POPULATION GENETICS OF CARIBOU IN THE BRIGHTSAND RANGE OF ONTARIO

By

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An Undergraduate Thesis Submitted in
Partial Fulfillment of the Requirements for the
Degree of Honours Bachelor of Environmental Management

Faculty of Natural Resources Management

Lakehead University

April, 2022

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ABSTRACT

Boreal woodland caribou are listed as threatened in Ontario. This thesis examined whether the genetic diversity of boreal woodland caribou in the Brightsand Range differs comparing populations in portions of the range with contrasting levels of disturbance. The GenAlex software was used to run three Analysis of Molecular Variances that showed that there was no significant difference in allele frequencies of the populations of caribou in the Brightsand range, but that the genetic diversity was lower in southern populations with more cutting of trees and road access causing fragmentation of the habitat. The lack of difference may be due to gene flow that is high enough to prevent loss of genetic diversity in the southern portion of the range.

ACKNOWLEDGEMENTS

Special thanks to the supervisor of this thesis, Dr. Ashley Thomson, and to the second of this thesis, Dr. Brian McLaren, for providing their expertise and help in many areas. Also, special thanks to Dr. Ashley Thomson for providing the data that made this thesis possible.

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INTRODUCTION

This thesis will examine the population genetics of woodland caribou (*Rangifer tarandus*) in the Brightsand range. The population genetics of woodland caribou should be examined because woodland caribou are listed as threatened in Ontario (Ministry of the Environment, Conservation and Parks 2021). Woodland caribou habitat in the Brightsand Range is currently considered as fragmented due to anthropogenic disturbances (Government of Canada 2015). When habitats are fragmented, the effective population size decreases because instead of one large population, there are multiple small populations (Frankham et al. 2010). Small populations lose genetic diversity faster than large populations because the effects of genetic drift are stronger in small, fragmented populations.

In a related case, populations of caribou in the Lake Superior Coastal Range have lower genetic diversity due to habitat fragmentation (Drake et al. 2018). Similarly, Thompson et al. (2019) found that caribou in the southern boreal forest had less genetic diversity than caribou in the northern boreal forest, implying that the reason is that the southern portion of the range was more disturbed (Thompson et al. 2019). The anthropogenic disturbance in the Brightsand range may also mean less genetic diversity in southern populations. The objective of this study is to examine whether population genetic diversity of woodland caribou differs significantly between managed and unmanaged portions of the Brightsand Range. I expect that genetic diversity of populations of caribou in the northern range is higher than the genetic diversity of the

populations of caribou in the southern range, because the northern range has less disturbance (Figure 1). The population estimates of caribou within the Brightsand range show that most of the caribou are in the northern portion of the range. There is a minimum of 224 individuals within the Brightsand range (Ministry of the Environment, Conservation and Parks 2021).

LITERATURE REVIEW

IMPORTANCE OF GENETIC DIVERSITY

Genetic diversity refers to the number of alleles and genotypes that can be found in a population. Genetic diversity is important because the it reflects the potential for adaptive evolution to changing environmental conditions (Frankham et al. 2010). For example, if a population has low genetic diversity, it may lack the capacity to adapt to increasing global temperatures or the introduction of novel pests or pathogens (Frankham et al. 2010; Bürger and Lynch 1995; Keller and Waller 2002; Bijlsma and Loeschcke 2012). Moreover, when genetic diversity is lowered, the fitness of individuals decreases (Thompson et al. 2019). These two effects cause a population to be more susceptible to extinction (Mimura et al. 2017).

One major way that genetic diversity decreases is through inbreeding. Inbreeding causes genetic diversity to decrease because it reduces heterozygosity (Frankham et al. 2010). Increased homozygosity associated with inbreeding exposes negative deleterious alleles, leading to reduction in the

mean fitness of individuals in the population, referred to as inbreeding depression. Inbreeding depression often acts to reduce reproductive fitness, which can lead to further population size reductions in already small, fragmented populations. The highest levels of inbreeding occur for small, genetically isolated populations that also lose alleles due to genetic drift at a higher rate than larger, more contiguous populations (Thompson et al. 2019).

Genetic diversity can only be acquired through mutations or brought into populations through migration (Frankham et al. 2010). However, somatic mutations occur at such a low rate that genetic diversity can only be regenerated through mutation over very long time periods (hundreds or thousands of generations). This means that once a population has lost a significant amount of genetic diversity, it can only be regenerated via gene flow from genetically differentiated populations. Thus, management strategies aimed at maintenance of genetic diversity for wild populations are generally focused on increasing heterozygosity through outbreeding by maintaining gene flow by ensuring habitat connectivity (Courtois et al. 2003). For example, a study of boreal caribou in Ontario and Manitoba found that caribou in more fragmented areas had lower genetic diversity than caribou living in less fragmented areas (Thompson et al. 2019).

