

Population size and major valleys explain microsatellite variation better than taxonomic units for caribou in western Canada

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Abstract

Identifying conservation units below the species level is becoming increasingly important, particularly when limited resources necessitate prioritization for conservation among such units. This problem is exemplified with caribou, a mammal with a circum-Arctic distribution that is exposed to a broad spectrum of ecological conditions, but is also declining in many parts of its range. We used microsatellite markers to evaluate the suitability of existing intra-specific taxonomic designations to act as population units for conservation and contrasted this with landscape features that were independent of taxonomy. We also quantified the relationship between genetic differentiation and subpopulation size, a factor that has been under-represented in landscape genetic research. Our data set included three subspecies and three ecotypes of caribou that varied in population size by five orders of magnitude. Our results indicated that genetic structure did not correspond to existing taxonomic designation, particularly at the level of ecotype. Instead, we found that major valleys and population size were the strongest factors associated with substructure. There was a negative exponential relationship between population size and F_{ST} between pairs of adjacent subpopulations, suggesting that genetic drift was the mechanism causing the structure among the smallest subpopulations. A genetic assignment test revealed that movement among subpopulations was a fraction of the level needed to stabilize smaller subpopulations, indicating little chance for demographic rescue. Such results may be broadly applicable to landscape genetic studies, because population size and corresponding rates of drift have the potential to confound interpretations of landscape effects on population structure.

Keywords: caribou, ecotype, genetic drift, landscape genetics, metapopulation, population size, *Rangifer tarandus*

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Introduction

In the current era of rapid loss of biological diversity (Sala *et al.* 2000), designating population units for conservation below the species level continues to gain importance (Moritz 1994; Crandall *et al.* 2000; Fraser &

Bernatchez 2001; Green 2005), particularly when decision-makers are forced to prioritize which units to conserve (Schneider *et al.* 2010). The Evolutionarily Significant Unit (ESU) is a common term used to describe groups of organisms that have undergone sufficient genetic divergence from conspecifics to represent unique and significant adaptive potential relative to the species as a whole (Ryder 1986). How to designate these units continues to be debated (Moritz 1994;

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Paetkau 1999; Fraser & Bernatchez 2001), yet recognized subspecies, varieties and ecotypes (*sensu* Turesson 1922) are often candidates for unique recognition.

Although existing taxonomic units can act as a basis for listing, in most cases, there is incomplete genetic information to validate these designations. To deal with this uncertainty, additional criteria were developed to define units, including demographic or geographic isolation, bio-geographic uniqueness and conservation status (Green 2005). Variants of these criteria have been reflected in several legal frameworks (Green 2005). Given the diverse approaches used to define intra-specific units, it has become increasingly important to understand the geographic and ecological factors that influence population genetic structure, so that population units and corresponding conservation status can be appropriately defined. By contrasting the relative influence of geographic features (mountain ranges, valleys or distance) with existing taxonomic designations, we can learn whether current classifications are appropriate or whether new ones should be considered.

Gauging the importance of existing taxonomy and landscape features is complicated by population size, a factor that is particularly relevant when dealing with endangered species. Among larger populations, where the rate of genetic drift is low, classical genetic methods would not be sensitive to connectivity declines in the last two or three generations, but in smaller populations one might encounter significant changes in allele frequencies from one generation to the next (Wright 1931). The high rate of genetic drift in small populations might mask the historic contributions of taxonomic boundaries or landscape features to population structure. To date, most landscape genetics studies have incorporated factors that are limited to geographic correlates (Manel *et al.* 2003) and may not have considered how population size could affect these interpretations.

Caribou (*Rangifer tarandus*) in North America are a good example of the challenges faced when identifying conservation units. They have multiple subspecies designations, are broadly distributed across a variety of ecosystems and exist in subpopulations that range in abundance over five orders of magnitude, many of which are in rapid decline (Wittmer *et al.* 2005a; Festa-Bianchet *et al.* 2011). Caribou are distributed from treeless deserts of the high arctic (<20 cm precipitation/year) to temperate rainforests where precipitation exceeds 200 cm/year, with most falling as snow (14 m/year). These climatic extremes are matched by differences in foraging and antipredator behaviour (Bergerud *et al.* 2008) and influenced their taxonomic classification. Subspecies in North America include migratory Peary (*R. t. pearyi*) and barren ground (*R. t. groenlandicus*; Banfield 1961), animals that spend

at least part of the year north of treeline, and woodland caribou (*R. t. caribou*), which live in forested ecosystems. In addition, there are three recognized ecotypes of woodland caribou in western North America (Heard & Vagt 1998): (i) Boreal caribou, which are found across the boreal forest and dig through shallow snow to access terrestrial lichens during winter, (ii) Shallow-snow mountain caribou, which spend most of the year in mountains and also dig through shallow snow for terrestrial lichens, either on mountain ridges or adjacent dry forests and (iii) deep-snow mountain caribou, which remain high on the mountains during winter where they walk on top of a very deep snowpack (2–5 m) to access arboreal lichen in the canopy of conifers (Seip & McLellan 2008). The latter group's exclusive use of arboreal lichens has led to their designation as a unique ecotype (Edwards *et al.* 1960), but it remains unknown whether there is genetic support for this classification. This ecotype is the most endangered of the three, where 20 years of radio-telemetry has revealed 18 subpopulations (Wittmer *et al.* 2005a), two having recently been extirpated and another 11 numbering <100 individuals.

Our goal with this paper was to present caribou as case study to evaluate existing taxonomy using microsatellite markers, but to contrast the magnitude of this pattern with population size, a mechanism that can clearly influence spatial structure. Our specific objectives were threefold. The first was to assess existing classifications of subspecies and ecotypes using microsatellite alleles and to contrast taxonomy with landscape features that were independent of taxonomic designation. If allele frequencies corroborate the taxonomy, then assessing conservation status for each of these groupings is supported. The second objective was to investigate the relationship between genetic differentiation (F_{ST}) and population size. We expected that smaller populations would be more genetically distinct relative to their neighbours compared with pairs of populations that were large, while controlling for geographic distance. If this result was confirmed, then population size would be of importance to incorporate into estimates of spatial population structure. Our final objective was to determine whether the structure estimated by microsatellite markers was supported by tracking a large proportion of the endangered deep-snow ecotype using radio-telemetry. Comparing estimates of movement using marked individuals relative with indirect estimates of gene flow (e.g. Ehrlich *et al.* 1975; Slatkin 1987) can be revealing because indirect estimates will lag behind contemporary barriers to movement, particularly for larger populations where the rate of drift is lower. Yet, if the population genetics confirm the structure identified using radio-telemetry,

the implication is that the structure is older, perhaps predating recent anthropogenic landscape changes. Thus, contrasting the two approaches provides more complete understanding of the timing and thus mechanisms governing population structure (Boulet *et al.* 2007; McDevitt *et al.* 2009).

