CONSERVATION GENETICS OF ROOSEVELT ELK IN BRITISH COLUMBIA

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DEDICATION

For my wife Debra, without whose support I would never have attempted a return to school. Your bravery and perseverance, in the face of adversity, inspired me to reach beyond my preconceptions and self-imposed limits.

And for my parents, Fred and Nadine. Your constant love and support throughout my early years, despite the challenges, is appreciated more than you know… but I'd bet you would never have thought you would see the day!

ABSTRACT

Species reintroductions have the potential to cause bottleneck events resulting in increased genetic drift, reduced genetic diversity and increased inbreeding, with potentially negative fitness consequences. Wildlife managers must consider how a species' ecology may affect its genetic diversity. Roosevelt elk, once widespread along the West Coast, were extirpated from the mainland and experienced a substantial population bottleneck on Vancouver Island. The species was reintroduced to the BC mainland in the 1980s, and their descendants used for subsequent reintroductions within the region. To understand genetic diversity in extant and reintroduced populations of Roosevelt elk, we analyzed genetic variation in 355 elk from 13 populations. Molecular analyses showed reduced genetic diversity, genetic isolation of southern Vancouver Island, increased genetic drift resulting in significant differentiation between source and reintroduced herds, and very low effective population size in multiple populations indicating a potential for inbreeding and associated negative fitness consequences.

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LIST OF ABBREVIATIONS

˚C	Degrees Celsius
µL	Microlitre
µM	Micromolar
1x	One times
5x	Five times
χ^2	Chi squared statistic
a	Alpha
A_r	Allelic richness
avg.	Average
BC	British Columbia
bp	Base pair
BRIT	Brittain-Skwawka group
BSA	Bovine serum albumin
CC	Central Coast group (Deserted-Narrows-Clowholm)
D	Jost's measure of genetic distance
df	Degrees of freedom
DNA	Deoxyribonucleic acid
EPU	Elk population unit
FDR	Benjamini-Höchberg false discovery rate
FLNRO	British Columbia Ministry of Forests, Lands, Natural Resource Operations and Rural Development
F_{st}	Fixation index
G_{is}	Inbreeding coefficient
H_e	Expected heterozygosity
H_o	Observed heterozygosity
H_t	Total heterozygosity expected across all populations
$_uH_e$	Unbiased expected heterozygosity
HO	Homathko-Southgate-Orford group
HWE	Hardy-Weinberg Equilibrium
IAM	Infinite alleles model
K	Genetic cluster
ΔK	Delta K
Prob(K)	Probability of K
km	Kilometres
LD	Linkage disequilibrium
m	Metres
$MgCl_2$	Magnesium chloride
mM	Millimolar
mtDNA	Mitochondrial DNA
n / N	Sample size / Census population estimate
N_a	Number of observed alleles
N_e	Effective population size
P	Probability

PCR	Polymerase Chain Reaction
PITT	Pitt River group
PRN	Powell River North group
PRS	Powell River South group
RG	Rainy-Gray group
SMM	Step-wise mutation model
SP	Sechelt Peninsula group
SQ	Squamish-Mamquam-Indian group
STV	Stave-Chehalis-Lower Lillooet group
TOBA	Toba group
TPM	Two-phase mutation model
VIN	Vancouver Island North group
VIS	Vancouver Island South group

CHAPTER 1: GENERAL INTRODUCTION

1.1 Background

A common definition of biodiversity is the abundance, evenness and / or richness of organisms in a defined space: e.g. a single quadrat frame, sampling area, habitat, or ecosystem. However, biodiversity is more than measures of the number and distribution of species; at its fundamental root, biodiversity is genetic (Frankham 2005, Ralls et al. 2018). In viewing any population, be it bacterial, fungal, plant or animal, its greatest level of diversity is contained within the combined genomes of its individuals. Understanding the diversity characteristics of many species and their populations is critical for their long-term conservation, therefore, we must consider how management choices affect the genetic diversity of species we choose to manage (Frankham 2005, Charlesworth and Willis 2009, Balkenhol et al. 2016, Palsbøll et al. 2007, Funk et al. 2012).

1.1.1 Conservation Biology

An overarching goal for ecological conservation is maintaining viable, healthy and productive populations of native species (Koenig 1988, Frankham 2005). An important consideration in conserving species and ecosystem function, is the sustainability of individual populations (a group of interbreeding individuals of a species). Small populations are more vulnerable to catastrophic events such as drought, heavy snow, flooding and disease (Koenig 1988, Young 1994). The best mitigation against population loss is to maintain large populations. Connectivity between smaller demes (groups of interbreeding individuals of the same species; Gilmour & Gregor, 1939) through the dispersal of individuals, increases the overall effective population size (Nei & Tajima, 1981, Nei, Maruyama, & Chakraborty, 1975, Reed & Frankham, 2003, Wright, 1931).

Appropriately, wildlife managers have increasingly focussed on preserving suitable 'connected habitat' to ensure that populations have the greatest number of potential interbreeding individuals. Such connectivity is often assumed, without supporting empirical evidence of metapopulation dynamics (Lowe and Allendorf 2010, Betts et al. 2015). While direct observations of dispersal, such as sightings of marked individuals or global positioning system (GPS) collar tracking data, can verify individual movements, they rarely confirm if those individuals have successfully reproduced. At a fundamental level, conservation biology is focussed on understanding the long term viability of populations (Young 1994).

1.1.2 Population Genetics

1.1.2.1 Evolution

The field of population genetics is an extension of evolutionary theory and conservation biology, wherein our understanding of biodiversity includes the genetic similarities and differences within and among individuals, demes, populations and species or what we call genetic 'structure'. Population genetics involves understanding how the mechanisms of evolution (mutation, gene flow, drift, non-random mating and selection; Wright, 1931) individually and collectively influence the genetic structure of populations and species.

Mutation is the fundamental agent of genetic diversity, providing variation in the genomes of organisms upon which all other evolution mechanisms act (Wright 1931). The processes involved in mutation, while underlying much of what is discussed herein, are beyond the scope of this thesis; therefore, mutation will only be discussed in as much detail as is required for the understanding of a general audience. Gene-flow is the

dispersal of gametes among demes or populations and is synonymous with connectivity. Gene flow is affected by various characteristics of the organism in question such as fecundity or dispersal ability and interaction with its environment, including physical barriers to dispersal or distance to conspecifics. Drift is the stochastic change in frequency of alleles (specific variants of a genetic sequence) within a deme or population due to the effect of random sampling of gametes in reproduction, eventually leading to the loss or fixation of alleles. Selection is the differential survival and fitness (lifetime reproductive success) of individuals with different alleles interacting with their environment (climate, food, parasites, other individuals, etc.). Non-random mating can be due to isolation and small population, or assortative (preferential) mating.

A fundamental concept in evolutionary theory is Hardy-Weinberg Equilibrium (HWE). For populations in HWE allele frequencies can be predicted from one generation to the next. Such populations will have five characteristics: they are large (mitigating drift), individuals breed at random, there is no net migration or mutation, and alleles are not under selection (all genotypes equally fit). Departures from HWE may indicate a violation of one or more of the above characteristics. Figuring out which requires researchers to evaluate overall patterns, and carefully examine potential causes (Waples 2015).

1.1.2.2 Influences on Genetic Structure

The five main agents of evolution each play a role in the genetic structure and levels of variation observed in natural populations, however, in small populations drift may act much faster and with greater magnitude (Nei & Tajima, 1981; Nei et al., 1975). A large reduction in a population is referred to as a ‘population bottleneck’. Even when a

reduction in population size is of short duration, drift can alter allele frequencies quickly, creating a 'genetic bottleneck'. Consider a small population of 24 diploid organisms (Figure 1.1) each with two of four possible alleles at a single locus (blue, yellow, purple or green). If the population is randomly reduced by 75%, as might happen during a severe winter storm, the loss of alleles is also random. While it is statistically probable that alleles of high frequency will be present in the surviving individuals, changes in rare allele frequencies are more unpredictable. In Figure 1.1 the purple allele happened to have two copies carried into the new population, increasing its frequency from 6% to 16%, while the yellow allele (initially more than twice as common as purple) was lost. Let us assume that the surviving individuals all successfully breed and rebuild to the pre-bottleneck population within one year. Within two generations, one allele has been lost from the population, a rare allele became relatively common and then rare again, and the frequencies of the two most common alleles have flipped.

Like drift, the effects of non-random mating, mutation, and migration is increased in small populations. An important consideration in population genetics is the difference between the census population, or number of individuals (*N*), and the effective population (N_e), the number of breeding individuals in an idealized population (Frankham, 1995, Kimura & Crow, 1963, Wright, 1931). N_e is often much smaller than *N*, and this has important conservation implications. Small populations, as low values of N_e suggest, are greatly affected by drift and populations below a threshold may not be sustainable. Widely accepted guidelines for species conservation suggest populations that fall below $N_e = 50$ may be at high risk of inbreeding depression, and populations should be maintained over the long term at $N_e > 500$ to maintain adaptive diversity, known as the

50/500 rule (Franklin 1980, Soulé 1980). Since the 50/500 rule was adopted by the International Union for Conservation of Nature (IUCN), new research has suggested that these numbers are much too low for most wild populations (Keller and Waller 2002, Frankham 2005) and N_e values of 100/1000 likely represents reasonable minimum targets to conserve species at risk (Frankham et al. 2014).

The N_e:N ratio provides a metric for evaluating levels of genetic variation in a population (Hedrick 2005). High values (~1) would be indicative of populations with high diversity and therefore less vulnerable to stochastic events. In a review of published data in which both N and N_e estimates were available, Frankham (1995) found that natural populations across taxa (102 species of birds, mammals, insects, molluscs, amphibians, reptiles and plants), some at risk and many common, exhibited an average N_e:N value of 0.10-0.11, or we can consider it as one 'genetic individual' for every 9-10 counted individuals in a population. Such a low value has far reaching implications in managing small populations when we recall that long term sustainability goals require an N_e of 100 short term, and >1000 long term. The most significant influences on N_e estimates were fluctuations in population size, variance in reproduction (as expected in polygynous systems), and the census population value used (all individuals, only adults, only breeding individuals).

1.1.2.3 Species at Risk

Species at risk are, by definition, small populations. To ensure their continued survival, it is imperative to increase N_e as much as possible by maintaining or increasing gene flow between fragmented populations (Palsbøll et al. 2007, Frankham 2015, Ralls et al. 2018). Unfortunately, many populations are fragmented due to anthropogenic disturbance that is not easily remedied. Where populations have poor connectivity due to physical distance, using reintroductions to establish new populations between existing ones may be beneficial. Where connectivity is low due to the unsuitability of the landscape between populations, whether temporary or permanent, translocation of individuals among populations in a genetically informed approach can provide important new diversity to small populations (Frankham 2015, Giglio et al. 2018). However, increasing the overall population of a species is the pre-eminent goal (Allendorf and Luikart 2007).

1.2 Study Area

The southwest coast of British Columbia (BC) is divided into two management areas in relation to wildlife and forestry management, the West Coast (WCR) and South Coast (SCR) Natural Resource Regions (Figure 1.2). The WCR encompasses Vancouver Island and the mainland coast and islands from near Phillips Arm to north of Bella Coola and includes Haida Gwaii. The portion of the WCR included in the study area is limited to Vancouver Island, and a few elk population units (EPUs) on the adjacent mainland (Figure 1.2). The SCR is comprised of mainland areas and numerous small islands from the US border up to the north side of Bute Inlet. The southern coastal areas of BC are highly heterogenous landscapes, characterized by topographic and climatic extremes. The

region is typified by temperate rainforests, alluvial flood plains, subalpine forests, alpine meadows, glaciers and broad icefields, with mountain peaks exceeding 2500 m and broad icefields less than 10 km from the ocean. The mainland is a mosaic of high elevation rock and ice, broad glacier-formed forested valleys, and deep fjords reaching far inland from the Salish Sea. Large terrestrial mammals are found throughout the regions, including black-tailed deer (*Odocoileus hemionus columbianus*), cougar (*Puma concolor*), black bear (*Ursus americanus*), gray wolf (*Canis lupus*) and Roosevelt elk (*Cervus canadensis roosevelti*) in both regions, with mountain goat (*Oreamnos americanus*), grizzly bear (*U. arctos horribilis*) and occasional moose (*Alces alces*) only occurring on the mainland. Vancouver Island has a population of ~870,000 people, with almost half living in and around Victoria, and the remainder are mostly distributed in communities along the east coast (BC Government 2020). The SCR is home to ~2.8 million people, with most living in the highly developed Fraser River floodplain and surrounding uplands (Metro Vancouver Regional District and Fraser Valley Regional District). Approximately 90,000 live in the Squamish-Lillooet Regional District and developed coastline areas (BC Government 2020). Outside of urban and suburban development resource extraction, mainly forestry, has been the main disturbance agent the last two centuries. More recently the construction and operation of small hydroelectric facilities, and increasing wilderness recreation have created significant disturbance, both direct and indirect, on the landscape and wildlife of the SCR (Mountain Goat Management Team 2010).

Vegetation in the region is dominated by dense temperate rainforests of western hemlock (*Tsuga heterophylla*), red cedar (*Thuja plicata*), Sitka spruce (*Picea sitchensis*) in fluvial soils near sea level and Douglas fir (*Pseudotsuga menziesii*) occurring in drier,

well drained sites. Mountain hemlock (*T. martensiana*) and yellow cedar (*Chamaecyparis nootkatensis*) dominate subalpine forests at higher elevations giving way to alpine tundra and vast glaciers and icefields on the mainland, while deciduous forests of black cottonwood (*Populus trichocarpa*), red alder (*Alnus rubra*) and big leaf maple (*Acer macrophylum*) occur in pure and mixed stands on disturbed sites at low elevation and along numerous river valleys (Meidinger & Pojar 1991). Annual precipitation varies considerably throughout the region from ~700 mm on southern Vancouver Island to over 3500 mm at nearby Port Renfrew, and from 1200 mm at Powell River to over 2800 mm near Squamish on the mainland (Environment and Climate Change Canada 2020).

1.3 Study Species - Ecology, Distribution and History

Roosevelt Elk (*C. c. roosevelti*) are one of four disputed extant North American elk (*C. canadensis* Erxleben 1777) subspecies collectively known as wapiti. The other subspecies being: Rocky Mountain (*C. c. nelsoni*), tule (*C. c. nannodes*) and Manitoban (*C. c. manitobensis*) elk, along with two extinct subspecies, the Merriam (*C. c. merriami*) and Eastern (*C. canadensis*) elk. Until recently, wapiti were considered sub-species of the Eurasian red deer (*Cervus elaphus* Linnaeus 1758). Studies on pre and postzygotic isolation in *C. canadensis* and *C. elaphus* hybrids (Dratch 1986) and modern genetic analyses have shown that all *C. canadensis* taxa (which includes the Asian *C. c. sibericus, C. c. wallichi* and *C. c. songaricus*) are monophyletic and share a most recent common ancestor, and are sister clade to the Sika deer (*C. nippon*) of eastern Asia, not a subspecies or sister clade to *C. elaphus,* as long accepted (Polziehn and Strobeck 1998, Lorenzini and Garofalo 2015). In the following discussion the term *wapiti* is used when

discussing all subspecies collectively, and by the common term *elk* in reference to a singular population, e.g. Roosevelt Elk, Vancouver Island Elk, Sechelt Peninsula Elk, etc.

Recent research by Speller et al. (2014), using both contemporary and archaeological genetic samples, suggests that prior to their extirpation from most of North America wapiti occurred as a relatively continuous population from the Rocky Mountains eastward through the Great Plains, calling into question the validity of separating the Manitoban and Rocky Mountain subspecies. Using both mitochondrial and microsatellite loci Pohlzein et al. (1998; 2000) supported the subspecific designation of Roosevelt elk from Vancouver Island, as well as tule elk in California, under the phylogenetic species concept. The authors noted that Roosevelt elk in the northwest United States showed genetic introgression with elk translocated from Yellowstone National Park (NP) in the early 1900s, leaving the BC population as the only remaining 'pure' Roosevelt elk.

Wapiti shared a common fate with much of North America's wildlife; as Old World diseases killed the majority of the indigenous human population (Jones 2014), large ungulates that had been heavily utilized initially experienced a release from human predation and exploded in numbers to where descriptions from early European explorers defied belief (McHugh 1979, Gray 1995). Regardless of the numerical accuracy of those reports, it cannot be argued that central and western North America was awash with large mammals by the early 1800s. As the west was further explored, exploited and settled, wildlife was initially viewed as a resource to feed local settlers and communities, and a rapidly expanding urban population in eastern regions (Gray 1995). As cattle were moved into the west and land was used for crops, wildlife was persecuted as competitors of

livestock, pests upon agriculture, and to deprive remaining free indigenous peoples a way to continue to exist outside of government control (McHugh 1979).

Due to widespread anthropogenic change, by the turn of the twentieth century the legendary abundance of much of North America's wildlife was nothing but a recent memory. Many species that were once numerous beyond comprehension were now at risk of extinction; the buffalo (*Bison bison*) once numbered between 30-60 million 200 years after Europeans first set foot on the continent. By 1883, an estimated 325 plains bison (*B. b. bison*) remained, with only 25 on public lands, and approximately 500 wood bison (*B. b. athabascae*) near Slave Lake, NWT (McHugh 1979). Unfortunately other legendary species had already been lost, such as the passenger pigeon (*Ectopistes migratorius*) and Eskimo curlew (*Numenius borealis*) (Gray 1995, Barsness 2000). Many species of large ungulates were extirpated from former habitats and survived only as remnant populations in protected areas, like Yellowstone NP in the United States and Riding Mountain NP in Canada (Gray 1995, O'Gara and Dundas 2002). Other species, though greatly reduced, found refuge by virtue of their remoteness or difficulty of terrain. The Roosevelt elk fell into that category.

Roosevelt elk once ranged widely along the west coast of North America from northern California to southern BC (Figure 1.1; Spalding 1992, O'Gara and Dundas 2002). By the early 1900s only a handful of populations remained. In the province they were extirpated from the mainland, and the total population was reduced to as few as 375 individuals in four herds on Vancouver Island; Shawnigan Lake, Strathcona Park, Kyuquot Sound and Quatsino Sound (Spalding, 1992; Figure 1.2). Though no estimates exist of pre-colonial populations for the sub-species, historic records from the late 1700s

to late 1800s report elk occurring at low densities along coastal areas of the province from the Fraser River delta as far as 52° N (near Bella Coola), wherever suitable habitats (major river valleys, riparian zones, coastal estuaries and plains) were available (Brunt 1990, Spalding 1992).

The territorial government in BC began enacting various piecemeal legislation regarding the taking of fish and wildlife as early as 1859 (Begg 2007), however, the exploitation of wildlife continued, resulting in large reductions of many species until the early 1900s. To help stem the ongoing loss of wildlife, BC followed many other North American jurisdictions in introducing a consolidated wildlife act. The *Game Act* (1914) provided legislation to address and control the widespread exploitation of endemic species (Begg 2007). When introduced, the act immediately closed the hunting of elk on Vancouver Island, allowing the population to slowly increase from a substantial population bottleneck (Spalding 1992).

The population of elk on Vancouver Island increased moderately until the 1970s. Illegal harvest of elk had always been a concern on the island, however poaching increased substantially in the late 1970s and early 1980s, and combined with the extensive loss of low elevation old growth forests critical to winter survival, wildlife managers became worried about the species' persistence (Spalding 1992, Quayle and Brunt 2003). To address conservation concerns provincial biologists translocated 22 Roosevelt elk from the Qualicum and Campbell River areas on Vancouver Island to the Sechelt Peninsula on the mainland in the late 1980s, and moved an additional five elk in the mid 1990s (Figure 1.2; Spalding 1992). The new mainland populations grew rapidly. So much so that conflict with local residents became a political issue by the late 1990s. In

an effort to address both wildlife conflicts (agricultural depredation, vehicle accidents) and restore the species to other former habitats on the mainland, a multi-year translocation and reintroduction program was begun (Reynolds et al. 2018). The project involved trapping groups of elk living in close proximity to developed areas on the Sechelt Peninsula and Powell River, and translocating them to isolated high quality habitats within the South Coast Region. Simultaneously, a limited elk hunting season was initiated on the Sechelt Peninsula, the original site of the mainland introductions (Quayle and Brunt 2003, Reynolds et al. 2018).

