Does connectivity exist for remnant boreal caribou (*Rangifer tarandus caribou*) along the Lake Superior Coastal Range? Options for landscape restoration

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**Abstract:** Genetic analysis can provide important information on the dynamic and spatial structure of groups of animals or populations. Little is known of the genetic population structure of caribou that inhabit the Lake Superior Coastal Range (LSCR) and the level of gene flow between individuals within the range and beyond. From a landscape perspective, this range is spatially isolated and genetic connectivity within the range is presumed limited due to large water crossings on Lake Superior. This study aims to answer if animal movement can be discerned, using genetic population and relatedness analyses, within and beyond the LSCR. Faecal and hair samples collected between 2005 and 2015 in Pukaskwa National Park were analyzed for genetic markers and compared to 131 unique genotypes previously obtained from both within the LSCR and in the two next closest ranges. Animals from one nearshore island (i.e. Otter) were more closely associated with offshore islands than other mainland caribou, likely a result of past movement and translocation rather than ongoing movement. Conversely, on another nearshore island (i.e. Pic), individuals assigned to a different genetic cluster and were related to animals further north outside the range, demonstrating some connectivity through the discontinuous distribution to the coast. Long-term population declines have been observed in the LSCR despite genetic connectivity within the range and relatively low total habitat disturbance. Restoring connectivity of the LSCR so that it is not isolated from populations to the north is required for the recovery of the mainland portion of the coastal range. These genetic analyses provide some insights on where movements may occur and where landscape restoration efforts may best be directed to enhance connectivity.

**Key words:** population genetics; relatedness; connectivity; isolated populations; Lake Superior coastal range; woodland caribou; island biogeography; microsatellites.
Introduction
Habitat fragmentation and habitat loss is often responsible for the isolation of animal populations across landscapes, leading to lower effective population sizes and lower genetic diversity due to decreases in animal movement (Gaggiotti, 2003; Keyghobadi, 2007). Persistence of wide-ranging species of conservation concern in patchy habitat strongly depends on habitat quality and the ability of animals to move between habitat patches (Fahrig, 2003) as well as corridors for migration to allow for movement (Hale et al., 2001; Mech & Hallett, 2001). Therefore, the identification of dispersal events between source and isolated populations may aid conservation and habitat restoration efforts in gaining a better understanding of population connectivity and in determining favourable migration routes.

Boreal caribou (Rangifer tarandus caribou, COSEWIC, 2011) have inhabited the forests in and around Pukaskwa National Park (PNP) and the Lake Superior coast presumably since the last ice age. Over the past century, animals in the surrounding regions increasingly moved north in response to habitat change (Schaefer, 2003). The persistence of caribou in small numbers on the mainland portion of the Lake Superior Coastal Range (LSCR) is likely due to nearshore islands (i.e. within ~1 km of the mainland) that provide a means of escape from predators and safe parturition sites (Patterson et al., 2014; Bergerud et al., 2015), in addition to low total habitat disturbance (16%; Environment Canada, 2012). Today, an approximate 100 km distribution gap exists between the LSCR and the next closest distribution range. Now referred to as the “discontinuous distribution”, this area is being managed as a linkage to support temporary occupancy or movement between the continuous ranges to the north and the LSCR (Ministry of Natural Resources, 2009) (Figure 1). Between 1974 and 2009, the population in PNP, representing roughly one quarter of the most intact habitat in the LSCR, declined at approximately 4% per year and became increasingly isolated from neighbouring ranges (Patterson et al., 2014).

Although PNP’s population has recently been described as extirpated (Bergerud et al., 2015), an animal was observed in the north end of the Park in the spring of 2015 and an aerial survey completed in 2016 estimated that 55 (95% CI: 13-227) animals still inhabit the mainland and nearshore islands in the LSCR (Shuter et al., 2016). Until recently, two large offshore islands in the LSCR, the Slate Islands and Michipicoten Island, supported self-sustaining populations of caribou due in large part to being predator-free. Their far-from-shore distance (13 and 16 km, respectively), resulted in infrequent movement between the mainland/nearshore island portion of the LSCR and the offshore islands for both prey and predators alike, with crossings occurring irregularly in winters when adequate ice-bridges formed (Bergerud, 2001; Carr et al., 2012).