LOSS OF GENETIC DIVERSITY IN SMALL POPULATIONS

Small populations are always at high risk of extinction (Frankham et al. 2010). A leading factor is that genetic drift has a greater effect when the population size small (Frankham et al. 2010). Genetic drift is the process by which alleles are lost due to random chance (Frankham et al. 2010). Eventually, genetic drift causes all but one allele to be lost (known as fixation). When a population is fixed for one allele, the fitness of the population decreases (Lynch and Burger 1995). Another set of problems that small populations face are stochastic genetic, environmental, and demographic problems (Robert et al. 2004). Stochastic environmental effects are dangerous because they can destroy small and large populations (Lande 1993; Robert et al. 2004). The chance of a small population going extinct by environmental problems is higher than for a large population (Lande, 1993; Robert et al. 2004). For example, a study of woodland caribou from the Atlantic-Gaspésie population found that there was genetic substructure in the woodland caribou meaning the effective population size decreased by 53% within the last 15 years (Pelletier et al. 2019).

Habitat fragmentation is the process by which one large habitat becomes converted into many smaller habitats (Frankham et al. 2010). When habitats are fragmented, the size of populations decreases and there is less migration leading to less gene flow. Fragmented populations have a higher chance of going extinct because these populations have the same problems that are associated with a small population, such as the loss of genetic diversity and

inbreeding depression. For example, a study of black-footed rock wallabies showed that almost all the isolated island populations lacked alleles that were common to their mainland counterparts (Mason et al. 2009).

One way that populations can become smaller is through population bottlenecks, for which a frequent outcome is that a population is reduced sharply (Frankham et al. 2010). While many at-risk species have reduced genetic diversity, the greatest losses of genetic diversity occur for populations that have experienced low population sizes over long time periods. One reason is that genetic diversity is lost with every generation that a population remains at small size. Another way that populations become small is through the process of fragmentation (Laidre et al. 2018).

CURRENT KNOWLEDGE IN GENETIC DIVERSITY OF CARIBOU IN RELATION TO LANDSCAPE FRAGMENTATION

Habitat fragmentation affects populations of caribou negatively. For example, the ranges of boreal caribou populations become smaller in more fragmented populations (Thompson et al. 2019). Furthermore, stochastic events might cause caribou populations to go extinct and, to avoid that, the abundance of caribou should be increased if the population is small (Courtois et al. 2003; Mcfarlane et al. 2018). For example, there might be adaptive differences in disease resistance in different populations (Kennedy et al. 2010). Also, there could be behavioral differences leading to genetic differences in caribou (Mager

et al. 2014). However, gene flow might still occur between fragmented populations, which can prevent genetic diversity from declining (Drake et al. 2018). The number of alleles between different populations of caribou might not be significantly different due to a low sample size, so having a large sample size is critical to being able to understand the genetic diversity of caribou (Ball et al. 2010).

ADAPTIVE VARIATION OF CARIBOU

There has been adaptive variation in caribou in the past. For example, a study found that there has been introgression in the Late Pleistocene for the eastern migratory caribou (Klütsch et al. 2016). The introgression can stay in the population of caribou for many generations (Colson et al. 2014). Increasing the abundance of caribou herds is more important than protecting individual herds of caribou (Kuhn et al. 2010).

MATERIALS AND METHODS

STUDY AREA

The study area is the Brightsand range of Ontario (Figure 1). In the southern part of the Brightsand range, forest companies have fragmented the landscape with roads, and planted jack pine and black spruce forests to be harvested, whereas the northern part is unmanaged with conifer and conifer mixed-wood (MNRF 2014). The Brightsand range is 22,000 square kilometers and is located in the boreal forest region of northwestern Ontario. The area is characterized by a high number of lakes and by an aggressive fire regime. Caribou occur throughout the Brightsand range but are concentrated within Wabakimi Park.