Materials and methods

Sample collection

Our samples encompassed three of the world's eight recognized caribou/reindeer subspecies (Flagstad & Røed 2003): Peary, barren ground and woodland. These samples represented a broad diversity of ecosystems in western North America, ranging from northern Idaho, USA, to Bathurst Island, Nunavut, Canada, spanning a distance of >3000 km. This area included wet, old-growth forests of the Columbia Mountains where natural disturbances are rare, drier forests from the Rocky Mountains where fires are relatively common, boreal forests where fires are also frequent but the topography is subdued and areas north of treeline to the high Arctic.

We collected blood and tissue samples during caribou live capture as part of our previous research on deep-snow caribou (e.g. Wittmer *et al.* 2005a). Additional faecal samples were collected during winter within 48 h of deposition by swabbing the surface with cotton swabs. Samples from northern and western British Columbia and barren ground caribou were from tissues from hunter harvest, and samples from Peary caribou were from antler sheds. Samples from the boreal ecotype and the Purcells were provided by McLoughlin *et al.* (2004) and Zittlau (2004), but we increased the number of genotyped markers to 18.

DNA was extracted using DNeasy kits from Qiagen using their specified methods. Faecal swabs were clipped and treated as other tissue samples. Hair samples were processed by clipping roots from up to 10 hairs and then analysed following standard protocols (Paetkau 2003). Analysis of microsatellites used ABI's detection system on 310 automated sequencers, while genotypes were scored using Genotyper software (ABI). Genotype profiles were assigned a low-confidence score if they failed to satisfy thresholds for legibility and strength. The use of these thresholds has been shown in other studies from both hair and faecal samples to produce initial rates of genotyping error well below 1% per locus (Kendall *et al.* 2009; Poole *et al.* 2011). As per Paetkau (2003), samples that produced low-confidence scores for <50% of markers on the first attempt at analysis were culled from the data set based on the logic that their low DNA concentration would elevate rates of genotyping error (Taberlet *et al.* 1996). In other cases,

low-confidence scores were re-analysed to confirm the weak initial result, using 60% more template DNA than during the first attempt. Samples that still had low-confidence scores for >1 marker after this phase of re-analysis were also culled from the data set, once again eliminating the most error-prone samples. Eight PCRs were used to amplify the 18 loci. Markers used were Rt1, Rt5, Rt6, Rt7, Rt9, Rt24, Rt27 (Wilson *et al.* 1997), BL42, BM4513, BM6506 (Bishop *et al.* 1994), BMS1788, BMS745 (Stone *et al.* 1995), CRH (Moore *et al.* 1992), FCB193 (Buchanan & Crawford 1993), NV16, NV30 (Røed & Midthjell 1998), OhemD and OhemQ (Jones *et al.* 2000). We tested each marker for deviations from Hardy-Weinberg equilibrium and for linkage disequilibrium using Genepop v. 4.0.10 (Raymond & Rousset 1995).

Genetic structuring among subspecies and ecotypes

We conducted population genetic analyses at several spatial scales. The broadest scale included samples from all three caribou subspecies and the three ecotypes of the woodland subspecies. We used the program FITCH (Fitch & Margoliash 1967) within the PHYLIP 3.69 package (Felsenstein 2005) to represent a matrix of F_{ST} values as a bifurcating, hierarchical 'tree'. Within the tree we differentiated subspecies and ecotypes, and along with a map this enabled broad comparisons of genetic and geographic distances in relation to subspecies and ecotypes (Appendix S1, Supporting information represents F_{ST} divided by geographic distance). We included all available Peary and barren ground subpopulations within our sample area, but selected a subset of woodland subpopulations that were representative of their geographic area (i.e. centre of the range or geographic endpoints) to reduce complexity in the tree. Therefore, this analysis included one Peary caribou subpopulation (Bathurst Island), three barren-ground subpopulations (Southampton, Dolphin & Union and Qamanirjuaq), six deep-snow mountain subpopulations (Hart, North Cariboo, Wells Gray, Columbia North, Columbia South, Purcells), five shallow-snow mountain subpopulations [Jasper, Graham and Kennedy/Quintette/Moberly (the latter three were grouped; McDevitt *et al.* 2009 and see Results)] and five boreal subpopulations. Jasper samples have been classified as separate herds (Tonquin and Maligne) but were treated as one because they were similar based on microsatellite markers (McDevitt *et al.* 2009). The Southampton samples were from a caribou population restricted to an Island in Hudson Bay that was founded from 48 caribou that were transplanted from Coates Island (also *R. t. Groenlandicus*) in 1968. We excluded two deep-snow subpopulations that received transplants (Monashee South and South Sel-

kirk) because they originated from different ecotypes and therefore may confound interpretation.

Our second scale of investigation was restricted to one subspecies, woodland caribou from British Columbia and Alberta, but included samples from all putative subpopulations for which we had data, including those from the previous analysis. The additional subpopulations included 12 shallow-snow mountain herds (Atlin, Horseranch, Level-Kawdy, Rabbit, Muskwa, Frog, Tsenaglode, Pink Mountain, Wolverine, Spatsizi, Tweedsmuir, Itchas-Ilgatchuz) and five deep-snow mountain subpopulations (Barkerville, Frisby-Queest, Groundhog, Kinbasket, Central Selkirks/Duncan, South Selkirks), totalling 12 of the 18 deep-snow subpopulations identified by Wittmer *et al.* (2005a). To increase sample size, Horseranch and Level-Kawdy were grouped, as were Spatsizi and Tsenaglode (these were large herds with contiguous ranges).

We used the program STRUCTURE (Pritchard *et al.* 2000), which assumes no a priori group membership, to identify groups of individuals. STRUCTURE runs had a burn-in of 100 000, a Markov Chain of 800 000 and were based on the admixture model. We tested for K (the number of populations) from 1 to 10 and repeated these 15 times. We used the methods of Evanno *et al.* (2005) to estimate the number of populations (K) that best describes the system under study. We then plotted the population membership of each individual on a map that outlined previously assumed ecotype designations. For each individual, STRUCTURE provides the estimated proportion of ancestry in each putative population (q), so we categorized individuals based on their mean value of q (0–0.6, 0.6–0.8, >0.8) and report the proportion of assignments in each cluster where $q > 0.8$, as an index of cluster strength.