Following the recommendations of Komers and Curman (2000), the strategy used for Roosevelt elk reintroductions from 2000 to 2017 was to provide a founding population of at least 20 individuals (min = 4, max = 56), heavily biased towards females. In practice, the majority of elk trapped and moved were cows and calves (84.7%), with lesser numbers of immature bulls. An effort was made to translocate at least one mature bull (4+ years) into each new herd when possible (Reynolds et al. 2018). Through 84 translocations, of 601 elk into 27 new elk population units (EPUs), the mainland population grew from ~400 animals in two EPUs in 2001 to ~2050 animals by 2020. It bears repeating, the vast majority of elk used for mainland translocations were descendants of 27 elk from Vancouver Island.

In recent years at least two individuals in the Rainy-Gray EPU were observed with nontypical colouration, putatively the result of genetic mutations (Figure 1.3). Though hypothetical, the individuals were suspected to display partial leucism, wherein a mutation disrupts pigment production pathways (melanogenesis) in some cell types resulting in either uniform discolouration or partial discolouration (piebaldism) of the

skin or hair. The melanogenesis pathway is highly complex, involving multiple genes involved in regulation of both qualitative and quantitative expression, some recessive while others are dominant, and identical mutations in specific genes result in different phenotypes in different species (Barsh 1996). While some colour phase animals, such as the Kermode black bear, exhibit recessive alleles that interrupt the production of the black-brown pigment eumelanin (Ritland et al. 2001), it may be that the observed elk have mutations affecting the production of red-yellow pigment pheomelanin in the same *MCR1* gene. Further discussion of the genetic causes of pigment disorders are beyond the scope of this thesis, however, it should be noted that such mutations are very infrequently expressed in large, wild populations. As most mutations of the melanogenesis pathway are recessive (Barsh 1996) requiring an individual to have two copies of the mutant allele, and the observed cow does not appear to have reproduced any leucistic offspring, the observation of two affected individuals in the Rainy-Gray EPU raises concern regarding the genetic diversity and effective population size of at least this herd, if not all reintroduced populations.

1.4 Molecular Markers

In this study we used both maternally inherited mitochondrial DNA (mtDNA) and neutral, biparentally inherited microsatellite loci to evaluate genetic structure in the Vancouver Island source and mainland reintroduced populations of Roosevelt elk. The D-loop of the mtDNA control region is non-coding, and therefore mutates at a rapid rate relative to other mtDNA regions. Mitochondrial markers can be used to detect distinct lineages within and among demes (Brown et al. 1982) and are particularly sensitive to bottleneck events (Avise 1994). Microsatellites are highly variable due to their short

repeated sequences, which are prone to replication errors (Schlötterer 2000, Bhargava and Fuentes 2010). Being biparentally inherited and highly variable, microsatellites provide multi-locus genotypes for individuals, and thus allow the characterization of contemporary population differentiation and comparison of important genetic diversity statistics (Slatkin 1995, Pritchard et al. 2000, Allendorf and Luikart 2007). The combination of both mtDNA and microsatellites has been used in compliment to evaluate various questions about population genetics in wildlife (Graham & Burg, 2012; Larson, Jameson, Bodkin, Staedler, & Bentzen, 2002; Polziehn, Hamr, Mallory, & Strobeck, 1998; Polziehn, Hamr, Mallory, & Strobeck, 2000). Genetic patterns (structure) observed in a species using neutral markers provide evidence of the interactions between gene flow and drift that affect genome wide diversity (Funk et al. 2012).

1.5 Study Design

To understand how reintroduction strategy impacted the genetic structure of mainland Roosevelt elk herds, we acquired tissue, blood and faecal samples from biologists with the BC Ministry of Forests, Lands and Natural Resource Operations (FLNRO) across as many EPUs as possible. Faecal samples were collected to enhance the number of samples, and therefore the precision of our analyses, for four mainland populations of particular interest. Where individual EPU sampling was insufficient to provide the desired 25-30 samples to allow adequate characterization of population allele frequencies (Hale et al. 2012), samples were aggregated with adjacent EPUs where no barriers to dispersal were likely. Grouped samples were analysed utilizing various software resources to investigate population diversity and structure to understand changes associated with past bottlenecks, recent translocations, and current connectivity among

populations. Redundant analyses were run with alternative software, where available, to confirm calculations and summary statistics.

1.6 Thesis Overview

The data chapter of this thesis investigates the legacy of historic bottlenecks associated with overexploitation, and the current state of population genetics within Roosevelt elk in BC. Sequences from mtDNA are used to explore long-term genetic patterns on Vancouver Island, and search for a signal related to recent translocations. Patterns in the genetic data are considered in relation to known historic events, and to current population connectivity. Microsatellite genotypes are then used to further examine population genetics, changes in diversity, population differentiation, bottleneck signals, and effective population size. In the final chapter I summarize and review findings, explore observed genetic patterns to predict causation, and provide context and management options for populations of concern.

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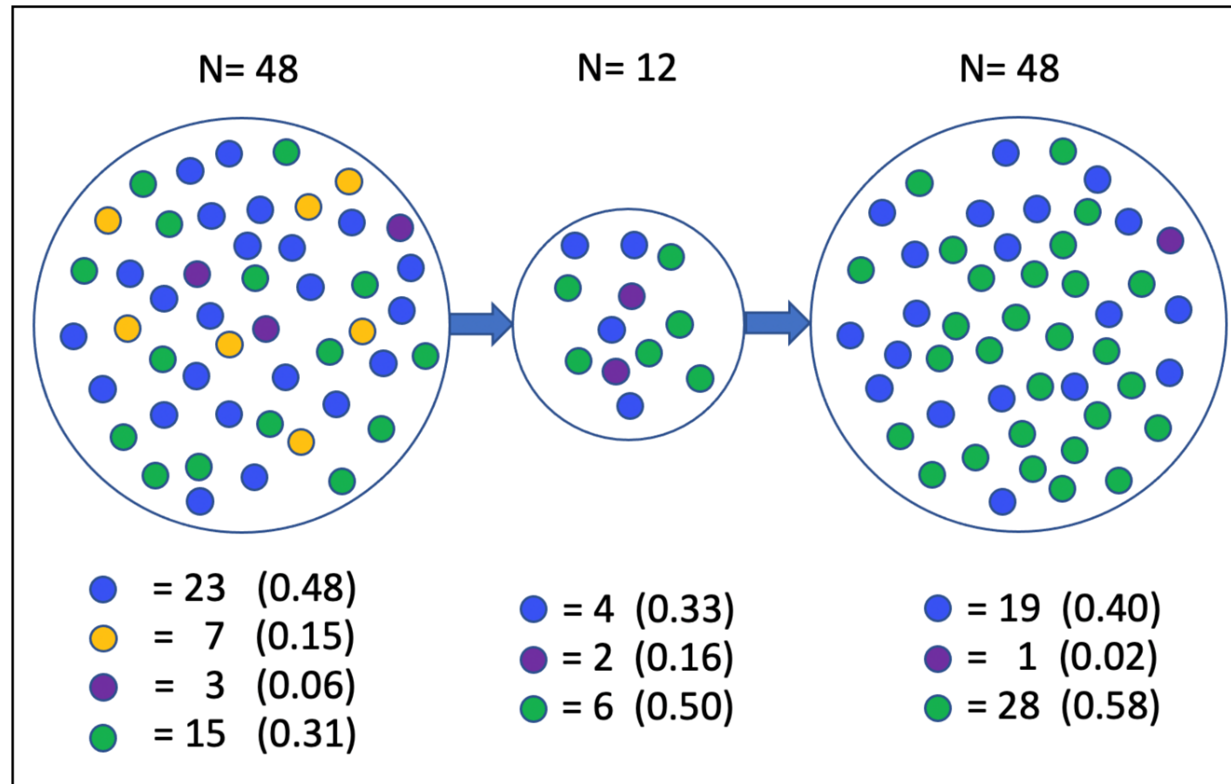

Figure 1.1: Stochastic effect of bottleneck events and drift. Theoretical changes in allele frequency for four alleles at a single locus, different alleles represented by different colours, allele frequency at each stage in brackets. Random loss of individuals from a population during a bottleneck may result in substantial changes in allele frequency.

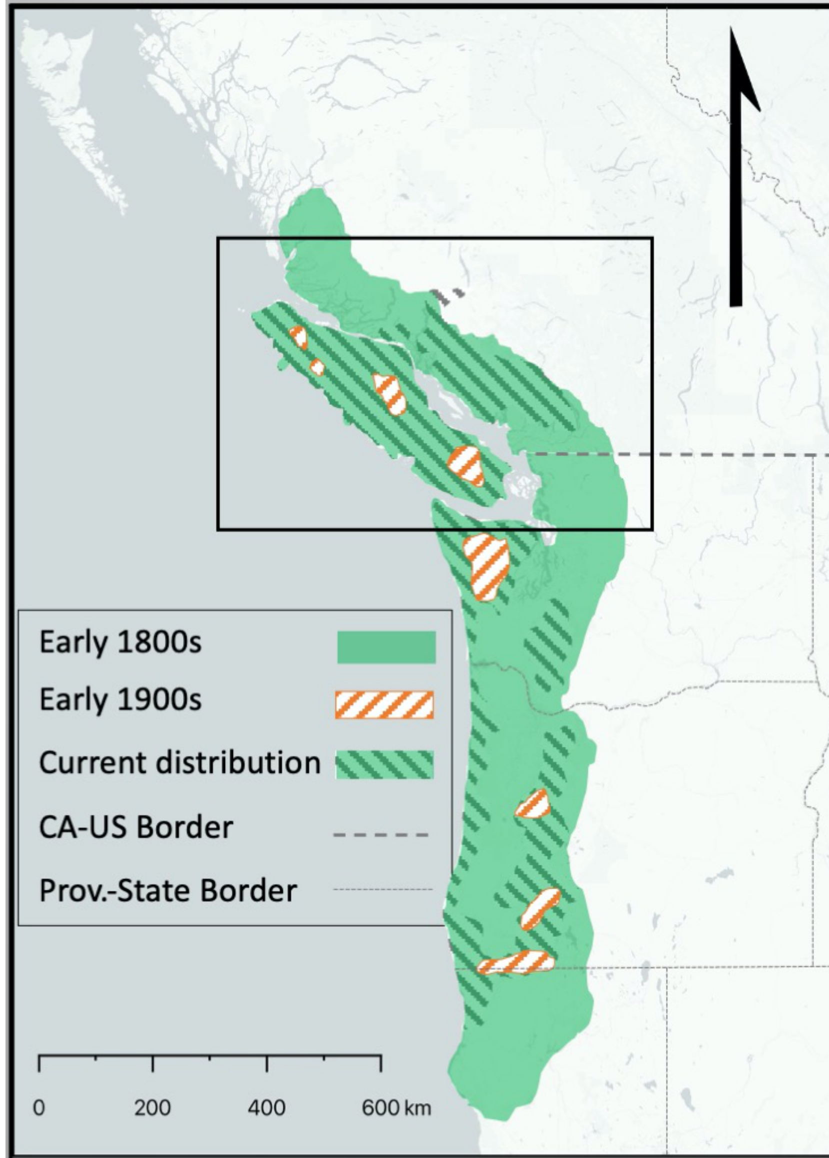

Figure 1.2: Historic and current distribution of Roosevelt elk (*C. c. roosevelti*) in North America; green shading shows species distribution prior to widespread European settlement along the Pacific Coast, orange and white diagonal polygons indicate refugia at the beginning of the 1900s, and green patterned areas show current distribution; map adapted from Spalding 1992, O'Gara and Dundas 2002, Quayle and Brunt 2003, Reynolds et al. 2018.

Figure 1.3: Observed Roosevelt elk (*C. c. roosevelti*) in the reintroduced Rainy-Gray Elk Population Unit on the mainland of British Columbia, with putative genetic mutations of the melanogenesis pathway: (L) mature bull elk exhibiting normal (L) and abnormal (R) colouration, photo by D. Reynolds 2017; (R) a mature cow elk with putative partial leucism, photo by D. Brackett 2018; photos used with permission.

CHAPTER 2: MULTI-MARKER GENETIC ANALYSIS IDENTIFIES METAPOPULATION STRUCTURE AND BOTTLENECK SIGNATURES IN ROOSEVELT ELK IN BRITISH COLUMBIA

2.1 Introduction

Where wildlife populations have been extirpated, or reduced to a relatively small number of individuals (population bottleneck), translocations have been used to augment extant populations or to re-establish species into historic habitats (Fritts and Carbyn 1995, Larson et al. 2002*b*, Olson et al. 2013, Greenhorn et al. 2018, West et al. 2018). Small reintroduced populations are at increased risk of inbreeding depression and reduced ability to adapt to changing environments; reintroduction strategy, social structure and mating system all potentially contribute to the loss of genetic diversity from even brief population bottleneck events (Keller et al. 2012, Keller and Waller 2002, Olson et al. 2013). Genetic bottlenecks coupled with genetic drift may result in further loss of genetic diversity (Wright 1931, Nei et al. 1975, Stockwell et al. 1996, Larson et al. 2002*a*, Allendorf and Luikart 2007). The increased impact of drift on small populations is well known (Charlesworth and Willis 2009, Frankham 2005; Keller and Waller 2002, Nei et al. 1975). Small populations experience increased loss of genetic diversity and increased frequency of deleterious alleles (Wright 1931, Nei et al. 1975, Aldridge and Boyce 2007, Frankham 2015). During a bottleneck event, the magnitude of any loss of genetic diversity is a result of the size of the remaining population and the duration of the reduction (Nei et al. 1975). Genetic bottlenecks may increase a population's vulnerability to inbreeding depression, lowering not only the fitness of the population but potentially

that of the entire species, putting it at increased risk of extinction (Franklin 1980, Reed and Frankham 2003).

The effect of landscape composition on genetic structure is complicated by the interplay between a species' evolutionary history (phylogeography), behavioural ecology (habitat niche, mating system, seasonal movement pattern, dispersal capabilities, etc.) and the abiotic environment (climate and geophysical features) in which it occurs (Manel et al. 2003, Cushman and Landguth 2012, Hindley et al. 2018). The relationship between landscape ecology and population genetics has become its own field of study known as landscape genetics (Manel et al. 2003, Storfer et al. 2007). The isolation of populations by barriers to dispersal (physical, ecological or behavioural) can result in further loss of diversity and increased risk of inbreeding depression (Reed and Frankham 2003, Frankham 2005, Ralls et al. 2018). The coastal areas of southern British Columbia (BC) represent extremes of topography and climate. Steep sided fjords reach far inland and elevation can range from sea level to over 2500 m in less than 10 km. This highly heterogenous landscape likely presents substantial barriers to dispersal for numerous organisms.

Roosevelt elk (*Cervus canadensis roosevelti*) are the largest subspecies of North American elk, historically occurring along the west coast of North America from northern California to southern BC (Figure 1.1; Bryant and Maser 1982, Spalding 1992). Roosevelt elk were extirpated from the mainland of the Province by the late 1800s during a time of market hunting and commercial exploitation of wildlife, though a small population at Phillips Arm (Figure 2.1) either survived in isolation or recolonized the area (Spalding 1992). The Vancouver Island population survived this period, experiencing a

substantial bottleneck event early in the 20th century with the population possibly reduced to as few as ~375 animals (Spalding 1992, Quayle and Brunt 2003).

Roosevelt elk on Vancouver Island are known to have low genetic diversity relative to other elk populations, likely due to small numbers of founding animals, geographic isolation, and at least one known population bottleneck (Polziehn et al. 1998*b*; Polziehn et al. 2000; Spalding, 1992). In BC Roosevelt elk are a provincially Blue Listed species; a species of special concern due to the degradation and loss of habitat and poaching (BC Conservation Data Centre, 2020). During the 1980s, biologists and conservation groups became interested in reintroducing the species to former habitat on the BC mainland. Initial reintroductions (1987-89) consisted of 22 animals translocated from Vancouver Island to the Sechelt Peninsula (Spalding 1992): 13 individuals (10 females, three males) from the Campbell River area, and nine individuals (eight females, one male) from the Qualicum area, with an additional five elk (unknown sex and age) moved onto the mainland near Powell River in 1994 from a site near Comox (Figure 2.1; Spalding 1992, Quayle and Brunt 2003).

A translocation and reintroduction program was initiated in 2001 with the goal of re-establishing viable populations throughout much of the subspecies' historic range in BC (Figure 1.1). During the 17 years the program was active, more than 600 elk were moved through 84 translocation events within the South Coast Region (Appendix I; Reynolds, Kelly, Tweddle, & Morrison, 2018). These translocations (Figure 2.2) helped the mainland Roosevelt elk population increase from approximately 400 individuals occurring in two elk population units (EPUs) in 2001 to more than 2050 in 27 EPUs by 2019 (Figure 2.2; D. Reynolds FLNRO personal communication, 2020).

Despite the overall success of this reintroduction program, and that of earlier Wapiti reintroductions across North America, concern has been raised about the potential for reduced genetic diversity in polygynous ungulate species generally (Stephen et al. 2005, Ortego et al. 2011, Olson et al. 2013, Hopken et al. 2015, Bérénos et al. 2016, Sattler et al. 2017, Giglio et al. 2018), and in reintroduced elk specifically (Eberhardt 1996, Hicks et al. 2007, Conard et al. 2010, Hundertmark and Van Daele 2010, Frankham et al. 2014, Muller et al. 2018). All elk used for South Coast Region (SCR) reintroductions from 2001-2017 were descendants of the initial 27 elk translocated to the South Coast Region from Vancouver Island between 1987-1996. Additional translocations occurred from Vancouver Island to the mainland in the West Coast Region in 2017 (Figure 2.1).

As mitochondrial markers represent an effective population size (N_e) one-quarter that of nuclear markers due to mitochondrial DNA being haploid and maternally inherited, they are more sensitive to stochastic events such as bottlenecks and specific lineages are more likely to be lost (Avise 1994). Nuclear microsatellite loci have been widely used to investigate contemporary population structure and relatedness in various taxa, from plants to vertebrates, due to their relatively high rates of mutation (Slatkin 1995, Schlötterer 2000, Balloux and Lugon-Moulin 2002). To discern how past bottlenecks, sequential translocations, and reintroduction strategy may have affected genetic diversity in British Columbia's Roosevelt elk, we evaluated both mitochondrial and nuclear diversity in extant Vancouver Island and reintroduced mainland populations.

Specifically, we:

1. evaluated the genetic diversity and structure of the source populations on Vancouver Island and reintroduced populations on the mainland,
2. compared reintroduced populations to determine if a relationship exists between the number of source populations, number of founding individuals, or years since founding and observed genetic diversity,
3. studied the role sequential population bottlenecks play in the genetic diversity of polygynous species, and
4. identified populations that may be vulnerable to inbreeding depression associated with low genetic diversity.

Understanding the genetic consequences and potential implications of past events is important for securing the future survival of sensitive species. This research was undertaken in partnership with the BC Ministry of Forests, Lands and Natural Resource Operations (FLNRO) to provide baseline genetic data important for informed stewardship of Roosevelt elk within the Province (Stockwell et al. 1996, Reynolds et al. 2018, West et al. 2018).

2.2 Methods

2.2.1 Sample Acquisition

Roosevelt elk samples (n = 357), collected between 2012 and 2019 from 48 EPUs in BC were acquired from FLNRO and analyzed in this study: 90 samples were from faeces, 22 from blood, and 245 from tissue (Appendix II). Of the 245 tissue samples, 243 were from incisor teeth collected for aging data during compulsory inspection of resident and indigenous hunter harvests. Tissue was cut or scraped from the tooth root using a

flame sterilized blade and collected in 1.5 ml microcentrifuge tubes and stored at ambient temperature until DNA extraction. Two additional tissue samples were provided by the BC Conservation Officer Service from euthanized animals. Faecal samples were opportunistically collected during census surveys similar to Ramón-Laca, Soriano, Gleeson, & Godoy (2015). A sterile flocked swab (Puritan PurFlock Ultra 25-3606-U) moistened with ASL buffer (stool lysis buffer - Qiagen GmbH cat.no. 1014755) was swabbed around pellets, focussing on areas not exposed to direct sunlight and areas with observable mucus. The swab tip was broken off into a 1.5 ml microcentrifuge tube containing 1.5 ml of ASL buffer, and stored at ambient temperature until DNA extraction (Hajkova et al. 2006).