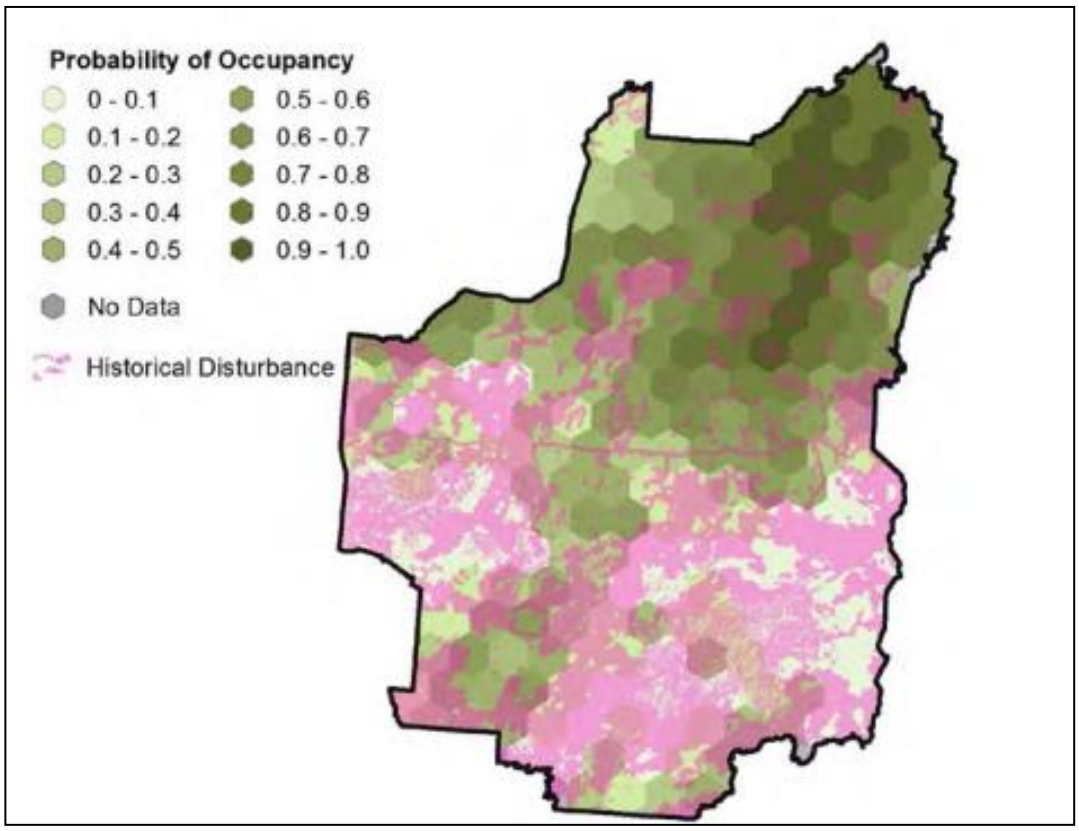

Figure 1: Disturbances within the Brightsand range. The southern part of the range has been changed while the northern part has less disturbance.

Source: MNRF 2014

GENETIC SAMPLING

Genetic data used in this study was obtained previously as part of a study aimed at estimating population size and structuring of woodland caribou in the Brightsand range (Anderson et al. 2021). Caribou feces were sampled two times, the first time in the first two weeks of February 2020, and the second in the first two weeks of March 2020. Two observers used global positioning systems (GPS) to record the coordinates of caribou activities while on a fixed wing aircraft. East-west transects spaced 5 km apart were flown across the

entire range (Figure 2). Another crew collected fecal pellet samples by landing a helicopter to sites with caribou activity. The samples of pellets were collected from locations where it was known that the sample was from a single animal. To maximize the chances of sampling different caribou individuals, sampling effort of the feces was spread across different areas so that there is a higher chance of sampling different animals. The crews collected samples that were equal to 1.5 times the estimated number of caribou in each site meaning they collected 1.5 times more scat than the number of caribou that was estimated to be at the site. Pellet samples were labeled with a unique number and the location that they were collected. During the collection of the fecal samples, the samples were kept frozen and at the end of the day, and then moved to a freezer. The researchers collected 358 samples in total from 28 locations in February. In March, 422 samples were collected from 35 locations. DNA was extracted and amplified at nine microsatellite loci using two multiplexed polymerase chain reactions (PCRs). A fluorescent tag was used to label one primer of each pair to enable microsatellite fragment size analysis. The data collected by Anderson et al. (2021) was used in the genetic analysis to identify the genetic diversity of the caribou in the Brightsand range in this thesis.

