THE UTILITY OF MOLECULAR DNA MARKERS FOR MONITORING POPULATION TRENDS AND GENETIC STRUCTURE OF WOODLAND CARIBOU

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ABSTRACT

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The use of non-invasive genetic sampling techniques is becoming frequent in the field of conservation. An examination of population trends of woodland caribou in Canada reveals the potential for fecal DNA analysis. Various sampling techniques have been practiced throughout multiple studies, using different protocols among species. The use of fecal matter to extract DNA has been done for terrestrial and aquatic species. There are three main methods for storing collected fecal matter, drying and lyophilisation, freezing and using solutions. For DNA extraction, two main brands were used for the process, Qiagen, and PureLink. This literature review focuses on the current understanding of woodland caribou population trends in Canada, and the potential for fecal DNA to close the knowledge gap between known knowledge and future trends. The use of fecal matter genetic sampling has potential to benefit the understanding and enhance current survey methodology of woodland caribou populations in Canada, and specifically, in the province of Ontario.

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INTRODUCTION

Ecosystems across the world is under pressure from biodiversity loss, the expansion of invasive species, and deteriorating ecosystem services due to anthropogenic activity (Beever et al. 2015). In response, many populations and species are experiencing decline, with increasing numbers of species being listed as at-risk. Biodiversity is critical for the sustainability of earth's ecosystems, as species provide beneficial resources for survival including food, recreation, and culture (Gascon et al. 2015). The scientific importance of species is undeniable, as many coexist to support biodiversity in ecological systems. Anthropogenic interference with ecological systems due to spatial variation have created challenges to monitoring wildlife demography and long-term viability (Fryxell et al. 2020). Managing conservation across large spatial landscapes requires the fundamental understanding of the three components of biodiversity. These include ecosystem diversity, species diversity and genetic diversity (Gugerli et al. 2008). Genetics is the primary foundation of ecosystem and species viability, and thus has become an essential tool for understanding the natural world (Frankham et al. 2013). The use of molecular genetics is not a new practice but has become an increasingly popular strategy for monitoring population trends across large spatial landscapes (Primmer 2009).

Conservation genetics applies genetic knowledge to reduce the risk of extinction in threatened species (Frankham et al. 2013). By understanding population trends though the use of genetics, the status and future survivability of a species can be predicted. In smaller populations, species face the impacts of genetic factors, such as a loss of genetic diversity and accumulation of deleterious mutations, leading to

extinction. Genetic diversity is described as the raw material upon which natural selection acts to bring about adaptive evolutionary change, and as the environment is constantly changing, it is essential to understand and monitor genetic diversity of wildlife populations. Genetic analysis can be used to determine historical, biological and the impacts of geographical information, and can be applied in various interpretations and models. Genetic monitoring has the potential to provide information on abundance, distribution, vital rates and genetic interchange (Sawaya et al. 2012).

To better understand long-term viability of at-risk populations and species, conservation biologists often study their population structure and trends using molecular genetic markers (Primmer 2009). To determine the reasoning behind why species at risk populations are deteriorating, it is important to understand the sensitivity of the habitat in which the animal is living in. (Corander et al. 2007). Determining whether or not a population is being influenced by an outlying population via gene flow is essential for understanding the health and dynamic of the species. This is one of the many ways genetic tools can be used to predict the survivability of the population. Genetics can be used in conservation biology to address causes of the population decline by determining how reproduction in the population is restricted. These tools can help determine whether the species is suffering from inbreeding due to geographical barriers or changing environmental conditions (Corander et al. 2007). Genetic tools can be applied to species at risk analysis to better understand population trends.

Methods of genetic sampling have common steps in which they follow. These include determining the methodology of collecting, storing, extracting and analysing. Determining the methodology of collection is typically decided upon budget and

resources available. In some cases, expertise is needed and excessive transportation such as a helicopter, boat or vehicle depending on the species being examined. Storing of samples is determined by the type and quality collected. Extraction and analysing are based off the information needed to conduct the study. Genetic diversity can be assessed in various ways, including both invasive and non-invasive sampling. Non-invasive sampling includes collecting DNA materials from the environment such as hair, feathers, eggshells and fecal matter. This provides an advantage over invasive collective sampling as it does not harm or disturb the animal. Invasive sampling is a traditional method of collecting DNA samples which involves physically extracting tissues, blood or saliva directly from the animal (Cronin et al. 2006). Invasive sampling provides higher DNA quality through direct contact with the animal. Many of these approaches have already been taken to analyze population structure of various different species, including those at risk.

Management of natural resources within Canada has been adapted to acknowledge the impact on endangered and at-risk species, with a focus on woodland caribou. Across Canada, woodland boreal caribou are estimated to number 31,000 to 39,000 within their entire distribution (Callaghan et al. 2010). Based on data collected by various jurisdictions, a total of 57 local population ranges of woodland caribou have been recognized (Callaghan et al. 2010). Of these local populations, 5.3% are increasing, 29.3% are declining, 28.1% are stable and the status of the remaining 36.8% are unknown (Callaghan et al. 2010). It has been predicted that the impacts of anthropogenic disturbance will be devastating to woodland caribou across Canada, with

at least 50% of all smaller populations facing extirpation in the future (Hebblewhite 2017).

The purpose of this study is to review the utility of molecular genetic studies to estimate population size and structure in boreal woodland caribou. The collection of fecal-DNA is an ideal solution to the predictions of caribou population trends, something that has not yet been conducted for an up-to-date status in the province of Ontario. This literature review provides a review of studies that aim to estimate population size or trends, with a focus on identifying current methodological best-practices and make recommendations for future studies.