2.2.2 DNA Extraction and Amplification

DNA from tissue and blood samples was extracted using a modified Chelex (Bio-Rad Chelex 100 resin) protocol (Walsh et al. 1991, Burg and Croxall 2001). Faecal DNA was extracted using a QIAamp® DNA Stool Mini Kit (Qiagen GmbH cat.no. 51504) following the published extraction protocol modified for the use of faecal swabs, where the microcentrifuge tube containing the swab and 1.5 mL ASL buffer was vortexed for 30 seconds, then a 1.2 mL aliquot was transferred to a new tube, and the standard extraction protocol followed.

A subset of samples was selected for mtDNA analysis (Appendix II); Vancouver Island samples (n=28) were selected to represent as many EPUs as possible, while mainland samples (total n=31) focussed on two of the oldest reintroduced populations, the Sechelt Peninsula (n=11) and Powell River (n=7), Pitt River (n=5) represented a rapidly growing population, and Squamish (n=5) was considered highly connected to

other populations. Remaining mainland samples were from peripheral Roosevelt herds (n=3) that may be connected to Rocky Mountain elk (*C. c. nelsoni*) populations occurring to the east and south.

A 567 bp portion of the mitochondrial D-loop known to be variable in Roosevelt elk (Polziehn et al. 1998) was amplified in 25 μL polymerase chain reactions (PCR). D-loop PCR reactions contained 5x Green GoTaq® Flexi 5x buffer (Promega), 0.2 mM dNTP, 2.5 mM $MgCl_2$, 0.4 μM primers EK-F23 and EK-R663 (Speller et al., 2014; Appendix III), 1 U GoTaq® Flexi polymerase, and DNA template. PCR conditions were as follows: one cycle of denaturing at 94°C for 120 seconds (s), annealing at 52°C for 45 s, extension at 72°C for 60 s; 35 cycles of 94°C for 30 s, 52°C for 30 s, and 72°C for 45 s, followed by a final extension step of 72°C for 300 s. To confirm successful amplification, 3 μL of PCR product was run on a 0.8% agarose gel. Samples that produced a clean band of approximately 600 bp were subsequently Sanger sequenced using the forward primer (EK-F23) at NanuQ, Genome Quebec (McGill University, Montreal, QC, Canada).

To further evaluate contemporary population structure and differentiation, 23 microsatellite loci (Appendix III) known to be variable in elk were individually screened using 10 μL PCR reactions containing GoTaq® Flexi 5x buffer (Promega), 0.2 mM dNTP, 2.0 mM $MgCl_2$ (see Appendix III), 0.2 μM forward primer, 0.4 μM reverse primer, 0.2 ng/μL BSA, 0.5 U GoTaq® Flexi polymerase, and DNA template. Forward primers were synthesized with a 5’ M13 tag sequence (CAC GAC GTT GTA AAA CGA C) to incorporate a fluorescent tag during amplification allowing PCR products to be visualized on a 6% acrylamide gel with a Li-COR 4300 DNA Analyzer (Li-COR Inc., Lincoln, NE, USA). Li-COR runs were conducted with three positive control samples, to

ensure consistent amplification and scoring, and one negative control. The positive controls provided a reference across multiple gels allowing consistent scoring of alleles.

Of the 23 loci screened, fourteen loci successfully amplified, and a subset of samples (n=12) was screened for variation; one locus appeared monomorphic (RT5) and one showed low levels of heterozygosity in the samples tested (RT27) and both were subsequently dropped from further analyses. Twelve polymorphic loci were retained for genotyping (BL42, BM203, BM888, BM4107, BM4513, BM6506, BMC1009, CSSM041, INRA107, RT7, RT13, OarFCB193; Appendix III). Loci RT13 and OarFCB193 later appeared highly sensitive to template DNA concentration in faecal samples and were subsequently removed from further genotyping. Primers for loci BM4107 and BM4513, originally developed for cattle (*Bos taurus*), produced PCR products with excessive stutter (additional bands) that made accurate scoring impossible; these primers were redesigned (BM4107RD and BM4513RD; Appendix III) while retaining the variable repeat motif. Final PCR amplification conditions were as follows: one cycle of denaturing at 94°C for 120 s, annealing at 55°C for 45 s, extension at 72°C for 60 s; 7 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 45 s, followed by 25 cycles of 89°C for 30 s, 57°C for 30 s, and 72°C for 45 s; and a final extension step of 72°C for 300 s. Four loci required different annealing temperatures: BM203 and CSSM041 at 52/54°C, BMC1009 at 48/50°C, and BM6506 at 60/62°C. When loci showed poor amplification for faecal samples, an additional three cycles were added at the higher annealing temperature (BM203, BM888, BM6506 and BMC1009).

Alleles were scored manually, cross checked multiple times, and scored by a second experienced individual. Samples with faint bands, substantial stutter, or

anomalous elements were reamplified until a clear genotype could be identified or were discarded from further analyses. A subset of samples from all gels was run together on a single load as an additional check to ensure consistent allele calls across runs.

2.2.3 Sequence Analyses

Study sequences were initially aligned to 1211 bp D-loop sequences for two Roosevelt and one tule elk (GenBank accession no. AF016970, AF016971 and AF016977 respectively) in MEGAX v10.1.7 (Kumar et al. 2018) to confirm subspecies and known polymorphic sites (Polziehn et al. 1998). All variable sites were confirmed or rejected through visual review of sequence chromatograms. Sequences were evaluated for standard population genetic measures (haplotype diversity H_D, nucleotide diversity - π) in DnaSP v6.12.03 (Rozas et al. 2017) to allow comparison between source and reintroduced populations. To evaluate population differentiation, pairwise genetic distances (Tajima and Nei 1984) were calculated in MEGAX with 1000 bootstraps to determine variance, and for F_{ST} (Φ_{ST}) in ARLEQUIN v.3.5.2 (Excoffier & Lischer, 2010).

2.2.4 Microsatellite Analyses

Previous studies have reported that a sample size of 25-30 individuals from a population is required to evaluate the genetic structure of a population using variable microsatellite loci (Hale et al. 2012), with some suggesting population differentiation detectable with smaller sample sizes (Landguth et al. 2012). To achieve a reasonable sample size for population analyses, EPUs with small sample size (<8) were grouped with adjacent units where no putative barrier to dispersal was suspected (Table 2.3). Where an EPU's sample size was low (8-12) and connectivity to adjacent herds was

unlikely, samples were analyzed as a separate group. Significance values for multiple tests were adjusted for false discovery rate (FDR; $\alpha = 0.05$) using the procedure detailed in Benjamini & Hochberg (1995) to reduce type 1 errors without greatly reducing power to detect potential differences.

To check for multiple sampling of individuals, important with non-invasive samples, a procedure to evaluate samples (Paetkau 2003) was undertaken for genotype matches with up to two mismatched loci identified using CERVUS v.3.0.7 (Marshall et al. 1998). Populations and loci were checked for deviation from Hardy-Weinberg equilibrium (HWE) in GENODIVE v.3.04 (Meirmans and Van Tienderen 2004) with 10,000 permutations via analysis of molecular variance (AMOVA). Deviations from linkage equilibrium (LD) between loci were checked with GENEPOP v.4.7.5 (Raymond and Rousset 1995, Rousset 2008). Standard genetic measures were calculated for loci and populations, including number of alleles per locus (N_a), effective number of alleles (N_{eff}), Shannon Information Index (I), observed heterozygosity (H_o), expected heterozygosity (H_e), unbiased expected heterozygosity (uH_e), and fixation index (F) were calculated in GenAlEx v6.5 (Peakall and Smouse 2012), allelic richness (A_r) was calculated with *hierfstat* (Goudet 2005) implemented in R (R Core Team 2013), and the inbreeding coefficient G_{is}, Nei's (1987) analogue of Wright's F_{is}, was calculated in GENODIVE.

To evaluate the effects of translocation on population diversity, changes in allele frequency from the primary source population(s) for each locus within each population and genetic diversity statistics were compared against population demographic history. Comparisons included: number of founding individuals, source population(s), initial male to female ratio, and years since founding (Appendix 1).

Pairwise population differentiation was calculated for both F_{st} (AMOVA; Excoffier, Smouse, & Quattro, 1992; Meirmans, 2006) and Jost's *D* in GENODIVE, with 5,000 permutations to determine significance. Jost's *D* provides a measure of differentiation that is independent of within population diversity (Jost 2008).

To further understand genetic differentiation between populations, genetic data were analyzed using the software STRUCTURE v.2.9.4 (Pritchard et al. 2000). STRUCTURE is a model-based Bayesian clustering software that infers ancestry of individuals based on their multi-locus genotypes. To elucidate population differences on Vancouver Island and in the South Coast Region, a hierarchical analysis was conducted using the *admixture* model with *locprior* setting. Settings were chosen assuming limited dispersal between populations, with some correlation of allele frequencies by location (Porras-Hurtado et al. 2013). The *locprior* setting has been shown to identify genetic structure in populations undergoing contemporary change (Hubisz et al. 2009, Porras-Hurtado et al. 2013). Five iterations for each genetic cluster value (K=1-5), with individual STRUCTURE runs consisting of 50,000 step burn-in and 200,000 Monte Carlo Markov Chain (MCMC) steps, were conducted. To determine the most likely number of genetic clusters the statistics ΔK (Evanno et al. 2005) and Prob(K) (Pritchard et al. 2000) were calculated in the online software CLUMPAK (Kopelman et al. 2015), and histograms were visually checked at each value of K.

As the source populations on Vancouver Island are known to have experienced at least one bottleneck event at the beginning of the 20th century, and reintroduced populations may have been subject to sequential bottlenecks associated with reintroduction, populations with a minimum of 20 samples were evaluated using the

software BOTTLENECK v.1.2.02 (Piry et al. 1999) for deviations from mutation / drift equilibrium and mode shift. BOTTLENECK implements four tests: *sign* test, *standard difference* test, *Wilcoxon sign rank* test, and *mode shift* test; a mode shift is indicated when there is a deviation from an L-shaped distribution and is suggested by the authors to be a reliable indicator of past bottleneck events. As the *standard difference* test requires at least 20 microsatellite loci to reliably detect a bottleneck, this test was not done. Both the *sign* and *Wilcoxon* tests were run for three mutation models: Infinite Alleles Model (IAM), Stepwise Mutation Model (SMM), and Two Phase Mutation model (TPM). The TPM model allows for adjustment of the ratio of strict single step mutation, as well as variance in size of multiple step mutations. As microsatellite loci rarely evolve under a strict SMM, the proportion of single step mutations was set at 0.90, with variance set at 0.12 (Garza and Williamson 2001, Hundertmark and Van Daele 2010).

To further understand how reintroduction strategy has affected genetic diversity in these recently established populations, the genetic effective population size (N_e) was calculated with the software NeESTIMATOR v.2.1 (Do et al. 2014) using the linkage disequilibrium model for random mating; this option provides an estimate for a single time point. Multiple tests were run for two thresholds for rare alleles (0.05, 0.01) to allow comparison of the model using different run parameters. As the N_e for each population is estimated independently, all populations were included.

2.3 Results

2.3.1 Sampling

Samples for 356 individuals (Figure 2.3) were successfully amplified and genotyped at seven or more microsatellite loci (Appendix II), with one additional sample that amplified with mtDNA primers but failed to amplify with most microsatellite loci. Genotype comparisons in CERVUS showed a total of 119 genotype matches with one or two mismatched microsatellite loci, and no matches with zero mismatched loci. Each genotype pair was reviewed to determine its potential for being the same individual sampled more than once. Out of 119 potential identity matches, all were rejected but one, as they were either tissue samples from different deceased individuals, tissue samples from dead elk and faecal samples collected at a later date, or Vancouver Island samples matched with mainland samples. One matched pair consisted of a faecal sample collected in spring and a tissue sample from an elk killed later that year at a nearby location. As a true match could not be excluded, the faecal sample was removed from the data set. All of the following analyses, except for mtDNA sequencing, were completed using the remaining 355 samples.

2.3.2 Sequencing

A subset of 58 samples were successfully sequenced and analyzed; all individuals (Vancouver Island n = 28; mainland n = 30) were determined to have Roosevelt elk mtDNA, presence of an A to G transition at site 493 (Figure 2.4b). We identified three variable sites and four Roosevelt elk haplotypes in our analysis (Table 2.2). Of the four haplotypes observed (Figure 2.4a), two haplotypes occurred exclusively south of Alberni Inlet on Vancouver Island (herein referred to as VIS; Hap-C and Hap-D) and two other

haplotypes to the north (VIN; Hap-A and Hap-B). Overall haplotype diversity (H_D) was 0.494 and overall nucleotide diversity (π) was 0.0011 (Table 2.2). VIN had the highest haplotype and nucleotide diversities (n = 18, *H*d = 0.523, π = 0.0009) compared to VIS (n = 10, H_D = 0.356, π = 0.0006). Among mainland populations all 30 individuals shared a single haplotype (H_D = 0, π = 0). This haplotype (Hap-A) was also found on VIN (Figure 2.4). Genetic distance among populations (Table 2.2) was highest between VIS and VIN (*d* = 0.00292, ±0.00201) and lowest between VIN and the mainland (*d* = 0.00079, ±0.00081), with intermediate values between VIS and mainland (*d* = 0.00213, ±0.00183). Pairwise F_{ST} difference was greatest between VIS and mainland (F_{ST} = 0.925, P <0.001), lowest between VIN and mainland (F_{ST} = 0.494, P <0.001), and intermediate between VIN and VIS (F_{ST} = 0.721, P <0.001).

2.3.3 Microsatellites

The 355 samples were aggregated into 13 populations in further analyses (Table 2.3, Figure 2.3): Vancouver Island South (VIS, n = 41), Vancouver Island North (VIN, n = 55), Sechelt Peninsula (SP, n = 48), Central Coast (CC, n = 35), Rainy Gray (RG, n = 53), Squamish – Indian River (SQ, n = 16), Pitt River (PITT, n = 29), Stave River – Tipella (STV, n = 16), Brittain – Skwawka (BRIT, n = 10), Powell River South (PRS, n = 17), Powell River North (PRN, n = 16), Toba River (TOBA, n = 11), and Homathko – Southgate – Orford (HO, n = 8).

Deviations from HWE were observed for two loci and four populations (Appendix IV), however, none remained significant after correcting for FDR (130 pairwise; adjusted P=0.0004). Two pair of loci showed significant LD (Appendix V) after controlling for FDR (45 pairwise; adjusted P = 0.0033): BM888 and Inra107 (χ^2 ≥116.77,

df = 26, $P < 1.81 \times 10^{-13}$; LD in VIN, SP and PITT populations, full population by locus-pair data not shown) and BM4107RD & Inra107 ($\chi^2 \geq 53.49$, *df* = 26, $P < 0.0012$; LD in four populations not significant after FDR adjustment). Bottlenecked populations often show deviations from HWE, as their characteristics often violate HWE assumptions (Nei et al. 1975, Avise 1994) and as such, all loci and populations were retained for further analyses.

Genetic diversity statistics were calculated to evaluate general patterns among populations (Table 2.4). The average number of alleles (N_a) showed the highest values in the primary source population VIN and the reintroduced (tertiary) population SQ (N_a = 3.0), the next highest value in the main secondary source population SP and tertiary RG, PITT and STV (N_a = 2.9), followed by CC (N_a = 2.8), primary source VIS, tertiary BRIT, PRS and TOBA (N_a = 2.7), and PRN and HO (N_a = 2.6). The effective number of alleles (N_{eff}) showed a similar pattern except STV had the highest value (N_{eff} = 2.29) followed by VIN (N_{eff} = 2.20), TOBA, SP, PITT, RG, and PRN (N_{eff} = 2.09-2.07), HO and CC (N_{eff} = 2.05-2.03), with BRIT, VIS, SQ, PRS with the lowest values (N_{eff} = 1.99-1.84). Allelic richness (A_r) was calculated for all populations, though it should be noted that this statistic is sensitive to small sample sizes and rare alleles. A_r showed a mixed pattern wherein four of the ten reintroduced populations (STV, SQ, PITT, TOBA) had equal or greater richness as VIN (A_r = 2.635), and an additional two (BRIT and HO) greater than secondary source SP (A_r = 2.577). The remaining four tertiary populations (CC, PRN, RG, PRS) had lower A_r values than SP, but greater richness than VIS (A_r = 2.229).

The information index (I) showed greatest diversity in STV and VIN (I = 0.866 and 0.843 respectively), followed by SP (I = 0.816) and tertiary populations (in

descending order TOBA, PITT, RG, PRN, CC, HO, SQ, BRIT (I = 0.807-0.763). VIS and PRS had the lowest values (I = 0.710 and 0.694 respectively). Observed (H_o), expected (H_e) and unbiased heterozygosity (uH_e) showed mixed patterns, however, four populations showed significant reductions in H_o as indicated by G_{is} (Table 2.5): VIS, RG, HO and TOBA (G_{is} = 0.111, 0.121, 0.227, 0.231, respectively). The fixation index (F) showed the same pattern as G_{is}, as they are both based on differences between observed and expected heterozygosity. Diversity statistics for microsatellite loci (Table 2.5) showed a pattern of lower observed heterozygosity than expected for all loci but two, BL42 and INRA107.

Comparisons of founding population demographics to allele frequency (Appendix VI) and diversity statistics (Table 2.5) failed to show any clear associations. Allele frequency changes ± 50% were common among the reintroduced populations when compared to VIN, the primary source population. All reintroduced populations lost at least one low frequency allele present in VIN, with PRN, TOBA and HO each losing four alleles. Four populations (RG, SQ, PITT and TOBA) had a unique allele at locus BM888 not observed on Vancouver Island or SP. Of note, VIS was missing three alleles, compared to VIN.

Pairwise population differentiation was similar with both F_{st} and Jost's D, with one exception (PRS x PRN significantly different with F_{st} only; not shown), therefore only F_{st} values are discussed (Table 2.7). After FDR correction (78 pairwise tests, adjusted P <0.0179), VIS was significantly different from all other populations except HO; primary source VIN was different from all populations except PRN, TOBA and HO. Among the reintroduced mainland populations, PRS showed significant differentiation

from five populations (SP, CC, PITT, STV and PRN), PITT was significantly different from three (SP, PRS and RG) as was SP (SQ, PITT and PRS). The only other significant difference was SQ and SP.

STRUCTURE analyses of microsatellite genotypes showed clear genetic differentiation between VIS and all other populations (Figure 2.5a). Analyses of all 13 populations showed ΔK = 2, as did Prob(K). A closer evaluation of the different ancestry plots, specifically K = 4, suggested further hierarchical structure may be present. To ascertain if there was further hierarchical structure, a series of STRUCTURE runs were conducted following the recommendations in Wang (2017), where the parameters for the model's prior assumptions were adjusted, namely allowing independent alpha values for each population, and lower initial values of alpha (a = 0.1-0.5). Adjusting these values allows STRUCTURE to more precisely assign individual ancestry contributions (Wang 2017). Using an initial value for alpha of 0.25, and allowing individual priors for each population, the estimate for ΔK increased to four, while Prob(K) remained at two (Figure 2.5a).

To evaluate the less developed genetic structure observed among the mainland populations further runs were conducted without VIS (Figure 2.5b). Including populations that are highly different from others can reduce the ability of the model to identify substructure. An initial run with *admixture*, *loc prior* and default settings resulted in ΔK = 3 and Prob(K) = 1 (not shown). Visual examination of ancestry plots indicated some population differentiation was evident at K = 3. In an attempt to reconcile the discrepancy between ΔK and Prob(K), parameters were set to allow individual alpha values and an initial alpha value of 0.25. At those settings both ΔK and Prob(K)

supported three clusters (Figure 2.5b). VIN is clearly differentiated, while the mainland populations show individuals assigned to two different ancestral populations (mostly orange, mostly light blue), regardless of their sampled population.

Populations run through BOTTLENECK (sample size >20) included VIS, VIN, SP, RG, PITT, and PR which included samples from three EPUs 2-12A, 2-12E and 2-12D with no barriers to dispersal between them other than distance. BOTTLENECK results (Table 2.7) showed significant deviations from expected heterozygosity at mutation / drift equilibrium (H_{ex}) for the IAM in all populations for both the *sign* test ($P \leq 0.006$) and *Wilcoxon sign rank* test ($P \leq 0.003$). In contrast, the strict SMM was only significant for two populations in the *Wilcoxon* test (VIN and SP, P = 0.009 and 0.012 respectively). For the TPM model both VIS and VIN were significant in the *sign* test (P = 0.018 and 0.017 respectively) with nine loci showing heterozygosity excess, and all populations except PITT were significant in the *Wilcoxon* test (P = 0.003-0.042). A mode shift was observed for SP, PITT and PR.

