuals. Red represents net immigration (positive genetic flux), and blue represents net emigration (negative genetic flux) from a node. Points represent node centroid locations and are sized according to density (large point = high bear density, small point = low bear density).

Table S2. Genetic flux calculations using different dispersal distances (d; 150, 200, 250, 300) to calculate the decay coefficient (k). Names of trap arrays are listed in the left most column.

	d = 150km	d = 200km	d = 250km	d = 300km
	k = -0.01997155	k = -0.01497866	k = -0.01198293	k = -0.009985774
325_Road	0.035061623	0.035721385	0.036121421	0.036389834
700_Rd	0.158454264	0.165110235	0.169241223	0.172054143
81_Rd	-0.085060848	-0.088764988	-0.091064549	-0.092630641
Anaconda	-0.313475181	-0.324650811	-0.331558786	-0.336250744
BeautyLakeRoad	0.182814656	0.189457836	0.193566231	0.196357553
BlackCreek	0.620552465	0.643565498	0.657805402	0.667483611
BogieAndClyde	-0.370199203	-0.38458598	-0.393500409	-0.399564351
Boreal	-0.121777775	-0.127558785	-0.131158634	-0.133614956
Borland	0.806312489	0.833781265	0.850738417	0.862246456
Caithness	0.029477176	0.030733335	0.031512218	0.03204226
Camp1Road	-0.167169894	-0.174351976	-0.17881156	-0.181849102
Cardiff	-0.163043916	-0.168612527	-0.172051759	-0.174386504
Cargill	0.116792443	0.120842064	0.123342134	0.125038869
CarpRd	-0.241634215	-0.250530444	-0.256035885	-0.259777981
CCGP	0.001188216	0.002293205	0.002988476	0.003465871
CedarNarrows	-0.207480623	-0.215657486	-0.22072256	-0.224167343
CenturyRoad	-0.331403149	-0.343436212	-0.350879602	-0.355937526
Crib_Road	-0.474225952	-0.491776481	-0.502635241	-0.51001497
DeerLakeRoad	-0.129849307	-0.135197868	-0.138512575	-0.140767589
Detour	0.054436982	0.056405274	0.057624301	0.058453302
Dorion	-0.099802587	-0.104567668	-0.107536895	-0.109563781
FredFlat	0.078379069	0.081427861	0.08331731	0.084602729
Fushimi	0.000589327	0.000400818	0.000280819	0.000197835
Garden_Lake_Road	-0.150150038	-0.156122041	-0.159822649	-0.16234002
GargMijnSand	0.359012682	0.373089292	0.3818125	0.387746759
Gibson_Lake_Rd	0.018015132	0.019422849	0.020305551	0.020910422
Goldfield_Road	-0.024596965	-0.02591994	-0.026744251	-0.027306906
Grassy	0.075195468	0.077927997	0.079619961	0.080770407
Grimsthorpe	-0.235010212	-0.243645108	-0.248987546	-0.252618293
Highway_631	0.052770608	0.05479132	0.056043084	0.056894443
Hwy_651	-0.395040751	-0.408307479	-0.416495743	-0.422052079
InglisLakeRoad	0.073232471	0.076211955	0.078059261	0.079316342

Killarney	-0.081159432	-0.083378269	-0.084739049	-0.085658736
Lampson	-0.220116032	-0.227783317	-0.232519246	-0.235734466
LarderRaven	0.463241789	0.482170788	0.49391625	0.501913015
Longlegged	-0.049716684	-0.052554319	-0.054321274	-0.055526867
MasseyTote	0.738149634	0.765268074	0.782048647	0.793453824
Mayburn	-0.002118268	-0.003166662	-0.00382786	-0.004282523
McConnell	0.027675849	0.028765905	0.02944082	0.029899701
Menet_Brent	-0.099217398	-0.102244607	-0.104108741	-0.105371879
MunroTower	0.214108594	0.223339877	0.229074262	0.23298113
NORTRoad	0.192563871	0.199431145	0.203678411	0.206564236
Oates	0.180323624	0.187136453	0.191349576	0.194211947
Ogoki	-0.219871166	-0.228169861	-0.233306402	-0.236798096
Opeepeesway	0.473554231	0.490344286	0.5007193	0.507764653
Opeongo	-0.333802485	-0.346761637	-0.354792045	-0.360254897
Pardo	0.000265298	0.001483311	0.002251037	0.002778748
PhillipCreek	-0.000324711	-0.000709386	-0.000952193	-0.001119238
Pickerel	-0.526691929	-0.546992091	-0.55957063	-0.568127064
Pineridge	-0.110679649	-0.116117338	-0.119508409	-0.121824414
Portelance_Rd	0.145793758	0.151961585	0.155787424	0.158391603
RedSquirrel	-0.070201863	-0.072322429	-0.07362695	-0.074510332
Road600	-0.292340446	-0.304344941	-0.311793841	-0.31686538
RobinsonLake	0.805134911	0.832428572	0.849281027	0.860719522
Round_Lake	0.082338234	0.084836441	0.086374863	0.087417328
SandEnglish	-0.425148061	-0.441190355	-0.451123775	-0.45787798
ShawRd	0.156750474	0.163814275	0.168204274	0.171196053
ShirleyLakeRd	-0.020316795	-0.020935454	-0.021316679	-0.02157511
South_EMU_Rd	-0.282097257	-0.29139045	-0.297124256	-0.30101423
Sowden	-0.199820218	-0.207503888	-0.212265354	-0.215504504
Translimit	-0.422074959	-0.436204074	-0.444928496	-0.45085035
TroutL	0.20115596	0.208849086	0.213615172	0.216856933
TurtleRiverRoad	-0.193997343	-0.20153297	-0.206200751	-0.209375307
TwoIslandLake	-0.041428593	-0.042630955	-0.043367605	-0.043865139
VermilionRiverRoad	-0.194455899	-0.202569162	-0.207605898	-0.211036133
Watabeag_Rd	0.153904744	0.160716179	0.164954691	0.167845572
Wenasaga	0.008444797	0.00787564	0.007510941	0.007257767
Wenebegon	0.191939615	0.199526737	0.204228463	0.207426975
WestEnd	-0.186885688	-0.194579201	-0.199351654	-0.202600319
WhitmanDam	0.078850317	0.082213794	0.084301684	0.085723548
WinterLake	-0.180388003	-0.185741932	-0.189037548	-0.191270118
WMU_54_Line	0.074581853	0.078393124	0.080775094	0.082404139

WMU_62	0.059284349	0.061484886	0.062848544	0.063776241
WMU50ParrySound	0.372770038	0.387122504	0.396016233	0.402066358

CHAPTER 3: POOLED WHOLE-GENOME ANALYSIS OF AMERICAN BLACK BEARS REVEAL COLD AND STARVATION RESPONSE-ASSOCIATED OUTLIERS AMONG FOREST AND CLIMATE ZONES

ABSTRACT

Understanding patterns of local adaptation among heterogeneous landscapes is critical to predicting how species respond to environmental change. Long-lived, vagile species are thought to have diminished capacities to adapt to rapidly changing selective pressures given longer generation times and patterns of high gene flow among populations, yet empirical assessments of these expectations in wildlife are lacking given the geographic scale of study required. We used pooled whole-genome sequencing to explore adaptive divergence in a long-lived, vagile, species, the American black bear (*Ursus americanus*), across a large area consisting of multiple forest and climatic zones, but no major physical barriers to bear dispersal. We characterized genetic differentiation in black bears across Ontario, Canada, among climate and forest zones, via outlier single nucleotide polymorphisms (SNPs). Given that differences in climate and forest zone likely represent differences in habitat quality for black bears, we screened for genetic variation in coding regions that may be under selection.

Genomic sequences were mapped to a black bear reference genome and we identified SNP outliers among regions associated with cellular responses to cold and starvation. We speculate that this genetic structure is driven by differences in temperature and food resources between forest and climate zones that subsequently impact hibernation that is key to black bear fitness. These data highlight candidate genes likely relevant to black bear responses to continued climatic changes and suggest balancing selection.

INTRODUCTION

Environmental heterogeneity across landscapes drives varying selective pressures that can shape patterns of local adaptation. Local adaptation influences the generation and maintenance of biodiversity (Gavrilets, 2003), contraction and expansion of species ranges (Atkins & Travis, 2010), and ecological and evolutionary dynamics of species interactions (Gandon & Van Zandt, 1998). At its core, local adaptation results from selective pressures driving genetic variation at loci underlying fitness-related phenotypes. Different selective pressures have varying influences on genetic variation. For example, positive selection reduces genetic variation by fixing beneficial alleles (Biswas & Akey, 2006; Cook & Saccheri, 2013). In contrast, balancing selection maintains genetic variation within a population (D. Koenig et al., 2019). Balancing selection can have several causes including heterozygote advantage, dependent selection, and environmental heterogeneity in space and time (Wu et al., 2017). Deciphering modes of selection and their influence on genetic variation and local adaptation provides key insight on what loci and traits influence fitness in wildlife populations. This information can be used to assess population viability under different scenarios of change in environment and selective pressures.

Local adaptation is not only influenced by variation in selective pressures, but also gene flow and genetic drift (Blanquart et al., 2013; J. L. Feder et al., 2012; Kawecki & Ebert, 2004). Homogenizing effects from gene flow and stochastic loss of variants through genetic drift can undermine local adaptation when effective population size (N_e) is large (Nosil, 2012). Thus, species with high dispersal capabilities, high N_e, and long

generation times are thought less likely to display patterns of local adaptation (Rosenheim & Tabashnik, 1991). Understanding processes that undermine or promote adaptation are key to predicting how wildlife populations may respond to environmental change and for informed management (Shafer et al., 2014).

Populations may also display phenotypic plasticity where single genotypes can manifest multiple phenotypes (DeWitt et al., 1998). Plasticity moves mean phenotypes toward environmental optimums favoured by selection without subsequent changes in the distribution of genotypes (Stortz & Scott, 2021). Examples of plasticity include intestinal enzyme changes in response to variation in diet in marsupials (Cortes et al., 2011), change in hibernation emergence timing in response to temperature and snowfall in ground squirrels (Lane et al., 2019), or alteration of reproductive timing with latitude in response to photoperiod in grizzly bears (Appleton et al., 2018). Thus, genetic studies across selective pressure gradients are essential in determining if populations demonstrate genetic adaptation or plasticity (Merilä & Hendry, 2014; Savolainen et al., 2013)**.**

Long-lived, vagile species are normally not expected to demonstrate strong patterns of local adaptation, particularly in large populations with a high N_e (Merilä & Hendry, 2014; Rosenheim & Tabashnik, 1991). However, genetic variation influencing functionally relevant phenotypes can exist when selective pressures overcome the diluting effects of gene flow (Bolnick & Fitzpatrick, 2007; J. L. Feder et al., 2012; Nosil, 2008, 2012; Tigano & Friesen, 2016). Under habitat matching theory, dispersal favours local adaptation when individuals disperse toward optimal habitats that maximize

performance and allow for new traits and underlying variants to be selected (Jacob et al., 2017; Sexton et al., 2014). Local adaptation has been observed in various species of fish, amphibians, plants, and insects with extensive gene flow (Dennenmoser et al., 2017; Hamala & Savolainen, 2019; Muir et al., 2014; Yadav et al., 2019), but also in long-lived species such as *Vincetoxicum hirundanaria* that demonstrate local adaptation to sympatric leaf herbivores (Kalske et al., 2012). Current rates of environmental change are faster than the rate at which many large mammals can adapt through changes in allele frequencies, given long generation times (Hetem et al., 2014). Assessing patterns of local adaptation in long-lived vagile species therefore becomes essential to predicting potential responses to rapid changes in selective pressures from ongoing climate and land-use change.

Ontario, Canada, contains a large heterogeneous landscape that includes multiple forest and climate zones likely exerting different selective pressures on species that inhabit them. Ontario is not divided by major physiogeographic barriers (ex. mountains or large bodies of water) expected to limit dispersal for large terrestrial mammals, whose distributions extend across these various forest and climate zones. Subtle genetic structure, largely compatible with isolation by distance, has been observed between northwestern portions and central portions of Ontario in several meso- and large carnivores, including in American black bears (*Ursus americanus*; Pelletier et al., 2011, 2012), Canadian lynx (Lynx canadensis; Row et al., 2014), and fisher (Pekania pennanti; Greenhorn & Bowman, 2018). In this region, the genetic structure of lynx is more pronounced than observed for other carnivores, coinciding with broad-

scale climatic gradients (Row et al., 2014). If broad environmental differences among Ontario's climatic and forest zones exert differing selective pressures on the inhabitants of these regions, they may drive adaptive divergence. Characterizing genetic structure of gene variants between zones could be used to infer such processes (Dennenmoser et al., 2017). These data are important in context of rapid environmental change that likely alter distributions of selective pressures on the landscape across both latitudinal and habitat gradients. Ontario's forest and climatic zonespresent a system to assess potential adaptive divergence in longer-lived, vagile species without obvious physiogeographic barriers to gene flow.

American black bears occupy much of North America's forested regions, exhibiting varying levels of genetic structure among populations and regions. In southern ranges, such as northern Mexico and southern United States, black bears exhibit increased genetic structure where habitat fragmentation inhibits gene flow (Dixon et al., 2007). In northern extents of the black bear range, genetic structure largely reflects isolation by distance patterns given a lack of major physical barriers that would impede gene flow (Puckett & Eggert, 2016). In addition to these broadscale genetic patterns, black bears display substantial biological and ecological variation across their range (Karelus et al., 2016), with vastly different levels of access to food resources (Boonstra et al., 2014). These resource differences influence bear life history traits such as hibernation timing and duration (Toien et al., 2011) and reproductive success (Samson & Huot, 1995).

Black bears occupy much of Ontario's Great Lake St. Lawrence (GLSL; mixed deciduous; Rowe, 1972) and boreal forests. Additionally, their range spans both the Continental and Atlantic climate zones. The forest zones differ in latitude, with GLSL forest spanning southern portions of the province and boreal forest occupying the north. Climate zones also influence broad weather patterns along a longitudinal gradient (Hurrell, 1996; Hurrell et al., 2003). Climate and forest zones influence factors such as precipitation and vegetation productivity (Holden et al., 2012; Obbard & Howe, 2014) that correlate with habitat quality for numerous herbivores and omnivores (Boonstra et al., 2014), including black bears (Howe et al., 2013).

When studying local adaptation, different genetic approaches have been successfully employed to identify loci underlying adaptive traits, including whole genome sequencing (WGS; Ng & Kirkness, 2010) and restriction site associated DNA sequencing (RAD-seq; Davey & Blaxter, 2010). More recently, pooled whole genome sequencing (pool-seq) has become an alternative to WGS and RAD-seq that entails pooling DNA from individuals (typically >44; Schlötterer et al., 2014) prior to sequencing. Sequenced reads are mapped to reference genomes and SNPs are called to characterize differences in allele frequencies (Ferretti et al., 2013; Schlötterer et al., 2014). Data from this approach reflect population-level polymorphism patterns as opposed to individual-level data (Schlötterer et al., 2014). Pool-seq data has noted limitations, such as the inability to isolate individual-level data or lack of reliability at low sample sizes (Cutler & Jensen, 2010; Schlötterer et al., 2014). However, studies on fish (Dennenmoser et al., 2017), mammals (Bertelsen et al., 2016), insects (Tilk et al., 2018), and birds (Neethiraj

et al., 2017) have reliably implemented this approach to obtain population-level data with high genome coverage (Fuentes-Pardo & Ruzzante, 2017).

In the current study, we employ pooled whole-genome sequencing to characterize genetic differentiation between black bears in boreal versus mixed deciduous forests and between Atlantic versus Continental climate zones. Bears inhabiting these regions are connected via ongoing gene flow but likely experience different selective pressures related to variation in winter precipitation and vegetation productivity. This research aims to enhance our understanding of the capacity of large, vagile, mammals to locally adapt despite high dispersal capabilities by identifying missense variants related to differences in climatic and forest zones. These data are also relevant in context of continued climate and land-use change that will likely influence resource distribution and selective pressures on black bears and other species.

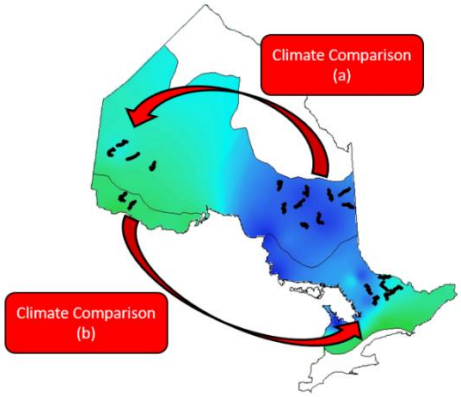
MATERIALS AND METHODS

Sample Collection

Genetic samples were collected as a part of the Ontario Ministry of Northern Development, Mines, Natural Resources and Forestry's (NDMNRF) black bear population monitoring program. This program uses genetic individual identification from non-invasive sampling to estimate population density across the province (Howe et al., 2013, 2022). In 2018, this project consisted of 35 networks of sampling stations (arrays) deployed across Ontario, spanning the GLSL and boreal forest regions, as well as the Atlantic (east) and Continental (west) climate zones. We used data from 21 arrays,

avoiding arrays that were on the border of forest or climate zone (Figure 3.1). Arrays consisted of at least 40 baited barbed-wire corrals (Woods et al., 1999) for a total of 847 unique sample locations, each separated by a minimum of 1.5km. Arrays were at least 25km apart to avoid sampling the same bear at multiple arrays (Tri, 2013). Traps were constructed according to Woods *et al*. (1999) by wrapping barbed wire around a series of trees, creating a corral. Black bears entering the corral snagged their hair on the barbed wire, and the hair was collected and subsequently used for DNA extraction.

a)



b)

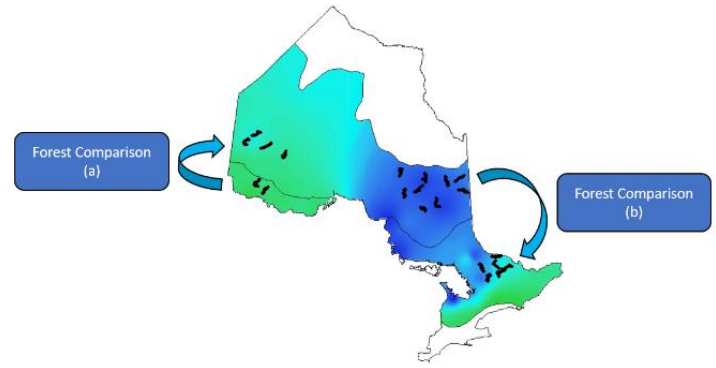


Figure 3.1. Sample locations relative to (a) the two climate comparisons we conducted and (b) the two forest zone comparisons we conducted. Blue represents high snowfall while green represents low snowfall (annual average from 1980-2010). Snowfall is considered the most accurate way to visualize the divide between climate zones located in north-central Ontario (Row et al., 2014). Lines dividing the province latitudinally represent differences in forest zone: north forest = boreal, south forest = GLSL/mixed deciduous. White areas represent unsampled forest regions (deciduous and Hudson Bay lowlands).