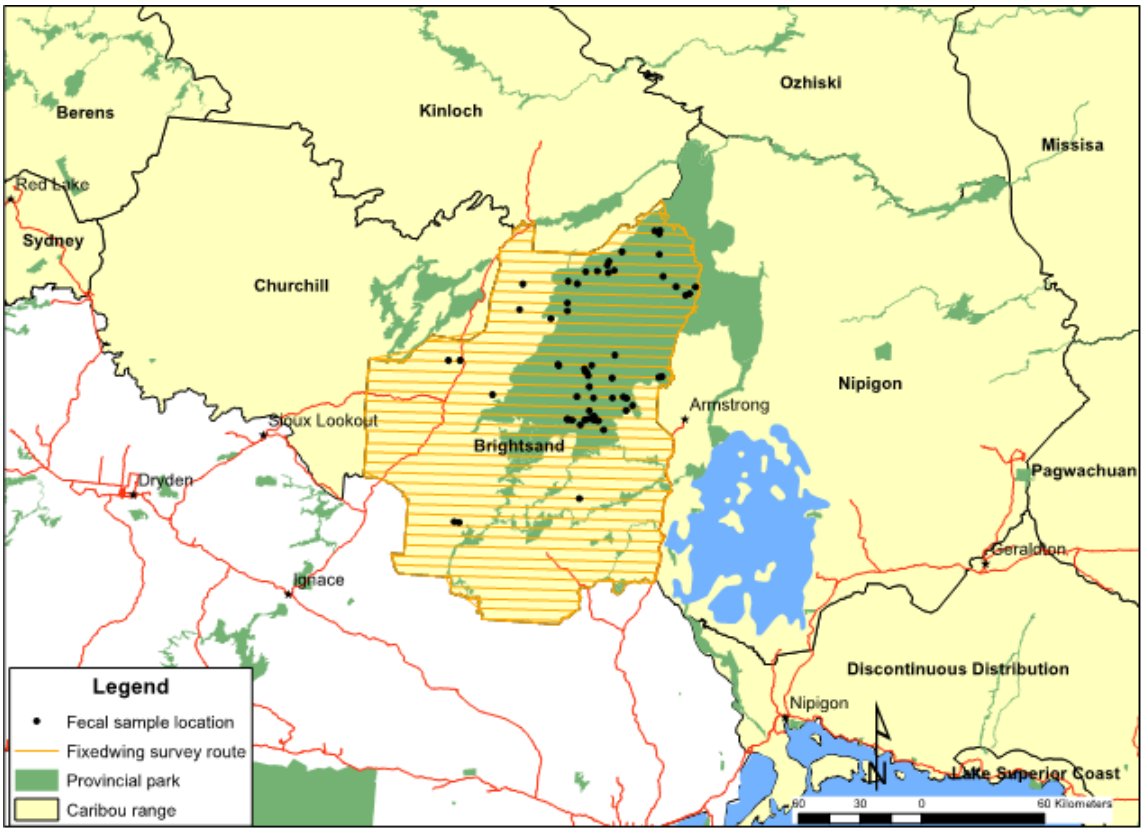

Figure 2. Location of the Brightsand Range, aerial survey route (5-km flight lines), and fecal DNA sample locations. The black dots are the locations where fecal samples were collected.

Source: Anderson et al. 2021.

GENETIC ANALYSIS

Three Analyses of Molecular Variation (AMOVA) were used to determine if there was significant variation in the frequencies of alleles in the northern versus southern populations. One AMOVA comparing the northern and southern portions of the range was completed to test for the genetic diversity of the caribou. Another AMOVA comparing the genetic diversity of caribou in the managed and unmanaged parts of the range was done. A third AMOVA

comparing the genetic diversity of caribou in the portions of the range that are not part of Wabakimi Provincial Park, northern portion of Wabakimi Provincial Park, and southern portion of Wabakimi Provincial Park was done. The AMOVA was done using the GeneAlex software (Peakall and Smouse 2012). The number of private alleles, expected heterozygosity (He), number of alleles (Na), and inbreeding coefficient (F) were calculated for each sampling location because these statistics help give insight into whether the populations have low genetic diversity.

RESULTS

NORTHERN AND SOUTHERN POPULATION GROUPS

There were two private alleles. These private alleles were found in the B06 population and the B08 population (Table 1). This means that the alleles that were only present in a single population were RT27, which was found in population B06, and RT24, which was found in population B08. The AMOVA for the North and Southern populations respectively showed that 97% of the molecular variation came from within individuals, 0% of the molecular variation came from among populations, and 3% of the molecular variation came from among individuals (Figure 3, Table 2). The number of replicates that was completed was 999.

Table 1: Table of the private alleles.