Estimates of effective population size were calculated for all 13 populations (Table 2.8). The N_e estimates for VIS and VIN were very similar at 58.5-59.7. N_e values for reintroduced populations ranged from a low of $N_e = 7$ (minimum allele frequency 0.05) for PITT to a maximum N_e=230 (minimum allele frequency 0.01) for RG, with an average N_e value of 72. Seven of the mainland populations (SP, RG, SQ, BRIT, PRS, TOBA and HO) had N_e estimates greater than their sample size, while four were lower (CC, PITT, STV and PRN). Confidence intervals (95%) for most N_e values were large, with maximum values of infinity in seven populations and a ten-fold difference between

minimum and maximum values. Only two populations (CC and PITT) had 95% CI values that differed roughly five-fold (5.3-24.5 and 2.9-13.8 respectively).

2.4 Discussion

Translocations have been used widely to reintroduce populations of wildlife to former habitat with varied measures of success (Lubow 1996, Wolf et al. 1996, Stephen et al. 2005). The reintroduction program undertaken by the British Columbia government between 2001 and 2017 has resulted in the reestablishment of Roosevelt elk to historic habitats, with a population increase of over 500% and species' range expansion of >30,000 km^2 from prior to initiation (Reynolds et al. 2018). While the program's overall success cannot be overstated, concern about genetic diversity loss associated with reintroductions, sequential translocations, and specific species life history requires careful evaluation of such projects, especially for species at risk (Stockwell et al. 1996, Larson et al. 2002*a*, West et al. 2018, White et al. 2020).

2.4.1 Population Structure

The results of this study suggest that population bottlenecks have reduced mitochondrial genetic diversity in both extant and reintroduced populations of Roosevelt elk in British Columbia. The initial reintroduction of 22 elk to the mainland occurred on the Sechelt Peninsula with eight females and one male from the Qualicum area on southern Vancouver Island; VIS has two unique haplotypes not shared with VIN. Our results suggest that either: 1) the translocated Qualicum area females shared the A haplotype common on VIN, or 2) the C and / or D haplotype was lost due to drift on the mainland within 25 years of translocation. Unfortunately, neither archival samples from the founding animals nor contemporary DNA samples from the Qualicum area were

available for analysis. Reviewing species distribution and density maps from near the time of translocation (Brunt 1990) suggest elk occurring near Qualicum were likely more connected to populations further south than those to the north (Billy Wilton FLNRO, personal communication 2020). Regardless of which haplotypes were present at the time of reintroduction, at best it appears mainland populations are lacking the mitochondrial diversity present on Vancouver Island, and at worst, there has been a substantial loss of diversity in mainland populations, despite a female bias which should have helped conserve mtDNA diversity.

Three of the haplotypes found in this study were previously identified on Vancouver Island in Polziehn et al. (1998). One haplotype from that study (*Roosevelt 33*), with an A to G transition at site 541, was not observed in our samples. Unfortunately, the source location of the *Roosevelt 33* sample is unknown (R. Polzeihn, University of Alberta, personal communication 2020). The D haplotype observed in the VI South population, with a T to C transition at site 441, has not been previously reported.

Multi-locus genotype analyses supported the mitochondrial pattern seen on Vancouver Island, showing the same differentiation between VIS and VIN associated with Alberni Inlet, both in pairwise differentiation (F_{ST}) and Bayesian clustering (STRUCTURE) analyses. This suggests that the Alberni Inlet represents a substantial barrier to both male and female dispersal on Vancouver Island. The Alberni Inlet runs southwest to northeast from Barkley Sound on the west coast of Vancouver Island to Port Alberni some 62 km inland. The inlet creates a barrier two-thirds of the island’s width, with areas from Port Alberni east to the Salish Sea comprised of mostly poor to very poor quality elk winter habitat (Quayle and Brunt 2003). Degradation of habitat quality is

likely associated with extensive loss of old growth forest, important in high snow years, transportation corridors, and development. Winter habitat appears to be the critical element of Roosevelt elk persistence in an area (Brunt 1990).

Analyses of multi-locus genotypes showed clear population differentiation between VIS and VIN, corresponding to the barrier represented by Alberni Inlet. Both pairwise F_{st} and Bayesian analyses show a marked difference between these two populations, suggesting very little gene flow is occurring. As the Alberni Inlet represents a barrier to dispersal on Vancouver Island, it is likely that the extensive inlets and the steep, rocky terrain of much of the mainland coast also represent significant barriers to Roosevelt elk dispersal. While the Alberni Inlet appears to be a somewhat obvious barrier to dispersal, the reasons underlying its status are not as obvious. Elk are good swimmers, as evidenced by occasional sightings on islands. A group of elk were translocated to the Chehalis EPU on the west side of Harrison Lake in 2013. A year and a half after release, a GPS collared female elk swam east across the lake, a distance of more than 3.2 km. Alberni Inlet is less than a kilometre wide for much of its length, and less than 700 m in a few spots, raising the question: is the inlet a substantive barrier to dispersal, or is the lack of gene flow more related to low population density in adjacent areas?

2.4.2 Reintroduction Effects

To understand the effect that reintroduction strategy has had on genetic diversity, we searched for patterns within the genetic data; changes in heterozygosity, allele frequency and richness were examined, and pairwise comparisons between populations were conducted (Tables 2.4, 2.6 and Appendix IV). Overall, rare alleles were lost in many populations, and allele frequencies varied substantially between them. Changes in

heterozygosity ($H_e - H_o$) between source and reintroduced populations often exceeded 20%, though changes were somewhat more likely to represent a loss of heterozygosity (~59%) than an increase. No clear pattern could be ascertained related to single vs. multiple source populations, number of founding individuals, or years since reintroduction, suggesting that the observed changes were likely the result of drift.

Pairwise differences were highly significant between both VIS, VIN and most mainland populations (Table 2.6). Differences among a handful of mainland populations were significant, with PRS showing five pairwise differences, and PITT and SP showing three each. Interestingly SP and to a lesser amount PRS, were the source populations for all of the other mainland populations sampled. Differentiation between the reintroduced populations and their source(s) is likely due to drift associated with small numbers of founding individuals, and / or the capture and translocation of closely related individuals. Unfortunately, little work has been conducted on the genetic relatedness of winter herds in elk, and none in Roosevelt elk. High home range fidelity and social influences on herd interactions (or lack thereof) suggests that elk social dynamics may contribute to genetic changes observed in reintroduced populations (Franklin et al. 1975, Larkin et al. 2004, Muller et al. 2018). The PITT represents a fast growing reintroduced population, which should have helped to minimize diversity loss and drift (Nei et al. 1975).

Bayesian analyses showed increasing differences between VIS, VIN and the mainland populations. Within the reintroduced groups, individual ancestry assignment appeared unrelated to its sampled population (Figure 2.5b), which may be indicative of founder effect, or could possibly be related to different levels of drift between overlapping generations. Roosevelt elk are long lived, and sampling may have captured

multiple generations. Also, some individuals with allele frequencies similar to source populations may have been sampled, or later cohorts may be more affected by inbreeding and drift making it appear as though there were two different source populations. Overlapping generations, unequal fecundity (as would be expected in polygynous mating systems), and variations in population size are all known to affect allele frequencies and population measures like N_e (Nunney 1993, Frankham 1995, Waples et al. 2014). The underlying model used in STRUCTURE assumes populations are in HWE, and loci are not linked (Wang et al. 2016). While no populations were significantly out of HWE and only two pairs of loci showed LD after adjusting for FDR, numerous loci in individual populations exhibited low probability values, just not enough to be significant. To see if specific loci were implicated, data sets were analyzed with the highly significant loci pair in LD (BM888 and INRA107), and a third locus possibly out of HWE (RT7) were removed one at a time (data not shown) and reanalyzed. The results of both pairwise differentiation and Bayesian analysis remained unchanged.

STRUCTURE is known to start ‘splitting’ assignments (assigning individuals to multiple populations) in groups when K values are higher than the likely number of populations (Lawson et al. 2018). While this is observed in many mainland individuals, at K=4 in Figure 2.5a and at K=3 in Figure 2.5b, the VIN population becomes differentiated from the mainland. Comparing STRUCTURE results to pairwise differences (Table 2.6) supported the conclusion that mainland populations are differentiated from VIN. The question of why different individuals in reintroduced herds sourced from a single population appear to be assigned to two, remains unanswered.

These results, taken as a whole, suggest that some mainland populations of Roosevelt elk are differentiating due to genetic drift, likely from a combination of factors including founder effect, small population size, and limited or complete isolation from other herds. The lack of reduced diversity, or even the increase in some diversity measures, in reintroduced populations is not unheard of. It appears that for species that have experienced substantial bottleneck events, the initial reduction of low frequency alleles results in only common alleles remaining, and as such they are more likely to be carried to new populations by founders (Clegg et al. 2002, Larson et al. 2002*a*, *b*).

The *Wilcoxon* test implemented in BOTTLENECK is reported to have the highest power of the program's test for detecting recent bottleneck events when using a moderate number of loci (Piry et al. 1999), and the TPM model is most representative of microsatellite mutation processes (DiRienzo et al. 1994). The results of the bottleneck analyses suggest that all Roosevelt elk populations in BC show some evidence of recent bottleneck events. Only the PITT herd was not significant, though close, in the *Wilcoxon* – TPM analysis (P=0.065), yet it still showed deviation from an L-shaped allele distribution *(mode shift),* suggesting that this population should also be considered bottlenecked. The authors of the program report that testing for bottleneck signatures is likely only detectable for $4N_e$ generations after the event; therefore, the greater the magnitude of the genetic bottleneck, the sooner it will become undetectable. The populations on Vancouver Island appear to still show the signature of a substantial bottleneck event after more than 100 years, which may be the result of a few hundred individuals surviving. In contrast, an insular population of Roosevelt elk introduced to an island in the Kodiak Archipelago, Alaska founded by five females and three males in

1929 showed no heterozygosity excess after 79 years when evaluated with various bottleneck detection software (Hundertmark and Van Daele 2010). The authors used genetic population models to show that the signature of the bottleneck may have been lost in the population within eight years. This surprising result was suggested to be due to a rapid loss of heterozygosity in the first year, slowing to virtually no change by year 10. The authors also reported that while other methods were also able to detect bottlenecks in the subspecies, they were unable to detect sequential bottlenecks.

The estimates effective population sizes calculated for Vancouver Island raises a number of important management considerations. Study data for Vancouver Island were less likely to be affected by the sampling of close relatives or family groups, and may be a complication in EPUs where non-invasive samples were included, because hunted samples were widely dispersed and could be considered relatively random. Both areas, VIS and VIN, had good numbers and quality of samples, yet N_e estimates were barely above the IUCN Red List criteria (Ne >50) for critically endangered. It should be noted that linkage disequilibrium estimators of N_e can become downwardly biased when sampling includes overlapping generations (Nunney 1993, Waples et al. 2014). While it is undoubtable study samples represented various age class animals, the extent to which this affected our N_e estimates is unknown as age class information was not available from the compulsory inspection data for teeth, which was the sole source of Vancouver Island samples. The unfortunate reality is that Roosevelt elk on Vancouver Island appear to have lost significant genetic diversity due to the bottleneck of the late 1800s, and moderate population growth in the decades that followed. N_e is also known to be adversely affected by fluctuations in population size and variance in individual reproductive success (Nei

and Murata 1966, Nunney 1993). Extreme weather events, specifically harsh and long winters, are known to have caused many Vancouver Island herds to have experienced substantial fluctuations in population size (Brunt 1990, Quayle and Brunt 2003).

The strategy undertaken in the reintroduction of Roosevelt elk to the mainland of BC resulted in the creation of numerous new herds in areas of high quality habitat, through translocation of a minimum of 20 individuals (Reynolds et al. 2018). The reintroduction of at least 20 individuals has been shown to result in increased population growth and increased reintroduction success in various artiodactyls (Komers and Curman 2000). From a demographic perspective, the reintroduction program can only be described as exemplary with all 27 mainland EPUs, except for one, showing healthy growth rates in the initial years after establishment (Reynolds et al. 2018). From a genetic diversity viewpoint, our results suggest that there is reason for concern. It is important to note that roughly 70% of all elk translocated within the region were captured on the Sechelt Peninsula, including 20 of the 25 founding individuals for the Powell River herd, the other source for mainland translocations, with the exception of the Phillips and Heydon EPUs discussed in the introduction. While Komers & Curman (2000) acknowledged that reintroduced species with polygynous mating systems could result in increased rates of inbreeding, only maintenance of heterozygosity was mentioned and dismissed, as "loss of heterozygocity [sic.] in particular can be virtually prevented if the founding population is allowed to increase rapidly".

More recent research has shown that even within reintroduced populations where the founding individuals were not closely related, and with roughly three times as many founders, differential reproductive success such as that expected with one or two males

dominating all breeding in a small population, could lead to the rapid loss of genetic diversity within the first few years of establishment (Wilson et al. 2005). Other researchers have found similar results in reintroduced, polygynous ungulates (Fitzsimmons et al. 1997, Slate et al. 2000, Zachos et al. 2007, Olson et al. 2013). In a recent study evaluating different approaches on maintaining genetic diversity in bison (*Bison bison*), a severely bottlenecked species, Giglio et al. (2018) found increased inbreeding if a male was a dominant breeder for two years. As bison and elk share a similar breeding system, the potential for increased loss of genetic diversity in reintroduced elk populations is likely exacerbated by strategies where only one or two mature males are present during initial herd establishment. Furthermore, as most male elk translocated with captured herds were predominantly yearlings or calves, any mature male present early in herd development was likely to dominate all breeding for multiple years, with subsequent increased levels of inbreeding and decreased levels of diversity. It is also possible, if not likely, that yearling males are closely related to at least some of the females in a captured herd (Clutton-Brock et al. 1988, Geist 2002), thus further compounding the potential for loss of diversity within these populations.

While historic bottleneck events may have reduced genetic diversity in British Columbia’s Roosevelt elk, it is critical that the remaining genetic diversity of the population is maintained. The mainland populations appear to be undergoing genetic differentiation from their Vancouver Island source. Mainland Roosevelt elk show a clear reduction in mitochondrial diversity and loss of multiple low frequency alleles in multiple herds. Bottlenecked populations often show a heterozygosity increase, however this effect is ephemeral and there is typically an eventual loss of heterozygosity (Cornuet and

Luikart 1996, Luikart and Cornuet 1998). Low heterozygosity is associated with reduced fitness of individuals, as well as populations and species (Aldridge & Boyce 2007, Bérénos et al. 2016, Keller et al. 2012, O'Brien & Evermann, 1988, Reed & Frankham, 2003, Slate et al. 2000; however, see Britten 1996). While the loss of microsatellite alleles on their own is likely inconsequential, as they are putatively neutral, it may be indicative of the loss of irreplaceable adaptive potential in the form of rare alleles at non-neutral loci. Roosevelt elk have low genetic diversity when compared to other elk subspecies (Polziehn et al. 1998, 2000)

For maternally inherited markers, rapid population growth is the critical element to reduce the effect of drift on mitochondrial diversity (Avise 1994). Between the initial reintroduction in 1987, and initiation of the mainland reintroduction project in 2000, the mainland population grew from 22 to over 400, likely representing maximum population growth for this subspecies. Despite this, it appears only a single mitochondrial haplotype occurs in mainland populations of Roosevelt elk. While mitochondrial sequencing occurred for only a subset of mainland elk, the sample size of 30 provided a reasonable chance of observing low frequency haplotypes, though it is possible that rare haplotypes are also present.

In practice, the majority of captures for Roosevelt elk reintroductions took place during winter months as attracting elk into traps is easier when food resources are scarce, and elk are more likely to congregate in larger groups (Reynolds et al. 2018). Relatedness within winter herds has not been studied for Roosevelt elk. Some work on other subspecies of elk showed herds are matrilineal (Clutton-Brock et al. 1988, Nussey et al. 2005), while others show lack of stability in family groups (Vander Wal et al. 2012). The

potential for loss of genetic diversity in elk may be complicated in reintroductions as genetic structuring has been shown to persist in herds with no physical barriers to dispersal for up to 13 years after release, at least partly due to social segregation among herds (Muller et al. 2018). Female elk are highly philopatric to a home range, and Roosevelt elk appear to be no exception. In 2001, two females were captured on the Sechelt Peninsula wearing VHF collars deployed during translocation from Vancouver Island between 1987-1989. Their capture occurred only a few hundred metres from their release location 12-14 years earlier, suggesting limited dispersal of established females in quality habitats (I. Gazeley personal observation, 2001).

2.5 Conclusions

This study used nuclear and mitochondrial molecular markers to reveal metapopulation structure of Roosevelt elk throughout much of their current range in British Columbia. Historic and contemporary bottlenecks have likely resulted in substantial losses of genetic diversity in the study populations, though the eventual extent of loss in the mainland populations will not be known for many years. To better understand genetic diversity changes in populations of Roosevelt elk, as well as other polygynous species, further monitoring and research is needed. Of particular interest, the Alberni Inlet and developed areas to the east on Vancouver Island represent a complete barrier to dispersal. Greater understanding of the role this landscape feature is playing in isolating elk on southern Vancouver Island is required. It should be recognized that Alberni Inlet was previously identified as a putative barrier dividing the Vancouver Island metapopulation into two main subpopulations (Brunt 1990, Quayle and Brunt 2003), however, this study represents the first genetic confirmation of that hypothesis.

As gene flow between populations is integral to maintaining genetic diversity, and therefore population fitness and persistence, an understanding of connectivity, in the context of the landscape, is required. While landscape genetics is effective and appropriate for understanding dispersal processes at the metapopulation level, in the case of mainland Roosevelt elk it is probably too soon for patterns to have become detectable. As individual elk herds have only been established from 3-23 years, it is unlikely that dispersal can be detected among populations that are mostly sourced from the same secondary population, with molecular markers similar to those used in this study. New molecular methods involving genome wide sequencing may be able to provide more power to identify population origin of suspected migrants.

The analyses presented here suggest that some populations of Roosevelt elk may require intervention. Elk in the southern part of Vancouver Island have very low genetic diversity compared to most other BC Roosevelt populations, including sequentially bottlenecked reintroduced herds on the mainland. On the mainland we suggest that the Rainy-Gray, Pitt, Stave, and Powell River North and South (Haslam, Lois, Eldred, Powell-Daniels, Theodosia EPUs) groups represent population units of concern due to loss of allelic diversity, increased inbreeding and fixation metrics, and / or low effective population sizes. Considering the samples sizes from Homathko-Southgate-Orford EPUs, and a single point sample (n=8) from the Toba EPU, we suggest additional efforts should be made to improve our knowledge of the genetic health of these herds. While the high inbreeding values in these EPUs are of concern, the small sample sizes increase uncertainty around these measures.

To make the most of scarce financial and human resources, any future translocations to augment genetically depauperate populations should consider a 'genetically informed' strategy (Wilson et al. 2005, Pemberton 2008, West et al. 2018, White et al. 2020) where source populations or candidate individuals are evaluated and targeted to maximize genetic differences from the receiving population. While human-mediated dispersal should be considered a last resort, and some projects have failed due to genetic swamping of locally adaptive alleles when source populations are poorly chosen, in the case of Roosevelt elk this is probably not a concern. It is unlikely that different populations of the subspecies in British Columbia express highly adaptive alleles or have evolved potential fitness reducing differences, such as major histocompatibility complex incompatibilities. In contrast, increased heterozygosity and allelic diversity have been shown to contribute to improved fitness in most animals (Lacy 1997, Reed and Frankham 2003, Johnson et al. 2010). It is our opinion that a strategy of translocating a few individuals per year, from areas on Vancouver Island with high frequency of other mitochondrial haplotypes (haplotypes B, C or D), would provide important additional genetic diversity to some, if not all, mainland populations.

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Table 2.1 Mitochondrial haplotypes identified in 58 British Columbia Roosevelt elk (*Cervus canadensis roosevelti*); nucleotide positions aligned to consensus sequence (OG) for North American Wapiti from Polziehn et al. (1998); † = diagnostic site for Roosevelt subspecies.