METHODS AND MATERIALS

This review will focus on the current understanding of caribou populations based on genetic data in Canadian jurisdictions and the best practice methodology for collecting fecal sample DNA. For this literature review, I will be using reliable academic sources and government reports to complete my research. To ensure accurate information and a thorough review, peer-reviewed journals from Canada and the world were examined. Key words such as non-invasive genetic sampling, woodland caribou population trends, fecal matter DNA, DNA genotyping were entered into the Google Scholar search engine.

LITERATURE REVIEW

TAXONOMY AND DESIGNATABLE UNITS

Caribou (*Rangifer tarandus caribou*) are medium sized mammals that belong to the deer (*Cervidae*) family. They are known for their characteristic antlers that grow on both male and female individuals; however, some female individuals may have only one antler, or in some cases lack them altogether (Cumming 1992, COSEWIC 2019). The antlers of the Caribou (Boreal Population) are characterized by their flatness and complexity (COSEWIC 2019). In comparison to other common Canadian large mammals, they are slightly larger in size than white-tailed deer (*Odocoileus virginianus*), but smaller than elk (*Cervus elaphus*) and moose (*Alces alces*) (Banfield 1974). This species requires special attention in Ontario as it constitutes as the only Indigenous cervid species north of Lake Superior (Cumming and Beange 1993).

There are multiple subpopulations of caribou found across Canada, including the Atlantic-Gaspesie (endangered), Barren-ground (threatened), Boreal (threatened), Central Mountain (endangered), Dolphin and Union (endangered), Eastern Migratory (endangered), Newfoundland (special concern), Northern Mountain (special concern), Southern Mountain (endangered), Torngat Mountains (endangered) and various Peary subpopulations (non-active status) (COSEWIC 2019). Forest-dwelling caribou are subdivided into five different woodland caribou populations in Canada. This review will focus on woodland caribou and its sub-populations, specifically the boreal population, which is currently listed as threatened in Ontario. Across Canada, 28 of 57 populations of boreal woodland caribou (*Rangifer tarandus caribou*) are declining (Hebblewhite 2017).

Figure 1. The distribution of Woodland Caribou, Boreal population in Canada displayed on a map (Government of Canada 2012).

THREATS TO POPULATION PERSISTENCE

Woodland caribou serve as an important aspect to biodiversity in the boreal forest. This species acts as an umbrella species, in which they occupy a narrow and specialized niche, occurring at low densities in large patches of old growth (Hebblewhite 2017).

Due to their specialized niche and the constant pressure of human disturbance on the boreal forest, it has been accepted that managing for woodland caribou has presented various challenges (Sleep 2007). Challenges are faced through climate change, predators, fire, parasites and hunting, and forestry (Thomas and Gray 2002). In the winter, the species can be found in high occupancy, deep in the boreal forest, whereas in spring and summer boreal caribou migrate short distances to calving areas (Sleep 2007).

Through an increase in anthropogenic changes to the environment, an indirect consequence of impacting the predator-prey balance within the woodland caribou range has driven the grey wolf (*Canis lupus*) to increase (St-Pierre et al. 2021). In a study by Dyer et al. (2001) the impacts of human disturbance on caribou were analyzed. It was found that anthropogenic disturbance such as roads and development had a negative impact on animals in the area (Dyer et al. 2001). Vehicular traffic and road building created major disturbance for caribou and allowed for higher predation rates (Dyer et al. 2001). Road building and vehicular traffic are common disturbances in northwestern Ontario where boreal woodland caribou take up permanent residence. The impacts of this disturbance are yet to be studied in the boreal population. Based on the scientific literature, genetic data has been used to determine what is known about woodland caribou in Ontario and across Canada.

APPROACHES TO POPULATION MONITORING OF WILDLIFE SPECIES

Measuring the population density of moving wildlife can be difficult and requires a large amount of time and resources (Witmer 2005). There are various factors to consider including budget, species and experience of the individuals performing the monitoring. Wildlife monitoring is applied in science and management for two main purposes (Pollock et al. 2002). Monitoring provides an estimation as to the current population trends, but also provides an insight as to how the population will react to future changes. Through monitoring, population trends and estimates can be obtained and applied through management to ensure successful methods are being applied to conserve wildlife.

Population size monitoring requires repeated sampling of variables to obtain accurate estimates (Block et al. 2001). With the challenge of working over large spatial scales, many different techniques have been tested to produce good quality estimates, and accurate population numbers. In a long-term study looking at the population of gray wolves (*Canis lupus Linnaeus*), a collection of scat and hair samples were collected for genetic analysis (Stansbury et al. 2014). Through this data, an estimated population size was produced with a single-session population estimator using two different recapture-coding methods (Stansbury et al. 2014). This estimate was then compared to population estimates produced through telemetry data. The results produced considerable variability in 95% confidence intervals between the two estimates (Stansbury et al. 2014). This study highlights the need for further development of a consistent population estimation method to be used across large spatial landscapes.

Monitoring programs are heavily influenced by the cost of methodology and time needed to execute the study. In a study by Phoebus et al. (2020), a low-cost, low-effort scat sampling methodology was tested against grid-based DNA hair-snag sampling for grizzly bears (*Ursus arctos horribilis*). It was found that there was a much higher success rate of identifying individuals from the hair samples when compared to the numbers estimated from the scat collection due to the amount and quality DNA extracted from the samples. This is an example of how budgeting plays a major role in the development of an accurate population monitoring model.

The estimation of population census size requires survey work, data collection, and further analysis through a matrix to conclude a final number (Besbeas et al. 2002). A number of different survey approaches have been used in population biology studies

and are generally determined by the time frame and resources available (Takashina et al. 2018). The chosen survey approach represents a trade-off between data accuracy, time and money (Takashina et al. 2018). Due to this trade-off, the sampling method, choice of scale, and data availability need to be carefully decided (Takashina et al. 2018). The resultant data must be accurate enough to be able to detect ecological change, especially when it comes to endangered species (Lindenmayer and Likens 2009).

