Evaluating Genomic Diversity for Caribou in Alberta to Allow for Effective Biodiversity Monitoring, Augmentation and Conservation

Final Report, New Results Only (Year 2017), AUPRF project

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Methodology

Sampling and study area

Archived samples of blood, muscle and skin of about 800 individual caribou were obtained from capture and hunting activities across western North America, representing various recognized caribou taxonomic identities: i) the Alaska caribou subspecies, ii) the Barren-Ground subspecies from eastern Northwest Territories, and iii) the Northern Mountain, Central Mountain, Southern Mountain and Boreal ecotypes of the woodland subspecies from British Columbia, Alberta, Yukon Territories, western Northwest Territories and central and eastern Canada (Fig. 1). Here we report on analyses of British Columbia and Alberta caribou, by comparing to the other populations.

Genomic data

Our genomic dataset consisted in sequencing of pools of individuals (Pool-seq)--an approach that provides genome-wide polymorphism data at considerably lower costs than sequencing of individuals (Schlötterer *et al.*, 2014). Genomic DNA was extracted from an average 15 individuals per spatially distinct population (Appendix I) using phenol-chloroform extraction protocol (Sambrook *et al*., 1989). Equal amounts of DNA from each individual were subsequently pooled for library preparation, which was carried out following Baird *et al.* (2008) and Etter *et al.* (2011). DNA libraries were sequenced on one lane of the Illumina HiSeq 2000 platform for 100-bp paired-end reads. We finally obtained genomic data for 18 pools (14 representing caribou populations and 4 formed by either sedentary or migratory individuals from the same geographical area – Central Rocky mountains) sequenced in two libraries.

Signature of natural selection – Population genomics approach

An empirical F_{ST} approach (Akey *et al.*, 2010; Feulner *et al.,* 2013) was used to identify SNPs potentially under selection among the 14 pools representing caribou spatially distinct populations and the four pools formed by migratory or sedentary individuals. When analyzing caribou populations using the empirical F_{ST} approach, SNPs were considered as outliers (under selection) when falling into the upper 0.5% tails of at least 10 of the 91 pairwise F_{ST} comparisons. When analyzing migratory vs. sedentary pools, SNPs were considered as outliers when falling into the upper 0.5% tails of at least one of the five pairwise F_{ST} comparisons.

Signature of natural selection - Landscape genomics approach

We explored association between SNPs and environmental variables only for 14 caribou populations since the pools formed by either sedentary or migratory individuals were from the same geographical area with similar environmental conditions. We first selected a set of 12 environmental layers: altitude, snow cover of October and November, Normalized Difference Vegetation Index (NDVI) of July and August, five variables representing temperature, three representing precipitation, land cover classes of 2010, and percentage of forest loss (for details Appendix II). A Zonal statistics (ARCGIS 10.3.1) was applied on continuous layers (all except land cover) to average the values of each variable across caribou ranges forming each pool. For each pool, the percentage of land cover classes was also calculated, and we used the R package "LABDSV" to conduct a PCA of these classes. The loadings of the first principal component were used for subsequent analyses. All variables were associated to the centroids of the caribou ranges forming each pool that was also used to estimate geographic coordinates.

To test the association of SNPs to environmental variables we used a Bayesian approach as implemented in BAYPASS 1.0 (Gautier, 2015) under the standard covariate model. We first

calculated the covariance matrix (Ω) of pools, then subsequently estimated the Bayesian Factor

(BF) for each SNP as a measure of the correlation between the marker and each environmental

factor. Jeffreys' rule was used to quantify the association between SNPs and environmental

variables: "strong evidence" when 10<BF<15, "very strong evidence" when 15<BF<20 and

"decisive evidence" when BF>20 (Jeffreys, 1961).

