Genetic, Genomic and Diet Diversity: Insight into the Persistence and Vulnerability of Muskoxen (*Ovibos moschatus*)

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Abstract

Genetic, Genomic and Diet Diversity: Insight into the Persistence and Vulnerability of Muskoxen (*Ovibos moschatus*)

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Molecular and genomic tools provide a deeper understanding of the ecology and evolution of species and their capacity to adapt to changing selective pressures, where diversity is presumedly linked to higher fitness and evolutionary potential. Molecular tools can also illustrate how historical processes affect contemporary genetic variation to predict how current population trends may influence future genetic diversity. Genomic investigations increasingly extend beyond variation within host genomes to include diversity of their associated microbiomes, recognized to influence host/environment interactions and adaptation.

Muskoxen (*Ovibos moschatus*) are iconic, Arctic herbivores of ecological, economic, and cultural significance. Demographically, most mainland muskox populations have remained stable or grown over the last century, yet the biggest herds, found on Victoria and Banks Islands (Nunavut and the Northwest Territories, Canada) have experienced recent and drastic population declines. These Arctic island population declines have been associated with warming trends leading to shifting ranges of forage biodiversity, and pathogen expansions directly associated with increased mortality. Genomic investigations have the potential to enhance understanding of these contrasting trends and the adaptive capacities of muskox to cope with rapid ecological change.

In this thesis, I assess genetic, genomic, microbiome and diet diversity to better understand the ecology, and evolution of muskoxen. I found extremely low levels of genetic variation associated with population bottlenecks coinciding with major glaciation events and contemporarily low levels of gene flow among populations. Whole genome analyses identified signatures of selection between muskox populations, providing a genetic basis for the

divergence of two previously proposed muskox subspecies. Significant differences in diversity, effective population size and inbreeding among subspecies suggests animals from Arctic islands and Greenland are more vulnerable to environmental change. Molecular investigations of diet and microbiome diversity reflected unique capacities of muskoxen to survive on high-fiber forage and exploit shifts in Arctic vegetation that may include continued shrubification. Overall, these data provide insight into the complex relationship between genetic diversity and changing environments, setting a foundation for expanded future investigations of muskox seeking to promote the future viability of this species.

Keywords: Muskox, *Ovibos moschatus*, Genome Assembly, Conservation, Arctic, Genetic Diversity, Microbiome, Metabarcoding, Microsatellites, Resequencing, Persistence.

Preface

This thesis has been written in manuscript format. Chapter 2 was published in the *Biological Journal of the Linnean Society*, Chapter 3 was published in *Genes,* Chapter 4 is *in prep* for submission, and Chapter 5 was published in *Ecology and Evolution*.

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List of Abbreviations

Abbreviation	Definition
AR	Allelic Richness
BOLD	Barcode of Life Database
BWA	Burrows-Wheeler Alignment
COI	Cytochrome C Oxidase Subunit 1
dN/dS	Non-synonymous to Synonymous Substitution Rate
ENM	Ecological Niche Model
EVM	EVidence Modeler
F_{is}	Coefficient of Inbreeding
FROH	Run of Homozygosity Based Coefficient of Inbreeding
GEA	Genotype-Environment Associations
GO	Gene Ontology
H_O	Observed Heterozygosity
H_E	Expected Heterozygosity
HTS	High Throughput Sequencing
HRR	Heterozygous Rich Region
ITS	Internal Transcribed Spacer Region
IUCN	International Union for Conservation of Nature
Kya	Thousand Years Ago
LnPd	Raw Posterior Likelihood
LGM	Last Glacial Maximum
mtDNA	Mitochondrial DNA
Mya	Million Years Ago
NA	Number of Alleles

N_c	Census Population Size
NCBI	National Centre for Biotechnology Information
N_E	Effective Population Size
N_E/N_c	Ratio of Effective Population Size and Census Population Size
PCR	Polymerase Chain Reaction
P_{ID}	Probability of Identity
P_{sib}	Probability of Identify Between Siblings
PSMC	Pairwise Sequentially Markovian Coalescent
QIIME2	Quantitative Insights into Microbial Ecology 2
RBCL	Ribulose 1,5-Biphosphate Carboxylase Region
ROH	Runs of Homozygosity
SMM	Stepwise Mutation Model
SNP	Single Nucleotide Polymorphism
TPM	Two Phased Model
TRNL	P6 Loop of the Chloroplast trnl UAA Intron Region
UCE	Ultra Conserved Region
$\theta\pi$	Nucleotide Diversity

Chapter 1: General Introduction

There is a general recognition that biodiversity is being lost across the globe at an alarming rate (Erdelen, 2020; Fernández-Palacios et al., 2021; Raven & Wackernagel, 2020). Biodiversity losses extend beyond species extinctions and include processes such as local extirpations from demographic declines (Palombo, 2021; Sandor et al., 2022). As local genetic variation is often associated with adaptation and the capacity to respond to an array of changing selective pressures, there is a need to preserve not only species, but also local populations and their often unique genetic attributes (Fraser & Bernatchez, 2001; Mable, 2019). A spectrum of direct and indirect factors like habitat loss or exploitation, climate variability, and globalisation can affect biodiversity through their impact on species viability, fecundity, dispersal patterns and ecosystem dynamics (Blaustein et al., 2010; Johnston et al., 2019; Muluneh, 2021; Weiskopf et al., 2019, 2020; Williams & Newbold, 2020). Climate change in the form of environmental warming has been acknowledged as a driver of biodiversity loss, through its directs effects that include heat stress, immunosuppression, and extreme weather events (Blaustein et al., 2010; Coristine & Kerr, 2011; Weiskopf et al., 2019, 2020; Williams & Newbold, 2020). Indirectly, environmental warming has also disrupted interspecific interactions, for example, through the spread of invasive species and disease (Blaustein et al., 2010; Coristine & Kerr, 2011; Weiskopf et al., 2019, 2020; Williams & Newbold, 2020). While all these factors can have deleterious effects on native species, their responses to environmental change are variable and dependant on species-specific adaptive mechanisms (Bernatchez, 2016; Catullo et al., 2019).

1.1 Responses to Change

Understanding how populations respond to rapid changes in their environment has become a topic of great interest in ecology as it pertains to species survival and conservation (Bernatchez, 2016; Fox et al., 2019). Identifying mechanisms that allow populations to adapt to novel or

changing stressors can provide insight into the sustainability of these populations under future conditions (Johnston et al., 2019). When faced with new environmental pressures, species may adopt several adaptive mechanisms, including behavioural and/or phenotypic plasticity, and evolutionary adaptation (Bernatchez, 2016; Catullo et al., 2019; Ghalambor et al., 2007; Romero-Mujalli et al., 2021).

Behavioural plasticity is the ability of an individual, population or species to change their behaviour across different environmental conditions or stimuli, thereby reducing their exposure to stressors (Hall & Chalfoun, 2019). Due to the speed at which behavioural alterations can be made, behavioural flexibility is often the first line of defense in responding to variable environments and critical for survival (Gabor et al., 2022; Refsnider & Janzen, 2012). Behavioural adaptation includes range shifts, whereby populations will migrate to track their ideal climatic niches, such as higher latitudes or elevations (Atkins & Travis, 2010; Hetem et al., 2014; Parmesan, 2006; Romero-Mujalli et al., 2021; Rubenstein et al., 2023). Migration as an adaptive mechanism, however, requires that suitable habitats are accessible, or within reasonable traveling distances (Hetem et al., 2014). For example, over the last decade, scimitar horned oryx (*Oryx dammah*) have become extinct in their natural habitat as a consequence of climate change, where reaching a suitable environment would have required emigrations of a travel thousands of kilometers; a distance that would have been impossible without human assistance (Hetem et al., 2014). While species translocations and migrations may sometimes be suitable, when species move to environments that they do not naturally inhabit, they can alter or challenge the stability of local populations by introducing new diseases, as well as competing for resources with native species (Hoffmann, 2010; Rubenstein et al., 2023). Behavioural plasticity can also minimize the negative impact of new environmental pressures when populations maintain their geographic distribution, despite environmental disturbances (Hetem et al., 2014;

Wolff et al., 2020). For instance, white-tailed deer (*Odocoileus virginianus*) have taken advantage of spatial and temporal variations in temperature, to reduce the potential of heat stress by favouring forage in shaded areas, or by foraging at night (Wolff et al., 2020).

Phenotypic plasticity is another adaptive mechanism than can allow for trait modification or variation within an individual's lifetime (Diamond & Martin, 2020; Fox et al., 2019). Phenotypic plasticity is the ability for organisms to display variable phenotypes in response to different environmental conditions (Bernatchez, 2016; Fox et al., 2019; Ghalambor et al., 2007; Valladares et al., 2014). For instance, body mass declines are expected to occur in response to environmental warming, as a smaller body mass is associated with increased heat loss (Hetem et al., 2014). Such patterns have been observed in Soay sheep (*Ovis aries*), with documented declines in body mass over the past twenty years reaching 0.3% per year in adults (Hetem et al., 2014). Phenotypic plasticity in response to climate change is also evident in the timing of seasonal life events (Boutin & Lane, 2014; Parmesan, 2006). This is illustrated by the change in vegetation phenology brought on by warmer springs, resulting in the earlier emergence of hibernating ground squirrels (*Urocitellus paryii*), as well as earlier rutting, calving and antler casting in reindeer (*Rangifer tarandus;* Boutin & Lane, 2014). While phenotypic plasticity may allow individuals or species to have higher fitness in new or changing environments, it can also shield populations from natural selection, thereby undermining evolutionary adaptation in the long term (Fox et al., 2019; Ghalambor et al., 2007).

Evolutionary adaptation occurs over generations, where selection acts on populations, increasing frequencies of favourable genotypes or phenotypes (Charlesworth et al., 2017; Frankham et al., 2010). These adaptive changes can help reduce a population's genetic load (defined herein as the amount of deleterious variation, or less optimal genotypes, that result in the loss of individual and population fitness), which is often necessary for long term population

survival (Catullo et al., 2019; Dussex et al., 2023; Flanagan et al., 2017; Forester et al., 2022). For example, Tibetan yaks (*Bos grunniens*) live in high altitude environments, facing low oxygen availability (Qiu et al., 2012). As such, there is evidence of selection in yak genes involved in hypoxia responses, as well as increased hemoglobin concentrations (Qiu et al., 2012). When selective pressures vary over space and time, species will be comprised of diverging populations, each locally adapted to their respective environmental conditions (Fernandes et al., 2019; Valladares et al., 2014; Zimmerman et al., 2023). This, in turn, leads to a mosaic of adaptations across their habitat range (Fernandes et al., 2019). The historical conditions to which many populations have become locally adapted, however, are rapidly changing. As a result, extant adaptations may no longer improve fitness and may even result in maladaptation (Diamond & Martin, 2020; Forester et al., 2022; Magnan et al., 2016). For example, ribwort plantain (*Plantago lanceolata*) plants became more regionally abundant with the introduction of cattle ranching (Diamond & Martin, 2020). Accordingly, checkerspot butterflies (*Euphydryas editha*) evolved to exclusively use ribwort plantain plants for larval growth and development (Diamond & Martin, 2020). Once cattle ranching was discontinued, there was a resurgence in the growth of long grasses that led to ground level cooling and eliminated areas near the ribwort plantain plants where thermophilic butterfly larvae could bask in sunlight (Diamond & Martin, 2020). The loss of these areas led to the extirpation of the checkerspot butterfly population (Diamond & Martin, 2020). However, in order for natural selection to occur when exposed to new selective pressures, beneficial genotypes must already exist in the population. As such, a species' adaptive capacity is affected by its level of genetic variation (Parmesan, 2006; Torda & Quigley, 2022; Zimmerman et al., 2023).

1.2 Genetic Diversity as a Metric for Population Health and Vulnerability

Genetic variation is an important metric of population health and vulnerability, with increased genetic diversity associated with improved population fitness and evolutionary potential (Forester et al., 2022; Fox et al., 2019; Hoban et al., 2021; Hoffmann, 2010; Kardos et al., 2021; Zimmerman et al., 2023). Increased genetic diversity has been found to affect population productivity and resilience (Forester et al., 2022; Gibson, 2022; Hoban et al., 2021; Kardos et al., 2021; Zimmerman et al., 2023). Influences of diversity have been extensively studied in agriculture, where low crop diversity has been found to increase the rate of disease transmission and reduce crop yield, with crop diversification efforts made to counter the variation lost from monoculture practices over the past century (Gibson, 2022; Hoban et al., 2021; Hughes et al., 2008; Khoury et al., 2022; Lin, 2011). The effects of genetic variation on population health have also been studied in diverse wildlife species, such as honey bees (genus *Apis*), where genetically diverse colonies were associated with increased foraging, food storage, colony growth, and survival (Mattila & Seeley, 2007). Greater variability increases the chance that allelic variation required for evolution via natural selection exists and may allow populations to respond faster to environmental change (Parmesan, 2006; Torda & Quigley, 2022; Zimmerman et al., 2023). For example, standing genetic variation in marine mussels (*Mytilus galloprovincialis*) appears to expedite adaptation to ocean acidification, as genotypes that improve fitness in future ocean conditions were present in the existing population (Bitter et al., 2019). Increasing a population's genetic diversity has become a common conservation strategy, where introducing new variation to a population can improve fitness (Barmentlo et al., 2018; Teixeira & Huber, 2021). While mixing populations can lead to outbreeding depression, or a loss in fitness in mixed offspring, increasing diversity nearly always increases viability, growth and survival in changing environments (Houde et al., 2011; Teixeira & Huber, 2021).

Maintenance of genetic diversity is not only important at the individual, population, or species level, but also for the structure and function of ecosystems (Crutsinger et al., 2006; Hughes & Stachowicz, 2004). Increased genetic diversity within keystone species, or species with important functional roles in an environment, has been found to improve ecosystem health and overall adaptive capacity (Crutsinger et al., 2006; Hughes & Stachowicz, 2004). Eelgrass (*Zostera marina*) with increased genetic diversity, recovered more quickly from environmental disturbances, and had improved post disturbance shoot densities (Hughes & Stachowicz, 2004). Correspondingly, animal abundance was higher in plots with more diverse eelgrass, as increased shoot density allowed for improved grazing and protection from predators (Hughes & Stachowicz, 2004). Similarly, genetic diversity in tall goldenrod (*Solidago altissima*), was positively correlated with plant productivity and resource availability for arthropod species (Crutsinger et al., 2006). As a result, plots with more diverse tall goldenrod plants had higher arthropod abundance and richness, demonstrating the effect that intraspecific variation can have on interspecific diversity (Crutsinger et al., 2006). Loss of community biodiversity can also have negative impacts on ecosystem health. Declines in shark populations have been shown to disturb community balance, by increasing the abundance of shark prey like cownose rays (*Rhinoptera bonasus;* Somaweera et al. 2020; Heithaus et al. 2008). Rapidly expanding ray populations resulted in the extirpation of local bay scallops, demonstrating that the loss of top predators can cause declines in important resource species, and impact several trophic levels (Heithaus et al., 2008). At the individual level, microbiota biodiversity has been found to influence host immunity, metabolism, and adaptive plasticity (Bello et al., 2018). Specifically, decreases in gut microbiome diversity have been associated with human diseases including obesity, and type 2 diabetes (Bello et al., 2018; Deng et al., 2019).

Populations exhibiting low or declining genetic variation are assumed to warrant conservation concern (Markert et al., 2010; Willoughby et al., 2015; Zimmerman et al., 2023). Willoughby et al. (2015) explored over 5000 published articles to assess if genetic diversity could be correlated with conservation status and found threatened species had lower genetic diversity than those of lesser concern. As a reduction in genetic diversity can negatively impact ecosystem resilience and a species' evolutionary potential, monitoring changes in variation can help identify vulnerable populations. While variation is generally accepted to play an important role in population fitness, there are species and populations that persist despite extremely low levels of diversity (Benazzo et al., 2017; Milot et al., 2007; Paetkau et al., 1998; Robinson et al., 2016).

Several studies have demonstrated that species with low genetic diversity and longer generation times can survive, and sometimes thrive, despite conservation theory suggesting otherwise (Benazzo et al., 2017; Milot et al., 2007; Paetkau et al., 1998; Robinson et al., 2016; Teixeira & Huber, 2021; Yates et al., 2019). Cheetahs (*Acinonyx jubatus*), despite having low genetic diversity, show no evidence of increased disease impact when compared to other wild carnivores (Mable, 2019). Critically endangered species like the Apennine bear (*Ursos arctos marsicanus*) and the Channel Island fox (*Urocyon littoralis*) contradict the assumption that low genetic variation compromises sustainability, as they have persisted in spite of small population sizes and low genetic variation (Benazzo et al., 2017; Robinson et al., 2016). Kodiak bears (*Ursos arctos middendorffi*) were found to have genetic diversity estimates lower than other brown bears, as well as many critically endangered species, and yet are known for their large body size, high population densities, and productivity (Paetkau et al., 1998). Long term survival with low genetic diversity has also been demonstrated in albatrosses (*Diomedea exulans*), which have thrived for millions of years despite being long lived species with extremely low diversity (Milot

et al., 2007). In some instances, the manner or rate of diversity declines can have a role on subsequent population health as seen in endangered mountain gorillas (*Gorilla beringei beringei*; van der Valk et al., 2019). Although mountain gorillas have likely had small population sizes for the past ~10 thousand years, they do not exhibit signs of negative genomic consequences, like increased genetic load or inbreeding, which has been attributed, in part, to gradual population declines (van der Valk et al., 2019). As such, determining what is a sufficient or healthy level of genetic variation may not only be dependant how much genetic variation is present, but also the ecological, evolutionary, and demographic forces that have shaped it (Kardos et al., 2021; Lawton-Rauh, 2008; Mable, 2019; Teixeira & Huber, 2021; Yates et al., 2019; Zimmerman et al., 2023).

1.3 Drivers of Genetic Diversity and Population Vulnerability

Levels and distribution of genetic variability are dependent on the rate of mutations, genetic drift, natural selection, and gene flow occurring within and between populations (Hughes et al., 2008; Slatkin, 1987; Torda & Quigley, 2022; Wright, 1946; Zimmerman et al., 2023). Mutation, or the alteration of a DNA sequence, is the source of all variation, and typically originates from random errors during DNA replication (Brown, 2002; Frankham et al., 2010). As the average mutation rate in mammals is 2.2×10^{-9} per base pair, per year, regenerating genetic diversity can take thousands of generations (Frankham et al., 2010; Kumar & Subramanian, 2002). Mutations can, in rare instances, produce beneficial phenotypes but are more likely to be deleterious or neutral, resulting in invisible phenotypes (Ghani et al., 2022). In contrast to mutation, genetic drift is the random change in allelic frequencies which results in the loss of rare alleles and genetic diversity (Frankham et al., 2010; Willi et al., 2022). Over time, drift can lead to the eventual fixation of alleles (Willi et al., 2022). Allelic frequencies can also move towards fixation via natural selection (Fernandes et al., 2019; Valladares et al., 2014;

Zimmerman et al., 2023). Where natural selection is directional, allele frequencies will shift in favour of a beneficial genotype, reducing genetic diversity, though this is typically advantageous to population fitness (Mäkinen et al., 2008). Purifying selection can also lower genetic diversity through the purging of deleterious alleles; however, as this decreases genetic load, it can also be beneficial to population health (Cvijović et al., 2018; Dussex et al., 2023; Pečnerová et al., 2024). Alternatively, balancing selection can occur, maintaining high diversity and heterozygosity longer than would be expected in a population, as is often seen in immune genes (Koenig et al., 2019). Diversity may also be introduced and maintained in a population through gene flow, whereby individuals from different populations will interact and reproduce, transferring genetic information from one population to another (Forester et al., 2022; Frankham et al., 2010). This transfer of genetic information can undermine any previous losses of variation, by introducing new genotypes to a population (Forester et al., 2022; Willi et al., 2022).

The impact that genetic diversity has on population vulnerability is driven by demographic and population processes, such as effective population size and inbreeding. Effective population size (N_E) is the size of an ideal population experiencing the same rates of genetic change as a population of interest (Beissinger & McCullough, 2002; Palstra & Ruzzante, 2008; Willi et al., 2022). As natural populations typically do not exist under ideal conditions, effective population sizes are generally smaller than census population sizes (Palstra & Ruzzante, 2008; Willi et al., 2022). Effective population size is correlated to the strength of genetic drift, where a smaller N_E results in increased drift and faster fixation rates (Palstra & Ruzzante, 2008; Willi et al., 2022). Smaller N_E also results in weaker selection, and can reduce the rate that deleterious mutations will be purged from a population (Andrews et al., 2016; Pérez-Pereira et al., 2022; Willi et al., 2022). Therefore, a low N_E can be indicative that a population is genetically compromised as it results in an increased fixation of deleterious alleles and reduced viability and

fitness (Forester et al., 2022; Torda & Quigley, 2022; Willi et al., 2022). Classically, minimum effective population sizes have been proposed for long- and short-term maintenance of endangered species (Frankham et al., 2014; Pérez-Pereira et al., 2022). A minimum N_E of 500 is thought to be required to maintain evolutionary potential, and any lower is indicative of poor long-term survival (Frankham et al., 2014; Pérez-Pereira et al., 2022). Alternatively, effective population sizes below 50 are likely to negatively impact short term viability due to inbreeding depression (Frankham et al., 2014; Pérez-Pereira et al., 2022). Inbreeding is the mating between related individuals, resulting in offspring with increased homozygosity (Mable, 2019; Pekkala et al., 2014; Teixeira & Huber, 2021). Inbreeding depression occurs when inbreeding leads to increased mortality rates, and reduced reproductive success, fitness, and adaptive capacity (Forester et al., 2022; Hoban et al., 2021; Mable, 2019; Willi et al., 2022). In outbred populations, when deleterious alleles arise via mutation, they may be purged by selection or remain masked by heterozygosity (Zhu et al., 2022). When populations are highly inbred, however, these deleterious alleles are more likely to become homozygous and fixed (Teixeira & Huber, 2021; Wang et al., 1999). Inbreeding depression significantly contributes to a species' extinction risk, with mortality rates of inbred offspring increased by 30-40%, and fitness expected to decrease by as much as 50% (Willi et al., 2022).

While stochastic events, environmental conditions, evolutionary history, and mating systems can also affect population health, general predictions can be made as to how low variation affects population outcomes (Banks et al., 2013; Forester et al., 2022; Hoban et al., 2021; Markert et al., 2010). Environmental disturbances, such as climate change, habitat loss, and pollution, can result in population bottlenecks and fragmentation, reducing N_E (Banks et al., 2013; Forester et al., 2022; Hoban et al., 2021; Markert et al., 2010). Decreases in N_E lead to more pronounced drift, the accumulation of deleterious mutations, inbreeding depression and a

loss in adaptive potential, all of which limit long term population viability (Forester et al., 2022; Hoban et al., 2021; Markert et al., 2010). This then causes the population to become less stable, further reducing population size and, eventually, creating what is known as an extinction vortex (Forester et al., 2022; Markert et al., 2010).

Species persistence, despite low genetic diversity, high inbreeding and small effective population sizes challenges broadly accepted conservation paradigms, suggesting mechanisms may exist to allow for the continued survival of populations at risk (Benazzo et al., 2017; Robinson et al., 2016). In island populations, like Apennine bears and Channel Island foxes, a lack of gene flow and small population sizes have led to inbreeding, low diversity and increased homozygous deleterious alleles (Benazzo et al., 2017; Robinson et al., 2016). In these instances, however, their island environments are thought to be comparatively benign to that of the mainland, facing fewer competitors or predators, which allows them to tolerate higher genetic loads (Benazzo et al., 2017; Robinson et al., 2016). Behavioural mechanisms, like inbreeding avoidance, have been identified in species like the albatross, to prevent inbreeding depression and genomic erosion (Milot et al., 2007). Even in species with extremely low levels of genetic diversity, high diversity hotspots have been identified in functionally relevant regions, like the immune system in Apennine bears, and the olfactory receptor in island foxes (Benazzo et al., 2017; Robinson et al., 2016). Additionally, purifying selection can reduce a population's genetic load via the purging of deleterious alleles, which has been found to negate the detrimental effects of long term small N_E in mountain gorillas (van der Valk et al., 2019). Beyond the host, the gut microbiome has been found to change in response to seasonal vegetation in American bison (*Bison bison*), improving gut health and digestion (Bergmann et al., 2015). Adaptive mechanisms used by these low diversity species are not well understood and can be unique to each population of interest due to complex ecosystem dynamics. A growing wealth of molecular

tools and analyses have provided unique insights into assessing wildlife vulnerability and persistence in study species that have low genetic diversity (Hoban et al., 2021; Rehnus & Bollmann, 2016). These data can provide the knowledge necessary to accurately assess conservation statuses and create effective management strategies (Hoban et al., 2021).

1.4 Measuring Diversity as a Monitoring Tool

Conservation genetics employs molecular techniques to measure the distribution and levels of genetic variation in order to better preserve diversity, evolutionary potential, and reduce extinction risk (De Kort et al., 2021; Willi et al., 2022). Traditional conservation genetic measurements have relied on a small number of markers, like microsatellite genotyping and Sanger sequencing of shorter DNA regions, up to 800-1000bp in length (Gupta & Gupta, 2014; Hohenlohe et al., 2021; Hughes et al., 2008). Despite a limited number of loci, these data are able to provide insight into evolutionary processes, population history, and population demographics (Ouborg et al., 2010; Wenne, 2023). Genotyping small numbers of microsatellite markers can provide robust discrimination between individuals, identify population structure and inbreeding, and assess neutral genetic diversity (Wenne, 2023). Genotyping of 16 microsatellite markers in beetles, for example, identified evidence of population bottlenecks and informed optimal conservation strategies by measuring the relationship between genetic variation, population connectivity, effective population size and habitat (Kajtoch et al., 2014). Sequencing mitochondrial DNA (mtDNA) markers can also be relevant in creating phylogenetic trees, providing taxonomic clarity, and establishing conservation units (Mable, 2019). For example, sequencing of the mtDNA cytochrome c oxidase subunit 1 (COI) of Australian freshwater macroinvertebrates detected cryptic species, and biodiversity hotspots in streams likely to experience future environmental disturbances (Baker et al., 2004). As such, these

analyses helped to identify areas where conservation efforts should be prioritized in order to preserve community diversity (Baker et al., 2004).

With technological advancements, conservation genetics continues to shift towards using high throughput sequencing (HTS), that can measure diversity at thousands of loci at once (Hoban et al., 2021; Hohenlohe et al., 2021; Zimmerman et al., 2023). As the cost of HTS decreases, the number of non-model organism genomes have increased, aiding in the conservation of diverse wildlife species (Hoban et al., 2021; Hohenlohe et al., 2021; Hu et al., 2023; Kardos et al., 2021; Zimmerman et al., 2023). Access to whole genome data allows for the identification of single nucleotide polymorphisms (SNPs) across an organism's complete set of DNA. These genome-wide SNPs provide a better representation of neutral and functional genetic variation within individuals, populations, and species, as opposed to traditional markers (Bernatchez, 2016; Zimmerman et al., 2023). Genome-wide SNPs also yield a higher resolution in genetic assessments of population history, selection, adaptive potential, and demographic processes like gene flow, inbreeding, and genetic drift (Hohenlohe et al., 2021; Zimmerman et al., 2023). For example, historical changes in N_E can be assessed via pairwise sequentially Markovian coalescent (PSMC) analyses, and have allowed for the identification of population bottlenecks in diverse species including moose (*Alces alces*), red pandas (*Ailurus fulgens*) and ibis (*Nipponia nippon*; Dussex et al., 2020; Hu et al., 2020; S. Li et al., 2014).

HTS also allows for the assessment of community level diversity, informing ecology, evolution, and biodiversity (Pascher et al., 2022). Metabarcoding utilises high throughput sequencing and conserved markers to identify diverse taxa within the same sample (van der Loos & Nijland, 2021). Metabarcoding can assess bacterial, plant, and fungal communities and, when applied to different DNA sources, have a myriad of applications (Blabolil et al., 2021; Pascher et al., 2022). Sequencing of the 12S mtDNA region from environmental water samples

was used to reconstruct fish communities, improving understanding of freshwater biodiversity and ecosystem interactions (Blabolil et al., 2021). HTS of the 16S ribosomal RNA gene in bacteria has been used to assess bacterial diversity within an individual's digestive tract, also known as their gut microbiome, which plays an important role in gut health and digestion, and can help individuals adapt to a changing diet (Trevelline et al., 2019). Comparing the diversity of an individual's microbiome to that of their diet has been used to assess the capacity for individuals to rapidly adapt to changing food composition and availability (Li et al., 2016). Overall, conservation genetics tools, utilising both traditional and high throughput sequencing methods, provide insight into a species' vulnerability to rapid environmental change which can inform and help prioritize management actions (Hoban et al., 2021; Hohenlohe et al., 2021; Zimmerman et al., 2023).

1.5 Study System

1.5.1 Muskoxen Importance

Muskoxen are an iconic Arctic species that play important environmental, economic, nutritional, and cultural roles in Arctic Indigenous communities (Cuyler et al., 2019; Tomaselli, Gerlach, et al., 2018; Tomaselli, Kutz, et al., 2018). As one of the only large herbivore Arctic inhabitants, muskoxen contribute to soil health through nutrient and element turnover, which can affect plant growth (Mosbacher et al., 2016; Tomaselli, Gerlach, et al., 2018). Muskox foraging and grazing reduces nitrogen concentrations in the soil that is then redistributed in their feces and urine, fertilising plants, stimulating plant and microbial growth, and increasing herbivory (Mosbacher et al., 2016). Muskox harvesting, sport hunting, and processing, provide employment opportunities to local communities, while the sale of muskox by-products provide additional revenue and bolster economic development (Tomaselli, Gerlach, et al., 2018). Meat

harvested from muskoxen has historically and contemporarily been a reliable source of food and an important resource for local food security, especially when caribou abundances are low (Tomaselli, Gerlach, et al., 2018). Muskox by-products have traditionally contributed to the creation of clothing, tools, shelter, and the arts. Muskox bones have been fashioned into hunting, scraping and drilling tools, sled runners, bone arrows, and games for children, while art was created from their carved horns (Tomaselli, Gerlach, et al., 2018). Their hides and wool have been used to create highly insulated bedding, boots, and parkas (Tomaselli, Gerlach, et al., 2018). Beyond the aforementioned contributions, muskoxen have clear connections to the Indigenous community's cultural identity, and their presence carries deep spiritual meaning (Tomaselli, Gerlach, et al., 2018).

1.5.2 Muskox Population History

Muskoxen are the only living members of the *Ovibos* genus and, despite their name and appearance, are more closely related to sheep and goats than they are to oxen (Groves & Shields, 1997; Pasitschniak-Arts et al., 1992; Shafer & Hall, 2010). Muskoxen are thought to have originated in Eurasia and migrated to North America in the early Pleistocene (Campos et al., 2010; MacPhee et al., 2005). By the late Pleistocene, muskoxen had a Holarctic distribution (Campos et al., 2010; MacPhee et al., 2005). Between the Last Glacial Maximum (LGM) and the mid-Holocene, multiple population bottlenecks occurred, that also decreased the overall geographic range of muskoxen (Campos et al., 2010; Hansen et al., 2018; MacPhee et al., 2005). In the early 1900's, excessive harvesting and hunting led to the near extirpation of muskoxen from the Canadian mainland, Victoria Island and Banks Island (Northwest Territories and Nunavut; Barr, 1991). Nearly 50 years of harvesting moratoriums allowed for population recovery and expansion, with endemic muskox populations now found in the Canadian Arctic

mainland, the Arctic Archipelago, and north east Greenland (Barr, 1991; Gunn & Forchhammer, 2022). Global distributions of muskoxen have also grown with the translocation of muskoxen to Quebec (Canada), Alaska (USA), Western Greenland, Russia, and Norway (Gunn & Forchhammer, 2022). Currently, muskoxen are considered a species of least concern by the International Union for Conservation of Nature (IUCN) due to their abundance (estimated at 170,000 individuals; 71% endemic), and broad geographic distribution (Gunn & Forchhammer, 2022). Global muskox populations have been further classified into two subspecies distinguished by their size, colouring and most notably, their geographic location (Cuyler et al., 2019; Groves & Shields, 1997; Hansen et al., 2018). It should be noted that the IUCN does not recognize these subspecies given a lack of mtDNA genetic variation observed between groups; a factor that somewhat undermines their conservation status by pooling population sizes and not recognizing contrasting population trends observed among the subspecies (Groves, 1997). Barren-ground muskoxen (*Ovibos moschatus moschatus*) , found in the mainland of the Canadian Arctic, are larger in size, and their faces and saddles are brown in colour (Cuyler et al., 2019; Groves, 1997; Groves & Shields, 1997). White-faced muskoxen (*Ovibos moschatus wardii*) are smaller in size and have white wool colouring their faces and saddles (Cuyler et al., 2019; Groves & Shields, 1997). White-faced muskoxen are endemically found across the Arctic Archipelago and eastern Greenland, though all translocated muskox populations are also white-faced muskoxen (Cuyler et al., 2019; Gunn & Forchhammer, 2022). As such, white-faced muskoxen make up most of the overall muskox population at ~132 000 individuals, while only ~36 000 muskoxen are of the barren-ground subspecies (Figure 1.1; Cuyler et al. 2019; Gunn & Forchhammer 2022).

1.5.3 Muskox Adaptations

The Arctic is a harsh environment with extreme photoperiods of sustained light or dark, temperature lows reaching -60°C in the winter, limited vegetation, and severe weather events (Berger et al., 2018; Blix, 2016; Callaghan et al., 2004). These conditions generate low levels of biodiversity, where species that do inhabit the Arctic require a host of key adaptations. As ice age survivors and the largest Arctic herbivore, muskoxen have developed several adaptations in order to improve their fitness to the harsh Arctic environment (Blix, 2016; Flood et al., 1989; Klein, 1992; Li et al., 2023). Two of the main Arctic stressors affecting muskoxen are the extreme cold, and limited seasonal vegetation availability (Blix, 2016). The body morphology of muskoxen reflects an adaptation to cold, where short legs and a stout body shape reduce surface area and heat loss (Ytrehus et al., 2008). Muskoxen conserve energy by decreasing the temperature of their peripheral tissues, which helps prevent hypothermia (Ribeiro et al., 2019). Muskoxen also have a unique pelage that is a highly effective insulator (Flood et al., 1989; Klein, 1992). Muskox pelage is separated into a fine underwool called qiviut, alongside very long and coarse guard hairs (Flood et al., 1989). The ratio of underwool to guard hairs is extremely high (37:1), in comparison to other ruminants, with the nearest ratio at 20:1 in domestically enhanced merino sheep (*Ovis aries*; Flood et al., 1989). Muskoxen also have high follicle density, similar to highly selected breeds of wool-producing sheep; however, in muskoxen the pattern of coat growth allows for high physical activity in summer feeding and breeding seasons to reduce heat stress (Flood et al., 1989). In addition to their unique wool, muskoxen are insulated by means of a thick layer of subcutaneous fat (Ytrehus et al., 2008). Li et al. (2023) found that genes associated with brown adipose tissue were under selection in muskoxen. Given its high vascularity, brown adipose tissue is associated with increased heat production that can be more easily distributed to other bodily tissues, allowing for more efficient heat exchange (Li et al., 2023). Muskox

hemoglobin is also acclimated to the cold by reducing the amount of heat released when binding to oxygen (Brix et al., 1989, 1990). This reduces hemoglobin sensitivity to temperature and allows oxygen to be unloaded, despite low ambient temperatures (Brix et al., 1989, 1990). Muskoxen also have one of the lowest metabolic rates in ungulates, which can be seasonally altered (Adamczewski et al., 1994; Schmidt et al., 2020). Resting metabolic rates are reduced 50% in winter, allowing muskoxen to maintain as much energy reserves as possible from slow digestive passage rates and high digestive efficiency (Schmidt et al., 2020). Similarly, muskoxen can alter their rumen efficiency to account for different seasonal volumes of food intake (Ungerfeld et al., 2018). They do so by managing rumen pH, osmolality, and digesta flow rates, which in turn impacts the microbial activity of their microbiome (Ungerfeld et al., 2018). The muskox microbiome has been found to be made up mostly of *Firmicutes* and *Bacterioidetes* (Bird et al., 2019; Cheon et al., 2022), a microbiotic adaptation that aids in the digestion of fibre and allows muskox to niche eat highly fibrous forage, including large volumes of low-quality roughage are available in summer months (Bird et al., 2019; Cheon et al., 2022; Qi et al., 2011). The muskox microbiome has also been found to vary, based on geography, seasonality, and population genetic structure; this plasticity is thought to allow muskoxen to rapidly adapt to changing vegetation quality and quantities (Bird et al., 2019). However, as climate change is rapidly altering the Arctic environment, this may undermine muskox Arctic adaptations.