We used mantel analyses to test the hypothesis that ecotypes within the woodland subspecies were genetically distinct units, while controlling for geographic distance. This test measures the variance of alleles among subpopulations relative to the total variance (similar to F_{ST} variance). Rousset's (1997) genetic distance ($F_{ST}/(1 - F_{ST})$) was the response metric. Major landscape features were included as covariates and included the North Thompson Valley, the Peace River Valley, the Fraser River Valley and the Rocky Mountains (Table 1). All analyses involving landscape features or ecotype membership were based on a matrix created for each covariate, with 1s indicating pairs of subpopulations that were on the same side of a landscape feature or ecotype membership and 0s indicating pairs of populations that did not share that feature. This coding ensured that if a feature was stronger than the effect of geographic distance (if distance was indeed a factor), the mantel r -value would be negative.

Table 1 Description of landscape features within the study area and included in the mantel, multiple regression and AMOVA analyses

Landscape feature	Description of features within the study area bounds
Rocky mountains	Mountain range that varies from 874 m to 3954 m in elevation, with adjacent valleys as low as 600 m.
North Thompson Valley	Broad valley surrounded by mountains and includes the North Thompson river, Highway 5, a railway, and several settlements with <10 000 people.
Fraser Valley	Broad valley surrounded by mountains and includes the Fraser river, Highway 16, a railway and several settlements with <10 000 people.
Peace River valley	Broad valley including the Peace River, with a major dam and reservoir in British Columbia, free flowing in Alberta but with extensive agriculture.

We combined the mantel analyses with multiple regressions (Legendre *et al.* 1994) to clarify the importance of individual factors and present r^2 estimates using Legendre *et al.*'s (1994) method. Mantel and regression analyses were performed using the ecodist package (Goslee & Urban 2007) in R (R Development Core Team 2008). We also used Program Arlequin version 3.11 (Excoffier *et al.* 2005) because this software partitions variance within subpopulations (F_{ST}), among ecotypes (F_{CT}) and subpopulations within ecotypes (F_{SC}). As a post hoc test, we used Arlequin and grouped caribou by landscape features (instead of ecotype) identified as important from the mantel and MR analyses. This was done to act as a check of the mantel and MR analyses, but also to compare the AMOVA-based variance explained from the ecotype (F_{CT}) grouping, to groupings based on landscape features (F_{CT}). Arlequin was also used for F_{ST} estimates with 1000 permutations for significance tests, and all values presented are significant ($P < 0.05$) unless specified (P -values are in Appendix S2, Supporting information). If subpopulations contained fewer than five samples, they were not included in mantel or Arlequin analyses (McDevitt *et al.* 2009).

Effects of population size on differentiation and heterozygosity

We conducted two analyses using population size to predict genetic patterns. First, we investigated how population size could affect differentiation among neighbouring subpopulations. For each subpopulation,

we selected the subpopulation that was its nearest geographic neighbour, calculated Rousset's F_{ST} divided by the geographic distance between population centres and used this index of geography-adjusted genetic distance as the dependent variable. The independent variable was the harmonic mean population size between pairs, which is commonly used in genetics because it weighs smaller populations more heavily (Hartl & Clark 2007). We did a permutation test by treating the subpopulation as the sample unit (because it may occur >1 in nearest-neighbour comparisons), similar to a mantel test, to estimate the uncertainty of this relationship. Our second analysis was to quantify the relationship between expected heterozygosity (H_E) and population size. We used the nls function (nonlinear regression) in R, weighting each subpopulation by the inverse of the H_E variance, and bootstrapped subpopulations to estimate whether the slope was different from 0. Both analyses used data from all subspecies and ecotypes within our sampling area, except those that received transplants (Monashee, South Selkirk, Southampton) or those with few samples (<5). In cases where our sample collection covered a period of time when populations changed rapidly, the mid-point of the subpopulation size was used.

Genetic structuring and migrant estimation within deep-snow mountain caribou

To determine whether population genetics supported the population structure of deep-snow caribou estimated by Wittmer *et al.* (2005a) using telemetry, we used program STRUCTURE with the same simulation parameters as mentioned earlier but included one neighbouring outgroup for comparison (Jasper, a shallow-snow ecotype that occurs adjacent to the deep-snow range). We also sought to estimate the number of first-generation migrants using an assignment test (Pritchard *et al.* 2004) with GeneClass 2.0 (Piry *et al.* 2004) to determine the chance of demographic rescue of smaller and declining subpopulations. This analysis was limited to estimating movement from the larger northern populations to the smaller southern populations of this ecotype of woodland caribou. However, such an analysis was only possible if there was sufficient genetic structuring to assign individuals to their natal population, so we conducted this analysis post hoc after we completed the STRUCTURE analyses and if there was structure between more abundant northern and less abundant southern subpopulations.

To clarify the population structure of caribou at the ecotone between the deep- and shallow-snow ecotypes of mountain caribou in British Columbia, we used factorial correspondence analysis (FCA) with program

Genetix (Belkhir *et al.* 1996). FCA is similar to principal components analysis and provides an objective depiction of groupings of similar individual genotypes with no a priori assumptions of group membership. We included four shallow-snow subpopulations (Burnt, Graham, Quintette, Moberly and Kennedy) that were nearby or adjacent to the northern distribution of deep-snow mountain caribou (Wells Gray, N. Cariboo Mountains and Hart subpopulations).

Results

A total of 606 unique genotypes were identified, 590 from blood or tissue and 16 from scat (all scat samples were from the deep-snow ecotype). We had 20, 149 and 437 genotypes from Peary, barren ground and woodland samples, respectively. Sample sizes from each subpopulation are listed in each figure where appropriate. None of the 18 markers showed significant deviation from HWE. There were 127 cases of linkage disequilibrium out of 2753 comparisons (4.6%; 18 markers across subpopulations) which is expected by chance at a critical value of 0.05.