DNA Extraction, Microsatellite Amplification, and Individual Identification

A minimum of 5 (average 20) hairs were used for DNA extraction and were genotyped at 15 microsatellite loci (described by Howe *et al*., 2022) plus the amelogenin locus for sex determination (see Appendix A. S1 for details). Reaction specific cycling conditions are included in Appendix A. Table 1. Individual identifications were performed per Howe *et al.,* (2022), with PCR products size separated on an ABI 3730 with Genescan 500 LIZ (Applied Biosystems). Scoring was performed using GeneMarker v.2.6.4 (SoftGenetics). When inconsistencies in genotype could not be confirmed as scoring errors, allelic pullup, or dropout, samples were re-amplified. If re-amplification did not remedy inconsistencies, samples were discarded (see Appendix A. S2 for details)

Sample Pooling and Sequencing

A total of 50 samples yielding full genotypes were used for sample pooling and whole genome re-sequencing from each of two climate zones (Figure 3.1a) and two

forest zones (Figure 3.1b): Boreal/Atlantic, Boreal/Continental, GLSL/Atlantic, GLSL/Continental. To provide enough DNA for pooled sequencing using hair samples, all extracted DNA belonging to a single individual was combined and concentrated using a Vacufuge plus (Eppendorf). 1ul from each individual was used for quantification using a Qubit dsDNA HS Assay Kit (Life Technologies, Carlsbad, CA, USA). Each individual sample was normalized to 4.2ng/ul, 2.5ng/ul, 4.5ng/ul, and 3.0ng/ul, respectively, for each of the aforementioned four sample region categories. Equal quantities of DNA from each individual were pooled for each sample region category per standard pool-seq protocol (Schlötterer et al., 2014). Pools were then concentrated using a Vacufuge plus and purified via ethanol precipitations (10% 3M sodium acetate and 2.5X 100% ethanol, followed by three 2.5X 70% ethanol washes). Pooled samples were resuspended in 40ul $TE_{0.1}$ and incubated at 55°C for 5 minutes to evaporate remaining ethanol. After purification, samples were quantified using a Qubit dsDNA HS Assay Kit, normalized to 20ng/ul, and run on a Tape Station 4200. Each library was prepared using PCR-free library prep. The four pools were sequenced across 6 lanes on the Illumina HiSeq X to achieve approximately 50x coverage.

Read Processing and Mapping

Raw read quality was assessed using FastQC (S. Andrews, 2010) and trimmed using trimmomatic (Bolger et al., 2014) with a minimum length of 36bp, and then quality filtered using bbduk, part of the bbmap suite (Bushnell, 2014), for a minimum quality of 20. Duplicate reads were removed using FastUniq (Xu et al., 2012). Reads were mapped

to the reference black bear genome (Srivastava et al., 2019) using BWA-MEM (H. Li, 2013). After sorting, uniquely mapped reads were realigned using sambamba (Tarasov et al., 2015). We used GenomeAnalysisTK (Van der Auwera et al., 2013) to perform local realignment around indels. A total of 4 mpileup files were generated for two pairwise climate comparisons and two pairwise forest comparisons (Figure 3.1). Mean and standard deviation of position coverage across the genome were assessed using samtools depth (H. Li et al., 2009). Masked regions were subsequently removed prior to the identification of indel regions. Identified indel regions were then removed using Popoolation2 (Kofler et al. 2011).

Analyses

Mpileup files were converted into sync format, a popoolation2-specific format, using Popoolation2's mpileup2sync.jar. We then calculated Fisher's exact test (FET) and fixation index (Fst) for all pairwise comparisons in 50kb non-overlapping windows. To test for broad-scale genetic differences between forest types and climate zones, pairwise comparisons were performed that consisted of two climate comparisons (one comparison within each forest zone; Figure 3.1a) and two forest comparisons (one comparison within each climate zone; Figure 3.1b). Minimum and maximum coverage cutoffs were set as mean coverage +/- 2 standard deviations (Appendix B; Figure S1). We tested the correlation between FET and F_{ST} results using a Spearman's Rank Correlation. For all pairwise comparisons, we found that FET and Fst were highly correlated ($r > 0.89$; Appendix B; Figure S2). We proceeded to subsequent analyses with only the top 0.5% of

F_{ST} windows and FET windows that met a significance threshold of $p<0.05$. We then ran bedtools intersect (Quinlan, 2014) to identify genomic outlier regions that were consistent in both climate comparisons and genomic regions that were consistent in both forest comparisons. Genomic regions that were not consistent outliers among comparisons were discarded. For subsequent analyses, we used both FET and Fst results, running them in replicate pipelines. Thus, to address critiques regarding uncertainty of pool-seq outlier detection methods (Cutler & Jensen, 2010), we only retained SNPs that were present in both comparisons (for either climate or forest) and were present within outlier regions based on both FET and Fst results. Replicates and multiple outlier detection methods are strategies commonly employed in pool-seq studies (e.g., Nielsen et al., 2018; Phair et al., 2019)

To identify significantly differentiated SNPs, we employed Popoolation2's Cochtan-Mantel-Haenszel (CMH) test to SNPs located within FET and Fst outlier windows. Again, SNPs were only retained if they were outliers within both FET and Fst windows. When running Popoolation2's cmh-test.pl, we used a significance threshold of $p<0.01$ and a minimum allele count of 7. Minimum and maximum coverage cutoffs were set to mean coverage +/- 2 standard deviations (15x-71x for the climate comparison, and 18x-72x for the forest comparison). If the same SNP was identified as an outlier in both climate and forest comparisons, it was discarded.

Once candidate SNPs were identified, we assessed their functional relevance using snpEff (Cingolani et al., 2012), retaining missense variants, or variants in 50kb flanking regions of known coding regions. Of the missense variants, we further filtered

based on if chemical characteristic of resulting amino acid change was different (e.g., acidic to basic, or polar to non-polar). For all genes containing missense variants or variants 50kb up/downstream, we acquired gene ontology (GO) pathway data from UniProt (The Uniprot Consortium, 2021). We used GOWINDA (Kofler & Schloterrer, 2012), setting the simulation count to 100000, to identify GO pathways that were enriched with our outlier genes (genes containing missense or flanking variants within 50kb of coding regions). Results were visualized using REVIGO (Supek et al., 2011) and WEGO (Ye et al., 2018).

RESULTS

Sequence reads were mapped to a high-quality black bear reference genome achieving 40-53x coverage for each of the 4 pools (Boreal/Atlantic = 47x; Boreal/Continental = 53x; GLSL/Atlantic = 40x; GLSL/Continental = 44x). Filtering for the top 0.5% of F_{ST} results (F_{ST} = 0.07 and 0.1 for climate comparisons; F_{ST} = 0.07 and 0.05 for forest comparisons), averaged across 50kb non-overlapping windows, 163 and 165 outlier windows were identified for the two climate comparisons (Figure 3.1a; comparisons a and b respectively), and 165 and 169 outlier windows for the two forest comparisons (Figure 3.1b; comparisons a and b respectively). Filtering for FET windows, with a significance of $p<0.05$, 494 and 1486 outlier windows were identified for the two climate comparisons (Figure 3.1a; comparisons a and b respectively), and 17 and 78 outlier windows for the two forest comparisons (Figure 3.1b; comparisons a and be respectively). We only retained outlier regions that were present in both climate

comparisons (Figure 3.1a), filtering separately with FET and Fst results. Similarly, we only retained outlier regions that were present in both forest comparisons (Figure 3.1b). Twenty outlier regions were consistently identified in both climate comparisons, based on Fst results, and 196 outlier regions based on FET results. Forest comparisons yielded no outlier regions based on FET, but five were detected in both forest comparisons based on Fst results. Given the small number of replicated outlier regions in the western Ontario forest comparison (Figure 3.1b; forest comparison a), we performed subsequent analyses using only one forest comparison in eastern Ontario (Figure 3.1b; forest comparison b; 169 outlier regions based on FET results, and 78 outlier regions based on FET results).

Outlier windows identified via both FET and Fst analyses were carried throughout subsequent analyses, with final SNPs retained when present in both FET and Fst outlier windows. Within FET outlier windows, 13724 and 10791 SNPs were identified for climate and forest comparisons, respectively. Within Fst outlier windows, 2081 and 16606 SNPs were identified for climate and forest comparisons (Appendix B; Table S1). To identify the most differentiated SNPs within outlier regions, we ran Popoolation2's CMH test that accounts for overdispersion ($p<0.01$, min allele count = 7). This multifaceted approach, identifying outlier windows and SNPs within them, was performed to limit the potential for false positives and is utilized in other pool-seq studies (Anserson et al., 2022). We identified 5845 (within FET windows) and 1309 (within Fst windows) outlier SNPs for the climate comparison, and 4717 (within FET windows) and 6461 (within Fst windows) for the forest comparison. Mean allele

frequency difference of outlier SNPs was calculated using a custom R script. Between climate zones, mean allele frequency difference was 0.31. Between forest zones, mean allele frequency difference was 0.29.

We ran snpEff (Cingolani et al., 2012) to determine proximity of these SNPs to known coding regions. SNPs were defined as 'upstream' or 'downstream' if they were within 50kb of these coding regions. Of the outlier SNPs within outlier FET windows, 2316 upstream variants, 1147 downstream variants, and 15 missense variants were identified for the climate comparison; 1609 upstream variants, 753 downstream variants and 21 missense variants were identified for the forest comparison (Appendix B; Table S1). Of the outlier SNPs within outlier Fst windows, 385 upstream variants, 260 downstream variants, and 7 missense variants were identified for the climate comparison; 2337 upstream variants, 1011 downstream variants, and 15 missense variants were identified for the forest comparison (Appendix B; Table S1). Of the identified missense variants, we retained only those present in both FET and Fst outlier regions and that resulted in a change in chemical characteristic of the resulting amino acid. This resulted in 5 final missense variants for the climate comparison, and 7 missense variants for the forest comparison (Appendix B; Table S1). Of the genes identified using snpEff, genes in the climate comparison five contained missense variants after filtering for consistency between comparisons and change in amino acid chemical characteristic. The same procedure identified six genes of interest in the forest comparison (Table 3.1). We then searched these genes in UniProt and GeneCards (Genecards – the human gene database) to determine function. Further, we searched

literature on these genes to help contextualize them in relation to the different environments we sampled, as well as in relation to relevant black bear life history traits. We found that genes containing missense variants in the climate comparison, such as TFEB, NBN, RPL31, and Hnrnpa1 were all functionally related to the cellular response to starvation. Between climate zones, mean frequency difference for final missense variants was calculated to be 0.31. Genes containing missense variants in the forest comparison, such as Itm2a, RBBP6, IFI44, and L3MBTL4 were all functionally relevant to the cellular response to cold. Between forest zones, mean frequency difference of final missense variants was calculated to be 0.26.

Ontology analyses of all genes containing flanking or missense variants produced similar results. Although many GO terms enriched with our genes were broad, such as “cell”, more specific terms that were enriched include “regulation of metabolic processes” and “response to stimulus” (Appendix B; Figure S4).

Table 3.1. Genes containing missense variants and their functional relevance to their respective environmental comparison. Final missense variants were retained if they were significantly differentiated (based on CMH test), present in both FET and Fst outlier windows, consistent between climate comparisons (does not apply for forest comparison), and the resulting amino acid incurs in a change in chemical characteristic (ex. acidic to basic). Itm2a and CES4A are listed as *potentially* relevant to immune and starvation responses as they not well documented relative to other genes we identified.

Comparison	Gene Name	Brief Description	Gene Relevance	Ensembl Transcript ID	Amino Acids	Reference
Climate	TFEB	Transcription factor EB	Response to starvation	ENSUAMT00000025911	Gln75Pro	P. J. Lee et al., 2020
Climate	NBN	Nibrin	Response to starvation	ENSUAMT00000025911	Gly258Ser	Yancey, 2018
Climate	RPL31	60S ribosomal protein L31	Response to starvation	ENST00000409000	Gly5Arg	Federov et al., 2009
Climate	Hnrnpa1	Heterogeneous nuclear ribonucleoprotein A1	Response to starvation	ENSMUST00000036004	Gly164Arg	Jobava et al., 2021
Climate	GBA3	Cytosolic beta-glucosidase	Relevance currently unknown	ENSUAMT00000032849	Lys226Glu	
Forest	RBBP6	E3 ubiquiting protein ligase RBBP6	Response to cold	ENST00000613729	Pro340Thr	do Amaral et al., 2020
Forest	IFI44	Interferon-induced protein 44	Response to cold	ENSUAMT00000026996	Cys179Arg	Zimmermann et al., 2019
Forest	IFI44	Interferon-induced protein 44	Response to cold	ENSUAMT00000026996	Tyr296His	Zimmermann et al., 2019
Forest	L3MBTL4	Lethal(3)malignant brain tumor-like protein 4	Response to cold	ENSUAMT00000008431	Asn52Asp	Falvo et al., 2021
Forest	IL12RB2	Interleukin-12 receptor subunit beta-2	Immune Response	ENSUAMT00000030842	Asn135Lys	Roffler et al., 2016
Forest	Itm2a	Integral membrane protein 2A	Immune Response (potential)	ENSUAMT00000011926	Gln32Arg	Kirchener & Bevan, 1999
Forest	CES4A	Carboxylesterase 4A	Response to starvation (potential)	ENSUAMT00000014997	Pro3Gln	Nunez, 2021

DISCUSSION

Identifying drivers of local adaptation within panmictic populations living on heterogeneous landscapes are critical to predicting population viability under different scenarios of environmental change (M. E. Feder & Mitchell-olds, 2003; Nosil, 2008; Rosenheim & Tabashnik, 1991). Understanding these processes can be particularly relevant for long-lived, vagile species where long generation times and high dispersal capabilities can reduce the likelihood of adapting to local selective pressures (Hetem et al., 2014). We used pooled whole-genome sequencing to examine patterns of genetic variation in American black bears from different forest and climate zones, sampled across ~1 000 000 km^2. Despite black bears having long generation times (~5-7 years; Murphy et al., 2019) and low levels of neutral genetic structure between sampled regions in this study, we identified genetic differentiation from SNP outliers between both forest and climate zones in coding regions of genes mostly related to cold and starvation responses (Table 3.1). Cold and starvation response processes are key to successful hibernation, a fundamental life history trait in bears that varies across environmental gradients (Hellgren, 1998; Lariviere, 2001).

Although these findings do not provide definitive evidence of local adaptation related to hibernation, they reflect balancing selection induced by climate and food availability. Balancing selection can act through a variety of mechanisms, one of which being spatial and temporal variation in selective pressures (Wu et al., 2017); in the case of this study, differences in food productivity and temperature across climate and forest zones likely influencing hibernation success. These results enhance our understanding of

the relative influence of gene flow versus selection in a large mammal and highlight that long-lived, vagile mammals may display patterns of balancing selection when populations experience varying environmental conditions that relate to important life-history traits.

Our analyses highlight genes that may be adaptive in black bears and associated with hibernation processes. Hibernation is employed by many mammalian species to survive periods of cold temperatures and subsequent low food availability (Luu et al., 2018). During hibernation, bears reduce metabolic processes, core body temperature, and numerous other physiological parameters including heartbeat, respiration, and organ perfusion (Luu et al., 2018; Schwartz et al., 2013). Numerous transcriptome studies identify differentially expressed genes in hibernating versus active states, potentially demonstrating phenotypic plasticity related to environmental stress (Fedorov et al., 2011, 2012; Goropashnaya et al., 2021; Zhao et al., 2010).

Climate Zone Comparison

Comparisons of genetic diversity between climate zones revealed significant genetic differentiation consistent between FET, Fst, and both climate comparisons. These consistent results between climate comparisons, but not forest comparisons, are likely related to increased geographic distances with less gene flow and greater differences in selective pressures between sampled locations (Dool et al., 2021; M. E. Feder & Mitchell-olds, 2003; Kawecki, 2008; Kawecki & Ebert, 2004). Differentiated genes between climate zones were functionally relevant to cellular responses to

starvation; a process key to hibernation (Fedorov et al., 2011; Gerisch et al., 2020; P. J. Lee et al., 2020).

Of the outliers identified, TFEB (Transcription factor EB) acts as a cellular metabolic sensor and is up-regulated during hibernation (P. J. Lee et al., 2020). Similarly, up-regulation of NBN (Nibrin) is noted in other mammals during hibernation, acting as a DNA repair protein in liver and muscle (Yancey, 2018). RPL31 (ribosomal protein L31), along with other RPL genes, are involved in protein biosynthesis and are well established in their importance to hibernation (Federov et al., 2009; Yan et al., 2006). Hnrnpa1 (Heterogeneous nuclear ribonucleoprotein A1) is involved in RNA pausing, which limits ATP supply and consumption, effectively conserving energy. This process is common under severe stress, such as prolonged starvation (Jobava et al., 2021).

Although variation in climate zone should represent differences in selective pressures for black bears, the specific mechanisms influencing observed genetic variation are somewhat equivocal. The Atlantic climate zone is largely governed by the north Atlantic oscillation (NAO), the most prominent and reoccurring pattern of atmospheric variability in the northern hemisphere (Hurrell et al., 2003). The NAO has profound influence over vegetation productivity and winter precipitation. Relative to western Ontario, where the effects of the NAO fade, eastern Ontario receives more snow annually. Snowfall is a large determinant in black bear hibernation timing and duration, where bears frequently den before snowfall makes excavation difficult and cold temperatures reduce food availability (Kolenosky & Strathearn, 1987). Additionally, females and cubs most often emerge from dens post-melt (Kolenosky & Strathearn,

1987; Tietje & Ruff, 1980). Therefore, bears in environments that experience increased snowfall are more likely to den for a longer duration, and by extension, undergo prolonged periods without food.

The precipitation governed by the NAO also influences vegetation productivity that black bears rely on for > 90% of their diet (Mosnier et al., 2008; Welch et al., 1997). This relationship between winter precipitation and vegetation is highly relevant to black bears as population-level reproductive failures have occurred in instances where berry crops similarly fail (Obbard & Howe, 2008). Within Ontario, crop failures occur more frequently in the Atlantic climate zone relative to the Continental climate zone. These crops include mountain ash (*Sorbus spp.)*, juneberry (*Amelanchier spp.*), dogwood (*Cornus spp.*), nannyberry (*Viburnum spp.*), and cherries (*Prunus spp.*; Howe et al., 2012), all of which are key to the black bear diet. Given that most large, long-lived, slow reproducing, frugivores and herbivores cannot directly track short-term fluctuations in resources by modulating reproductive efforts (Potts et al., 2009), the lack of food resources in select years may be another prominent mechanism influencing observed genetic variation. We speculate this is the most likely mechanism influencing observed variation in genes related to starvation response (Figure 3.2).