Haplotype	Nucleotide position			
	441	450	476	493†
OG	T	G	A	A
A	T	G	A	G
B	T	G	G	G
C	T	A	A	G
D	C	A	A	G

Table 2.2 Summary of mitochondrial diversity for 58 Roosevelt elk D-loop sequences (*C. c. roosevelti*) and pairwise F_{st} values; *** P <0.001.

Population	Number of individuals (n)	Haplotype diversity (H_D)	Nucleotide diversity (π)	Number of haplotypes observed	Pairwise F_{ST}	
					VIN	VIS
Vancouver Island North	18	0.523	0.0009	A (n = 10) B (n = 8)	-	-
Vancouver Island South	10	0.356	0.0006	C (n = 8) D (n = 2)	0.721***	-
Mainland	30	0.000	0.0000	A (n = 30)	0.494***	0.925***
Total	58	0.494	0.0011	-	-	-

Table 2.3 Population groupings and associated management units for Roosevelt elk (*C. c. roosevelti*) study samples (n=355) used in microsatellite and mitochondrial analyses.

Grouping	WMU / EPU	Number of samples	MSAT success (min 7/10 loci)	D-loop Sequence
VIS	1-03A-B	9		
	104A-C	26		
	1-05A-C	9		
	Total	44	41	10
VIN	1-06A-B/D	9		
	1-09A-D	12		
	1-10A-H	20		
	1-11A-B	9		
	1-12C-E	5		
	Total	55	55	18
SP	2-05A	49	48	11
CC	2-05D	8		
	2-05E-H	27		
	Total	35	35	-
RG	2-05I	55	53	0
SQ	2-06A/2-08A	17	16	5
PITT	2-08B	29	29	5
STV	2-08C/2-09A	16	16	2
BRIT	2-12E/Q	10	10	-
PRS	2-12A/S	18	17	7
PRN	2-12D/F	17	16	-
TOBA	2-13A	17	11	-
HO	2-14A-B/2-15A	10	8	-
TOTAL		372	355	58

Table 2.4 Genetic diversity statistics at 10 microsatellite loci for 13 populations of Roosevelt elk (*Cervus c. roosevelti*) in British Columbia, including number of alleles per locus (N_a), effective number of alleles (N_{eff}), Shannon Index (I), observed heterozygosity (H_o), expected heterozygosity (H_e), unbiased expected heterozygosity (uH_e), fixation index (F), allelic richness (A_r), and the inbreeding coefficient G_{is}, * indicates significance at a = 0.05, adjusted for FDR ($P \leq 0.015$).

Pop	**n**	**Na**	**Neff**	**Ar**	**I**	**Ho**	**He**	**uHe**	**F**	**Gis**
VIS	41	2.7	1.94	2.229	0.710	0.423	0.469	0.475	0.101	*0.111
VIN	55	3.0	2.20	2.635	0.843	0.538	0.519	0.524	-0.020	-0.027
SP	48	2.9	2.08	2.577	0.816	0.500	0.507	0.512	0.008	0.024
CC	35	2.8	2.03	2.549	0.787	0.481	0.484	0.492	0.013	0.021
RG	53	2.9	2.07	2.489	0.792	0.444	0.499	0.504	0.125	*0.121
SQ	16	3.0	1.96	2.689	0.765	0.471	0.455	0.470	-0.005	-0.003
PITT	29	2.9	2.08	2.635	0.804	0.491	0.485	0.493	-0.012	0.004
STV	16	2.9	2.29	2.729	0.866	0.504	0.535	0.554	0.074	0.093
BRIT	10	2.7	1.99	2.633	0.763	0.480	0.468	0.493	-0.007	0.027
PRS	17	2.7	1.84	2.487	0.694	0.448	0.423	0.436	-0.019	-0.028
PRN	16	2.6	2.07	2.512	0.788	0.497	0.498	0.514	0.005	0.035
TOBA	11	2.7	2.09	2.640	0.807	0.409	0.499	0.525	0.199	*0.231
HO	8	2.6	2.05	2.600	0.782	0.413	0.493	0.526	0.159	*0.227
Mean	27.1	2.8	2.05	2.570	0.786	0.469	0.487	0.501	0.048	0.066

Table 2.5 Averaged diversity statistics for 10 microsatellite loci in 13 populations of Roosevelt elk (*C. c. roosevelti*) in British Columbia, including number of alleles per locus (N_a), effective number of alleles (N_{eff}), observed heterozygosity (H_o), expected heterozygosity (H_e), corrected expected heterozygosity (H_t), and inbreeding coefficient G_{is}.

Locus	N_a	N_{eff}	H_o	H_e	H_t	G_{is}
BL42	3	2.437	0.628	0.606	0.621	-0.037
BM203	3	1.842	0.448	0.471	0.504	0.048
BM4107RD	4	2.098	0.527	0.539	0.558	0.022
BM4513RD	4	2.027	0.472	0.523	0.532	0.097
BM6506	2	1.891	0.437	0.487	0.483	0.101
BM888	3	1.549	0.318	0.366	0.367	0.132
BMC1009	3	1.789	0.413	0.455	0.469	0.092
CSSM041	2	1.849	0.449	0.473	0.470	0.050
Inra107	5	2.831	0.682	0.665	0.681	-0.026
RT7	2	1.734	0.317	0.439	0.467	0.278
Overall	3.1	2.005	0.469	0.502	0.515	0.066

Table 2.6 Pairwise genetic difference for 13 populations of Roosevelt elk (*C. c. roosevelti*) in British Columbia; F_{st} values below diagonal (significant values in bold), *P* values above diagonal (FDR adjusted *a* = 0.05, *P* = 0.0179).

POP	VIS	VIN	SP	CC	RG	SQ	PITT	STV	BRIT	PRS	PRN	TOBA	HO
VIS	--	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.002	0.043
VIN	**0.067**	--	<0.001	<0.001	<0.001	<0.001	<0.001	0.016	0.007	<0.001	0.365	0.018	0.485
SP	**0.074**	**0.020**	--	0.216	0.056	0.001	0.010	0.649	0.233	<0.001	0.085	0.212	0.326
CC	**0.086**	**0.037**	0.004	--	0.071	0.035	0.873	0.777	0.601	0.002	0.282	0.114	0.102
RG	**0.065**	**0.028**	0.008	0.009	--	0.040	0.007	0.582	0.543	0.027	0.063	0.926	0.210
SQ	**0.103**	**0.054**	**0.038**	0.020	0.019	--	0.132	0.037	0.699	0.078	0.030	0.051	0.108
PITT	**0.105**	**0.049**	**0.016**	-0.007	**0.018**	0.011	--	0.294	0.887	0.003	0.134	0.096	0.019
STV	**0.067**	**0.023**	-0.005	-0.008	-0.004	0.029	0.004	--	0.408	0.007	0.331	0.505	0.493
BRIT	**0.092**	**0.035**	0.008	-0.005	-0.003	-0.01	-0.015	0.001	--	0.076	0.542	0.397	0.100
PRS	**0.090**	**0.041**	**0.040**	**0.035**	0.021	0.019	**0.041**	**0.044**	0.025	--	0.017	0.087	0.124
PRN	**0.071**	0.001	0.012	0.004	0.015	0.031	0.011	0.004	-0.004	**0.036**	--	0.163	0.405
TOBA	**0.052**	0.028	0.008	0.016	-0.017	0.035	0.019	-0.003	0.002	0.024	0.017	--	0.311
HO	0.035	-0.001	0.005	0.022	0.012	0.031	0.046	-0.004	0.037	0.023	0.002	0.010	--

Table 2.7 Bottleneck analysis results for six populations of Roosevelt elk (*C. c. roosevelti*) in British Columbia for the Sign test, Wilcoxon Sign Rank test, and deviation from an L-shaped allele frequency distribution for three different mutation models: Infinite Allele model (IAM), Two Phase Mutation model (TPM; strict stepwise mutation=90%, variance=0.12), and Stepwise Mutation model (SMM) for populations with sample size >20: Hex = expected number of loci with heterozygosity excess at mutation-drift equilibrium, He = observed number of loci with heterozygosity excess, L = expected "L" shaped allele frequency distribution, * denotes significance at a=0.05.

Test	**VIS**	n=41	**VIN**	n=55	**SP**	n=48	**RG**	n=53	**PITT**	n=29	**PR**	n=26
Sign	Hex\He	*P*	Hex \ He	*P*	Hex \ He	*P*	Hex \ He	*P*	Hex \ He	*P*	Hex \ He	*P*
IAM	4.67 \ 9	*0.006	4.77 \ 10	*0.001	4.78 \ 10	*<0.001	4.35 \ 10	*<0.001	4.94 \ 10	*0.001	4.84 \ 10	*<0.001
TPM	5.38 \ 9	*0.018	5.23 \ 9	*0.017	5.19 \ 8	0.067	5.33 \ 8	0.080	5.56 \ 7	0.088	5.35 \ 7	0.234
SMM	5.45 \ 8	0.091	5.40 \ 8	0.087	5.20 \ 7	0.205	5.41 \ 8	0.088	5.61 \ 6	0.528	5.41 \ 7	0.246
Wilcoxon												
IAM		*0.003		*<0.001		*<0.001		*<0.001		* <0.001		*<0.001
TPM		*0.042		*0.005		*0.003		*0.007		0.065		*0.016
SMM		0.053		*0.009		*0.012		0.053		0.080		0.065
Dist. Shape		L		L		Shifted		L		Shifted		Shifted

Table 2.8 Effective population size (N_e) for 13 populations of Roosevelt elk (*C. c. roosevelti*) in British Columbia, linkage disequilibrium model for a single time-point with two minimum allele frequency cut-off values (0.05, 0.01) with 95% confidence intervals, with *N* values calculated from census estimates (Wilson 2015, Reynolds et al. 2018).

Pop'n Name	Sample Size	Crit. Value	Weighted Mean	N_e	CI (95%) Min	Max	N_e:N Values	N_e:N Ratio
VIS	41	0.05	40.9	58.5	18.9	Infinite	58.5:1400	0.04
		0.01		44.4	17.0	1302.3		
VIN	55	0.05	53.7	59.7	26.4	389.6	68.9:4870	0.01
		0.01		68.9	28.7	1187.2		
SP	48	0.05	46.9	52.9	21.2	935.7	55.4:300	0.18
		0.01		55.4	22.7	887.5		
CC	35	0.05	34.5	11.9	5.3	24.8	18.8:356	0.05
		0.01		18.8	8.8	49.3		
RG	53	0.05	47.6	143.0	34.4	Infinite	40.2:101	0.40
		0.01		229.7	40.2	Infinite		
SQ	16	0.05	15.5	161.5	12.2	Infinite	51.7:170	0.30
		0.01		51.7	9.5	Infinite		
PITT	30	0.05	28.1	7.0	2.9	13.8	9:79	0.11
		0.01		9.0	3.6	18.4		
STV	15	0.05	14.6	10.9	2.9	90.9	12:120	0.10
		0.01		12.0	3.1	164.7		
BRIT	10	0.05	10	24.1	2.7	Infinite	24.1:130	0.18
		0.01		24.1	2.7	Infinite		
PRS	17	0.05	16	22.4	5.2	Infinite	35.7:120	0.30
		0.01		35.7	7.0	Infinite		
PRN	16	0.05	15.5	10.7	2.7	178.3	11.4:123	0.09
		0.01		11.4	2.7	368		
TOBA	11	0.05	9.6	131	3.6	Infinite	-	-
		0.01		99.2	3.3	Infinite		
HO	8	0.05	8	18.6	1.9	Infinite	18.6:130	0.14
		0.01		18.6	1.9	Infinite		

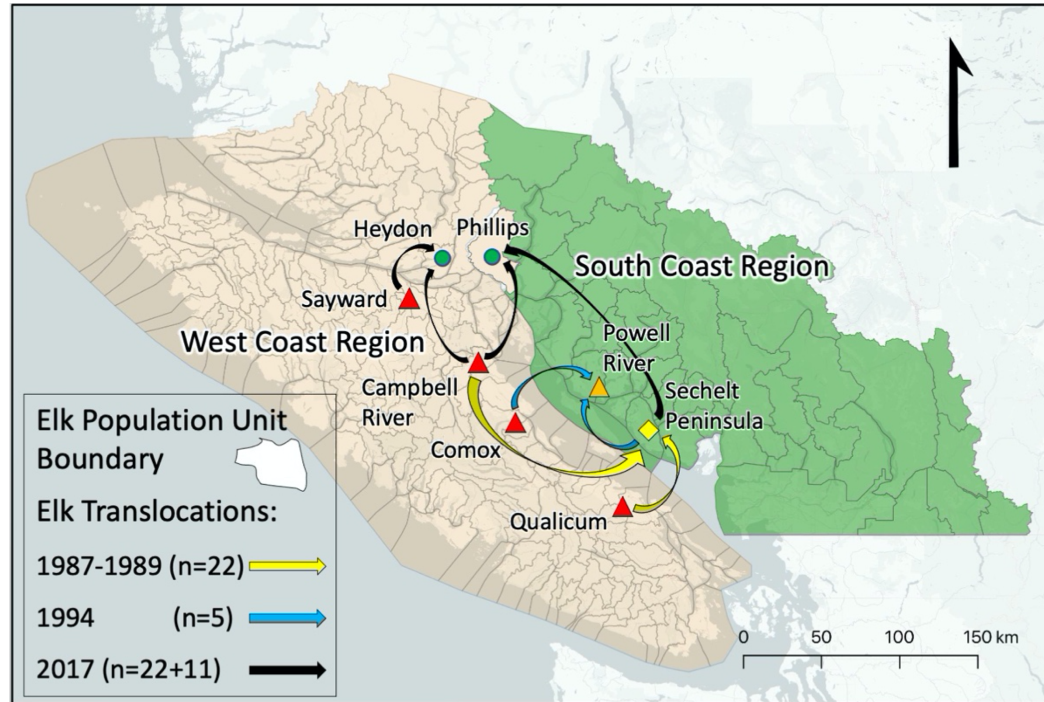


Figure 2.1: Study area and translocations of Roosevelt elk (*Cervus canadensis roosevelti*) from Vancouver Island to the mainland of British Columbia between 1986 and 2017; red triangles = primary source populations, yellow diamond = Sechelt Peninsula secondary source population, orange triangle = Powell River (Haslam) tertiary source population; translocations to Phillips and Heydon EPUs (green circles) occurred in 2017.

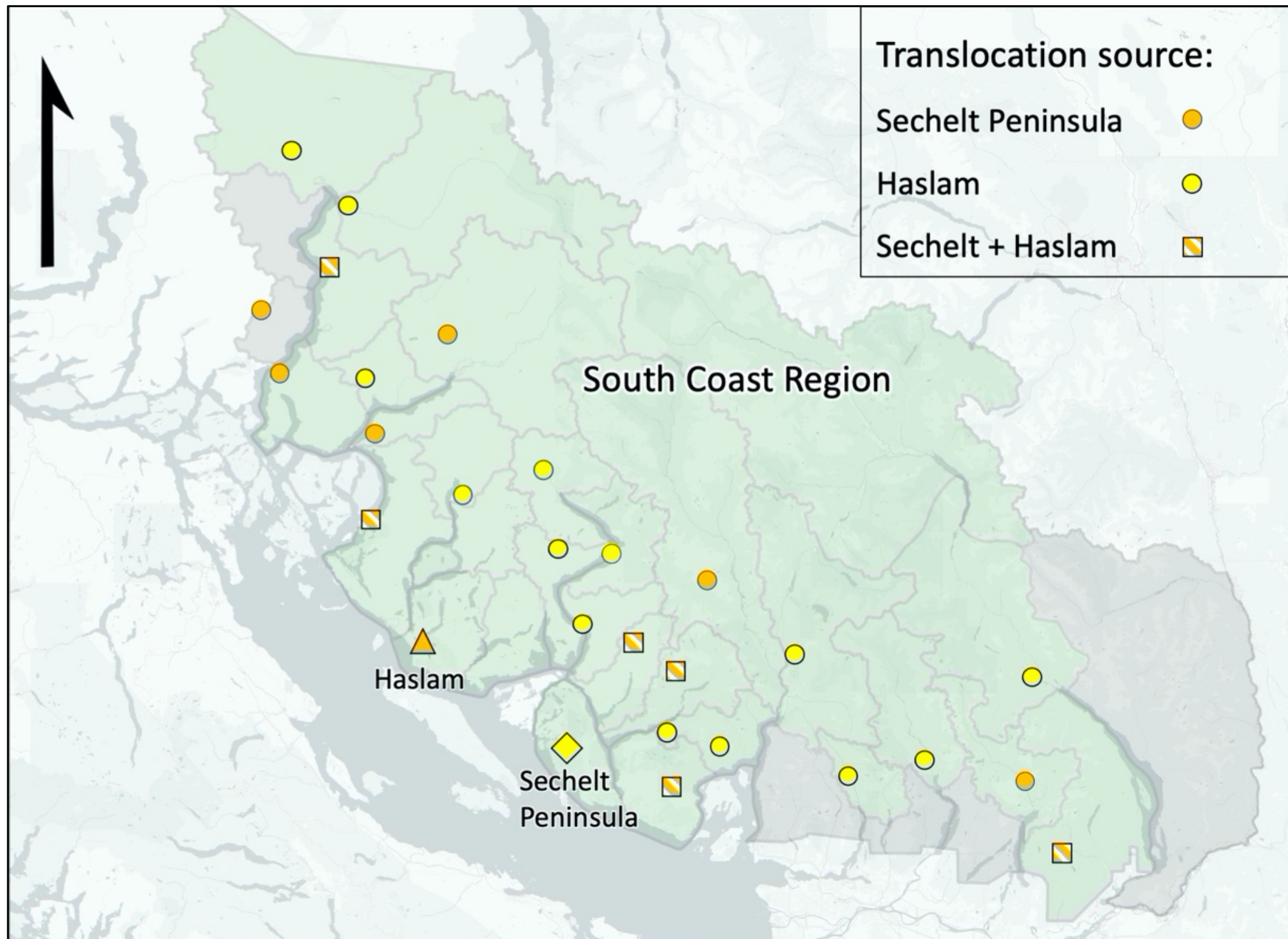


Figure 2.2: Translocations of Roosevelt elk (*C. c. roosevelti*) in the South Coast Region of British Columbia from 2001 – 2017.