Genetic structuring among subspecies and ecotypes

At the broadest scale of analysis, only the Peary subspecies formed a distinct clade ($F_{ST} = 0.07$ with its nearest neighbour) in the phylogenetic tree; all other subspecies and ecotypes were interleaved (Fig. 1). The genetic distance between some adjacent deep-snow mountain caribou subpopulations were much larger than differences among ecotypes and even among subspecies (Fig. 1). For example, the genetic distance of the Purcell subpopulation to its neighbours <250 km away ($F_{ST} = 0.15$) was greater than between some of the tundra/taiga herds that were separated by >1000 km (i.e. Peary to Qamanirjuaq $F_{ST} = 0.07$; Fig. 1). Even the directly adjacent deep-snow mountain subpopulations of Columbia North and Columbia South (5 km of separation, $F_{ST} = 0.04$) were more genetically distinct than large migratory herds separated by almost 1000 km (e.g. Qamanirjuaq vs. Dolphin & Union, $F_{ST} = 0.02$). Furthermore, the difference between Columbia North and Columbia South was of the same magnitude as differences between subspecies (e.g. Graham vs. Qamanirjuaq; $F_{ST} = 0.04$; Fig. 1). Similarly, the genetic distance between Wells Gray and Columbia North subpopulations, which live as close as 5 km apart, was large relative to differences among ecotypes and subspecies. Populations south of the North Thompson Valley were relatively distinct from those to the north ($F_{ST} \geq 0.04$, Appendix S2, Supporting information). The Peace River was also associated with population differentiation,

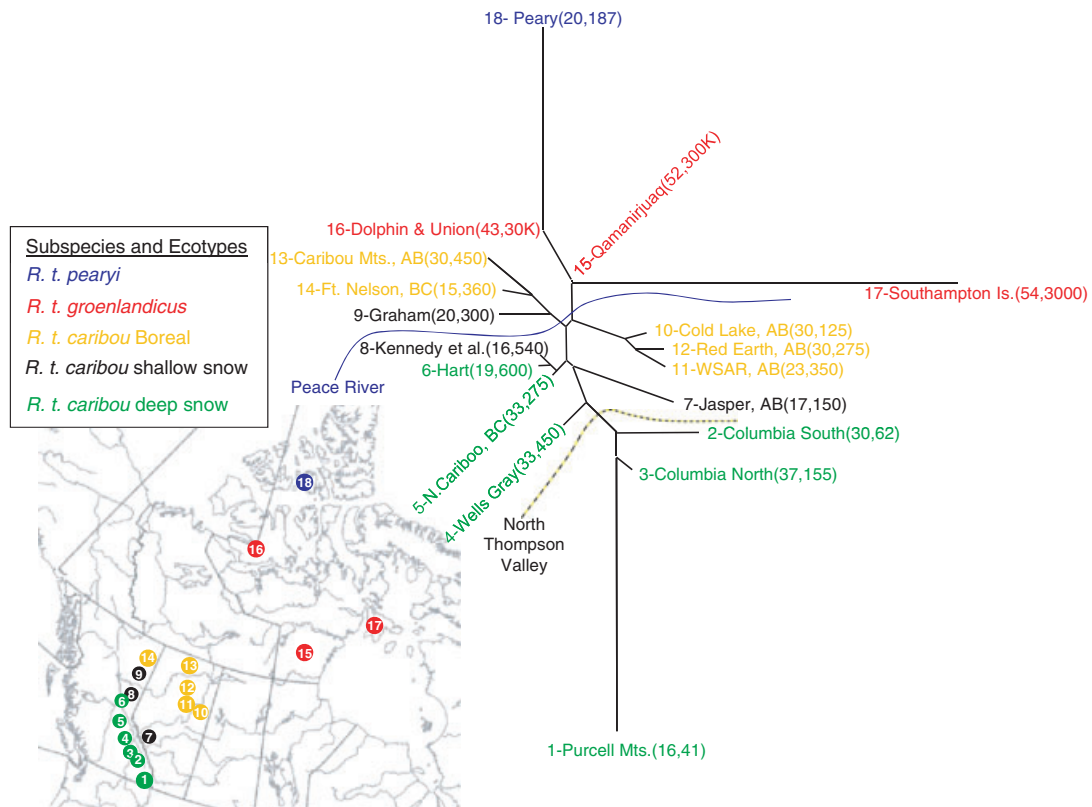


Fig. 1 Classification tree based on genetic distance of pre-defined caribou subpopulations in western Canada. Legend shows colour for three caribou subspecies, and three ecotypes within the woodland subspecies. Map shows collection locations, highlighting major departures of geographic vs. genetic distances. The North Thompson Valley and the Peace River are highlighted (see Table 2 for significance of these features). The F_{ST} value between the Purcells and Peary was 0.22. Southampton samples were founded based on transplants from 48 barren ground caribou. Sample size, followed by population size ($K = \times 1000$) are shown in brackets.

regardless of whether caribou were separated by the portion flooded by the WAC Bennet Dam in British Columbia (shallow-snow mountain ecotype) or the undammed portion in Alberta (boreal ecotype).

Our finer-scale analysis was restricted to woodland caribou and program STRUCTURE suggested that the optimal number of clusters was five (Fig. 2), although some subpopulations were not well classified in any of these clusters, e.g. Jasper and South Selkirks. Four of 11 samples from the South Selkirks contained highly admixed individuals ($q < 0.6$); this deep-snow subpopulation received transplants in the 1990s from the shallow-snow ecotype. The five clusters represented boreal caribou from north of the Peace River, boreal caribou from south of the Peace River, shallow-snow mountain caribou samples from north of the Peace River, the mix of shallow-snow and deep-snow mountain caribou between the Peace River and the North Thompson Valley and the deep-snow mountain caribou to the south of the North Thompson Valley (Fig. 2). The proportion of samples that were strongly assigned ($q > 80\%$) to their respective clusters was always $\geq 70\%$ (70, 78, 92,

72 and 92% for Clusters 1–5, respectively). However, two of 15 runs provided a moderately different clustering pattern because iterations converged to a different (lower) local maximum on the likelihood surface. In these runs 3 clusters were unaffected, but Boreal samples north of the Peace River in Alberta (Cluster 1) were lumped with shallow-snow mountain caribou samples from north of the Peace River in British Columbia, and samples from Wells Gray formed a distinct cluster (Appendix S3, Supporting information). Because these alternate outcomes occurred in $< 15\%$ of the runs and produced lower likelihoods, we used average q -values from the runs that produced the more common pattern, as shown in Fig. 2. The F_{ST} values for these clusters ranged from 0.09 between cluster 3 and 5 to 0.03 for between cluster 1 and 2 (Fig. 2 and Appendix S4, Supporting information). At this scale, the boreal ecotype of woodland caribou was clearly separated into two clusters, on either side of the Peace River, consistent with the phylogenetic tree (Fig. 1). The shallow-snow mountain ecotype of woodland caribou was also separated into two clusters, again with an apparent break associ-