1.5.4 Muskox Population Drivers

Historically, population dynamics in muskoxen have mainly been driven by recruitment and were therefore most influenced by factors that impact calf and adult survival, and vital rates for calf births (Cuyler et al., 2019; Desforges et al., 2019; Eikelenboom et al., 2021; Schmidt et al., 2015, 2023). Muskoxen are capital breeders, meaning that for reproduction they rely upon the

resource reserves gained earlier in the year, rather than during the current breeding season (Gustine et al., 2010; Kerby & Post, 2013). As such, accessibility to forage in winter greatly affects muskox reproductive success, with snow cover being the dominant driver for muskox population sustainability (Desforges et al., 2019; Schmidt et al., 2015, 2023). Desforges et al. (2019) found that reproductive success and first year calf survival rates were impacted by snow depth and the length of the snow-covered season, where even small increases in snow depth resulted in population declines with long population recovery times. A reduction in accessible forage can deplete energy reserves in gestational females, resulting in increased abortions (Desforges et al., 2019; Schmidt et al., 2015, 2023). A lack of forage can also lead to reductions in the volume and quality of milk when calves are born, which has a negative impact on early life growth and development (Desforges et al., 2019). Low quality forage also results in insufficient protein, as well as a lack of essential nutrients and minerals, which can negatively impact muskox health (Afema et al., 2017; Mosbacher et al., 2016).

1.5.5 Muskox Threats

Climate change poses a major threat to wildlife and biodiversity on a global scale, impacting the structure and function of diverse ecosystems by rapidly altering the selective pressures organisms face in their environments (De Kort et al., 2021; Espunyes et al., 2022; A. A. Hoffmann et al., 2019; Parmesan, 2006; Rubenstein et al., 2023). Environmental warming has caused a shift in the phenology and distributions of insects, plants, and animals, altering ecosystem ecology (Parmesan, 2006; Refsnider & Janzen, 2012). The effects of climatic change a particularly pronounced in Arctic environments, where warming is occurring at nearly four times the global rate, introducing additional threats and stressors to muskox populations (Cuyler et al., 2019; Ernakovich et al., 2014; Gunn & Forchhammer, 2022; Kutz et al., 2015; Olofsson et al.,

2009; Speed et al., 2021). For example, plant phenology is changing due to environmental warming, with advancing springs resulting in earlier green up and leaf emergence times, which further impacts herbivore life events, fecundity, and fitness (Parmesan, 2006). Arctic warming is also associated with Arctic shrubification, where shrub abundances and distributions have accelerated, altering ecosystem carbon exchange, nutrient cycling, and permafrost degradation (Mekonnen et al., 2021). Additionally, with increased seasonal temperatures, there is a higher probability of extreme weather events which result in increased rain and icing, as well as deeper snow (Cuyler et al., 2019). This limits access to winter forage and increases the burden of foraging, leading to poor calf recruitment and increased mortalities in muskoxen (Cuyler et al., 2019; Desforges et al., 2019; Schmidt et al., 2015, 2023). Climate change related mammal migration is also expected to intensify resource competition for native mammalian species like the muskox, further exacerbating shifts in vegetation availability (Parmesan, 2006). Increasing temperatures are expected to result in heat stress as well as heat-induced immunosuppression, which was likely a contributing factor to outbreaks of fatal pneumonia in muskoxen in Norway (Cuyler et al., 2019; Ytrehus et al., 2008). Similarly, disease dynamics and ranges of other muskox pathogens have changed due to Arctic warming (Kafle et al., 2020; Kutz et al., 2015; Tomaselli et al., 2016). A specific example of the impacts are warming are illustrated by the substantial northward expansion of two lungworm species that impact muskox, *Umingmakstrongylus pallikuukensis* and *Varestrongylus elegunenniensis*, though higher abundances are still seen in warmer, more southern latitudes (Kafle et al., 2020). Additionally, ORF virus, a member of the parapoxvirus family, was recently confirmed in wild Canadian muskox populations (Dalton et al., 2023, 2024; Tomaselli et al., 2016). ORF virus is known to cause ecthyma in muskoxen and can lead to lethargy, starvation, and mortalities due to feeding avoidance, as the painful legions often form on their nose and mouth (Dalton et al., 2023, 2024;

Tomaselli et al., 2016). Further, *Brucella suis* is a bacterium that typically causes stillbirths, abortions, reproductive failure and lameness in reindeer and is now being found in muskoxen with relatively high prevalence in Victoria Island populations (Aguilar et al., 2022; Tomaselli et al., 2019). Increased seroprevalence of the zoonotic bacterium *Erysipelothrix rhusiopathiae* has also been found in Canadian muskoxen, with high prevalence in Victoria Island and Banks Island populations tied to high mortality rates and deaths in individuals with otherwise good body condition (Kutz et al., 2015; Mavrot et al., 2020). This increase in disease morbidity or, in some cases, widespread disease-related muskox mortalities, further indicates the threat that pathogens pose to muskox populations.

Adding to environmental stressors, are extremely low levels of genetic diversity found in muskoxen (Campos et al., 2010; Groves & Shields, 1997; Hansen et al., 2018; Holm et al., 1999; MacPhee et al., 2005; Thulin et al., 2011; Van Coeverden De Groot & Boag, 2004). Genetic diversity in muskoxen has been measured via microsatellite genotyping and mitochondrial DNA sequencing, all of which found limited variation (Campos et al., 2010; Groves & Shields, 1997; Holm et al., 1999; MacPhee et al., 2005; Thulin et al., 2011). More recently, Hansen et al. (2018) performed genotyping by sequencing via ddRadSeq to gain a more thorough understanding of the neutral genetic diversity in muskoxen across their range. They found levels of genetic variation were lower than many endangered species (Hansen et al., 2018). Even whole genome sequencing has revealed muskox populations have the lowest levels of heterozygosity found in ungulates, largely due to historical bottlenecks, and post glacial recolonisations (Pečnerová et al., 2024). Low diversity has led to concerns over the adaptive capacity of muskox populations in response to changing selective pressures (Cuyler et al., 2019; Gunn & Forchhammer, 2022). These concerns are substantiated by the recent population collapses on Victoria and Banks Islands, which previously held 61% of Canada's muskoxen (Cuyler et al., 2019; Gunn &

Forchhammer, 2022; Kutz et al., 2015). It should be noted that outside of these observed island die-offs, other muskox populations have remained stable or even grown in recent years, with the reason for this dichotomy unclear (Cuyler et al., 2019; Gunn & Forchhammer, 2022).

1.6 Thesis objectives

The overall objective of this thesis was to use molecular tools to better understand mechanisms contributing to long-term persistence in a long lived, genetically depauperate species experiencing rapid environmental change. I aimed to assess genetic, genomic, and diet diversity in muskoxen to identify and measure drivers of muskox health and vulnerabilities.

In **Chapter 2,** I assessed the degree and distribution of neutral genetic variation in two endemic muskox populations undergoing opposing demographic trends using microsatellite data. I used demographic modelling to assess the effects of historical bottlenecks on contemporary levels of genetic variation as well as to predict the how differing demographic trends may affect muskox genetic diversity in the future.

In **Chapter 3**, I assembled the first muskox genome using high throughput sequencing and cross-species scaffolding. I used these data to estimate the divergence time of this unique genus from other ungulate species as well as to model trends in historical effective population size.

In **Chapter 4,** I resequenced genomes of 22 muskox individuals from across their natural range to provide a more thorough estimate of genome wide diversity. I reconstructed the phylogeny of muskoxen using both mitochondrial genes and ultra conserved nuclear regions to better understand the divergence of muskox populations and subspecies. I assessed the levels gene flow between muskox populations using admixture analyses. I measured inbreeding,

effective population size, runs of homozygosity and heterozygous rich regions to better estimate drivers of population health and identify vulnerable muskox populations.

In **Chapter 5,** I performed plant metabarcoding to assess diet composition and diversity in muskoxen in context of a changing Arctic environment. Plant diversity measurements were compared to that of the muskox microbiome to gain insight into the microbiome plasticity and the capacity for muskoxen to survive off poor quality and low quantity forage.

In **Chapter 6,** I synthesize these data to make inferences on the source of low genetic diversity in muskoxen, the mechanisms that have assisted in their persistence and conservation concerns in the face of a rapidly changing Arctic. I also provide insights into the benefits and limitations of different molecular tools that have been used to assess genetic diversity in muskoxen. Finally, I provide recommendations for future research in the context of my findings and other recent genomic literature. Overall, these chapters provide a better understanding of the relationship between genetic diversity, population vulnerability and persistence in an iconic Arctic species, experiencing rapid environmental change.

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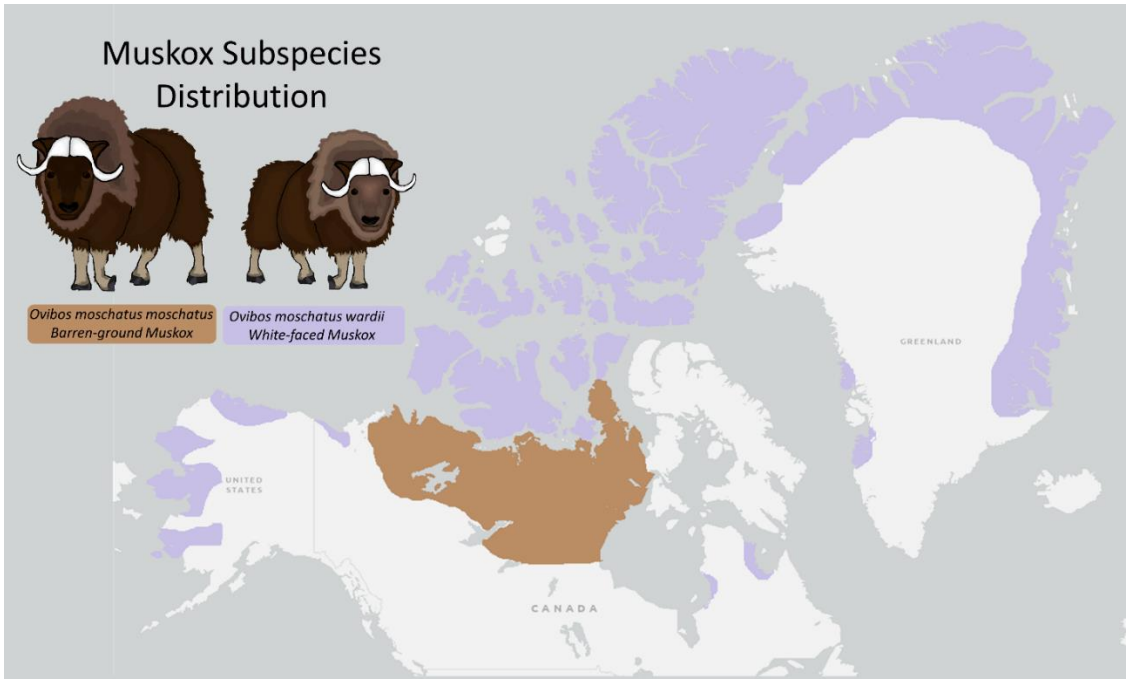

Figure 1.1. Map showing the distribution of the muskoxen. Colour differences on the map represent the distribution of the different subspecies, *Ovibos moschatus moschatus* and *Ovibos moschatus wardii*.

Chapter 2. Already at the bottom? Demographic declines are unlikely further to undermine genetic diversity of a large Arctic ungulate: muskox, *Ovibos moschatus* (Artiodactyla:Bovidae)

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2.1 Abstract

Low genetic diversity is associated with low fitness and evolutionary potential, yet the demographic and life-history traits of some species contribute to low genetic diversity, without empirical evidence of negative impacts on fitness. Modelling past and future trajectories of genetic diversity under different demographic scenarios can provide insight into how genetic variation might impact population fitness. The muskox is an Arctic species that has undergone multiple population bottlenecks and although populations have rebounded repeatedly, two large populations have recently declined by > 50%. It is unclear how these demographic patterns influence muskox genetic diversity and fitness. We compared the genetic diversity of Canadian muskox populations undergoing opposing population trends. Genotyping 84 mainland and 244 Victoria Island individuals at ten microsatellite loci revealed low genetic variation (Victoria Island, mean allelic richness 1.66, expected heterozygosity 0.16; mainland, mean allelic richness 2.58, expected heterozygosity 0.41), with no evidence of further reductions in diversity subsequent to recent demographic declines. Bayesian modelling showed that historical bottlenecks contributed to the lack of diversity in contemporary populations, and forward-in-time simulations suggested little effect on genetic diversity over the next 100 years. Muskoxen might have reached a genetic diversity minimum, and additional research will be needed to determine their capacity to adapt to rapid changes in selective pressures in a rapidly changing Arctic.

2.2 Introduction

Understanding the maintenance and spread of genetic variation within and among populations is a central theme in wildlife conservation, where higher genetic variation has been associated with increased fitness and evolutionary potential (e.g. Parra et al., 2018). Drivers of genetic

diversity include gene flow, selection and genetic drift, where smaller populations are greatly influenced by effective population size (N_E) and genetic drift (Frankham, 1996). In smaller populations, diversity can be undermined by the following factors: (1) low and fluctuating census sizes, such as population bottlenecks, which lead to genetic drift (Hoelzel, 1999); (2) small numbers of founding individuals, which undermine genetic diversity via genetic drift, but also inbreeding among the founders (Frankham, 1996; Parra et al., 2018); and (3) population fragmentation and isolation, which prevent the influx of genetic variation via gene flow (Corti et al., 2011; Frankham, 1996). Although the reduction of genetic diversity is thought to decrease overall fitness and increase the risk of extinction of local populations (Finlay et al., 2017), some species with low genetic diversity as a result of founder events and multiple population bottlenecks persist and thrive (e.g. Johnson et al., 2009; Meiri et al., 2004; Terrell et al., 2016). These variable effects of low genetic diversity demonstrate the need to study how demographic histories impact each system and potentially influence species persistence.

Beyond assessments of contemporary levels of genetic variation, population genetic modelling and simulations provide tools to understand how historical processes have impacted contemporary genetic variation and to predict how population trends will impact genetic diversity in the future (Attard et al., 2015; Chaves et al., 2011). Forward-in-time simulations are particularly important in conservation studies to help predict the genetic consequences of different environmental and demographic scenarios (García-Navas et al., 2015; Pelletier et al., 2017).

The muskox (*Ovibos moschatus*) is an Arctic species that has evolutionary and demographic histories expected to lead to lowered fitness and adaptive capacity. In the early Pleistocene, muskoxen originating from Eurasia arrived in North America and by the late Pleistocene had a Holarctic distribution and higher genetic diversity than contemporary

populations (Campos et al., 2010; MacPhee et al., 2005). However, from the Last Glacial Maximum to the mid-Holocene, multiple population contractions and expansions occurred, leading to population and genetic diversity declines (Campos et al., 2010; Hansen et al., 2018; MacPhee et al., 2005). In the early 1900s, muskox populations were extirpated from most of their natural range, with only two main pockets of animals (Thelon and Great Bear Lake, Nunavut) remaining on the Canadian mainland and numbers on Victoria Island and Banks Island dropping to nearly zero (Barr, 1991). After a 50-year harvest moratorium, and active management in the form of translocations to Greenland, Alaska, Russia and Quebec, muskox populations began to recover, and they are now found across the Canadian Arctic, Alaska and Greenland (Figure 2.1; Barr, 1991). Two of the largest endemic contemporary populations are found on Banks Island and Victoria Island, with population estimates of 70 000 and 40 000 individuals, respectively in the 1990s (Barr, 1991; Dumond, 2006). However, around 2001, the Banks Island and Victoria Island populations started to decline, with the rate of decline becoming more severe by 2010, and by 2014 both islands had lost > 50% of their populations (Kutz et al., 2015). In contrast, during this time, most endemic mainland populations remained stable or continued to grow (Kutz et al., 2015). Although a thorough review of muskox population statuses was recently completed by Cuyler et al. (2019) to determine population trends, population drivers, including anthropogenic changes, and stressors, such as extreme weather events, it is still unclear why these contrasting population trends exist. However, given that the largest remaining populations were on the mainland subsequent to the last population bottleneck, and Banks Island and Victoria Island populations are from very small founding populations, low genetic diversity and an ability to cope with new selective pressures are hypothesized as factors to explain these differences.

Evidence of low genetic diversity has been reported in several muskox genetic studies in terms of allelic richness and heterozygosity (Hansen et al., 2018; Holm et al., 1999; Laikre et al., 1997; MacPhee et al., 2005; Thulin et al., 2011; Van Coeverden De Groot & Boag, 2004), markers directly associated with fitness (e.g., a single MHC DRB3 allele observed; Mikko et al., 1999) and whole-genome scans (Hansen et al., 2018), revealing a general lack of genetic variation in this species, with one of the lowest levels of intergenic variation observed in mammals. Given these data, it is unclear why some muskox populations show confounding demographic growth patterns. It is also unclear whether recent population declines on Victoria Island will further reduce genetic diversity, undermining the capacity of these animals to adapt, as would be expected from extremely low effective population sizes, where genetic drift can become a stronger force than natural selection and gene flow.

Herein, we assess the degree and distribution of standing genetic variation of two endemic muskox populations using a large subset of non-invasive samples. Samples were acquired before and after recent major population declines on Victoria Island to determine whether recent trends could account for ongoing declines in genetic diversity (pre- and post-2009). Modelling was used to determine whether the most recent or more historical bottlenecks account for the low contemporary levels of genetic variation in muskox populations. We also modelled how future muskox genetic diversity would be affected under differing demographic predictions. Finally, we used these data to gain a better understanding of the contrasting population trends observed between endemic mainland and Victoria Island muskox populations (Leclerc, 2015; Tomaselli et al., 2018), and as an indicator of future population viability.

2.3 Materials and Methods

2.3.1 Sample collection and DNA extraction

Muskox tissue samples and faecal pellets were collected through community-based muskox health-monitoring programmes, guided outfitted harvests, commercial harvests, and purposeful collections (faecal samples), between 2007 and 2016 from Victoria Island (Nunavut and Northwest Territories; N = 544) and west of the Coppermine River from mainland Nunavut (N = 103; Figure 2.1), with individuals identified via genotyping (see below). Samples were also collected from Banks Island but provided insufficient DNA for analyses. Faecal pellets were swabbed, and swabs were placed into 500 µL of 1× lysis buffer with negatives to assess for contamination. For tissue, 0.2 cm2 of hide and muscle tissue was cut and placed into 500 µL of 1× lysis buffer. Twenty microlitres of 20 mg/mL proteinase K was added to faecal and tissue samples and incubated at 56 °C for 2 h. Sample extraction was performed using both a modified version of the Qiagen DNeasy blood and tissue extraction kit and a magnetic bead extraction using 400 µL of lysed sample (Magnesil, according to the manufacturer's protocol). Negative and positive extraction controls were included to detect contamination and determine extraction efficiency. Extracted samples were quantified with Picogreen using a BMG FluoStar Galaxy plate reader and subsequently standardized to 1.25 ng/µL.

2.3.2 Microsatellite amplification and genotyping

Twelve primers were selected for multiplex genotyping (Table S2.1). Polymerase chain reaction (PCR) conditions were optimized using temperature and magnesium gradients, altering primer concentrations and varying PCR cycles (Table S2.2). The PCR products, including positive and negative controls, were resuspended in Hi-Di, formamide and 6-carboxy-X-rhodamine (ROX)-labelled internal size standards (Applied Biosystems). Genotyping was performed on an

automated ABI 3730 DNA analyser (Applied Biosystems), and GeneMarker v.2.6.3 was used to identify alleles. Sample replicates (~10%) were analysed to assess for consistent genotypes relative to potential artifacts, such as allelic dropout. Any loci consistently missing data or containing only a single allele across all samples tested were removed. Individuals were determined by removing duplicate genotypes within the region and year sampled.

Sex determination was performed using primers developed by Fain & LeMay (1995) and Aasen & Medrano (1990 ; Tables S2.1 and S2.2). Amplified products were separated and scored via 1.5% agarose gel electrophoresis stained with ethidium bromide in the context of positive and negative control samples.

2.3.3 Genetic analyses

Genetic diversity

Evidence of null alleles, scoring errors or allelic dropout was assessed using MICRO-CHECKER v.2.2.3 (Van Oosterhout et al., 2004). Loci were assessed for linkage disequilibrium, deviation from Hardy–Weinberg equilibrium and genetic variation using GENEPOP v.4.2 (Raymond & Rousset, 1995). Allelic richness and inbreeding coefficients were calculated using FSTAT v.2.9.3, and probability of identity (PID) and probability of identity between siblings (PSIB) were calculated using CERVUS v.3.0.7 (Goudet, 1995; Kalinowski et al., 2007). Default settings were used for all programs. To compare estimates of genetic variation between populations and significance, two-tailed t-tests were performed, assuming inequal variance.

Genetic structure

STRUCTURE v.2.3.1 was used to perform population genetic structure analyses on all individuals using admixture ancestry and independent frequency models (Pritchard et al., 2000). Burn-ins were set at 50 000, and repetitions were set at 500 000. K, or the number of clusters, was set

from one to ten, and ten iterations were run per K. The posterior probability of the data [LnP(D)] for each run was used to calculate ΔK values to determine the optimal K (Figure S2.1). Population assignment probabilities for the resulting clusters were used for structure plots, with individuals organized chronologically within their collection region.

Bottleneck and simulation of variation loss

Population bottlenecks were tested for populations across all years using BOTTLENECK v.1.2.02 software (Piry et al., 1999). Two mutation models recommended for microsatellite data were used to run BOTTLENECK: stepwise mutation (SMM) and two phased (TPM), with a 95% stepwise mutation model and 5% multistep mutations. Wilcoxon signed rank tests identified heterozygote excess for each population and year tested using 10 000 iterations. BEASTvntr assessed the demographic history of both Victoria Island and mainland regions separately, by constructing an extended Bayesian skyline plot (Bouckaert et al., 2014). A strict clock model was used because all samples were contemporaneous and from the same population and, therefore, assumed to evolve at the same rate. A mutation rate of 0.001 per generation was used, based on muskox microsatellite mutation rates provided by Gordeeva et al. (2009), with a Sainudiin model developed for modelling microsatellite data and a generation time of 7 years. TRACER v.1.6 was used to visualize the convergence of the Markov chain Monte Carlo chains and R software to visualize the skyline plot.

BottleSim v.2.6.1 predicted future losses in genetic diversity if the Victoria Island population were to continue to decline (Kuo & Janzen, 2003). The bottleneck scenarios simulated maintained 100, 75, 50 and 25% of the current consensus population size on Victoria Island, estimated at 22 000 individuals.

2.4 Results

2.4.1 Genetic diversity

Of the 12 microsatellite loci tested, one was monomorphic despite being polymorphic in the study by Van Coeverden De Groot & Boag (2004) and one did not consistently amplify across populations, leaving ten variable loci. After removal of duplicate genotypes, 244 individuals were identified from Victoria Island and 85 from the mainland. No loci had null alleles, although two sampling years from the mainland (2015 and 2016) deviated significantly from Hardy–Weinberg equilibrium after Bonferroni correction. These deviations occurred at one locus (OM55-04) for the 2015 population and two loci (OM53-12 and Modias3) for the 2016 population. Linkage disequilibrium between each pair of microsatellites over all populations found three significant values after Bonferroni corrections; however, different pairs of loci were involved in these cases, implying that genotypes from these loci were independent.

Allele frequencies for each locus are reported for both the mainland and Victoria Island populations in Table 2.1. Of note, for Victoria Island, seven of ten loci had the most common allele frequencies either fixed or nearing fixation (> 0.9), whereas only one locus on the mainland had an allele at a frequency > 0.9. The PID and PSIB were higher for Victoria Island populations (PID = 7.43 × 10−3 and PSIB = 5.94 × 10−2) than for mainland populations (PID = 1.33 × 10−6 and PSIB = 1.31 × 10−3).

Allelic variation was measured using the average number of alleles (NA) and average allelic richness (AR) for each year for both the mainland and Victoria Island. Victoria Island averaged NA = 2.05 alleles per locus, and the mainland averaged NA = 2.50. Allelic richness for the mainland was 2.47 and for Victoria Island AR was 1.66. The AR was significantly different (P = 1.95 × 10−3) between the mainland and Victoria Island.

Observed (Ho) and expected (He) heterozygosity differed between mainland and Victoria Island populations, with mainland metrics > 2.5 times larger than those of Victoria Island across all years. Mainland populations had an average Ho of 0.374, and Victoria Island populations were significantly different, averaging 0.147 ($P = 2.93 \times 10{-}5$). Likewise, expected heterozygosity for mainland populations averaged 0.410 and was significantly different from Victoria Island at 0.160 ($P = 4.81 \times 10{-}8$). The coefficient of inbreeding for the mainland populations averaged 0.0838, with 2015 having the highest rate of inbreeding at 0.216, and Victoria Island had a similar overall average at 0.0773, with the highest rate of inbreeding in 2014 at 0.201; however, differences between these populations were not significant.

2.4.2 Genetic structure

Raw posterior likelihood (LnPd) values produced by STRUCTURE for K = 1–10 were used to calculate ΔK (Supporting Information, Figure S2.1), where K = 2 was optimal, with mainland samples and Victoria Island samples respectively pooling into discrete, separate clusters, with no further structuring within the clusters (Figure 2.2). Two mainland samples were cross-assigned to the Victoria Island genetic cluster, and some Victoria Island samples showed admixture with the mainland cluster, indicating that there might be low levels of gene flow occurring between regions.

2.4.3 Demographic histories and forward-in-time simulations

Under the two mutation models recommended for microsatellite data in BOTTLENECK, only one year (mainland 2016) showed heterozygote excess using Wilcoxon signed rank tests ($P = 0.01$ for TPM; $P = 0.04$ for SMM). BEASTvntr analyses of extended Bayesian skyline plots showed that both mainland and Victoria Island populations remained at stable effective population sizes (Ne) of ~500 before starting an increasing trend ~100 generations ago (generation time = 7 years;

Figure 2.3). The mainland population increased at a consistent rate, from an N_E of ~500 to 42 000 starting 60 generations ago, whereas the Victoria Island population increased more rapidly between 75 and 50 generations ago, after which the rate of growth slowed until 2016, reaching an N_E of 13 000 (Figure 2.3).

Forward-in-time simulations of genetic diversity performed on the Victoria Island population predicted no loss in genetic diversity over the next 100 years (Figure 2.4). With an estimated population size of ~22 000 in the early 2010s on Victoria Island (Davison & Williams, 2013; Leclerc, 2015), 99% of diversity in terms of observed allele diversity and observed heterozygosity was maintained with all simulated bottlenecks ranging from 100 to 25% of the current population size.

2.5 Discussion

In the present study, we evaluated levels of genetic variation from two endemic North American muskox populations with contrasting recent demographic trends, in order to gain a better understanding of how past and contemporary population declines have impacted genetic variation in this species. We also modelled future levels of genetic diversity under different demographic models as an indicator of the future viability of muskoxen in a rapidly changing Arctic environment. Non-invasive sampling techniques were used to increase sample sizes and obtain an enhanced spatial and temporal distribution of samples from before and after the most recent population declines observed on Victoria Island that started ~2009. Similar to previous work (Holm et al., 1999; Van Coeverden De Groot & Boag, 2004), we found very low levels of variation in comparison to other ungulates and imperilled species. Modelling these data suggests that currently observed levels of genetic variation most closely align with historical population bottlenecks, but that significant differences between mainland and Victoria Island

populations exist in terms of both allelic richness and heterozygosity. Furthermore, modelling projections of genetic diversity suggest that continued trends of decreasing demographic population size will have only marginal effects on effective population size, hence genetic diversity in these populations, indicating that genetic variation in this species might have approached a minimum. These findings are relevant in that on Victoria Island, poor body condition, hoof abnormalities and a variety of other abnormalities in addition to other demographic changes, such as observably smaller group sizes and changing age and sex structures, have been reported through local knowledge (Tomaselli, Gerlach, et al., 2018; Tomaselli, Kutz, et al., 2018). Although direct links have not been established, Victoria Island animals might also have a higher exposure to pathogens that were previously excluded by harsh environmental conditions that are now more accommodating to different diseases as the Arctic landscape continues to warm (Kutz et al., 2004).

With expected heterozygosity values ranging from 0.41 on the mainland to 0.16 on Victoria Island (Table 2.2), muskoxen have among the lowest values documented in the literature, and notably less than many other ungulates, including imperilled species such as Dall's sheep (0.722; Worley et al., 2004) and barren-ground and Peary caribou (0.43–0.86; Cronin et al., 2003, 2005; Jenkins et al., 2018). Additionally, Hansen et al. (2018) found that genetic diversity declined as populations continued northwards to the high Arctic islands and Greenland. Given that our results follow this trend, the values reported from the mainland and Victoria Island might be the highest expected in this species.

The low genetic variation observed in muskox populations has been hypothesized to be the result of repeated historical bottlenecks, specifically during the Pleistocene (MacPhee et al., 2005), compounded by more recent declines in the early 1900s. Aligning with evidence of historical bottlenecks, BEAST 2 results show low effective population sizes on Victoria Island

occurring until ~75 generations ago (~525 years ago), after which N_E increased rapidly to ~13 000 (Figure 2.3; Hansen et al. 201). For comparison, census size estimates of > 25 000 individuals are reported for Victoria Island (Cuyler et al., 2019). Mainland population BEAST 2 results are also consistent with historical demographic trends, with low N_E until ~60 generations ago (~420 years ago), after which it grew consistently, tripling that of the Victoria Island at 42 000 (Figure 2.3). Estimates of mainland N_E are larger than the reported census size of ~8000 (Leclerc, 2015). There was no indication of a contemporary population bottleneck for either population based on analyses within BOTTLENECK (Table 2.2). This suggests the low levels of genetic diversity in muskoxen are likely to be the result of historical bottlenecks rather than the contemporary population declines; however, the long generation times of muskoxen might undermine our ability to detect more contemporary genetic impacts of recent population declines.

In addition to historical population bottlenecks, fragmented or island populations, such as the one on Victoria Island, are expected to have reduced genetic diversity owing to small population sizes and inbreeding. STRUCTURE results show that there is little gene flow between the mainland and Victoria Island populations, leaving Victoria Island isolated (Figure 2.2). For the Victoria Island population, although the inbreeding coefficient was low, the fixation or near fixation of seven loci indicates that genetic drift is acting more strongly on the island population than on the mainland population, which had only one locus near fixation (Table 2.1). However, because the probability of identity between siblings is very low, this could have led to the removal of siblings with the same genotype, resulting in a low inbreeding coefficient. The extremely low genetic variation on Victoria Island is believed to be the result of both a historical founder effect from the colonization of muskoxen on the islands and a more contemporary founder effect after the population bottleneck that occurred 100 years ago (Hansen et al., 2018).

Additionally, if the current rate of population declines were to continue on Victoria Island, even with an assumption of a 70% decrease in population size, BOTTLESIM software showed that there would be a minimal additional loss in genetic diversity over the next 100 years (Figure 2.4). Although we predicted that we would see a decrease in genetic diversity as a result of the lower effective population size, the comparatively large census population size might help to maintain what diversity remains in this population.

Although it appears that no genetic losses would be associated with the recent population declines on Victoria Island, genetic diversity is already very low, and thus this should still be cause for concern. Genetic diversity is often used as a proxy for population health and evolutionary potential (Reed & Frankham, 2003), meaning that the extremely low genetic variation on Victoria Island and within muskox populations as a whole might indicate that they lack the capacity to adapt to new selective pressures in a changing Arctic environment. Historically, muskoxen, especially those in the high Arctic, have faced fewer selective pressures from human interference, competition and pathogen exposure (Kutz et al., 2009). However, owing to climatic changes, the range of selective pressures to which they must respond is increasing rapidly (Hoffmann & Sgrò, 2011). Arctic warming has led to increased icing events that reduce food availability, heat stress and the undermining of dispersal barriers for southern species and pathogens, leading to more competition and disease outbreaks (Berger et al., 2018; Di Francesco et al., 2017; Kutz et al., 2009). A potential parallel comes from research on woolly mammoths on Wrangel Island, which found that inbreeding, population bottlenecks and low genetic diversity led to a build-up of deleterious alleles thought to have contributed to the consequent extinction of the species (Palkopoulou et al., 2015). However, the mammoth survived for thousands of years with low genetic diversity before any evidence of so-called genomic meltdowns were observed (Palkopoulou et al., 2015). In fact, evidence has suggested

that the extinction of the Wrangel Island mammoths was attributable to sudden rather than gradual environmental change, to which mammoths could not adapt, with one hypothesis being the emergence of a novel pathogen (Nyström et al., 2010). As such, it should be noted that in muskoxen, the isolated Victoria Island population, with lower genetic diversity, has recently been experiencing disease die-offs.

Overall, muskoxen display low levels of variation, and there is an indication of potential inbreeding effects and increased susceptibility to disease on Victoria island. That said, adaptation to local selective pressures can occur via different genetic mechanisms, which might be identified by future whole-genome studies (Harrisson et al., 2014; Primmer, 2009), such as rapid adaptation via modulated methylation patterns (Harrisson et al., 2014). Continued genetic and genomic studies will be crucial in assessing how to best manage for the long-term survival of this Arctic species.

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Table 2.1. Allele frequencies for each locus, probability of identity and probability of identity between siblings for Mainland and Victoria Island samples

Locus	Allele	Mainland	Victoria Island	Locus	Allele	Mainland	Victoria Island
MoDIAS 1	120	0.527	1.00	OM58-06	144	0.012	0.00
	133	0.260	0.00		146	0.939	0.015
	135	0.185	0.00		150	0.006	0.00
	147	0.027	0.00		152	0.043	0.985
OM51-19	205	0.756	0.842	OM53-38	140	0.530	0.011
	207	0.012	0.002		152	0.101	0.00
	209	0.232	0.094		158	0.345	0.599
	211	0.00	0.062		160	0.018	0.388
OM50-8	148	0.355	0.009		162	0.006	0.002
	156	0.645	0.991	OM54-23	93	0.099	0.00
OM53-12	197	0.00	0.002		95	0.197	0.004
	199	0.281	0.00		97	0.00	0.006
	201	0.152	0.372		101	0.00	0.037
	203	0.146	0.00		103	0.053	0.950
	205	0.421	0.626		107	0.651	0.002
OM55-04	245	0.319	0.011	OM51-16	258	0.007	0.015
	249	0.406	0.015		260	0.066	0.964
	251	0.025	0.013		262	0.529	0.002
	253	0.244	0.941		264	0.338	0.019
	255	0.006	0.021		266	0.059	0.00
MoDIAS 2	204	0.00	0.002	MoDIAS3	170	0.728	0.013
	206	0.177	0.669		176	0.00	0.004
	208	0.824	0.330		178	0.271	0.983
Probability of Identity (PID)						*1.33* x10 $^{-6}$	*7.43* x10 $^{-3}$
Probability of Identity siblings (Psib)						*1.31* x10 $^{-3}$	*0.059*

Table 2.2. Microsatellite diversity for Victoria Island and mainland (Kitikmeot, Nunavut) muskox populations from 2007-2016. Sample size (N), average number of alleles per locus (A), mean allelic richness (AR), observed heterozygosity (Ho), expected heterozygosity (He), Coefficient of inbreeding (Fis), Heterozygote Excess (HE) from Bottleneck using two models; (TPM), (SMM).