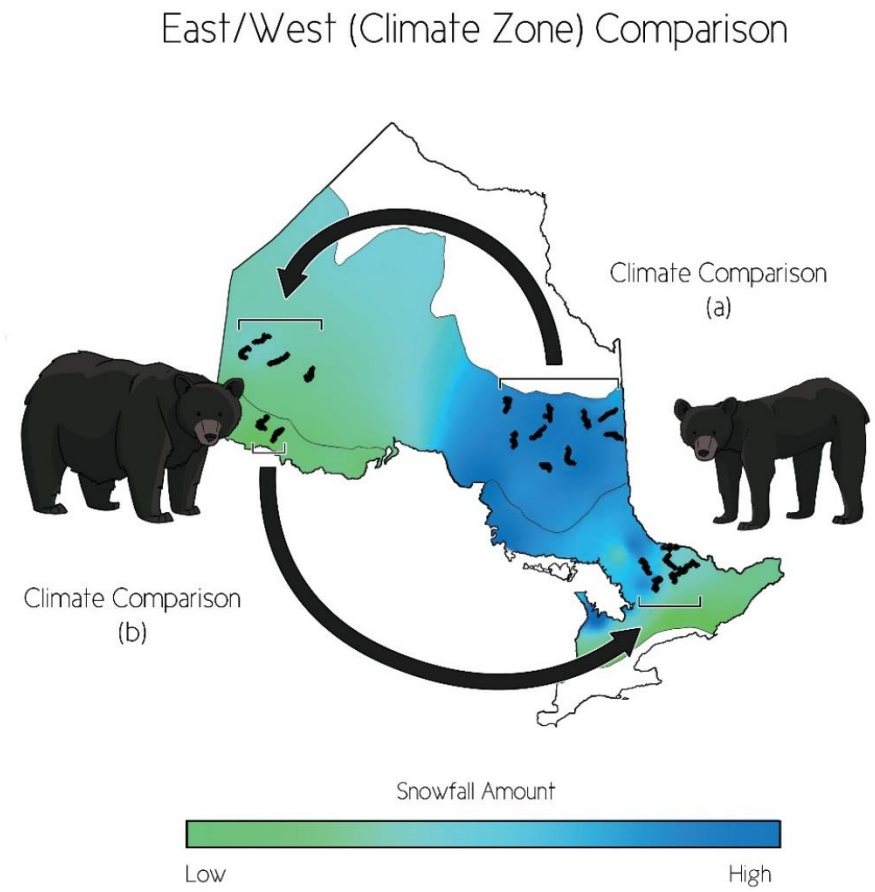


Figure 3.2. Frequent poor-food years in eastern Ontario (Atlantic climate zone) appear to drive genetic variation in genes related to the cellular response to starvation, selecting for resilience against food shortages. Colour gradient represents annual precipitation, which is considered the most accurate way to visualize difference in climate zone, within Ontario.

Other explanations for genetic variation related to starvation response exist, however. Years where seed and berry crops fail can also result in synchronization of seed and berry production, something that has been observed in eastern Ontario, but

not western Ontario (Obbard et al., 2014). This synchrony has been documented to sustain higher population densities in large-bodied frugivores, potentially suggesting higher habitat quality relative to areas with more consistent but asynchronous seed and fruit production (Potts et al., 2009). Additionally, maximum snow-water equivalent has been shown to explain 70% of variability in serviceberry productivity, which is also important to the black bear diet (Holden et al., 2012). Snow depth and density are significant determinants in the composition of plant species (Leffler et al., 2016; Rixen et al., 2008), where dense snow can delay plant development (Rixen et al., 2008). While the influence of snowmelt on vegetation productivity is well studied in other systems (Kelsey et al., 2021; Leffler et al., 2016; P. Li et al., 2020; Vankoughnett & Henry, 2014), the specific influence of snow depth and density in Ontario is not well documented. Some studies in forested systems suggest that snow depth shelters plant and root systems from cold temperatures, facilitating nitrogen uptake in spring and subsequent increases in productivity (Fitzhugh et al., 2001).

Given the genetic differentiation we observed as well as environmental differences, between climate zones in Ontario, there could be numerous mechanisms driving generic variation in genes related to starvation response. The lack of observable phenotypes related to outlier genes, and lack of measured fitness consequences of variants, make it difficult to draw concrete conclusions regarding specific mechanisms driving observed genetic variation. We speculate that more frequent poor food years in the Atlantic climate zone are the most likely force driving variation in starvation

response genes, given their correlation with population-level reproductive failures in black bears (Obbard & Howe, 2008).

Forest Zone Comparisons

Boreal and GLSL forests provide different food resources for black bears and presumably represent significant differences in habitat quality for this species. We observed little genetic variation between forest zones in western Ontario, a finding that likely relates to the geographic proximity and gene flow between the two pools that would outweigh any effects of local selective pressures. Additionally, the GLSL forest in western Ontario is located at a higher latitude, relative to the GLSL forest in eastern Ontario. In context of the cold response-related genes we identified, it is likely that genetic differences observed in western Ontario would be less pronounced as both pools were located further north. We did observe significant genetic variation, both at a broad-scale and SNP level, in the second forest comparison located in eastern Ontario.

The boreal forest is generally a less productive environment for black bears, relative to southern forest zones, where conifer tree stands do not provide the fatty mast used by bears in southern latitudes to gain weight before denning (Mosnier et al., 2008; Rogers, 1987). We expected this to be the key driver in genetic variation between forest zones. Instead, our results suggest that genetic differentiation relates to temperature along a latitudinal gradient, likely allowing bears to cope with the longer and colder winters that characterize the boreal forest (Mosnier et al., 2008). Of the genes containing outlier SNPs between the forest zones, most were related to cellular

responses to cold (Figure 3.3). Other genes displaying genetic variation in coding regions relate to immunity and lipid metabolism, but two of these genes are not well documented in wildlife systems. Variation in cold response genes is not entirely unexpected given forest zones vary along a latitudinal gradient, and the influence of temperature on hibernation timing, duration, and other adaptive mechanisms, is well documented (Geiser, 2013).

Genes containing missense variants between forest zone include RBBP6 (E3 ubiquitin protein ligase RBBP6) which acts as a regulator in response to stress (Mbita et al., 2021) and is associated with regulation of muscle mass. Hibernation studies in numerous animals have documented differential expression in this gene in response to cold (do Amaral et al., 2020; Velickovska & van Breukelen, 2007). IFI44 (Interferon Induced Protein 44), belongs to the IFN family and is associated with the cellular cold-induced stress response (Peschke et al., 2014; Zimmermann et al., 2019). L3MBTL4 (L3MBTL Histone Methyl-Lysine Binding Protein 4) contributes to p38/JNK activation (Hu et al., 2020), and the JNK (c-Jun N-terminal kinase) pathway aids in neuroprotective adaptations that are essential to successful hibernation (Falvo et al., 2021; M. Lee et al., 2002; Luu et al., 2018).

Additional genes exhibiting genetic variation between forest zones, such as IL12RB2 (Interleukin-12 receptor protein 2A) and Itm2a (integral membrane protein 2A) are involved in immunity (Kirchner & Bevan, 1999; Rofflerm Gretchen H. et al., 2016). Itm2a is not well documented in wildlife systems, however. Variation in immune-related genes along latitudinal gradients is not uncommon as pathogen exposure and

abundance typically decrease with latitude and decreasing temperatures (Rofflerm Gretchen H. et al., 2016). CES4A (Carboxylesterase 4A) was identified as being differentiated between forest zones and is involved in lipid metabolism affected by fasting (Carellini IV, 2018; Nunez, 2021) but CES4A is not well documented in wildlife systems.

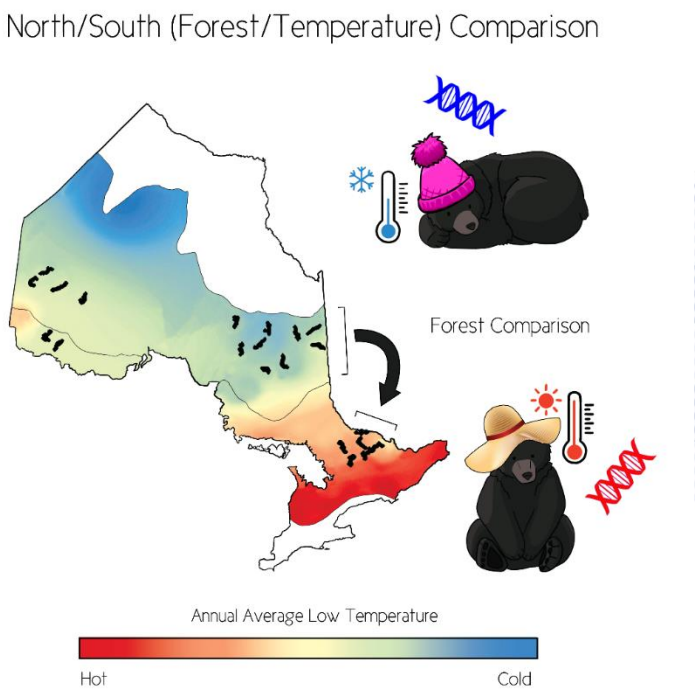


Figure 3.3. In American black bears in eastern Ontario, temperature differences along a latitudinal gradient likely drive genetic variation in genes influencing the cellular response to cold. Colour gradient represents annual average low temperature, where red represents warmer temperatures and blue represents colder temperatures (Range = -7 to +6 degrees Celsius).

Conclusion

Using pooled whole-genome sequencing, we tested the capacity of a large mammal to exhibit genetic variation across heterogeneous landscapes within an otherwise panmictic population. In doing so, we identified genetic variation between climate zones in genes related to the cellular response to starvation. Further, we identified variation between forest zones in genes related to the cellular response to cold. While these results do not provide definitive evidence of local adaptation, they do identify candidate genes for future study that are likely experiencing balancing selection.

There are, however, alternative explanations for documented genetic variation that should be taken into consideration: under linked selection, ancestral variation is reduced, and differentiation is elevated in regions of low recombination while the remainder of the genome may share high amounts of genetic variation and show little differentiation. Predictions for linked selection include a negative relationship between recombination and genetic differentiation (ex. F_{ST}; Burri et al., 2015). If black bears in Ontario exhibit low rates of recombination, this could explain highly differentiated regions of the genome. Indeed, recombination rates, demography, and selection can influence the patterns investigated here (Shang et al., 2021). It is suggested that joint genome scans measuring $F_{ST,}$ genetic divergence (d_{xy}), and nucleotide diversity (π) may be a more robust means of disentangling selection from neutral patterns of evolution (Campbell et al., 2018; Shang et al., 2021).

Although further study is required, these data are still valuable in that they suggest balancing selection can occur in large panmictic mammal populations when selective pressures across heterogeneous landscapes act on important life history traits. This is particularly important as unprecedented rates of climate and land use change continue to rapidly alter environments and have already driven range shifts and local extinctions in many large mammal populations (Hetem et al., 2014; Thuiller et al., 2006).

Particularly in southern North America, the American black bear range represents a small fraction of its historical range, largely owing to human-induced habitat fragmentation (Obbard, Howe, et al., 2010; Scheick & Mccown, 2014). Changing environments coupled with severe habitat fragmentation have resulted in dramatic population reductions to the point where several of these southern black bear populations are now of conservation concern (Dixon et al., 2007; Freedman et al., 2003; Lariviere, 2001). Similar patterns are observed in southern Ontario, such as the fragmented Bruce Peninsula black bear population, and in many large mammal populations worldwide. In such instances, understanding mechanisms of adaptation provide insight on what key traits are being selected for or against. This information can be used to ensure relocation and reintroduction candidates are well suited to their target environment, promoting the development of robust populations.

In central and northern Ontario, black bears are not limited in their dispersal capabilities and may therefore be more resilient to environmental change compared to fragmented populations such as the Bruce Peninsula. However, our data suggest that some black bears, likely those within the continental climate zone, may be more

susceptible to food shortages relative to those in the Atlantic climate zone where more frequent poor-food years may select for optimal responses to starvation. Additionally, black bears who are more resilient to cold temperatures may extend the Ontario black bear range further north as temperatures drop within suitable ranges. This pattern has been documented in numerous mammalian species (Williams & Blois, 2018). Overall, our study highlights the importance of monitoring for evidence of local adaptation in large mammal populations, whether they are fragmented or not. Resulting data will be valuable as continued climate and land use change alter environments faster than most large mammals can adapt.

REFERENCES

Andrews, S. (2010). *FastQC: a quality control tool for high throughput sequence data*.

Anserson, S. J., Cote, S. D., Richard, J. H., & Shafer, A. B. A. (2022). Genomic Architecture of phenotypic extremes in a wild cervid. *BMC Genomics*, *23*(1), 1–13.

Appleton, A. D., Van Horn, R. C., Noyce, K. V., Spady, T. J., Swaisgood, R. R., & Arcese, P. (2018). Phenotypic plasticity in the timing of reproduction in Andean bears. *Journal of Zoology*, *305*(3), 196–202.

Atkins, K. E., & Travis, J. M. J. (2010). Local adaptation and the evolution of species' ranges under climate change. *Journal of Theoretical Biology*, *266*(3), 449–457.

Bertelsen, H. P., Gregersen, V. R., Poulsen, N., Nielsen, R. O., Das, A., Madsen, L. B., Buitenhuis, A. J., Holm, L. E., Panitz, F., Larsen, L. B., & Bendixen, C. (2016). Detection of genetic variation affecting milk coagulation properties in Danish Holstein dairy cattle by analyses of pooled whole-genome sequences from phenotypically extreme samples (pool-seq). *Journal of Animal Science*, *94*(4), 1365–1376. https://doi.org/10.2527/jas.2015-9884

Biswas, S., & Akey, J. M. (2006). Genomic insights into positive selection. *TRENDS in Genetics*, *22*(8), 437–446.

Blanquart, F., Kaltz, O., Nuismer, S. L., & Gandon, S. (2013). A practical guide to measuring local adaptation. *Ecology Letters*, *16*(9), 1195–1205.

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*, *30*(15), 2114–2120.

Bolnick, D. I., & Fitzpatrick, B. M. (2007). Sympatric speciation: models and emirical evidence. *Annual Review of Ecology, Evolution, and Systematics*, *38*, 459–487.

Boonstra, R., Dantzer, B., Delehanty, B., Fletcher, Q. E., & Sherif, M. J. (2014). Equipped for Life in the Boreal Forest : The Role of the Stress Axis in Mammals. *Arctic*, *67*, 82–97.

Burri, R., Nater, A., Kawakami, T., Mugal, C. F., Olason, P. I., Smeds, L., Suh, A., Dutoit, L., Bures, S., Garamszegi, L. Z., Hogner, S., Moreno, J., Qvarnstrom, A., Ruzic, M., Saether, S.-A., Saetre, G.-P., Torok, J., & Ellegren, H. (2015). Linked selection and recombination rate variation drive the evolution of the genomic landscape of differentiation across the speciation continuum of Ficedula flycatchers. *Genome Research*, *25*(11), 1656–1665.

Campbell, R. C., Poelstra, J. W., & Yoder, A. D. (2018). What is Speciation Genomics? The roles of ecology, gene flow, and genomic architecture in the formation of species. *Biological Journal of the Linnean Society*, *124*(4), 561–583.

Carellini IV, F. (2018). *Targeted Disruption of Zebrafish Carboxylesterase Genes Differentially Alters Liver and Blood Lipid Levels in a Sexually-dimorphic Manner*.

Cavedon, M., Gubili, C., Heppenheimer, E., vonHoldt, B., Mariani, S., Hebblewhite, M., Hegel, T., Hervieux, D., Serrouya, R., Steenweg, R., Weckworth, B. V., & Musiani, M. (2019). Genomics, environment and balancing selection in behaviourally bimodal populations: The caribou case. In *Molecular Ecology* (Vol. 28, Issue 8). https://doi.org/10.1111/mec.15039

Cingolani, P., Platts, A., Wang, L. L., Coon, M., Nguyen, T., Wang, L., Land, S. J., Lu, X., & Ruden, D. M. (2012). A program for annotating and predicting the effects of a signle nucleotidy polymorphism, SnpEff. *Fly*, *6*(2), 80–92.

Cook, L. M., & Saccheri, I. J. (2013). The peppered moth and industrial melanism: evolution of a natural selection case study. *Heredity*, *110*(3), 207–212.

Cortes, P. A., Franco, M., Sabal, P., Quijano, S. A., & Nespolo, R. F. (2011). Bioenergetics and intestinal phenotypic flexibility in the microbiotherid marsupial (Dromiciops gliroides) from the temperate forest in South America. *Comparative Biochemistry and Physiology Part A: Molecular and Integratice Physiology*, *160*(2), 117–124.

Cutler, D. J., & Jensen, J. D. (2010). Commentary To Pool, or Not to Pool? *Genetics*, *186*, 41–43. https://doi.org/10.1534/genetics.110.121012

Davey, J. W., & Blaxter, M. L. (2010). RADSeq: next-generation population genetics. *Briefings in Functional Genomics*, *9*(5–6), 416–423.

Dennenmoser, S., Vamosi, S. M., Nolte, A. W., & Rogers, S. M. (2017). Adaptive genomic divergence under high gene flow between freshwater and brackish-water ecotypes of prickly sculpin (Cottus asper) revealed by Pool-Seq. *Molecular Ecology*, *26*(1), 25–42. https://doi.org/10.1111/mec.13805

DeWitt, T. J., Sih, A., & Wilson, D. S. (1998). Costs and limits of phenotypic plasticity. *Trends in Ecology & Evolution*, *13*(2), 77–81.

Dixon, J. D., Oli, M. K., Wooten, M. C., Eason, T. H., McCown, J. W., & Cunningham, M. W. (2007). Genetic consequences of habitat fragmentation and loss: The case of the Florida black bear (Ursus americanus floridanus). *Conservation Genetics*, *8*(2), 455–464. https://doi.org/10.1007/s10592-006-9184-z

do Amaral, M. C. F., Frisbie, J., Crum, R. J., Goldstein, D. L., & Krane, C. M. (2020). Hepatic transcriptome of the freeze-tolerant Cope's gray treefrog, Dryphytes chrysoscelis: response to cold acclimation and freezing. *BMC Genomics*, *21*(1), 1–18.

Dool, S. E., Picker, M. D., & Eberhard, M. J. B. (2021). Limited dispersal and local adaptation promote allopatric speciation in a biodiversity hotspot. *Molecular Ecology*.

Falvo, S., Santillo, A., Maddalena Di Fiore, M., Rosati, L., & Chieffi Baccari, G. (2021). JNK/Elk1 signaling and PCNA protein expression in the brain of hibernating frog Pelophylax esculentus. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, *335*(5), 529–536.

Feder, J. L., Egan, S. P., & Nosil, P. (2012). The genomics of speciation-with-gene-flow. *Trends in Genetics*, *28*(7), 342–350. https://doi.org/10.1016/j.tig.2012.03.009

Feder, M. E., & Mitchell-olds, T. (2003). Evolutionary and ecological functional genomcis. *Nature Review Genetics*, *4*(8), 649–655.

Federov, V. B., Goropashnaya, A. v., Toien, O., Stewart, N. C., Gracey, A. Y., Chang, C., Qin, S., Pertea, G., Quackenbush, J., Showe, L. C., Showe, M. K., Boyer, B. B., & Barnes, B. M. (2009). Elevated expression of protein biosynthesis genes in liver and muscle of hibernating black bears. *Physiological Genomics*, *37*(2), 108–118.