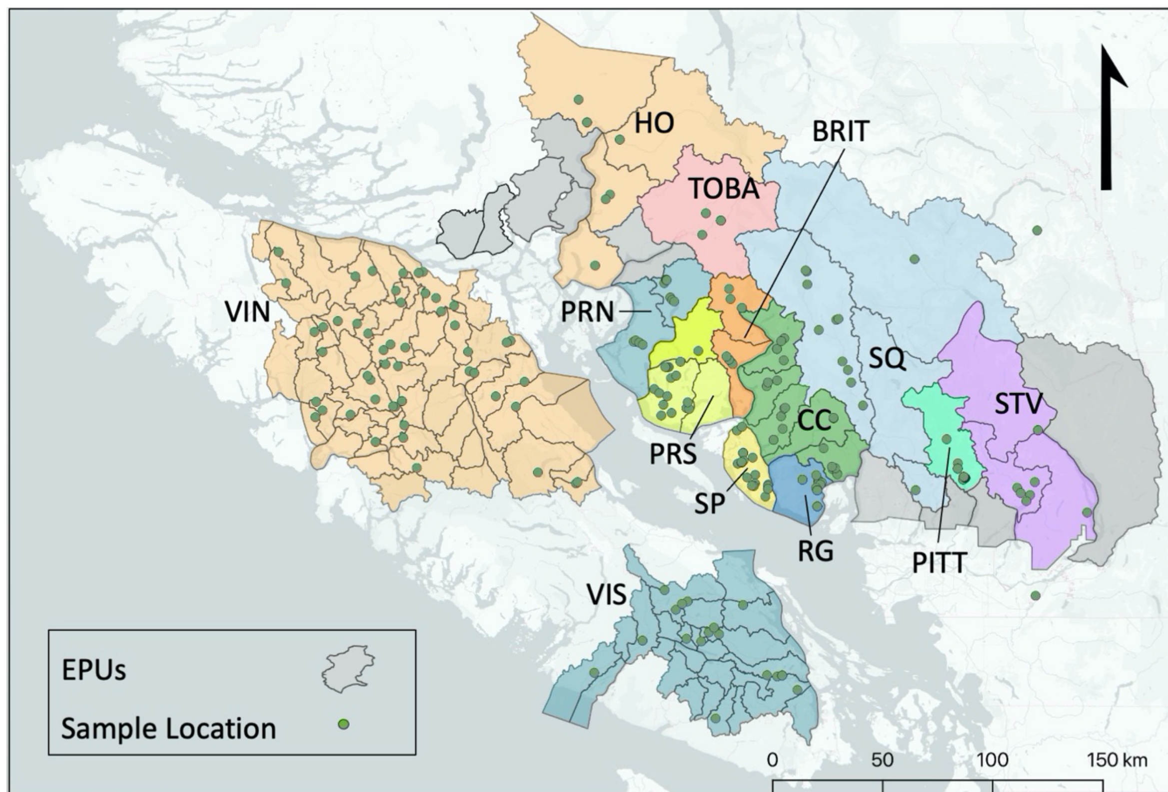
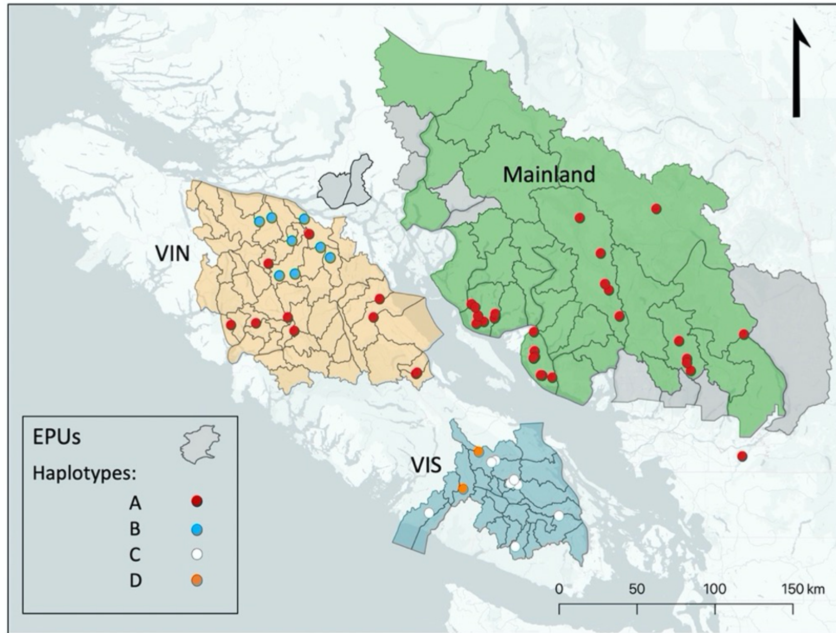
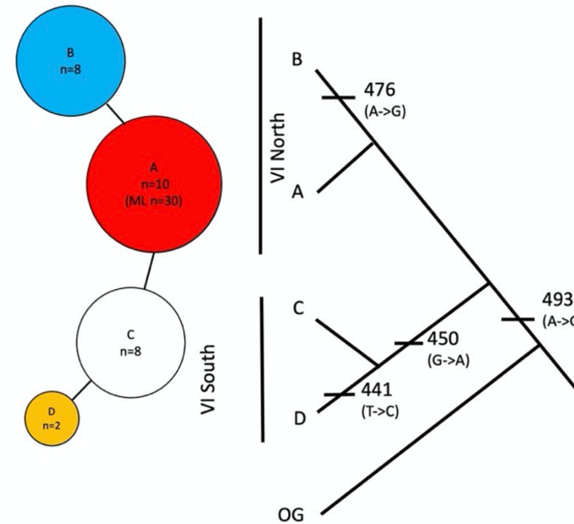


Figure 2.3: Elk population groupings used for analyses of Roosevelt elk (*C. c. roosevelti*) genetic analyses, and locations of samples (n = 355) collected between 2012 and 2019 in British Columbia.



A



B

Fig. 2.4: A) Observed haplotypes and associated sampling locations for Roosevelt elk (*C. c. roosevelti*) in British Columbia, based on 566 bp mitochondrial D-loop sequences (n = 58). B) Phylogenetic tree and haplotype network for D-loop sequences observed in Roosevelt elk (*C. c. roosevelti*) in British Columbia; consensus sequence for *C. c. nelsoni* (Polziehn et al., 1998) used as the outgroup; numbers are transition sites with nucleotide transition in brackets, letters on branch tips are observed haplotypes.

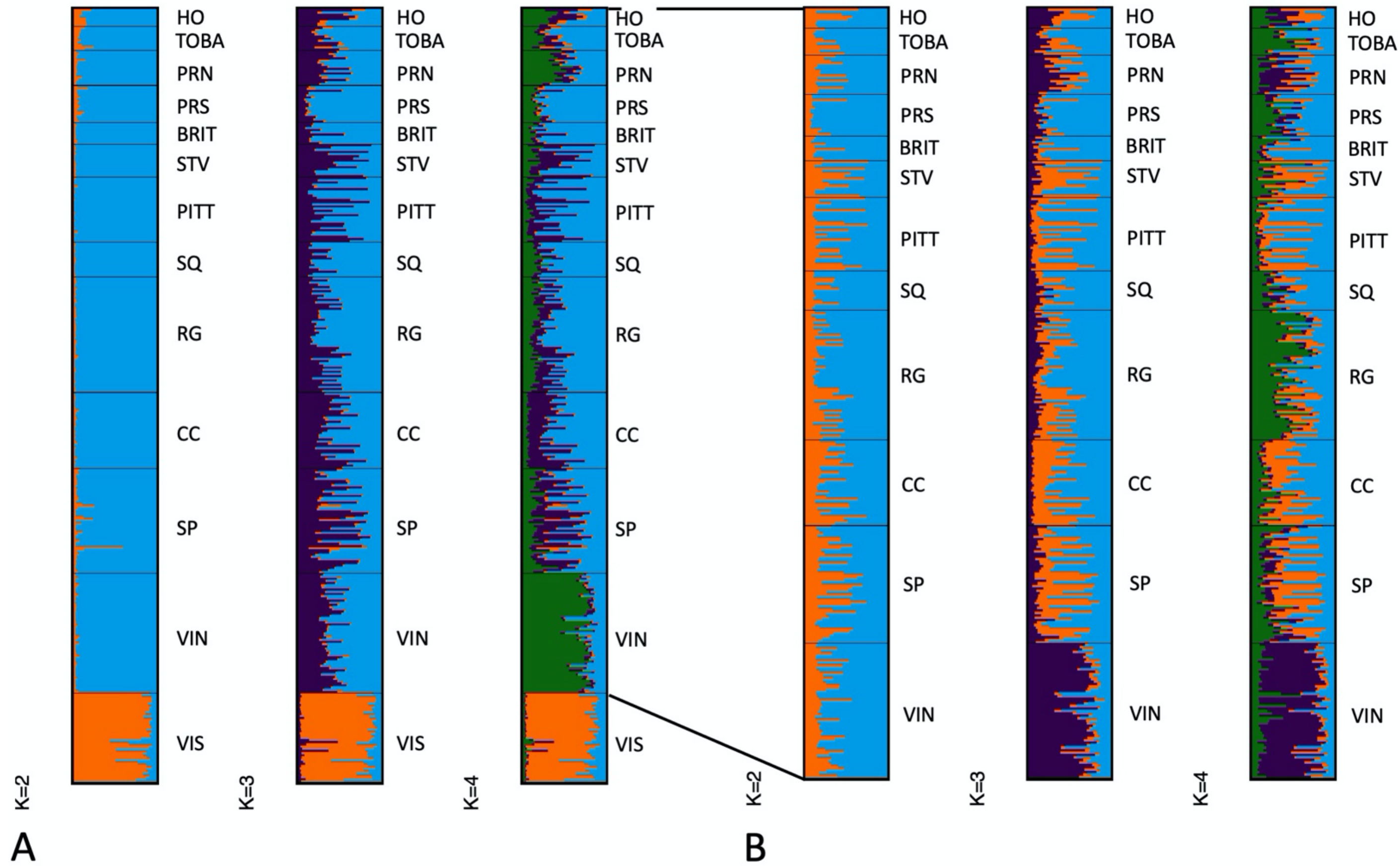


Figure 2.5 STRUCTURE ancestry plots for 355 Roosevelt elk (*C. c. roosevelti*) in British Columbia: A) for 13 populations ΔK = 2 and Prob(K) = 4; B) for 12 populations ΔK = 3 and Prob(K) = 3. STRUCTURE settings = 50,000 burn in and 200,000 MCMC steps, with *admixture* and *locprior* model, infer *a*, independent values for *a*, initial *a* = 0.25; plots and Best K values calculated in CLUMPAK (Kopelman et al., 2015).

CHAPTER 3: GENERAL DISCUSSION

The reintroduction of species into the landscapes they have been extirpated from is a challenging undertaking. The science behind reintroductions, and the augmentation of extirpated and / or endangered populations, has shifted towards understanding the underlying factors that influence the establishment and long term success of those species, including the important role of genetics (Franklin 1980, Frankham 1995, Ralls et al. 2018). It is to this body of knowledge I hope this study makes a contribution.

For many at risk species former habitats have become unsuitable (Fritts et al. 1997), while for others the underlying cause of their extirpation, such as direct exploitation or isolation from conspecifics, is still a concern (Wolf et al. 1996). In a review of 180 reintroduction and translocation projects, Fischer & Lindenmayer (2000) identified three main aims of successful reintroduction and translocation projects: to address human – wildlife conflicts, to restock game species, and to conserve species. The reintroduction of Roosevelt elk (*Cervus canadensis roosevelti*) from Vancouver Island to their former habitat on the mainland was initiated to address conservation concerns; low species diversity, struggling populations and ongoing illegal hunting. When the subspecies population grew rapidly and began to find conflict with the area's human residents, a long term reintroduction and translocation project was initiated to mediate conflict, create sustainable harvest opportunities for resident and indigenous hunters, solidify the return of Roosevelt elk, and restore ecosystem function to the coastal mainland of BC (Reynolds et al. 2018). However, despite a ~2.5x increase in the provincial population since 2001, and range expansion of over 30,000 km^2 (Wilson 2015,

Reynolds et al. 2018), there remains concern about the future of this economically and culturally important, charismatic animal.

3.1 Management Implications

Genetic evaluation and monitoring of reintroduced populations of Roosevelt elk have been identified as areas of need, as *Recommendation 6* in Reynolds et al. (2018). Our assessment has identified numerous reintroduced populations with reduced diversity compared to the core population on northern Vancouver Island. Mitochondrial analyses showed that mainland populations have half the diversity of northern Vancouver Island elk, and only one quarter the diversity of Roosevelt elk across the island metapopulation, with a complete division of haplotypes north and south of the Alberni Inlet (Table 2.2, Figure 2.4a). This finding, on its own, should be enough to initiate a review of past management strategies and careful consideration of potential mitigations.

Further analyses with multi-locus nuclear genotypes identified more cause for concern; as seen with mtDNA, microsatellite genotypes revealed Roosevelt elk populations are highly structured between the northern and southern areas of Vancouver Island (Figure 2.5a), with low effective population sizes (Table 2.8). The southern island population exhibits less diversity, loss of low frequency alleles, and substantial shifts in allele frequencies compared to northern Vancouver Island (Appendix VI). The mainland population is genetically isolated from Vancouver Island populations, and without mitigation, this is likely to continue. Loss of alleles and changes in allele frequencies among the mainland populations is likely the combined result of pre-existing low diversity, polygynous breeding system, and translocation of limited numbers of founding individuals (Nunney 1993, Polziehn et al. 2000).

Currently the threshold for a Roosevelt elk population unit to be considered 'recovered' is a population of >50 animals, with a bull to cow ratio 20:100 or greater, with 30% of bulls branch antlered (Wilson 2015), which is usually seen in two year-old and older bulls. In practice, most populations have a more even bull:cow ratio by the time the population has reached 50. Once designated as recovered, hunting opportunities are allocated first to indigenous communities, and then through a controlled limited draw system for resident hunters (Wilson 2015). The low effective population (N_e) values observed in many sampled populations, while probably downward biased somewhat due to sampling multiple years and potentially multiple generations (Waples et al. 2014), suggest that the census threshold for recovered status may currently be too low. However, the observed N_e estimates for Vancouver Island are also very low, equating to N_e:N ratios of approximately 0.04 and 0.01 for VIS and VIN, respectively (Table 2.8). These values are extremely concerning and require further evaluation.

The observed overall low genetic diversity, low N_e, reduced allelic diversity, rapid differentiation due to drift, and increased levels of inbreeding, serve to underline the status of Roosevelt elk as a "species of special concern". Individually these signals are problematic, in combination they require a strategy to mitigate against further genetic degradation.

3.2 Future Directions

The reintroduction of Roosevelt elk to the British Columbia mainland should be heralded as a success in the restoration of species at risk and ecosystem function. The increased population size and range expansion represent significant steps towards securing the future of this species. The identification of populations of concern should be

used as an opportunity to reinvigorate continued management of Roosevelt elk, and conservation of their critical habitats. Research is currently underway to identify ecological drivers of important habitats for elk survival. This critical work is being done to assist in identification and designation of ungulate winter range on Vancouver Island, and aid in forestry management planning (A. Ford, University of British Columbia, personal communication 2021).

An important element in the reintroduction strategy for Roosevelt elk has been an assumption of gene-flow among mainland populations. Long-distance dispersal along the coast by male elk, most likely from the Haslam herd, was anecdotally supported in the late 1990s and early 2000s. Likewise, a single female translocated into the Chehalis EPU travelled over 120 km, including a 3.2 km swim across a lake, before being killed in a motor vehicle collision (D. Reynolds, FLNRO, personal communication 2021). These events support the assumption that gene-flow among many mainland groups is possible, if at low rates. Low rates of dispersal between populations can mitigate drift, preventing or reducing differentiation between populations (Wright 1951, Balloux and Lugon-Moulin 2002, Allendorf and Luikart 2007). It is therefore important to understand the landscape level connectivity in the South Coast Region.

While current genetic diversity and close relatedness of mainland populations is not likely conducive to identification of migrants, it may be possible to use genetic studies of other species in the region to predict important multi-species corridors for dispersal (Cushman and Landguth 2012, Brennan et al. 2020). Currently unpublished genetic connectivity analyses have been previously been conducted in the South Coast Region for wolverine (*Gulo gulo*) and grizzly bears (C. Neivelt and S. Rochetta, FLNRO

personal communication 2019). Additional species that may be suitable as proxies for modelling population connectivity could include black bear, black-tailed deer, grey wolf, or even ruffed-grouse (*Bonasa umbellus*).

The provincial government has set a goal of removing Roosevelt elk from the Blue List of species at risk by 2025 (Wilson 2015, Reynolds et al. 2018) through the following objectives:

> "1. Maintain self-sustaining populations of Roosevelt elk throughout their current range in the West Coast and South Coast Regions,
>
> 2. Re-establish Roosevelt elk in their historic range where ecological conditions are suitable,
>
> 3. Maintain or restore the contribution of Roosevelt elk to natural biodiversity and ecosystem function."

To this list I suggest adding a fourth objective in support of the first: to maintain and / or improve genetic diversity in Roosevelt elk within individual population units, and for the subspecies as a whole. The maintenance of genetic diversity in reintroduced populations has been identified as a critical element in their long term survival and adaption to environmental change (IUCN/SSC 2013, Frankham et al. 2014). As such, it is recommended that a mitigation strategy be developed to improve the genetic diversity of Roosevelt elk within mainland populations, through the use of translocation of individuals from populations of high or different diversity (Hogg et al. 2006, Frankham 2015, Poirier et al. 2019). Occasional translocations of elk from herds in conflict with residents on Vancouver Island could be partially or wholly diverted to reinforce genetic diversity among mainland populations. Of considerable genetic value would be individuals with mitochondrial haplotypes not found on the mainland, such as those that

occur on northern Vancouver Island in the vicinity of the Adam, Eve, Tsitika, Nimpkish, White and Salmon Rivers, near Sayward. Risks associated with further translocations between Vancouver Island and the mainland, such as outbreeding or disease, are far outweighed by the potential benefits for the subspecies (Frankham 2015).

3.3 Conclusions

A growing number of studies on the genetics of reintroduced polygynous ungulates have shown potential negative consequences due to drift, isolation and subsequent inbreeding depression (Stephen et al. 2005, Olson et al. 2013, Bérénos et al. 2016, Sattler et al. 2017). The results of this study add to the current body of knowledge around genetic changes associated with species reintroductions; identifying the potential for bottlenecks, founder effects, isolation, and source population characteristics to contribute to increased drift and population differentiation. Populations of at-risk species benefit from our understanding of the fundamental biodiversity that population genetic studies provide. The continued persistence and successful expansion of many species depends on maintaining their capacity to adapt to changing environments, and without knowing which species or populations are vulnerable, it is impossible to know where wildlife managers should prioritize their resources. This author proposes that Roosevelt elk should be considered high priority for additional management, through ongoing translocations from Vancouver Island to the mainland, to prevent continued loss of genetic diversity.

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Appendix I: Translocation history of Roosevelt elk (*Cervus canadensis roosevelti*) for mainland reintroductions between 1987-2017 (Reynolds et al. 2018); Sechelt Peninsula served as the main translocation source from 2000-2017 (72.3% of individuals), followed by Haslam (near Powell River, 19.3%), Vancouver Island (2017 translocations into Heydon and Phillips EPUs, 5.3%) and Rainy-Gray EPU (3.1%); * this value is reported in Spalding (1992) and Quayle and Brunt (2003), however, it is later reported as 11 in Wilson (2015) and Reynolds et al. (2018).

Year	Source Location	Release EPU	Released (n)
1987	Vancouver Island (Campbell R)	Sechelt Peninsula	7
1988	Vancouver Island (Campbell R)	Sechelt Peninsula	6
1989	Vancouver Island (Qualicum)	Sechelt Peninsula	*9
1994	Vancouver Island (Fanny Bay)	Haslam	5
1996	Sechelt Peninsula	Haslam	9
1996	Sechelt Peninsula	Haslam	5
1996	Sechelt Peninsula	Haslam	6
2000	Sechelt Peninsula	McNab	25
2001	Sechelt Peninsula	Skwawka	12
2001	Sechelt Peninsula	Rainy-Gray	6
2002	Haslam	Narrows	7
2002	Sechelt Peninsula	McNab	1
2002	Sechelt Peninsula	Skwawka	7
2002	Sechelt Peninsula	Narrows	4
2003	Haslam	Narrows	9
2003	Haslam	Rainy-Gray	3
2003	Sechelt Peninsula	Clowhom	5
2003	Sechelt Peninsula	Narrows	2
2003	Sechelt Peninsula	Rainy-Gray	2
2004	Haslam	Clowhom	7
2004	Sechelt Peninsula	Clowhom	8
2004	Sechelt Peninsula	Rainy-Gray	2
2004	Sechelt Peninsula	Deserted	13
2005	Sechelt Peninsula	Brittain	20
2005	Sechelt Peninsula	Deserted	7
2005	Sechelt Peninsula	Pitt	23
2006	Haslam	McNab	8
2006	Haslam	Vancouver	11
2006	Sechelt Peninsula	Indian	20
2006	Sechelt Peninsula	Vancouver	10
2007	Haslam	Squamish	26
2007	Haslam	Quatum	12
2007	Sechelt Peninsula	Stave	19
2008	Haslam	Quatum	6
2008	Sechelt Peninsula	Powell-Daniels	17
2008	Sechelt Peninsula	Quatum	1
2008	Sechelt Peninsula	Stave	1
2008	Sechelt Peninsula	Theo	3

2009	Haslam	Homathko	2
2009	Haslam	Orford	19
2009	Sechelt Peninsula	Brem	14
2009	Sechelt Peninsula	Homathko	18
2009	Sechelt Peninsula	Orford	1
2009	Sechelt Peninsula	Toba	10
2010	Haslam	Theo	3
2010	Sechelt Peninsula	Powell-Daniels	7
2010	Sechelt Peninsula	Toba	10
2011	Sechelt Peninsula	Mamquam	8
2011	Sechelt Peninsula	Rainy-Gray	5
2011	Sechelt Peninsula	Southgate	20
2011	Sechelt Peninsula	Theo	13
2012	Sechelt Peninsula	Brem	10
2012	Sechelt Peninsula	Rainy-Gray	8
2013	Sechelt Peninsula	Chehalis	5
2013	Sechelt Peninsula	Lower Lillooet	14
2014	Sechelt Peninsula	Chehalis	24
2015	Sechelt Peninsula	Chehalis	5
2015	Sechelt Peninsula	Lower Lillooet	14
2015	Sechelt Peninsula	Chehalis	10
2016	Rainy-Gray	Chehalis	12
2016	Rainy-Gray	Sechelt Peninsula	4
2016	Sechelt Peninsula	Eldred	14
2017	Sechelt Peninsula	Phillips	11
2017	Vancouver Island (Campbell R)	Phillips	11
2017	Vancouver Island (Lower Salmon)	Heydon	18
2017	Vancouver Island (Campbell R)	Heydon	4

Appendix II: Summary of 356† samples from Roosevelt elk (*C. c. roosevelti*) in British Columbia; BC WID = wildlife identification number for compulsory inspection of resident, alien and indigenous hunter kills (6 digits) or wildlife health identification number for samples from elk captured for global positioning system collar deployment (7 digits), or Sample (ID) for faecal samples (3 digits); one sample (SP465) was successfully sequenced, but failed to amplify at 7+ microsatellite loci and was excluded from further analyses.