Telemetry

Telemetry is a conventional technique in which radio-collared animals are tracked from the ground or aircraft (Curatolo 1986). Radio collars require trapping of the animal and attachment of the collar, which can be costly and requires experienced technicians (Kolenosky and Johnston 2015). The use of radio collars has been a common method for tracking wildlife migration patterns and daily movements (Curatolo 1986). Multiple studies have taken place using radio collars to track caribou, timber wolves, fruit-bats, whitetailed deer, and water voles (Leuze 1979, Tester et al. 1964, Spencer et al. 1991, Rasiulis et al. 2014). The use of collars is effective to monitor population dynamics but has consequences and negative impacts to the animal it is being used for. The literature suggests the need for a less impactive alternative to wildlife population monitoring. When working with species at risk, it is crucial to have the least amount of impact and stress on the animal.

Aerial Surveys

To estimate the population size of large terrestrial mammals, it has been common to use aerial surveys as the primary method (Caughley 1977, Gasaway et al. 1985). This survey methodology has been used all over the world for various types of

wildlife, such as elephants, pronghorns, elk, and caribou (Vermeulen et al. 2013, Firchow et al. 1990, Svancara et al. 2002, Carr et al. 2010). Within Pukaska National Park, an aerial survey methodology was used to estimate the current population trend of woodland caribou (Patterson et al. 2014). The results showed a gradual decline of 3.7% per year since 1974 (Patterson et al. 2014). Aerial surveys are typically flown out of a helicopter or fixed wing-aircraft, with an experienced crew of observers on board, documenting animal tracks and sign along transect flights (Patterson et al. 2014). To save costs on operation, the use of unmanned aircraft systems (UAS) is being developed (Vermeulen et al. 2013).

MARK-RECAPTURE USING GENETICS

Genetic analysis is used as a tool for determining information about species dynamics and populations. Using genetic mark-recapture data has proven effective for understanding smaller population dynamics in terms of size and trends (Miller and Waits 2005).

Studies that have introduced the use of genetic analysis into traditional mark-recapture models are becoming more popular as the benefits from these studies are becoming more evident (Miller and Waits 2005). This proposes benefits to distinguishing individuals within small, rare populations such as the woodland caribou. Some techniques and software used in various literature could be applied to future caribou research. The estimating software *Capwire,* was used by Miller and Waits (2005) in a study looking at different biological mark-recapture population datasets of European badgers (*Meles meles*), red wolves (*Canis rufus*), forest elephants (*Loxodonta cyclotis*) and northern hairy-nosed wombats (*Lasiorhinus krefftii*). This software's intent

is to develop a method for estimating population size when the data may contain multiple observations of an individual within a session (Miller and Waits 2005). This software has potential to expand current knowledge of woodland caribou in Ontario.

The need for accurate genotyping is extremely important when collecting genetic data to use in capture-recapture analysis (Hettinga et al. 2012). The recent study done by Hettinga et al. (2012) executes the use of genetic data and from fecal matter DNA and capture-recapture models to estimate the population size of the North Interlake woodland caribou population.

NON-INVASIVE GENETIC SAMPLING

To obtain quality DNA, it is critical to collect adequate samples. Traditional sampling techniques are invasive to the animal, usually disrupting natural behaviours and taking tissues from the body. However, it is possible to obtain quality DNA from samples without disrupting or handling animals. Non-invasive techniques include extracting DNA from hair, feces, urine, feathers, shed skin, saliva and eggshells (Waits and Paetkau 2005).

Information extracted from DNA is important to the conservation of species and can be applied to behavior ecology (Taberlet and Luikart 1999). Non-invasive genetic sampling allows for the study of large mammals without disturbance; and includes not having to catch, injure, risk individual safety, or observe the wildlife (Taberlet and Luikart 1999). Non-invasive genetic sampling has become common practice for the monitoring of black bear (*Ursus americanus*) populations in Canada (Woods et al. 1999). Using barbed wire to collect samples of hair at baiting stations has proven effective for determining sex and population estimates (Woods et al. 1999).

There are limitations to non-invasive genetic sampling when it comes to genetic analyses critical for aiding conservation efforts (Ball 2010). An article written by Ball (2010) highlights some of the challenges that conservation efforts face with the limitations of non-invasive genetic sampling. Currently there is no method to determining the age of individuals through non-invasive genetic sampling. This is an attribute that would be beneficial to conservation efforts in estimating population trends of caribou. This paper suggests an alternative to using genetic analyses and suggests implementing the method of determining age class based on fecal pellet size. Applying this methodology to determine age classes of the caribou population could guide genetic analysis where known age is needed. A study by Flasko et al. (2017), obtained similar results

HANDLING AND STORAGE OF FECAL MATTER

Various techniques have been used in the handling and collection of fecal matter for genetic analysis (Lindquist and Wictum 2015, Gillet et al. 2008, Murphy et al. 2002). To ensure maximum quality of DNA, and to reduce the possibility of genotypic error, it is critical to employ sample handling standards. Throughout research studies, there has been a tendency to use one type of storage method, as opposed to executing multiple different techniques to reduce degradation. Very few studies have compared the effectiveness of different handling techniques of various fecal matter (Murphy et al. 2002). There are multiple factors to consider when deciding how to handle and store fecal samples. Variables such as seasonal timing of the collection, wild versus captive animals, the environment in which the sample will be collected, and the time available to process and transport. Throughout the literature, there have been a multitude of

techniques including different types of drying and lyophilisation, freezing and the use of solutions. These techniques have been used for various species from aquatic marine mammals such as whales to domesticated canines (Lindquist and Wictum 2015).