Restoring habitat within the LSCR and in the adjacent discontinuous distribution is necessary to recover the mainland coastal and nearshore island populations (herein after coastal populations) over the long-term (Gonzales et al., 2015). Focusing restoration efforts in areas where movement occurred historically could improve chances of recovery. However, our understanding of movement extent and pattern within and beyond the LCSR is limited. A collaring program in the 90’s showed one animal moving inland to the north >50 km (Neale, 2000), one animal being sedentary throughout the year staying on the calving island (Neale, 2000), and a few others moving south and east along the Lake Superior coast (Bergerud, 1985; Neale, 2000).

Here, we used genetic analysis from faecal material to examine population association and relatedness of individuals from the offshore islands, the Lake Superior coast, and the main-
land north of the discontinuous distribution (i.e., the Nipigon and Pagwachuan ranges) to provide information on movement and dispersal patterns. With this information, we characterized and spatially identified movement corridors that could be used to focus habitat restoration efforts.

Methods
Faecal pellet and hair samples were collected in PNP in the winter seasons of 2005, 2009, 2011 and early spring of 2015 (Figure 1). Samples \( n=28 \) were mainly collected on Otter Island, a nearshore island in the south end of the park regularly used for calving. One sample was collected on the mainland at the north end of the park in 2015. Otter Island samples were collected during aerial surveys being completed as part of PNP’s regular caribou monitoring program (Patterson et al., 2014). Faecal and hair samples were bagged and shipped frozen to Trent University for laboratory analysis.

In the laboratory, DNA was collected from the tissue present in the outer mucosal layer of each sample using a sterile cotton swab. A two-step digestion was carried out using 20 units of proteinase K (Roche Applied Science) with an incubation period of 2 hours at 65°C followed by a second incubation period (12 hours at 37°C) after adding an extra 20 units of proteinase K. A DNeasy Blood and Tissue Kit (QIAGEN) was used for DNA extractions following the manufacturer’s protocol. Samples were eluted in preheated (~70°C) 65.0 µl of 0.1 M TE buffer. DNA sample concentrations were determined by PicoGreen and samples were normalized to 2.5 ng/ul to ensure reliable amplification of samples.

Extracted DNA was amplified at nine microsatellite loci following Ball et al., (2007, 2010). Amplification reactions contained: 1x PCR buffer; 2.0 mM MgCl; 0.2 µg/ml of BSA; 0.4 µM of each primer pair; 0.2 µM of each dinucleotide triphosphate; 0.5 units of Taq polymerase (Invitrogen Life Technologies); and 5 ng of DNA template. The thermocycling protocol consisted of a denaturation step at 95°C
for 10 min, followed by 30 cycles of 94°C for 30s, an annealing step for 60 s at multiplex specific temperatures (Klütsch et al., 2016), and an extension period at 72°C for 1 min. A final extension time of 65°C for 15 min was added to complete extension of fragments.

All samples were scored by two independent scorers to check for atypical profiles and potential contamination. Allele scores were compared by an in-house database to check for scoring errors. Allele scores that showed any signs of above mentioned issues, were set to ‘-99’ to indicate that the locus was not scored. Only samples that had at least 8 out of 9 loci amplified were used for statistical analyses and unique individual identification.

Unique genotypes were identified using the program Allelematch 2.5 (Galpern et al., 2012) and COLONY 2.0.6.4 (Wang, 2016). The individual identification conducted with ALLELEMATCH and COLONY gave congruent results and only unique genotypes were used in subsequent analyses. PNP results were compared to 131 unique genotypes previously obtained from seven surrounding areas (Figure 1; Cochrane, Hearst, Kapuskasing, Nipigon, Pic Island, Michipicoten Island, and the Slate Islands) (Klütsch et al., 2016).

We calculated summary statistics (i.e., number of alleles (NA), observed (HO) and expected heterozygosity (HE), Fis estimates, and standard errors (SE)) with the program GENEALEX version 6.5 (Peakall & Smouse, 2012). The program HP-RARE version June 2006 (Kalinowski, 2004; 2005) was used to estimate allelic richness and private allelic richness with a rarefaction method to account for uneven sample sizes. The program GenePop 4.2.2. (Rousset, 2008) was used to test for linkage disequilibrium and heterozygosity deficiency and pairwise Fst and P values were calculated in Arlequin 3.5 (Excoffier and Lischer 2010).