Sample ID	BC WID / WHID / ID	EPU	Group	Latitude dd	Longitude dd	Sample Type	Haplotype
VIS120	127569	103A	VIS	48.593907	-124.124541	T	C
VIS410	140604	103A	VIS			T	
VIS414	144174	103A	VIS			T	
VIS421	137991	103A	VIS			T	
VIS422	141443	103A	VIS			T	
VIS427	144182	103A	VIS			T	
VIS420	144159	103B	VIS			T	C
VIS429	144184	103B	VIS			T	
VIS121	127554	104A	VIS	48.763174	-123.748715	T	C
VIS122	127587	104A	VIS	48.709152	-123.624961	T	
VIS123	131907	104A	VIS	48.767542	-123.718139	T	
VIS124	135905	104A	VIS	48.767544	-123.718140	T	
VIS125	143882	104A	VIS	48.767546	-123.718141	T	
VIS126	142430	104A	VIS	48.768191	-123.811036	T	
VIS127	149892	104A	VIS	48.767548	-123.718143	T	
VIS413	147259	104A	VIS			T	
VIS415	144172	104A	VIS			T	
VIS416	144230	104A	VIS			T	
VIS419	144162	104A	VIS			T	
VIS451	144101	104A	VIS			T	
VIS452	142465	104A	VIS			T	
VIS456	142449	104A	VIS			T	
VIS128	127411	104B	VIS	49.049792	-123.956777	T	
VIS129	143915	104B	VIS	48.904039	-124.212684	T	
VIS130	143919	104B	VIS	48.914796	-124.302660	T	
VIS409	144238	104B	VIS			T	
VIS423	142471	104B	VIS			T	
VIS176	137396	104C	VIS	48.907486	-124.572274	T	D
VIS177	139516	104C	VIS	48.907475	-124.572284	T	
VIS426	144207	104C	VIS			T	
VIS428	144183	104C	VIS			T	
VIS449	144161	104C	VIS			T	
VIS181	127575	105C	VIS	48.933008	-124.103835	T	C
VIS182	142439	105C	VIS	48.940110	-124.166757	T	
VIS412	144235	105C	VIS			T	C
VIS417	144171	105C	VIS			T	C
VIS180	127585	105B	VIS	49.064286	-124.296341	T	C
VIS455	144144	105B	VIS			T	C
VIS178	127584	105A	VIS	49.031167	-124.367789	T	
VIS179	140843	105A	VIS	49.109949	-124.437192	T	D
VIS457	144143	105A	VIS			T	
VIC186	140979	106B	VIN	49.535297	-124.980065	T	A
VIC458	144204	106B	VIN			T	A

VIC187	121597	106D	VIN	49.579056	-125.212720	T	
VIC453	144121	106D	VIN			T	
VIC183	140969	106A	VIN	49.879228	-125.470202	T	
VIC184	140974	106A	VIN	49.936597	-125.294321	T	
VIC185	143880	106A	VIN	49.838666	-125.347393	T	A
VIC408	144222	106A	VIN			T	A
VIC454	144092	106A	VIN			T	
VIN213	121588	110F	VIN	50.054299	-125.639279	T	
VIN214	121594	110F	VIN	49.978404	-125.630855	T	
VIN215	137389	110F	VIN	49.971325	-125.601293	T	
VIN211	133683	110E	VIN	50.097263	-125.379139	T	
VIN212	137056	110E	VIN	50.090736	-125.403515	T	
VIN206	121582	110C	VIN	50.234533	-125.724426	T	
VIN207	127413	110C	VIN	50.157658	-125.719591	T	B
VIN208	121581	110D	VIN	50.264731	-125.835128	T	
VIN209	127414	110D	VIN	50.212338	-125.802853	T	B
VIN210	127421	110D	VIN	50.282934	-125.899883	T	A
VIN218	127573	110H	VIN	50.367497	-125.914568	T	
VIN219	140810	110H	VIN	50.363295	-125.941925	T	B
VIN203	121589	110B	VIN	50.060980	-126.157265	T	B
VIN204	121592	110B	VIN	50.083334	-126.114549	T	
VIN205	140850	110B	VIN	50.070755	-126.022860	T	B
VIN200	121587	110A	VIN	50.362152	-126.035592	T	
VIN201	121591	110A	VIN	50.293592	-126.080989	T	
VIN202	121600	110A	VIN	50.247060	-126.049118	T	B
VIN216	127417	110G	VIN	50.370232	-126.223748	T	B
VIN217	127571	110G	VIN	50.349684	-126.329504	T	B
VIN220	127406	111A	VIN	50.322964	-126.753457	T	
VIN221	137381	111A	VIN	50.445098	-126.798917	T	
VIN222	121580	111B	VIN	50.174741	-126.437482	T	
VIN223	127403	111B	VIN	50.150557	-126.526540	T	
VIN224	127407	111B	VIN	50.132278	-126.580187	T	
VIN225	127408	111B	VIN	50.164133	-126.319784	T	
VIN226	127574	111B	VIN	50.053837	-126.530734	T	
VIN227	127599	111B	VIN	50.125509	-126.251962	T	A
VIN228	132025	111B	VIN	50.174750	-126.437490	T	
VIN229	140973	112C	VIN	49.807220	-126.359405	T	A
VIN230	121590	112D	VIN	49.824137	-126.531014	T	
VIN231	127578	112D	VIN	49.857999	-126.569837	T	
VIN232	127596	112D	VIN	49.797168	-126.575105	T	A
VIN233	127422	112E	VIN	49.699694	-126.205696	T	
VIC196	121578	109D	VIN	49.943950	-126.236673	T	
VIC197	127409	109D	VIN	49.960230	-126.255307	T	
VIC198	127416	109D	VIN	49.867130	-126.206848	T	
VIC199	140799	109D	VIN	49.765476	-126.028782	T	A
VIC188	121579	109A	VIN	49.838596	-126.084910	T	A
VIC189	131928	109A	VIN	50.005403	-126.145032	T	
VIC190	133682	109A	VIN	49.997462	-126.068508	T	
VIC191	140849	109A	VIN	49.840044	-126.098668	T	
VIC192	121598	109B	VIN	49.597055	-125.953028	T	
VIC193	127420	109B	VIN	49.715655	-126.035082	T	
VIC194	127410	109C	VIN	49.860887	-126.043541	T	
VIC195	127418	109C	VIN	49.860950	-126.043543	T	
SP031	031	205A	SP	49.656194	-123.957273	F	
SP032	032	205A	SP	49.656195	-123.957274	F	
SP033	033	205A	SP	49.656196	-123.957275	F	

SP034	034	205A	SP	49.656197	-123.957276	F	
SP035	035	205A	SP	49.656198	-123.957277	F	
SP036	036	205A	SP	49.656199	-123.957278	F	
SP037	037	205A	SP	49.656200	-123.957279	F	
SP038	038	205A	SP	49.656201	-123.957280	F	
SP039	039	205A	SP	49.656202	-123.957281	F	
SP040	040	205A	SP	49.656203	-123.957282	F	
SP041	041	205A	SP	49.656204	-123.957283	F	
SP042	042	205A	SP	49.656205	-123.957284	F	
SP043	120689	205A	SP	49.569727	-123.883227	T	
SP044	120680	205A	SP	49.559306	-123.932889	T	
SP047	120688	205A	SP	49.535257	-123.884633	T	
SP048	120679	205A	SP	49.656206	-123.957285	T	
SP050	120691	205A	SP	49.656207	-123.957286	T	
SP054	120690	205A	SP	49.656208	-123.957287	T	
SP057	120701	205A	SP	49.744814	-123.994676	T	
SP058	120702	205A	SP	49.656209	-123.957288	T	
SP061	120706	205A	SP	49.525291	-123.892384	T	
SP062	120707	205A	SP	49.525350	-123.892401	T	
SP063	120708	205A	SP	49.613849	-123.989777	T	
SP065	120710	205A	SP	49.656210	-123.957289	T	
SP066	120711	205A	SP	49.656211	-123.957290	T	
SP067	120712	205A	SP	49.656213	-123.957292	T	
SP068	120713	205A	SP	49.620565	-123.963517	T	
SP069	120714	205A	SP	49.530660	-123.803154	T	
SP070	120715	205A	SP	49.530665	-123.803180	T	
SP074	135953	205A	SP	49.483573	-123.820467	T	
SP075	135955	205A	SP	49.618141	-123.966165	T	A
SP076	135956	205A	SP	49.656212	-123.957291	T	A
SP079	135960	205A	SP	49.618143	-123.966167	T	
SP080	135961	205A	SP	49.744820	-123.994686	T	
SP081	135962	205A	SP	49.618145	-123.966169	T	
SP082	135963	205A	SP	49.626565	-123.953517	T	A
SP085	135966	205A	SP	49.525295	-123.892400	T	A
SP090	135971	205A	SP	49.618149	-123.966173	T	
SP094	100851	205A	SP	49.514189	-123.805327	T	A
SP095	101622	205A	SP	49.525310	-123.892320	T	A
SP098	101625	205A	SP	49.514195	-123.805330	T	
SP099	113911	205A	SP	49.525313	-123.892323	T	A
SP111	120428	205A	SP	49.618147	-123.966171	T	A
SP249	135945	205A	SP	49.933975	-123.296253	T	
SP281	135994	205A	SP	49.620785	-123.966293	T	A
SP283	135996	205A	SP	49.526821	-123.903543	T	A
SP288	143754	205A	SP	49.637941	-123.898290	T	
SP290	1813835	205A	SP	49.759472	-123.963353	B	
SP465[†]	140610	205A	SP			T	A
JIE045	120686	205E	CC	49.793618	-123.404113	T	
JIE071	120720	205E	CC	49.793625	-123.404121	T	
JIE100	113913	205E	CC	49.793618	-123.404113	T	
JIE108	113925	205E	CC	49.793610	-123.404111	T	
JIE287	143752	205E	CC	49.795274	-123.404427	T	
JIE010	1811031	205D	CC	49.801059	-123.724938	B	
JIE078	135959	205D	CC	49.708212	-123.771019	T	
JIE097	101624	205D	CC	49.831717	-123.705270	T	
JIE102	113915	205D	CC	49.708220	-123.771010	T	
JIE103	113917	205D	CC	49.708225	-123.771015	T	

JIE234	135993	205D	CC	49.752015	-123.714669	T
JIE279	1813509	205D	CC	49.834638	-123.700405	B
JIE002	1811023	205H	CC	49.919399	-123.813037	B
JIE003	1811024	205H	CC	49.942349	-123.749369	B
JIE060	120705	205H	CC	49.934669	-123.805042	T
JIE092	135973	205H	CC	49.525289	-123.805000	T
JIE433	147706	205H	CC			T
JIE072	120721	205F	CC	49.674948	-123.465045	T
JIE091	135972	205F	CC	49.592937	-123.410079	T
JIE093	000093 no WID recorded	205F	CC	49.573578	-123.381571	T
JIE112	120429	205F	CC	49.573573	-123.381575	T
JIE114	120431	205F	CC	49.573583	-123.381577	T
JIE115	120432	205F	CC	49.573593	-123.381579	T
JIE116	120433	205F	CC	49.573603	-123.381561	T
JIE235	99635	205F	CC	49.572259	-123.381265	T
JIE236	135903	205F	CC	49.595792	-123.393722	T
JIE237	135937	205F	CC	49.601914	-123.413880	T
JIE432	135917	205F	CC			T
JIE096	101623	205G	CC	50.065680	-123.750360	T
JIE059	120703	205G	CC	50.065675	-123.750378	T
JIE101	113914	205G	CC	50.065670	-123.750380	T
JIE110	120427	205G	CC	50.065680	-123.750372	T
JIE238	137390	205G	CC	50.107232	-123.705816	T
JIE280	1813510	205G	CC	50.096438	-123.723998	B
JIE284	135997	205G	CC	50.021158	-123.708499	T
RG011	011	205I	RG	49.549977	-123.594104	F
RG012	012	205I	RG	49.549978	-123.594105	F
RG013	013	205I	RG	49.549979	-123.594106	F
RG014	014	205I	RG	49.549980	-123.594107	F
RG015	015	205I	RG	49.549981	-123.594108	F
RG016	016	205I	RG	49.549982	-123.594109	F
RG017	017	205I	RG	49.549983	-123.594110	F
RG018	018	205I	RG	49.549984	-123.594111	F
RG020	020	205I	RG	49.533129	-123.498200	F
RG021	021	205I	RG	49.533130	-123.498201	F
RG022	022	205I	RG	49.533131	-123.498202	F
RG023	023	205I	RG	49.533132	-123.498203	F
RG024	024	205I	RG	49.533133	-123.498204	F
RG025	025	205I	RG	49.533134	-123.498205	F
RG026	026	205I	RG	49.548348	-123.496457	F
RG027	027	205I	RG	49.548349	-123.496458	F
RG028	028	205I	RG	49.548350	-123.496459	F
RG029	029	205I	RG	49.548351	-123.496460	F
RG030	030	205I	RG	49.548352	-123.496461	F
RG077	135957	205I	RG	49.548355	-123.496451	T
RG084	135965	205I	RG	49.548360	-123.496465	T
RG107	113924	205I	RG	49.533150	-123.498195	T
RG117	117	205I	RG	49.511031	-123.503915	F
RG118	118	205I	RG	49.511032	-123.503916	F
RG119	119	205I	RG	49.511033	-123.503917	F
RG239	121593	205I	RG	49.567830	-123.510077	T
RG240	135944	205I	RG	49.538420	-123.475611	T
RG241	136418	205I	RG	49.521037	-123.499105	T
RG242	136451	205I	RG	49.531113	-123.485156	T
RG278	1813508	205I	RG	49.511076	-123.504265	B

RG286	146928	205I	RG	49.518362	-123.511083	T	
RG303	163	205I	RG	49.443980	-123.503430	F	
RG304	164	205I	RG	49.443990	-123.503440	F	
RG305	165	205I	RG	49.444000	-123.503450	F	
RG320	163	205I	RG	49.443985	-123.503451	F	
RG321	164	205I	RG	49.444010	-123.503460	F	
RG322	165	205I	RG	49.444015	-123.503462	F	
RG323	166	205I	RG	49.444020	-123.503464	F	
RG324	167	205I	RG	49.444025	-123.503466	F	
RG325	168	205I	RG	49.444030	-123.503468	F	
RG326	169	205I	RG	49.444035	-123.503470	F	
RG327	170	205I	RG	49.444040	-123.503472	F	
RG328	171	205I	RG	49.444045	-123.503474	F	
RG329	172	205I	RG	49.444050	-123.503476	F	
RG330	173	205I	RG	49.508335	-123.504050	F	
RG331	174	205I	RG	49.508336	-123.504052	F	
RG332	175	205I	RG	49.508337	-123.504054	F	
RG333	333	205I	RG	49.508338	-123.504056	F	
RG334	334	205I	RG	49.508339	-123.504058	F	
RG335	335	205I	RG	49.508340	-123.504060	F	
RG336	336	205I	RG	49.508341	-123.504062	F	
RG337	337	205I	RG	49.508342	-123.504064	F	
RG338	338	205I	RG	49.508343	-123.504066	F	
SQ001	1811022	206A	SQ	50.016654	-123.350003	B	
SQ073	135951	206A	SQ	49.843409	-123.223693	T	A
SQ244	135902	206A	SQ	50.179613	-123.387268	T	
SQ245	135904	206A	SQ	50.318761	-123.564271	T	
SQ246	135936	206A	SQ	50.368372	-123.567841	T	
SQ247	135940	206A	SQ	50.373019	-123.574470	T	
SQ248	135943	206A	SQ	50.139770	-123.494396	T	
SQ250	135947	206A	SQ	49.983129	-123.316231	T	A
SQ251	135949	206A	SQ	50.183489	-123.373858	T	
SQ401	401	206A	SQ	50.529136	-122.157748	F	
SQ405	1912074	206A	SQ	50.416898	-122.907282	T	A
SQ430	146927	206A	SQ			T	A
SQ448	135907	206A	SQ			T	A
SQ460	140625	206A	SQ			T	A
SQ253	135946	208A	SQ	49.509039	-122.900124	T	
SQ466	135923	208A	SQ			T	
FVN434	141478	208B	PITT			T	A
FVN437	146983	208B	PITT			T	A
FVN438	135922	208B	PITT			T	
FVN439	135918	208B	PITT			T	
FVN440	147255	208B	PITT			T	A
FVN441	143858	208B	PITT			T	A
FVN442	143618	208B	PITT			T	
FVN443	143619	208B	PITT			T	
FVN447	143666	208B	PITT			T	A
FVN462	140609	208B	PITT			T	
FVN463	140601	208B	PITT			T	
FVN467	143743	208B	PITT			T	
FVN252	140698	208B	PITT	49.615312	-122.642281	T	
FVN254	143564	208B	PITT	49.710629	-122.711023	T	
FVN291	1813836	208B	PITT	49.550601	-122.610351	B	
FVN292	1813837	208B	PITT	49.594265	-122.643159	B	
FVN295	141	208B	PITT	49.550602	-122.610352	F	

FVN296	142	208B	PITT	49.550603	-122.610353	F	
FVN297	143	208B	PITT	49.550604	-122.610354	F	
FVN298	144	208B	PITT	49.550605	-122.610355	F	
FVN299	145	208B	PITT	49.550606	-122.610356	F	
FVN300	147	208B	PITT	49.594267	-122.643160	F	
FVN301	148	208B	PITT	49.594500	-122.643100	F	
FVN306	149	208B	PITT	49.594502	-122.643105	F	
FVN307	150	208B	PITT	49.594505	-122.643106	F	
FVN308	151	208B	PITT	49.594508	-122.643107	F	
FVN309	152	208B	PITT	49.594511	-122.643108	F	
FVN310	153	208B	PITT	49.594514	-122.643109	F	
FVN311	154	208B	PITT	49.594517	-122.643110	F	
FVN009	1811030	208C	STV	49.518087	-122.279600	B	
FVN255	140840	208C	STV	49.489570	-122.201137	T	
FVN256	143562	208C	STV	49.497676	-122.258562	T	
FVN257	143557	208C	STV	49.540640	-122.172087	T	
FVN403	1911923	208C	STV	49.087048	-122.167567	T	A
TI008	1811029	208C	STV	49.419160	-121.851985	B	
TI302	161	208C	STV	49.746017	-122.152777	F	
TI312	155	208C	STV	49.746018	-122.152770	F	
TI313	156	208C	STV	49.746020	-122.152780	F	
TI314	157	208C	STV	49.746022	-122.152790	F	
TI315	158	208C	STV	49.746024	-122.152800	F	
TI316	159	208C	STV	49.746026	-122.152810	F	A
TI317	160	208C	STV	49.746028	-122.152820	F	
TI318	161	208C	STV	49.746030	-122.152830	F	
TI319	162	208C	STV	49.746032	-122.152840	F	
FVN461	140897	208C	STV			T	
PRT004	1811025	212Q	BRIT	50.300607	-124.042384	B	
PRT243	135984	212Q	BRIT	50.226043	-123.963411	T	
PRT267	140828	212Q	BRIT	50.260762	-124.033982	T	
PRT431	146926	212Q	BRIT			T	
PRT046	120681	212E	BRIT	50.012996	-124.028309	T	
PRT264	135978	212E	BRIT	50.023873	-124.042931	T	
PRT289	1813834	212E	BRIT	50.005834	-124.020573	B	
PRT293	137	212E	BRIT	50.023885	-124.042932	F	
PRT294	138	212E	BRIT	50.023890	-124.042934	F	
PRT444	144219	212E	BRIT			T	
PRT051	120693	212S	PRS	50.057557	-124.230664	T	
PRT055	120695	212S	PRS	49.814152	-124.394257	T	A
PRT056	120700	212S	PRS	49.951556	-124.397890	T	
PRT087	135968	212S	PRS	49.994525	-124.411924	T	
PRT088	135969	212A	PRS	49.994300	-124.420000	T	
PRT089	135970	212S	PRS	49.994450	-124.430000	T	
PRT104	113919	212S	PRS	49.994455	-124.432500	T	
PRT105	113920	212S	PRS	49.804198	-124.457136	T	A
PRT106	113922	212S	PRS	49.961139	-124.366110	T	
PRT109	120426	212S	PRS	49.833414	-124.302408	T	
PRT113	120430	212S	PRS	49.833410	-124.302418	T	A
PRT258	135991	212S	PRS	49.829618	-124.286573	T	
PRT259	135992	212S	PRS	49.856334	-124.295400	T	A
PRT260	135990	212S	PRS	49.846546	-124.444193	T	A
PRT268	135986	212S	PRS	49.908105	-124.500042	T	A
PRT269	135988	212S	PRS	49.892162	-124.468615	T	A
PRT273	1813828	212S	PRS	49.880079	-124.420377	B	
PRT049	120696	212D	PRN	50.266832	-124.401644	T	