Drying and Lyophilisation

Drying is a common method of preserving fecal matter. In a study looking at the comparison of breed, age and diet for canines to develop a yield of canine DNA, the drying method was used (Lindquist and Wictum 2015). Samples were collected from each canine, and a portion of samples were dried out for a total of 24 hours for immediate extraction. Due to the risk of exposure of the feces immediately after defecation to mold growth, environmental conditions and active bacteria, beginning the drying process as soon as possible is critical to prevent degradation. DNA was extracted from the outer surface of the dried stool, and the results produced sufficient quality and quantity of DNA. Drying is an ideal approach for land mammals, where stool can easily be collected, but poses a challenge for marine collection. In a similar study looking at the DNA of highly endangered North Atlantic right whales, lyophilisation was used as the storage method for the whale fecal matter to remove any hydration from being collected in a marine environment (Gillet et al. 2008). Fecal matter was freeze-dried at -20 degrees Celsius for a total of seven days. Lyophilisation is carried out in extremely low temperature, allowing for the critical characteristics of the product (stool) to be preserved (Baressi et al. 2018). This technique is common practice in pharmaceuticals and provides longer stability and shelf life of the feces. The freezing temperature of -20 degrees Celsius is the baseline for most research, as a similar study extracting DNA from bottlenose dolphins by Parsons (2005), used the same approach.

Freezing

In a local study done in Manitoba, Canada, an alternative approach for storage was taken as conditions allowed for easy cold storage of samples. The cold climate allowed for the samples to be stored with no risk of exposure to contaminants. Hettinga et al. (2012) used cold storage when collecting fecal samples for the North Interlake woodland caribou population (Hettinga et al. 2012). Samples were collected using disposable wooden sticks and placed in sterile bags (Hettinga et al. 2012). The sterile bags were then placed in a cooler on board the aircraft that was used to access the remote locations of the population (Hettinga et al. 2012). Once the samples were delivered to the lab, they were frozen at -20^{o}C until thawed for extraction (Hettinga et al. 2012).

Similar techniques were used in Klütsch et al. (2012) examining mitochondrial DNA data to determine postglacial expansion from multiple glacial refugia in caribou (Klütsch et al. 2012). Fecal matter was collected and bagged from caribou cratering sites. These cratering sites were determined by flying aerial transects in winter (Klütsch et al. 2012). Samples were kept frozen until processed for DNA extraction in the lab (Klütsch et al. 2012). Exact temperatures were not mentioned for reference but can be assumed to be similar to Hettinga et al. (2012). The cold storage method allows for less complications in sample contamination as heat does not pose a threat for the rapid growth of microbes, as well as less materials are required allowing for decreases in costing. This method is ideal in cold climate environments, such as the boreal forest where different populations of caribou are found.

When determining effectiveness and practicality in the field, the use of silica pouches have been preferred over any other type of storage (Murphy et al. 2002). Determining how to create the most cost-effective handling procedure, silica pouches were used in the collection of brown bear fecal matter. This produced the lowest quality DNA when compared to other storage methods such as using solutions. It is recommended that silica not be used during wet seasons as the drying power of the pouches is not strong enough to prevent mold from occurring. Murphy et al. (2002) also found that the use of resealable plastic bags was effective for storing silica and the sample, and no noticeable leaks were noted from the bags.

Solutions

Another technique of preserving the quality of feces involves using different types of solutions, such as ethanol or DET (DMSO/EDTA/Tris). In a study looking at the amplifiability of mitochondrial, microsatellite and amelogenin DNA loci of red brocket deer (*Mazama americana*), fecal samples were stored in 100% ethanol solution in 50 ml centrifuge tubes with screw caps (Oliveira and Duarte 2013). The amplification of DNA was successful, but it was found that the freshness of the sample impacted the quality of the DNA, suggesting that preservation begins immediately when stored in ethanol, making timing of sample collection a major factor in DNA quality. Ethanol is commonly used in biological specimen preservation, as it dehydrates tissue and slows the degradation process (Vesper et al. 2017). In a study examining the comparison of bone mechanical properties from mice, a sample preserved in ethanol was analyzed against a control sample preserved through wrapping in gauze soaked in phosphate buffered saline and frozen for seven days. The results showed that the bone preserved in

ethanol produced significant differences in stiffness at the structural and tissue level, leading to decreased elastic deformation and elastic strain. Based off this literature, storing in ethanol solution would be ideal for preventing degradation of fecal samples for DNA extraction.

Solutions have been used as a preservation method across the globe. In a study done by Bhagavatula and Singh (2006) looking at Bengal tigers (*Panthera tigris tigrsi*), in India, fecal samples were stored in ethanol and silica (Bhagavatula and Singh 2006). Samples were broken into two portions, each of which were stored separately in ethanol (90%) and silica pouches (Bhagavatula and Singh 2006). The solution and samples were then encapsulated into 50 ml screw-cap tubes and kept at room temperature during transportation (Bhagavatula and Singh 2006). DNA was extracted from the samples in lab within a week of collection (Bhagavatula and Singh 2006).

Frantzen et al. (1998) also tested the use of ethanol for preservation of DNA. The goal of this study was to determine if the quality of DNA was dependent on the preservation method used. This study should be used as a fundamental basis for deciding upon which method is best suited for the type of DNA extraction that is being done. Frantzen et al. (1998) tested two different uses of solutions for the preservation of mitochondrial DNA. Fecal samples collected from free-ranging baboons (*Papio cynocephalus ursinus)* were stored in two different solutions, 70% ethanol and DMSO/EDTA/Tris/salt solution (DETs). It was found that samples stored in DETs produced the best results for amplification, compared to the ethanol solution.
This finding was contrasted in a study by Murphy et al. (2002) where the comparison of handling methods showed that samples stored in ethanol for up to one week amplified as

well as samples in stored in DETs, but that ethanol better-preserved DNA quality over longer time periods (Murphy et al. 2002). In a study by Srbek-Araujo et al. (2018), the amplification rates of DNA from samples stored in DETs was compared against the DNA from samples stored in silica. It was found that overall, the DETs provided a higher amplification success rate of 94% compared to silica at an 81% amplification success rate.