Pop	Locus	Allele	Freq
B06	RT27	159	0.269
B08	RT24	213	0.100

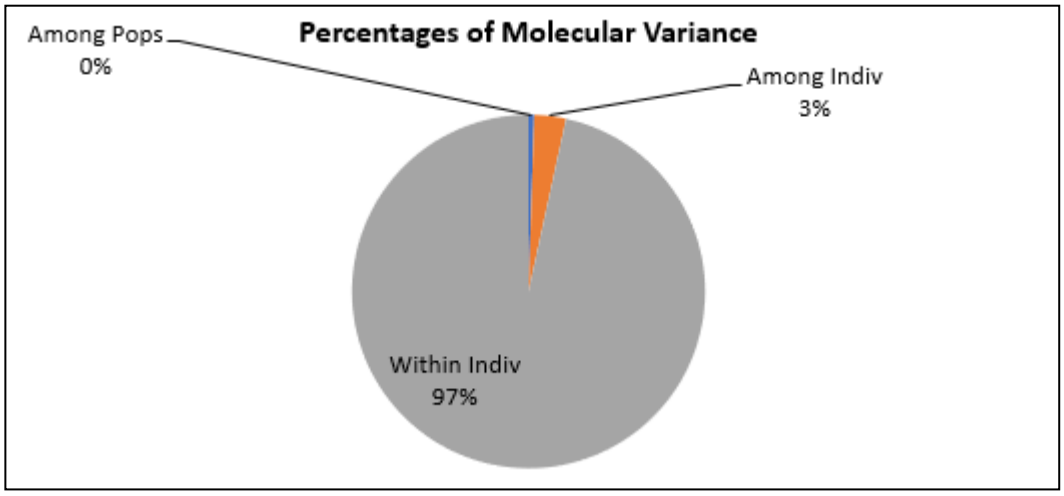

Figure 3: Percentages of molecular variances for among individuals, among populations, and within individuals for the AMOVA comparing the North and South populations. Most of the variance is within individuals.

Table 2: Partitioning of molecular variance among populations, within populations, and within individuals for the North and South populations. The calculated P values and critical p values are also in the table.

Source	df	SS	MS	Est. Var.	% Var.	Calc.P-Value	Crit. P-value
Among Populations	1	15.31	15.31	0.02	1%	0.005	0.001
Among Individuals	785	2512.47	3.20	0.09	3%	0.029	0.002
Within Individuals	787	2376.00	3.02	3.02	97%	0.034	0.001
Total	1573	4903.8		3.13	100%		

The average number of alleles per locus was 8.2 for both northern and southern populations, indicating that northern and southern populations had similar levels of genetic diversity (Table 3). The average expected heterozygosity for northern populations (0.692) was slightly higher than that of southern populations (0.678). Inbreeding was low across the sampled populations, with an average inbreeding coefficient (F) of 0.001.

Table 3: Table of the means and standard error of the average number of alleles, expected heterozygosity, and inbreeding coefficient.

Pop		Na	He	F
North	Mean	8.222	0.692	-0.017
	SE	0.596	0.029	0.024
South	Mean	8.222	0.678	0.019
	SE	0.683	0.032	0.021
Average	Mean	8.222	0.685	0.001
	SE	0.440	0.021	0.016

MANAGED AND UNMANAGED POPULATION GROUPS

The AMOVA results for the managed versus unmanaged sections of the Brightsand range, meaning the populations that are not in Wabakimi Provincial Park, and the populations within Wabakimi Provincial Park, indicated that most (97%) of the variation occurred within individuals, with 0% of the allele frequency variation attributed to among-population differences (Figure 4, Table 4). The average number of alleles per locus for unmanaged populations (8.44) was slightly higher than that of unmanaged populations (7.56) (Table 5). Expected heterozygosity was similar between unmanaged and managed populations, with values of 0.68 and 0.69, respectively. Inbreeding coefficients were low across the range (average = 0.009) but slightly higher for managed than unmanaged populations.

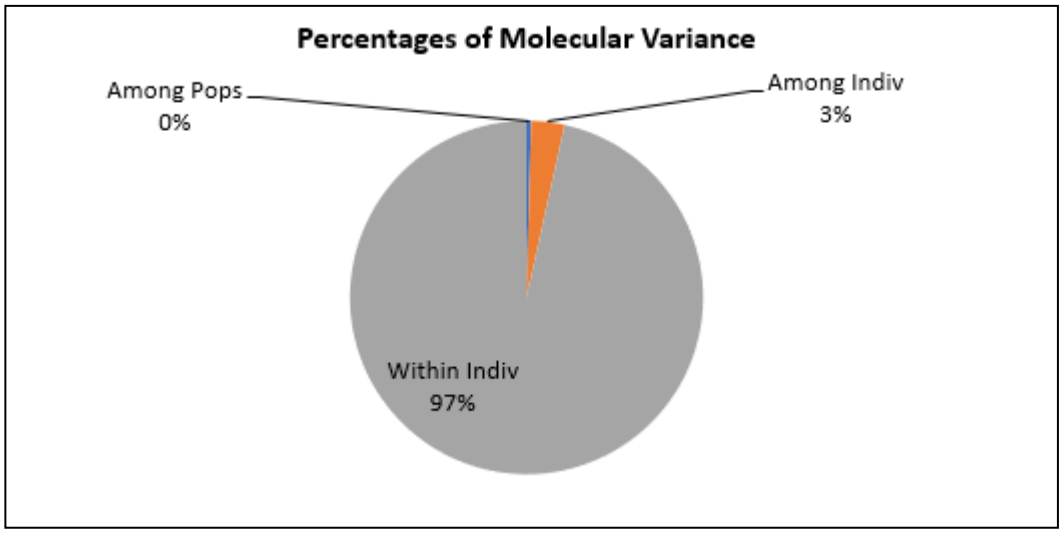

Figure 4: The percentage of molecular variances for among populations, among individuals, and within individuals for the AMOVA that compared the managed and unmanaged locations of caribou.