Populations structure, organization of ecotypes and differentiation of migratory vs. sedentary caribou

We determined population structure of the 14 caribou pools on the basis of the pairwise

F_{ST} matrices, estimated by POPOOLATION2. To visualize the multi-locus patterns of population

differentiation, a Principal Coordinate Analysis (PCoA) plot was generated using the R package

labdsv (http://ecology.msu.montana.edu/labdsv/R/) based on average F_{ST} values. Population

structure was also determined on the basis of a covariance matrix on of population allele

frequencies. Finally, a Principal Coordinate Analysis plot based on F_{ST} comparison across 14

populations was also conducted examining only SNPs potentially under selection. Patterns of

genetic differentiation between migratory vs. sedentary caribou were examined by estimating

pairwise F_{ST} values. F_{ST} values between migratory vs. sedentary pools were also compared to

values estimated across caribou populations.

We used previously developed nonlinear movement modelling methods (Bunnefeld *et al.*, 2011)

to classify migratory behaviour of all individual caribou (DeCesare *et al.*, 2012). Briefly,

individual caribou were defined as migratory or sedentary based on ungulate migration and their

movement between seasonally nonoverlapping, allopatric ranges (Craighead *et al.*, 1972). The

overlap between summer ranges (1 July– 15 September; Dyer *et al.*, 2001, 2002) and winter

ranges (1 December–30 April; Smith *et al.*, 2000) for collared individuals was calculated.

Caribou were considered migratory when showing nonoverlapping ranges, and sedentary when ranges overlapped seasonally (McDevitt *et al.*, 2009).

Results

Environmental factors and candidate SNPs under selection in caribou populations

Using the empirical outlier detection approach (Population genomics approach), 28 SNPs were found at least once in the top 0.5% of pairwise F_{ST} values between populations, suggesting selection. Using the program BAYPASS (which uses Bayesian statistics and correlation to environmental variables – Landscape genomic approach), a total of 54 SNPs (four of which also found as outliers with empirical F_{ST}) were shown as being associated with environmental factors (Supporting information 2), also suggesting selection. Out of the 54 SNPs, 36 were associated with a variable and 18 with multiple variables; whereas variables could be associated with multiple SNPs. The variables associated with the greatest number of SNPs were minimum temperature of coldest month (n=15 SNPs), temperature seasonality (n=12), precipitation of coldest quarter (n=11), and annual precipitation (n=10). Snow cover and altitude were associated with eight SNPs, whereas mean annual temperature, maximum temperature of warmest month, mean diurnal temperature range, precipitation seasonality and NDVI (defined above) were associated with fewer SNPs. We also found two SNPs associated with percentage of forest loss.

Population structure and organization of ecotypes

The organization of ecotypes and their relationships were remarkably clarified by a covariance matrix of population allele frequencies resulting from their (possibly unknown and complex) shared history. Its Hierarchical Tree Cluster (Fig. 2) clearly indicated separation of Barren-Ground, Alaskan and Northern Mountain populations; the other populations were on a separate branch. The latter was also divided into Boreal groups and, on another sub-branch, Central Mountain and Southern Mountain populations. The only exception to this clear organization was

the Little Smoky population (Boreal ecotype in theory, but of disputed assignment) as this was together with Central Mountain and Southern Mountain. However, when candidate SNPs under selection were used (including outlier SNPs obtained with the empirical F_{ST} approach and environmentally correlated SNPs), a PCoA plot indicated differentiation of some populations only, including Pool 1 (Banff and Jasper), Pool 18 (Columbia South, Duncan, Kinbasket and Purcell) and Pool 13 (Redstone/North West Ter.), likely reflecting local patterns of selection (Fig. 3) .

Differentiation of migratory and sedentary caribou

Pairwise F_{ST} values across the genome yielded an overall average pairwise F_{ST} estimate of 0.08644 (SE = 0.00408) between pools of individual caribou that performed migratory vs. sedentary behavior (2 replicate pools for each), while belonging to the same or to different populations (range = 0 to 0.47693; median = 0.08488). Using the empirical F_{ST} approach, we identified 155 outlier SNPs potentially under selection.