DNA EXTRACTION METHODS

Along with adequate storage and handling of samples, successful DNA extraction is necessary for producing quality DNA (Waits and Paetkau 2005). Often, fecal DNA extracts contain high concentrations of PCR inhibitors, and extraction methods are designed to minimize inhibitors while maximizing DNA yield (Waits and Paetkau 2005). There are numerous different commercial kits available for fecal DNA extraction, and various brands have been used successfully within the recent literature. Some kits are compatible with other biological sample types such as saliva, blood and tissue. Major brands on the market include Qiagen and PureLink, which retail kits specifically for extracting DNA from fecal matter.

Fecal DNA Extraction Kits

In a study done by Srbek-Araujo et al. (2018), a QIAamp DNA Stool Mini Kit was used to extract DNA from *Panthera onca* fecal matter. Qiagen is an international company known in various parts of the world for producing molecular testing products (Qiagen 2020). Qiagen offers a range of testing kits for DNA and RNA testing, for various types of samples such as blood, cell tissue, soils, and stool. The QIAmp DNA Stool Mini Kit was also used in a similar study extracting DNA from *Puma concolor*

feces, in which the results showed a successful extraction rate of 80% (Miotto et al. 2007). This kit is sold at retail price of approximately $285 US and is capable of 50 DNA preps (Qiagen 2020). The QIAmp DNA Stool Mini Kit is the most commonly employed fecal DNA extraction technique to date (Waits and Paetkau 2005).

Another popular brand for DNA extraction kits is PureLink (Uda-Shimoda et al. 2014). PureLink offers DNA Purification kits, retailing anywhere between $200-$1000, depending on the number of samples to be processed (Thermofisher 2020). One study by Uda-Shimoda et al. (2014), conducting DNA extraction from cysts found in human stool concluded that the PureLink purification kit outperformed the Qiagen kit mentioned earlier. This study used the PureLink PCR purification kit and found that it produced a larger amount of DNA, executed the extraction in a shorter amount of time, and had a lower cost for a higher amount of sampling (Uda-Shimoda et al. 2014).

DNA PROCESSING

The extraction of DNA is a critical component to any genetic analysis, especially when using non-invasive techniques involving very limited quantity. Two types of DNA can be extracted from animals, mitochondrial deoxyribonucleic acid (mtDNA) and nuclear deoxyribonucleic acid (nDNA). Through the use of polymerase chain reactions (PCR), the amplification of DNA can be done from minute amounts of fresh, alcohol-preserved, or dried tissues (Bacon et al. 1999). PCR is ideal for small samples because it replicates only the DNA region of interest (Frankham et al. 2013). The process involves extracting the DNA and further purifying it from the biological sample, such as fecal matter. The extracted DNA is then mixed with reagents which include oligonucleotide primers, DNA polymerase, magnesium, DNA nucleotides and PCR

buffer. Three major components go into the PCR process, denaturing, annealing and extension (Garibyan and Avashia 2013). Denaturing involves heating the reagent solution above the melting point, allowing for two strands of DNA to separate (Garibyan and Avashia 2013). Annealing is the next part of the process when the temperature is lowered to allow specific primers to bind to the target DNA (Garibyan and Avashia 2013). Finally, the temperature is raised again, allowing for the DNA polymerase to extend the primers by adding nucleotides to the developing DNA strand (Garibyan and Avashia 2013).

Genotyping

Failures and potential error are always a possibility in any experiment. In genetic analysis there are various steps in which potential errors can occur. Poor DNA quality produces two main types of genetic error, allelic dropout and false alleles (Adams and Waits 2007). Genotypic error occurs when the observed genotype of an individual does not correspond to the true genotype (Pompanon et al. 2005). A study focused on the DNA extraction of Bonobo (*Pan paniscus)* suggests that samples found homozygous at one or more loci should be genotyped repeatedly for verification (Gerloff et al. 1995). In this study, the authors found it was difficult to produce high quality DNA from *Pan paniscus* feces. They highlighted the limitations to handling limited amounts of extracted DNA and found that a second extraction was required a majority of the time to produce positive amplifications (Gerloff et al. 1995).

RESULTS

POPULATION SIZE AND TRENDS

A comprehensive review of the literature reviewed only two studies that have used genetic data to estimate population size and trends in woodland caribou (Carr et al. 2010, Hettinga et al. 2012). Genetic sampling can be used to provide population estimates with high confidence (Carr et al. 2010). This was demonstrated and tested on a small, closed area in the Slate Island Provincial Park (Figure 4) where the population was estimated from a collection of fecal matter DNA. Using a period of two different sample collections in 2007 and 2009, the population declined within 2 years (Carr et al. 2010). In 2007 a total of 49 unique individual genotypes were identified, resulting in a total population estimate of 151 individuals (Carr et al. 2010). In 2009 a total of 57 unique individual genotypes were identified resulting in an estimate of 99 individuals (Carr et al. 2010).