	N	A	Ar	Ho	He	Fis	HE (TPM/SMM)
Victoria Island							
2008	12	2	1.88	0.151	0.164	0.078	N/N
2009	40	1.5	1.44	0.141	0.143	0.028	N/N
2013	70	2.9	1.94	0.192	0.192	0.004	N/N
2014	66	2.4	1.66	0.126	0.157	0.201	N/N
2015	37	1.9	1.45	0.122	0.123	0.003	N/N
2016	19	1.6	1.58	0.149	0.182	0.150	N/N
Average	40.667	2.05	1.66	0.147	0.160	0.077	
Mainland							
2007	19	2.7	2.59	0.370	0.419	0.123	N/N
2014	15	2.4	2.33	0.400	0.393	-0.019	N/N
2015	12	2.8	2.75	0.334	0.424	0.216	N/N
2016	38	2.4	2.20	0.398	0.404	0.015	Y/Y
Average	21	2.58	2.47	0.375	0.410	0.084	

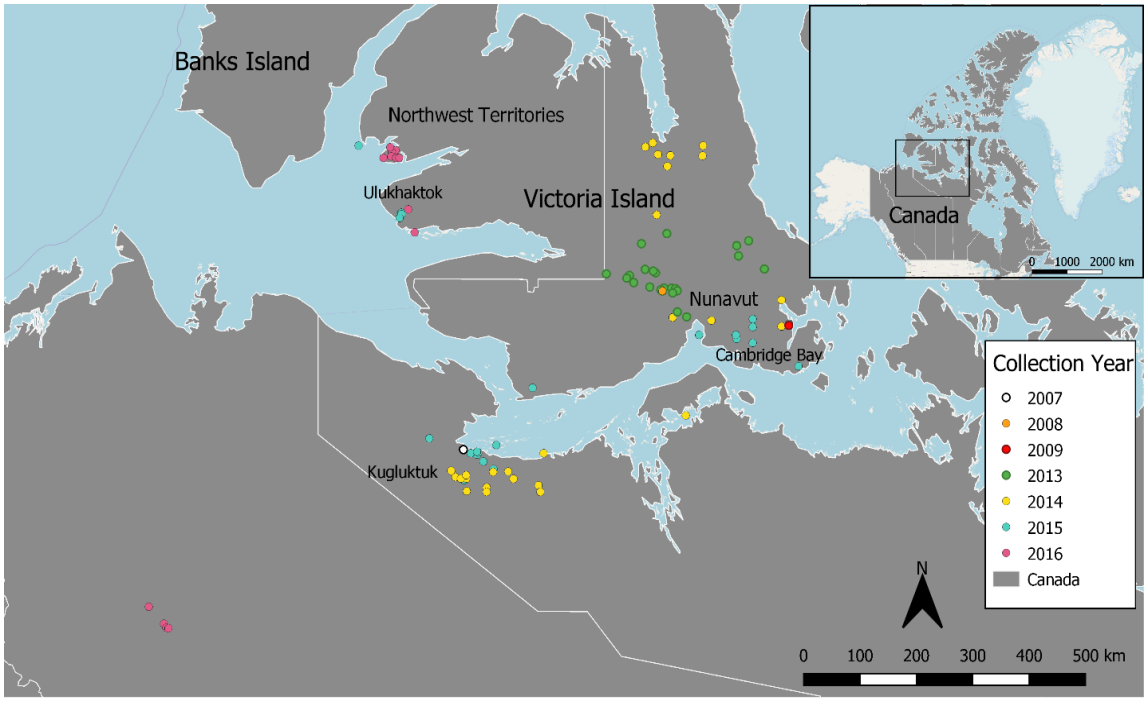

Figure 2.1. Map showing the locations or communities from which samples were collected. Samples from Ulukhatok, 19 of 47 samples from Kugluktuk and 61 of 218 samples from Cambridge Bay were coarsely defined within the general regions.

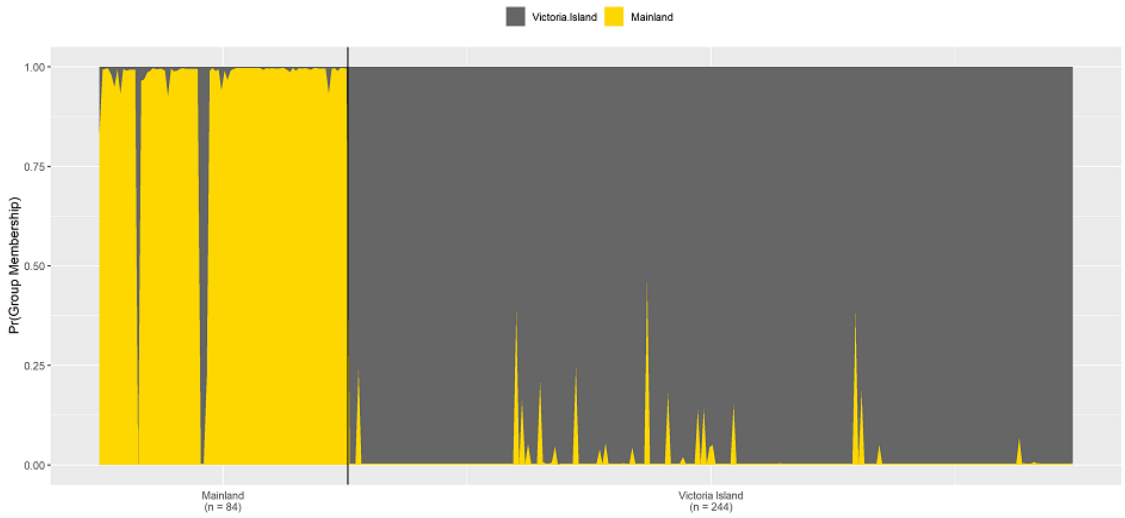

Figure 2.2. STRUCTURE plot showing the probabilities of assignment of individuals to different clusters based on STRUCTURE analyses. Each column represents a different individual, and samples are grouped by sampling location (Victoria Island or mainland). Two inferred clusters are represented by different colouring.

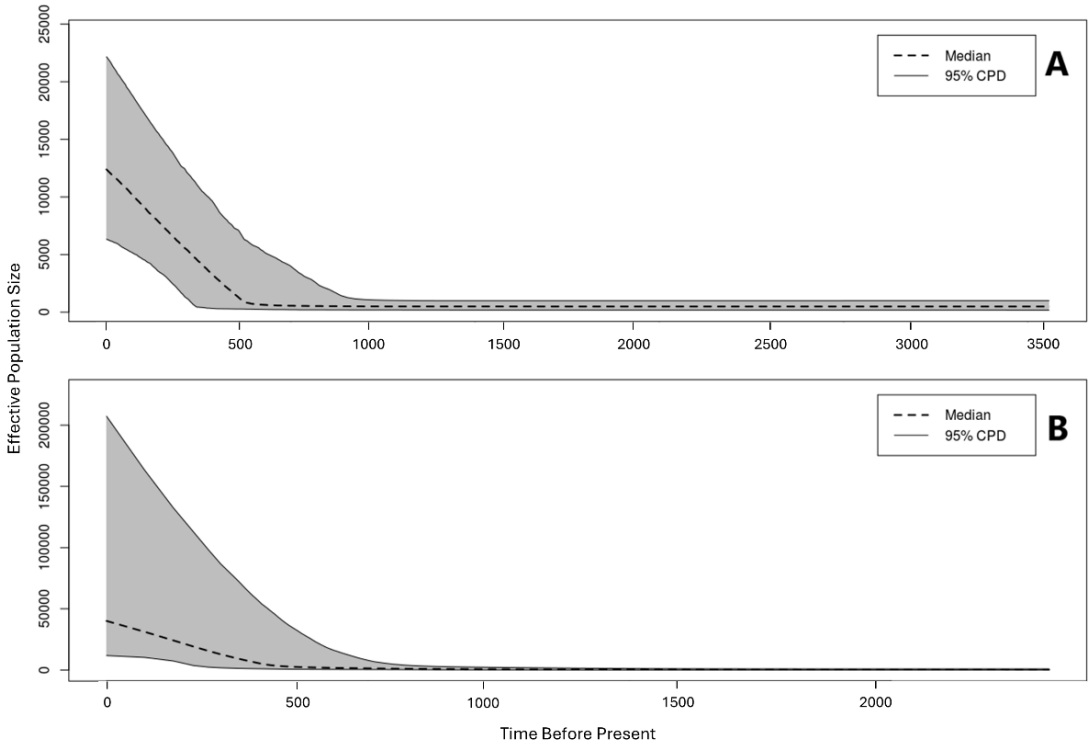

Figure 2.3. BEAST results of effective population size of Victoria Island (A) and mainland (B) muskox populations through years before present. Effective population sizes remain low in muskoxen until ~500 years ago on Victoria Island and ~400 years ago on the mainland. Final effective population sizes are estimated at ~14,000 on Victoria Island and ~42,000 on the mainland.

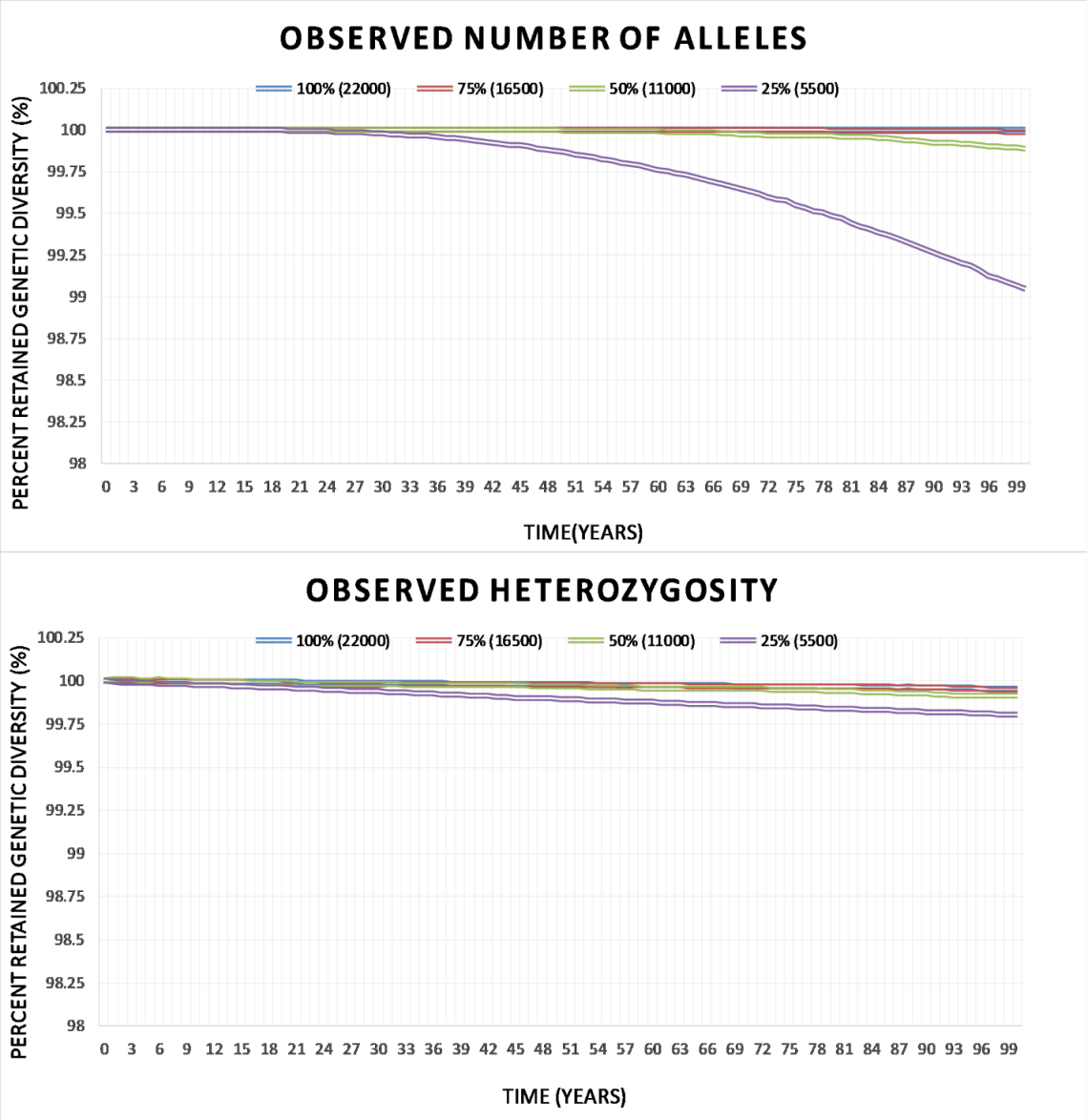

Figure 2.4. BOTTLESIM results of simulated genetic diversity of the Victoria Island muskox populations over the next 100 years. Neither the observed number of alleles (A; OA) nor the observed heterozygosity (B; H_o) was projected to decline in any bottleneck scenario.

Chapter 3: Draft Genome Assembly of an Iconic Arctic Species: Muskox (*Ovibos moschatus*).

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Contributions: CJK and EP conceived and designed the study. SK, LML and CJK provided samples, and resources. EP performed the formal analyses and prepared draft manuscript. CJK, SK, LML edited and reviewed the final manuscript. CJK and SK provided funding acquisition.

3.1 Abstract

Muskoxen (*Ovibos moschatus*) are an Arctic species within the Caprinae subfamily that are economically and culturally significant to northern Indigenous communities. Low genetic diversity from repeated genetic bottlenecks, coupled with the effects of Arctic warming (e.g., heat stress, changing forage, pathogen range expansions), present conservation concerns for this species. Reference genome assemblies enhance our ecological and evolutionary understanding of species, which in turn aid conservation efforts. Herein, we provide a full draft reference muskox genome using Illumina Hiseq data and cross-species scaffolding. The final reference assembly yielded a genome of 2,621,890,883 bp in length, a scaffold N50 of ~13.2 million, and an annotation identifying ~19.3 k genes. The muskox genome assembly and annotation were then used to reconstruct a phylogenetic tree which estimated muskoxen diverged from other ungulate species~12 Mya. To gain insight into the demographic history of muskoxen we also performed pairwise sequentially Markovian coalescent (PSMC) that identified two population bottlenecks coinciding with major glaciation events contributing to the notoriously low genetic variation observed in muskoxen. Overall, this genome assembly provides a foundation for future population genomic studies, such as latitudinal analyses, to explore the capacity of muskoxen to adapt to rapidly changing environments.

3.2 Introduction

Muskoxen (*Ovibos moschatus*) are an iconic Arctic species closely related to sheep and goats within the *Caprinae* subfamily and the only living member of the *Ovibos* genus. Endemic muskoxen are found on the mainland and Arctic Archipelago of the Northwest Territories (NWT) and Nunavut (NU), Canada and east Greenland (Denmark). Reintroduced or translocated populations of muskoxen are currently found in west Greenland, Russia, Alaska (USA), and

portions of the Yukon and Quebec (Canada; Cuyler et al., 2019). Muskoxen are keystone Arctic species, facilitating nitrogen and soil nutrient turnover while also having significant cultural, nutritional, and economic roles for Indigenous People of the Arctic (Mosbacher et al., 2016; Tomaselli, Gerlach, et al., 2018). Muskoxen contribute to community identity in the creation of art, tools, and clothing, but also local food security, employment, and revenue through sport hunting and sales of muskox by-products such as its specialized wool, qiviut (Tomaselli, Gerlach, et al., 2018). Harvests were suspended in 2012 due drastic declines in muskox populations on Banks Island (NWT) and Victoria Island (NU; Kutz et al., 2015; Tomaselli, Kutz, et al., 2018). These population declines occurred concurrently with changing climatic conditions including severe icing events, vegetation shifts, and northward range expansions of pathogens (Cuyler et al., 2019; Mavrot et al., 2020; Tomaselli et al., 2016, 2019). As Banks and Victoria Island populations were two of the largest endemic populations, these declines incited concern for muskox health and sustainability (Cuyler et al., 2019; Kutz et al., 2015).

Muskox populations have notoriously low levels of genetic diversity resulting from a combination of population bottlenecks, founder effects, and population fragmentation across their range (Groves & Shields, 1997; Holm et al., 1999; MacPhee et al., 2005; Prewer et al., 2019; Van Coeverden De Groot & Boag, 2004). Confounding the premise that low genetic diversity may be associated with population declines on Victoria and Banks Island is the fact that other populations across Canada remained stable or grew with unknown reasons for this dichotomy (Cuyler et al., 2019). Past diversity estimates from microsatellite studies (Holm et al., 1999; Prewer et al., 2019; Van Coeverden De Groot & Boag, 2004), as well as genotyping by sequencing by Hansen et al. (2018), focused on neutral regions of the genome. While informative, these studies did not elucidate information related to the adaptive capacity of muskoxen that might be associated with demographic trends in context of changing selective

factors on the landscape (such as those from climate change and a warming Arctic). Elucidating the functional genomic variation across muskox populations may provide insight into muskox population health and vulnerabilities but requires a better genomic foundation. Access to a reference genome would offer the ability to assess the genetic diversity of genes linked to evolutionary and local muskox adaptations such as increased digestion, cold resistance, and immune response (Adamczewski, Flood, et al., 1994; Adamczewski, Kerr, et al., 1994; Brix et al., 1990). As such, genome assemblies have the potential to provide insight into how evolutionary and demographic histories have influenced patterns of genomic diversity in muskoxen as well as their capacity to adapt to a rapidly changing environment.

Herein, we report the first assembly and annotation of the muskox genome using high throughput Illumina sequencing and cross-species in silico mate pair library construction. Our final genome assembly and annotation was used to reconstruct a phylogenetic tree and estimate divergence times of this unique genus from other ungulate species. The genome was also used to perform pairwise sequentially Markovian coalescent (PSMC) analyses to identify historical trends in an effective population size. Our main goal was to provide a reference genome from which further population genomic studies could be performed, such as assessing the distribution of genetic diversity in muskoxen, identifying genes involved in their unique Arctic adaptations, and to better understand their capacity to respond to rapid environmental change.

3.3 Materials and Methods

3.3.1 Genome Sequencing

Genomic DNA for the reference genome assembly was extracted from the hide of a male muskox from Holman, Victoria Island, Canada using the Qiagen DNeasy blood and tissue kit

(Qiagen, Mississauga, ON, Canada). Quality of extracted DNA was assessed on a 1.5% agarose gel and quantified using PicoGreen. Extracted DNA was shipped on dry ice to the Centre for Applied Genomics at Sick Kids hospital, Toronto, ON, CA for library preparations and sequencing. Four paired-end libraries were prepared using Illumina TruSeq Nano DNA kit (Illumina, San Diego, CA, USA) with inserts sizes of 200 bp, 350 bp, 550 bp and 700 bp and two mate-pair libraries were created using the NxSeq Long Mate Pair Library Kit with insert sizes of 5 kbp and 8 kbp. Two paired-end libraries, with insert sizes 200 bp and 350 bp, were sequenced on the HiSeqX which produced approximately 940 million (2 × 151 bp) paired reads. The two additional, paired-end libraries with insert sizes of 550 bp and 700 bp, as well as two mate-pair libraries, were sequenced on the HiSeq2500 producing approximately 237 million (2 × 126 bp) paired reads.

3.3.2 De Novo Genome Assembly

We performed a series of data filtering steps to remove read contamination, low quality reads, and duplicate reads. We used FastQC v0.11.5 (Andrews) to check overall quality of the libraries after each step to assess how much data was removed and effectiveness of each program. First, we removed adapter and primer sequence contamination using scythe v0.994 (Buffalo) and EA utils v. 805 (Aronesty, 2011), with mate-pair libraries undergoing additional processing to split and remove chimera code, junction code and linker sequences as per the Lucigen NxSeq long mate-pair library kit protocol. Next, we performed low quality base trimming using sickle v. 1.33 (Joshi & Fass, 2011) with a quality score cut off set at Q = 25 and a minimum length set at 70% of the original read length. We then removed duplicate read pairs using FastUniq v. 1.1 (Xu et al., 2012) in order to remove any technical duplicates which may affect downstream scaffolding. Finally, we used bbsplit from the bbmap v37.22 (Bushnell) package to remove sequences

belonging to known lab contaminants using viral, bacterial, fungal and human database created by DeconSeq (Schmieder & Edwards, 2011). After data preprocessing, approximately 1.23 × 1011 bp remained from paired-end libraries and approximately 4.93 × 106 bp remained from mate-pair libraries for a theoretical coverage of ~85× based on the genome size of the domestic goat (2.9 Gb).

We performed an initial de novo assembly using SOAPdenovo2 v.240 software (Luo et al., 2012), which is a De Bruijn graph-based de novo assembler and then ran Gapcloser v.1.12-r6 (Luo et al., 2012) which uses short read library data to fill gaps that occur during scaffolding. To further improve the de novo genome assembly, we used cross-species-scaffolding v 1.0.1 (Grau et al., 2018). This reference-based scaffolding method uses genomes of closely related species to create scaffolding libraries in-silico that can then be used to re-perform scaffolding on assembled genomes. Cross-species-scaffolding requires a closely related genome, so we tested both domestic goat (GCA_001704415.1) and domestic sheep genomes (GCA_002742125.1) to create two separate sets of 20 mate-pair libraries with inserts ranging from 500 bp to 50 kbp. To determine which in silico library (goat or sheep based) produced the best overall assembly we used both datasets to scaffold and assemble multiple genomes with SOAPdenovo2, with kmers ranging from 51 to 127 bp. These genomes were then ranked using metrics estimated by Quast v4.4 (Gurevich et al., 2013) and BUSCOv3 (Simão et al., 2015), including NG50, number of Ns per 100 kbp and completed BUSCOs (Table S3.2) to identify which kmer value, and reference-based mate pair library, was optimal for downstream analyses.

Large sections of the muskox's mitochondrial genome were found within several long scaffolds of the top assembly. To address this issue, we further filtered cleaned paired-end and mate-pair reads to remove reads that corresponded to the mitochondrial genome. Mitochondrial reads were extracted from cleaned sequence libraries using the bbsplit tool from

bbmap by binning reads that mapped to the reference mitochondrial genome (GenBank FJ207536.1). Then, to create the mitochondrial genome, these reads were aligned to the reference (FJ207536.1) and the vcfutils.pl tool from the samtools package was used to call consensus sequences using default parameters. Finally, we assembled a new whole nuclear genome using SOAPdenovo2 with the newly filtered clean read dataset. This genome assembly was then used to re-perform genome scaffolding with the previously optimized cross-species-scaffolding library and kmer value. The final genome assembly was completed by a final round of Gapcloser.

3.3.3 Genome Annotation

We used Repeat Modeler v.1.0.10 (Smit & Hubley, 2008) software to create repeat libraries for the muskox genome assembly. We then used these libraries to perform hard masking of repetitive regions in the muskox genome using Repeat Masker v. 4.0.7 software (Smit et al., 2013). We retrained Augustus v. 3.2.3 software (Stanke & Morgenstern, 2005) to perform gene prediction on the masked genome. In order to create a set of training genes for Augustus, we used Gmap v2017-08-15 (Wu & Watanabe, 2005), Genemark v4.35 (Besemer & Borodovsky, 2005), Exonerate v. 2.2.0 (Slater & Birney, 2005) and EVidenceModeler v.1.1.1 software (Haas et al., 2008). Genemark-ES was used to perform ab initio gene predictions on the masked genome. We downloaded all sheep and goat EST (Expressed Sequence Tags) sequences from GenBank and used them to perform evidence-based gene prediction on the masked genome using Gmap software. We also downloaded all sheep and goat SWISS-PROT reviewed protein sequences from the UniProt-Kb database and performed evidence-based gene prediction of the masked genome using exonerate software. We used EVidenceModeler v.1.1.1 software to create a set of consensus genes from outputs produced by Genemark v4.35, Exonerate v. 2.2.0 and Gmap

v2017-08-15 software (Besemer & Borodovsky, 2005; Haas et al., 2008; Slater & Birney, 2005; Wu & Watanabe, 2005) with more weight given to the evidence-based predictions. This set of consensus genes was used to retrain Augustus software, creating a new set of parameters for muskoxen. These parameters were used to perform gene prediction on the masked genomes with the consensus gene set used as hints.

3.3.4 Evolutionary and Demographic History

Pairwise Sequentially Markovian Coalescent (PSMC) model was used to estimate effective population history following the pipeline outlined by Li et al. (2011). Sequences from cleaned paired-end libraries were aligned to the muskox genome using Burrows-Wheeler alignments (BWA) v. 0.7.17 (Li & Durbin, 2009). BAM alignments from each library were merged and duplicates were removed using MergeBamAlignment and MarkDuplicates from the Picard Toolkit (Broad Institute). The combined alignment was used to create consensus sequences using samtools v.0.1.15, vcfutils and bcftools (Li et al., 2009). Option -d was set to 20, as recommended to be set to a third of the coverage, while -D was set at 170, as recommended to be twice the average coverage. We then performed PSMC v.0.6.5 analyses using -p parameter suitable for modern humans (4 + 25*2 + 4 + 6) and bootstrapped using 100 iterations. The mutation rate 2 × 10−9 was chosen as the average mutation rate in mammals with a generation time of 10 years (Kumar & Subramanian, 2002).

To construct the phylogenetic tree, 1:1 orthologous genes were identified among 8 species (muskox-*Ovibos moschatus*, sheep-*Ovis aries*; GCA_000298735.1, goat-*Capra hircus*; GCA_001704415.1, cow–*Bos taurus*; GCA_002263795.2, horse -*Equus caballus*; GCA_002863925.1, pig–*Sus scrofa*; GCA_000003025.6, bison–*Bison bison bison*; GCA_000754665.1, yak–*Bos gruniens*; GCA_000298355.1). PorthoMCL (Tabari & Su, 2017) was

used to identify the 1:1 orthologous genes and corresponding coding sequences were aligned using Clustal-Omega. All alignments were concatenated using FasConCat v.1.04 software (Kück & Meusemann, 2010), and Jmodeltest v.2.1.10 was used to predict the best-fit model of nucleotide substitution (Darriba et al., 2012). A maximum likelihood tree was then generated in RAxMLv8 (Stamatakis, 2014) using the GTR+I+G model with 1000 bootstrap replicates and horse as the outgroup. MCMCtree from the PAMLv4 package (Yang, 2007) was used to convert the tree to an ultrametric format and to calibrate the nodes with known split times. Calibration minimums and maximums were given for sheep-goat divergence (3.9–8.1 Mya) from Chen et al. (2019) and for sheep-cow divergence (18.3–28.5 Mya) from Benton and Donoghue (2007).

3.4 Results & Discussion

3.4.1 Genome Sequencing and Assembly

We sequenced the draft genome of a male muskox from Victoria Island using 4 paired-end libraries and 2 mate-pair libraries. Across all 6 libraries, a total of 1,178,984,124 paired reads were generated. After filtering for low quality, duplicate and contaminant reads, 78.9% (843,572,921) paired reads remained for paired-end libraries, however only 0.04% (39,179) paired reads remained for mate-pair libraries (Table S3.1). Loss of mate pair library data was the result of duplicate read removal steps. Mate pair sequencing failure likely relates to a lack of sufficient quality DNA for mate pair library preparations, and thus PCR bias when sequencing. The overall result was a sequencing coverage of ~85× for the muskox genome based on a goat genome size of 2.9 Gb.

An initial draft assembly was performed by SOAPdenovo2 software v.2.04–r240 (Luo et al., 2012) using all paired-end libraries and low coverage of mate pair reads that remained after filtering. The resulting genome was fragmented with over ~822 K contigs and an N50 of 26,107

bp, meaning that half of the genome was made up of contigs of this length or larger, and a complete BUSCO score of 65.6% which is below that of other ungulate genomes (Chen et al., 2019). We assumed the lower genome quality as reflected by the metrics of # of contigs, N50 and BUSCO scores was likely associated with the lack of mate pair libraries, preventing SOAPdenovo2 v.240 (Luo et al., 2012) from combining contigs, especially those separated by large gaps or repetitive regions, into scaffolds. In order to improve genome quality, we used in silico mate-pair libraries created by Cross-Species-Scaffolding (v.2.2; Grau et al., 2018) by aligning the cleaned muskox reads to those of closely related species. Both sheep and goat genomes were used as a reference, after which assemblies were compared based on resulting N50, scaffold number and BUSCO scores amongst other common quality metrics (e.g., Ns per 100 kbp) to determine which genome would produce the best in silico mate-pair libraries, (Table S3.2). Cross-species scaffolding libraries created by both reference genomes greatly improved genome quality, but the assembly with the goat genome as reference, using a kmer value of 121, ranked highest. The best ranked genome had 6495 contigs, a complete BUSCO score of 87.5% and an N50 of 1,705,149 bp, which is 65× higher than the assembly without cross-species scaffolding. The use of cross-species scaffolding allowed us to create large scaffolds without further sequencing of long read data, mate pair libraries, and/or sequencing by ligation, but there are potential drawbacks when using related species scaffolding methods. First, cross-species scaffolding is limited by the quality of the initial genome assembly as well as the existence of a high-quality genome from a related species, though the increased availability of genomic resources may allow this method to be more widely used (Grau et al., 2018; Prasad et al., 2022). For example, in silico mate pair libraries have been used to improve genomes of fin whales, narwhals, gray's beaked whales as well as addax that similarly used the goat genome as a reference (Westbury, Petersen, Garde, et al. 2019; Westbury, Petersen & Lorenzen 2019;

Westbury et al.; Hempel et al. 2021). Additionally, as the arrangement of the contigs within the scaffolds are based on the genome of another species, this can limit analyses of genomic architecture, such as gene copy number and gene rearrangements (Grau et al., 2018; Prasad et al., 2022). Previous karyotype mapping has found muskoxen to be highly homologous to ancestral Pecora chromosomal arrangements, with five fusions of different chromosome arms forming submetacentric chromosomes (Biltueva et al., 1995; Desaulniers et al., 1989; Pasitschniak-Arts et al., 1994; Proskuryakova et al., 2019). However, G−, C− and R− banding found many muskox chromosomes to be either identical or strikingly similar to those of goats. These data further strengthen the validity of using the goat genome as a reference for cross-species scaffolding of muskoxen, and likely explain why we found goats produced a better muskox assembly than the sheep genome (Biltueva et al., 1995; Desaulniers et al., 1989; Pasitschniak-Arts et al., 1994; Proskuryakova et al., 2019). The removal of mitochondrial reads did not greatly improve the quality of the initial genome assembly with a complete BUSCO score of 64.2% and an N50 of 26,274 however using this genome as the base to reperform cross-species scaffolding had a large impact on the final genome assembly quality.

The final draft genome produced 8659 scaffolds for a cumulative length of 2,621,890,883 bp, with a contig and scaffold N50 of 38,369 bp and 13,200,690 bp, respectively. The scaffold N50 was 7× better than our previous assembly that used cross-species scaffolding prior to mitochondrial read filtering. The longest scaffold was 50,595,910 bp long, where 2,601,612,364 bp of the assembly was made up of contigs over 25,000 bp in length. Based on cumulative length and Nx plots (Figure S3.1) these quality metrics show that the draft genome was mainly composed of large scaffolds with few short sequences. Completeness of the draft genome was further assessed with Benchmarking Universal Single-Copy Orthologs (BUSCO v.3; Simão et al. 2015) vertebrata gene set. Of the 3023 genes in the BUSCO gene set, 2658 (87.9%)

complete single copy orthologs were identified in the muskox draft genome, indicating that gene prediction would identify a large percentage of completed genes in muskoxen. Additional BUSCO scores and basic statistics of the assemblies, pre- and post-cross-species scaffolding, as well as pre- and post- mitochondrial read removal, are shown in Table 3.1. The draft assembly was deposited in GenBank (JACAUE000000000).

3.4.2 Genome Annotation

Repeat masking was performed prior to gene predictions using RepeatMasker (v.4.0.7; Smit et al. 2013) that identified 42.39% of sequences were made up of interspersed repeats. Once repeats were masked, both homology and ab initio predictions were used to identify protein coding genes. Genes predicted by both methods were combined using EVidence Modeler (EVM). These genes were then used as a training set for Augustus (v.3.2.3; Stanke & Morgenstern 2005) which performed the final gene prediction with the combined consensus gene set as hints. A total of 19,132 genes were predicted with an average of 24.9 kb per gene, 1461 bp per coding DNA sequence, and 154 bp per exon. Of the predicted genes, 12,848 (67%) were annotated by Interpro for Gene Ontology and 11,349 genes (59%) aligned to the National Centre for Biotechnology Information (NCBI) non-redundant protein database (Figure S3.2). In comparison to the genome annotations of other ungulate species, the number of genes and average coding sequence length of the muskox genome assembly are within the norm (Chen et al., 2019). We also compared genomes using quality metrics of contig and scaffold N50s, and complete BUSCO scores. The quality of the draft muskox genome assembled herein falls within those presented by Chen et al. (2019) for other ruminant species. Genome assembly and annotation metrics of the final muskox genome are shown in Table 3.2 in comparison with the highest quality

ruminant genomes assembled by Chen et al. (2019) as well as their assembly of another Arctic ruminant (reindeer).

3.4.3 Evolutionary and Demographic History

In order to compare coding sequences of muskoxen to orthologous genes among Caprinae and other mammals, PorthoMCL was used to identify orthologs amongst 8 ungulate species. The ortholog set was then filtered to contain only 1:1 orthologs and resulting in 893 genes used for the phylogenetic tree. As expected, the ultrametric and time calibrated phylogenetic tree from mcmctree shows that muskoxen are more closely related to sheep and goats than cows (Figure 3.1; Groves & Shields 1997; Shafer & Hall 2010; Pasitschniak-Arts et al. 1992). From this tree, muskoxen diverged from sheep and goats approximately 12 million years ago (Mya) and this sister clade diverged from cows approximately 21.6 Mya. The divergence time of the sheep and goats' clade is comparable with times previously estimated in the literature, as are the branch dates of other species included in the tree (Chen et al., 2019). Previous studies using the mitochondrial genome estimate muskoxen diverged from sheep and goats ~8–15 Mya (Bibi, 2013; Toljagić et al., 2018), values that fall within our confidence intervals of 8–17 Mya. Mitochondrial gene sequences have been found to have lower resolving power over nuclear exons when investigating phylogeny reconstructions, therefore the analyses performed based on the nDNA genome assembly should be more accurate (Springer et al., 2001) in describing the phylogeny and divergence times of this unique genus.

We used the Pairwise Sequentially Markovian Coalescent (PSMC) model to assess historical patterns of effective population size (N_E) (Figure 3.2) (Li & Durbin, 2011). At 3 Mya, there is a relatively low N_E at ~35,000, and over the next 500 thousand years, N_E increases until it reaches 100 k. At 1 Mya, N_E begins to steadily decline until it reaches an N_E of ~2 k. This low N_E

remains from ~20—40 thousand years ago (Kya) before showing a slight recovery ~15 Kya, reaching an N_E ~5 k. Low points in muskox N_E starting at 3 Mya and 40 Kya coincide with major glaciation events. The start of the PSMC occurred during a major glaciation event occurring ~3.15–2.75 Mya, referred to as the climate crash (Bartoli et al., 2005) and we see N_E recovery follows immediately after this period. The second low point represents a bottleneck occurring during the last glacial maximum (LGM) ~26.5—19 Kya where once again a recovery is observed only after the LGM ended [62], though the decline of N_E was continuous from 1 Mya onward.

Previous muskox diversity analyses performed by Hansen et al., (2018) used ddRAD sequencing to assess neutral genome wide variation in muskox populations, where stairway plot analyses were used to assess N_E through time. The analyses by Hansen et al. (2018) of the mainland west population, the closest geographical population to the sample used in this study, showed that N_E was at a stable high of ~13 k at 50 Kya until ~27 Kya when N_E began to steadily decline. Hansen et al. (2018) showed the population decline started to plateau at ~15 Kya with N_E estimates remaining at ~9 k at 10 Kya. In comparison to the PSMC analyses performed herein, N_E estimates at 10 Kya are nearly doubled in analyses by Hansen et al. (2018). Additionally, where Hansen et al. (2018) found population declines occurred between ~27 Kya and ~15 Kya, the PSMC analyses performed in this study found N_E stable or increasing in size between this time frame. Finally, the high N_E reported in Hansen et al. (2018) between ~27 Kya and 50 Kya coincides with an N_E low according to PSMC analyses with N_E estimates 6× higher in Hansen et al. (2018). Discrepancies between these two results likely relate to the difference in reference genomes used to call genotypes, where Hansen et al. (2018) used the sheep genome, and the PSMC analyses performed herein used the cross-species scaffolded muskox genome. Prasad et al. (2022) performed variant calling using the reference genomes of related species of varying phylogenetic distance and compared them to variants called using cross-species scaffolded

genomes. When used for downstream analyses such as PSMCs, analyses using cross-species scaffolded genomes were more reliable than mapping directly to the reference genome of related species, even if the cross-species genome was highly fragmented (Prasad et al., 2022). Overall, Prasad et al. (2022) found that if a suitable reference genome did not exist for a species of interest, cross-species scaffolding provides a good reference genome alternative. As such, the use of the muskox genome assembled herein may provide a better reference for demographic analyses such as the PSMCs than published sheep and goat genomes.