Table 4: Partitioning of molecular variance among populations, within populations, and within individuals for the managed versus unmanaged populations. The calculated P values and critical p values are also in the table.

Source	df	SS	MS	Est. Var.	% Var.	Calc.P-Value	Crit. P-value
Among Populations	1	7.81	7.81	0.01	0	0.004	0.001
Among Individuals	785	2519.97	3.21	0.1	3	0.031	0.001
Within Individuals	787	2376.00	3.02	3.02	97	0.035	0.001
Total	1573	4903.8		3.13	100		

Table 5: Table of the means and standard error of the average number of alleles, expected heterozygosity, and inbreeding coefficient.

Pop		Na	He	F
Unmanaged	Mean	8.44	0.68	0.005
	SE	0.67	0.03	0.019
Managed	Mean	7.56	0.69	0.013
	SE	0.65	0.03	0.033
Average	Mean	8.00	0.69	0.009
	SE	0.46	0.02	0.018

MANAGED FOREST, NORTHERN WABAKIMI, AND SOUTHERN WABAKIMI POPULATION GROUPS

The analysis of molecular variance for the three population groups (populations outside of Wabakimi Provincial Park, Northern Wabakimi meaning the populations in the northern portion of Wabakimi Provincial Park, and Southern Wabakimi meaning the populations in the southern portion of Wabakimi Provincial Park) indicated that the majority of allele frequency variation (97%) occurred within individuals (Figure 5). Allele frequencies did not vary significantly between the three population groups (Table 6). Allelic richness values were highest for the Northern Wabakimi population group (8.22), followed by the Southern Wabakimi group (7.78), and the managed forest group (7.56) (Table 6). Heterozygosity values were high across population groups (average = 0.685), while inbreeding coefficients were low (average = 0.005).

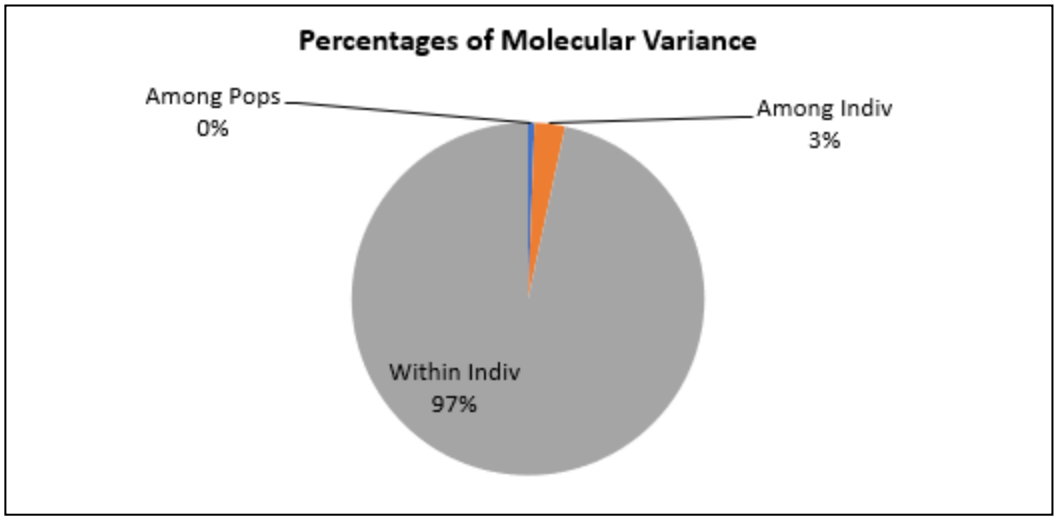

Figure 5: Partitioning of molecular variance among populations, within populations, and within individuals for the AMOVA that compared population groupings from the managed forest area, Wabakimi Provincial Park North, and Wabakimi Provincial Park South.

Table 6: Partitioning of molecular variance among populations, within populations, and within individuals for the managed, North WabakimiProvincial Park, and South WabakimiProvincial Park populations. The calculated P values and critical p values are also in the table.

Source	df	SS	MS	Est. Var.	% Var.	Calc.P-Value	Crit. P-value
Among Populations	2	23.63	11.82	0.02	1%	0.006	0.001
Among Individuals	784	2504.15	3.19	0.09	3%	0.028	0.001
Within Individuals	787	2376.00	3.02	3.02	97%	0.034	0.001
Total	1573	4903.8		3.13	100%		

Table 7: Table of the means and standard error of the average number of alleles, expected heterozygosity, and inbreeding coefficient.