Fedorov, V. B., Goropashnaya, A. v., Toien, O., Stewart, N. C., Chang, C., Wang, H., Showe, L. C., Showe, M. K., & Barnes, B. M. (2011). Modulation of gene expression in heart and liver of hibernating black bears (Ursus americanus). *BMC Genomics*, *12*(1), 1–15.

Fedorov, V. B., Goropashnaya, A. v., Tøien, Ø., Stewart, N. C., Chang, C., Wang, H., Yan, J., Showe, L. C., Showe, M. K., Donahue, S. W., & Barnes, B. M. (2012). Preservation of bone mass and structure in hibernating black bears (Ursus americanus) through elevated expression of anabolic genes. *Functional and Integrative Genomics*, *12*(2), 357–365. https://doi.org/10.1007/s10142-012-0266-3

Ferretti, L., Ramos-Onsins, S. E., & Pérez-Enciso, M. (2013). Population genomics from pool sequencing. *Molecular Ecology*, *22*(22), 5561–5576. https://doi.org/10.1111/mec.12522

Fitzhugh, R. D., Driscoll, C. T., Groffman, P. M., Tierney, G. L., Fahey, tomothy J., & Hardy, J. P. (2001). Effects of soil freezing disturbance on soil solution nitrogen, phosphorus, and carbon chemistry in a northern hardwood ecosystem. *Biogeochemistry*, *56*(2), 215–238.

Freedman, A. H., Portier, K. M., & Sunquist, M. E. (2003). Life history analysis for black bears (Ursus americanus) in a changing demographic landscape. *Ecological Modelling*, *167*(1–2), 47–64. https://doi.org/10.1016/S0304-3800(03)00171-6

Fuentes-Pardo, A. P., & Ruzzante, D. E. (2017). Whole-genome sequencing approaches for conservation biology: Advantages, limitations and practical recommendations. *Molecular Ecology*, *26*(20), 5369–5406. https://doi.org/10.1111/mec.14264

Gandon, S., & Van Zandt, P. A. (1998). Local adaptation and host-parasite interactions. *Trends in Ecology & Evolution*, *13*(6), 214–216.

Gavrilets, S. (2003). Perspective: models of speciation: what have we learned in 40 years? *Evolution*, *57*(10), 2197–2215.

Geiser, F. (2013). Hibernation. *Current Biology*, *23*(5), R188–R193.

Gerisch, B., George Tharyan, R., Mak, J., Denzel, S. I., Popkes-van Oepen, T., Henn, N., & Antebi, A. (2020). HLH-30/TFEB Is a Master Regulator of Reproductive Quiescence. *Developmental Cell*, *53*(3), 316–329.

Goropashnaya, A. v., Tøien, Ø., Ramaraj, T., Sundararajan, A., Schilkey, F. D., Barnes, B. M., Donahue, S. W., & Fedorov, V. B. (2021). Transcriptional changes and preservation of bone mass in hibernating black bears. *Scientific Reports*, *11*(1), 1–9. https://doi.org/10.1038/s41598-021-87785-9

Greenhorn, J. E., & Bowman, J. (2018). Genetic monitoring suggests increasing structure following recolonization by fishers. *The Journal of Wildlife Management*, *82*(7), 1403–1406.

Hamala, T., & Savolainen, O. (2019). Genomic Patterns of Local Adaptation under Gene Flow in Arabidopsis lyrata. *Molecular Biology and Evolution*, *36*(11), 2557–2571.

Hellgren, E. C. (1998). Physiology of hibernation in bears. *Ursus*, *10*, 467–477.

Hetem, R. S., Fuller, A., Maloney, S. K., & Mitchell, D. (2014). Responses of large mammals to cliamte change. *Temperature*, *1*(2), 115–127.

Holden, Z. A., Kasworm, W. F., Servheen, C., Hahn, B., & Dobrowski, S. (2012). Sensitivity of berry productivity to climatic variation in the cabinet-yaak grizzly bear recovery zone, Northwest United States, 1989-2010. *Wildlife Society Bulletin*, *36*(2), 226–231. https://doi.org/10.1002/wsb.128

Howe, E. J., Obbard, M. E., & Bowman, J. (2012). Prior reproduction and weather affect berry crops in central Ontario, Canada. *Population Ecology*, *54*(2), 347–356. https://doi.org/10.1007/s10144-011-0301-6

Howe, E. J., Obbard, M. E., & Kyle, C. J. (2013). Combining data from 43 standardized surveys to estimate densities of female American black bears by spatially explicit capture–recapture. *Population Ecology*, *55*, 595–607. https://doi.org/10.1007/s10144-013-0389-y

Howe, E. J., Potter, D., Beauclerc, K. B., Jackson, K. E., & Northrup, J. M. (2022). Estimating animal abundance at multiple scales by spatially explicit capture-recapture. *Ecological Applications*.

Hu, C., Zuo, K., Li, K., Gao, Y., Chen, M., Hu, R., Liu, Y., Chi, H., Wang, H., Qin, Y., Liu, X., Zhong, J., Cai, J., Yang, X., & Li, J. (2020). p38/JNK Is Required for the Proliferation and Phenotype Changes of Vascular Smooth Muscle Cells Induced by L3MBTL4 in Essential Hypertension. *International Journal Og Hypertension*, *2020*.

Hurrell, J. W. (1996). Influence of variations in extratropical wintertime teleconnections on Northern Hemisphere temperature. *Geophysical Research LEtters*, *23*(6), 665–668. https://doi.org/10.1029/96GL00459

Hurrell, J. W., Kushnir, Y., Ottersen, G., & Visbeck, M. (2003). An overview of the north atlantic oscillation. *Geophysical Monograph Series*, *134*(January), 1–35. https://doi.org/10.1029/134GM01

Jacob, S., Legrand, D., CHaine, A. S., Bonte, D., Schtickzell, N., Huet, M., & Clobert, J. (2017). Gene flow favours local adaptation under habitat choice in ciliate microcosms. *Nature Ecology and Evolution*, *1*(9), 1407–1410.

Jobava, R., Mao, Y., Guan, B.-J., Hu, D., Krokowski, D., Chen, C.-W., Shu, X. E., Chikwurah, E., Wu, J., Gao, Z., Zagore, L. L., Merrick, W. C., Trifunovic, A., Hsieh, A. C., Valadkhan, S., Zhang, Y., Qi, X., Jankowski, E., Topisirovic, I., … Hatzoglou, M. (2021). - Adaptive translation pausing is a hallmark of the cellular response to severe environmental stress. *Molecular Cell*, *81*(20), 4191–4208.

Karelus, D. L., McCown, J. W., Scheick, B. K., Kerk, M. van de, & Oli, M. K. (2016). Home Ranges and Habitat Selection by Black Bears in a Newly Colonized Population in Florida. *Southeastern Naturalist*, *15*(2), 346. https://doi.org/10.1656/058.015.0215

Kawecki, T. J. (2008). Adaptation to Marginal Habitats. *Annual Review of Ecology, Evolution, and Systematics*, *39*(1), 321–342. https://doi.org/10.1146/annurev.ecolsys.38.091206.095622

Kawecki, T. J., & Ebert, D. (2004). Conceptual issues in local adaptation. *Ecology Letters*, *7*(12), 1225–1241. https://doi.org/10.1111/j.1461-0248.2004.00684.x

Kelsey, K. C., Pedersen, S. H., Leffler, J. A., Sexton, J. O., Feng, M., & Welker, J. M. (2021). Winter snow and spring temperature have differential effects on vegetation phenology and productivity across Arctic plant communities. *Global Change Biology*, *27*(8), 1572–1586.

Kirchner, J., & Bevan, M. J. (1999). ITM2A is induced during thymocyte selection and T cell activation and causes downregulation of CD8 when overexpressed in CD4+ and CD8+ douple positive thymocytes. *The Journal of Experimental Medicine*, *190*(2), 217–228.

Koenig, D., Hagmann, J., Li, R., Bemm, F., Slotte, T., Neuffer, B., Wright, S. I., & Weigel, D. (2019). Long-term balancing selection drives evolution of immunity genes in Capsella. *Elife*, *8*, e43606.

Kofler, R., & Schloterrer, C. (2012). Gowinda: unbiased analysis of gene set enrichment for genome-wide association studies. *Bioinformatics*, *28*(15), 2084–2085.

Kolenosky, G. B., & Strathearn, S. B. (1987). Winter Denning of Black Bears in East-Central Ontario. *International Association for Bear Research and Managment*, *7*, 305–316.

Lane, J. E., Zenon, J. C., Findlay-Robinson, R., & Bayen, E. (2019). Phenotypic Plasticity and Local Adaptation in a Wild Hibernator Evaluated through Reciprocal Translocation. *American Naturalist*, *194*(4), 516–528.

Lariviere, S. (2001). Ursus amencanus,. *American Society of Mammalogists*, *647*, 1–11.

Lee, M., Choi, I., & Park, K. (2002). Activation of stress signaling molecules in bat brain during arousal from hibernation. *Journal of Neurochemistry*, *82*(4), 867–873.

Lee, P. J., Cho, N., Yoo, H. M., & Kim, J. P. (2020). Active Turnover of Heme in Hibernation Period in Mammals. *Fronteirs in Physiology*, *10*, 1585.

Leffler, J. A., Klein, E. S., Oberbauer, S. F., & Welker, J. M. (2016). Coupled long-term summer warming and deeper snow alters species composition and stimulates gross primary productivity in tussock tundra. *Oecologia*, *181*(1), 287–287.

Li, H. (2013). *Aligning sequence reads, clone sequences, and assembly contigs with BWA-MEM*.

Li, H., Handsacker, B., Wysocker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., & Durbin, R. (2009). The Sequence Alignment/Map format and SAMtools. *Bioinformatics*, *25*(16), 2078–2079.

Li, P., Sayer, E. J., Jia, Z., Liu, W., Wu, Y., Yang, S., Wang, C., Yang, L., Chen, D., Bai, Y., & Liu, L. (2020). Deepened winter snow cover enhances net ecosystem exchange and stabilizes plant community composition and productivity in a temperate grassland. *Global Change Biology*, *26*(5), 3015–3017.

Luu, B. E., Wijenayake, S., Zhang, J., Tessier, S. N., Quintero-Galvis, J. F., Gaitan-Espitia, J. D., Nespolo, R. F., & Storey, K. B. (2018). Strategies of biochemical adaptation for hibernation in a South American marsupial Dromiciops gliroides: 1. Mitogen-activated protein kinases and the cell stress response. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *224*, 12–18.

Mbita, Z., Hull, R., Mokoena, F., Lai, C.-H., & Dlamini, Z. (2021). RBBP6 interactome: RBBP6 isoform 3/DWNN and Nek6 interaction is critical for cell cycle regulation and may play a role in carcinogenesis. *Informatics in Medicine Unlocked*, *23*, 100522.

Merilä, J., & Hendry, A. P. (2014). Climate change, adaptation, and phenotypic plasticity: The problem and the evidence. *Evolutionary Applications*, *7*(1), 1–14. https://doi.org/10.1111/eva.12137

Mosnier, A., Ouellet, J.-P., & Courtois, R. (2008). Black bear adaptation to low productivity in the boreal forest. *Ecoscience*, *15*(4), 485–497. https://doi.org/10.2980/15-4-3100

Muir, A. P., Biek, R., Thomas, R., & Mable, B. K. (2014). Local adaptation with high gene flow: temperature parameters drive adaptation to altitude in the common frog (Rana temporaria). *Molecular Ecology*, *23*(3), 561–574.

Murphy, S. M., Hast, J. T., Augustine, B. C., Weisrock, D. W., Clark, J. D., Kocka, D. M., Ryan, C. W., Sajecki, J. L., & Cox, J. J. (2019). Early genetic outcomes of American black bear reintroductions in the Central Appalachians, USA. *Ursus*, *29*(2), 119–133.

Neethiraj, R., Hornett, E. A., Hill, J. A., & Whear, C. W. (2017). Investigating the genomic basis of discrete phenotypes using a Pool-Seq-only approach: New insights into the genetics underlying colour variation in diverse taxa. *Molecular Ecology*, *26*(19), 4990–5002.

Ng, P. C., & Kirkness, E. F. (2010). *Whole Genome Sequencing*.

Nielsen, E. S., Henriques, R., Toonen, R. J., Knapp, I. S. S., Guo, B., & von der Heyden, S. (2018). Complex signatures of genomic variation of two non-model marine species in a homogeneous environment. *BMC Genomics*, *19*(1), 1–16. https://doi.org/10.1186/s12864-018-4721-y

Nosil. (2008). Speciation with gene flow could be common. *Molecular Ecology*, *17*(9), 2103–2106.

Nosil, P. (2012). *Ecological Speciation*. Oxford University Press.

Nunez, A. J. A. (2021). *Exploring the Prox1 SUMO-switch in Hepatocytes*.

Obbard, M. E., & Howe, E. J. (2008). Demography of Black Bears in Hunted and Unhunted Areas of the Boreal Forest of Ontario. *Journal of Wildlife Management*, *72*(4), 869–880. https://doi.org/10.2193/2006-158

Obbard, M. E., & Howe, E. J. (2014). *Ontario Wildlife Food Survey , 2014*. https://doi.org/10.13140/RG.2.1.1855.9522

Obbard, M. E., Howe, E. J., & Kyle, C. J. (2010). Empirical comparison of density estimators for large carnivores. *Journal of Applied Ecology*, *47*(1), 76–84. https://doi.org/10.1111/j.1365-2664.2009.01758.x

Obbard, M. E., Howe, E. J., Wall, L. L., Allison, B., Black, R., Davis, P., Dix-gibson, L., Gatt, M., & Hall, M. N. (2014). Relationships among food availability , harvest , and human – bear conflict at landscape scales in Ontario , Canada. *Ursus*, *25*(2), 98–110. https://doi.org/10.2192/URSUS-D-13-00018.1

Peschke, K., Friebe, F., Zimmermann, N., Wahlicht, T., Schumann, T., Achleitner, M., Berndt, N., Luksch, H., Behrendt, R., Lee-Kirsch, M. A., Roers, A., & Gunther, C. (2014). Deregulated Type I IFN Response in TREX1-Associated Familial Chilblain Lupus. *The Journal of Investigative Dermatology*, *134*(5), 1456.

Phair, N. L., Toonen, R. J., Knapp, I., & von der Heyden, S. (2019). Shared genomic outliers across two divergent population clusters of a highly threatened seagrass. *PeerJ*, *7*, e6806. https://doi.org/10.7717/peerj.6806

Potts, K. B., Chapman, C. A., & Lwanga, J. S. (2009). Floristic heterogeneity between forested sites in Kibale National Park, Uganda: insights into the fine-scale determinants of density in a large-bodied frugivorous primate. *Journal of Animal Ecology*, *78*(6), 1269–1277.

Puckett, E. E., & Eggert, L. S. (2016). Comparison of SNP and microsatellite genotyping panels for spatial assignment of individuals to natal range: A case study using the American black bear (Ursus americanus). *Biological Conservation*, *193*, 86–93. https://doi.org/10.1016/j.biocon.2015.11.020

Quinlan, A. R. (2014). BEDTools: the Swiss-army tool for genome feature analysis. *Current Protocols in Bioinformatics*, *47*(1), 11–12.

Rixen, C., Freppaz, M., Stoeckli, V., Huovinen, C., Huovinen, K., & Wipf, S. (2008). Altered Snow Density and Chemistry Change Soil Nitrogen Mineralization and Plant Growth. *Arctic, Antarctic, and Alpine Research*, *40*(3), 568–575.

Rofflerm Gretchen H., Amish, S. J., Smith, S., Cosart, T., Mardos, M., Schwartz, M. K., & Luikart, G. (2016). SNP discovery in candidate adaptive genes using exon capture in a free-ranging alpine ungulate. *Molecular Ecology*, *16*(5), 1147–1164.

Rogers, L. L. (1987). Factors influencing dispersal in the black bear. In *Mammalian dispersal patterns: the effects of social structure on population genetics* (pp. 75–84).

Rosenheim, J. A., & Tabashnik, B. E. (1991). Influence of generation time on the rate of response to selection. *The American Naturalist*, *137*(4), 527–541.

Row, J. R., Wilson, P. J., Gomez, C., Koen, E. L., Bowman, J., Thorton, D., & Murray, D. L. (2014). The subtle role of climate change on population genetic structure in Canada lynx. *Global Change Biology*, *20*, 2076–2086.

Rowe, J. S. (1972). *Forest Regions of Canada*.

Samson, C., & Huot, J. (1995). Reproductive Biology of Female Black Bears in Relation to Body Mass in Early Winter. *Journal of Mammalogy*, *76*(1), 68–77.

Savolainen, O., Lascoux, M., & Merila, J. (2013). Ecological genomics of local adaptation. *Nature Reviews Genetics*, *14*, 807–820.

Scheick, B. K., & Mccown, W. (2014). Geographic distribution of American black bears in North America. *Ursus*, *25*(1), 24–33. https://www.jstor.org/stable/24643793

Schlötterer, C., Tobler, R., Kofler, R., & Nolte, V. (2014). Sequencing pools of individuals-mining genome-wide polymorphism data without big funding. *Nature Reviews Genetics*, *15*(11), 749–763. https://doi.org/10.1038/nrg3803

Schwartz, C., Hampton, M., & Andrews, M. T. (2013). Seasonal and regional differences in gene expression in the brain of a hibernating mammal. *PLoS One*, *8*(3), e58427.

Sexton, J. P., Hangartner, S. B., & Hoffman, A. A. (2014). Genetic isolation by environment or distance: which pattern of gene flow is most common? *Evolution*, *68*(1), 1–15.

Shafer, A. B. A., Nielsen, S. E., Northrup, J. M., & Stenhouse, G. B. (2014). Linking genotype, ecotype, and phenotype in an intensively managed large carnivore. *Evolutionary Applications*, *7*(2), 301–312. https://doi.org/10.1111/eva.12122

Shang, H., Rendon-Anaya, M., Paun, O., Field, D. L., Hess, J., Vogl, C., Liu, J., Ingvarsson, P. K., Lexter, C., & Leroy, T. (2021). Conserved genomic landscapes of differentiation across Populus speciation continuum. *BioRxiv*.

Srivastava, A., Kumar Sarsani, V., Fiddes, I., Sheehan, S. M., Seger, R. L., Barter, M. E., Neptune-Bear, S., Lindqvist, C., & Korstanje, R. (2019). Genome assembly and gene expression in the American black bear provides new insights into the renal response to hibernation. *DNA Research*, *26*(1), 37–44. https://doi.org/10.1093/dnares/dsy036

Stortz, J. F., & Scott, G. R. (2021). Phenotypic plasticity, genetic assimilation, and genetic compensation in hypoxia adaptation of high-altitude vertebrates. *Comparative Biochemistry and Physiology Part A: Molecular and Integratice Physiology*, *253*, 110865.

Supek, F., Bosnjak, M., Skunca, N., & Smuc, T. (2011). REVIGO Summarizes and Visualizes Long Lists of Gene Ontology Terms. *PLoS ONE*, *6*(7), e21800.