Discussion

Existence of genetic markers under selection between caribou populations

Some caribou populations are declining and nearing extinction at the moment, while other populations became extinct recently, for example the Banff population (Hebblewhite *et al.*, 2009, 2017). It is therefore important to determine local patterns of selection, to understand whether these too might be lost as populations disappear (Hoffman & Willi 2009, Allendorf *et al.*, 2010). Our results indicated existence of candidate SNPs under selection in caribou populations. In addition, double the number of SNPs were shown as being associated with environmental factors and also under selection. Our results therefore supported selection of SNPs in the spatially distinct populations that we sampled in Alberta and British Columbia in this project and compared to populations ranging from Alaska to the southern Canadian Rockies. This finding was consistent with our staple hypothesis that populations were subject to different environmental conditions (we strategically sampled areas remarkably different environmentally [Fig. 1], within the uninterrupted distribution of the species) and could hold gene to environment associations under selection.

Climate and environment as candidate drivers of genetic divergence between caribou populations

The variables associated with the greatest number of SNPs were all climatic including temperature indices, precipitation indices and altitude (which could obviously be correlated with climatic variables). Other non-climatic variables were also significantly associated with SNPs (although with fewer) including Normalized Difference Vegetation Index (NDVI, an index of plant productivity - Pettorelli *et al.*, 2011; Guay *et al.*, 2014) and forest loss (Hansen *et al.*, 2013). Our findings therefore confirmed that different populations of caribou lived and perhaps

are adapted to different climates and different plant communities (Schoville *et al.*, 2012). Caribou is known to have evolved in extremely cold, iced, and harsh environments, and evolution of specific traits have been proposed to cope with such conditions (Timisjarvi *et al.*, 1984; Geist 1998; Hummel & Ray 2008) . A thick insulating coat, feet suitable to walk on ice and snow, hairs covering muzzles as well as tails and feet bottom, specialized digestive and urinary metabolisms are just few example of those traits. Glacial interglacial cycles and successive post glacial expansions also allowed caribou to live in different environments, sometimes less harsh (e.g. boreal forests). In addition, dramatically different environments exist also along the Southern Rockies of Alberta and British Columbia, where caribou ranges include boreal forest and alpine tundra in different proportions. Evolutions of traits under selection might therefore have occurred to let caribou survive in different environments (McDevitt *et al.*, 2009; Weckworth *et al.*, 2012) and the allelic variation along a cline of our SNPs supports this idea.

Ecotype organization confirms COSEWIC's with some exceptions

Our work could help defining caribou ecotypes based on concordant distributions of traits of adaptive significance (potentially), including genetic and behavioural variation (migratory, partially migratory or sedentary groups), therefore adhering to COSEWIC's designations (COSEWIC 2011). At finer scale, caribou ecotypes were also defined based upon differences in foraging ecology: on terrestrial or arboreal lichens (COSEWIC 2011). This study's genetic information supports such distinctions with a notable exception. Our findings supported separation of Barren-Ground, Alaskan and Northern Mountain populations; the other populations were on a separate branch (Fig. 2). The latter was also divided into Boreal groups and, on another sub-branch, Central Mountain and Southern Mountain populations. However, the only exception to this organization in our study was the Little Smoky population as this was together

with Central Mountain and Southern Mountain (Fig. 2 – see below).

Southern Mountain, Central Mountain and Boreal (Albertan) ecotypes organization

In our study, Pool 18 (Columbia South, Duncan, Kinbasket and Purcell) and Pool 1 (Banff and Jasper) were found in the same group, whereas they are designated into two separated DUs – ectypes- (Central and Southern Mountain) according to COSEWIC (COSEWIC 2011). However, when considering candidate SNPs under selection (Fig. 3), genetic distance between these two pools was evident (although both were at one end of the variation spectrum), indicating, perhaps, possible adaptations to distinct habitats, which would support COSEWIC's interpretation of DUs. Indeed Pool 1 and Pool 18 are known to have different feeding strategies (relying on terrestrial vs. arboreal lichens), which are needed to cope with different snow conditions (shallow and deep snow, respectively), and are also known to have different movement ecology (Pool 1 conducting seasonal altitudinal migration, and Pool 18 remaining at high altitude all year round). Therefore our results indicated that COSEWIC's designations found meaning when selective patterns were accounted for.