Pop		Na	He	F
Northern Wabakimi	Mean	8.222	0.692	-0.017
	SE	0.596	0.029	0.024
Southern Wabakimi	Mean	7.778	0.672	0.017
	SE	0.619	0.033	0.021
Managed Forest	Mean	7.556	0.692	0.013
	SE	0.648	0.031	0.033
Average	Mean	7.852	0.685	0.005
	SE	0.349	0.017	0.015

DISCUSSION

The AMOVA for the populations of caribou showed that there was no significant difference in allele frequencies between caribou occupying the managed and unmanaged portions of the Brightsand Range. This result could have occurred because there were not enough samples in the managed parts of the range to have the statistical power to detect potentialallele frequency variations. Private alleles are alleles that are only present in one population (Szpiech and Rosenberg 2011). The presence of only two private alleles suggests that the populations are connected by gene flow, preventing their divergence due to genetic drift. This means that the populations are not very distinct from each other (Thompson et al. 2019).

More fragmented populations of Boreal caribou have lower genetic diversity (Thompson et al. 2019). As such, genetic diversity of the caribou in the southern side of the Brightsand range was expected to be lower than in the northern portion because the southern portion was more fragmented. I showed that there is a difference in genetic diversity, but no difference in allele frequencies. This might differ from Thompson et al. (2019) because the AMOVA was done for populations across a much smaller geographic area than the study area of Thompson et al.'s (2019) paper. Another reason that the results could have been different was because the sample size of Thompson et al's (2019) was larger and covered a greater area whereas this study only covered the Brightsand range. I would expect a higher degree of genetic differentiation among populations in that study because the longer distances between sampled

populations should limit gene flow among populations compared to when distances among sampled populations are relatively short (Rousset 1997). Another reason why the result was not significantly different was that the gene flow was high enough to prevent the loss of genetic diversity in the southern portion of the range.

In the future, habitat fragmentation might cause some populations of caribou to become more isolated and the genetic diversity might decrease. Therefore, future studies should compare the genetic diversity of the caribou if the range continues to be fragmented.

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APPENDICES

Appendix 1: Table of the means and standard errors for each population for the average number of alleles, expected heterozygosity, and inbreeding coefficient.

Pop		Na	He	F
A1	Mean	4.778	0.629	-0.031
	SE	0.434	0.034	0.071
A10	Mean	4.556	0.621	-0.027
	SE	0.338	0.034	0.053
A11	Mean	3.667	0.597	-0.515
	SE	0.289	0.025	0.052
A12	Mean	3.889	0.582	-0.197
	SE	0.261	0.040	0.090
A13	Mean	3.444	0.556	-0.311
	SE	0.242	0.051	0.103
A14	Mean	5.222	0.646	-0.052
	SE	0.521	0.035	0.070
A15	Mean	5.333	0.662	-0.040
	SE	0.373	0.031	0.055
A16	Mean	4.889	0.636	-0.060
	SE	0.484	0.040	0.061

A17	Mean	5.444	0.646	0.003
	SE	0.412	0.055	0.022
A18	Mean	4.444	0.658	0.014
	SE	0.475	0.029	0.073
A19	Mean	4.111	0.653	-0.210
	SE	0.539	0.048	0.052
A2	Mean	5.333	0.647	-0.089
	SE	0.441	0.033	0.068
A20	Mean	3.333	0.568	-0.232
	SE	0.333	0.061	0.053
A21	Mean	4.444	0.628	-0.129
	SE	0.294	0.042	0.055
A22	Mean	4.333	0.680	-0.092
	SE	0.408	0.022	0.078
A23	Mean	2.556	0.528	-0.259
	SE	0.242	0.046	0.190
A24	Mean	3.889	0.584	-0.231
	SE	0.484	0.056	0.065
A25	Mean	3.111	0.520	-0.204
	SE	0.351	0.078	0.080
A26	Mean	5.000	0.586	-0.123
	SE	0.624	0.056	0.058

A27	Mean	4.222	0.611	0.047
	SE	0.364	0.041	0.118
A28	Mean	4.222	0.664	-0.057
	SE	0.324	0.031	0.090
A3	Mean	5.000	0.667	-0.087
	SE	0.408	0.026	0.074
A4	Mean	4.444	0.620	-0.223
	SE	0.556	0.067	0.067
A5	Mean	5.667	0.655	-0.002
	SE	0.441	0.042	0.068
A6	Mean	5.111	0.642	-0.107
	SE	0.790	0.045	0.081
A7	Mean	4.111	0.630	-0.174
	SE	0.455	0.042	0.078
A8	Mean	5.333	0.713	-0.045
	SE	0.333	0.026	0.072
A9	Mean	5.778	0.633	-0.003
	SE	0.494	0.041	0.047
B01	Mean	3.667	0.616	-0.258
	SE	0.408	0.041	0.123
B02	Mean	4.333	0.611	-0.110
	SE	0.333	0.035	0.062