Tarasov, A., Vilella, A. J., Cuppen, E., Nijman, I. J., & Prins, P. (2015). Sambamba: fast processing of NGS alignment formats. *Bioinformatics*, *31*(15), 2032–2034.

The Uniprot Consortium. (2021). UniProt: the universal protain knowledgebase in 2021. *Nucleic Acid Research*, *49*(D1), D480–D489.

Thuiller, W., Broennimann, O., Hughes, G., Robert, J., Alkemande, M., Midgley, G. F., & Corsi, F. (2006). Vulnerability of African mammals to anthropogenic climate change under conservative land transformation assumptions. *Global Change Biology*, *12*(3), 424–440.

Tietje, W. D., & Ruff, R. L. (1980). Denning Behaviour of Black Bears in Boreal Forest of Alberta. *The Journal of Wildlife Managment*, *44*(4), 858–870.

Tigano, A., & Friesen, V. L. (2016). Genomics of local adaptation and gene flow. *Molecular Ecology*, *25*(10), 2144–2164.

Tilk, S., Bergland, A., Goodman, A., Schmidt, P., Petrov, D., & Greenblum, S. (2018). High accuracy haplotype-derived allele frequencies from ultra-low coverage pool-seq samples. *BioRxiv*, 1–26. https://doi.org/10.1101/244004

Toien, O., Blake, J., Edgar, D. M., Grahn, D. A., Heller, C., & Barnes, B. M. (2011). Hibernation in Black Bears: Independence of Metabolic Supression from Body Temperature. *Science*, *331*(6019), 906–909.

Tri, A. (2013). *Temporal, Spatial, and Environmental Influences on the Demographics and Har aphics and Harvest Vulnerability of American Black Bears ability of American Black Bears (Ursus americanus) in Urban Habitats in New Jersey, Pennsylvania and West Virginia*. West Virginia University.

van der Auwera, G. A., Carneiro, M. O., Harlt, C., Poplin, R., del Angel, G., Levy-Moonshine, A., Jordan, T., Shakir, K., Roazen, D., Thibault, J., Banks, E., Garimella, K. v., Altshuler, D., Gabriel, S., & DePristo, M. A. (2013). From FastQ data to high-confidence variant calls: the genome analysis toolkit best practices pipeline. *Current Protocols in Bioinformatics*, *43*(1), 11–10.

Vankoughnett, M. R., & Henry, H. A. L. (2014). Soil freezing and N deposition: transient vs multi-year effects on plant productivity and relative species abundance. *New Phytologist*, *202*(4), 1277–1285.

Velickovska, V., & van Breukelen, F. (2007). Ubiquitylation of proteins in livers of hibernating golden-mantled ground squirrels, Spermophilus lateralis. *Cryobiology*, *55*(3), 230235.

Welch, C. A., Keay, J., Kendall, K. C., & Robbins, C. T. (1997). Constraints on frugivory by bears. *Ecology*, *78*(4), 1105–1119.

Williams, J. E., & Blois, J. L. (2018). Range shifts in response to past and future climate change: Can climate velocities and species' dispersal capabilities explain variation in mammalian range shifts? *Journal of Biogeography*, *45*(9), 2175–2189.

Woods, J. G., Paetkau, D., Lewis, D., McLellan, B. N., Proctor, M., & Strobeck, C. (1999). Genetic Tagging of Free-Ranging Black and Brown Bears. *Wildlife Society Bulletin*, *27*(3), 616–627.

Wu, Q., Han, T.-S., Chen, X., Chen, J.-F., Zou, Y.-P., Li, Z.-W., Xu, Y.-C., & Gou, Y.-L. (2017). Long-term balancing sleection contributes to adaptation in Arabidopsis and its relatives. *Genome Biology*, *18*(1), 1–15.

Xu, H., Lou, X., Qian, J., Pang, X., Song, J., Qian, G., Chen, J., & Chen, S. (2012). FastUniq: a fast de novo duplicates removal tool for paired short reads. *PLoS ONE*, *7*(12).

Yadav, S., Stow, A. J., & Dudaniec, R. Y. (2019). Detection of environmental and morphological adaptation despite high landscape genetic connectivity in a pest grasshopper (Phaulacridium vittatum). *Molecular Ecology*, *28*(14), 3395–3412.

Yan, J., Burman, A., Showe, L. C., Showe, M. K., Boyer, B. B., Barnes, B. M., & Marr, T. G. (2006). Detection of differential gene expression in brown adipose tissue of hibernating ground squirrels with mouse microarrays. *Physiological Genomics*, *25*(2), 346–353.

Yancey, K. L. (2018). *Shining light on hibernator genomes: using radiation to reveal DNA damage and repair dynamics in Arctic ground squirrels*.

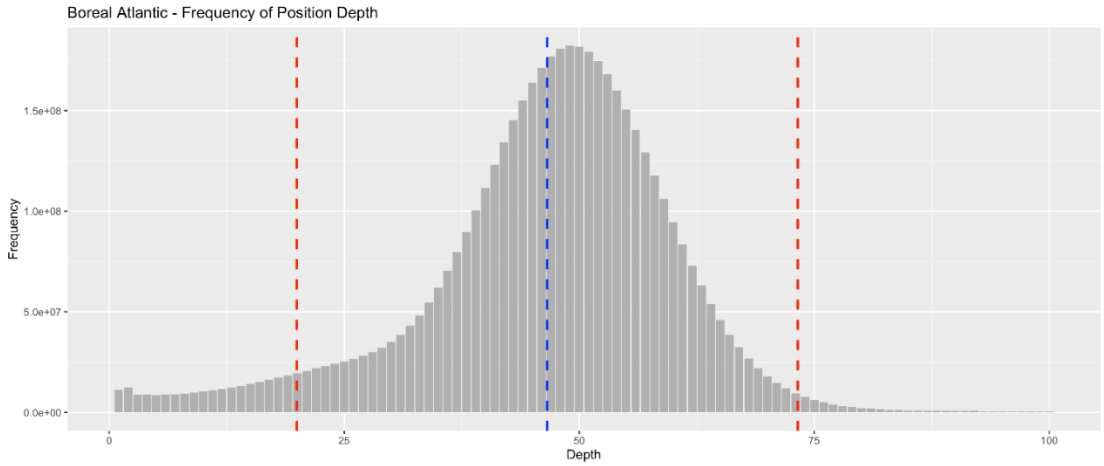
Ye, J., Zhang, Y., Cui, H., Liu, J., Wu, Y., Chen, Y., Xu, H., Huang, X., Li, S., Zhou, A., Zhang, X., Bolund, L., Chen, Q., Wang, J., Yang, H., Fang, L., & Shi, C. (2018). WEGO: a web tool for plotting GO annotations, 2018 update. *Nucleic Acids*, *46*(W1), W71–W75.

Zhao, S., Shao, C., Goropashnaya, A. v., Stewart, N. C., Xu, Y., Toien, O., Barnes, B. M., Federov, V. B., & Yan, J. (2010). Genmoic analysis of expressed sequence tags in American black bear Ursus americanus. *BMC Genomics*, *11*(1), 1–16.

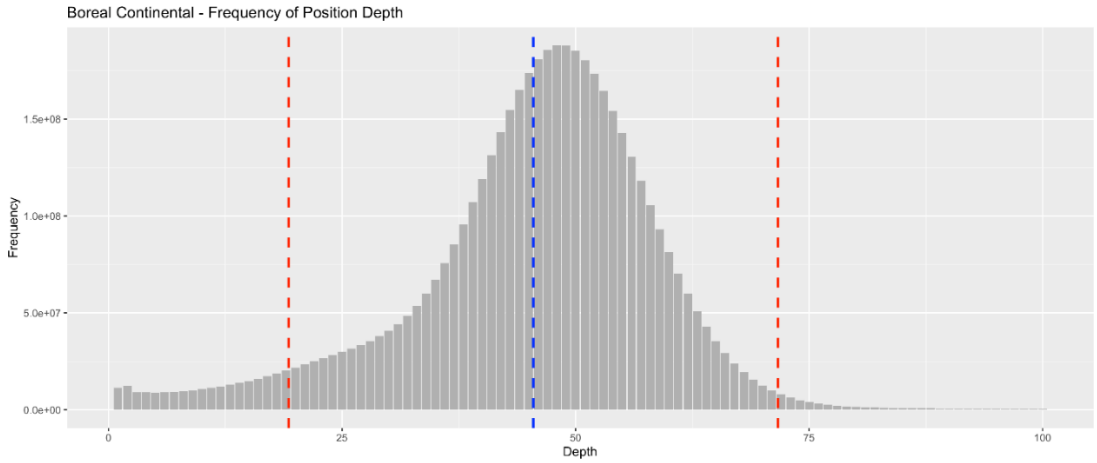
Zimmermann, N., Wolf, C., Schewnke, R., Luth, A., Schmidt, F., Engel, K., Lee-Kirsch, M. A., & Gunther, C. (2019). Assessment of Clinical Response to Janus Kinase Inhibition in Patients With Familial Chilblain Lupus and TREX1 Mutation. *JAMA Dermatology*, *155*(3), 342–346.

APPENDIX B

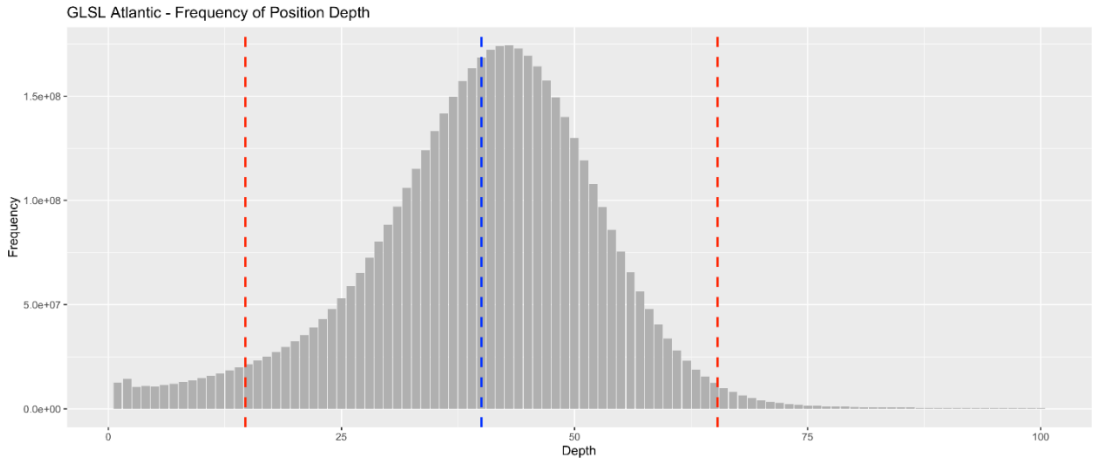
a)



b)



c)



d)

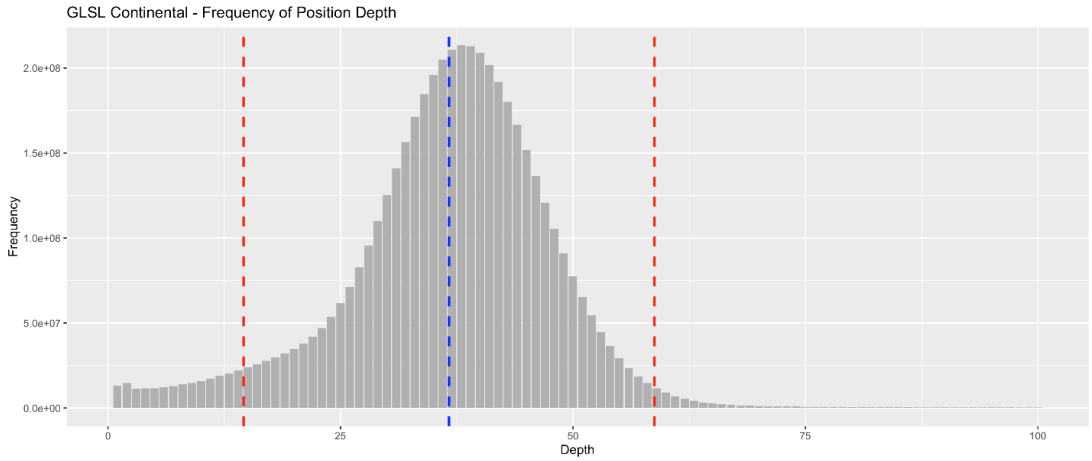
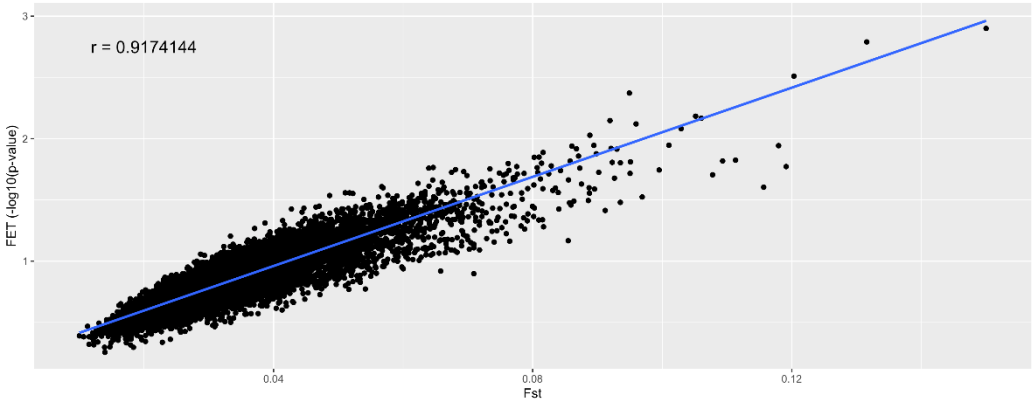
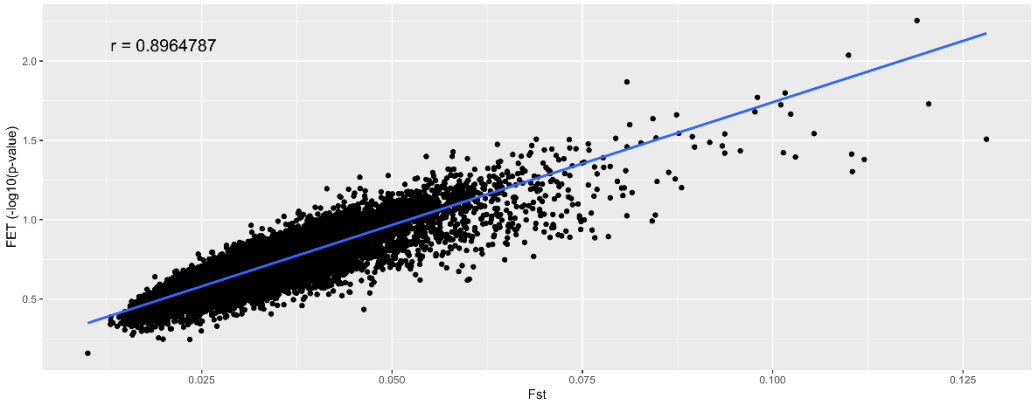


Figure S1. Frequency of covered position depth, with maximum depth set to 100x, for (a) Boreal Atlantic, (b) Boreal Continental, (c) GLSL Atlantic, and (d) GLSL Continental pools. Dotted blue lines indicate mean coverage; red lines indicate mean coverage +/- 2 standard deviations, which were the values used as the minimum and maximum coverage cutoffs when calculation FET and Fst in 50kb non-overlapping sliding windows

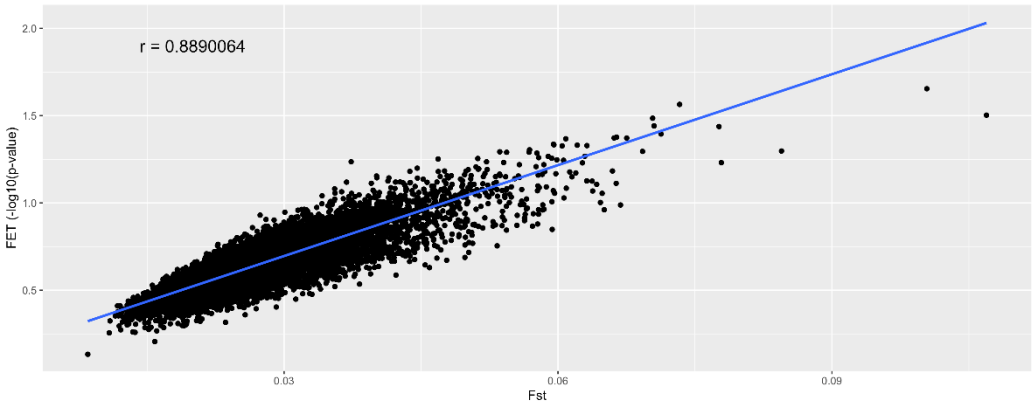
A Boreal Atlantic v. Boreal Continental: FET v. Fst (50kb non-overlapping wondow)



B Boreal Atlantic v. GLSL Atlantic: FET v. Fst (50kb non-overlapping wondow)



C Boreal Continental v. GLSL Continental: FET v. Fst (50kb non-overlapping wondow)



D GLSL Atlantic v. GLSL Continental: FET v. Fst (50kb non-overlapping wondow)