Pools 1 and 18 (defined above) were close to Central Mountain pools (Pools 5 and 6 formed by individuals from local populations: A La Peche, Redrock-Prairie Creek, Narraway, Red Willow and Quintette, Kennedy, Moberly, Scott and Parsnip). However, Pool 5 and 6 were also in the same genetic group of Pool 4 (Little Smoky) of Boreal ecotype, in theory.

Therefore, the only exception to this organization in our study was the Little Smoky population as this was together with Central Mountain and Southern Mountain populations (Fig. 2). Interestingly, this population is of Boreal ecotype in theory, but of disputed assignment, because for example some caribou do migrate and because there is gene flow with Central Mountain populations (McDevitt *et al.*, 2009; Weckworth *et al.*, 2012; Gubili *et al.*, 2016).

Other Boreal pools from Alberta (Pools 9, 10, 11 and 12; Fig. 2) formed a distinct branch, and

this organization was somehow confirmed when analyzing candidate SNPs under selection, as all

these populations were at one margin of the distribution (Fig. 3).

Genetic differentiation of migratory and sedentary caribou

Our results indicated differentiation between migratory and sedentary caribou, regardless of

populations belonging. The overall average pairwise F_{ST} estimate was about double when the

sample was divided into migratory and sedentary individuals (while belonging to the same or to

different populations) than into distinct populations. All the outliers SNPs were also uniquely

found in the pool of caribou with known movement behaviour (if migratory or not) and did not

correspond to the outliers found in distinct populations.

Management implications

- A fundamental step toward caribou management and conservation is the delineation of caribou DUs based upon distinctiveness criteria. However, some inconsistencies were identified in the past and further investigation based upon genomic criteria too was demanded (COSEWIC 2011). Our population structure analysis supported DUs designation when considering all the available SNPs with few exceptions, indicating the robust basis of DUs designation according to COSEWIC but also the possibility of refinement. The Little Smoky population of caribou does not seem to be of typical boreal type, while other patterns for other Alberta and British Columbia populations are less extreme.

- Our findings also inform plans regarding biodiversity monitoring and augmentation. Biodiversity monitoring should continue for the units so far delineated, with a particular focus on populations that have some divergent characteristics from their DU, for example the Little Smoky. In the future, if augmentation actions will be considered, ideal source populations will be those that are closer genetically (information available from this study and others) and ecologically (see Discussion above for concordance with ecological traits).

- Our findings indicate that unique genetic traits under selection, including those underlying migratory behavior, might exist in different caribou populations. These findings have conservation applications. For example, previous data revealed that landscape resistance (of inhospitable habitat), geographic distances and predation risk were correlated with genetic distances, with correlations threefold higher for sedentary than for migratory caribou (Gubili *et al.*, 2016). Thus, sedentary caribou are in theory

more affected by population isolation. Our study indicates that these caribou might harbor unique genetic traits that might get lost in the process (e.g. due to drift or local extinctions). Overall, preserving connectivity could be key to maintaining unique gene to environment associations and biodiversity within the species (Allendorf *et al.*, 2010; Harrison *et al.*, 2014).

- Management and conservation plans have long been applied, nonetheless caribou are still declining (Hervieux *et al.*, 2013) and doubts on their effectiveness are arising (Hebblewhite 2017). Triage solutions are starting to be proposed with the new challenge being to identify specific thresholds to determine which populations can be saved and which cannot (Schneider *et al.*, 2010; Hebblewhite 2017). If management will move forward to triage, our information on traits under selection can help establishing thresholds by being incorporated in demographic models to determine current and future pattern of decline (Hoban *et al.*, 2012; Harrison *et al.*, 2014; Pacifici *et al.*, 2015).

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