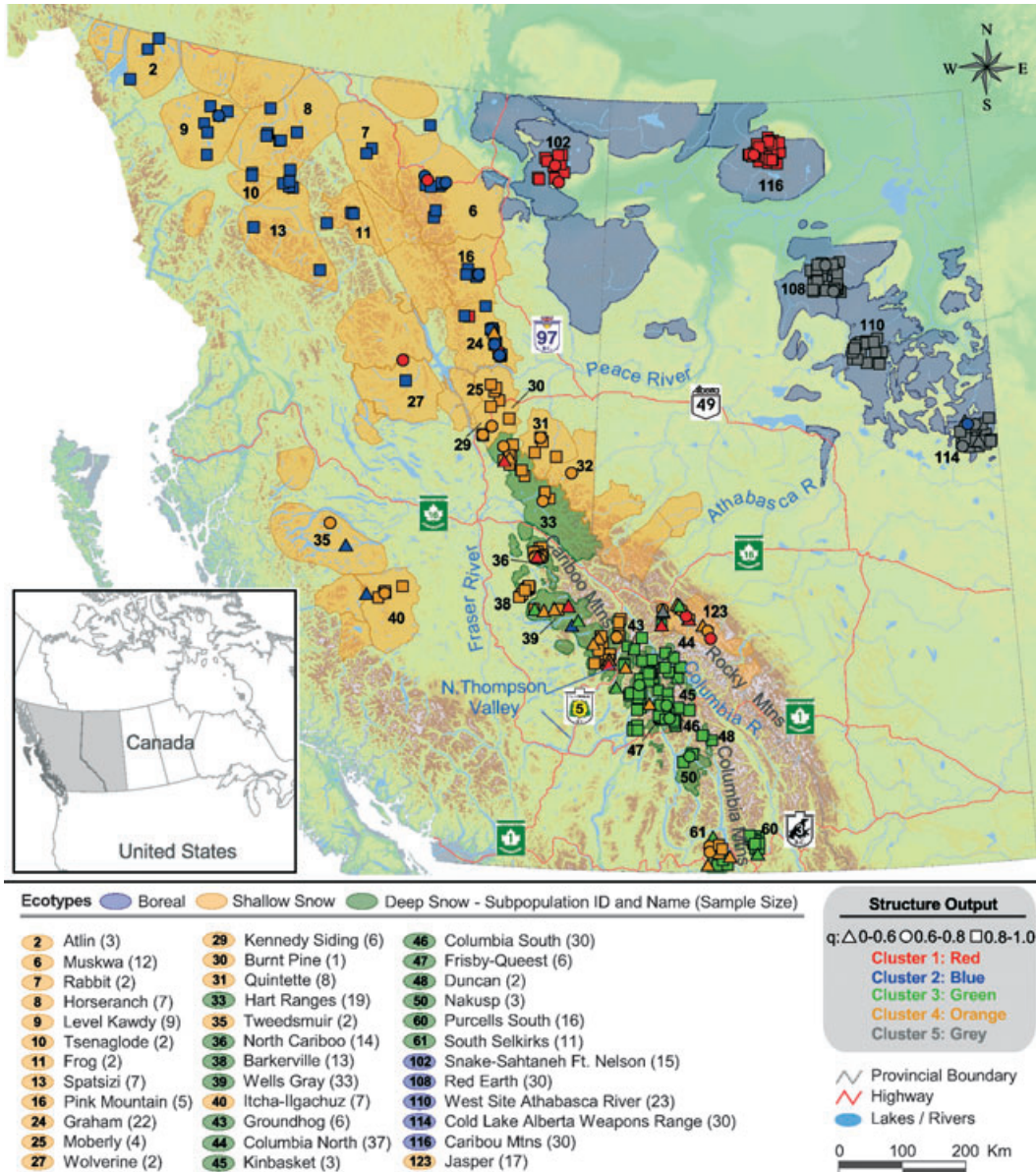


Fig. 2 Genetic clusters of individual woodland caribou samples from British Columbia and Alberta using program STRUCTURE. Cluster membership is shown by colour, and classification uncertainty (q) is shown by symbols. Also shown are a priori defined ecotypes (colour-shaded polygons) and subpopulation names of woodland caribou. For reference, the F_{ST} value for cluster 2 vs. cluster 3 is 0.07 (see Appendix S3, Supporting information). Sample sizes are shown in brackets, and locations are approximate for the boreal ecotype.

ated with the flooded portion of the Peace River in British Columbia. Samples from western British Columbia (the Itcha-Ilgachuz and Twuidsmuir) more closely resembled the shallow-snow ecotype south of the Peace River. The Wells Gray deep-snow mountain subpopulation contained animals from both the southern and northern cluster. The main discontinuity in the deep-snow mountain caribou was the North Thompson Valley. A significant break appeared at this location, despite adjacent subpopulations having <5 km of sepa-

ration. F_{ST} values for all woodland subpopulations defined a priori are provided in Appendix S2 (Supporting information).

Mantel analyses within woodland caribou revealed that the ecotype designation of deep-snow mountain was weak ($r^2 = 0.13$) but significant (mantel $r = -0.36$, CI = -0.55 to -0.27 ; Table 2, model 12). When corrected for geographic distance, the correlation was weaker than its independent effect (mantel $r = -0.26$, model 10), but the CIs between the two models overlapped.

No.	Model		Mantel r	Lcl [†]	Ucl [†]	MR r^2
1	Geo_dist**		0.41	0.29	0.53	0.17
2	Peace_riv*	Geo_dist**	0.27	0.18	0.33	0.23
3	Boreal_ecot	Geo_dist**	0.14	-0.02	0.25	0.18
4	Shallow_ecot	Geo_dist**	0.10	0.01	0.23	0.18
5	Peace_riv		-0.02	-0.16	0.06	0.00
6	Boreal_ecot		-0.03	-0.17	0.05	0.00
7	Shallow_ecot		-0.03	-0.20	0.08	0.00
8	Rockies**		-0.16	-0.32	-0.04	0.03
9	Fraser_riv*	Geo_dist*	-0.21	-0.48	-0.02	0.20
10	Deep_ecot**	Geo_dist**	-0.26	-0.48	-0.10	0.22
11	Fraser_riv**		-0.36	-0.53	-0.19	0.13
12	Deep_ecot**		-0.36	-0.55	-0.27	0.13
13	NT_valley**	Geo_dist**	-0.57	-0.68	-0.48	0.44
14	NT_valley**		-0.60	-0.71	-0.54	0.36

* $P < 0.05$, ** $P < 0.01$

[†]Upper and lower 95% CIs (Ucl and Lcl).