3.5 Conclusions

In this study, we present the first draft genome for muskoxen, the only living member of the *Ovibos* genus. A combination of paired-end and in silico mate-pair libraries resulted in an assembly with an N50 of 13 Mb and a BUSCO score of 88.7%. The application of in silico mate pair libraries greatly improved genome scaffolding and genome quality but does not fully the replace the need for further sequencing of long read data, mate pair libraries, and/or sequencing by ligation to further enhance the assembly of the muskox genome. Beyond the development of a draft genome assembly and annotation, these data were used to reconstruct a phylogenetic tree, estimate the divergence time of this unique genus, and assess trends in historical effective population sizes via pairwise sequentially Markovian coalescent (PSMC) analyses. Divergence estimates were consistent with previous studies using mitochondrial DNA, while PSMC analyses found effective population lows coinciding with major glaciation events. With the addition of genomes from diverse muskox populations, future research should include the identification of positively selected genes to gain insight into the muskox's key Arctic adaptations whose genetic underpinnings remain unknown. Overall, these data provide a solid foundation for further genome sequencing to elucidate patterns of gene flow, drift, and

selection to better understand the muskox's varying demographic and evolutionary histories and their capacity to adapt to rapid environmental change.

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Table 3.1 Comparison of quality metrics for genome assemblies pre- and post-cross-species-scaffolding and pre- and post-mitochondrial read filtering. Mx1 is the initial scaffold level assembly prior to both cross-species scaffolding and mitochondrial read filtering; Mx2 is the scaffold level genome assembly after cross-species scaffolding, but prior to mitochondrial DNA filtering; Mx3 is the scaffold level genome after mitochondrial read filtering, but prior to cross-species scaffolding; Muskox Final is the final genome assembly with columns for both contig and scaffold levels of assemblies.

Program	Quality metric	Mx1	Mx2	Mx3	Muskox Final (contig)	Muskox Final (scaffold)
QUAST	Assembly length	2,528,575,139	2,704,466,437	2,527,322,807	2,473,404,411	2,621,890,883
	Assembly length over 25000 bp	1,310,898,307	2,687,729,513	1,275,654,848	1,683,252,947	2,601,612,364
	Number of contigs	822,935	6,495	868,473	114,189	8,659
	Longest contig	328,372	9,453,317	301,459	388,735	50,595,910
	L50	27,014	494	26,422	18,651	61
	N50	26,107	1,705,149	26,274	38,369	13,200,690
	N per 100kbp	5056.29	10,272	5,035	0.93	5,664
	GC content	41.56	41.81	41.55	44.53	44.53
BUSCO	Completed BUSCO (out of 3023)	1,984 (65.6%)	2,646 (87.6)	1,953 (64.6%)		2,673 (88.4%)
	Single Copy Completed BUSCO (out of 3023)	1,973 (65.2%)	2,623 (86.7)	64.2(1941)		2,658 (87.9%)
	Duplicated BUSCO (out of 3023)	14 (0.4%)	14 (0.4%)	0.4(12)	N/A	15 (0.5%)
	Fragmented (out of 3023)	448 (14.8%)	223 (7.1%)	15.2(458)		212 (7%)
	Missing (out of 3023)	591 (19.5%)	163 (5.3%)	20.2 (612)		138 (4.6%)

Table 3.2 Comparison of the final muskox genome assembly to both another Arctic ruminant (reindeer) and the highest quality wild ruminant genomes assembled by Chen et al.

	Ovibos moschatus	***Okapia johnstoni***	***Rangifer tarandus***	***Moschus berezovskii***	***Procapra przewalskii***	***Capra ibex***	***Ammotragus lervia***
Common name	Muskox	Okapi	Reindeer	Forest musk deer	Przewalski's gazelle	Ibex	Barbary Sheep
Scaffold N50	13,200,690	3,620,116	1,059,113	2,509,225	5,152,914	15,190,720	1,263,981
Contig N50	38,369	58,892	91,805	57,721	20.,018	24.835	18,541
BUSCO %	88.4%	90.10%	90.40%	85.50%	89.10%	92.50%	90.40%
Number of genes	19,132	19,568	21,555	22,475	23,562	21,204	23,439
Average cds	1,461	1,518	1,440	1,489	1,150	1,544	1,671

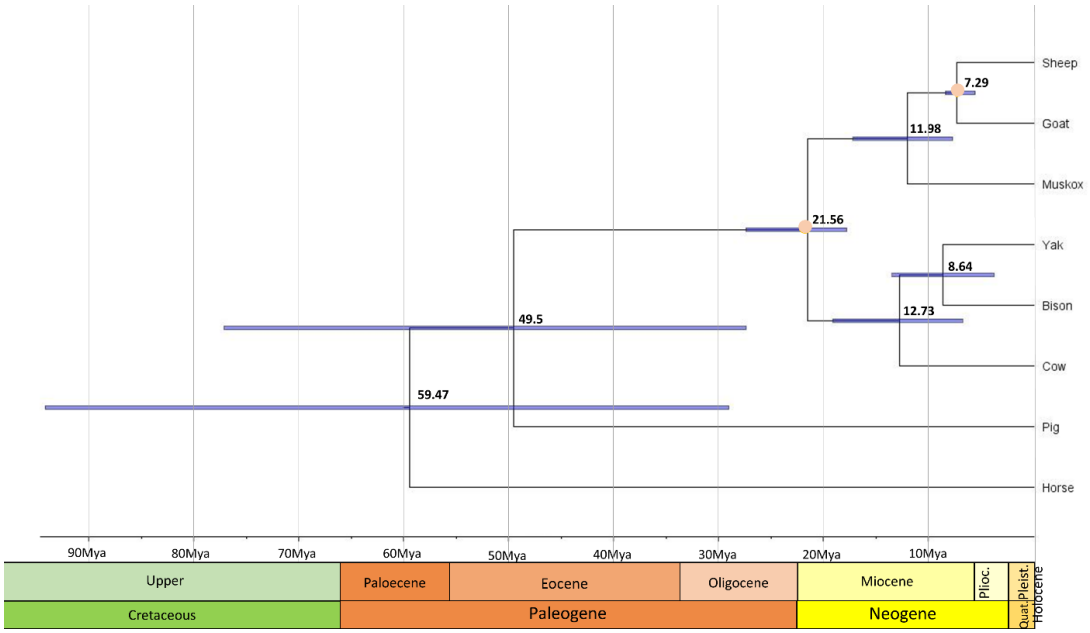

Figure 3.1. Evolution of gene families among muskoxen and non-Arctic relatives. The phylogenetic tree was constructed using 893 single copy orthologs across all 8 ungulate species. Divergence times are in black, orange dots represent the calibration points, and 95% credible intervals are represented by blue node bars.

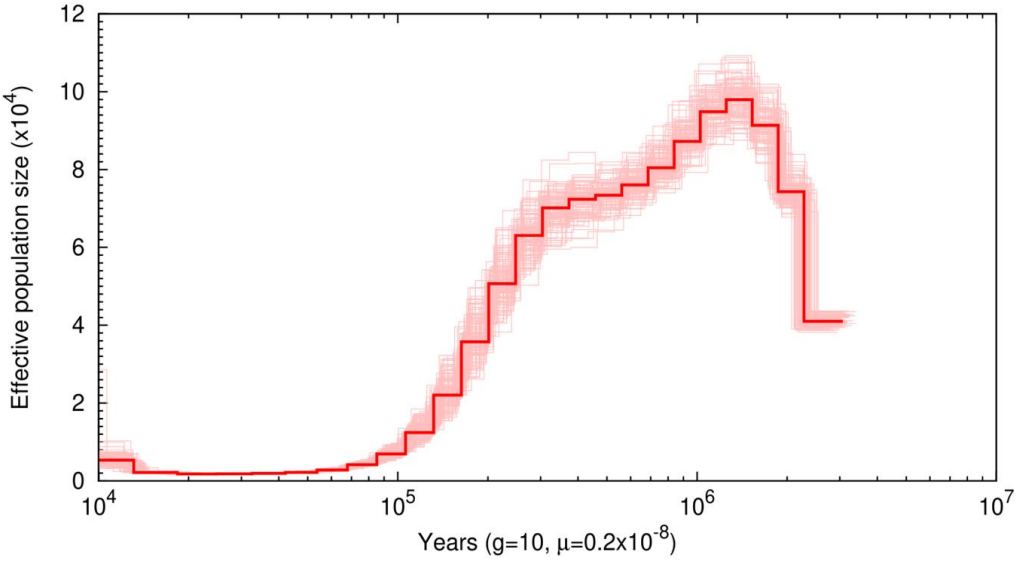

Figure 3.2. Pairwise sequentially Markovian coalescent (PSMC) plot of the muskox genome with 100 bootstrap repetitions assuming a generation time of 10 and substitution rate of 0.2 × 10−8 The x-axis represents time before present on a log scale and y axis represents effective population size.

Chapter 4. Whole genome resequencing of *Ovibos moschatus* reveals divergence and relative vulnerabilities among muskox subspecies

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A version of this chapter is *in prep* for submission.

Contributions: CJK and EP conceived and designed the study. SK, LML, MF, CC and CJK provided samples, and resources. EP performed the formal analyses and prepared draft manuscript. CJK edited the final manuscript. CJK and SK provided funding acquisition.

4.1 Abstract

Genetic diversity is intricately associated with the adaptive and evolutionary potential of species. Thus, understanding the distribution and drivers of genetic diversity, both historical and contemporary, can reveal a species' vulnerabilities to rapid changes in environmental selective pressures and inform effective conservation strategies. Genome sequencing improves the resolution of traditional diversity metrics and introduces new measurements that make use of information stored in long stretches of DNA. Combined, traditional and genomic diversity metrics offer insights into processes of genetic drift, gene flow, selection, and population history relevant to varied fitness across landscapes, subpopulations, and subspecies.

Muskoxen are iconic Arctic ungulates with notoriously low genetic diversity. Changing suites of selective pressures from invasive pathogens and rapid Arctic warming pose major threats to muskox persistence. Phenotypic variation delineates two subspecies that vary in their high Arctic (white-faced) and mainland (barren-ground) distributions, yet limited genetic diversity among groups has undermined these subspecific designations. Southern distributions of white-faced muskoxen have recently undergone severe population declines. In contrast, barren-ground populations have remained stable or grown in size.

Twenty-two muskox genomes were used to assess genetic diversity and differences among subspecies and provide insight into the evolutionary potential of populations from Canada and Greenland. Phylogenetic analyses, using ultra conserved nuclear DNA regions, identified monophyletic clades associated with subspecific divergence and geographic region. Admixture analyses clustered individuals by subspecies, lineage, and geographic region, with evidence of subspecific gene flow between Victoria Island and mainland Canada. White-faced muskoxen, relative to their barren-ground counterparts, had much lower estimates of

nucleotide diversity, effective/census population size ratios, observed heterozygosity, and heterozygous rich regions, with concomitantly higher estimates of inbreeding and runs of homozygosity. Overrepresented GO terms within overlapping runs of homozygosity of white-faced muskoxen provided insight into the genetic basis of their distinctive morphology. These data suggest white-faced muskoxen may be more vulnerable to rapid changes in selective pressures, though genetic rescue may be possible via gene flow with barren-ground populations. Overall, this study provides insight into the mechanisms that contribute to genetic diversity maintenance, population persistence and vulnerability.

4.2 Introduction

Genomic investigations provide unprecedented insight into otherwise cryptic aspects of the drivers and distributions of genetic variation (Hohenlohe et al., 2021; Zimmerman et al., 2023). Metrics such as the loss of genetic variation over time, effective population size (N_E) estimates, selection, inbreeding, admixture, genetic load, and demographic histories of populations, all have important implications in assessing the relative vulnerabilities of species and their subpopulations to changing selective pressures (Hohenlohe et al., 2021; Leroy et al., 2017; Willi et al., 2022; Zimmerman et al., 2023). For example, genome sequencing of Scandanavian wolverines (*Gulo gulo luscus*) revealed a long-term decline in effective population size, spanning thousands of years, suggesting contemporary pressures do not fully explain observed population declines (Ekblom et al., 2018). Sequencing the Tasmanian devil (*Sarcophilus harrisii*) genome, a species of conservation concern threatened by a facial cancer disease, identified genomic regions associated with tumour regression, data that has been integrated into genetic management and conservation of this species (Epstein et al., 2016; Hohenlohe et al., 2021; Hubert et al., 2018). Beyond refined assessments of effective population sizes and identifying genes directly associated with fitness, whole genomic sequencing also provides access to

analyses and metrics that inform species diversity, divergence, and health; data derived from information stored within long stretches of DNA (Bizarria dos Santos et al., 2021; Weng et al., 2022; Zimmerman et al., 2023).

Runs of homozygosity (ROH), continuous homozygous regions in the genome, have become a key diversity metric by providing insight into inbreeding, selection, and genetic drift (Bizarria dos Santos et al., 2021; Cesarani et al., 2021; Colpitts et al., 2022; Fabbri et al., 2021; Mulim et al., 2022; Selli et al., 2021; Sun et al., 2015; Weng et al., 2022). The percentage of the genome covered by ROHs can assess inbreeding levels (FROH), while the length of the ROHs can indicate the timing of inbreeding events (Bizarria dos Santos et al., 2021; Cesarani et al., 2021; Dzomba et al., 2021; Fabbri et al., 2021; Selli et al., 2021; Sun et al., 2015). Specifically, short ROHs connote more ancient inbreeding, whereas longer ROHs point to more recent inbreeding, as fewer generations of recombination have occurred (Bizarria dos Santos et al., 2021; Cesarani et al., 2021; Dzomba et al., 2021; Fabbri et al., 2021; Selli et al., 2021; Sun et al., 2015). In addition, ROHs occurring at the same genomic region across a large proportion of a population (ROH islands) can be indicative of directional selection (Bizarria dos Santos et al., 2021; Colpitts et al., 2022; Fabbri et al., 2021; Mulim et al., 2022). For instance, ROH islands in Maremmana cattle contained genes involved in the regulation of body weight, fat, and energy metabolism, as well as climate adaptation (Biscarini et al., 2020; Selli et al., 2021). In contrast to the genomic metric ROH, heterozygous rich regions (HRRs) are sections of the genome with increased variability (Biscarini et al., 2020; Selli et al., 2021). Genomic regions where HRRs are present in a high frequency within a population represent HRR islands and are suggestive of balancing selection, where high diversity is beneficial to overall fitness (Bizarria dos Santos et al., 2021; Colpitts et al., 2022; Dzomba et al., 2021; Fabbri et al., 2021; Mulim et al., 2022). HRR islands identified in pigs, for example, are made up of genes essential to disease resistance and fertility

(Biscarini et al., 2020; Chen et al., 2022). HRR islands can thus reveal genes important to species survival, interactions with the environment, and act as a complement to ROH islands in identifying genes associated with positive selection and physiological adaptations (Bizarria dos Santos et al., 2021; Colpitts et al., 2022; Dzomba et al., 2021; Fabbri et al., 2021; Mulim et al., 2022).

Beyond the ability to contextualise the distribution of genetic diversity, timing of inbreeding, and improve the resolution of gene flow, structure, and admixture estimates, genome-wide data provides insight into deeper phylogenetic interactions (Young & Gillung, 2020; Zhang et al., 2019). Traditionally, phylogenetic trees are reconstructed using a limited number of loci or genes, which can result in stochastic or sampling errors and affect the inference of backbone nodes with poor resolution or support (Young & Gillung, 2020; Zhang et al., 2019). Genomic data allows for complex phylogenetic reconstructions with hundreds to thousands of loci at a time, reducing stochastic error and improving node resolution (Young & Gillung, 2020; Zhang et al., 2019). For example, genome scale data helped resolve conflicting nodes and detect novel tribal relationships among rodents within the *Sigmodontinae* family (Parada et al., 2021). Thus, using genomic data in this way improves understanding of historical and contemporary gene flow patterns; data that inform wildlife management actions that may erode genetic differentiation or impact local adaptation (Ghani et al., 2022; Woodruff, 2001). Overall, genomic analyses become increasingly important as climatic changes introduce new selective pressures to environments at rates that may be incompatible with the adaptive capacity of species, notably species with low genetic diversity and long generation times (Diamond & Martin, 2020; Forester et al., 2022; Parmesan, 2006).

Muskoxen are an iconic Arctic species most closely related to sheep and goats (Groves & Shields, 1997; Pasitschniak-Arts et al., 1992; Prewer et al., 2022; Shafer & Hall, 2010). Muskoxen

have both obvious Arctic environment adaptations, such as a specialised wool, qiviut, highly prized for its thermal and textural qualities, and more cryptic adaptations, such as unique gut microbiomes for Arctic forage (Bird et al., 2019; Prewer et al., 2023). The global population size of muskoxen is ~ 127,000 individuals, comprised of endemic and introduced herds distributed across the Canadian Arctic, Greenland, Alaska, and Russia (Gunn & Forchhammer, 2022). The International Union for Conservation of Nature (IUCN) conservation status of muskoxen is "least concern" given their current global population size and broad geographic distribution, despite some populations being in steep decline (Cuyler et al., 2019; Gunn & Forchhammer, 2022; Kutz et al., 2015). Morphological differences have led to two proposed subspecies of muskoxen (Cuyler et al., 2019; Groves, 1997; Hansen et al., 2018; Tener, 1963). White-faced muskoxen (*Ovibos moschatus wardii*) are endemic to Arctic islands and Greenland, with white pelage colouring their saddle and face (Cuyler et al., 2019; Groves, 1997). White-faced muskoxen are typically smaller in comparison to barren-ground muskoxen (*Ovibos moschatus moschatus*). Barren-ground muskoxen have brown pelage across their body and face and are distributed across the Canadian Arctic mainland (Cuyler et al., 2019; Groves, 1997). While muskox subspecies are not officially recognized by the IUCN due to a lack of both genetic divergence at mitochondrial DNA markers and significant morphometric differences (Groves, 1997; Gunn & Forchhammer, 2022; Tener, 1963), the recent genetic work using genotyping by sequencing suggests subspecific status may be warranted (Cuyler et al., 2019; Groves, 1997; Gunn & Forchhammer, 2022; Hansen et al., 2018). Subspecific designations of muskoxen are potentially pertinent in context of differing respective population trends across the landscape. There have been relatively recent and severe declines of two white-faced muskox populations at the southern end of their distribution, resulting in losses as high as 80% in 3 generations (Cuyler et al., 2019; Gunn & Forchhammer, 2022). These declines occurred on Banks Island and Victoria

Island that previously held ~ 61% of the global muskox population. Declines in these populations have been attributed to changing selective pressures associated with Arctic warming, including range expansions of pathogens, extreme icing, shifting vegetation and nutritional deficits (Afema et al., 2017; Cuyler et al., 2019; Gunn & Forchhammer, 2022; Kafle et al., 2020; Kutz et al., 2015; Prewer et al., 2019; Tomassini et al., 2019). Concurrently, more southern, but proximally adjacent, barren-ground muskox populations have grown in size, despite longer term exposure to these same presumed, selective pressures (Cuyler et al., 2019; Gunn & Forchhammer, 2022). In contrast, northern white-faced muskox populations outside of Banks and Victoria Islands have thus far remained stable (Cuyler et al., 2019; Gunn & Forchhammer, 2022).

Genetic studies of muskoxen, using a spectrum of molecular markers, have all found very low genetic diversity, observations attributed to repeated bottlenecks, and founder effects subsequent to glacial retreats (Campos et al., 2010; Hansen et al., 2018; Pečnerová et al., 2024; Prewer et al., 2019). Mitochondrial DNA (mtDNA) analyses showed only 1.4% of sites were variable in the control region, with cytochrome b gene diversity even lower (Groves, 1997; Groves & Shields, 1997; MacPhee et al., 2005). Nuclear DNA microsatellite marker analyses by Prewer et al. (2019) found heterozygosity values as low as 0.16, notably lower than other at-risk ungulates (Cronin et al., 2005; Worley et al., 2004), but also that muskoxen may have reached a genetic diversity minimum based on forward in time simulations. One of the most revealing aspects of low diversity in muskoxen comes from the immunogenetic, major histocompatibility complex DRB2 region of the genome, expected to have high diversity maintained by balancing selection, where only one allele was found among 43 individuals (Mikko et al., 1999). Broader scale assessments of genetic diversity, using genotyping by sequencing via ddRadSeq and whole genome sequencing, found muskox heterozygosity estimates as low as many endangered

species, with a decline in genetic diversity along a northeastern expansion front (Hansen et al., 2018; Pečnerová et al., 2024). Most recently, whole genome resequencing of 108 muskoxen revealed white-faced muskox populations had the lowest rates of heterozygosity recorded in ungulates (Pečnerová et al., 2024). Further, comparisons between contemporary and ancient muskox genomes revealed current day muskoxen have one third of the diversity, likely shaped by Pleistocene refugia, recolonisations and inbreeding (Pečnerová et al., 2024). Together, these data suggest muskox populations may be vulnerable to changing selective pressures in their environment, as their limited genetic diversity, relatively long generation times, and restricted northern distributions likely undermine their capacity to adapt (Kardos et al., 2021; Parmesan, 2006; Pečnerová et al., 2024; Torda & Quigley, 2022; Zimmerman et al., 2023).

Previous genetic assessments of muskoxen have been consistent in suggesting all measured populations are genetically depauperate (Groves, 1997; Hansen et al., 2018; Mikko et al., 1999; Pečnerová et al., 2024; Prewer et al., 2019). With reference genomes now available for muskoxen (Prewer et al., 2022), there is the capacity to more fully assess genetic factors impacting population vulnerabilities using whole genome resequencing. Herein, 22 muskox genomes were resequenced from across their natural range, including individuals from the Canadian mainland, the Arctic Archipelago and Greenland. This sample scheme allowed for comparisons of muskox diversity across a latitudinal gradient and in both subspecies. Importantly, this study is the first to perform population genomics with samples from Victoria Island, one of the two muskox populations undergoing severe declines. The objectives of this study were to: a) create phylogenetic trees to gain insight into the divergence of muskox populations and to add to the taxonomic data available for muskoxen overall; b) perform admixture analyses to assess historical and contemporary connectivity between populations; c) develop a more thorough estimate of genome wide diversity for endemic white-faced muskox

populations relative to barren-ground individuals; d) indirectly infer genomic vulnerability by assessing inbreeding, N_E, HRR and ROH metrics; and e) detect signatures of balancing and directional selection acting on muskoxen across the Arctic landscape by identifying ROH islands and HRR islands. Cumulatively, these genomic metrics and enhanced phylogenetic analyses will help identify unique and vulnerable muskox populations, as well as provide insight into which populations are more suitable for reintroduction or gene flow facilitation, improving our ability to conserve this unique Arctic species.

4.3 Methods

4.3.1 Genome sequencing

Muskox hide and tissue samples were collected from 22 muskoxen across most of their natural Arctic range (Figure 4.1). A minimum of 2 individuals were selected from each island in this study, providing 18 individuals from the proposed white-face muskox group, and 4 from the barren-ground muskox group. Selected samples could also be subdivided into population clusters identified by Hansen et al. (2018) with a minimum of 4 individuals per cluster for comparison. DNA extraction, library preparation, and sequencing were performed in two separate runs. For the first run, high molecular weight genomic DNA was extracted from 4 individuals (Kugluktuk 1, Kugluktuk 2, Victoria Island 3, and Victoria Island 4) using a standard phenol chloroform extraction. For the second run, the remaining 18 samples (Greenland 1, Greenland 2, Greenland 3, Greenland 4, Bathurst Island 1, Bathurst Island 2, Cornwallis Island 1, Cornwallis Island 2, Ellesmere Island 1, Ellesmere Island 2, Kent Peninsula 1, Kent Peninsula 2, Victoria Island 1, Victoria Island 2, Victoria Island 5, Victoria Island 6, Devon Island 1, Devon Island 2) were extracted using the Qiagen DNeasy blood and tissue kit. DNA quality and quantity for all samples were estimated using a Tapestation 4200. Extracted DNA was shipped on dry ice

to the Centre for Applied Genomics at the Hospital for Sick Children, in Toronto, for library preparation and sequencing. All samples were sequenced on a HiSeqX, producing ~500 bp insert paired end libraries of 150bp in length. The first genome sequencing run had 4 muskox libraries across 4 flow cells, aiming for an estimated coverage of ~20x. The second run included all remaining 18 muskox libraries across 6 flow cells, aiming for an estimated coverage of ~10x. Basic quality and quantity statistics of the raw genome sequences for each muskox library were assessed via FastQC (v 0.11.9; Andrews). Adapter and primer sequences were removed from the reads using bbduk, a bbmap package (v. 38.86; Bushnell). Low quality base trimming was performed using sickle (v. 1.33; Joshi and Fass 2011), with a minimum length requirement of 120 bp, and a quality score cut off set at Q25. Contaminating reads from known lab viral, bacterial, fungal and human databases were removed using bbsplit, a bbmap package (v. 38.86 Bushnell).

4.3.2 Phylogenetics

Phyluce Tree

Phyluce (v1.7.1; Faircloth 2016) was used to identify ultra conserved regions (UCE) in the muskox genome, assembled by Prewer et al (2022; JACAUE000000000.2). Common UCEs amongst muskoxen and 5 ungulate species (sheep-*Ovis aries*; GCA_000298735.1, goat-*Capra hircus*; GCA_001704415.1, cow–*Bos taurus*; GCA_002263795.2, horse -*Equus caballus*; GCA_002863925.1, bison–*Bison bison bison*; GCA_000754665.1) were identified using the Phyluce "harvesting UCE loci from genomes" workflow. These UCE sequences were extracted from the muskox genome and used as reference to extract, align and create corresponding UCE sequences for the 22 resequenced genomes in this study via the bbsplit package from bbmap (v. 38.86; Bushnell) and ATRAM (v2.0; Allen *et al.* 2015). The Phyluce "UCE phylogenomics" workflow was then used to align UCEs from the resequenced muskoxen and 6 other ungulate

species. Assembled UCE loci were aligned using mafft and trimmed using Gblocks. Alignments were filtered for completeness (percent of taxa present at each locus), creating an alignment where 95% of taxa were present at each locus. This alignment, as well as an alignment with only muskox sequences, were used by RAXML (v 8.2.12; Stamatakis 2014) to estimate a phylogenetic tree with 1000 bootstraps. Low bootstrap branches or nodes (<85%) were collapsed.

Mitochondrial Tree

Mitochondrial reads were extracted from cleaned raw reads for each resequenced individual using the bbsplit package from bbmap (v. 38.86; Bushnell) using the muskox mitochondrial genome as reference (NC_020631.1), and aligned using bwa mem (v. 0.7.17; Li and Durbin 2009). The resulting bam file was used by samtools (v1.16; Li *et al.* 2009) to create a consensus mitochondrial genome with the consensus function. Bedtools (v2.29.2; Quinlan and Hall 2010) was used to extract muskox mitochondrial coding regions which were then aligned following the Phyluce "UCE phylogenomics workflow". A phylogenetic tree was then created using RAXML (v 8.2.12; Stamatakis 2014) with 1000 bootstraps, where individuals with identical coding region sequences concatenated and where low confidence branches or nodes were collapsed.

4.3.3 Diversity Statistics

Satsuma synteny (v2.1; Grabherr *et al.* 2010) was employed to identify and remove scaffolds aligning to sex chromosomes from the reference muskox genome assembly. Seqtk (v1.3; Li) was then used to remove scaffolds from the reference muskox genome below 10 kbp in length. Remaining long, autosomal scaffolds were then used as reference for read alignment with cleaned reads from resequenced muskox genomes using bwa-mem (0.7.17; Li and Durbin 2009). As each individual was sequenced across 4-6 lanes, each lane was aligned individually and markduplicates from picardtools was used to remove duplicate reads (v. 2.26.3; Picard Tools

2022). Markduplicates was then used to combine lane data for each individual and remove duplicate reads from the combined datasets. Sambamba (v. 0.8.0; Tarasov *et al.* 2015) was then used to select uniquely mapped reads, followed by indel realignment using GATK (v. 4.2.4.0; Van der Auwera and O'Connor 2020). ANGSD (v. 0.939; Kim *et al.* 2011; Korneliussen *et al.* 2014) was used to call genotype likelihoods using the following parameters: - doVcf 1 -doGeno -4 -doPlink 2 -doPost 1 -gl 2 -SNP_pval 1e-6 -minMapQ 20 -minQ 20 -doCounts 1 -skipTriallelic 1 -doGlf 2 -doMajorMinor 1 -doMaf 2 -minmaf 0.05 -baq 1 -C 50 -uniqueOnly 1 -remove_bads 1 -trim 0. Resulting plink and vcf files were filtered to keep only scaffolds 100 kbp or larger in length. Genotype data from remaining scaffolds were used in structure analyses with admixture software (v. 1.3.0; Alexander *et al.* 2009). Filtered genotype data was then used to calculate nucleotide diversity ($\theta\pi$) and Tajimas D with VCFtools (v. 0.1.16; Danecek *et al.* 2011). A window size of 10,000 base pairs was run for each scaffold over 100 kbp with individuals grouped by subspecies, where individuals identified as mixtures by admixture were grouped separately. Nucleotide diversity and Tajimas D were also calculated for genic and intergenic regions of the genome using the bed function of vcftools. Genotype data from scaffolds over 100kbp were merged via Plink (v. 2.00-10252019-avx2; Purcell *et al.* 2007), that was also used to calculate expected and observed heterozygosity and inbreeding (F_{is}) for each individual using Plinks –het flag.

4.3.4 Mutation rate and Effective population size

To calculate the muskox specific mutation rate, 1:1 orthologous genes were identified among the reference muskox genome (JACAUE000000000.2) and 5 other ungulate species (sheep-*Ovis aries*; GCA_000298735.1, goat-*Capra hircus*; GCA_001704415.1, cow – *Bos taurus*; GCA_002263795.2, horse -*Equus caballus*; GCA_002863925.1, bison – *Bison bison bison*;

GCA_000754665.1). PorthoMCL was used to identify and extract 1:1 orthologous genes, followed by Clustal-Omega to align corresponding coding sequences (Tabari & Su, 2017). Individual gene alignments were concatenated using FasConCat software (v1.04; Kück and Meusemann 2010), followed by RaxML (Stamatakis, 2014) to create a maximum likelihood based phylogenetic tree. The GTRGAMMA model was used with 1000 bootstrap replicates, and horse as the outgroup. The tree was then time calibrated and converted to an ultrametric format, using MCMCtree from the PAML package (Yang, 2007). Calibration minimums and maximums for sheep-goat divergence (3.9 – 8.1 Mya), and for sheep-cow divergence (18.3 – 28.5 Mya) were used. The branch model from the CodeMl package from PAML then calculated the non-synonymous to synonymous substitution rate (dN/dS) of each branch of the phylogenetic tree. From these data, the muskox specific mutation rate was estimated by dividing synonymous substitution rate by its divergence time and a generation time of 10 years based on their age of first reproduction and reproductive life span (Hansen et al., 2018; Pacifici et al., 2013). Ancestral N_E was calculated across all muskoxen sampled, as well as for each subspecies, using the equation $\theta\pi = 4Ne\mu$, followed by SNeP to calculate contemporary N_E (Barbato et al., 2015). To reduce inflation of R2 estimates generated from genome scale data, -maxsnp was set at 10,000 and run 100 times for each subspecies and across all muskoxen to obtain a mean N_E. Census population sizes were calculated based on surveys compiled by Cuyler et al. (2019), where only populations or management units sampled herein were included in estimates.

4.3.5 Runs of Homozygosity and Heterozygous rich regions

Plink formatted genotype files for scaffolds larger than 10Mbp were used to identify runs of homozygosity (ROH) using a window-based method via Plink (v. 2.00-10252019-avx2; Purcell *et*

al. 2007). The minimum number of SNPs constituting an ROH was calculated using a method adapted by Purefiled et al. (2012; Meyermans *et al.* 2020). Plink was used to detect runs of homozygosity for each individual and each scaffold using the following parameters: --homozyg --homozyg-window-snp 50 --homozyg-window-het 1 --homozyg-window-missing 5 --homozyg-window-threshold 0.05 --homozyg-snp 50 --homozyg-kb 500 --homozyg-density 50 --homozyg-gap 1000. The inbreeding coefficient, FROH was calculated for each scaffold by totalling ROH length for that scaffold and dividing it by the total length of the scaffold. Individual FROH was calculated by averaging FROH over all scaffolds. Detectruns (v.0.96; Biscarini *et al.* 2018) was used to identify heterozygous rich regions (HRR) for each scaffold of each individual using the sliding windows based method with the following parameters: windowSize = 10, threshold = 0.05, minSNP = 5, ROHet = TRUE, maxOppWindow = 2, maxMissWindow = 1, maxGap = 10^6, minLengthBps = 500000, minDensity = 1/70, # SNP/kbp, maxOppRun = NULL, maxMissRun = NULL. ROH and HRR islands were identified for different clusters/groupings of muskox individuals (white-faced, barren-ground, northern islands, southern islands, Victoria Island, Greenland, all islands) for each scaffold. In this study, if a grouping of muskoxen had 4 individuals, all individuals had to have the common ROH or HRR for it to be considered an ROH or HRR island. If the grouping was larger than 4 individuals (white-faced, Island-only), then the ROH or HRR region was considered an island if over 50% of individuals had overlapping regions (i.e. 9/16 of white-face muskoxen, 7/12 of island muskoxen). The annotation file for the reference muskox genome used in this study was used to identify genes found within these ROH and HRR islands, and corresponding cow ortholog ids for these genes were pulled from ensemble as the closest relatives to muskoxen with the most complete functional annotation. The cow ensemble IDs were then used to perform overrepresentation analyses for ROH and HRR islands of each muskox grouping via PANTHER software (Mi et al., 2019; Thomas et al., 2022).

4.4 Results

4.4.1 Genome sequencing

Muskox genomes from across the Arctic Archipelago and Greenland were resequenced, yielding 3,657,340,752 raw paired reads across 22 individuals, with coverage ranging from ~ 12x – 25x per individual (Table S4.1). Data filtering of low-quality bases, adapter sequences and contaminating sequences resulted in 3,008,800,671 paired reads with coverage ranging from ~ 10x – 22x.

4.4.2 Phylogenetics

3148 common Ultra Conserved Regions (UCE) were identified across muskoxen and five representative reference ungulate genomes (sheep-*Ovis aries*; GCA_000298735.1, goat-*Capra hircus*; GCA_001704415.1, cow–*Bos taurus*; GCA_002263795.2, horse -*Equus caballus*; GCA_002863925.1, GCA_000003025.6, bison–*Bison bison bison*; GCA_000754665.1). Reads that aligned to UCEs were used to create UCE consensus sequences via ATRAM (Allen et al., 2015) and aligned to construct a phylogenetic tree (Figure 4.2A). Mainland and barren-ground muskoxen each formed monophyletic clades and diverged early in the tree. Victoria Island, as well as the southern Arctic Islands (i.e., Bathurst Island and Cornwallis Island) diverged next, although sequences did not cluster to individual islands. Greenland samples branched together, separating from an ancestral muskox population before the northern Arctic Island muskox samples, which also clustered amongst their respective islands (Figure 4.2A).

Coding regions of muskox mitochondrial genomes were used to create a phylogenetic tree (Figure 4.2B). Several individuals had the same gene sequences upon alignment and were thus concatenated so that one representative individual was left in the alignment to create the tree. The final tree displayed all individuals, with those that had the same sequences grouped

together on the same branch. The mitochondrial gene tree did not yield monophyletic clades for barren-ground and white-faced muskoxen given several barren-ground and white-faced muskoxen shared the same mitochondrial gene sequences, including individuals from southern Arctic Islands, Victoria Island and Kent Peninsula. Greenland muskox mitogenomes diverged earlier than most of the other island individuals, followed by a grouping of Victoria Island, southern Arctic Islands and remaining mainland samples, with the northern Arctic Islands last to diverge.

4.4.3 SNP Calling and Admixture

ANGSD identified 1,767,668 SNPs across autosomal scaffolds larger than 100 kbp in length for all individuals, which were then used to perform admixture analyses (Figure 4.3). At K=2, clear structuring occurred between barren-ground and white-faced muskoxen, with admixture occurring in two Victoria Island individuals (Figure 4.3A). K=3 showed samples clustering between barren-ground muskoxen, Greenland individuals, and remaining white-faced muskoxen (Figure 4.3B). There was admixture within samples from Devon Island and Ellesmere Island, with membership to both Greenland and the Canadian Island clusters. The two Victoria Island samples that were admixed at K=2 showed the same pattern at K=3. At K=4, a northern Arctic Island cluster was formed by Devon Island and Ellesmere Island individuals with southern Arctic Island samples showing membership to both northern Arctic Island and Victoria Island samples. At K=5, samples clustered into northern Arctic Islands, southern Arctic Islands, Victoria Island, and barren-ground muskoxen (Figure 4.3C). There was no admixture between clusters other than the two Victoria Island samples, that were admixed at all K values. Victoria Island 1 was a mixture of 63% Victoria Island, 15% barren-ground, and 22% southern Arctic Island clusters; while Victoria Island 4 was a mixture of 66% Victoria Island, and 34% barren-ground clusters.