B03	Mean	5.667	0.661	-0.112
	SE	0.441	0.023	0.065
B04	Mean	5.222	0.700	0.021
	SE	0.494	0.027	0.112
B06	Mean	3.889	0.569	-0.334
	SE	0.389	0.068	0.063
B07	Mean	3.222	0.580	-0.168
	SE	0.324	0.049	0.127
B08	Mean	4.667	0.603	-0.145
	SE	0.408	0.052	0.064
B09	Mean	3.000	0.512	-0.347
	SE	0.236	0.050	0.058
B10	Mean	4.111	0.589	-0.027
	SE	0.389	0.041	0.090
B11	Mean	1.778	0.389	-1.000
	SE	0.147	0.073	0.000
B12	Mean	6.333	0.675	-0.022
	SE	0.687	0.027	0.057
B122	Mean	1.667	0.333	-1.000
	SE	0.167	0.083	0.000
B13	Mean	5.222	0.643	-0.132
	SE	0.434	0.030	0.056

B14	Mean	2.667	0.556	-0.526
	SE	0.289	0.047	0.137
B15	Mean	2.778	0.519	-0.291
	SE	0.364	0.049	0.101
B16	Mean	3.667	0.603	-0.123
	SE	0.373	0.041	0.074
B17	Mean	2.556	0.528	-0.467
	SE	0.176	0.040	0.109
B18	Mean	5.556	0.619	0.004
	SE	0.377	0.049	0.077
B19	Mean	4.000	0.559	-0.180
	SE	0.408	0.069	0.075
B20	Mean	1.333	0.167	-1.000
	SE	0.167	0.083	0.000
B21	Mean	5.778	0.639	0.061
	SE	0.572	0.041	0.053
B22	Mean	3.778	0.574	-0.335
	SE	0.364	0.053	0.076
B23	Mean	3.667	0.562	-0.211
	SE	0.289	0.061	0.062
B24	Mean	4.889	0.636	-0.082
	SE	0.611	0.042	0.047

B26	Mean	3.889	0.584	-0.158
	SE	0.455	0.056	0.059
B27	Mean	3.556	0.573	0.017
	SE	0.294	0.064	0.103
B29	Mean	3.778	0.573	-0.039
	SE	0.222	0.035	0.090
B30	Mean	3.556	0.554	-0.150
	SE	0.294	0.055	0.076
B31	Mean	2.778	0.559	-0.375
	SE	0.364	0.051	0.134
B32	Mean	3.111	0.544	-0.115
	SE	0.309	0.050	0.094
B323	Mean	1.667	0.333	-1.000
	SE	0.167	0.083	0.000
B33	Mean	4.222	0.628	-0.069
	SE	0.222	0.029	0.057
B35	Mean	1.889	0.444	-1.000
	SE	0.111	0.056	0.000
B36	Mean	4.111	0.612	-0.033
	SE	0.484	0.040	0.074
B37	Mean	3.000	0.489	-0.178
	SE	0.373	0.060	0.145

B52	Mean	1.778	0.389	-1.000
	SE	0.147	0.073	0.000

Appendix 2: The number of suspected animals in each site and the samples collected in each site from February 2020.

Site ID	Suspected Animals	Total Samples
A1	7+	14
A2	12+	15
A3	8+	20
A4	6-7	14
A5	20+	30
A6	12	11
A7	5-7	8
A8	10+	15
A9	12+	18
A10	5+	9
A11	5+	9
A12	5+	8
A13	5	7
A14	15-20	22
A15	7	17
A16	10+	16
A17	20	32
A18	5+	10
A19	3	6
A20	3	3
A21	7	11
A22	5+	9
A23	2	4
A24	4+	8
A25	4+	5

A26	6+	15
A27	3-4	6
A28	10+	16
TOTAL		358

Appendix 3: The number of suspected animals in each site and the samples collected in each site from March 2020.

Site ID	Suspected Animals	Total Samples
B1	6+	5
B2	12+	11
B3	25+	36
B4	10+	19
B6	8+	13
B7	5+	3
B8	8+	9
B9	12+	3
B10	15+	9
B11	12+	1
B12	30-50	46
B13	30+	45
B14	20+	2
B15	3	3
B16	6	6
B17	4	2
B18	25+	43
B19	7	11
B20	2	2
B21	25+	40
B22	4	6
B23	7	5
B24	9	14
B26	5	8
B27	5	5
B29	10+	15

B30	8	7
B31	8	3
B32	4+	7
B33	10+	19
B35	2	2
B36	13+	19
B37	13+	3