Geo_dist, geographic distance; Peace_riv, the Peace River; Rockies, the Rocky Mountains; NT_valley, the North Thompson Valley; Fraser_riv, Fraser River Valley; Deep_ecot, deep-snow mountain ecotype; Shallow_ecot, shallow-snow mountain ecotype; Boreal_ecot, Boreal ecotype.

Neither the boreal or shallow-snow ecotype designations of woodland caribou were significant (mantel r overlapping 0, models 6 and 7, $r^2 = 0$). When geographic distance was accounted for, the r^2 for these models (Table 2, Models 3 and 4) was similar to the r^2 for geographic distance alone ($r^2 = 0.17$), so boreal and shallow-snow ecotype designations did not add any explanatory power. The effect of the Fraser River Valley was almost identical to caribou classified as the deep-snow ecotype (Model 11), even though this valley transected the northern 20% of deep-snow caribou range (see Fig. 2). In contrast to the ecotype designations, the North Thompson valley had the clearest influence on allele variation within all woodland caribou from sampled subpopulations. The mantel correlation value (r) remained negative even when corrected for geographic distance, and the r^2 was 0.44, suggesting that a high proportion of allelic variation was explained by this valley. This valley splits the range of deep-snow caribou into northern and southern halves, although *c.* 80% of the current population of this ecotype live north of this valley. Finally, the Rocky Mountains were associated with population structure but the amount of variation explained was minimal (Table 2). The variance partitioning from the AMOVA suggested that 2.68% of the allelic variance was explained by the ecotype designations (F_{CT}) for woodland caribou. This value compared with 91.33% for variance within subpopulations (F_{ST}) and 5.98% for among subpopulations but within ecotypes (F_{SC} ; all values significant at $P < 0.001$). With caribou grouped by landscape feature (south of the North Thompson, between the North Thompson and the

Table 2 Factors affecting the genetic distance ($F_{ST}/(1 - F_{ST})$) among woodland caribou subpopulations in BC and Alberta. Analyses were conducted using both Mantel and multiple regressions (Legendre *et al.* 1994). Mantel analyses are represented by the r -value (with 95% CIs), whereas Legendre *et al.*'s (1994) multiple regression was used to calculate r^2 (MR r^2). Asterisks denote significance of independent factors based on the multiple regression approach. The effect of caribou ecotype is contrasted with major landscape features (described in Table 1). For partial mantel tests (e.g. when geographic distance was included as a covariate), the interpretation is the effect of 'X' while controlling for geographic distance

Peace River and N of the Peace River), 41% more variation was explained compared with ecotype grouping ($F_{CT} = 3.80\%$).

Effects of population size on differentiation

Using data from all subpopulations including the three subspecies and ecotypes, there was a negative exponential relationship between the harmonic mean abundance of adjacent pairs of subpopulations, and genetic distance (Fig. 3; $r = 0.74$, CIs 0.72–0.77). The outlier at the bottom left was from a population pair that van

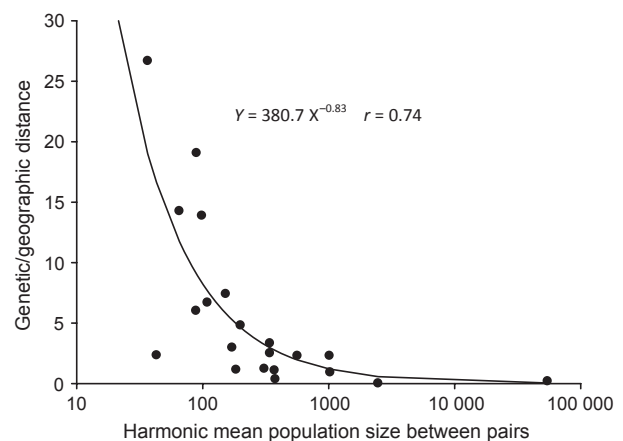


Fig. 3 Genetic distance, adjusted for geographic distance, as a function of the harmonic mean population size between pairs of caribou subpopulations. Pairs of subpopulations were based on their nearest geographic neighbour from sampled populations in western Canada.

Oort *et al.* (2011) found was connected based on telemetry (Groundhog and Columbia North, i.e. updated data from Wittmer *et al.* 2005a), so excluding this data point would increase the r -value to 0.91. Population size (N) positively affected heterozygosity (Fig. 4), where $H_E = 0.014 \times \ln(N) + 0.68$ and the 95% CI of the slope was 0.010–0.023 (genetic diversity data by subpopulation are in Appendix S5, Supporting information).

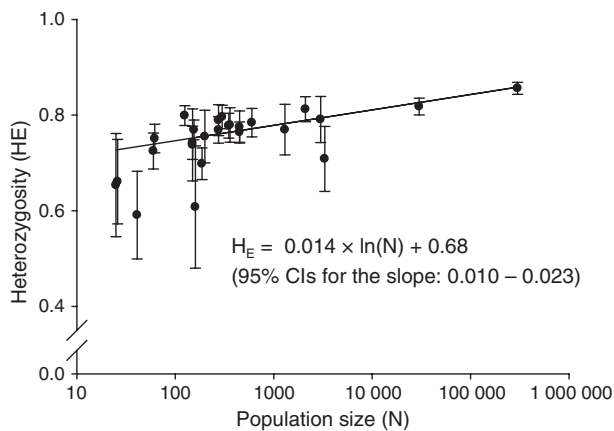


Fig. 4 Expected heterozygosity (H_E) as a function of population size for caribou in western Canada. Parameters were estimated using nonlinear regression, weighted by the inverse of the H_E variance, and bootstrapped each subpopulation ($n = 1000$) to estimate uncertainty. Error bars are 95% CI.

Genetic structuring and migrant estimation within deep-snow mountain caribou

Program STRUCTURE suggested six clusters within this ecotype of woodland caribou, with Jasper, a shallow-snow ecotype included as an outgroup, adding a seventh cluster (Fig. 5). Program STRUCTURE grouped the 10 separate subpopulations estimated by Wittmer *et al.* (2005a) using radio-telemetry into six clusters. No meaningful genetic differentiation was detected between the Hart Ranges and the North Cariboo, nor among the Columbia North, Groundhog, Frisby-Queest and Nakusp/Duncan subpopulations, but in the remaining cases, the genetic analysis supported the population structure estimated by Wittmer *et al.* (2005a).