PRT086	135967	212D	PRN	50.266835	-124.401650	T
PRT261	121584	212D	PRN	50.015356	-124.339855	T
PRT263	135989	212D	PRN	50.323950	-124.439176	T
PRT274	1813829	212D	PRN	50.251394	-124.377361	B
PRT275	1813830	212D	PRN	50.337601	-124.427449	B
PRT285	143751	212D	PRN	50.335193	-124.426162	T
PRT407	144120	212D	PRN			T
PRT445	144226	212D	PRN			T
PRT052	120698	212F	PRN	50.093910	-124.609228	T
PRT053	120699	212F	PRN	50.093919	-124.609235	T
PRT064	120709	212F	PRN	50.093950	-124.609350	T
PRT265	127565	212F	PRN	50.094328	-124.627012	T
PRT266	135987	212F	PRN	50.099185	-124.613787	T
PRT276	1813832	212F	PRN	50.090680	-124.592228	B
PRT406	124764	212F	PRN			T
PRT005	1811026	213A	TOBA	50.512057	-124.206327	B
PRT006	1811027	213A	TOBA	50.595810	-124.183924	B
PRT339	339	213A	TOBA	50.567324	-124.092812	F
PRT340	340	213A	TOBA	50.567327	-124.092814	F
PRT341	341	213A	TOBA	50.567330	-124.092816	F
PRT342	342	213A	TOBA	50.567333	-124.092818	F
PRT344	344	213A	TOBA	50.567339	-124.092822	F
PRT345	345	213A	TOBA	50.567342	-124.092824	F
PRT346	346	213A	TOBA	50.567345	-124.092826	F
PRT348	348	213A	TOBA	50.567351	-124.092830	F
PRT349	349	213A	TOBA	50.567354	-124.092832	F
HO270	127415	214A	HO	50.668139	-124.771313	T
HO271	127419	214A	HO	50.650106	-124.798452	T
HO277	1813833	214A	HO	50.392028	-124.862724	B
HO436	144225	214B	HO			T
HO272	121595	215A	HO	50.946868	-124.912139	T
HO424	144123	215A	HO	50.946875	-124.912140	T
HO425	144124	215A	HO			T
HO007	1811028	215A	HO			B

Appendix III: Mitochondrial and microsatellite primers used in this study; all microsatellite forward primers contained an M13 primer sequence (CAC GAC GTT GTA AAA CGA C) added to allow incorporation of a fluorescent M13 tag during polymerase chain reaction amplification; allele size (in bp), number of alleles observed (#A), expected heterozygosity (H_e), observed heterozygosity (H_o), accession number in GenBank (if known), PCR temperature = 8 cycles_25 cycles at temp[1]_temp[2] °C, and magnesium chloride in millimolar.

Primer ID	sequence 5' to 3'	source	size	# A	He	Ho	Rpt	Acces. #	PCR Temp	MgCl2
EK-F23 EK-R663	TAC CAA TCA CCA GCA CAAT CG CGG GTT GCT GGT TTC ACG	Speller et al 2014							54	
BL42 F BL42 R	CAA GGT CAA GTC CAA ATG CC GCA TTT TTG TGT TAA TTT CAT GC	Bishop et al 1994	269-279	3	0.620	0.628	Di	G18455	55_57	2.5mM
BM203 F BM203 R	GGG TGT GAC ATT TTG TTC CC CTG CTC GCC ACT AGT CCT TC	Bishop et al 1994	246-250	3	0.502	0.448	Di	G18500	52_54	2.0mM
BM4107 F BM4107 R	AGC CCC TGC TAT TGT GTG AG ATA GGC TTT GCA TTG TTC AGG	Bishop et al 1994		4		NA	Di	G18519	55_57	2.0mM
BM4107RD F BM4107RD R	GCATTGTTCAGGGTTCTCTA GCTATTGTGTGAGGCAATTC	Redesign	176-190	4	0.557	0.527	Di		55_57	2.0mM
BM4513 F BM4513 R	GCG CAA GTT TCC TCA TGC TCA GCA ATT CAG TAC ATC ACC C	Bishop et al 1994		4		NA	Di	G18507	55_57	2.0mM
BM4513RD F BM4513RD R	CTCATGCACTTTTCCTTCTG GCTTATTCAAGTGGTGTAGGC	Redesign	143-149	4	0.531	0.472	Di		55_57	2.0mM
BMC1009 F BMC1009 R	GCA CCA GCA GAG AGG ACA TT ACC GGC TAT TGT CCA TCT TG	Bishop et al 1994	302-306	3	0.468	0.413	Di		48_50	2.0mM
BM888 F BM888 R	AGG CCA TAT AGG AGG CAA GCT T CTC GGT GAG CTC AAA ACG AG	Bishop et al 1994	199-209	3	0.367	0.318	Di	G18484	55_57	2.0mM
BM6506 F BM6506 R	GCA CGT GGT AAA GAG ATG GC AGC AAC TTG AGC ATG GCA C	Bishop et al 1994	226-234	2	0.483	0.437	Di	G18455	60_62	2.0mM
CSSM041 F CSSM041 R	AAT TTC AAA GAA CCG TTA CAC AGC AAG GGA CTT GCA GGG ACT AAA ACA	Moore et al 1994	144-148	2	0.47	0.449	Tet	U03816	52_54	2.0mM
INRA107 F INRA107 R	TCC CAG ATA CAG ATG CAA CAG GGA GAG CCG AGG GCT TCA G	Vaiman et al 1994	177-193	5	0.679	0.682	Di	X71577	55_57	2.0mM

Rt7 F Rt7 R	CCT GTT CTA CTC TTC TTC TC ACT TTT CAC GGG CAC TGG TT	Wilson et al 1997	234-235	2	0.465	0.317	Di	U90740	55_57	2.0mM
AML-1 F AML-1 R	CAG CCA AAC CTC CCT CTG C CCC GCT TGG TCT TGT CTG TTG C	Pajeres et al 2007	211/205 or 255	2	0.5	NA	Sex	X/Y	Failed	
BM415 F BM415 R	GCT ACA GCC CTT CTG GTT TG GAG CTA ATC ACC AAC AGC AAG	Bishop et al 1994		X		NA			Failed	
BM848 F BM848 R	TGG TTG GAA GGA AAA CTT GG CCT CTG CTC CTC AAG ACA C	Bishop et al 1994		X		NA			Failed	
BM1225 F BM1225 R	TTT CTC AAC AGA GGT GTC CAC ACC CCT ATC ACC ATG CTC TG	Bishop et al 1994		X		NA			Failed	
Rt1 F Rt1 R	TGC CTT CTT TCA TCC AAC CAT CTT CCC ATC CTC TTT AC	Wilson et al 1997		X		NA	Di	U90737	Failed	
RT5 F RT5 R	CAG CAT AAT TCT GAC AAG TG AAT TCC ATG AAC AGA GGA G	Wilson et al 1997							Failed	
Rt13 F Rt13 R	GCC CAG TGT TAG GAA AGA AG CAT CCC AGA ACA GGA GTG AG	Wilson et al 1997		X		NA	Di	U90743	55_57	
OvirH F OvirH R	AAG TCT ACA ATC CAT GGG CTT GC GTT CTT TAC CAC CTG CAC CA	DeWoody et al 1995	133-143	2		NA			Failed	
OarFCB193 F OarFCB193 R	TTCATCTCAGACTGGGATTCAGAAAGGC GCTTGGAAATAACCCTCCTGCATCCC	Buchanan Crawford 1993	114			NA	Di	LO1533	58_60	
C32 F C32 R	CATCACCTCCACTAGCTTTG ATCTGAGCCACTAGGGAAAC	Meredith et al 2005				NA	Tet		Failed	
C36 F C36 R	TATGGTGGAGATGTAGGTG CCATTATGTGTAACCCTCCA	Meredith et al 2005				NA	Tet		Failed	
ELK_T115 F ElK_T115 R	TGGTTATCTGGGTCATGAAG TTGCTATTGAGCCATAGG	Meredith et al 2005				NA	Tet		Failed	

Appendix IV: Deviations from Hardy-Weinberg Equilibrium for 13 populations of Roosevelt elk (*C. c. roosevelti*) at 10 microsatellite loci, AMOVA with 10000 permutations to estimate significance adjusted for FDR (*P*=0.015). Significant values in bold.

Pop'n	BL42	BM203	BM4107 RD	BM4513 RD	BM6506	BM888	BMC1009	CSSM041	Inra107	Rt7	Multi-locus
VIS	0.190	0.141	-0.040	0.192	0.264	0.149	0.032	-0.115	0.071	0.250	***0.111**
VIN	-0.142	-0.008	0.029	0.094	-0.095	0.294	0.114	-0.174	-0.189	-0.032	-0.027
SP	0.133	-0.035	0.009	-0.073	0.020	-0.025	0.276	-0.177	0.103	-0.04	0.024
CC	-0.192	0.120	0.102	0.112	0.200	-0.142	-0.091	0.005	-0.040	0.202	0.021
RG	0.172	0.200	0.124	0.029	0.138	***0.395**	0.196	-0.224	-0.009	0.324	***0.121**
SQ	-0.389	-0.161	0.178	-0.091	-0.037	0.038	0.236	0.400	-0.115	0.200	-0.003
PITT	-0.107	-0.096	0.147	-0.222	0.164	0.293	0.066	0.016	-0.033	-0.174	0.004
STV	-0.217	0.346	-0.045	-0.011	0.101	0.200	-0.077	-0.077	-0.021	***0.860**	0.093
BRIT	0.027	0.153	-0.019	0.385	-0.370	-0.059	-0.301	0.043	-0.086	0.640	0.027
PRS	0.083	-0.435	-0.129	0.255	0.050	0.458	-0.091	0.125	-0.258	0.050	-0.028
PRN	-0.007	-0.206	0.011	0.336	0.084	0.118	0.352	-0.132	-0.250	0.063	0.035
TOBA	0.196	0.137	0.125	0.161	0.226	0.302	0.429	0.386	0.200	0.310	***0.231**
HO	-0.135	0.276	-0.296	0.282	0.548	-0.273	0.164	0.517	0.300	0.774	***0.227**
Overall	-0.020	0.040	0.018	0.033	0.058	***0.149**	0.090	-0.070	-0.062	***0.128**	0.028

Appendix V: Linkage disequilibrium for 10 microsatellite loci in 13 populations of Roosevelt elk (*C. c. roosevelti*) in British Columbia, significance adjusted for FDR (*P*=0.002), * *a* = 0.05, *** *a* = 0.001.

Locus pair	Chi2	df	P-Value
BL42 & BM203	29.814155	26	0.275382
BL42 & BM4107RD	21.586875	26	0.711074
BM203 & BM4107RD	21.805299	26	0.699301
BL42 & BM4513RD	28.455439	26	0.336419
BM203 & BM4513RD	27.194096	26	0.399211
BM4107RD & BM4513RD	20.984569	26	0.74276
BL42 & BM6506	26.958661	26	0.411504
BM203 & BM6506	33.842002	26	0.139049
BM4107RD & BM6506	26.73083	26	0.423552
BM4513RD & BM6506	34.011405	26	0.134737
BL42 & BM888	17.5218	26	0.892473
BM203 & BM888	19.74874	26	0.803312
BM4107RD & BM888	23.199969	26	0.621611
BM4513RD & BM888	33.185286	26	0.15679
BM6506 & BM888	25.356648	26	0.498866
BL42 & BMC1009	27.094049	26	0.404414
BM203 & BMC1009	29.85085	26	0.273836
BM4107RD & BMC1009	22.38148	26	0.667655
BM4513RD & BMC1009	34.650226	26	0.119422
BM6506 & BMC1009	34.483764	26	0.123272
BM888 & BMC1009	23.064513	26	0.629295
BL42 & CSSM041	27.860356	26	0.365364
BM203 & CSSM041	32.333618	26	0.18232
BM4107RD & CSSM041	16.454449	26	0.924641
BM4513RD & CSSM041	21.796943	26	0.699754
BM6506 & CSSM041	37.17449	26	0.072091
BM888 & CSSM041	45.274708	26	0.01098
BMC1009 & CSSM041	25.368371	26	0.498208
BL42 & Inra107	27.573406	26	0.379764
BM203 & Inra107	15.80768	26	0.94072
BM4107RD & Inra107	53.492337	26	***0.001176**
BM4513RD & Inra107	20.941219	26	0.744992
BM6506 & Inra107	36.703372	26	0.079472
BM888 & Inra107	>116.7698	26	*****<1.81e-13**
BMC1009 & Inra107	27.610948	26	0.377864
CSSM041 & Inra107	35.995345	26	0.091755
BL42 & Rt7	18.788961	26	0.845215
BM203 & Rt7	15.746589	26	0.942109
BM4107RD & Rt7	12.069678	26	0.990773
BM4513RD & Rt7	33.899137	26	0.137583
BM6506 & Rt7	23.185709	26	0.622421
BM888 & Rt7	25.987624	26	0.463785
BMC1009 & Rt7	17.996824	26	0.875889
CSSM041 & Rt7	25.574413	26	0.486679
Inra107 & Rt7	35.685261	26	0.097611

Appendix VI: Allele frequency for 13 loci in 10 populations of Roosevelt elk (*C. c. roosevelti*) in British Columbia; primary source population VIN and VIS, secondary source populations SP and PRS; shaded cells indicate missing alleles or frequency reduced by ~40% or more from VIN.

Locus	Allele	VIS	VIN	SP	CC	RG	SQ	PITT	STV	BRIT	PRS	PRN	TOBA	HO
BL42	**n**	41	55	48	35	52	16	28	16	10	17	16	10	8
	269	0.463	0.509	0.271	0.329	0.433	0.375	0.268	0.344	0.300	0.500	0.594	0.450	0.500
	277	0.037	0.145	0.125	0.143	0.087	0.344	0.214	0.125	0.150	0.206	0.094	0.100	0.250
	279	0.500	0.345	0.604	0.529	0.481	0.281	0.518	0.531	0.550	0.294	0.313	0.450	0.250
BM203	**n**	41	55	48	35	51	16	29	16	10	16	16	11	8
	246	0.171	0.273	0.219	0.171	0.382	0.156	0.224	0.313	0.250	0.313	0.156	0.500	0.188
	248	0.341	0.691	0.698	0.786	0.608	0.813	0.741	0.656	0.700	0.656	0.750	0.364	0.688
	250	0.488	0.036	0.083	0.043	0.010	0.031	0.034	0.031	0.050	0.031	0.094	0.136	0.125
BM4107RD	**n**	41	55	47	35	52	16	28	15	10	17	15	8	8
	176	0.524	0.391	0.638	0.629	0.692	0.719	0.536	0.667	0.600	0.824	0.400	0.625	0.688
	186	0.463	0.255	0.181	0.229	0.250	0.188	0.214	0.200	0.200	0.118	0.233	0.250	0.250
	188	0.000	0.091	0.011	0.000	0.010	0.094	0.000	0.033	0.000	0.000	0.000	0.000	0.063
	190	0.012	0.264	0.170	0.143	0.048	0.000	0.250	0.100	0.200	0.059	0.367	0.125	0.000
BM4513RD	**n**	41	55	48	35	49	15	28	16	10	16	16	11	8
	143	0.110	0.100	0.240	0.257	0.265	0.233	0.214	0.281	0.350	0.094	0.313	0.227	0.125
	145	0.012	0.173	0.115	0.143	0.143	0.100	0.143	0.188	0.150	0.094	0.094	0.182	0.063
	147	0.012	0.018	0.021	0.014	0.061	0.033	0.054	0.031	0.000	0.000	0.000	0.045	0.000
	149	0.866	0.709	0.625	0.586	0.531	0.633	0.589	0.500	0.500	0.813	0.594	0.545	0.813
BM6506	**n**	41	53	47	33	51	15	28	16	10	17	15	9	8
	226	0.573	0.604	0.617	0.591	0.618	0.533	0.589	0.500	0.550	0.765	0.567	0.722	0.500
	234	0.427	0.396	0.383	0.409	0.382	0.467	0.411	0.500	0.450	0.235	0.433	0.278	0.500
BM888	**n**	41	55	47	35	50	16	29	16	10	17	16	11	8
	199	0.000	0.000	0.000	0.000	0.010	0.031	0.017	0.000	0.000	0.000	0.000	0.045	0.000
	207	0.695	0.791	0.734	0.700	0.770	0.813	0.707	0.625	0.900	0.882	0.781	0.773	0.750
	209	0.305	0.209	0.266	0.300	0.220	0.156	0.276	0.375	0.100	0.118	0.219	0.182	0.250

Locus	Allele													
BMC1009	**n**	41	54	48	35	52	16	25	16	10	17	15	11	8
	302	0.012	0.019	0.000	0.014	0.000	0.031	0.020	0.000	0.050	0.000	0.000	0.000	0.063
	304	0.634	0.472	0.594	0.686	0.721	0.813	0.760	0.625	0.700	0.618	0.567	0.818	0.438
	306	0.354	0.509	0.406	0.300	0.279	0.156	0.220	0.375	0.250	0.382	0.433	0.182	0.500
CSSM041	**n**	41	55	48	35	51	16	29	16	10	16	16	10	8
	144	0.610	0.564	0.615	0.757	0.608	0.469	0.672	0.719	0.550	0.594	0.688	0.650	0.625
	148	0.390	0.436	0.385	0.243	0.392	0.531	0.328	0.281	0.450	0.406	0.313	0.350	0.375
Inra107	**n**	41	54	47	35	48	16	29	16	10	16	16	11	8
	177	0.683	0.306	0.213	0.286	0.354	0.375	0.259	0.281	0.350	0.469	0.281	0.273	0.375
	179	0.012	0.009	0.021	0.071	0.000	0.063	0.103	0.156	0.000	0.031	0.000	0.000	0.000
	185	0.000	0.148	0.287	0.200	0.167	0.094	0.224	0.156	0.100	0.063	0.188	0.273	0.375
	191	0.305	0.463	0.436	0.443	0.438	0.438	0.414	0.344	0.500	0.375	0.500	0.455	0.250
	193	0.000	0.074	0.043	0.000	0.042	0.031	0.000	0.063	0.050	0.063	0.031	0.000	0.000
Rt7	**n**	40	50	46	33	48	15	27	15	10	17	16	11	8
	243	0.563	0.520	0.457	0.288	0.417	0.200	0.167	0.367	0.150	0.235	0.344	0.455	0.563
	245	0.438	0.480	0.543	0.712	0.583	0.800	0.833	0.633	0.850	0.765	0.656	0.545	0.438