4.4.4 Runs of Homozygosity (ROH) and Heterozygous Rich Regions (HRR)

In scaffolds larger than 10 Mb, Plink (Purcell et al., 2007) identified 7,854 ROHs with a wide range of ROHs per sample. A minimum of five ROHs were identified in Victoria Island 1, and a maximum of 646 ROHs were identified in Victoria Island 3 (Figure 4.4). 7,826 ROHs were between 0.5 – 2 Mb in length, with the remaining 28 ROHs between 2 – 4 Mb. Detectruns (Biscarini et al., 2018) identified heterozygous rich regions (HRR) across the same scaffolds, and also found a wide range of HRRs, with Greenland 2 having 0 HRRs and Victoria Island 4 having a high of 591 HRRs (Figure 4.4). All HRRs were between 0.5 – 2 Mb in length. Barren-ground individuals all showed relatively low numbers of ROHs, ranging from 19 – 52 ROHs, in comparison to the 368 – 646 ROHs observed in white-faced muskoxen. Many more HRRs were identified in barren-ground muskoxen than in white-faced muskoxen, ranging from 263 – 374 and 0 – 67, respectively. Within white-faced muskoxen, there was a difference between the number of ROHs in the non-admixed Victoria Island muskoxen and remaining white-faced muskoxen, with higher ROHs consistently noted among the Victoria Island individuals. The highest number of ROHs in the remaining white-faced muskoxen was lower than the lowest number of ROHs in non-admixed Victoria Island individuals at 532 and 605, respectively.

4.4.5 ROH and HRR Islands

ROH and HRR regions shared across > 50% of all muskoxen, 50% of white-faced muskoxen, and across all four barren-ground muskoxen, were defined as ROH or HRR islands. 24 ROH islands were identified in all muskoxen that ranged from 110,077 – 755,243 bp in length, across 21 scaffolds (Table S4.4). These regions contained 52 annotated genes (Table S4.12). No HRR islands were identified across all muskoxen. No ROH islands were found across all barren-ground muskoxen; however, 14 HRR islands were detected, ranging from 237,770 and 494,530 bp in

length, across 13 scaffolds (Table S4.11). Within the barren-ground HRR islands, 58 annotated genes were discovered (Table S4.11 – 4.12). In white-faced muskoxen, no HRR islands were detected; however, 279 ROH islands were detected (Table S4.5). ROH islands ranged from 148,877 – 876,173 bp in length, along 65 scaffolds, containing 842 annotated genes (Table S4.5 & S4.12). Genes found in ROH islands across all muskoxen, in ROH islands within white-faced muskoxen, and in HRR islands within barren-ground muskoxen, were tested for Gene Ontology (GO) term and pathway overrepresentation using Panther. Genes from ROH islands within white-faced muskoxen were the only grouping to have statistically significant overrepresented GO terms, including: 28 biological processes, two molecular functions, and 11 cellular component terms (Table S4.13). These overrepresented terms included anatomical structure morphogenesis, epithelium development, animal organ development, anterior/posterior pattern specification, and several terms related to metabolism (Table S4.13). While ROH and HRR islands were identified in other groupings of muskoxen that included: all four Greenland individuals (Table S4.10), all four barren-ground individuals (Table S.4.11), northern Arctic muskoxen (Table S4.7), southern Arctic muskoxen (Table S4.8), all pure white-faced muskoxen from Victoria Island (Table S4.6), and over 50 % of Arctic Island muskoxen (Table S4.9), none of these groupings had statistically significant overrepresented GO terms or pathways.

4.4.6 Genome Diversity statistics

For all subspecies level calculations, individuals Victoria Island 1 and Victoria Island 4 were removed from white-faced muskox calculations as admixture analyses demonstrated they had membership to both subspecies. After filtering to include only scaffolds over 100 kbp, the number of observed heterozygous SNPs found within muskoxen ranged from 195,577 to 666,564, with an average of 346,950 across all individuals. Within barren-ground muskoxen,

observed heterozygous SNPs ranged from 601,344 to 666,564, with an average of 637,280. White-faced muskoxen ranged from 195,577- 305,759, with an average of 239,166. The heterozygosity rate across muskoxen ranged from 1.3E-4 – 4.67E-4 (Table S4.3) with an average of 2.43E-4, while barren-ground muskoxen had an average rate of 4.47E-4 and white-faced muskoxen averaged 1.68E-4 (Table 4.1). Across the entire scaffold, there was an average 12.2 alleles per 10 kbp window across all muskoxen, 10.03 per window in barren-ground muskoxen, and 9.76 in white-faced muskoxen. When comparing intergenic and genic regions of the scaffolds, there were ~ 3 alleles more per 10 kbp window in intergenic regions of scaffolds. Nucleotide diversity ($\theta\pi$) was 3.96E-4 across all muskoxen, while $\theta\pi$ in barren-ground and white-faced muskoxen was 3.45E-4 and 4.56E-4, respectively (Table 4.1). Intergenic $\theta\pi$ was higher across all muskoxen, and within subspecies, when compared to genic $\theta\pi$. Heterozygosity across all muskoxen was estimated at 2.43E-4, 4.47E-4 in barren-ground muskoxen, and 1.68E-4 in white-faced muskoxen (Table 4.1).

A total of 893 orthologs were identified across five ungulate species and the reference muskox genome. Corresponding coding sequences were used to create a time calibrated phylogenetic tree (Figure 4.2) and dN, dS and dN/dS ratios calculated for each branch of the resulting phylogenetic tree, provided ratios ranging from 0.005 – 0.057, 0.013 – 0.326 and 0.175 – 0.591, respectively (Table S4.2). Using dS of muskoxen, a mutation rate of 4.874E-8 mutations/site/generation was calculated with a generation time of 10 years (Hansen et al., 2018). This value represents 4.874E-9 mutations/site/per year and falls within the range of values observed in other mammals. Using the muskox specific mutation rate and genomic diversity, ancestral effective population size was calculated for muskoxen overall, barren-ground muskoxen, and white-faced muskoxen, as 2031, 2339 and 1770, respectively. Contemporary effective population size was calculated by SNeP (Barbato et al., 2015) which provided an

effective population size of 22 across all muskoxen, 37 in white-faced muskoxen and 15 in barren-ground muskoxen (Table 4.1). The ratio of effective population size and census size (N_E/N_C) was calculated for both ancestral and contemporary metrics for the muskox subspecies and muskoxen overall. All N_E/N_C ratios were smaller than 1. Ancestral N_E/N_C ratios for all muskoxen was 0.031, 0.029 for white-faced muskoxen and 0.401 for barren-ground, while contemporary N_E/N_C ratios were much smaller at 3.34E-4, 6.16E-4 and 2.57E-3, respectively (Table 4.1).

Genomic inbreeding (FROH) was calculated by dividing the length of the genome covered by runs of homozygosity, by the total length of the genome for each individual, with the results ranging from 0.0024 to 0.3794 (Figure 4.4). FROH was consistently lower in barren-ground muskoxen, with FROH reaching a high of 0.0257, while the lowest FROH in white-faced muskoxen was 0.1962. For barren-ground muskoxen, the average FROH was 0.0177, 15x lower than that of the white-faced muskoxen at 0.2669. Across all muskoxen, average FROH was 0.2034. The inbreeding coefficient (F_{is}) was also calculated based on expected and observed heterozygosity for each individual. F_{is} ranged from 0.004 to 0.34 across in white-faced muskoxen (Table S4.3) with an average of 0.221 (Table 4.1). In barren-ground muskoxen, F ranged from -0.14 to -0.028 (Table S4.3), with an average of -0.09 (Table 4.1). Tajima's D was estimated over a window size of 10 kbp in scaffolds larger than 100 kbp across all muskoxen, and within subspecies. Tajima's D estimates were all greater than 0, with 0.698, 0.646 and 0.560 found across all muskoxen, barren-ground muskoxen, and white-faced muskoxen, respectively (Table 4.1). Tajima's D was also calculated within genic and intergenic regions of the scaffolds, where intergenic regions had higher values when compared to genic regions.

4.5 Discussion

Twenty-two wild muskox genomes were resequenced in this study to better assess levels of genetic diversity across the natural range of the species, in context of their demographic and evolutionary history. These data not only confirmed the lack of genetic diversity previously observed in this species, but clarified how genetic variation was distributed temporally, geographically, and within the genome, yielding further insight into population genetic structure, effective population sizes, subspecific delineations, and populations that are potentially vulnerable to changing selective pressures. These data also suggest that Victoria Island may be a key population in maintaining diversity and for potential evolutionary rescues of the northern subspecies. Specifically, phylogenetic analyses of nuclear DNA supported subspecies delineations between white-faced and barren-ground muskoxen. These data conflict with the phylogenetic findings of the mitochondrial genome. However, mito-nuclear discordance could be the result of the muskox's mitochondrial coding region lacking sufficient diversity to accurately infer backbone nodes that would distinguish between subspecies, as has been observed in previous studies (Groves, 1997). Finer-scale, SNP admixture analyses also found muskoxen cluster by subspecies and geographic region, with evidence of limited gene flow between white-faced and barren-ground populations at their interface on Victoria Island. Diversity estimates were low across all muskox populations, yet distinctly different between subspecies. White-faced muskox individuals had lower nucleotide diversity, N_E/N_C ratios, observed heterozygosity and heterozygous rich regions (HRR), while also having higher inbreeding estimates and runs of homozygosity (ROH). Significantly overrepresented GO terms were exclusively discovered within genes located in the ROH islands of white-faced muskoxen and are potentially associated with the distinctive morphological differences observed among muskox subspecies, such as their stature. The lack of diversity in white-faced muskoxen,

combined with precipitous declines at their southern range, suggests they may be more vulnerable to rapid environmental changes and require prioritised conservation management. Overall, these data indicate muskoxen may be more imperiled than is currently recognised by their conservation status.

4.5.1 Phylogenetics

Phylogenetic trees based on coding regions of the mitochondrial genome and nuclear ultra-conserved regions in muskoxen had very different topologies. Mito-nuclear discordance was notably observed in the placement of the Greenland clade, where the nuclear Phyluce tree yielded monophyletic clades associated with geographic distribution and subspecific delineations, while the mitochondrial DNA (mtDNA) tree did not. Mito-nuclear discordance is often the result of asymmetrical introgression from demographic expansions and hybridization events that, in extreme cases, can result in the replacement of one species' mtDNA by another, without evidence of nuclear introgression (Andersen et al., 2021; Good et al., 2008; Leavitt et al., 2017; Mao & Rossiter, 2020; Toews & Brelsford, 2012; Wiens et al., 2010; Wright et al., 2022). Differing nuclear and mito-genome patterns have been observed in several species and associated with Pleistocene glacial cycles (Andersen et al., 2021; Harris et al., 2018; Jiang et al., 2016). At glacial maximas, populations in distinct refugia accumulate mutations in both nuclear and mitochondrial genomes (Andersen et al., 2021; Harris et al., 2018; Jiang et al., 2016). Upon glacial retreat, populations disperse back to historical ranges, experiencing repeated founder events with secondary contact, gene flow and back crossing in hybridization zones (Andersen et al., 2021; Jiang et al., 2016; Mao & Rossiter, 2020; Toews & Brelsford, 2012; Wright et al., 2022). In muskoxen, archaeological and genetic evidence propose two refugia existed that were fully isolated around the last glacial maximum (Campos et al., 2010; Groves, 1997; Hansen et al.,

2018). As ice sheets melted, muskoxen surviving in a refugium north of the ice (white-faced muskoxen) dispersed throughout the islands and Greenland, while those from a southern refugium (barren-ground muskoxen) dispersed northward (Campos et al., 2010; Groves, 1997; Hansen et al., 2018). These patterns likely resulted in multiple founder events with prolonged small effective population sizes (Hansen et al., 2018). When asymmetric introgression occurs, it usually remains in a narrow geographic range near the hybridisation zone, and mtDNA of one population does not extend beyond 50% of the other population's range (Good et al., 2008). This appears true of the discordance in the muskox phylogenies, as shared mitochondrial gene sequences only occur between the barren-ground muskoxen and individuals from the southern Arctic islands.

Differences between mitochondrial DNA and nuclear DNA phylogenetic trees show the improved resolution that can result from genome level data. Clades formed in the nuclear genome Phyluce tree are similar to population structures previously identified using genome-level data (Hansen et al., 2018; Pečnerová et al., 2024), where Greenland, the northern Arctic Islands (Devon and Elsmere), the southern Arctic Islands (Bathurst and Cornwallis), and mainland individuals formed their own distinct clusters. Victoria Island individuals were not included in these studies, but it is not unreasonable to suggest that they would similarly group with the other southern Arctic Islands. Additionally, Hansen et al. (2018) found that muskox populations diverged along a northeastern expansion front which was supported by the Phyluce tree herein. The Phyluce tree also identified a clear separation between the muskox subspecies, with the first internal node forming two monophyletic subspecies groups, while the mitochondrial tree did not. Paraphyletic subspecies groupings in the mitochondrial phylogenetic tree were not altogether unexpected, as previous mtDNA sequence analyses using cytochrome b and hypervariable control regions were also unable to distinguish between subspecies, likely

given a lack of variation (Campos et al., 2010; Groves, 1997; Groves & Shields, 1997). Groves (1997) found only 1.4% of mtDNA sites were variable within the control region of muskoxen (8 haplotypes/37 individuals), whereas in other species, variation ranges from 1.6-20%. Similarly, low genetic variation was observed herein, as several individuals shared identical mtDNA coding sequences, including individuals from different subspecies. These phylogeny results further demonstrate the need for genome level phylogenetic analyses, as an increased number of nuclear loci can better clarify cryptic branches, populations, or subspecies.

4.5.2 Population Structure

Admixture analyses were performed using genome-wide SNPs to identify genetic structure across muskoxen along the latitudinal gradient of their distribution. We found muskoxen clustered geographically, with limited admixture between populations, as has been found in previous muskox research (Bird et al., 2019; Hansen et al., 2018; Pečnerová et al., 2024; Prewer et al., 2019). However, this study is the first to include whole genome sequence data from Victoria Island muskoxen, which are one of the biggest herds of muskoxen that have recently experienced dramatic population declines. In this study, Victoria Island animals formed their own cluster, separate from the other southern Arctic Islands. The gene flow between Victoria Island and barren-ground populations found in this study has previously been detected through microsatellite analyses by Prewer et al. (2019), as well as through microbiome diversity analyses by Bird et al. (2019). These admixture results confirm that while there is some movement between Victoria Island and barren-ground muskoxen, the remaining white-faced muskox populations are relatively isolated between islands, thus limiting their genetic diversity and prospects for increasing diversity.

4.5.3 Distribution and Drivers of Genome Diversity

Estimates of muskox genomic diversity, including nucleotide diversity (Θπ) and heterozygosity rates were lower than most species. Θπ in muskoxen (3.96E-4) were within those of endangered species like Tasmanian devils and Amur tigers which had Θπ estimates of 3.2E-4 and 4.9E-4, respectively (Cho et al., 2013). Among other large Arctic ungulates, muskox Θπ estimates were much lower than those of Arctic caribou, where nucleotide diversity was measured at 2.5E-3 (Taylor et al., 2024). Similarly, muskox heterozygosity rates (2.43E-4) were lower than other ungulates such as yak (8.9E-4), reindeer (2.05E-3), the endangered Przewalskis horse (3.9E-4), and Pere David's deer (5.4E-4). Muskox heterozygosity rates were also lower than the average estimates of IUCN endangered animals (~ 7E-4; Liu et al., 2022; Weldenegodguad et al., 2020). Clear differences in diversity were found between subspecies; white-faced muskoxen had an average heterozygosity rate nearly two times lower than barren-ground muskoxen. Similar differences in subspecific heterozygosity estimates have previously been noted in muskoxen using whole genome sequencing, genotyping by sequencing and microsatellite analyses (Hansen et al., 2018; Pečnerová et al., 2024; Prewer et al., 2019). Beyond conventional estimates of diversity, white-faced muskoxen sequenced herein were found to have more runs of homozygosity (ROH) and fewer heterozygous rich regions (HRR) than barren-ground individuals. In white-faced muskoxen, there was an average 480 ROHs per individual, higher than most estimates found in ungulate genomic literature (Wu et al., 2021). At an average of 37.8, the number of ROHs in barren-ground muskoxen are similar to livestock species like sheep, goats and cows, whose average number of ROHs range from 24-90 (Macciotta et al., 2021). The average number of HRRs in barren-ground muskoxen (323) was notably greater than those of white-faced muskoxen (27), as well as estimates in sheep (154) and cattle (83; Mulim et al., 2022; Selli et al., 2021).

Low genetic diversity in muskoxen has previously been attributed to repeated bottlenecks and historical founder events, leading to extended periods of low effective population size (N_E; Campos et al., 2010; Hansen et al., 2018; Prewer et al., 2019, 2022). These theories appear to be supported by Tajima's D analyses, which estimate population genetic neutrality from allele frequency distributions (Carlson et al., 2005; Gattepaille et al., 2013). Positive Tajima's D estimates, as found in muskoxen in this study, indicate an excess of high frequency variation, and may be the result of balancing selection or population contractions such as ancient range fragmentations, founder events and population bottlenecks (Carlson et al., 2005; Gattepaille et al., 2013). These factors are further exacerbated by inbreeding in populations with smaller N_E (Carlson et al., 2005; Gattepaille et al., 2013). Two measures of muskox inbreeding were calculated, with F_{IS} based on heterozygosity estimates, and FROH based on runs of homozygosity. F_{IS} was 0.338 and FROH was 0.2034; relatively high values when compared to other ungulate species. For example, across livestock cattle populations, the highest F_{IS} estimate was at 0.086, and the largest FROH estimate was 0.075. When examining inbreeding estimates at the individual level in muskoxen, there were again marked differences between subspecies. Estimates in barren-ground muskoxen were indicative of either no inbreeding (FROH = 0.0177) or even an excess of heterozygotes (F_{IS} = -0.215). In contrast, an average F_{IS} of 0.544 in white-face muskoxen was higher than that observed in endangered species like Eurasian lynx (max 0.480), as well as values previously estimated in muskoxen (Hansen et al., 2018; Mueller et al., 2022; Prewer et al., 2019). FROH estimates further demonstrated that inbreeding was occurring in white-faced muskoxen but not barren-ground muskoxen, with an FROH in white-faced muskoxen of 0.267 and 0.0177 in barren-ground muskoxen. Sequencing of a Siberian muskox genome from ~ 21Kya found a lack of inbreeding, alongside heterozygosity estimates up to 14 times higher than those in modern muskoxen,

indicating that population fragmentation since the last glacial maximum likely contributed to diversity declines (Pečnerová et al., 2024). While the patterns of genetic diversity observed in this study can be largely explained by demographic trends (bottlenecks, founder events), strong selection on standing genetic variation could also influence the patterns observed (von Seth et al., 2021).

Selection can affect patterns of diversity throughout the genome. Purifying selection purges deleterious alleles from the population, reducing overall diversity but also reducing a population's genetic load (Cvijović et al., 2018; Dussex et al., 2023; Pečnerová et al., 2024). As muskox populations are fragmented with low genetic diversity, compounded by inbreeding in some populations, genetic drift is expected to be more pronounced. As a result, deleterious alleles are likely to accumulate and become fixed in muskox populations, leading to inbreeding depression and reduced population viability (Forester et al., 2022; Willi et al., 2022). Evidence of purifying selection has been found in muskoxen, acting on both strongly and moderately deleterious alleles, likely contributing to the continued persistence and low genetic diversity in muskoxen (Pečnerová et al., 2024). Selective pressures leading to local adaptations can also impact levels of diversity throughout the genome, where balancing selection can help maintain diversity and directional selection can reduce it (Biscarini et al., 2020). As such, ROH and HRR islands provide insight into selective pressures acting on muskoxen. In this study, only barren-ground muskoxen had HRR islands, although there were no statistically significant overrepresented GO terms or pathways associated with genes annotated within those regions. ROH islands were present across all muskoxen, yet only ROH islands found in white-faced muskoxen had statistically significant overrepresented GO terms (Table S4.13). Within these significantly overrepresented GO terms, several were likely related to distinctive morphological differences identified between muskox subspecies. For example, anatomical structure

morphogenesis (GO:0009653), epithelium development (GO:0060429), tissue development (GO:0009888), animal organ development (GO:0048513) and anterior posterior pattern specification (GO:0009952), may be related to the smaller stature in white-faced muskoxen. Additionally, several terms associated with metabolic processes, for example, macromolecule metabolic process (GO:0043170); primary metabolic process (GO:0044238); and nitrogen compound metabolic process (GO:0006807), were overrepresented in white-face muskoxen. We speculate that these patterns may be related to the limited food availability and poorer quality of plant species found in more northern ecozones (CAVM Team, 2003; Northwest Territories et al., 2012). For instance, Canadian white-faced muskoxen sampled in this study all inhabit the northern Arctic ecozone, while the barren-ground muskoxen inhabit the southern Arctic ecozone with higher plant diversity (CAVM Team, 2003; Northwest Territories et al., 2012). Differences in plant diversity and seasonal variation could therefore act as a selective pressure on genes involved in the metabolism of white-faced muskoxen. Other overrepresented GO terms identified included regulation of neurogenesis (GO:0050767) and regulation of nervous system development (GO:0051960), where causative selective pressures remain unclear with respect to subspecific adaptations. These results show that there are significant differences between these muskox subspecies, yet more research is needed to determine how these homozygous regions affect fitness, and more specifically, how these homozygous SNPs directly affect phenotypes.

4.5.4 Conservation Implications

Phylogenetic, diversity and selection analyses carried out in this study contribute to growing evidence that reconsideration of muskox subspecies may be required. Even if subspecific designations are not warranted, these data suggest that there are likely two key designatable

units of muskoxen that should be assessed and managed separately, with clear implications for conservation management and population sustainability. Though census population sizes in muskoxen remain large, the impact low diversity may have on species vulnerability and persistence in the face of rapidly changing landscapes and selective pressures should be considered (England et al., 2010; Ferchaud et al., 2016; Palstra & Ruzzante, 2008; Peart et al., 2020; Stoffel et al., 2018). Effective population size (N_E) provides insight into evolutionary factors such as genetic drift, and the capacity for species to adapt to changing selective pressures and is considered a key metric for conservation (England et al., 2010; Ferchaud et al., 2016; Palstra & Ruzzante, 2008; Peart et al., 2020; Stoffel et al., 2018). In muskoxen, a contemporary N_E of 22 is below the minimum threshold proposed to reduce inbreeding depression (~ 50), and to preserve evolutionary or adaptive potential (~ 500; Palstra & Ruzzante, 2008). When separated into subspecies, white-faced muskoxen have a higher N_E than barren-ground muskoxen with 37 and 15, respectively. This is unexpected, given the diversity differences found between the two groups. However, the census size, or number of breeding individuals, is also lower in barren-ground muskoxen. The ratio of N_E to census population size (N_E/N_C) can provide a better measure of a species' or population's genetic risk, with smaller N_E/N_C ratios associated with increased rates of genetic diversity loss, and deleterious mutation fixation (England et al., 2010; Ferchaud et al., 2016; Palstra & Ruzzante, 2008; Peart et al., 2020; Stoffel et al., 2018). N_E is often expected to be smaller than census size (ratio of ~ 0.5) and other factors, including unequal sex ratios and variance in reproductive success can further reduce this ratio, where ~ 0.1 is typical in wildlife species (Palstra & Ruzzante, 2008). In muskoxen, contemporary N_E/N_C ratios are extremely low (3.34E-4), though the ratio in barren-ground muskoxen was the highest at 2.57E-3. While this low N_E/N_C ratio is concerning for muskox vulnerability, it has been suggested that contemporary N_E may not accurately reflect inbreeding

or losses of genetic variation as it fails to account for population history, is sensitive to migration, and estimates can often be stochastic (Dedato et al., 2022; Peart et al., 2020). Instead, the ancestral or coalescent N_E/N_C ratio can account for evolutionary forces that have impacted the genetic variation currently observed in populations (Dedato et al., 2022; Peart et al., 2020). Ancestral estimates of N_E/N_C are found to be less sensitive to recent changes in population size, which can change rapidly across few generations. Therefore, ancestral N_E/N_C ratios can be a useful metric to assess a population's adaptive potential (Dedato et al., 2022; Peart et al., 2020). Barren-ground muskoxen had an ancestral N_E/N_C ratio higher than typical wildlife populations at 0.401 yet it was still low in white-faced muskoxen (0.029) and muskoxen as a whole (0.031). Based on estimates of diversity, inbreeding and N_E/N_C ratios measured in this study, white-faced muskoxen may be more vulnerable to rapid environmental changes occurring in the Arctic. Population fluctuations have been seen in Greenland and high Arctic island populations, where drastic declines are followed by a slow recovery (Desforges et al., 2021; Gunn & Forchhammer, 2022). Alongside the recent population collapse on Victoria and Banks Islands, these patterns observed in Greenland and high Arctic island populations would suggest the capacity for white-faced muskoxen to adapt to changing selective pressures could be compromised (Gunn & Forchhammer, 2022).

Population vulnerability can be addressed through the facilitation of gene flow between healthier, or more variable stock populations (Woodruff, 2001). Gene flow between barren-ground and white-faced muskoxen could allow for evolutionary rescue to occur, by increasing genetic variation in white-faced populations, thereby improving adaptive potential (Khan et al., 2021). Barren-ground muskox populations may be an ideal stock population as they have higher estimates of genetic diversity, lower estimates of inbreeding, and have grown despite long-term exposure to the selective pressures thought to be negatively impacting white-faced muskoxen.

For example, *Erysipelothrix rhusiopathiae* was found to be the cause of several mortality events on Victoria and Banks Island contributing to the nearly 80% decline in population size (Kutz et al., 2015; Mavrot et al., 2020). Alternatively, adjacent barren-ground muskox populations have also been exposed to the same pathogen without similar die-off events (Kutz et al., 2015; Mavrot et al., 2020). Therefore, increasing gene flow between subspecies may not only improve diversity estimates in white-faced muskox populations, but also introduce the variation needed for white-faced muskoxen to respond to their changing environment. Furthermore, there is already evidence that gene flow is occurring naturally between white-faced and barren-ground populations. In this study, the two admixed individuals had increased heterozygosity and heterozygous rich regions, as well as decreased runs of homozygosity and inbreeding, in comparison to pure white-faced individuals. Nonetheless, increasing the gene flow between barren-ground and white-faced muskoxen may result in the introduction of deleterious alleles into white-faced populations (Dzomba et al., 2021; Khan et al., 2021; Pečnerová et al., 2024; Woodruff, 2001). Pečnerová et al. (2024) found that relative mutational load was higher in barren-ground muskoxen than in white-faced muskoxen. Additionally, although offspring resulting from mixing barren-ground and white-faced populations may have increased heterozygosity rates, which could mask deleterious alleles and mitigate their potentially negative impacts, this may undermine subspecific adaptations, like those indicated by ROHs in white-face muskoxen (Chen et al., 2022; Northwest Territories et al., 2012; Young & Gillung, 2020). For example, one of the admixed white-faced muskox individuals, Victoria Island 1, had nearly no runs of homozygosity. While increased heterozygosity rates could enhance population viability, it may also result in the loss of biodiversity overall if homozygous regions contribute to what makes these subspecies unique. Additional genome sequencing may help determine if these muskox subspecies or populations are locally adapted to their environment and whether

increased gene flow will result in outbreeding depression or provide the variation needed for adaptations to the changing landscape.

4.6 References

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Table 4.1. Diversity metrics for all muskoxen were sampled, as well as for barren-ground and white-face subspecies. N represents the number of samples per grouping; F_{IS} represents the inbreeding coefficient based on heterozygosity; FROH represents the inbreeding coefficient based on runs of homozygosity; Θπ represents nucleotide diversity; N_C represents census population size; N_E represents effective population size; N_E/N_C represents the ratio of effective population size to census size.

	Barren-ground	**White-faced**	**All**
N	4	16	22
Heterozygosity Rate	4.47E-4	1.68E-4	2.43E-4
F_{IS}	-0.215	0.544	0.338
FROH	0.0177	0.2669	0.2034
Θπ	4.56E-4	3.45E-4	3.96E-4
θπ (Genic)	3.09E-4	2.09E-4	2.48E-4
θπ (Intergenic)	4.34E-4	3.38E-4	8.99E-3
Tajima's D	0.646	0.560	0.698
Tajima's D (genic)	0.627	0.487	0.578
Tajima's D (intergenic)	0.636	0.562	0.691
N_C	5,831	60,094	65,925
Ancestral Ne	2,339	1,770	2,031
N_E/N_C (Ancestral)	0.401	0.029	0.031
Contemporary N_E	15	37	22
N_E/N_C (Contemporary)	2.57E-3	6.16E-4	3.34E-4

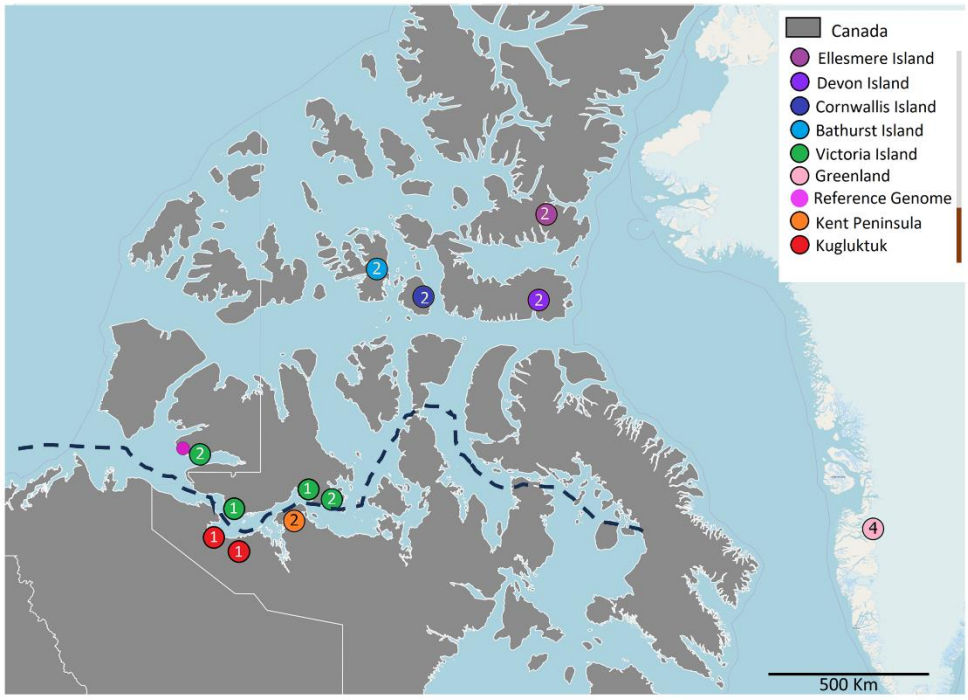

Figure 4.1. Map of the sampling locations of muskox. Sampling regions are differentiated via circle colour and the number of samples collected per sampling location is indicated by the number inside. Grey and brown bars in the legend indicate which sampling locations correspond to each subspecies with the brown bar indicating barren-ground muskox locations and grey bar representing white-face muskox locations. Subspecies are delineated on the map by the dotted line with barren-ground muskoxen found below the line and white-faced muskoxen found above the line and in Greenland.

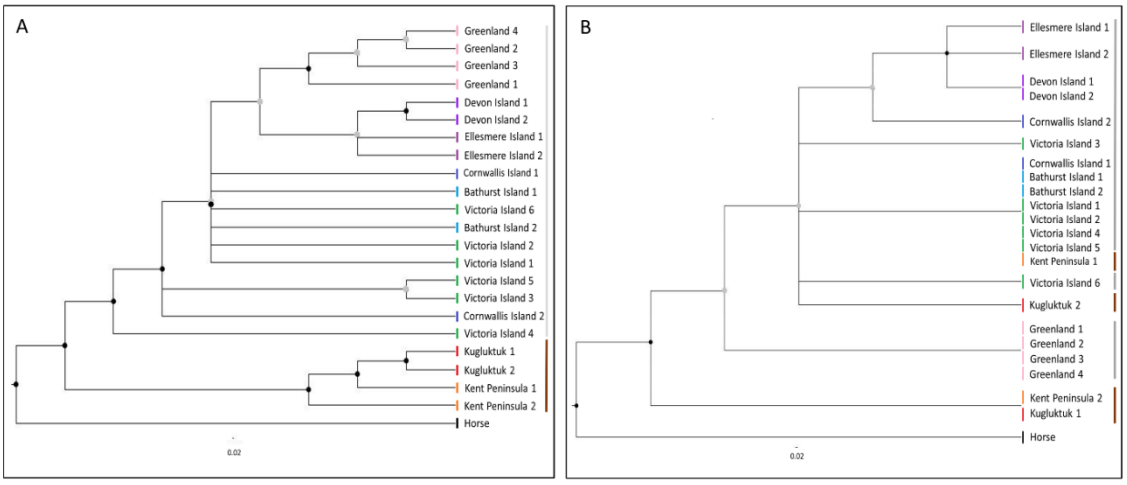

Figure 4.2. Phylogenetic tree constructed using A) the coding region of the mitochondrial genome and B) 3148 common ultra-conserved regions across the genome via Phyluce. Grey dots represent nodes with a bootstrap value between 85-95% while black dots represent nodes with >95% support. Coloured lines before the sample name represent the sampling region, while the brown and grey lines to the right of sample names delineate subspecies with grey representing white-faced muskoxen, and brown representing barren-ground muskoxen. Major differences between the topology of two trees included the placement of the Greenland clade, and the identification of monophyletic vs paraphyletic subspecies clades, in the Phyluce and mitochondrial tree respectively.

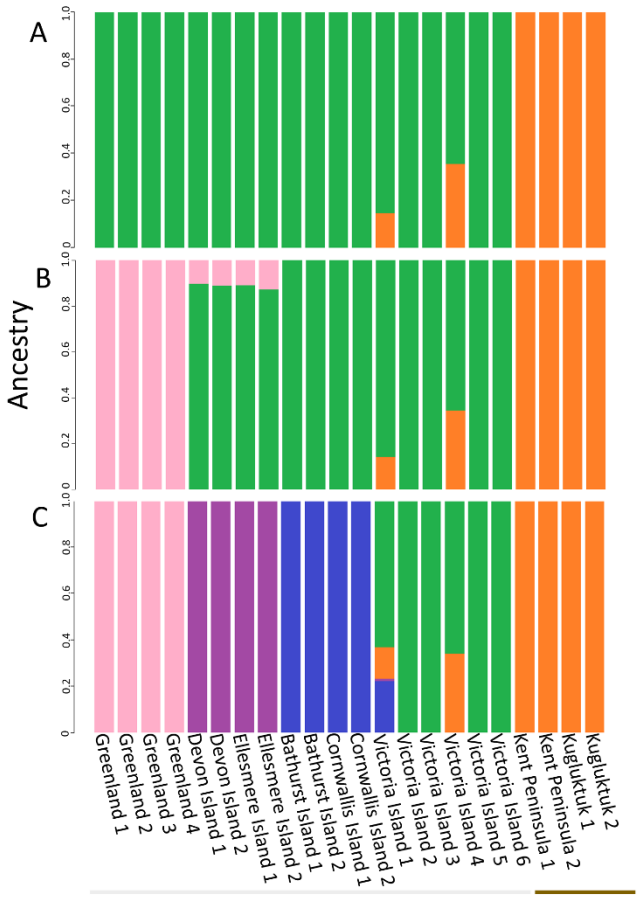

Figure 4.3. Admixture plot of the muskox samples at K=2(A), K=3 (B) and K=5(C). Each bar represents a different sample, with the samples arranged by geographic location and ancestry percentages along the y axes. Subspecies are delineated by the grey and brown lines below the sample names, with grey indicating white-faced muskoxen, and brown indicating barren-ground muskoxen.