We again expressed the strength of each cluster as a proportion of individuals that were strongly ($q > 0.8$) assigned to their own cluster. Cluster 2 (Barkerville, 87%) was the strongest, followed by Cluster 3 (Jasper, 84%), whereas Cluster 4 (Columbia North/Groundhog/Frisby-Queest/Nakusp, 42%) and Cluster 7 (Columbia South, 54%) had fewer individuals that were strongly assigned to their own cluster.

The structure within deep-snow caribou confirmed that the North Thompson Valley was a barrier to gene flow (Fig. 5). Our post hoc analysis was to estimate the migration rate across and to the south of this valley (i.e. from the Wells Gray subpopulation), where caribou are declining more rapidly (Wittmer *et al.* 2005a). Of 48 samples collected from subpopulations south of the North Thompson Valley (i.e. Groundhog, Columbia

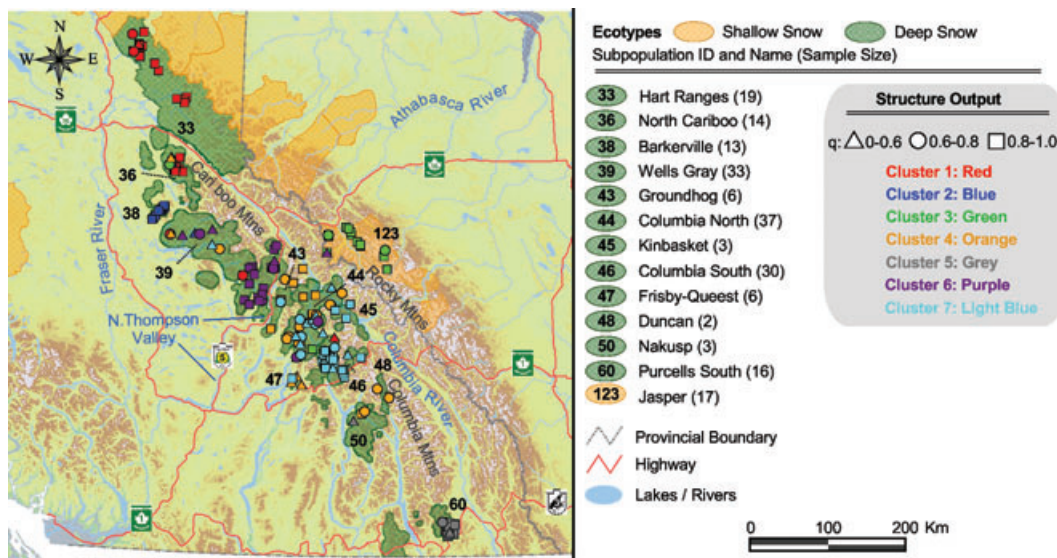


Fig. 5 Genetic clusters of individual woodland caribou samples from the deep-snow ecotype, including one outgroup subpopulation from Jasper, AB. Program STRUCTURE assuming no a priori group membership. Cluster membership is shown by colour, and classification uncertainty is shown by symbols.

North and Frisby-Queest), only one potential first-generation migrant originated from Wells Gray during this 17-year time span (1992–2008; $P = 0.002$, all other $P > 0.50$).

Our analysis of the ecotone between deep- and shallow-snow subpopulations revealed that the Graham subpopulation, north of the Peace River, was distinct from populations to the south. South of the Peace River, several subpopulations appear indistinguishable and these include both deep- (Hart Ranges, North Cariboo) and shallow-snow ecotypes (Kennedy, Quintette, Moberly). To the south of these, the Wells Gray subpopulation was distinct (Fig. 6).

Discussion

There appears to be little support for ESU designation using current taxonomic definitions for caribou below the species level. With the exception of Peary caribou, branches within the phylogenetic tree did not correspond to existing taxonomic designations, as branch lengths among units were often shorter than lengths within units. This lack of pattern was most pronounced at the level of ecotype within the woodland subspecies, whereby genetic differentiation (F_{ST}) indicated less demographic isolation than expected for populations with independent evolutionary trajectories. Additional analyses suggested that landscape features and in particular major valleys explained variation better than ecotype designation. The AMOVA, mantel and multiple regression approaches support this conclusion. Finally, a visual inspection of the STRUCTURE outputs (Fig. 2, Appendix S3, Supporting information) reveals

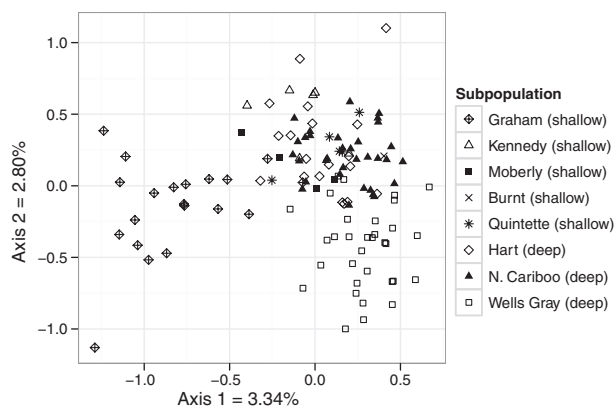


Fig. 6 Ecotone between deep- and shallow-snow mountain caribou ecotypes (brackets). All sub populations are on the edge of the deep- and shallow-snow ecotypes (see Fig. 2 for sub population locations). The Peace River lies between the Graham subpopulation and those to the south.

breaks at two major valleys and less so at ecotype boundaries.

Mitochondrial analyses of caribou suggest two main lineages, northern and southern, roughly corresponding to the barren ground and woodland caribou subspecies (Cronin *et al.* 2005), and both lineages have been documented within subpopulations (Cronin *et al.* 2005; McDevitt *et al.* 2009). These lineages probably reflect refugia north and south of continental ice sheets present during the Pleistocene (Flagstad & Røed 2003). Although we sampled fewer subpopulations across subspecies relative to ecotype, Weckworth *et al.*'s (2012) recent analyses at the mtDNA and nDNA level support our findings that little differentiation occurs among some of the woodland and adjacent barren ground subpopulations. Furthermore, there is little evidence that ecotype designations within the woodland caribou subspecies evaluated herein are supported by mitochondrial analyses (McDevitt *et al.* 2009; Weckworth *et al.* 2012). Behavioural differences in foraging and seasonal migration patterns between ecotypes (Jones 2007) are likely too recently derived and genetically complex to be manifested through mtDNA and may be rooted in phenotypic plasticity.