The Bayesian clustering program STRUCTURE 2.3.4 (Pritchard et al., 2000) was used to assess the most likely number of population clusters (K) and to assign individuals to the inferred population. Run parameters included a burn-in of 1 x 10^6 and a MCMC chain of 1 x 10^7 as well as an admixture model with correlated allele frequencies (Falush et al., 2003) to test K = 1 to K = 15 with five iterations each. No a priori assignment of individuals according to sample location was included. We applied the ΔK method (Evanno et al., 2005) in the program STRUCTURE HARVESTER v. 0.6.93 (Earl & von Holdt, 2012) to identify structure at the highest hierarchical level. Additional STRUCTURE analyses within each first level clusters were ran to identify any substructure. The software programs CLUMPP v.1.1.2 (Jakobsson & Rosenberg, 2007) and DISTRACT v.1.1 (Rosenberg, 2004) were used to retrieve averaged individual and population membership q values.

In addition, the MEMGENE (Galpern et al., 2014) package in R (http://www.cran.r-project.org/) was used to visualize patterns of spatial genetic variation that may not have been detected with STRUCTURE. Moran’s eigenvector maps (MEM) were selected from the geographic locations of individuals and fit against genetic distance data to determine the amount of genetic variation (R^2 adj.) that can be attributed to spatial patterns.

Finally, we estimated relatedness relationships (i.e. full-sibling and parent-offspring relationships) with the program ML-Relate (Kalinowski et al., 2006) in order to assess whether there are potential close relationships between groups that would indicate recent gene flow.

Results
The program COLONY calculated an allele dropout rate ranging from 0.01 – 0.08. Most loci had an estimated genotyping error rate of 0, with two markers, BMS888 and RT5 having an error rate of 0.02 and 0.01, respectively.

Only 4/72 tests for Hardy-Weinberg devia-
tions showed significant heterozygosity deficits at the 0.05 level of which none remained significant after Bonferroni correction. Similarly, inbreeding coefficients (Table 1) were close to zero indicating that there were no signs of heterozygosity deficits. For linkage disequilibrium, 9/251 pairwise comparisons were significant at the 0.05 level but again none of those remained significant after Bonferroni correction.

According to the mean L(K) approach, four genetic clusters were identified providing a more detailed picture. The first cluster consisted of the Slate Islands, Michipicoten Island, and Pukaskwa as identified with the Evanno method. However, Pic Island was identified as a group together with Hearst and the one animal found in the north end of PNP in 2015. The remaining mainland populations were assigned to two different clusters with Cochrane and Kapuskasing in one cluster and Nipigon in a separate cluster. To confirm the structure found at K = 4, additional STRUCTURE analyses of each cluster (K = 2) were performed and confirmed the genetic clusters found with the mean L(K) approach (Figure 4). No additional clusters (K = 1) were identified in the STRUCTURE analysis of the second (red) cluster (PNP’s nearshore calving island (Otter Island), Michipicoten and Slate Islands).

The population genetic analysis revealed that mainland populations generally showed higher (private) allelic richness than offshore island populations (Table 1). These results are consistent with higher genetic distances (FST, Table 2 and 3) of island populations to mainland pop-

### Table 1. Summary of genetic diversity estimates, averaged across 9 microsatellite loci, for sampling sites. Number of samples (N), number of alleles (NA), allelic richness (AR), private allelic richness (ARP), observed and expected heterozygosity (HO, HE), FIS estimates, and standard errors (SE) for each of the estimates are given.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>NA</th>
<th>AR</th>
<th>ARP</th>
<th>HO</th>
<th>HE</th>
<th>FIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>COCH</td>
<td>25</td>
<td>7.11 (0.261)</td>
<td>3.6</td>
<td>0.6</td>
<td>0.72 (0.03)</td>
<td>0.74 (0.01)</td>
<td>0.03 (0.04)</td>
</tr>
<tr>
<td>HEAR</td>
<td>4</td>
<td>3.00 (0.373)</td>
<td>2.7</td>
<td>0.2</td>
<td>0.61 (0.13)</td>
<td>0.51 (0.07)</td>
<td>-0.18 (0.18)</td>
</tr>
<tr>
<td>KAPU</td>
<td>14</td>
<td>6.00 (0.236)</td>
<td>3.3</td>
<td>0.4</td>
<td>0.79 (0.04)</td>
<td>0.68 (0.02)</td>
<td>-0.16 (0.06)</td>
</tr>
<tr>
<td>NIPI</td>
<td>22</td>
<td>5.78 (0.40)</td>
<td>3.1</td>
<td>0.4</td>
<td>0.62 (0.02)</td>
<td>0.68 (0.02)</td>
<td>0.08 (0.04)</td>
</tr>
<tr>
<td>PNP</td>
<td>5</td>
<td>3.11 (0.35)</td>
<td>2.6</td>
<td>0.1</td>
<td>0.49 (0.07)</td>
<td>0.50 (0.06)</td>
<td>0.00 (0.09)</td>
</tr>
<tr>
<td>SLAT</td>
<td>46</td>
<td>3.89 (0.39)</td>
<td>2.7</td>
<td>0.1</td>
<td>0.62 (0.06)</td>
<td>0.59 (0.06)</td>
<td>-0.05 (0.02)</td>
</tr>
<tr>
<td>PIC</td>
<td>10</td>
<td>2.78 (0.28)</td>
<td>2.4</td>
<td>0.1</td>
<td>0.55 (0.09)</td>
<td>0.52 (0.05)</td>
<td>-0.04 (0.13)</td>
</tr>
<tr>
<td>MICH</td>
<td>10</td>
<td>3.00 (0.29)</td>
<td>2.4</td>
<td>0.1</td>
<td>0.54 (0.05)</td>
<td>0.52 (0.05)</td>
<td>-0.05 (0.06)</td>
</tr>
</tbody>
</table>