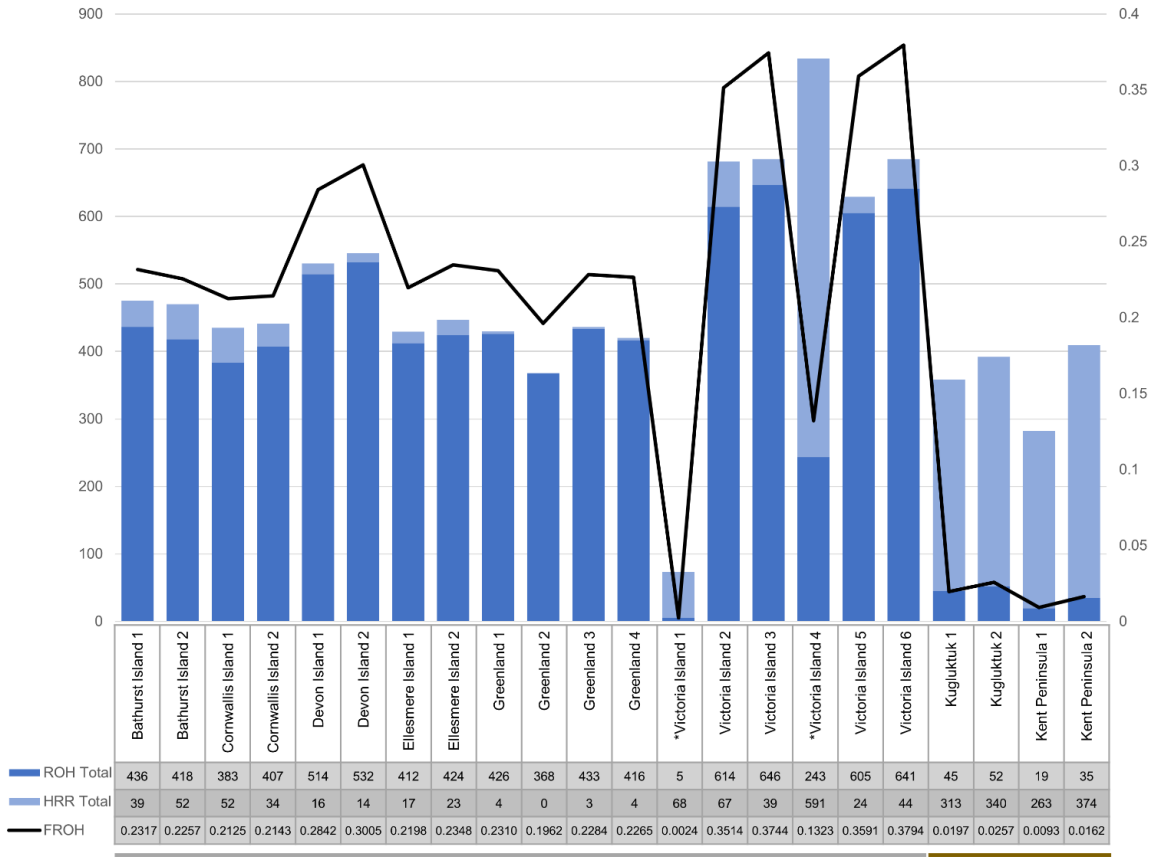

Figure 4.4. Number of runs of homozygosity (ROH=dark blue) and runs of heterozygosity (HRR=light blue) in each muskox genome sampled. The number of ROH and HRR is represented by the y-axis on the left side of the plot, as well as within the table below the plot. The FROH, or percentage of the genome covered by runs of homozygosity for each individual, is represented by the black line corresponding to the y-axis on the right side of the plot and can be found in the table below the plot. Subspecies are delineated by the grey and brown lines below the table, with grey representing white-faced muskoxen, and brown representing barren-ground muskoxen. Starred samples, Victoria Island 1 and Victoria Island 4 represent admixed barren-ground and white-faced individuals.

Chapter 5: Muskox diet metabarcoding

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Contributions: STV, CJK, EP and SB designed the research; CJK, SK and LML provided samples and resources; EP, SB and STV performed the research and analysed data; EP and CJK wrote the paper with review and editing by all authors; CJK and SK provided funding acquisition.

5.1 Abstract

Microbiome diversity and diet composition concomitantly influence species health, fitness, immunity, and digestion. In environments where diet varies spatially and temporally, microbiome plasticity may promote rapid host adaptation to available resources. For northern ungulates in particular, metabarcoding of noninvasively collected fecal pellets presents unprecedented insights into their diverse ecological requirements and niches by clarifying the interrelationships of microbiomes, key to deriving nutrients in context of altered forage availability in changing climates. Muskoxen (*Ovibos moschatus*) are Arctic-adapted species that experience fluctuating qualities and quantities of vegetation. Geography and seasonality have been noted to influence microbiome composition and diversity in muskoxen, yet it is unclear how their microbiomes intersect with diet. Following observations from other species, we hypothesized increasing diet diversity would result in higher microbiome diversity in muskoxen. We assessed diet composition in muskoxen using three common plant metabarcoding markers and explored correlations with microbiome data. Patterns of dietary diversity and composition were not fully concordant among the markers used, yet all reflected the primary consumption of willows and sedges. Individuals with similar diets had more similar microbiomes, yet in contrast to most literature, yielded negative relationships between microbiome and diet alpha diversity. This negative correlation may reflect the unique capacities of muskoxen to survive solely on high fiber Arctic forage and provide insight into their resiliency to exploit changing dietary resources in a rapidly warming Arctic altering vegetation diversity.

5.2 Introduction

Climate change has dramatically changed both terrestrial and aquatic ecosystems, affecting the ecology and evolution of many organisms and communities (Espunyes et al., 2022; Hoffmann et

al., 2019). Increasing global temperatures have resulted in changing and novel selective pressures for many species, which can lead to population declines, local extirpations, and overall lowered biodiversity (Bright Ross et al., 2021; Hamann et al., 2021; Lovari et al., 2020). Vegetation is particularly sensitive to changes in precipitation and temperature, where climate change has been associated with decreased nutrient availability, droughts, increased pests, soil erosion, and increased competition (Akram et al., 2022; Bright Ross et al., 2021; Elbasiouny et al., 2022; Espunyes et al., 2022; Hamann et al., 2021). Reduced plant productivity and quality, as well as altered plant phenology, have a cascading negative effect on herbivore species that depend on these resources (Espunyes et al., 2022; Hamann et al., 2021; Lovari et al., 2020). For example, environmental warming is expected to reduce the availability of snow bed vegetation, an important food source for Apennine chamois (*Rupicapra pyrenaica*), and is thus predicted to increase juvenile mortalities, potentially leading to local extirpations (Lovari et al., 2020). While some alpine herbivore species, like the ibex (*Capra ibex*) or alpine chamois (*Rupicapra rupicapra*) can adjust to changing vegetation availability via behavioral changes (e.g., foraging bite and step rates) or range shifts to higher elevations (Espunyes et al., 2022; Lovari et al., 2020), these changes are thought to compromise fitness (Bright Ross et al., 2021). Alternatively, some species have the capacity to adapt to environmental modifications brought about by climate change (Fulgione & Buglione, 2022). For example, beech (*Fagus sylvatica*) yields have increased in Sweden; an important food source for wild boars (*Sus scrofa*; Fulgione & Buglione, 2022), where additional food availability is expected to result in population growth given increases in lifespan, earlier female sexual maturity, and decreased piglet mortality rates (Fulgione & Buglione, 2022). As outcomes of climate change vary, there is a demonstrated need to better understand the capacity of affected species to adapt to rapid environmental changes.

Gut microbiome compositions are increasingly recognized as contributors to the ability of species to respond to rapidly changing environments by combating pathogens, expanding dietary niches through digestion and individual metabolic function, and enhancing heat tolerance (Bolnick et al., 2014; Buglione et al., 2022; Hicks et al., 2018; Li et al., 2016; Loo et al., 2019; Rothschild et al., 2018; Wu et al., 2018). It is not always clear, however, how microbiome diversity and composition are impacted by changing diet in influencing individual health, fitness, nutritional status, and even population dynamics (Kartzinel et al., 2019; Zeineldin et al., 2018). The relationships between the microbiome and diet are especially important in herbivore species as microbiome bacteria (commonly *Bacteroidetes* and *Firmicutes* species) help extract nutrients from plant materials that can otherwise be unsustainable or nutrient poor, and break down toxic plant compounds (Bergmann et al., 2015; Dearing & Kohl, 2017; Ungerfeld et al., 2018). Further, extended fermentation by bacteria in ruminants increases the production of short-chain fatty acids; metabolic end products absorbed by ruminants to help fulfill energy requirements that are also important signaling molecules with antimicrobial and anti-inflammatory properties that regulate energy metabolism and macromolecule synthesis (Andersen-Ranberg et al., 2018; Shabat et al., 2016). By contrast, altered or imbalanced microbiomes can lead to both decreased digestion and gastrointestinal disease that threaten nutritional status and survival (Belanche et al., 2021). In cows, high energy feed containing increased levels of starch or carbohydrates can result in dysbiosis, or the imbalance of bacteria, which negatively affects gastrointestinal tract absorption capacity (Neubauer et al., 2020). In natural systems, metabarcoding of American Bison (*Bison bison*) diets from fecal samples found *Tenericutes* bacteria increased in abundance in response to the seasonal availability of high caloric and high protein content plants (Bergmann et al., 2015). Thus, the plasticity of the microbiome in responding to external biotic and abiotic factors (e.g., season, geography,

disease, and diet), may allow individuals to adjust to their environment and exploit different ecological niches (Loo et al., 2019; Ng et al., 2018; Reese & Dunn, 2018). The extent and effect that diet has on microbiome composition varies greatly by species, as do the physiological effects that microbiome plasticity can have on individuals; thus impacting their capacity to acclimate to their environment (Diaz & Reese, 2021). The culmination of the aforementioned studies suggests health, fitness, and sustainability assessments of wild populations would be further enhanced through a better understanding of the seasonal and geographical dietary factors that influence microbiome compositions and diversity in natural systems.

The Arctic is an extreme environment characterized by temperatures regularly ranging from −35 to 15°C, extreme photoperiods, and severe weather events leading to reduced vegetation biodiversity throughout the year (Berger et al., 2018; Blix, 2016; Callaghan et al., 2004). The Arctic is warming at nearly four times the global rate, reducing plant diversity, shifting plant dispersal patterns, and altering plant phenology (Ernakovich et al., 2014; Olofsson et al., 2009; Speed et al., 2021); drastic changes threatening the sustainability of Arctic herbivores. Muskoxen (*Ovibos moschatus*) are large Arctic ruminants whose range encompasses the Arctic Archipelago, Greenland, Russia, and Alaska (Cuyler et al., 2019), and are culturally, economically, and nutritionally significant to the Indigenous Peoples around the Arctic (Prewer et al., 2022; Tomaselli et al., 2018). Muskoxen are keystone species that contribute to ecosystem health via soil nutrient turnover (Mosbacher, Michelsen, et al., 2016). While muskoxen are currently considered species of least concern by the International Union for Conservation of Nature (IUCN) given their current global population size, there are fears that the long-term sustainability of this iconic Arctic species is threatened by continued, rapid environmental warming (Cuyler et al., 2019; Gunn & Forchhammer, 2022). These concerns are substantiated by population collapses on Victoria and Banks Island, which previously held 61% of Canada's overall

muskox population (Cuyler et al., 2019; Gunn & Forchhammer, 2022; Kutz et al., 2015). Muskoxen are long-lived species with notoriously low levels of genetic diversity, characteristics predicted to reduce their capacity to adapt on timescales reflective of rapidly changing Arctic environments (Hansen et al., 2018; Prewer et al., 2019). As such, other adaptive mechanisms such as microbiome plasticity may be key to the long-term survival of muskoxen (Bird et al., 2019; Hansen et al., 2018; Prewer et al., 2019).

Muskox microbiomes consist mainly of *Firmicutes* and *Bacteroidetes* that digest carbohydrates and fiber, consistent with observations in other ruminant species like cattle, bison, goats, and sheep (Bird et al., 2019). The ability of muskoxen to digest a high fiber diet is key to their survival in the Arctic as it allows them to eat vegetation that other Artic herbivores, like caribou and hares, avoid given their low-nutrient status (Bird et al., 2019; Salgado-Flores et al., 2016; Schmidt et al., 2018). Variations in wild muskox microbiomes have been associated with environmental and host factors such as body mass and population of origin (Andersen-Ranberg et al., 2018; Bird et al., 2019). Additionally, Bird et al. (2019) found geography and seasonality had strong effects on abundance, diversity, and richness of bacterial species in muskoxen, with higher bacterial diversity observed in more northern regions and winter months (Bird et al., 2019). While the diversity and abundance of ingested vegetation in muskoxen are thought to have concomitant effects on the microbiome, this hypothesis has not been directly tested in wild populations.

Muskoxen are grazers, where previous studies suggest they have a narrow dietary niche made up mostly of graminoids, sedges, and willows, all of which are considered low-quality forage (Adamczewski, Flood, et al., 1994; Adamczewski, Kerr, et al., 1994; Schmidt et al., 2018; Staaland et al., 1997; Ungerfeld et al., 2018). In Canada, muskoxen span a wide latitudinal range from the mainland of Nunavut and Northwest Territories to the high Arctic islands, consisting of

several ecozones that vary in vegetation types and abundances (Figure 5.1). This variation, as well as clinal changes in vegetation morphology and nutritional status, is thought to result in latitudinal shifts in diet, especially between mainland and island populations (Smith et al., 2002). Muskox diet has also been found to vary seasonally, as access to vegetation is restricted during winter, where icing and snowfall reduces the quantity of food available (Ihl & Klein, 2001; Larter & Nagy, 1997, 2004; Mosbacher, Michelsen, et al., 2016). Ihl and Klein (2001) found that while muskoxen select for graminoids, suitable snow conditions led them to feed predominantly in high lichen cover areas with their feces containing high occurrences of lichen. As climate change impacts Arctic forage abundance and distributions, it is unclear whether vegetation changes will influence muskox gut bacteria and undermine fitness. Direct comparisons of muskox microbiomes and diet diversity may help in understanding how muskoxen interact with their environments and the influence climate change may have on their health.

Previous descriptions of muskox diet variability have been mostly based on broad vegetation indexes, microhistology, and stable isotope analyses. While informative, these approaches are limited in resolving finer-scale changes in diet (Ihl & Klein, 2001; Larter & Nagy, 1997, 2004; Mosbacher, Michelsen, et al., 2016). Metabarcoding allows for species identification in mixed samples through high throughput sequencing (Bush et al., 2019; Ruppert et al., 2019; Zepeda Mendoza et al., 2015), where a variety of genetic markers are used to inform a myriad of applications such as ecosystem biomonitoring, environmental responses to pollution, and the characterization of the gut microbiome (Bird et al., 2019; Ruppert et al., 2019). DNA metabarcoding of plant markers has also been established as a highly sensitive, accurate, and non-invasive method to assess diet, particularly from fecal samples (Boukhdoud et al., 2021). Diet metabarcoding was performed on 20 muskoxen from Greenland by Schmidt et al. (2018), where their diet was found to consist mainly of forbs and graminoids, consistent with previous

muskox diet analyses. The validation of diet analyses via fecal plant metabarcoding in muskoxen therefore allows for the integration of microbial and vegetation metabarcoding data, with the potential to better describe and elucidate relationships between muskox diet and microbiome diversity.

In this study, we amplify three common plant markers, the P6 loop of the chloroplast trnL (UAA) intron region (TRNL), ribulose 1,5-biphosphate carboxylase region (RBCL), and Internal Transcribed Spacer region (ITS) from fecal samples to assess the diet of wild muskoxen across much of their Canadian range. We compared the diversity and composition of the diet to that of the microbiome to determine how vegetation in different landscapes contributes to bacterial variation in muskox gut microbiomes. Data provided herein offer insight into the plasticity of microbiomes relative to dietary composition, which in turn also allow for predictions of the long-term impact climate change and concomitant vegetation changes have on muskox viability.

5.3 Methods

5.3.1 Plant DNA Analysis

Fecal pellet samples and DNA used in this study were previously selected and extracted by Bird et al. (2019) as part of a gut microbiome investigation of 78 wild male muskoxen. These samples were collected from six regions, encompassing two genetically distinct populations (Victoria Island and mainland individuals), three ecozones (taiga plains, southern Arctic, and northern Arctic), and three seasons (spring, summer, and winter) (Figure 5.1). The extracted DNA from these 78 samples was used herein for plant metabarcoding in order to directly compare microbiome and vegetation diversity.

We employed three primer pairs commonly used for plant metabarcoding including chloroplast TRNL region using the g/h primer set from Taberlet et al. (2007); chloroplast RBCL region using the z1aF/hp2R primer set from Hofreiter et al. (2000); and an Internal Transcribed Spacer region, ITS, using 2F/3R primers from Chen et al. (2010). These three markers were chosen as they sequenced both chloroplast and nuclear DNA and had different amplicon lengths, which provided the ability to amplify degraded DNA while still retaining the sequence diversity to differentiate between species. Amplicon lengths were ~150, 300, and 500 bp, respectively. Genes were amplified and libraries were prepared using a modified version of the 16S metagenomic Illumina protocol (Illumina). Total amplification reaction volumes were halved using 6.25 µL of Kappa HiFi mastermix (Roche), 0.38 µL of 10 µM forward and reverse primer, 3.5 µL of water, and 2 µL of stock extracted DNA for a total volume of 12.5 µL per sample. Amplification temperatures and cycle numbers were optimized on pure soy DNA as a control, as well as a subset of muskox fecal samples using temperature gradients. TRNL and RBCL were amplified at 62°C, and ITS was amplified at 60°C with 35 cycles to amplify all markers. All muskox samples were amplified in triplicate and visualized on an ethidium bromide-stained 1.5% agarose gel along with negative controls of ultrapure water and positive controls consisting of soy DNA. Triplicate samples were pooled, cleaned, and size-selected using AMPure XP beads (Beckman Coulter Life Sciences) with a bead ratio of 1.2 for TRNL and 0.8 for RBCL and ITS. Samples were indexed for sequencing using dual 8 bp combinations following the Illumina 16S metabarcoding protocol where volumes were once again halved for a total of 25 µL. Following a final AMPure XP bead clean up, Quant-IT dsDNA Picogreen kit (ThermoFisher) was used on a FLUOstar Omega microplate reader to determine the DNA concentration of each sample for each marker. Samples were normalized to a standard concentration per marker and pooled to obtain a final library for each marker. Libraries were then sent to the University of Guelph's AAC

genomics facility for sequencing on an Illumina MiSeq using a 600-cycle V3 kit to produce 2 × 300 bp reads.

5.3.2 Plant library filtering

Data were analyzed using the Quantitative Insights into Microbial Ecology 2 (QIIME2) pipeline v.2019.7 (Bolyen et al., 2019). QIIME2 was first used to demultiplex samples after which primer and adapter sequences were trimmed using cutadapt wrapper followed by denoising performed using the dada2 algorithm. A reference database of TRNL sequences was created from sequences uploaded to the NCBI database. Sequences were then filtered to remove duplicates and only contain plant and fungi nucleotide sequences from the respective chloroplast regions. Taxon IDs were extracted from sequences to create a corresponding lineage database for each taxon. The final database contained 288,520 sequences from 161,845 taxa. For RBCL, full data records were downloaded from the Barcode of life database (BOLD), which included taxonomy and nucleotide sequences for all plant species. The sequences were filtered to only contain sequences that were from RBCL markers and had taxonomy information down to the family level at minimum. The taxonomy and sequence information was then extracted and filtered further to remove duplicates and low-quality sequences. This resulted in 128,125 sequences of 47,665 taxa. A high-quality QIIME2 formatted database created by Banchi et al. (2020) was used as a reference for the ITS marker using the ITS2 dataset. Sequences for the RBCL and TRNL markers were then compared with their respective databases to create amplicon sequence variants (ASVs) that were taxonomically classified using BLAST+. ITS sequences were taxonomically classified using the sklearn algorithm. Libraries from each marker were then rarified for sequencing depths of 2000 for RBCL, 4000 for TRNL, and 1000 for ITS.

5.3.3 Plant data analysis

Rarefied and nonrarefied datasets were imported into R (v4.2.1; R Core Team, 2022) for statistical analyses. First, phyloseq (v1.40.0; McMurdie & Holmes, 2013) was used to create relative abundance bar plots for all three markers using the rarefied dataset. Beta diversity correlation between ASVs and Shannon index of TRNL, RBCL, ITS, and the microbiome were compared using Bray–Curtis dissimilarity and Jaccard dissimilarity using Spearman correlations with the Vegan package (Oksanen et al., 2020). Rarefied data was used for these analyses to allow for sample and marker comparisons where sample size and sampling depth varied (Cameron et al., 2021). Nonrarefied data from the three diet markers, as well as from Bird et al. (2019), was then used to compare alpha diversity between the diet and microbiome observed ASVs and Shannon index using Spearman's rank correlation using Vegan v.2.5-7 (Oksanen et al., 2020). The nonrarefied dataset was used to calculate alpha diversity statistics to reduce the risk of bias and provide a more thorough representation of diversity by detecting all differentially abundant ASVs (Bird et al., 2019; McMurdie & Holmes, 2013). Correlation was measured between ASVs of TRNL and microbiome, RBCL and microbiome, and ITS and microbiome and repeated with the Shannon indexes of these samples. Additionally, these comparisons were made between TRNL, RBCL, and ITS markers to determine how similar data were between the three markers.

5.4 Results

5.4.1 Sequencing and library analysis

A total of 4,882,634 paired reads were sequenced for TRNL, ranging from 223 to 227,408 paired reads per individual. RBCL had a total of 1,682,655 paired reads sequenced, ranging from 22 to 93,399 paired reads per individual. ITS had a total of 882,042 paired reads ranging from 8 to

92,073 per individual. Denoising left 2,109,690 (43%) sequences for TRNL, with an average of 25,418 reads per individual, RBCL retained 1,335,114 (82%) sequences with an average of 16,689 reads per individual and ITS had 186,475 (21%) sequences left with an average of 2330 sequences per individual. Remaining TRNL reads were clustered into 227 Amplicon Sequence Variants (ASVs), where RBCL clustered into 188 ASVs, and ITS reads were clustered into 212 ASVs. The final rarefied dataset had an equal sequencing depth of 4000 reads for TRNL, 2000 reads for RBCL and 1000 reads for ITS.

5.4.2 Muskox diet

Two metabarcoding markers found a similar number of species, with RBCL identifying 178 species in comparison to 188 species for TRNL while ITS only identified 66 species. At the family level, there was a large difference in diversity with RBCL having over double that of TRNL with 38 and 17 families, respectively, and ITS having the least at 13 families. The muskox's diet was mainly composed of two families, *Cyperaceae* (sedges), and S*alicaceae* (willows). Based on the overall number of reads associated with each family, TRNL had both willows and sedges as its top two families; however, RBCL only had sedges as a dominant family while for ITS the dominant family was willows (Figure 5.2). Specifically, willows only made up 0.33% of RBCL sequences while sedges were not identified in ITS at all. In addition to willows and sedges, *Fabaceae* (legumes) and *Betulaceae* (birch) were also prominent families based on TRNL sequences. For RBCL, *Rosaceae* (rose), *Betulaceae* (birch), *Ericaceae* (heaths), and *Saxifragaceae* (saxifrages) were dominating families in addition to sedges. Finally, ITS had *Fabaceae* (legumes) and *Rosaceae* (rose) as the dominating families in addition to willows. TRNL and RBCL shared 10 families making up 87.5% of reads in RBCL and 98.8% of reads in TRNL. All of the families found in ITS were also found in both TRNL and RBCL. At a species level, only one species (*Stellaria*

longipes) was shared across all three markers, with seven species shared between at least two markers, demonstrating that comparisons among markers should only be performed at higher taxonomic levels.

5.4.3 Relationship between alpha diversity of diet and microbiome

We tested the alpha diversity of microbiomes to the corresponding alpha diversity of diets and found a significant, negative correlation between diet and microbiome alpha diversity with R2 ranging from −0.259 to −0.519 (Figure 5.3). This significant negative relationship was consistent for all markers based on Shannon indices and ASVs with the exception of TRNL and 16S alpha diversity, which was not significant (Figure 5.3b). We also tested alpha diversity between diet markers to determine whether they were correlated despite not sharing similar species. There was a significant positive correlation between diet alpha diversities of ITS and RBCL R2 values at 0.52 for ASVs and 0.285 for the Shannon index (Figure S5.1c,f). However, only observed ASVs were significant between ITS and TRNL with an R2 value of 0.111 and only Shannon index was significant between RBCL and TRNL with an R2 value of 0.335 (Figure S5.1a,e).

5.4.4 Relationship between beta diversity measures

We tested beta diversity between microbiomes and diet for correlations, where beta diversity is a measure of the degree to which samples differ from one another. We found significant positive correlations between beta diversity of microbiome and diet, as well as between diet markers (Figure 5.4). Samples with similar diet were found to have more similar microbiomes where R2 ranged from 0.298 to 0.657 between diet and microbiome (Figure 5.4).

5.5 Discussion

We assessed wild muskoxen plant diet composition and diversity via DNA metabarcoding from environmental fecal samples across ecozones and seasons. These data were then compared with observed microbiomes from the same fecal samples to evaluate correlations between diet diversity on that of the microbiome. We observed a lack of concordance between metabarcoding markers employed, with divergent plant species identified, as well as varying plant family abundances within individuals, yet there were consistent general trends found across markers. When comparing diet and microbiome, alpha diversity was negatively correlated, indicating that as diet diversity increases, microbiome diversity decreases. Diet beta diversity and microbiomes were positively correlated, indicating samples with similar diets had more similar microbiomes. These data enhance our understanding of relationships between muskox diet and their microbiome; relationships relevant to assessing the long-term sustainability of this iconic species in response to climate change and a warming Arctic.

5.5.1 Muskox Diet

This study aimed to elucidate muskox diet from three commonly employed plant metabarcoding markers (RBCL, TRNL, and ITS) in an attempt to avoid ascertainment biases that can result from single marker studies and add to the breadth of dietary items identified. While the three markers identified a wide range of species and families, the lack of concordance in the number of families identified and familial abundances indicate clear ascertainment biases among markers and demonstrate the importance of using multiple markers and marker choice for metabarcoding approaches (da Silva et al., 2019; Srivathsan et al., 2015). The lack of concordance observed among the markers could partially be attributed to primer specificity biases, where different primers preferentially bind to specific species due to mismatches and

can change the relative abundances of those families in the reads (da Silva et al., 2019; Deiner et al., 2017). Additionally, universal primer designs for metabarcoding must balance high taxonomic coverage and high taxonomic resolution, which can lead to concordance issues as primer pairs designed to bind to highly conserved sites will likely amplify a larger variety of species but lack the variation to distinguish between said species (da Silva et al., 2019; Hollingsworth, 2011; Moorhouse-Gann et al., 2018). For example, the use of ITS has been criticized due to its lower universality in comparison to other markers. ITS, however, has a higher power to discriminate where TRNL has been found to have ambiguity problems at the genus and species level (da Silva et al., 2019; Moorhouse-Gann et al., 2018). The level of sample degradation can also affect the concordance of metabarcoding results where markers with shorter amplicon sizes better amplify degraded samples but have lower power to distinguish between species (Coghlan et al., 2021; Mallott et al., 2018; Ruppert et al., 2019). To illustrate this point, Mallott et al. (2018) used metabarcoding of the TRNL (~150 bp) and RBCL (~500 bp) markers to study diets of capuchin monkeys. They found that TRNL produced a higher number of sequences, more unique sequences, and that more sequences were identified to the order or family level, while RBCL had more reads that were identifiable to species level (Mallott et al., 2018). Similarly, we amplified and retained more sequences with the TRNL marker in our study, while the RBCL marker identified more families. A lack of reference reads across the databases could also partly account for the lack of concordance and overlapping species between markers. To assess this possibility, we searched the databases for sequences of species that were highly represented by one marker and missing in the others. Representative sequences were found across all markers indicating that this was not the cause of minimal overlapping species. Despite differences in diversity and abundance of plant families between markers in this study, our results do align to previous observations that found willow (*Salicaceae*) and sedge (*Cyperaceae*)

families comprise up to 95% of the muskox's diet using both metabarcoding and histological methods (Ihl & Klein, 2001; Larter & Nagy, 1997, 2004; Schmidt et al., 2018).We also found legumes (*Fabacea*), rose (*Rosaceae*), heath (*Ericaceae*), birch (*Betulaceae*), and saxifrage (*Saxifragacea*) families to be large contributors outside of willows and sedges, though no marker found all these families in high abundance. Overall, with both sedges and willows identified as the major contributors to diet, TRNL provided results most consistent with previous histological studies that sampled from similar regions and seasons but lacked family-level diversity identified by RBCL (Ihl & Klein, 2001; Larter & Nagy, 1997, 2004). While histological studies (Ihl & Klein, 2001; Larter & Nagy, 1997, 2004) have sampled muskoxen across multiple seasons and geographic regions, improved taxonomic resolution and sensitivity in detecting low-abundance plant families were observed with metabarcoding tools. Previous metabarcoding of muskox diet performed by Schmidt et al. (2018), similarly found that muskoxen ate mainly shrubs, forbs, and graminoids, though they did not find a high abundance of sedges. While findings from Schmidt et al. (2018) differ from the results found herein, all 20 samples from their study were collected in winter from a 7-km radius area in Greenland. As such, data from Schmidt et al. (2018) would not be expected to encompass the variation of plants eaten by muskoxen across multiple seasons and ecozones in the Canadian Arctic (Schmidt et al., 2018). Although the sampling scheme herein is also limited by the distribution of samples over season and ecozones, results from the current study may provide a more complete picture of muskox diet diversity in the Canadian Arctic. While metabarcoding has improved the ability to detect low-abundance plants, the use of fecal samples to assess diet may be biased against species that are highly digestible. This might result in an artificially high abundance of fibrous plants like willows and sedges as they are harder to digest. Overall, despite a lack of consensus between these three markers

regarding specific diet composition, there were consistent trends identified from the metabarcoding data from this study in relation to diet diversity and the microbiome.

5.5.2 Diet and microbiome diversity correlations

Samples used in this study were the same as those used by Bird et al. (2019), providing an opportunity to directly compare microbiome diversity to diet diversity in wild muskoxen. We compared both beta diversity (diversity between samples) and alpha diversity (diversity within samples) of muskox diets and microbiomes. We found significant positive correlations between beta diversities of microbiomes and diet, where individuals with more similar diets had more similar microbiomes. When comparing alpha diversities, however, there was a significant negative correlation between these data types. This was unexpected, as a more diverse diet is thought to introduce more diverse microbiota and nutrients to the gut and therefore require, or lead to, higher microbiome diversity (Bolnick et al., 2014). Few studies have yielded similar negative correlations between microbiome and diet diversity, such as Bolnick et al. (2014) who found a negative correlation of alpha diversity in wild and laboratory-raised freshwater fish, where generalist fish, expected to have more diverse diets, had lower microbiome diversities. Li et al. (2016), compared the microbiome and diet of pika and found that while there was a positive correlation of beta diversity, alpha diversity was not correlated in this species. Both Bolnick et al. (2014) and Li et al. (2016) suggest several hypotheses as to how more diverse diets could lead to reduced gut microbiome diversity. First, certain foods may contain chemical inhibitors that reduce or eliminate certain bacteria, therefore the more diverse the diet, the more inhibitors they would ingest. It was also hypothesized that diet influences host health and immunity, which affects an individual's ability to respond to novel microbial species or microbial infections (Bolnick et al., 2014; Li et al., 2016). Finally, these authors proposed that some

dominant species of microbes function or survive better on a wider range of nutrients derived from more diverse diets, as opposed to more rare microbes that have more specific nutrient needs (Bolnick et al., 2014; Li et al., 2016). While previous studies provide general explanations for unusual microbiome and diet correlations, there may be factors specific to muskoxen that contribute to the trends observed in the current study.

As stated, the muskox diet consists primarily of willows and sedges (up to 95%) (Ihl & Klein, 2001; Larter & Nagy, 1997, 2004; Mosbacher, Michelsen, et al., 2016), suggesting this species is selective in their diet, if only at the familial level. Similarly, Schmidt et al. (2018) found that when food availability was low, the diets of muskoxen, rock ptarmigans, and Arctic hares did not overlap, and muskoxen continued to consume the lowest diversity diet (Schmidt et al., 2018). This might suggest microbiomes of muskoxen are adapted primarily to these two plant families, rather than the presence of other families in low abundance. Therefore, the muskox microbiome may be more influenced by the relative abundance of the ingested plant families, rather than the diet diversity in terms of plant families present. Additionally, sedges and willows are high-fiber plants, thus muskox microbiome compositions may be adapted to the digestion of fiber specifically. Bird et al. (2019) found the major bacterial orders in muskox samples were *Firmicutes* and *Bacteroidetes*, species known to help fiber and carbohydrate digestion. However, if willows and sedges were key families affecting microbiome diversity, we would expect to see similar results to that of Li et al. (2016), where beta diversity between microbiome and diet was correlated, but alpha diversity was not, as these families were consistently found to make up a large percentage of the muskox diet. While the presence of these plant families may be consistent within the muskox diet, the quality of the willows and sedges can vary across ecozones, seasons, and years (Larter & Nagy, 2001), which we speculate may also affect the muskox microbiome. Another factor that could impact the relationships between diet and

microbiome of muskoxen is the amount of food eaten. Metabarcoding methods have the potential to be more sensitive and accurate in their detection of plant species, but they are unable to measure the volume of food eaten and how this impacts a species' microbiome. Food intake is known to affect the concentration of fermentation acids, ruminal pH, and bacterial conditions in muskoxen that in turn may limit microbial communities and suppress more active microbes (Barboza et al., 2006). In species that have high-fiber diets, a slow passage rate allows for increased food breakdown and nutrient extraction (Barboza et al., 2006). However, hyperphagia results in an increased or fast passage of food and therefore may be more detrimental to digestion when it occurs in muskoxen (Barboza et al., 2006). Decreased food intake in winter has been documented in captive muskoxen where the same food is available throughout the seasons, indicating further that low food intake over winter is beneficial/adaptive for muskoxen (Adamczewski, Flood, et al., 1994). Decreases in food volume also lower the number of bacteria in their rumen overall, rather than just the number of species present (Barboza et al., 2006; Crater & Barboza, 2007). This helps maintain ideal digestion rates for decreased food intake but also makes it easier to detect lower quantity bacteria species that may have previously been outcompeted and consequently, increases measures of microbiome diversity.

5.5.3 Muskox diet, microbiome, and Arctic warming

Arctic warming is changing the diversity and composition of Arctic plant communities (Mekonnen et al., 2021; Mod & Luoto, 2016) and specifically, results in shrubification, a rise in shrub abundance via increased growth and progressive shrub lines (Mekonnen et al., 2021; Mod & Luoto, 2016). As shrubification continues, increased competition for native Arctic plant species is predicted to reduce tundra plant biodiversity (Mekonnen et al., 2021; Mod & Luoto,

2016). This in turn could impact the survival and biodiversity of Arctic herbivores that fall within dietary niches that consist of native Arctic plants (Mekonnen et al., 2021; Mod & Luoto, 2016; Mosbacher, Kristensen, et al., 2016; Schmidt et al., 2018). Some examples of shrub families increasing in abundance include willows, consistently found to play an important role in muskox diet, and birch, which muskoxen are thought to typically avoid but were found in relatively high abundances herein. Shrubification may then have the potential to aid muskox populations by providing additional resources (Mekonnen et al., 2021; Mod & Luoto, 2016; Mosbacher, Kristensen, et al., 2016). In Greenland, muskox grazing reduced the extent of shrubification occurring, diminishing the effects of Arctic warming on Arctic vegetation and increasing plant biodiversity in grazed areas (Mosbacher, Kristensen, et al., 2016; Post et al., 2021; Post & Pedersen, 2008). While an increased availability of shrubs like willows and birch may be promising, increasing their relative abundance in the muskox diet may not be ideal, as nutrient content affects diet selection and their microbiome. Lawler and White (1997) investigated the effects of browse, like willows and birch, to muskox diet and found muskoxen rejected feed containing over 60% browse, while feed with over 40% browse resulted in a post-ingestion energy loss (Lawler & White, 1997). These results were thought to be linked to energy required for the detoxification of poisonous secondary compounds like tannins found in leaves and twigs of willows (Lawler & White, 1997). Further, these findings indicate that though muskoxen could benefit from an abundance of shrubs in the Arctic, they require access to graminoid species as well. The loss of other plant species, particularly graminoid species from shrubification, could eventually result in diets that are more harmful and cost more energy to muskoxen. Arctic warming not only affects Arctic plant species diversity but the phenology of local vegetation. Availability and reliability of vegetation play a role in muskox demographics with earlier green-up times associated with increased population sizes and plant consumption (Eikelenboom et al.,

2021; Koltz et al., 2022; Post et al., 2019). Muskoxen are capital breeders and are expected to be relatively insensitive to changing plant phenology as their breeding is reliant on their body reserves from the past year, rather than resources obtained during the breeding period (Gustine et al., 2010; Kerby & Post, 2013). As green-up times occur earlier, there would be an expected increase in trophic match, where longer growing seasons are associated with increased muskox abundance (Kerby & Post, 2013; Koltz et al., 2022; Post et al., 2019). Additionally, with advancing green-up, there is improved calf recruitment in muskoxen, as earlier and more abundant food lowers the cost of lactation on the mothers and calves have higher quality forage when they transition to grazing (Eikelenboom et al., 2021). However, increases in muskox populations from early green-ups are expected to eventually stabilize or decline as winters become more wet and snow depths increase (Eikelenboom et al., 2021). High snow depth and coverage limit muskox accessibility to willows and can result in periods of starvation and weight loss (Desforges et al., 2021; Mosbacher, Kristensen, et al., 2016). Body stores that would normally aid calf growth then become necessary for the survival of cows, leading to poor calf recruitment (Mosbacher, Kristensen, et al., 2016). Thus, while speculative, changes to vegetation diversity and phenology may benefit muskoxen in the short term, but as effects continue, muskoxen populations are at risk of decline in the long term.