GENETIC DIVERSITY AND POPULATION STRUCTURE

With the increase in anthropogenic disturbance, habitat is becoming fragmented across large landscapes, causing a distribution change in terrestrial animals (Thompson et al. 2019). This distribution is generally associated with a decrease in genetic diversity (Thompson et al. 2019). The trend of decreasing diversity was found through a study done by analyzing the boreal population of caribou in Manitoba and Ontario (Figure 2) (Thompson et al. 2019). Using genetic data from more than 1000 caribou and nine microsatellite loci, patterns of genetic erosion were revealed suggesting a range retraction of the boreal population (Thompson et al. 2019). Further examining the boreal population in Saskatchewan and Manitoba have shown similar results, suggesting a

noticeable separation between the northern and southern areas (Ball et al. 2010). The use of genetics in this region shows a decrease in genetic diversity.

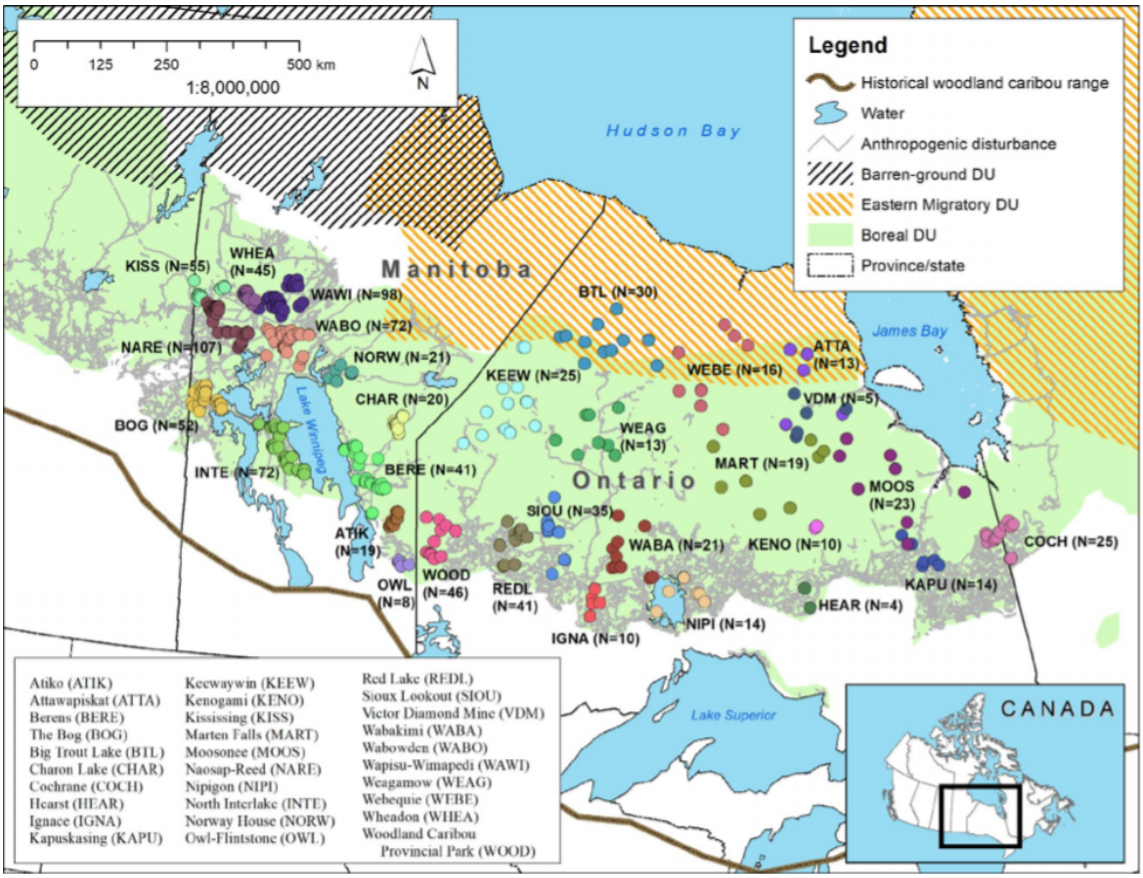

Figure 2. A map of the study area showing the sampling areas of the boreal woodland caribou (Thompson et al. 2019).

In a study done by Klütch et al. (2012) the use of genetic analysis of mitochondrial DNA from 1600 individuals, was able to estimate population structure of woodland caribou in Canada. The results were interpreted to determine if glacial refugia contributed to the phylogeographical structure in this species of caribou (Klütch et al. 2012). It was determined that woodland caribou most likely originated from a distinct area south of the Laurentide ice sheet and that the other four subspecies originated in northern refugia. These results can be applied to the future conservation of caribou and

planning for adaptation to climate change by modelling historical movement and interpreting future climate conditions in Canada (Klütch et al. 2012).

In Manitoba, the impact of major highways, hydro transmission lines, and smaller roads have reduced the intermingling of smaller woodland populations, such as the Upper and Lower North Interlake groups (Hettinga et al. 2012). Population genetic structure analysis revealed a significant level of fragmentation between these two populations and neighbouring populations within the area (Hettinga et al. 2012). Results of population trending models showed a 0.90 decline of the North Interlake population (Figure 3) from 2005-2009 (Hettinga et al. 2012).