Of 28 samples collected in PNP, only 13 (46%) could be successfully amplified (12 of 26 faecal pellets and one hair sample) at 8 out 9 loci, belonging to five individuals. All animals were female (likely due to collection occurring for the most part on a calving island). Of the four individuals identified on Otter Island, each was observed in only one year with the exception of one animal that was present in both 2005 and 2009. STRUCTURE analysis on the complete dataset (n = 136) revealed a likelihood of 2 genetic clusters in these eight areas according to the Evanno method (Figure 2). The first cluster corresponded to the mainland (Cochrane, Kapuskasing, Hearst, Nipigon Hearst) and one nearshore island (Pic Island) (Figure 3a and b, green), and the second cluster corresponded to PNP’s nearshore calving island (Otter Island) and both offshore islands (Michipicoten and Slate Islands) (Figure 3a and b, red). One individual sampled in PNP (from 2015) was most closely genetically associated with the mainland animals further north (Figure 3a and b, green).
Figure 2. a) Mean likelihood for each K including standard deviation, and b) number of genetic clusters identified by the Evanno method (Evanno et al., 2005) as calculated with Structure Harvester v0.6.94 (Earl & von Holdt, 2012).

Table 2. Mean likelihood for each K including standard deviation and number of genetic clusters identified by the Evanno method (Evanno et al., 2005) as calculated with Structure Harvester v0.6.94 (Earl & von Holdt, 2012).

Discussion
These results show that boreal caribou on a nearshore island in PNP (sampled between 2005-2010) are genetically more closely associated with offshore island animals (i.e. the Slate Islands and Michipicoten Island) than mainland animals further north; whereas Pic Island animals are more closely associated with northern mainland populations outside of the LSCR than the offshore or nearshore island animals in PNP. The origin of the Slate Islands population is natural and believed to have been established in the 1940s after animals crossed over on an ice-bridge (Bergerud, 2001). The population from Michipicoten Island was established when eight animals were translocated from the Slate Islands in 1982, to supplement one bull that had moved naturally onto the island (Bergerud, 1985). As this was approximately only four generations ago (or perhaps as low as two generations if animals are living longer than average due a predator-free existence on the islands) (Thomas & Gray, 2002), it is not surprising that these two populations are genetically similar and that relatedness relationships are many (i.e. because the Michipicoten Island animals are descendants from Slate Islands animals).
mals). It has always been assumed that due to the distance between the offshore islands and the mainland coast, as well as the rarity of ice-bridge events on Lake Superior, there is little immigration/emigration. There are rare exceptions however, as in 2014 when animals sighted by local snowmobilers were seen crossing the ice in both directions (ca. 13 km) between the Slate Islands and the mainland (Kingston, unpublished reports). For the Slate Islands, the genetic results corroborate the rare observations of crossings, as only one animal originating from the Slate Islands was sampled on the coastal mainland in the Nipigon range (Figure 3b). Also, as indicated by the relatedness relationship, this animal had a parent off-spring relationship to an animal on the Slate Islands (Table 4a), indicating this may have been a relatively recent connection. Connectivity via rare ice-bridge events will likely become even rarer.
in future; total ice cover on Lake Superior has declined at approximately 2% per year since 1979 and this warming trajectory is expected to continue (Wang et al., 2012; Mason et al., 2016).