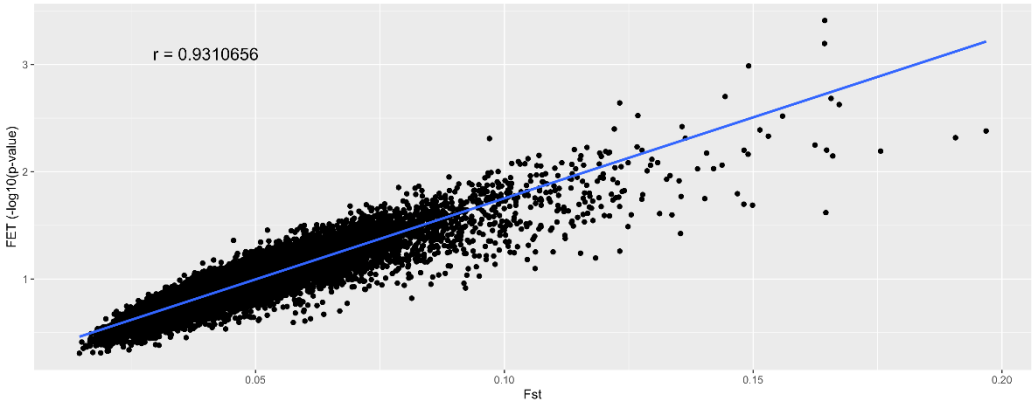
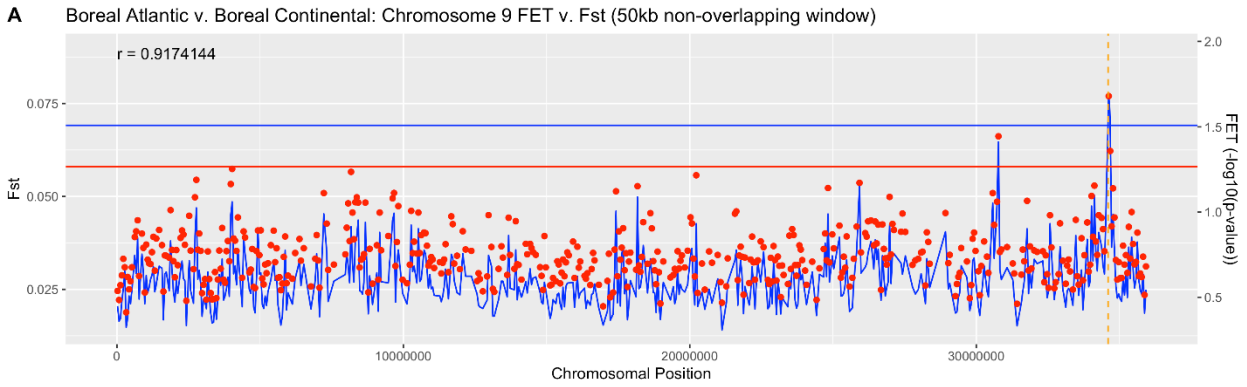
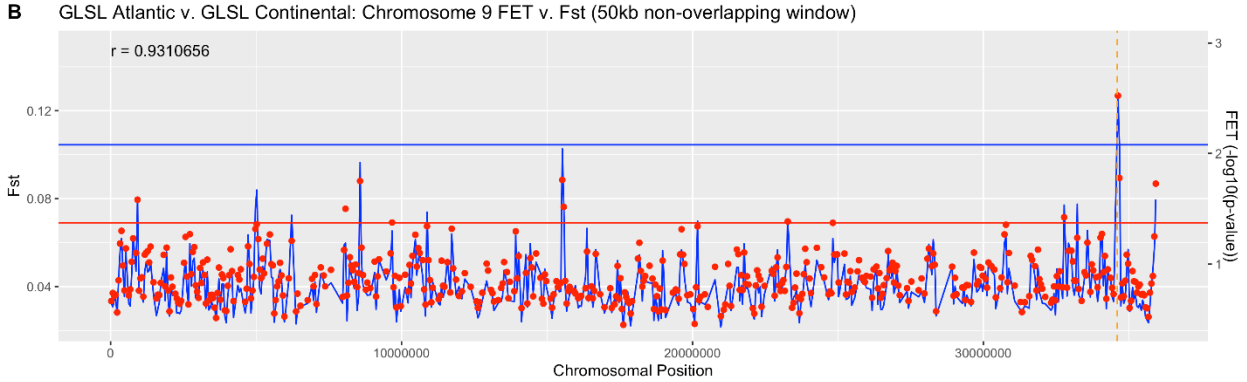
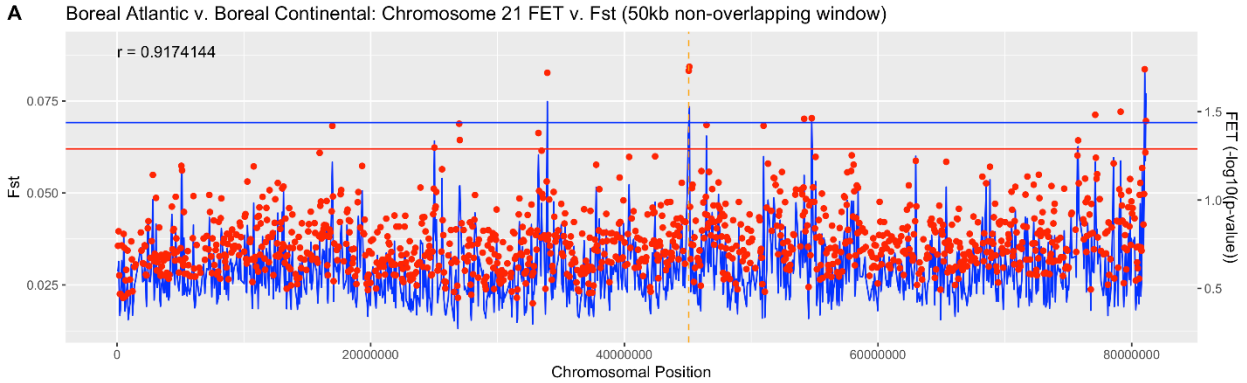
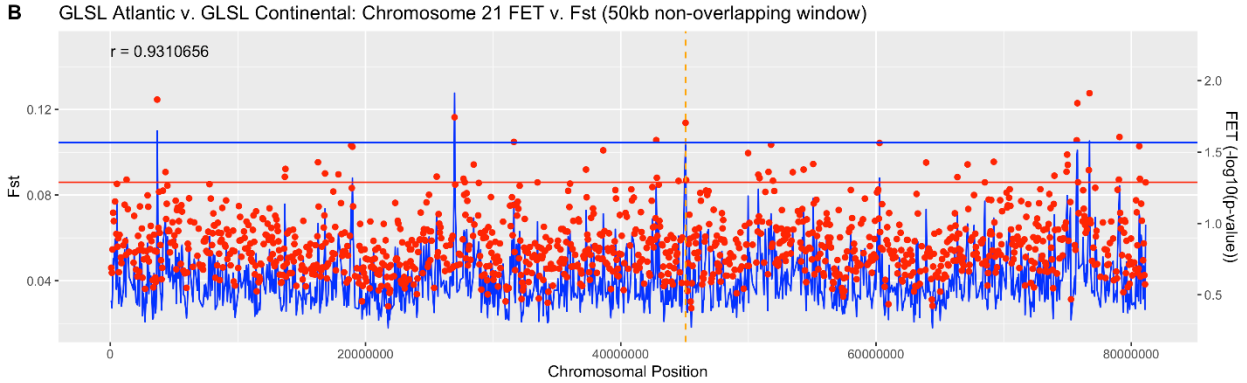


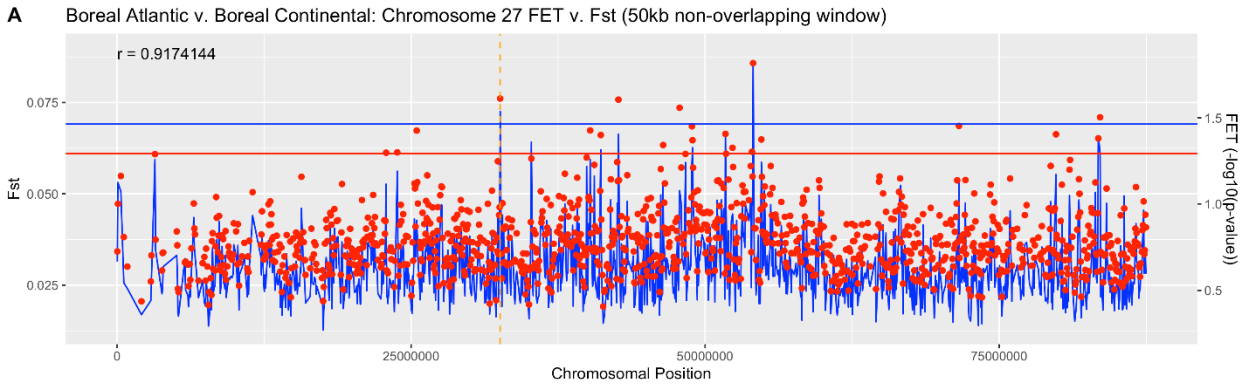
Figure S2. Scatterplot of FET versus Fst (50kb non-overlapping sliding window) results for (A) Boreal Atlantic versus Boreal Continental, (B) Boreal Atlantic versus GLSL Atlantic, (C) Boreal Continental versus GLSL Continental, and (D) GLSL Atlantic versus GLSL Continental. Spearman's Rank Correlation coefficient is indicated in the top left of each plot, and the relationship between FET and Fst results is plotted as the red trend line (calculated using a simple linear regression).

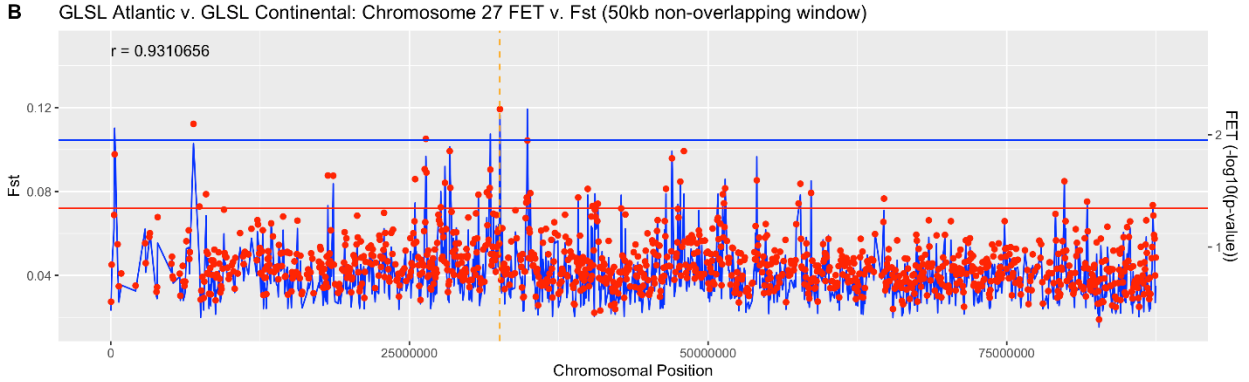

A
Boreal Atlantic v. Boreal Continental: Chromosome 9 FET v. Fst (50kb non-overlapping window)
r = 0.9174144
Fst
FET (-log10(p-value))
Chromosomal Position

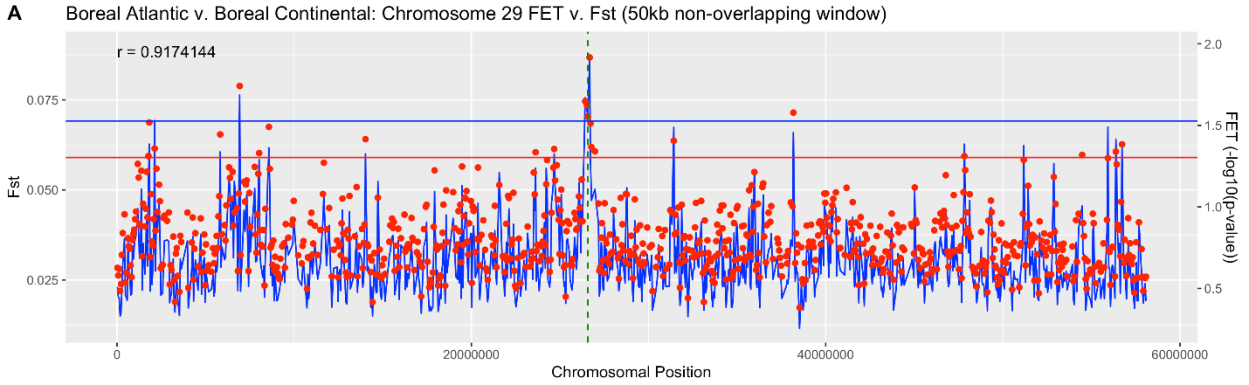

B
GLSL Atlantic v. GLSL Continental: Chromosome 9 FET v. Fst (50kb non-overlapping window)
r = 0.9310656
Fst
FET (-log10(p-value))
Chromosomal Position

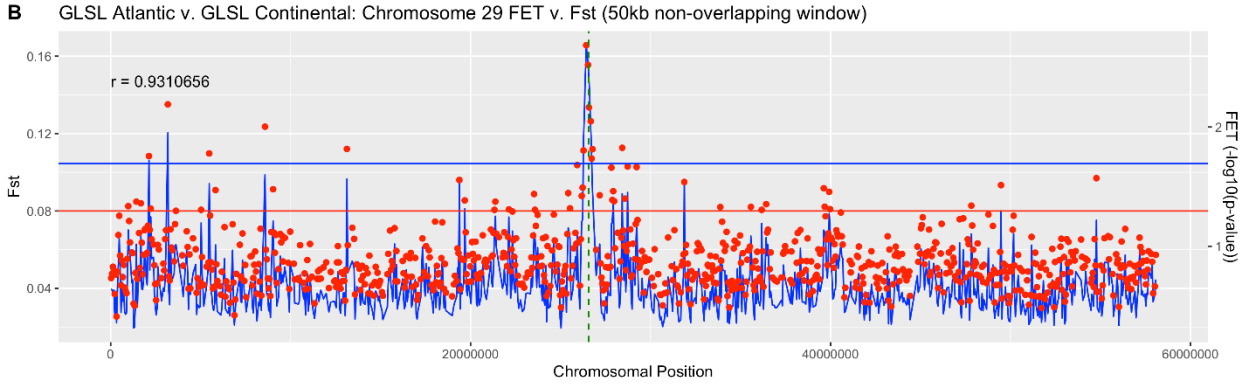

A
Boreal Atlantic v. Boreal Continental: Chromosome 21 FET v. Fst (50kb non-overlapping window)
r = 0.9174144
Fst
FET (-log10(p-value))
Chromosomal Position


B
GLSL Atlantic v. GLSL Continental: Chromosome 21 FET v. Fst (50kb non-overlapping window)
r = 0.9310656
Fst
FET (-log10(p-value))
Chromosomal Position


A
Boreal Atlantic v. Boreal Continental: Chromosome 27 FET v. Fst (50kb non-overlapping window)
r = 0.9174144
Fst
FET (-log10(p-value))
Chromosomal Position


B
GLSL Atlantic v. GLSL Continental: Chromosome 27 FET v. Fst (50kb non-overlapping window)
r = 0.9310656
Fst
FET (-log10(p-value))
Chromosomal Position


A
Boreal Atlantic v. Boreal Continental: Chromosome 29 FET v. Fst (50kb non-overlapping window)
r = 0.9174144
Fst
FET (-log10(p-value))
Chromosomal Position


B
GLSL Atlantic v. GLSL Continental: Chromosome 29 FET v. Fst (50kb non-overlapping window)
r = 0.9310656
Fst
FET (-log10(p-value))
Chromosomal Position

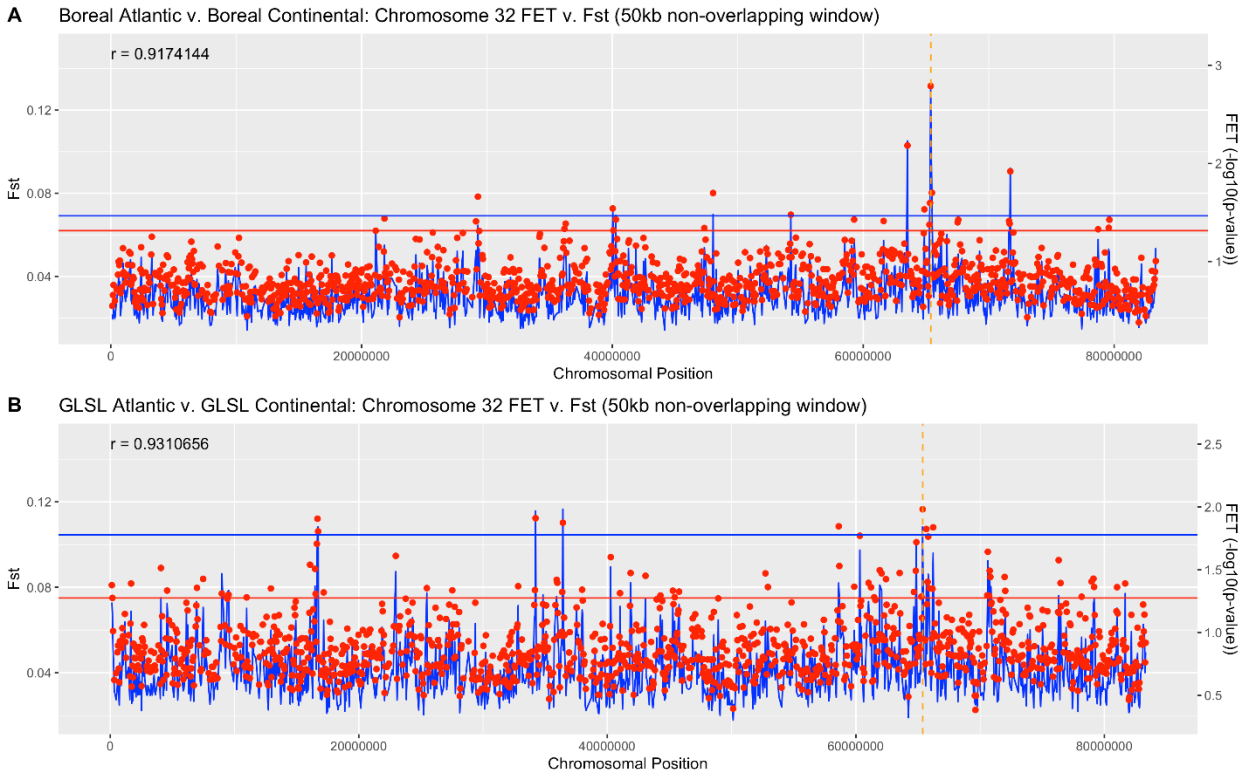
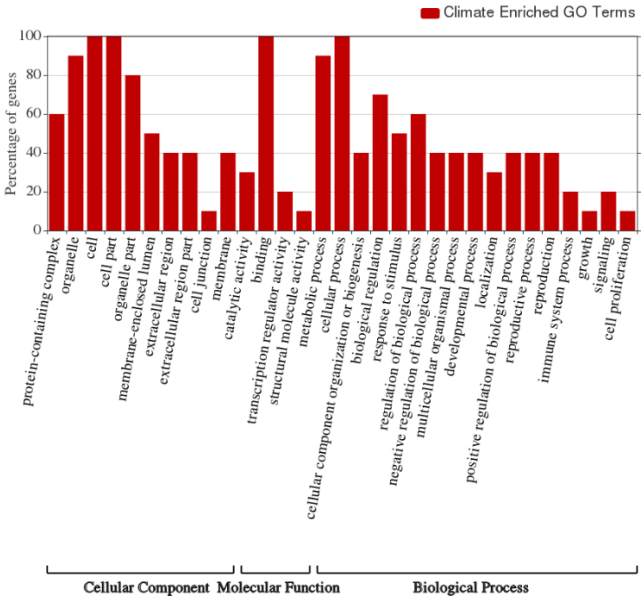


Figure S3. Manhattan plots indicating FET (red dots) and Fst (blue vertical lines) results across chromosomes that contain final outlier variants (location indicated by orange vertical dotted line) for the climate comparisons: (A) Boreal Atlantic versus Boreal Continental and (B) GLSL Atlantic versus GLSL Continental. Horizontal red lines indicate the log transformed p-value (right y axis) that corresponds to p=0.05 which we used as a filtering cutoff. Horizontal blue lines represent the 99.5th percentile of Fst results, which we also used as a filtering cutoff.