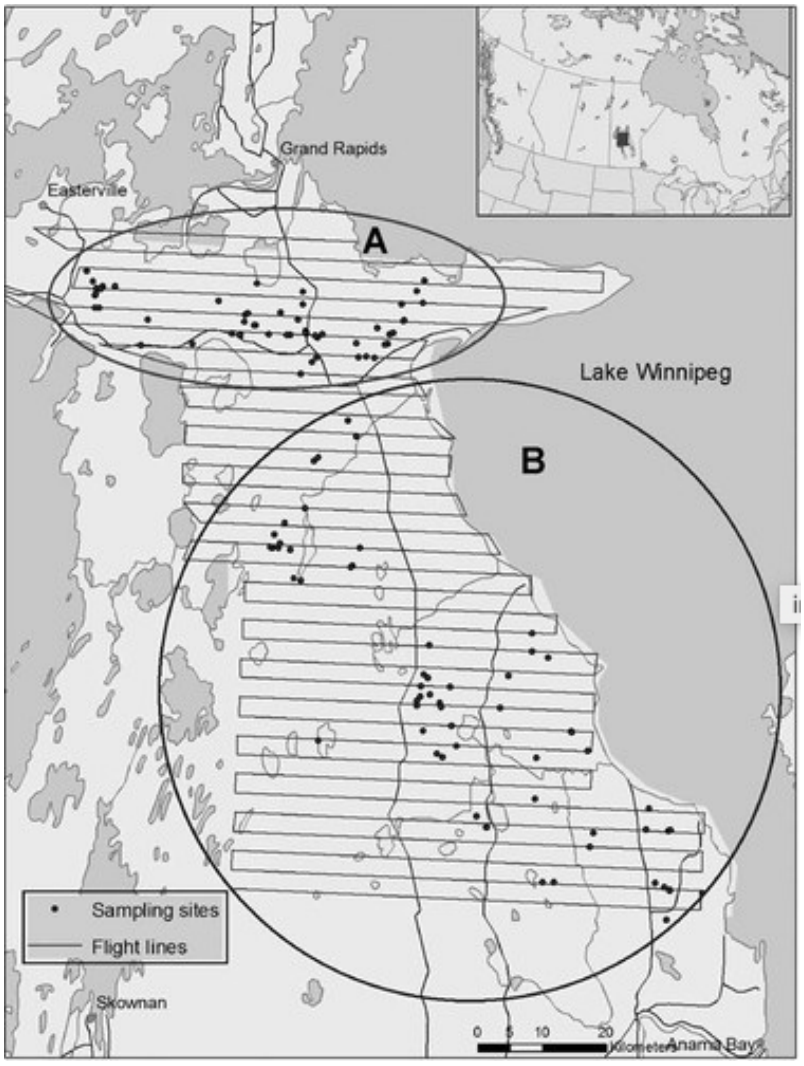

Figure 3. A diagram showing the North Interlake population sampling sights and flight lines used (Hettinga et al. 2012).

Another study in Ontario was done using the analysis of fecal matter was done to analyze the population of woodland caribou within the Lake Superior Coastal Range and to determine the gene flow (Figure 5) between individuals (Drake et al. 2018). Samples were collected between 2005-2015 in Pukaskwa National Park, and long-term population decline was observed for the population despite the genetic connectivity within the range (Drake et al. 2018). Genetic analysis was used to identify areas of movement and suggestions for habitat improvement (Drake et al. 2018).

Figure 4. A map in the Slate Island Provincial Park marking the fecal matter collection sites and transects flown (Carr et al. 2010).

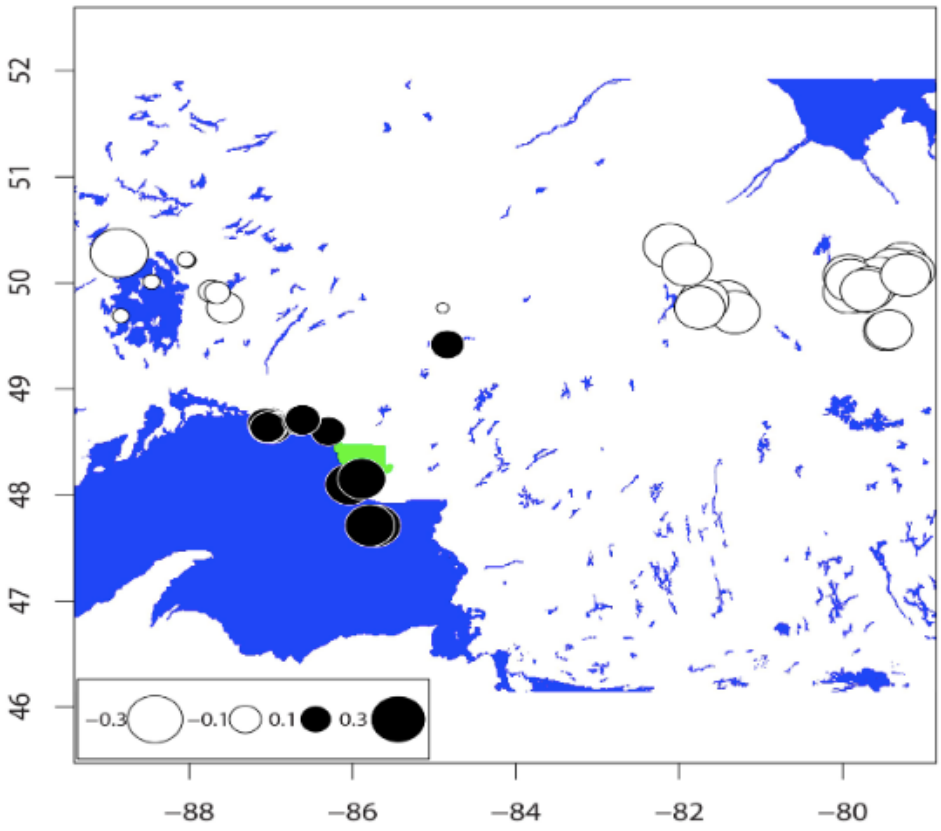

Figure 5. A visual representation of the genetic variation of clusters within the Lake Superior Coastal Range. The dark large circles represent the greatest genetic variation (Drake et al. 2018).