Although we found genetic association and parent/sibling relatedness between and within both offshore islands and Orter Island in PNP, we believe this is likely a founder affect rather than recent genetic connectivity; in the case of Michipicton as an artifact of translocation, and in the case of the Slate Islands, as a result of a one-time crossing event where only a few animals colonized the island. This is supported by the following three results: first, we did not see strong evidence of animals from offshore islands in the northern contiguous ranges (only one animal). Presumably if animals did cross at the rate suggested by the relatedness results, more related individuals would have found...
further north. Second, if animals had crossed from offshore islands to the mainland but remained close to the coast, we would most likely have seen them in areas along the coast from time to time, particularly if they were travelling between Michipicoten and the Slate Islands (ca. 200 km distance). This was not the case in PNP, where range recession has been documented over the last 40 years (Patterson et al., 2014), and animals were found in later years only in the highest quality habitat (i.e. calving grounds). Finally, there were no genetic associations between the offshore islands and Pic Island animals, an area where animals travelling between the offshore islands would have had to use, and presumably reproduce or be sampled there.

Given the above, the most plausible interpretation is that offshore island animals, as well as the Otter Island animals in PNP have remained relatively sedentary, restricted to their respective areas of Lake Superior and have not bred with other animals for many generations. Such philopatry was demonstrated with radio collared caribou in a 1996-97 research project in PNP where four animals made summer migrations from Otter Island up to ca. 50 km, then returned by rutting season, with one animal even staying year-round on Otter Island for three years (Neale, 2000). Signs of inbreeding in animals on Otter Island were observed by Park staff in the form of small and malformed antlers (Figure 6). This is a poignant observation given the last caribou to be photographed by remotely deployed wildlife cameras on Otter Island was in the winter of 2011 (unpublished data, Parks Canada).

Also interesting in our results is that Pic Island, a nearshore coastal island, shows high genetic differentiation relative to all other island populations in both the $F_{ST}$ and relatedness results, indicating that it is isolated from other is-
land populations but has some connectivity to mainland animals. The 2015 sample from the north end of PNP genetically associated with the Pic Island/Hearst group as shown in the STRUCTURE and relatedness results, likely wandering into PNP en route to or from those areas (perhaps not coincidentally, that same year there was extensive land-fast ice from Lake Superior being completely frozen over that may have facilitated movement from Pic Island). The Pic Island/Hearst group also had genetic association to one animal in the Nipigon range (Figure 4), which was supported by the relatedness analysis (Table 4b) that showed Pic Island having full sibling and parent-offspring relationships in this range. If animal movement between Hearst and Pic Island or Nipigon and Pic Island occurred, that would mean migrating through the discontinuous distribution, which includes a mosaic of disturbed/undisturbed habitat (e.g., small communities, forestry roads, mining developments, hydro corridors, etc.). Albeit a small sample size, these results point to some level of recent connec-
tivity between northern ranges and the LSCR (i.e. between Hearst and Pic Island/PNP and Nipigon and Pic Island/PNP). Restoration efforts directed towards establishing corridors or habitat may be most successful between these locations for recovery of mainland animals in the LSCR.

Conclusion
Boreal caribou have persisted along the Lake Superior coast despite being separated by a discontinuous distribution or gap from other populations of boreal caribou further north for many decades (Schaefer, 2003). Despite that the majority of habitat in the LSCR is undisturbed (Environment Canada, 2012), a large portion of this range has experienced a steady decline in the past 40 years (Patterson et al., 2014). Our study found two main genetic clusters in the LSCR: the first between one nearshore island (Otter Island in PNP) and off-shore islands, most likely a result of sedentary behaviour and translocation; and the second, between another nearshore island (Pic Island) and animals to the north, part of the mainland continuous distribution. These results indicate that gene flow has occurred relatively recently across the discontinuous distribution (i.e. a distance >100 km) and provide a clue to where restoration efforts focused on improving habitat connectivity may be successful. Our study also shows that without connection to a source population, the fate of the remaining animals in the mainland portion of the LSCR is unequivocally grim.

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Figure 6. Signs of inbreeding, such as small and malformed antlers, were observed in some of the last caribou captured on wildlife cameras deployed in Pukaskwa National Park.
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