	FET		Fst	
	Climate	Forest	Climate	Forest
Total SNPs within outlier regions	13724	10791	2081	16606
Outlier SNPs (CMH test; p<.01, MAC=7)	5845	4714	1306	6461
50kb upstream	2316	1609	385	2337
50kb downstream	1147	753	260	1011
missense variants	15	21	7	15
chemical characteristic changes	8	11	5	8
consistent b/w FET & Fst	5	7	5	7

Table S1. SNPs identified within outlier FET and Fst outlier windows for climate and forest comparisons at progressive filtering steps. Rows indicate total SNPs within outlier regions, SNPs identified as significantly differentiated via CMH test, SNPs identified within 50kb of known coding regions via snpEff, number of SNPs whose codon product also results in a difference in chemical characteristic, and the final number of missense variants consistent between FET and Fst results.

a)



b)

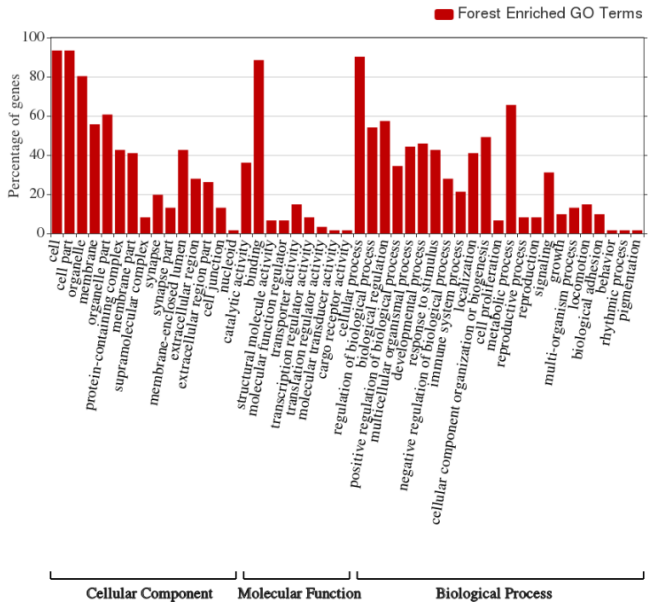


Figure S4. Bar plot highlighting GO terms that were enriched with identified outlier genes. Outlier genes were defined as any gene that contained a significantly differentiated SNP within a coding region, or within 50kb of a coding region. Results were obtained using GOWINDA and visualized using WEGO.

GENERAL DISCUSSION

Given current rates of global environmental change, driven by climate and land-use change (Hetem et al., 2014), understanding how wildlife populations interact with their surroundings are key in predicting responses to further change and what can be done to mitigate negative consequences (Blanchong et al., 2016; Morin et al., 2004). Large mammals are thought to be more vulnerable to environmental change given large spatial requirements and long generation times (Hetem et al., 2014; Thuiller et al., 2006). Yet, they can be logistically difficult to study appropriately on both temporal and spatial scales (Howe et al., 2013, 2022; Stokes et al., 2010). Genetic studies offer viable alternatives to traditional approaches to assess responses to environmental change, such as from radio telemetry or direct observation, providing inferences to underling ecological process shaping genetic structure where non-invasive samples can reflect generational changes in a cost-effective manner over appropriate spatial and temporal scales. In this thesis, microsatellite data and pooled whole-genome sequencing (pool-seq) were used to characterize patterns of gene flow directionality and functional genetic variation in Ontario black bear populations. Ontario was used as a study system given the climatic and environmental gradients that bears observe across this landscape to enhance understanding of underlying patterns influencing dispersal and local adaptation. These data were used to make inferences on how these populations may respond to continued ecological and climate changes towards enhancing conservation and management practices for this species. Further, these data provide insight as to how large mammals in general may/may not adapt to rapid ecological change.

Summary of Findings: Chapter 2 – Black Bear Dispersal

In my first data chapter, I aimed to further understanding of key factors influencing black bear dispersal. Specifically, I sought to assess the influence of population density, sex, harvest pressure, and food productivity gradients on dispersal patterns. To do so, I used microsatellite data to characterize patterns of gene flow directionality, using results in spatial models to test the influence of potential drivers of dispersal. Consistent with my hypotheses, I found black bear dispersal was positively density dependent (i.e., bears dispersed from areas of high density to areas of low density), male biased, and influenced by broad food productivity gradients. Although we hypothesized dispersal would be influenced by harvest pressures, we did not identify significant relationships between harvest density, nor harvest rate, and gene flow directionality. However, the area of the Ontario that is subject to some of the highest harvest pressures across the province also exhibits the highest genetic influx. These results reflect density-dependent source-sink dynamics. We speculate this pattern is driven by harvest pressures maintaining low bear density in a highly productive region of the province. Subsequently, bears moved from high density, less productive source regions towards this large population sink in central Ontario.

Numerous studies on dispersal in black bears have yielded contrasting results (Costello et al., 2008; Moore et al., 2014; Roy et al., 2012), potentially owing to smaller spatial extents sampled than what was assessed in this study, making results difficult to apply broadly. Our results remedy these inconsistences by sampling a large number of bears across a vast and heterogeneous landscape, effectively characterizing patterns of

dispersal and underlying drivers. Results have significant implications for management of large mammal populations, particularly those subject to high harvest pressures. Source-sink dynamics have been suggested in numerous wide-raining species (Andreasen et al., 2012; Jeke et al., 2019; Naranjo & Bodmer, 2007). In such systems, understating which sub-populations serve as sources and sinks is critically important for management efforts. Wildlife management traditionally monitors demographic rates; however, our study joins a growing body of research suggesting this can be misleading when source-sink dynamics are not considered (Naranjo & Bodmer, 2007; Novaro et al., 2005; Pulliam, 1988; Robinson et al., 2008a). Indeed, understanding patterns of source-sink dynamics are key for informed and sustainable population management where establishing large and regularly distributed source regions for hunted species may be more efficient than regulating harvest size based solely on demographic rates (Novaro et al., 2005). Based solely on population density, certain low-density regions of the Ontario black bear population may warrant reduced hunting allocations. However, our results suggest some of these low-density regions may be sustained by a continual influx of dispersing individuals. Therefore, ensuring the health of adjacent source populations may be a more effective and sustainable option.

Summary of Findings: Chapter 3 – Black Bear Functional Genetic Variation

In my second data chapter, I sought to better understand the capacity of a large mammal (American black bear) to demonstrate patterns of local adaptation across heterogeneous landscapes without significant barriers to dispersal. To do this, I used

pool-seq to characterize patterns of genetic variation between climate zones and between forest zones across Ontario, Canada. We expected this environmental variation would represent significant differences in habitat quality for black bears and would drive genetic differentiation in functionally relevant genes. Given the panmictic nature of the Ontario black bear population, we did not expect to see high levels of genetic differentiation between climate zones. This was largely true, as genome-wide patterns of differentiation based on Fisher's Exact Test (FET) and Fixation Index (Fst) were generally low. Yet, select regions of the genome were significantly differentiated between climate zones. I found outlier SNPs associated with genes related to cellular responses to starvation similar to findings among numerous hibernation studies (Federov et al., 2009; P. J. Lee et al., 2020; Yan et al., 2006; Yancey, 2018), suggesting balancing selection. Given known differences in precipitation (Hurrell, 1996; Hurrell et al., 2003) and annual vegetation synchrony (Howe et al., 2012) between Atlantic and continental climate zones in Ontario, we speculate that identified genetic variation relates to broad-scale differences in food productivity for black bears and thus different selective pressures on their ability to withstand periods of limited food. Adaptive mechanisms to withstand periods of limited food are fundamental for successful hibernation and reproduction in black bears (Srivastava et al., 2019; Toien et al., 2011). Food productivity differences between climate zone have not been quantified within our system, so exact mechanisms influencing observed genetic variation remain unclear.

We identified significant genetic differentiation between forest zones in our eastern comparison, but not in our western comparison. Differences between eastern and

western comparisons were likely due to the close geographic proximity of compared sample regions, in western Ontario, where gene flow was sufficiently high to dilute selective pressures. Additionally, the GLSL forest in western Ontario is located at higher latitudes compared to the GLSL forest in eastern Ontario. This is relevant as most outlier SNPs influenced genes relevant to cellular response to cold. Thus, both pools in western Ontario being located further north likely reduces differentiation in genes related to cold response. In our eastern forest comparison, differentiated genes related primarily to cellular responses to cold. Similar to our climate comparison, results of the forest comparison also reflect balancing dispersal. While we expected differences in food productivity would be the key selective pressure between forest zones (Mosnier et al., 2008), temperature variation along a latitudinal gradient better explains observed genetic variation in cold response genes. Influence of temperature on hibernation timing, duration, and other adaptive mechanisms, is well documented (Geiser, 2013; Hellgren, 1998; Toien et al., 2011). These data suggest that genetic variation in coding regions of cold response genes may serve to optimize hibernation, where it is necessary to withstand periods of prolonged cold and limited food availability in northern latitudes.

Variation in genes related to starvation response suggest that some bears in Ontario may be more resilient to food shortage than others. Therefore, bears who are less resilient may experience reduced fitness if ongoing climate and land-use change reduce food productivity in areas where food is currently plentiful. Black bears in central and northern Ontario do not have major barriers to dispersal and may be able to track

suitable habitats should food productivity decrease in any region. However, this is not the case for bears in southern latitudes, such as northern Mexico and southern United States, who experience increased habitat fragmentation and reduced dispersal capabilities (Costello, 2010; Dixon et al., 2007). In such cases, understanding which populations are more or less resilient to reduced food productivity, or any other selective pressure for that matter, can aid in relocation/restoration efforts where matching genotype/phenotype to environment can optimize relocation success (Weeks et al., 2011). Results of my adaptation study also indicate that some black bears in Ontario are more resilient to cold temperatures than others. Northern extents of the black bear range could extend further for these bears as temperatures drop within suitable ranges, as has been documented in numerous other mammal species (Williams & Blois, 2018).

Data obtained in this chapter demonstrate genetic variation reflective of balancing selection in a large mammal across a panmictic population. This suggests that local adaptation can occur when selective pressures across heterogeneous landscapes act on important life history traits, even when dispersal and subsequent gene flow are not limited. These data are important in context of environmental change and the influence it is projected to have on large mammal populations worldwide (Hetem et al., 2014). Understanding what key life history traits are being acted upon by selective pressures can be used to predict population viability if selective pressures change. Further, characterizing patterns of local adaptation can be used to optimize management efforts, such as relocation/re-introduction into fragmented populations, where matching

genotype and environment can promote development and maintenance of healthy wildlife populations (Zhao et al., 2013).

Conclusion

Genetic studies like the ones conducted herein are becoming increasingly valuable in elucidating how wildlife populations interact with each other, and their environments, across heterogeneous landscapes. Data obtained from such efforts can be used to predict responses to environmental change and can also be used in conjunction with demographic data to inform wildlife management practices. This is particularly true for large mammals that are frequent targets of wildlife management efforts as they are often more vulnerable to environmental change given long generation times and large spatial requirements (Gantchoff et al., 2020; Hetem et al., 2014).

My dispersal study indicates dispersal in black bears is complex and influenced by a range of factors. Identified source-sink dynamics suggest more complex mechanisms of dispersal than previously understood. Given emerging challenges in managing wildlife populations due to changing landscapes, understanding source-sink dynamics is of key importance, especially in harvested large mammal populations. In metapopulations exhibiting source-sink patterns, establishing designated source regions can be more effective than regulating harvest size based solely on demographic rates (Novaro et al., 2005). Additionally, results from my adaptation study suggests that balancing selection has driven variation in starvation response genes across the Ontario

black bear population. Should ongoing climate and land use change reduce food availability in areas where bears lack resilience to food shortages, that population may suffer negative concequences. Bears in our study system are not currently listed as an at-risk species and may be able to track suitable habitats given a lack of barriers to dispersal (Hetem et al., 2014). Thus, in context of climate and land-use change, these results have more significant implications for other black bear populations of conservation concern in southern latitudes who may be locally adapted but lack necessary dispersal and rapid adaptation capabilities required to withstand changes in selective pressures. This is true of large mammal populations in general, many of whom are facing similar situations (Hetem et al., 2014; Thuiller et al., 2006). Together, the studies conducted in this thesis indicate that black bears in our study system may be more susceptible to changing landscapes than previously thought due to being locally adapted. However, black bears in Ontario may be able to track suitable habitats that optimize fitness given high dispersal capabilities. Understanding patterns of dispersal and underlying drivers can be used to project likely range shifts and adjust management practices accordingly.

Limitations

While this thesis effectively characterized patterns of gene flow directionality and functional genetic variation in the Ontario black bear population, certain limitations exist. Some limitations can be attributed to differences in scale of metrics used in modelling genetic flux. Further limitations exist due to lacking data on phenotypes and

vegetation productivity that may help explain observed genetic variation in genes associated with starvation response.

In my dispersal study, I did not observe a relationship between gene flow directionality and harvest rate, or harvest density, although I speculate they are directly tied to bear density which demonstrates a negative relationship with genetic flux (i.e. low bear density results in high genetic influx). In this analysis, differences in scale when measuring genetic flux versus harvest may explain the lack of observed relationship between the two. Harvest was measured at the wildlife management unit (WMU) level where harvest data was collected by voluntary hunter reporting. Within these WMUs, there were often multiple study areas on which density was estimated, each having an independent genetic flux value. Harvest likely varies in nuanced ways within WMUs. Thus, measuring harvest on a broader scale relative to genetic flux represents a limitation that may explain the lack of observed relationship.

My pooled sequencing approach achieved high genome coverage and identified candidate genes likely experiencing balancing selection, but greater confidence in results could be achieved by employing joint genome scans of F_{ST}, d_{xy}, and π. Further, allele frequency estimates can be validated using individual-based methods such as qPCR. Pool-seq thus represents an important and valuable step in identifying candidate genes likely under selection but cannot be used as the sole means of addressing this complex issue. Further, I speculate as to what mechanisms are likely driving observed genetic variation. Although identified genes have obvious functional relevance to hibernation, the success of which directly influences reproductive success (Elowe &

Dodge, 1989), I cannot definitively say variation in identified genes work to optimize hibernation unless phenotypic differences and associated fitness consequences for missense variants are known. I also present different arguments regarding the way climate zones influence food productivity for black bears. While features such as increased snow depth and more frequent poor food years are documented in the Atlantic climate zone, I lack data on specific influences of vegetation synchrony and snow depth on food productivity for black bears. Ultimately, further investigation is required to determine whether the Atlantic or continental climate zone provides more optimal food resources for black bears and thus which bears have become more locally adapted to food shortages.

REFERENCES

Amarasekare, P. (2006). Productivity, dispersal and the coexistence of intraguild predators and prey. *Journal of Theoretical Biology*, *243*(1), 121–133.

Andrade-Restrepo, M., Champagnat, N., & Ferriere, R. (2019). Local adaptation, dispersal evolution, and the spatial eco-evolutionary dynamics of invasion. *Ecology Letters*, *22*(5), 767–777.

Andreasen, A. M., Stewart, K. M., Longland, W. S., Beckmann, J. P., & Forister, M. L. (2012). Identification of source-sink dynamics in mountain lions of the Great Basin. *Molecular Ecology*, *21*(23), 5689–5701. https://doi.org/10.1111/j.1365-294X.2012.05740.x

Andrews, K. R., Good, J. M., Miller, M. R., Luikart, G., & Hohenlohe, P. A. (2016). Harnessing the power of RADseq for ecological and evolutionary genomics. *Nature Reviews Genetics*, *17*(2), 81–92.

Baguette, M., Benton, T. G., & Bullock, J. M. (2012). *Dispersal ecology and evolution*. Oxford University Press.

Baguette, M., Clobert, J., & Schtickzelle, N. (2011). Metapopulation dynamics of the bog fritillary butterfly: experimental changes in habitat quality induced negative density-dependent dispersal. *Ecography*, *34*(1), 2011.

Blanchong, J. A., Robinson, S. J., Samuel, M. D., & Foster, J. T. (2016). Application of genetics and genomics to wildlife epidemiology. *Journal of Wildlife Management*, *80*(4), 593–608. https://doi.org/10.1002/jwmg.1064

Bonan, G. B., Pollard, D., & Thompson, S. L. (1992). Effects of boreal forest vegetation on global climate. *Nature*, *359*(6397), 716.

Boonstra, R., Dantzer, B., Delehanty, B., Fletcher, Q. E., & Sherif, M. J. (2014). Equipped for Life in the Boreal Forest : The Role of the Stress Axis in Mammals. *Arctic*, *67*, 82–97.

Costello, C. M. (2010). Estimates of dispersal and home-range fidelity in American black bears. *Journal of Mammalogy*, *91*(1), 116–121.

Costello, C. M., Creel, S. R., Kalinowski, S. T., Vu, N. v., & Quigley, H. B. (2008). Sex-biased natal dispersal and inbreeding avoidance in American black bears as revealed by spatial genetic analysis. *Molecular Ecology*, *17*(21), 4713–4723.

Coster, S. S., & Kovach, A. I. (2012). Anthropogenic influences on the spatial genetic structure of black bears. *Conservation Genetics*, *13*(5), 1247–1257. https://doi.org/10.1007/s10592-012-0368-4

de Bona, S., Bruneaux, M., Lee, A. E. G., Reznick, D. N., Bentzen, P., & López-Sepulcre, A. (2019). Spatio-temporal dynamics of density-dependent dispersal during a population colonisation. *Ecology Letters*, *22*(4), 634–644. https://doi.org/10.1111/ele.13205

Dixon, J. D., Oli, M. K., Wooten, M. C., Eason, T. H., McCown, J. W., & Cunningham, M. W. (2007). Genetic consequences of habitat fragmentation and loss: The case of the Florida black bear (Ursus americanus floridanus). *Conservation Genetics*, *8*(2), 455–464. https://doi.org/10.1007/s10592-006-9184-z

Dyer, R. J., Nason, J. D., & Garrick, R. C. (2010). Landscape modelling of gene flow: Improved power using conditional genetic distance derived from the topology of population networks. *Molecular Ecology*, *19*(17), 3746–3759. https://doi.org/10.1111/j.1365-294X.2010.04748.x

Elowe, K. D., & Dodge, W. E. (1989). Factors affecting black bear reproductive success and cub survival. *The Journal of Wildlife Managment*, *53*(4), 962–968.

Eriksson, A., Elías-Wolff, F., Mehlig, B., & Manica, A. (2014). The emergence of the rescue effect from explicit within and between-patch dynamics in a metapopulation. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1780). https://doi.org/10.1098/rspb.2013.3127

Feder, M. E., & Mitchell-olds, T. (2003). Evolutionary and ecological functional genomcis. *Nature Review Genetics*, *4*(8), 649–655.

Federov, V. B., Goropashnaya, A. v., Toien, O., Stewart, N. C., Gracey, A. Y., Chang, C., Qin, S., Pertea, G., Quackenbush, J., Showe, L. C., Showe, M. K., Boyer, B. B., & Barnes, B. M. (2009). Elevated expression of protein biosynthesis genes in liver and muscle of hibernating black bears. *Physiological Genomics*, *37*(2), 108–118.