5.6 Conclusion

Metabarcoding markers employed in this study did not provide fully concordant diversity and composition results yet revealed willows and sedges as major dietary contributors for muskoxen; a finding consistent with previous metabarcoding and microhistology studies (Ihl & Klein, 2001; Larter & Nagy, 1997, 2004; Schmidt et al., 2018). Comparisons of diet and microbiome diversity found similar diets had more similar microbiomes; however, alpha diversity between microbiome and diet was negatively correlated. The negative correlation was

unexpected and may be due to muskox adaptation to high-fiber diet when access to vegetation is limited, as it is in non-summer months in the high Arctic. Marker inconsistencies undermined the capacity to directly measure how diet composition impacted microbiome and future studies with additional markers could help clarify these patterns. In particular, the addition of markers that can amplify lichen species, which muskoxen are known to ingest, could provide insight into how these organisms contribute to microbiome diversity. Testing the efficiency of the markers binding to the most abundant plant families could also be performed in future to strengthen diet metabarcoding analyses and assess marker reliability (Buglione et al., 2018). Further, adding more individuals to provide even sampling across ecozones and seasons would allow for analyses of spatial and temporal differences in the muskox diet. With extremely low levels of genetic variation across muskox populations, there are concerns that muskoxen cannot rapidly adapt to the environmental changes brought on by climate change (Cuyler et al., 2019; Gunn & Forchhammer, 2022; Hansen et al., 2018; Prewer et al., 2019). In other systems of long-lived, low genetic diversity species microbiome plasticity has been postulated as a mechanism to rapidly adapt to climate change and thus may play a role in long-term muskox sustainability (Bird et al., 2019; Cuyler et al., 2019; Gunn & Forchhammer, 2022; Kutz et al., 2015). Overall, the data from this study provides insight into the relationship between muskox diet and microbiome, which will further our understanding of how muskoxen can adjust to shifting vegetation due to rapid Arctic warming.

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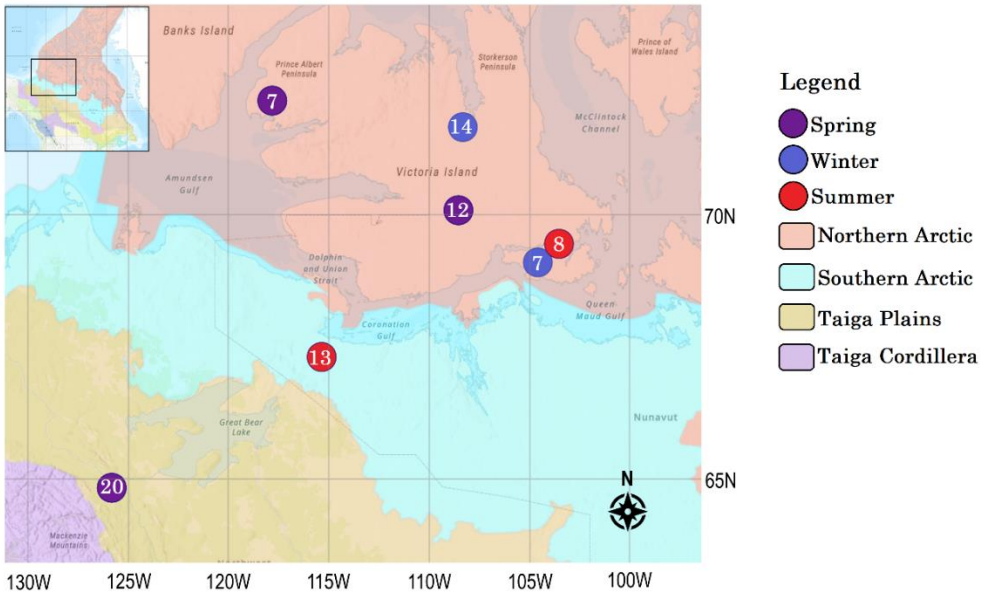

Figure 5.1. Map of sampling sites included in this study. Ecozones are differentiated by base map colour (ESRI Canada), season of collection is indicated via circle colour and the number of samples collected per sampling location is indicated by the number inside circle.

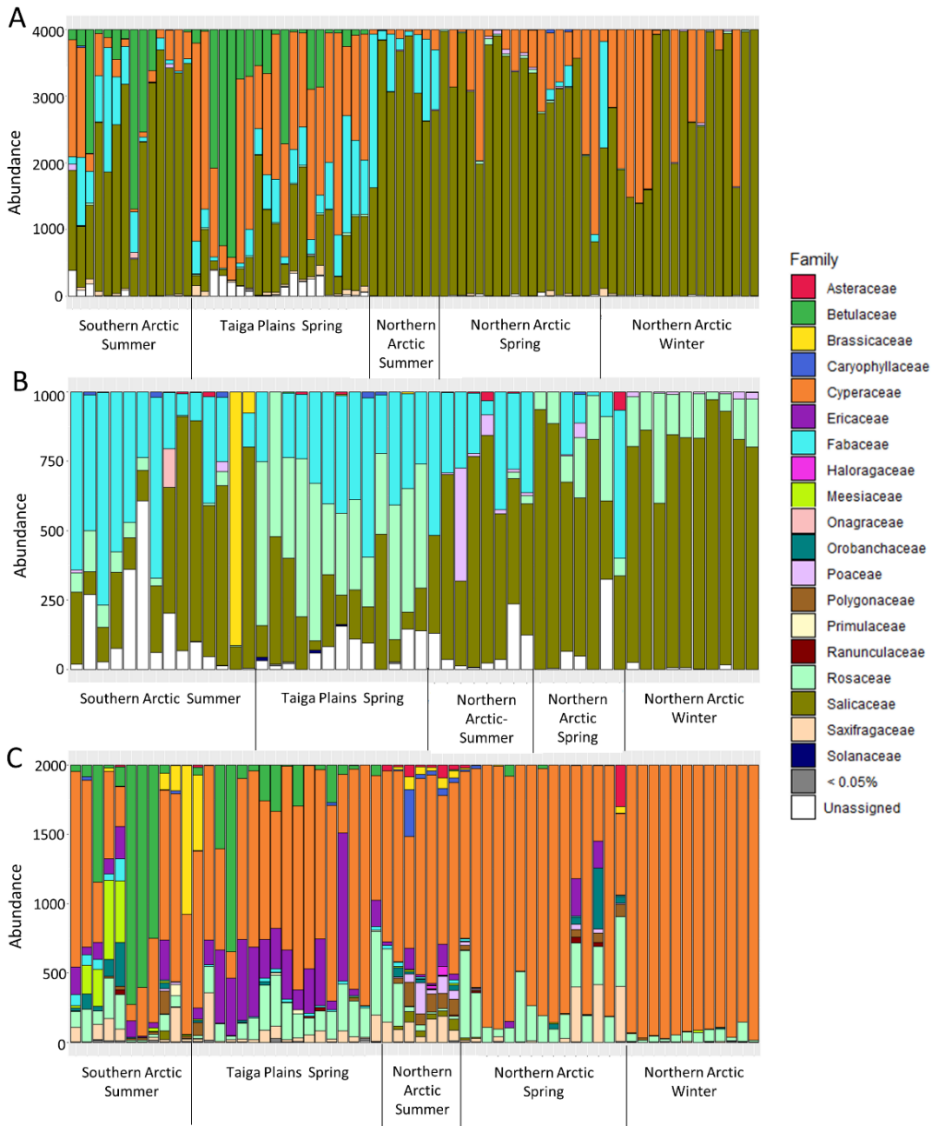

Figure 5.2. Taxonomic abundance plot for the top plant families from: a) TRNL, b) ITS and c) RBCL markers classified in QIIME2. Each bar represents an individual with colours showing ratio of each family found in the reads. Plant families that made up under 0.05% of the overall reads across all three markers were combined and represented by a single bar colour. Samples are organised by season for each ecozone. RBCL found 38 families with the most abundant family being *Cyperaceae*, TRNL found 18 families with *Salicaceae* most abundant and ITS found 13 families with *Salicaceae* also being the more abundant.

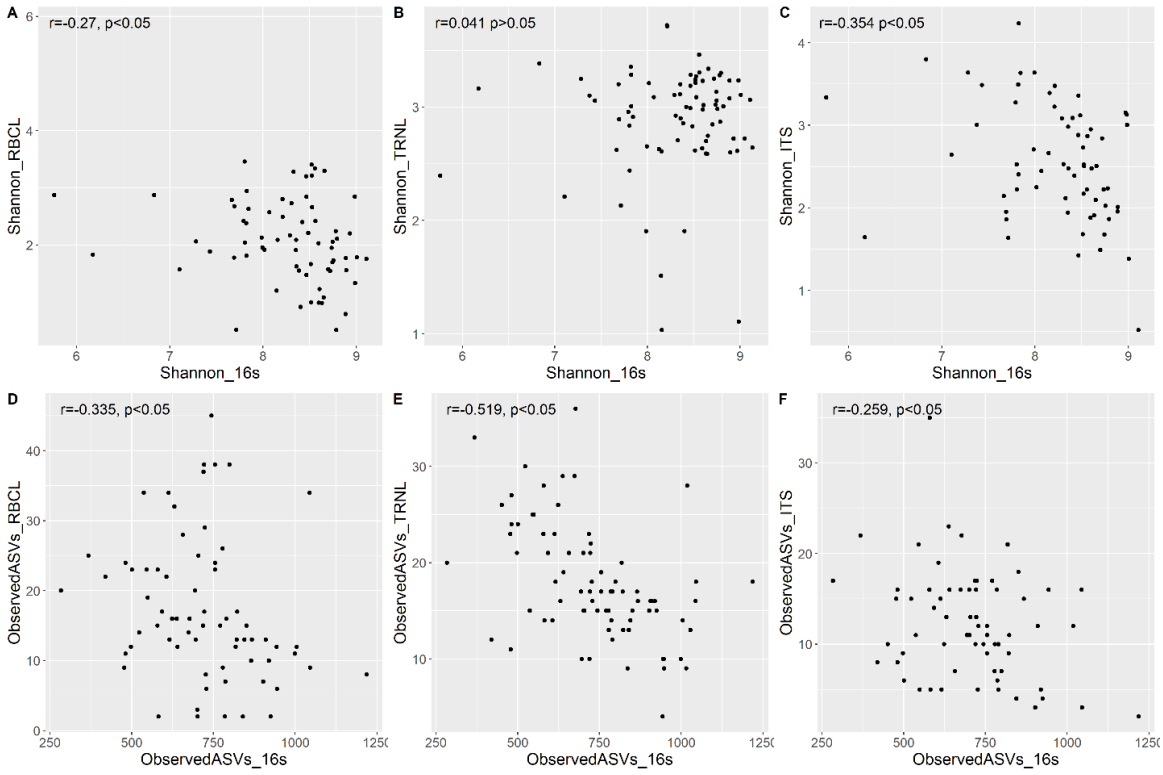

Figure 5.3. Relationships between alpha diversity metrics of the diet and microbiome metabarcoding from 78 male muskox individuals. (A) RBCL diet Shannon index and 16S microbiome Shannon index. (B) TRNL diet Shannon index and 16S microbiome Shannon index. (C) ITS diet Shannon index and 16S microbiome Shannon index. (D) RBCL diet ASVs, and 16S microbiome ASVs. (E) TRNL diet observed ASVs, and 16S microbiome ASVs. (F) ITS diet ASVs, and 16S microbiome ASVs.

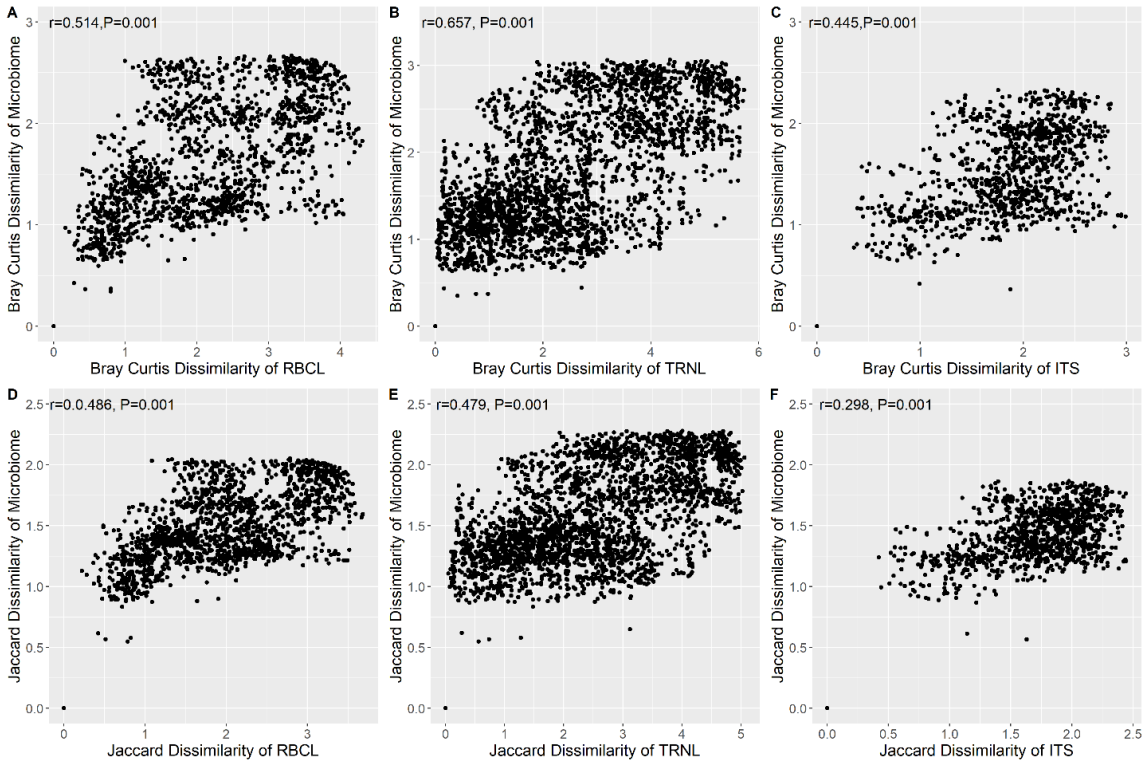

Figure 5.4. Relationship between beta diversity for of (A) RBCL diet Bray Curtis dissimilarity and 16S microbiome dissimilarity. (B) TRNL diet Bray Curtis Dissimilarity and 16S microbiome Bray Curtis Dissimilarity. (C) ITS diet Bray Curtis Dissimilarity and 16S microbiome Bray Curtis Dissimilarity. (D) RBCL diet Jaccard Dissimilarity and 16S microbiome Jaccard Dissimilarity. (E) TRNL diet Jaccard Dissimilarity and 16S microbiome Jaccard Dissimilarity. (F) ITS diet Jaccard Dissimilarity and 16S microbiome Jaccard Dissimilarity.

Chapter 6. General Discussion

In this thesis, I used molecular tools to assess the levels and drivers of genetic diversity in muskoxen and how diversity impacts their viability. Overall, I found a lack of genetic diversity across muskoxen as a result of historic bottlenecks, population fragmentation and inbreeding. Even with muskox diversity potentially reaching a minimum, muskoxen have repeatedly recovered from population lows and many populations currently thrive due, in part, to local adaptations and purging of deleterious alleles. Differences in muskox microbiome diversity across variable landscapes also reflect its plasticity to diet variation and demonstrates a potential mechanism for rapid responses to changing environments. Despite their long-term persistence, the data from my thesis holistically suggests that muskoxen may be vulnerable to the rapid environmental change they are now facing, given the lack of genetic variation that may be necessary to respond to new selective pressures. Further, within muskoxen, two distinct groups arose, aligning with proposed subspecies and were found to have different levels of conservation concern due to opposing trends in genetic diversity, inbreeding and effective population sizes. These conclusions were supported by findings in: Chapter 2, where microsatellite markers identified significant genetic diversity differences between two muskox populations currently undergoing opposing population trends and revealed genetic variation in muskoxen may already be at a minimum; Chapter 3, where the first muskox genome was assembled, providing a foundation for future muskox genetic research as well as a better understanding of the muskox's demographic and evolutionary histories; Chapter 4, where 22 muskox genomes were sequenced from across their natural range, improving muskox phylogenies and better measuring the distribution and drivers of genetic diversity in muskoxen; Chapter 5, where plant metabarcoding was used to assess muskox diet composition and diversity in relation to their microbiome in order to gain insights into their microbiome plasticity

and unique capacity to digest a high fiber diet. More broadly, the findings from this thesis can help provide a better understanding of the relationship between genetic diversity and population persistence and vulnerability in the face of a changing environment.

6.1 Drivers of muskox persistence and vulnerability

Genetic diversity has been used as a measure of population fitness and evolutionary potential, where species or populations with low genetic diversity are often assumed to warrant conservation concern (Forester et al., 2022; Fox et al., 2019; Hoban et al., 2013; Hoffmann, 2010; Kardos et al., 2016; Zimmerman et al., 2023). Stochastic events resulting in population bottlenecks and fragmentation can lead to small census population sizes associated with inbreeding depression, poor population performance and low variation (Banks et al., 2013; Forester et al., 2022; Hoban et al., 2013; Markert et al., 2010). This low variation is also associated with a reduced response to selection and increased drift, further contributing to declines in census population sizes (Andrews et al., 2016; Pérez-Pereira et al., 2022; Willi et al., 2022). Muskoxen are estimated to have diverged from their most recent common ancestor 8-17 Mya, emigrating from Eurasia to North America (Chapter 3; MacPhee et al. 2005; Campos et al. 2010). They have since undergone multiple population cycles, where repeated population bottlenecks have reduced their once Holarctic distribution. Pairwise sequentially Markovian coalescent (PSMC) analyses performed in Chapter 3 showed two historical population lows aligning with major glaciation events. The second, and more extensive population decline, ranged from 1 Mya to 20 Kya, when effective population sizes fell from 100 thousand individuals to 2000 individuals. Further, sequencing of a 21-thousand-year-old muskox genome found modern muskox genetic diversity estimates were up to 14 times lower than those of the ancient muskox, suggesting diversity continued to decline since the last glacial maximum (Pečnerová et al., 2024). Upon glacial retreats, muskoxen are thought to have repopulated their historic ranges

via northward expansions and successive founder events (Hansen et al., 2018; Pečnerová et al., 2024). This was previously found by Hansen et al. (2018), and further supported by phylogenetic trees constructed in Chapter 4 using ultra conserved regions of the nuclear genome. In the early 1900s, over-hunting and harvesting led to the near extirpation of several southern muskox populations, though harvesting moratoriums allowed for recoveries in census population sizes and BEAST2 analyses showed their effective population sizes steadily increased since ~500 years ago (Chapter 2; Barr 1991). While the current global muskox census size is estimated at ~ 170,000 individuals, population structure and admixture analyses found that muskox populations are fragmented, with low gene flow leading to high levels of inbreeding in island populations (Chapter 2; Chapter 4; Gunn & Forchhammer 2022). The demographic history of muskoxen has therefore led to the loss of genetic variation.

Extremely low levels of genetic diversity have been found in muskoxen using both traditional and high throughput sequencing methods (Chapter 2; Chapter 4; Hansen et al. 2018; Holm et al. 1999; Van Coeverden et al. 2004; MacPhee et al. 2005; Thulin et al. 2011; Campos et al. 2010; Pečnerová et al. 2024; Lok et al., 2024). Sanger sequencing of the mitochondrial control region in muskoxen identified only 1.4% site variation, where in other species, site variation typically ranges between 1.6-20% (Groves, 1997). The assembly of the mitochondrial genomes in Chapter 4 also revealed low genetic variation, with individuals from different muskox populations and subspecies sharing the same mitochondrial coding sequences. Assessments of expected and observed heterozygosity via microsatellite genotyping (Chapter 2), genotyping by sequencing (Hansen et al., 2018), and whole genome sequencing (Chapter 4; Pečnerová et al. 2024; Lok et al., 2024), all revealed estimates lower than critically endangered species. Heterozygosity and allelic richness from multi-locus microsatellite data can be used in tandem to assess temporal diversity changes and adaptive capacity (Zimmerman et al., 2023). Allelic

richness has been found to immediately change with population declines, as it is more sensitive to population disturbances than heterozygosity and can be indicative of long term adaptive capacity (Zimmerman et al., 2023). As low heterozygosity takes longer to arise in populations, it is indicative of more extensive population declines and poor short-term adaptive capacity (Zimmerman et al., 2023). This was seen in the forward in time simulations of microsatellite diversity performed in Chapter 2 on muskoxen from Victoria Island. The observed number of alleles declined more quickly than heterozygosity under different population bottleneck scenarios, though retained 99% of the population's genetic diversity. However, both heterozygosity and allelic richness were already extremely low in Victoria Island muskoxen, indicating that they might already be at a genetic diversity minimum. For to genome wide diversity estimates, it is unclear whether heterozygosity and allelic richness estimates calculated from bi-allelic SNPs provide the same ability to assess evolutionary capacity and temporal diversity trends as is found with multi-locus microsatellites (Zimmerman et al., 2023). Therefore, other measures of genomic diversity, like effective population size (N_E), may be more informative of population status and maintenance.

Estimates of N_E were performed using both microsatellite and whole genome resequencing data which provided varied results with slightly contrasting conservation implications. Contemporary N_E estimates based on microsatellite data through BEAST2 analyses (Chapter 2), found mainland muskoxen had an N_E at ~ 42,000 while Victoria Island muskoxen had an N_E of ~ 13,000. Contemporary N_E through whole genome resequencing was estimated at 22 in muskoxen overall, 37 in white-faced muskoxen and 15 in barren ground muskoxen (Chapter 4). Therefore, N_E estimates from microsatellites would imply that muskoxen are relatively healthy while those from whole genome resequencing would indicate they have lost evolutionary potential and have poor short term viability (Frankham et al., 2014; Pérez-Pereira et al., 2022). One reason for these

differences could be that microsatellites may not provide the data necessary to create extended Bayesian skyline plots as the microsatellite mutation process limits the ability to estimate coalescence events (Nikolic & Chevalet, 2014). The N_E estimated using microsatellite data may also be artificially high due to a poor capacity to identify individuals through microsatellite genotypes. As the fecal samples utilised in Chapter 2 were anonymously sampled, one of the data processing steps was to remove duplicate genotypes, assuming these were the same individual, sampled multiple times from a population. However, as 7 loci neared or reached fixation on Victoria island, there is a high likelihood that there were some samples that were removed despite coming from unique animals which would have led to increased estimates of expected and observed heterozygosity, N_E, admixture and reduced estimates of inbreeding. Alternatively, whole genome resequencing based contemporary estimates of N_E may be artificially low. SNeP was used in Chapter 4 to estimate contemporary N_E through linkage disequilibrium yet assumes that populations are panmictic, closed to geneflow and that the allele frequencies are not affected by selection (Barbato et al., 2015). As Chapter 4 analyses revealed population structure amongst white-faced muskoxen and evidence of directional selection, these assumptions may not have been met and may have contributed to the differences in N_E between Chapter 2 and Chapter 4. Contemporary N_E in general, can also be stochastic as it may not consider inbreeding, historic losses of genetic variation, migration and census population sizes. The ratio of ancient N_E/N_C can therefore be more informative as to population health by provided a better understanding of genetic risk, deleterious mutation fixation, genetic diversity loss and adaptive potential. Ancient N_E/N_C ratios in muskoxen fall below wildlife standards that would allow for population maintenance, suggesting that muskoxen are vulnerable to the rapid environmental changes they are now facing. However this is likely due to poor genetic diversity in white-faced muskoxen as, when considered separately,

ancient N_E/N_C estimates in barren-ground muskoxen are actually higher than what is typically seen in wildlife populations (Frankham et al., 2014; Pérez-Pereira et al., 2022). While low genetic diversity is typically a conservation concern, there are numerous examples of species or populations, like muskoxen, that survive and even thrive despite extremely low levels of genetic diversity.

Persistence in the face of low genetic diversity may be the result of complex ecosystem dynamics, demographic histories and adaptive strategies (Bernatchez, 2016; Catullo et al., 2019; Ghalambor et al., 2007; Romero-Mujalli et al., 2021). Therefore, behavioural, phenotypic or adaptive mechanisms, beyond standing genetic diversity, must also be considered when assessing muskox persistence. For example, the muskox's microbiome, known for its role in host immunity, fitness and digestion, was found to change in migrant muskoxen and in response to dietary shifts, demonstrating an ability to adapt within an individual's lifetime (Chapter 5; Bird et al. 2019). This plasticity could have allowed muskoxen to adjust to temporal and geographic changes in forage availability despite low genetic diversity (Chapter 5; Bird et al. 2019). Additionally, with the muskox's history of low N_E and poor genetic diversity, it is expected that populations would show signs of inbreeding depression and poor viability due to pronounced genetic drift (Chapter 4; Pečnerová et al. 2024). Despite high inbreeding estimates in endemic white-faced muskox populations, there has been no indication of inbreeding depression (Cuyler et al., 2019; Hansen et al., 2018; Pečnerová et al., 2024). Evidence of purging of strongly and moderately deleterious alleles has been found in muskoxen by Pečnerová et al. (2024) via comparison of modern and ancient muskox genomes. Purging of deleterious mutations through purifying selection can reduce genetic load and help mitigate fitness declines that result from inbreeding (Hedrick & Garcia-Dorado, 2016; Khan et al., 2021). Purging has also been found in other species that have persisted in the face of low genetic diversity, long term low N_E, and

repeated bottlenecks like the mountain gorilla (*Gorilla beringei beringei*) and kākāpō (*Strigops habroptilus;* Dussex et al., 2021; van der Valk et al., 2019). The degree of purging that occurs is a factor of inbreeding and genetic drift, where increased inbreeding also increases the expression of previously masked deleterious alleles, creating the opportunity for purifying selection to act against them, while high drift reduces the efficiency of that selection (Dussex, Morales, et al., 2023). Historically diverse species that also have a history of high gene flow and N_E, like caribou (*Rangifer tarandus*), have been found to have relatively high genetic loads and face higher risks of inbreeding depression as census populations decline due to climate change (Taylor et al., 2024). While muskoxen have extremely low levels of genetic diversity, the purging of both highly and moderately deleterious alleles has resulted in a lower masked load in white-faced muskoxen which would reduce the likelihood of inbreeding depression, even if rapid population declines continue on Vicotria Island (Pečnerová et al., 2024). Additionally, while island populations are typically at a higher risk for inbreeding and low genetic diversity due to relative isolation, this isolation is also thought to provide some relief from additional selective pressures like competition or predators, allowing them to tolerate higher genetic loads (Benazzo et al., 2017; Robinson et al., 2016). Variable selective pressures across the Arctic can also affect the distribution of diversity throughout the landscape. Directional selection can improve population fitness via local adaptations, while simultaneously reducing overall genetic diversity if the favourable allele reaches fixation (Assis et al., 2016; Hancock et al., 2010). For example, in Chapter 4, white-faced muskoxen were found to have a high number of runs of homozygosity (ROH). However, genes within the ROH islands of white-faced muskoxen were found to be overrepresented with GO terms associated with subspecific traits that may have improved their fitness to more northern, island environments, like their smaller size and metabolic processes (Chapter 4). Similar morphological and physiological traits have been found across Arctic or

island adapted species, indicating their adaptive importance. For example, Svalbard reindeer (*Rangifer tarandus platyrhynchus*), another large ungulate that, like white-faced muskoxen, inhabits an Arctic Archipelago, also have reduced body sizes and lowered metabolisms (Dussex, Tørresen, et al., 2023). Additionally, a chromosomal-level assembly performed by Lok et al. (2024) found that immune regions in the muskox genome actually had increased levels of variation, similar to that of caribou, which may represent a diversity hotspot and allow them to better respond to novel pathogens.

While muskoxen have long survived with low genetic diversity, climate change is rapidly changing the selective pressures they are now facing (Cuyler et al., 2019; Ernakovich et al., 2014; Gunn & Forchhammer, 2022; Kutz et al., 2015; Olofsson et al., 2009; Speed et al., 2021). With recent population declines occurring in two key muskox populations, concerns over their continued persistence have arisen, though most other muskox populations have remained stable or grown in size in the same time frame (Cuyler et al., 2019; Gunn & Forchhammer, 2022). The mosaic of selective pressures, diversity, and adaptive mechanisms across the Arctic landscape have likely contributed to variable muskox population dynamics. Amongst many stressors newly imposed on muskoxen, are changes to Arctic vegetation abundances, diversity, and phenology (Mekonnen et al., 2021; Mod & Luoto, 2016). Shrubification, or the increase of shrub abundance and distribution, has led to a loss in Arctic plant biodiversity (Mekonnen et al., 2021; Mod & Luoto, 2016). Losses in resource species can greatly affect ecosystem structure and are expected to negatively affect herbivore species (Heithaus et al., 2008; Mekonnen et al., 2021; Mod & Luoto, 2016). Plant metabarcoding performed in Chapter 5, assessed the composition and abundance of plants in the muskox diet and found several shrub families were in relatively high abundance. This indicates shrubification may actually provide additional food resources to muskoxen, despite negatively impacting Arctic biodiversity overall. Further, muskox

microbiome plasticity may allow the rapid changes in Arctic plant composition to have little effect on muskox population dynamics or health status. While promising for long term muskox sustainability, diet metabarcoding is unable to measure the volume of food ingested by muskoxen. As Arctic warming is expected to increase both extreme weather events and snow depth, it may further limit the muskox's access to winter forage (Cuyler et al., 2019; Desforges et al., 2019; Schmidt et al., 2015, 2023). As the muskox's reproductive success and first year calf survival rates are known to rely on resource reserves and accessible winter forage, Arctic warming still poses a significant threat to long-term muskox population growth or maintenance (Desforges et al., 2019; Schmidt et al., 2015, 2023).

Environmental warming also affects the dispersal of pathogens in the Arctic, which may pose more of a threat to muskoxen, as standing genetic variation has typically been associated with a population's capacity to respond to novel or changing pathogens. Not only has low diversity been identified throughout this thesis, but also in the sequencing of the MHC II DRB region in 43 muskoxen which found only one monomorphic locus (Mikko et al., 1999). This region of the MHC is involved in pathogen recognition and immune response and high diversity is typically maintained via balancing selection (Mainguy et al., 2007). In contrast to Mikko et al. (1999), Lok et al. (2024) found an increase in diversity within the immune genes of muskoxen, though their sampling scheme only included one individual from Banks Island and none from Victoria Island. Understanding MHC diversity within these two populations may be important as multiple die-off events occurred on Victoria and Banks Island with the cause of death associated with the zoonotic bacteria, *Erysipelothrix rhusiopathiae,* leading to population losses of ~80% (Cuyler et al., 2019; Kutz et al., 2015; Mavrot et al., 2020). In the same time period, however, adjacent barren-ground muskox populations were also exposed to *E*. *rhusiopathiae* , but did not experience similar mortality events (Mavrot et al., 2020). While pathogen abundance and stress

play a role in disease outcomes, significant differences in genetic diversity between muskox populations may also play a contributing role (Mavrot et al., 2020). White-faced muskox populations have lower diversity and higher inbreeding estimates than barren-ground muskoxen, suggesting they may have a higher susceptibility to the same diseases. As northward pathogen expansions are currently more concentrated to the more southern Arctic islands, this may clarify why more northern white-faced populations have not experienced die off events despite equally low levels of genetic diversity (Mavrot et al., 2020). The lack of gene flow between muskox populations might reduce the rate of disease dispersal across muskoxen; however, *E. rhusiopathia* can be spread by ticks and flies and may not be restricted by muskox migrations (Chapter 2; Chapter 4; Kutz et al., 2015). Therefore, as pathogens continue dispersing north, and climate related selective pressures grow, population declines are likely to occur in the more northern white-face muskox populations as well. Muskoxen are currently assessed by the IUCN without recognizing subspecies, due in part to a lack of diversity in muskox mitochondrial DNA (Gunn & Forchhammer, 2022). Archaeological and genetic evidence, including whole genome phylogenies and PCA analyses, indicate non-overlapping Pleistocene refugia separated white-faced and barren-ground muskoxen (Chapter 4; Pečnerová et al., 2024). When paired with a lack of gene flow, morphological differences and evidence of subspecific adaptations, these data suggest white-faced and barren-ground muskoxen warrant separate considerations by the IUCN (Chapter 4; Pečnerová et al., 2024). Further, based on the differing levels of population vulnerability identified between the subspecies In Chapter 4, white-faced muskoxen should be a higher conservation concern.

6.2 Benefits and Limitations of Molecular Monitoring Tools

The molecular tools used herein have provided insight into muskox population fitness and their capacity to adapt to a changing Arctic environment. These genetic tools allowed for the

estimation of population monitoring metrics like migration, population size and diet which can be challenging to attain in difficult terrains like the Arctic. For example, DNA based metrics can be calculated with noninvasively collected samples, which do not require access to, interactions with, or tracking of, the species of interest. While genetic data can be used to help monitor muskox persistence and vulnerabilities, the array of methods used in this thesis each came with their own set of benefits and limitations.

Microsatellite analyses performed in Chapter 2 allowed for the use of fecal DNA, which was non-invasively sampled and greatly expanded the number of samples available for downstream diversity investigations. Though genotyping data in Chapter 2 was limited by the number of neutral loci, these analyses provided insight into population and diversity trends of muskoxen from mainland Canada and Victoria Island that were in line with those using whole genome sequencing. However, while fecal samples are more readily available for collection, they are anonymously sampled and therefore a large number of duplicate individuals may need to be purged after genotypes are generated, as was seen in Chapter 2. Further, and perhaps more importantly, the microsatellite markers used herein had several loci where alleles nearly reached fixation across Victoria Island samples. Due to this low genetic variation, the probability that two muskox siblings from Victoria Island would share the same genetic profile (P_{sib}) was ~1 in 16, indicating this system has poor distinguishing power for this genetically impoverished population. Therefore, there is a likelihood that individuals were removed, as they were genotypically identical to another closely related individual. The removal of these individuals could result in artificially low inbreeding estimates and could account for the differences in the levels of inbreeding and N_E seen between Chapter 2 and Chapter 4. There is ongoing research to identify additional tetranucleotides throughout the genome to help address these concerns by

improving individual identifications, allowing for continued non-invasive research surrounding muskox diet, disease, diversity and distributions.

While the genome assembled in Chapter 3 provided a reference for further genome resequencing, the use of cross species scaffolding in its initial assembly limits its use in assessing structural rearrangements in muskoxen, relative to other ungulate species. Whole genome sequencing also requires higher quality DNA and as such limits the number of samples that are accessible across the muskox's range. Genome wide SNPs can also provide a more thorough and accurate understanding of the drivers of muskox diversity and population health. One of the most notable improvements that arose from using genomic data was to the muskox phylogeny. The phylogenetic tree based on nuclear DNA ultra conserved regions resolved the node of subspecies divergence, which was not accomplished with mitochondrial DNA due to a lack of genetic variation.

Plant metabarcoding (Chapter 5) provided insight into the composition and diversity of the muskox diet but was limited by the sample design. The samples included in Chapter 5 spanned several seasons and ecozones, but sampling was not equal across all geographic and seasonal groups. This meant that geographical and seasonal variation in diet could not be explicitly tested. Another limitation with this chapter is the variation in plant composition and abundances across the three markers used. As such, we were unable to determine whether the presence or abundance of specific plant families resulted in shifts in the microbiome. Additionally, as previously stated, diet metabarcoding does not measure the volume of ingesta and therefore cannot elucidate the effect that increased snow cover may have on muskox population health.