A study done by Cronin et al. (2005), the gene flow of 11 herds of caribou across Canada were analyzed through genetic data. Three different ranges of caribou were examined, the Alaskan barren ground caribou, the Canadian barren ground caribou and the woodland caribou (Cronin et al. 2005). Mitochondrial DNA (mtDNA) was extracted from tissues, and genotypes at 18 microsatellites loci were determined with PCR (Cronin et al. 2005). The genetic results revealed a high level of differentiation of mtDNA genotype and microsatellite allele frequencies, reflecting a limited gene flow due to geographic barriers (Cronin et al. 2005). Similar results have been found in other

studies where highways, roads and large bodies of water restrict the dispersal of boreal caribou (Ball et al. 2010, Fall et al. 2007, Priadka 2018).

The delineation of management units is dependent upon the genetic information available, specifically genetic diversity (Yannic et al. 2015). In a study to determine distinguished management units, the collection of genetic information was done for populations of woodland caribou from eastern Canada (Quebec and Labrador) (Yannic et al. 2015). The results showed a difference between the genetically based designation of management units and the presently defined ecological designation of management units.

A study done examining the population of the Atlantic-Gaspesie caribou compared data that suggested the dispersal of three separate subgroups from GPS telemetry to the genetic diversity of the population (Pelletier et al. 2019). The results of this study showed genetic substructure among groups based on their geographical location (Pelletier et al. 2019). It was also determined that the effective population size has decreased by 53% over last 15 years (Pelletier et al. 2019).

Maintaining genetic connectivity is essential for the conservation of woodland caribou (Priadka 2018). Using two different clustering methods (nonspatial and spatial) and the relative contribution of isolation by distance and isolation by resistance, the population genetic structure of boreal caribou across western Canada (clusters in Saskatchewan and Manitoba) was analysed (Priadka 2018). This study provides genetic tools to assess fine-scale spatial pattern of genetic variation, partition drivers of genetic variation and suggestions based on the use of these tools to improve management for maintaining genetic connectivity (Priadka 2018). The results of this study showed that

the significance of isolation by distance across the study area and within each genetic cluster supports the fact that boreal caribou maintain a natural clinal pattern of genetic structure (Priadka 2018). There were areas of discontinuity found across the study area, which could be led to the anthropogenic disturbance of roads in the area.

The Journal of Wildlife Management, Conservation Genetics, Rangifer, The Wildlife Bulletin, and Molecular Ecology resulted in the highest citations for this review (Table 1). These citations will also include information from project applications, committee reports, and company websites. Most resources were found using the Lakehead University Database, which has open access articles for enrolled students.

Table 1. A compiled list of cited academic journals and the number of referenced articles used within this literature review.

Name of Journal	Number of articles
The Journal of Wildlife Management	11
Conservation genetics	5
Rangifer	3
The Wildlife Bulletin	3
Molecular Ecology	3
PLos ONE	3
All other Journals	<2

DISCUSSION

Utilizing genetic tools to monitor woodland caribou population trends and population structure presents advancements in the current knowledge of caribou populations, especially in woodland caribou. Currently, there is a large unknown surrounding the population dynamics within Ontario, and even more so across Canada. Of the known populations of woodland caribou, still 36.8% of them have an unidentified status, leaving the effectiveness of our management strategies undisclosed (Callaghan et al. 2010). Protecting species as risk is important to the health of the environment and understanding the impacts of anthropogenic impacts on genetic diversity is essential to preserve species and ecosystem diversity.

Of the found literature, studies suggest that the overall population of woodland caribou in Canada is declining, mainly due to the continuous loss of genetic diversity (Cronin et al. 2005, Carr et al. 2010, Hettinga et al. 2012). These findings were determined with the use of genetic DNA analysis, similar to findings with the use of telemetry data. This presents an area of unclarity when it comes to range delineation and genetic diversity and clustering. Further advancement of genetic sampling could create a greater understanding of gene flow within the population.

The knowledge gap between what is known about woodland caribou substructure and range delineation could be lessened with the use of genetic tools and can be applied to the future of species at-risk. The importance of universal designatable units for the conservation of species at risk is essential when it comes to developing legislation for protection (Weckworth et al. 2018). Designatable units are used within Canada to address areas of ecological significance, and many are established for

woodland caribou (Weckworth et al. 2018). Finding a way to prevent overlapping of these units could be beneficial for the development of effective management practices. Incorporating the use of genetics into developing designatable units could be ideal for a greater understanding of woodland caribou. The use of capture-recapture non-invasive genetic sampling is effective in providing both population size and trend estimates (Fryxell et al. 2020). There is endless opportunity for further development of this methodology and use of its results to build a greater understanding of caribou population dynamics in Ontario.

In 2011, the Ontario Federation of Anglers and Hunters (OFAH) released a report on woodland caribou, recommending the use of telemetry data to provide herd-specific estimations of occupation and utilization to designate ranges of individual populations (Reid and Demille 2011). Going forward, using genetic tools may present a more feasible solution to understand subpopulations and substructure. It has been led to believe that the efforts to develop a strong conservation strategy for woodland caribou in Ontario have fallen short due to a lack of resources and validated diagnostic tools being used in the field (Serrouya et al. 2017). Due to the large amounts of helicopter time required, genetic sampling can be an expensive technique, however the collection of fecal samples can be easily added as an extra to traditional survey methods, such as aerial census (Carr et al. 2010). Genetic sampling can be made cost-effective when applied with traditional surveying and can enhance the data collected (Carr et al. 2010).

CONCLUSION

The use of non-invasive genetic sampling is an ideal alternative to traditional invasive genetic sampling of tissues when it comes to species at-risk. Understanding population trends and gene flow is essential to the proper management and population persistence of woodland caribou. Several advantages of genetic testing of fecal matter have been revealed, as it does not disrupt the life of the animal in any way and is effective in estimating population trends. The aid of genetics in understanding species at-risk should be further researched and applied to woodland caribou in Ontario.

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