Ferretti, L., Ramos-Onsins, S. E., & Pérez-Enciso, M. (2013). Population genomics from pool sequencing. *Molecular Ecology*, *22*(22), 5561–5576. https://doi.org/10.1111/mec.12522

Flanagan, S. P., Forester, B. R., Latch, E. K., Aitken, S. N., & Hoban, S. (2018). Guidelines for planning genmoic assessment and monitoring of locally adaptive variation to inform species conservation. *Evolutionary Applications*, *11*(7), 1035–1052.

Fuentes-Pardo, A. P., & Ruzzante, D. E. (2017). Whole-genome sequencing approaches for conservation biology: Advantages, limitations and practical recommendations. *Molecular Ecology*, *26*(20), 5369–5406. https://doi.org/10.1111/mec.14264

Gantchoff, M. G., Hill, J. E., Kellner, K. F., Fowler, N. L., Petroelje, T. R., Conlee, L., Beyer, D. E., & Belant, J. L. (2020). Mortality of a large wide-ranging mammal largely caused by anthropogenic activities. *Scientific Reports*, *10*(1), 1–11. https://doi.org/10.1038/s41598-020-65290-9

Geiser, F. (2013). Hibernation. *Current Biology*, *23*(5), R188–R193.

Hamala, T., & Savolainen, O. (2019). Genomic Patterns of Local Adaptation under Gene Flow in Arabidopsis lyrata. *Molecular Biology and Evolution*, *36*(11), 2557–2571.

Hawkins, B. A., Porter, E. E., & Alexandre, J. (2003). Productivity and history as predictors of the latitudinal diversity gradient of terrestrial birds. *Ecology*, *84*(6), 1608–1623.

Hellgren, E. C. (1998). Physiology of hibernation in bears. *Ursus*, *10*, 467–477.

Hetem, R. S., Fuller, A., Maloney, S. K., & Mitchell, D. (2014). Responses of large mammals to cliamte change. *Temperature*, *1*(2), 115–127.

Hohenlohe, P. A., Funk, C. W., & Rajora, O. P. (2020). Population genomics for wildlife conservation and managment. *Molecular Ecology*, *30*(1), 62–82.

Howe, E. J., Obbard, M. E., & Bowman, J. (2012). Prior reproduction and weather affect berry crops in central Ontario, Canada. *Population Ecology*, *54*(2), 347–356. https://doi.org/10.1007/s10144-011-0301-6

Howe, E. J., Obbard, M. E., & Kyle, C. J. (2013). Combining data from 43 standardized surveys to estimate densities of female American black bears by spatially explicit capture–recapture. *Population Ecology*, *55*, 595–607. https://doi.org/10.1007/s10144-013-0389-y

Howe, E. J., Potter, D., Beauclerc, K. B., Jackson, K. E., & Northrup, J. M. (2022). Estimating animal abundance at multiple scales by spatially explicit capture-recapture. *Ecological Applications*.

Hurrell, J. W. (1996). Influence of variations in extratropical wintertime teleconnections on Northern Hemisphere temperature. *Geophysical Research LEtters*, *23*(6), 665–668. https://doi.org/10.1029/96GL00459

Hurrell, J. W., Kushnir, Y., Ottersen, G., & Visbeck, M. (2003). An overview of the north atlantic oscillation. *Geophysical Monograph Series*, *134*(January), 1–35. https://doi.org/10.1029/134GM01

Ims, R. A., & Andreassen, H. P. (2005). Density-dependent dispersal and spatial population dynamics. *Proceedings of the Royal Society B: Biological Sciences*, *272*, 913–918.

Jeke, A., Chanyandura, A., Muposhi, V. K., Madhlamoto, D., & Gandiwa, E. (2019). Trophy Hunting and Possible Source-Sink Dynamics in Protected Areas: Insights from Trophy Size and Offtake Patterns in Southeast Zimbabwe. *International Journal of Zoology*, *2019*. https://doi.org/10.1155/2019/1313927

Johnson, R. N., O'Meally, D., Chen, Z., Etherington, G. J., Ho, S. Y. W., Nash, W. J., Grueber, C. E., Cheng, Y., Whittington, C. M., Dennison, S., Peel, E., Haerty, W., O'Neill, R. J., Colgan, D., Russell, T. L., Alquezar-Planas, D. E., Attenbrow, V., Bragg, J. G., Brandies, P. A., … Belov, K. (2018). Adaptation and conservation insights from the koala genome. *Nature Genetics*, *50*(8), 1102–1111. https://doi.org/10.1038/s41588-018-0153-5

Kawecki, T. J., & Ebert, D. (2004). Conceptual issues in local adaptation. *Ecology Letters*, *7*(12), 1225–1241. https://doi.org/10.1111/j.1461-0248.2004.00684.x

Koen, E. L., Bowman, J., & Wilson, P. J. (2016). Node-based measures of connectivity in genetic networks. *Molecular Ecology Resources*, *16*, 69–79.

Koenig, W. D., van Vuren, D., & Hooge, P. N. (1996). Detectability, philopatry, and the distribution of dispersal distances in vertebrates. *Trends in Ecology & Evolution*, *11*(12), 514–517.

Lariviere, S. (2001). Ursus amencanus,. *American Society of Mammalogists*, *647*, 1–11.

Lee, P. J., Cho, N., Yoo, H. M., & Kim, J. P. (2020). Active Turnover of Heme in Hibernation Period in Mammals. *Fronteirs in Physiology*, *10*, 1585.

Liu, C., Newell, G., White, M., & Bennett, A. F. (2018). Identifying wildlife corridors for the restoration of regional habitat connectivity: A multispecies approach and comparison of resistance surfaces. *PLoS One*, *13*(11), e0206071.

Luu, B. E., Wijenayake, S., Zhang, J., Tessier, S. N., Quintero-Galvis, J. F., Gaitan-Espitia, J. D., Nespolo, R. F., & Storey, K. B. (2018). Strategies of biochemical adaptation for hibernation in a South American marsupial Dromiciops gliroides: 1. Mitogen-activated protein kinases and the cell stress response. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *224*, 12–18.

Lv, F. H., Agha, S., Kantanen, J., Colli, L., Stucki, S., Kijas, J. W., Joost, S., Li, M. H., & Marsan, P. A. (2014). Adaptations to climate-mediated selective pressures in sheep. *Molecular Biology and Evolution*, *31*(12), 3324–3343. https://doi.org/10.1093/molbev/msu264

Matthysen, E. (2005). Density-dependant dispersal in birds and mammals. *Ecography*, *28*, 403–416.

McLaren, M., Dix-Gibson, L., Armstrong, T., Dawson, N., Obbard, M., Landriault, L., de Almeida, M., Dodwell, P., Black, R., Martin, G., & Radford, B. (2009). *An approach to assessment of harvested black bear populations in Ontario: Technical recommendations*.

Merilä, J., & Hendry, A. P. (2014). Climate change, adaptation, and phenotypic plasticity: The problem and the evidence. *Evolutionary Applications*, *7*(1), 1–14. https://doi.org/10.1111/eva.12137

Miller, S. D. (1990). Population Managment of Bears in North America. *Ursus*, *8*, 357–373.

Moore, J. A., Draheim, H. M., Etter, D., Winterstein, S., & Scribner, K. T. (2014). Application of large-scale parentage analysis for investigating natal dispersal in highly vagile vertebrates: A case study of American black bears (Ursus americanus). *PLoS ONE*, *9*(3). https://doi.org/10.1371/journal.pone.0091168

Morin, P. A., Luikart, G., & Wayne, R. K. (2004). SNPs in ecology, evolution and conservation. *Trends in Ecology and Evolution*, *19*(4), 208–216. https://doi.org/10.1016/j.tree.2004.01.009

Morozova, O., & Marra, M. A. (2008). Applications of next-generation sequencing technologies in functional genomics. *Genomics*, *92*(5), 255–264. https://doi.org/10.1016/j.ygeno.2008.07.001

Mosnier, A., Ouellet, J.-P., & Courtois, R. (2008). Black bear adaptation to low productivity in the boreal forest. *Ecoscience*, *15*(4), 485–497. https://doi.org/10.2980/15-4-3100

Muir, A. P., Biek, R., Thomas, R., & Mable, B. K. (2014). Local adaptation with high gene flow: temperature parameters drive adaptation to altitude in the common frog (Rana temporaria). *Molecular Ecology*, *23*(3), 561–574.

Naranjo, E. J., & Bodmer, R. E. (2007). Source-sink systems and conservation of hunted ungulates in the Lacandon Forest, Mexico. *Biological Conservation*, *138*(3–4), 412–420. https://doi.org/10.1016/j.biocon.2007.05.010

Ng, P. C., & Kirkness, E. F. (2010). *Whole Genome Sequencing*.

Novaro, A. J., Funes, M. C., & Walker, R. S. (2005). An empirical test of source-sink dynamics induced by hunting. *Journal of Applied Ecology*, *42*(5), 910–920.

Obbard, M. E., Coady, M. B., Pond, B. A., Schaefer, J. A., & Burrows, F. G. (2010). A distance-based analysis of habitat selection by american black bears (Ursus americanus) on the Bruce Peninsula, Ontario, Canada. *Canadian Journal of Zoology*, *88*(11), 1063–1076. https://doi.org/10.1139/Z10-072

Obbard, M. E., & Howe, E. J. (2014). *Ontario Wildlife Food Survey , 2014*. https://doi.org/10.13140/RG.2.1.1855.9522

Obbard, M. E., Howe, E. J., Wall, L. L., Allison, B., Black, R., Davis, P., Dix-gibson, L., Gatt, M., & Hall, M. N. (2014). Relationships among food availability , harvest , and human – bear conflict at landscape scales in Ontario , Canada. *Ursus*, *25*(2), 98–110. https://doi.org/10.2192/URSUS-D-13-00018.1

Obbard, M. E., Newton, E. J., Potter, D., Orton, A., Patterson, B. R., & Steinberg, B. D. (2017). Big enough for bears? American black bears at heightened risk of mortality during seasonal forays outside Algonquin Provincial Park, Ontario. *Ursus*, *28*(2), 182–194. https://doi.org/10.2192/ursu-d-16-00021.1

Paetkau, D., & Strobeck, C. (1994). Microsatellite analysis of genetic variation in black bear populations. *Molecular Ecology*, *3*(5), 489–495.

Pelletier, A., Obbard, M. E., Harnden, M., McConnell, S., Howe, E. J., Burrows, F. G., White, B. N., & Kyle, C. J. (2017). Determining causes of genetic isolation in a large carnivore (Ursus americanus) population to direct contemporary conservation measures. *PLoS ONE*, *12*(2), 1–23. https://doi.org/10.1371/journal.pone.0172319

Pelletier, A., Obbard, M. E., Mills, E. J., Burrows, F. G., White, B. N., & Kyle, C. J. (2012). Delineating genetic groupings in continuously distributed species across largely homogeneous landscapes: a study of American black bears (Ursus americanus) in Ontario, Canada. *Canadian Journal of Zoology*, *90*, 999–1014. https://doi.org/10.1139/Z2012-068

Poethke, H. J., & Hovestadt, T. (2001). Evolution of density- and patch-size-dependent dispersal rates. *Proceedings of the Royal Society of London. B: Biological Sciences*, *269*(1491), 637–645.

Pulliam, H. R. (1988). Sources , Sinks , and Population Regulation. *American Sociaety of Naturalists*, *132*(5), 652–661.

Qiu, Q., Zhang, G., Ma, T., Qian, W., Ye, Z., Cao, C., Hu, Q., Kim, J., Larkin, D. M., Auvil, L., Capitanu, B., Ma, J., Lewin, H. A., Qian, X., Lang, Y., Zhou, R., Wang, L., Wang, K., Xia, J., … Wang, J. (2012). The yak genome and adaptation to life at high altitude. *Nature Genetics*, *44*(8), 946–949. https://doi.org/10.1038/ng.2343

Rayfield, B., Fortin, M.-J., & Fall, A. (2011). Connectivity for conservation: a framework to classify network measures. *Ecology*, *92*(4), 847–858.

Reuter, J. A., Spacek, D. v., & Snyder, M. P. (2015). High-Throughput Sequencing Technologies. *Molecular Cell*, *58*(4), 586–597. https://doi.org/10.1016/j.molcel.2015.05.004

Robinson, H. S., Wielgus, R. B., Cooley, H. S., & Cooley, S. W. (2008). Sink populations in carnivore management: Cougar demography and immigration in a hunted population. *Ecological Applications*, *18*(4), 1028–1037. https://doi.org/10.1890/07-0352.1

Rogers, L. L. (1987). Factors influencing dispersal in the black bear. In *Mammalian dispersal patterns: the effects of social structure on population genetics* (pp. 75–84).

Ronce, O. (2007). How does it feel to be like a rolling stone? Ten questions about dispersal evolution. *Annual Review of Ecology, Evolution, and Systematics*, *38*, 231–253. https://doi.org/10.1146/annurev.ecolsys.38.091206.095611

Rowe, J. S. (1972). *Forest Regions of Canada*.

Roy, J., Yannic, G., Côté, S. D., & Bernatchez, L. (2012). Negative density-dependent dispersal in the American black bear (Ursus americanus) revealed by noninvasive sampling and genotyping. *Ecology and Evolution*, *2*(3), 525–537. https://doi.org/10.1002/ece3.207

Savolainen, O., Lascoux, M., & Merila, J. (2013). Ecological genomics of local adaptation. *Nature Reviews Genetics*, *14*, 807–820.

Sawaya, M. A., Kalinowski, S. T., & Clevenger, A. P. (2014). Genetic connectivity for two bear species at wildlife crossing structures in Banff National Park. *Proceedings of the Royal Society B: Biological Sciences*, *281*(1780). https://doi.org/10.1098/rspb.2013.1705

Scheick, B. K., & Mccown, W. (2014). Geographic distribution of American black bears in North America. *Ursus*, *25*(1), 24–33. https://www.jstor.org/stable/24643793

Schlötterer, C., Tobler, R., Kofler, R., & Nolte, V. (2014). Sequencing pools of individuals-mining genome-wide polymorphism data without big funding. *Nature Reviews Genetics*, *15*(11), 749–763. https://doi.org/10.1038/nrg3803

Shafer, A. B. A., Nielsen, S. E., Northrup, J. M., & Stenhouse, G. B. (2014). Linking genotype, ecotype, and phenotype in an intensively managed large carnivore. *Evolutionary Applications*, *7*(2), 301–312. https://doi.org/10.1111/eva.12122

Sheard, C., Neate-Clegg, M. H. C., Alioravainen, N., Jones, S. E. I., Vincent, C., MacGregor, H. E. A., Bregman, T. P., Claramunt, S., & Tobias, J. A. (2020). Ecological drivers of global gradients in avian dispersal inferred from wing morphology. *Nature Communications*, *11*(1). https://doi.org/10.1038/s41467-020-16313-6

Srivastava, A., Kumar Sarsani, V., Fiddes, I., Sheehan, S. M., Seger, R. L., Barter, M. E., Neptune-Bear, S., Lindqvist, C., & Korstanje, R. (2019). Genome assembly and gene expression in the American black bear provides new insights into the renal response to hibernation. *DNA Research*, *26*(1), 37–44. https://doi.org/10.1093/dnares/dsy036

Stapley, J., Reger, J., Feulner, P. G. D., Smadja, C., Galindo, J., Ekblom, R., Bennison, C., Ball, A. D., Beckerman, A. P., & Slate, J. (2010). Adaptation genomics: The next generation. *Trends in Ecology and Evolution*, *25*(12), 705–712. https://doi.org/10.1016/j.tree.2010.09.002

Stark, R., Grzelak, M., & Hadfield, J. (2019). RNA sequencing: the teenage years. *Nature Reviews Genetics*. https://doi.org/10.1038/s41576-019-0150-2

Stokes, E. J., Strindberg, S., Bakabana, P. C., Elkan, P. W., Lyenguet, F. C., Madzoke, B., Malanda, G. A. F., Mowawa, B. S., Moukoumbou, C., Ouakabadio, F. K., & Rainey, H. J. (2010). Monitoring Great Ape and Elephant Abundance at Large Spatial Scales: Measuring Effectiveness of a Conersvation Landscape. *PloS One*, *5*(4), e10294.

Thuiller, W., Broennimann, O., Hughes, G., Robert, J., Alkemande, M., Midgley, G. F., & Corsi, F. (2006). Vulnerability of African mammals to anthropogenic climate change under conservative land transformation assumptions. *Global Change Biology*, *12*(3), 424–440.

Toien, O., Blake, J., Edgar, D. M., Grahn, D. A., Heller, C., & Barnes, B. M. (2011). Hibernation in Black Bears: Independence of Metabolic Supression from Body Temperature. *Science*, *331*(6019), 906–909.

Travis, J. M. J., Murrell, D. J., & Dytham, C. (1999). The evolution of density dependent dispersal. *Proceedings of the Royal Society of London. B: Biological Sciences*, *266*(1432), 1837–1842.

Valladares, F., Matesanz, S., Guilhaumon, F., Araújo, M. B., Balaguer, L., Benito-Garzón, M., Cornwell, W., Gianoli, E., van Kleunen, M., Naya, D. E., Nicotra, A. B., Poorter, H., & Zavala, M. A. (2014). The effects of phenotypic plasticity and local adaptation on forecasts of species range shifts under climate change. *Ecology Letters*, *17*(11), 1351–1364. https://doi.org/10.1111/ele.12348

Weeks, A. R., Sgro, C. M., Young, A. G., Frankham, R., Mitchell, N. J., Miller, K. A., Byrne, M., Coates, D. J., Eldridge, M. D. B., Sannucks, P., Breed, M. F., James, E. A., & Hoffmann, A. A. (2011). Assessing the benefits and risks of translocations in changing environments: a genetic perspective. *Evolutionary Applications*, *4*(6), 709–725.

Whittle, A. J. (2009). Florida panther and black bear: a road and urban avoidance/utilization analysis and impacts of land use and climate change on large carnivore habitat in Florida. In *University of Kentucky*. https://uknowledge.uky.edu/gradschool_theses/618%0Ahttp://uknowledge.uky.edu/gradschool_theses/618

Williams, J. E., & Blois, J. L. (2018). Range shifts in response to past and future climate change: Can climate velocities and species' dispersal capabilities explain variation in mammalian range shifts? *Journal of Biogeography*, *45*(9), 2175–2189.

Yan, J., Burman, A., Showe, L. C., Showe, M. K., Boyer, B. B., Barnes, B. M., & Marr, T. G. (2006). Detection of differential gene expression in brown adipose tissue of hibernating ground squirrels with mouse microarrays. *Physiological Genomics*, *25*(2), 346–353.

Yancey, K. L. (2018). *Shining light on hibernator genomes: using radiation to reveal DNA damage and repair dynamics in Arctic ground squirrels*.

Zhao, S., Zheng, P., Dong, S., Zhan, X., Wu, Q., Guo, X., Hu, Y., He, W., Zhang, S., Fan, W., Zhu, L., Li, D., Zhang, X., Chen, Q., Zhang, H., Zhang, Z., Jin, X., Zhang, J., Yang, H., … Wei, F. (2013). Whole-genome sequencing of giant pandas provides insights into demographic history and local adaptation. *Nature Genetics*, *45*(1), 67–71. https://doi.org/10.1038/ng.2494