6.3 Future Research

This thesis has provided valuable knowledge on drivers of muskoxen sustainability while also creating new avenues for research into their ecology and evolution. The muskox genome assembled in this thesis, by Pečnerová et al. (2024) and most recently Lok et al. (2024), provide a solid foundation for future genomic work, with many possible applications. For example, additional muskox genomes from across their range could be sequenced to investigate local adaptation or functional differences between muskox populations and help define subspecific designations or ecologically significant units. While Chapter 4 of this thesis has provided initial insights into subspecific variation, a larger and more representative sampling scheme is needed. Additionally, with a chromosomal-level genome assembly now available, analyses performed in Chapter 4 that require long stretches of DNA, like runs of homozygosity (ROH), heterozygous rich regions (HRR), ROH islands and N_E estimates, could also be improved. Further sequencing of ancient muskox genomes, from different populations and time points could also improve our understanding of genomic erosions and purifying selection in muskoxen (Dehasque et al., 2020; Díez-del-Molino et al., 2018; Dussex, Kurland, et al., 2023; Rogers & Slatkin, 2017). A time series of genomic data can provide insight into selection and drift through time and can also provide insights into the loss of diversity in muskoxen, mechanisms of divergence and the origin of muskox subspecies (Dehasque et al., 2020; Díez-del-Molino et al., 2018; Dussex, Kurland, et al., 2023; Rogers & Slatkin, 2017). With whole genome assemblies available, the development of target capture assays for regions of interest, like immune genes, can expand our knowledge of functionally relevant diversity. Kessler et al. (2021) performed targeted DNA sequencing of ~ 1 kbp amplicons in alpine ibex (*Capra ibex*) to simultaneously monitor population structure, hybridisation, and immune diversity in order to inform conservation recommendations like translocations. With disease dispersal being a threat to muskox populations, it would be

valuable to study variation in a wider array of immune genes in muskoxen, relevant to their response to pathogen exposure. As noted, Lok et al. (2024) annotated muskox immune genes, including the MHC region, and found increased genetic variation indicating potential balancing selection however, they lacked samples from muskox populations experiencing disease die-offs. With access to annotated immune genes, there is an improved capacity to measure variation at these functionally relevant regions of the genome in populations experiencing population declines. Genotype-environment association (GEA) analyses can also be performed to identify local adaptations to climactic environmental variables, known to affect muskox stress and habitat suitability like maximum summer temperature, winter precipitation and spring snow (Razgour et al., 2019; Schmidt et al., 2015; Tomassini et al., 2019). These data can then be incorporated into ecological niche models (ENM) to better predict range losses for locally adapted muskox populations and landscape genetics can then be used to identify barriers of gene flow to areas that will become climactically suitable. This was done by Razgour et al., (2019) where they identified climate adapted SNPs in bats, which were then used to differentiate between cold/wet and hot/dry adapted populations. ENM analyses then determined that hot/dry populations would increase in suitability with predicted environmental changes, but cold/wet populations would be at risk (Razgour et al., 2019). As Lok et al. (2024) recently published a chromosomal-level assembly, there is a much-improved baseline for these potential projects, however, sequencing the muskox transcriptome could also improve the muskox genome annotation. Access to a more complete functional annotation with accurate exon coordinates would be key in designing assays and identifying functionally relevant genes.

Where access to high quality tissue may pose a problem for whole genome resequencing, alternative sequencing strategies that require lower quantities or qualities of DNA, like genotyping-in-thousands sequencing, can leverage the available reference genome to

identify genome wide variation from non-invasive samples like fecal pellets and wool (Burgess et al., 2022; Hayward et al., 2022). Genotyping-in-thousands has been shown to produce reliable population-level diversity data using fecal samples in species including polar bears (*Ursus maritimus*) and Sitka black-tailed deer (*Odocoileus hemionus sitkensis;* Burgess et al., 2022; Hayward et al., 2022). Where muskox diversity may be lacking in microsatellites, as seen in Chapter 2, genotyping-in-thousands could provide more informative variation data. Whole genome data can also be used to design additional microsatellite markers with more diversity within Victoria Island or white-faced muskox individuals in order to improve our ability to obtain unique individual genotypes. A new microsatellite system, which can more accurately estimate genetic diversity, inbreeding, N_E and gene flow across all muskoxen would be an incredibly important monitoring tool. Finally, recent advancements in sequencing technologies have allowed high quality genomes to be resequenced from fecal DNA, as has been done in caribou (*Rangifer tarandus;* Taylor et al., 2022). Feces are a much more readily available source of DNA for muskoxen, so the ability to perform genome resequencing on these types of samples would greatly improve future sample designs.

Other avenues of research could focus on understanding phenotypic plasticity as an adaptive mechanism in muskoxen given their extremely low levels of genetic variation. For example, comparative population transcriptomics, across individuals under different selective pressures or stressors, can be used to identify differentially expressed pathways or genes associated with an individual's plastic response. Sun et al. (2018) compared blood transcriptomes of forest musk deer (*Moschus berezovskii*) that were healthy to those with purulent sores in order to better understand their susceptibility to abscesses. They identified 113 differentially expressed unigenes, of which 13 upregulated genes were associated with immunodeficiency and responses to disease, indicating both intrinsic and extrinsic factors

played a role in forest musk deer susceptibility (Sun et al., 2018). Further research into the microbiome of muskoxen could also provide insights into their plastic response to changing vegetation availability and diversity in the Arctic. Although metabarcoding was performed in Chapter 5 to assess muskox diet and microbiome diversity, supplementing the sample design with more individuals from each ecozone and season could assess how their diets and microbiomes vary temporally and geographically. Female individuals can also be included in future metabarcoding runs to compare the diets and microbiomes of males and females, which have different nutritional requirements throughout the year. Finally, additional diet markers can be included to amplify lichen, which muskoxen are known to ingest and may play a role in their microbiome diversity.

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Chapter 5

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Appendix II. Supplementary Material for Chapter 2

Table S2.1. A summary of the primer pairs chosen for optimisation. Contains primer ID, forward and reverse primer sequences, number of alleles (N_A) detected from previous papers and the known product size of microsatellites.

Primer ID	*Forward Primer*	*Reverse primer*	*N_A in literature*	*Size of product*	*Reference*
OM50-8	CCTTTGTAGCCTCTTCAATAAC	ACCTTTAGTGCATATGAGTTCC	2	150, 160	1
OM53-38	CCATAGGGTGCAAAATAAATAA	GCAGTCACAAAAGAATCAGATA	4	150-160	1
OM58-06	GAGAATCACTGGGACAGAGAAG	GTGGACAGTGTTTGATGTCTTA	2	156-162	1
OM51-19	AAGAAAATAGCAACCTACTCCA	AGCATTAACCATCATCAGTGTA	5	201-209	1
OM53-12	CCAGAGATGGTCCATAGTTTAG	GGTTACCATTTTCTATTCTAAA	2	203-207	1
OM56-27	TGGAGAAAAGACACATGTAAAA	CGTAAATACTCTCAGTGCCTGT	2	213-215	1
OM55-04	NTTTAACTCTTNAAACGGCTG	GAGTAAGATTCGAGAAGACTGG	3	247-255	1
OM51-16	CCTTTGTAGCCTCTTCAATAAC	TGTTAGTTTTGAGATTCCACAT	5	262-556	1
OM54-23	TGGGATTTACATAGGAACAGAT	GTCAGTGGATGAGTAGACACCA	4	99-113	1
MoDIAS 1	GCACAGCTTAGACATTGTT	TTATTGGTGGTATCCTTTAG	3	122-148	2
MoDIAS 2	ACTGGCAGGTGGATTCTTAT	CCAAACTTTCTGTCATGACC	3	203-210	2
MoDIAS 3	TGCAGAGTCCAACACAACTT	ACTCATTCCAGTATACTTGC	1	176	2

1: Van Coeverden De Groot and Boag, 2004. 2: Holm et al. 1999

Table S2.2. Optimized reaction conditions for selected primers. Contains optimised magnesium concentrations, annealing temperature.

Locus	Annealing Temperature	Magnesium Concentration	Primer Concentration
Multiplex 1			
Modias 1	55	2mM	0.22uM
OM58-06	55	2mM	0.1uM
OM51-19	55	2mM	0.15uM
OM56-27	55	2mM	0.15uM
Multiplex 2			
OM54-23	55	2mM	0.1uM
OM53-38	55	2mM	0.1uM
OM53-12	55	2mM	0.1uM
OM55-04	55	2mM	0.3uM
Multiplex 3			
OM50-8	56	1.5mM	0.18uM
Modias3	56	1.5mM	0.28uM
Modias2	56	1.5mM	0.18uM
OM51-16	56	1.5mM	0.23uM

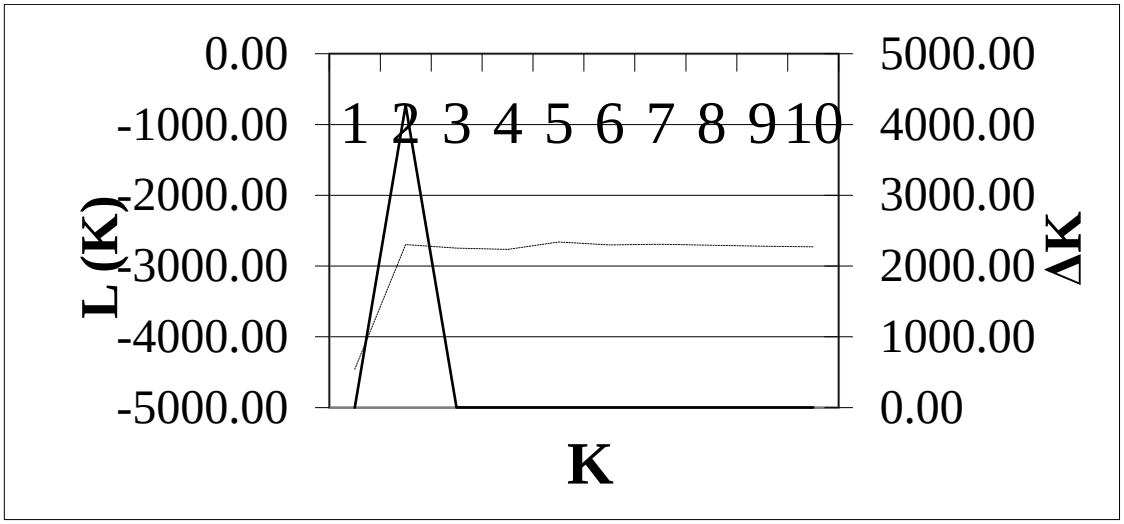

Figure S2.1. Graph of Delta K values. The X-axis consists of the number of K or inferred clusters. The Y-axis on the right represents delta K values which were used to determine the optimal K value. The highest peak on the graph is a 2 K, or clusters.

Appendix III. Supplementary Material for Chapter 3

Table S3.1. Library Sequencing statistics for reference genome assembly. Reads were filtered to remove adapters sequences, low quality bases and reads, duplicates and contaminating reads from other organisms.

Library Type	Platform	Read Length	Insert Size	Raw Number of reads	Number of filtered reads	percentage remaining
Paire end	HiSeqX	151	200bp	460981116	352008779	76.36%
Paire end	HiSeqX	151	350bp	480614298	385366379	80.18%
Paire end	HiSeq2500	126	550bp	40994290	30757253	75.03%
Paire end	HiSeq2500	126	700bp	87262637	75440510	86.45%
Mate Pair	HiSeq2500	126	5000bp	55518229	15888	0.03%
Mate Pair	HiSeq2500	126	8000bp	53613554	23291	0.04%

Table S3.2. Comparison of Genome assemblies using metrics including the NG50, the percent of contigs over 25 kbp, the longest scaffold, the number of Ns per 100 kbp and the percentage of complete BUSCOs with final rankings. Genomes were assembled using either the sheep or goat cross-species scaffolding mate pair libraries with kmer values also varying per assembly.

version	Reference	Kmer	number of contigs	N50	NG50	NG50 Broken	L50	LG50	over 25000	Percent over 25000	over 50000	percent over 50000	Absolute 25000	longest contig	assembly length	Length over 25000	Length over 50000	N's per 100kbp	% Busco Complete	Complete Busco	duplicated buscos	Fragmented	Missing
Cross29	Goat	121	6495	1705149	1707250	27242	494	493	2860	44%	2644	41%	1.2E+07	9453317	2704466437	2687729513	2680068058	10272	87.6	2632	14	214	163
Cross20	Goat	99	6408	1683990	1688620	27394	507	503	2935	46%	2682	42%	5270342	8250876	2712417191	2694729658	2685873025	10424	87.4	2627	16	214	166
Cross23	goat	127	6467	1721795	1728540	27203	493	491	2798	43%	2601	40%	9562189	7895471	2705950185	2690437811	2683525390	10357	87.5	2645	15	217	161
Cross31	Goat	123	6453	1698800	1701282	27236	503	501	2854	44%	2651	41%	1.2E+07	7895482	2704285376	2688342271	2681182056	10278	87.6	2633	14	216	160
Cross18	Goat	97	6582	1642599	1652705	27394	512	507	3028	46%	2736	42%	678187	9206847	2718261414	2699321813	2689176530	10595	87.1	2614	18	223	168
Cross30	sheep	117	9157	1712798	1715501	27504	484	481	2798	31%	2511	27%	2E+07	11605637	2710010080	2680361859	2670508020	10452	87.3	2624	16	218	165
Cross16	Goat	93	7020	1629153	1639960	27413	524	516	3165	45%	2820	40%	5937937	9254715	2728573848	2705937937	2693911016	10893	87.5	2627	18	210	168
Cross26	sheep	121	9041	1746734	1752182	27477	493	491	2711	30%	2462	27%	2.2E+07	9036664	2706292749	2677979702	2669322527	10325	87.3	2623	14	223	163
Cross19	sheep	99	9086	1649401	1704013	27636	511	506	2910	32%	2601	29%	1.1E+07	10117339	2718859589	2689231827	2678580405	10631	87.1	2617	16	224	166
Cross15	sheep	97	9189	1685717	1702063	27644	510	503	2943	32%	2617	28%	6493878	10118130	2724173683	2693506122	2692322608	10791	87.1	2619	15	218	171
Cross22	sheep	127	9136	1738337	1738337	27440	480	480	2685	29%	2455	27%	2.5E+07	8303812	2702521065	2674581079	2666621388	10233	87.3	2623	14	220	166
Cross11	sheep	93	9525	1661230	1661230	27295	521	513	3110	33%	2722	29%	7404282	10118397	2726463257	2692595718	2679479716	10846	87.2	2621	15	209	178
Cross24	sheep	113	9246	1683225	1688368	27500	511	507	2897	31%	2584	28%	1.7E+07	10030566	2714192114	2683480386	2672469378	10538	87.3	2622	17	221	163
Cross14	Goat	83	10081	1475596	1519772	27447	576	554	3871	38%	3170	31%	2E+07	7148492	2766365380	2719976027	2695811214	11998	86.7	2622	19	222	179
Cross27	sheep	101	10972	1494432	1514574	27553	562	547	3496	32%	2950	27%	112595	7407774	2745355475	2699887405	2681191046	11464	86.6	2599	19	229	185
Cross10	sheep	83	11476	1469034	1488168	27704	587	566	3698	32%	3033	26%	8671602	7258315	2759208795	2708671602	2685821123	11801	86.3	2595	15	220	193
Cross7	sheep	73	17757	1154576	1202625	27683	753	703	5301	30%	3853	22%	2E+07	5819131	2818054410	2720383806	2671186189	13530	85.5	2570	14	234	205
Cross5	sheep	63	26379	827151	889501	27660	1018	917	7478	28%	4973	19%	1.1E+07	7381590	2872101050	2710928420	2625446594	15046	69.8	2100	10	419	494
Cross13	Goat	73	18846	1214169	1297206	27427	715	657	5520	29%	3790	20%	3E+07	5949222	2844885485	2730379843	2670602124	14282	84.6	2540	19	257	207
Cross1	sheep	51	41774	544957	603891	27526	1511	1313	11058	26%	6586	16%	4.7E+07	3712418	2927077183	2652857373	2499522919	16562	81.1	2437	15	287	284
Cross4	Goat	51	46686	564392	638848	27308	1445	1229	11570	25%	6332	14%	5.8E+07	4765594	2960133571	2642191629	2642344183	17433	67.3	2026	10	429	558

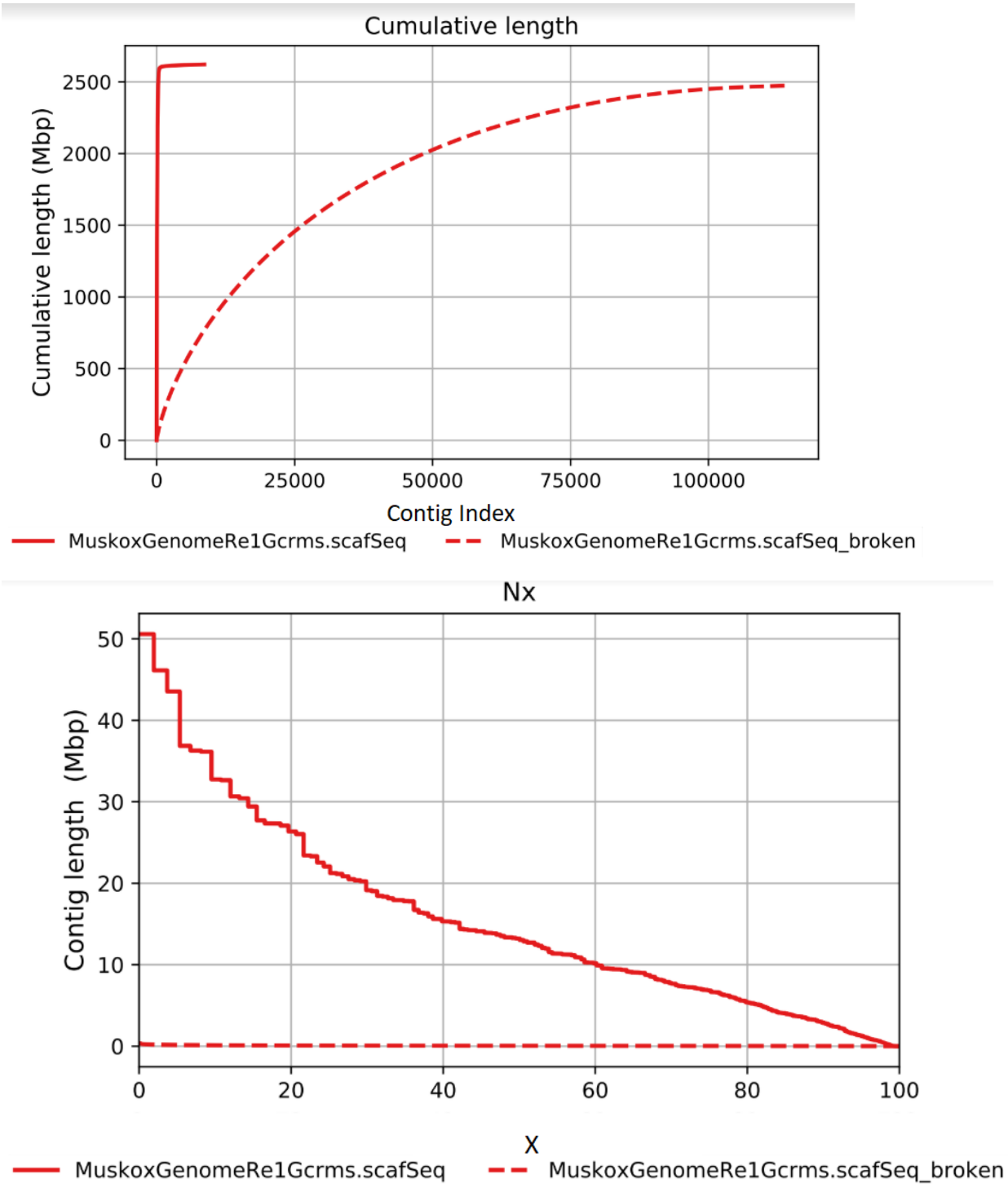

Figure S3.1. Nx and cumulative length plot of muskox reference genome assembly based on Quast analyses.

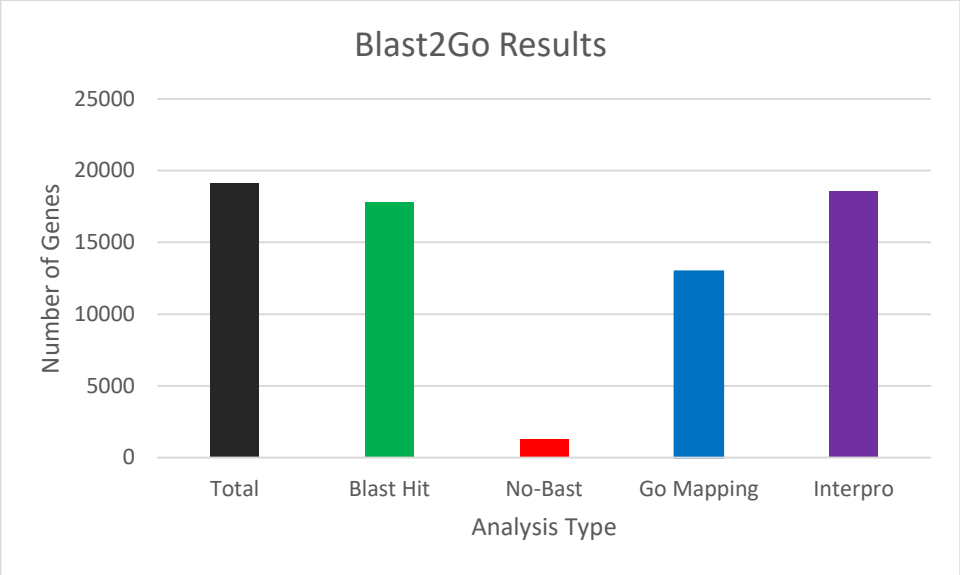

Figure S3.2. Barplot of number of genes with Blast2Go output

Appendix IV. Supplementary Material for Chapter 4

Table S4.1. The number of paired reads and estimated coverage for each sequenced genome for the raw data and final cleaned data sets.

Sample		Subspecies	Paired raw reads	Raw Coverage	Paired cleaned reads	Cleaned Coverage
Kugluktuk 1	KUG1	Barren-ground	213269412	24.56	180878732	20.83
Kugluktuk 2	KUG2	Barren-ground	220305746	25.37	186462819	21.47
Kent Peninsula 1	KP1	Barren-ground	105201678	12.11	85792453	9.88
Kent Peninsula 2	KP2	Barren-ground	168182783	19.37	134849310	15.53
Bathurst Island 1	BI1	White-faced	122803237	14.14	99081797	11.41
Bathurst Island 2	BI2	White-faced	148433291	17.09	120770233	13.91
Cornwallis Island 1	CWI1	White-faced	159711849	18.39	129365193	14.90
Cornwallis Island 2	CWI2	White-faced	111859298	12.88	91895666	10.58
Devon Island 1	DEV1	White-faced	144714296	16.66	117893948	13.57
Devon Island 2	DEV2	White-faced	189491916	21.82	155264374	17.88
Ellesmere Island 1	ELI1	White-faced	141618420	16.31	118314332	13.62
Ellesmere Island 2	ELI2	White-faced	157552507	18.14	128650071	14.81
Greenland 1	GL1	White-faced	154690416	17.81	127429303	14.67
Greenland 2	GL2	White-faced	157306507	18.11	125538360	14.46
Greenland 3	GL3	White-faced	208776590	24.04	173366851	19.96
Greenland 4	GL4	White-faced	169385438	19.51	140635107	16.19
Victoria Island 1	VIC1	Admixed	188196023	21.67	151813748	17.48
Victoria Island 2	VIC2	White-faced	181356146	20.88	148344582	17.08
Victoria Island 3	VIC3	White-faced	220518780	25.40	187702109	21.62
Victoria Island 4	VIC4	Admixed	212941977	24.52	179294836	20.65
Victoria Island 5	VIC5	White-faced	120348699	13.86	95943679	11.05
Victoria Island 6	VIC6	White-faced	160675743	18.50	129513168	14.91

Table S4.2. PAML results summary for the calculation of the muskox specific mutation rate. T represents time of divergence; dN represents nonsynonymous mutation rate; dS represents synonymous mutation rate.

Species	t	dN/dS	dN	dS
Cow	0.021	0.397274	0.005279	0.013288
Bison	0.052	0.544422	0.014558	0.026741
Horse	0.361	0.175388	0.057237	0.326345
Sheep	0.095	0.470729	0.025083	0.053285
Goat	0.024	0.257054	0.004764	0.018535
Muskox	0.143	0.590751	0.041148	0.069654

Table S4.3. Diversity and inbreeding statistics calculated per muskox individual. ID provides the individual ID, observed heterozygotes show the number of heterozygote SNPs, heterozygosity rate is the percentage of snps identified that were heterozygous. F_{IS} provides individual inbreeding coefficients based on heterozygosity estimates while FROH is the individual inbreeding coefficient based on runs of homozygosity.

Individual ID	Observed heterozygotes	heterozygosity rate	Fis	FROH
Bathurst Island 1	273462	0.000192	0.47844	0.2317
Bathurst Island 2	291991	0.000205	0.4431	0.2257
Cornwallis Island 1	305759	0.000214	0.41685	0.2125
Cornwallis Island 2	264432	0.000185	0.49567	0.2143
Devon Island 1	223796	0.000157	0.57317	0.2842
Devon Island 2	214563	0.00015	0.59078	0.3005
Ellesmere Island 1	259449	0.000182	0.50517	0.2198
Ellesmere Island 2	261291	0.000183	0.50166	0.2348
Greenland 1	208290	0.000146	0.60274	0.231
Greenland 2	202153	0.000142	0.61445	0.1962
Greenland 3	195577	0.000137	0.62699	0.2284
Greenland 4	208550	0.000146	0.60225	0.2265
Victoria Island 1	545427	0.000382	-0.04026	0.0024
Victoria Island 2	246192	0.000173	0.53045	0.3514
Victoria Island 3	216886	0.000152	0.58635	0.3744
Victoria Island 4	711686	0.000499	-0.35735	0.1323
Victoria Island 5	224537	0.000157	0.57176	0.3591
Victoria Island 6	229733	0.000161	0.56185	0.3794
Kent Peninsula 1	666564	0.000467	-0.27129	0.0197
Kent Peninsula 2	658191	0.000462	-0.25532	0.0257
Kugluktuk 1	601344	0.000422	-0.1469	0.0093
Kugluktuk 2	623021	0.000437	-0.18825	0.0162

Table S4.4-S4.12 are available at https://gitlab.com/Prewer93/Trent_Muskox_Genomics/-/tree/master/Prewer_PhD_Supplementals/Chapter%204?ref_type=heads

Table S4.4. ROH islands identified within all muskoxen sampled. Each ROH island was given its own Island ID. N individuals included provides the number of individuals included in this ROH island with the subpopulation information included as well. Scaffold and start and end coordinates were included along with any genes located within the island.

Table S4.5. ROH islands identified within all white-faced muskoxen sampled. Each ROH island was given its own Island ID. N individuals included provides the number of individuals included in this ROH island with the subpopulation information included as well. Scaffold and start and end coordinates were included along with any genes located within the island.

Table S4.6. ROH islands identified within all Victoria Island muskoxen sampled. Each ROH island was given its own Island ID. N individuals included provides the number of individuals included in this ROH island with the subpopulation information included as well. Scaffold and start and end coordinates were included along with any genes located within the island.

Table S4.7. ROH islands identified within all Northern Arctic Island (Devon Island and Ellesmere Island) muskoxen sampled. Each ROH island was given its own Island ID. N individuals included provides the number of individuals included in this ROH island with the subpopulation information included as well. Scaffold and start and end coordinates were included along with any genes located within the island.

Table S4.8. ROH islands identified within all Southern Arctic Island (Bathurst Island and Cornwallis Island) muskoxen sampled. Each ROH island was given its own Island ID. N individuals included provides the number of individuals included in this ROH island with the subpopulation information included as well. Scaffold and start and end coordinates were included along with any genes located within the island.

Table S4.9. ROH islands identified within all Canadian white-faced muskoxen sampled. Each ROH island was given its own Island ID. N individuals included provides the number of individuals included in this ROH island with the subpopulation information included as well. Scaffold and start and end coordinates were included along with any genes located within the island.

Table S4.10. ROH islands identified within all Greenland white-faced muskoxen sampled. Each ROH island was given its own Island ID. N individuals included provides the number of individuals included in this ROH island with the subpopulation information included as well. Scaffold and start and end coordinates were included along with any genes located within the island.

Table S4.11. HRR islands identified within muskoxen sampled. Each HRR island was given its own Island ID. N individuals included provides the number of individuals included in this HRR with the subpopulation information included as well. Scaffold and start and end coordinates were included along with any genes located within the island.

Table S12. Functional annotations for the genes identified within the ROH and HRR islands. The group indicates which populations were included in that ROH or HRR island, the Island ID corresponds to the ROH or HRR Islands identified in Table S4-S11. GeneID provides the gene id number in the muskox genome annotation. The description and name provide the functional annotation for that gene. COWID provides the corresponding gene ID from the cow genome which to then be used for enrichment analyses.

Table S4.13. Statistically significant overrepresented GO terms within the runs of homozygosity islands found in white-faced muskoxen. Terms are grouped by ontology and colour groupings represent related terms.

Ontology	Accession	Count	Name	P-value	FDR
Biological Process	GO:0009952	17	anterior/posterior pattern specification	7.36E-05	2.96E-02
Biological Process	GO:0003002	23	regionalization	1.93E-05	1.12E-02
Biological Process	GO:0007389	27	pattern specification process	3.40E-05	1.82E-02
Biological Process	GO:0007275	147	multicellular organism development	2.04E-07	2.95E-04
Biological Process	GO:0048856	175	anatomical structure development	6.99E-08	1.44E-04
Biological Process	GO:0032502	183	developmental process	3.93E-07	4.37E-04
Biological Process	GO:0050767	21	regulation of neurogenesis	5.08E-05	2.23E-02
Biological Process	GO:0051960	26	regulation of nervous system development	7.16E-06	5.18E-03
Biological Process	GO:2000026	94	regulation of multicellular organismal process	6.84E-05	2.83E-02
Biological Process	GO:0050789	393	regulation of biological process	9.70E-05	3.69E-02
Biological Process	GO:0065007	417	biological regulation	1.08E-04	3.90E-02
Biological Process	GO:0050794	374	regulation of cellular process	4.90E-05	2.44E-02
Biological Process	GO:0009653	101	anatomical structure morphogenesis	1.70E-09	4.10E-06
Biological Process	GO:0060429	46	epithelium development	1.33E-04	4.58E-02
Biological Process	GO:0009888	72	tissue development	2.33E-06	2.11E-03
Biological Process	GO:0048731	129	system development	2.72E-06	2.32E-03
Biological Process	GO:0030154	121	cell differentiation	8.47E-06	5.56E-03
Biological Process	GO:0048869	122	cellular developmental process	6.91E-06	5.26E-03
Biological Process	GO:0009987	523	cellular process	7.32E-06	5.04E-03
Biological Process	GO:0048523	167	negative regulation of cellular process	2.44E-07	2.94E-04
Biological Process	GO:0048519	184	negative regulation of biological process	1.07E-06	1.03E-03
Biological Process	GO:0048513	107	animal organ development	7.71E-05	3.01E-02
Biological Process	GO:0048522	191	positive regulation of cellular process	4.89E-06	3.93E-03
Biological Process	GO:0048518	215	positive regulation of biological process	6.33E-07	6.54E-04
Biological Process	GO:0043170	227	macromolecule metabolic process	3.82E-05	1.97E-02
Biological Process	GO:0071704	281	organic substance metabolic process	5.52E-05	2.35E-02
Biological Process	GO:0006807	249	nitrogen compound metabolic process	4.96E-05	2.24E-02
Biological Process	GO:0044238	265	primary metabolic process	1.03E-04	3.80E-02
Molecular Function	GO:0070851	15	growth factor receptor binding	6.33E-06	7.23E-03
Molecular Function	GO:0005515	250	protein binding	3.14E-06	4.79E-03
Cellular Component	GO:0005730	47	nucleolus	5.28E-05	9.06E-03
Cellular Component	GO:0031981	144	nuclear lumen	8.40E-05	1.32E-02
Cellular Component	GO:0043226	453	organelle	1.47E-08	1.39E-05
Cellular Component	GO:0110165	632	cellular anatomical entity	8.66E-08	3.27E-05
Cellular Component	GO:0043229	439	intracellular organelle	1.20E-07	3.76E-05
Cellular Component	GO:0005622	510	intracellular anatomical structure	5.84E-12	1.10E-08
Cellular Component	GO:0005634	267	nucleus	7.25E-08	3.42E-05
Cellular Component	GO:0043231	397	intracellular membrane-bounded organelle	1.34E-07	3.62E-05

Cellular Component	GO:0043227	415	membrane-bounded organelle	2.37E-08	1.49E-05
Cellular Component	GO:0005654	126	nucleoplasm	2.66E-04	3.87E-02
Cellular Component	GO:0005737	386	cytoplasm	1.29E-06	2.44E-04

Appendix V. Supplementary Material for Chapter 5

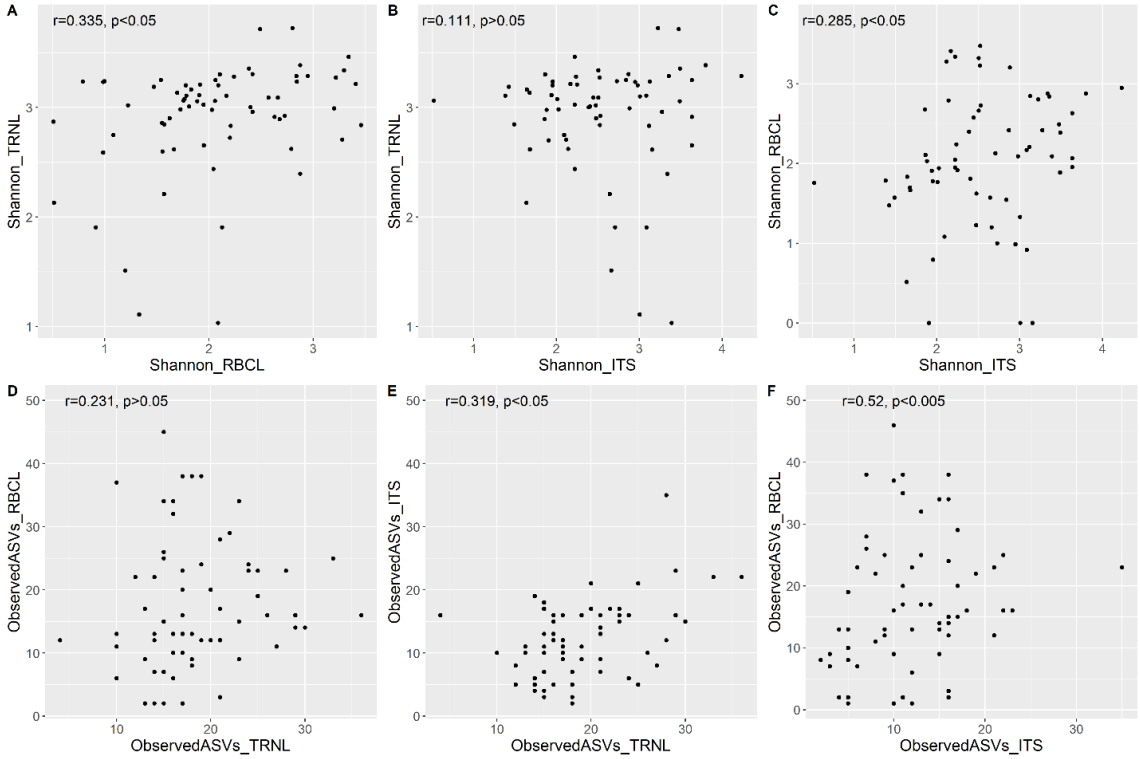

Figure S5.1. Relationships between alpha diversity of (A) RBCL and TRNL diet Shannon index. (B) TRNL and ITS diet shannon index. (C) RBCL and ITS diet Shannon index. (D) RBCL and TRNL diet observed ASVs. (E) ITS and TRNL diet observed ASVs. (F) RBCL and ITS diet observed ASVs

Raw sequence reads for diet metabarcoding and ASV tables are available from Dryad (https://doi.org/10.5061/dryad.h9w0vt4n3)

Raw sequence reads for microbiome metabarcoding and ASV tables are available from Dryad (https://doi.org/10.5061/dryad.fj6q573q2).