Population size and landscape features

In contrast to existing taxonomy, population size and specific landscape features were strongly associated with genetic variation among subpopulations. That population size had a nonlinear influence on population structure is a predictable result based on the theory of genetic drift (Wright 1931). Although the relationship may vary among species owing to possible differences between census and effective population sizes, there appears to be a threshold with caribou. Below a census population size of approximately 150 animals, the magnitude and variation of differentiation greatly increases between pairs of adjacent subpopulations. The fact that H_E was also affected by population size further suggests that drift was the mechanism that led to spatial structuring among neighbouring populations (and see Côté *et al.* 2002). These results demonstrate a genetic outcome of ecological factors affecting woodland caribou since at least the early 20th century (Seip 1992; Bergerud *et al.* 2008). Many subpopulations have and continue to decline from unsustainable predation because of apparent competition (Holt 1977) with moose (*Alces alces*) and deer (*Odocoileus* sp.) (Seip 1992). Continued declines are anticipated (Wittmer *et al.* 2010) because predator numbers are not linked to the abundance of caribou. The resulting small populations of caribou suffer more rapid genetic drift and populations become increasingly structured. Therefore, our results

demonstrate a link between community-level ecological processes and genetic population structure within caribou.

It is well established that heterozygosity is affected by population size (Frankham 1996; Courtois *et al.* 2003). However, the effect of small population size on population structure is an important consideration, particularly when estimating the relevance of factors such as highways and valleys settled by people that are often highlighted in landscape genetic research (Manel *et al.* 2003). Including population size as an explanatory factor may have broad applicability in the field of landscape genetics, particularly when dealing with endangered species. This approach could explain variation that was unaccounted for and more importantly, unmask spurious correlations that may have been erroneously attributed to landscape-level factors. For example, if formerly contiguous populations contracted and became isolated owing to overharvest, predation, or competition, a retrospective landscape analysis may conclude that landscape features such as human development caused the population fragmentation by reducing movement, when in reality the mechanism was simply reduced abundance and distribution (Gaston *et al.* 2000). Another consideration is that effects of population size on genetic distance are likely to be complicated by time, because the longer a population has been isolated, the greater the differentiation. Time at a given population phase was not accounted for in our analysis, yet our results seemed robust to this omission.

The effect of population size on genetic structuring helps contextualize other ecological factors affecting differentiation. Arguments of uniqueness are ubiquitous in the conservation literature and are often used to assign conservation priority to certain population units. Based on their unique behaviour among all Cervids of walking on top of the >2 m snowpack to access a food source that is otherwise out of reach (Serrouya *et al.* 2007), deep-snow mountain caribou were an interesting candidate to determine whether there was a genetic correlation with this foraging strategy as there is likely selective pressure on an ungulate living under such extreme conditions. These animals must respond not only to snowfall events outside the normal range for temperate ungulates, but also to snow texture and consolidation when deciding to migrate to high elevations in winter. Their limited dispersal behaviour and philopatry (van Oort *et al.* 2011), plus living in rugged mountains that may naturally fracture populations and restrict gene flow, provided intuitive support for taxonomic distinction. Yet, our results suggest that deep-snow mountain caribou as a group were not genetically unique, particularly when geographic distance and landscape features are accounted for. The southern-

most subpopulations such as the Purcells did stand out as unique (*sensu* Zittlau 2004), but this is likely due to their very small population size and consequent rapid drift.

Two major river valleys were associated with genetic discontinuities. The effect of the North Thompson Valley was reflected across a variety of spatial scales and analyses. The Peace River was also associated with differentiation, similar to what McLoughlin *et al.* (2004) documented. Population fractures at major valleys is likely a result of these caribou generally preferring higher elevations (Apps *et al.* 2001) for foraging as well as avoiding predation at lower elevations where deer, moose and their predators are more abundant (Stotyn 2007).

Historic vs. contemporary movement: demographic rescue within the deep-snow ecotype

Although there was little support for taxonomic subdivision in our data set, results suggest isolation of many subpopulations. Using an assignment test to directly identify putative migrants and validate contemporary telemetry information with longer-term measures of gene flow estimated by indirect methods, deep-snow caribou exhibit structure consistent with little movement among subpopulations. These subpopulations can therefore be considered separate management units as defined by Palsbøll *et al.* (2007). Furthermore, this structure suggests that fragmentation preceded telemetry studies which began in the early 1990s.

While direct and indirect genetic methods can be used to show relative demographic independence, a lack of genetic differentiation cannot be interpreted as proof of demographic integration. For example, the subpopulations of Frisby-Queest and Columbia North appear genetically similar, but van Oort *et al.* (2011) documented no interchange of individuals, despite intensive sampling (over time, >40% of the standing population size had been radio-tagged). A telemetry data set from the early 1980s, however, found four of nine collared animals moved between these two subpopulations (unpublished data from K. Simpson & G. Woods 1987). Therefore, the lack of genetic differentiation probably reflects a time lag resulting from a contemporary barrier to gene flow (van Oort *et al.* 2011). This contrast between genetic data and telemetry based movement is consistent with comparatively recent fragmentation. In a second case, at the ecotone between deep- and shallow-snow caribou, several populations of both ecotypes appear genetically indistinguishable. In this area, Jones (2007) found that individual caribou exhibited both foraging strategies (terrestrial and arboreal) depending upon local and

annual snow conditions, suggesting a degree of plasticity in foraging behaviour.

The population structuring among deep-snow mountain subpopulations has immediate implications for conservation. The genetic structuring, radio-tagged dispersal estimates (van Oort *et al.* 2011) and the assignment test all suggest insufficient movement to provide demographic rescue. From 1992 to 2008, 48 caribou adjacent to but south of the North Thompson Valley were sampled, yet only one potential immigrant was identified across this barrier from the large population (Wells Gray) immediately north of this valley, for an immigration rate of 2.1%. During the same time period, the populations south of the North Thompson valley declined from 272 to 170 (Wittmer *et al.* 2005a; B. N. McLellan and R. Serrouya, unpublished data). Therefore, the immigration rate estimated from the assignment test is approximately 1/17th the level needed to provide sufficient movement to stabilize the smaller populations directly south of this barrier. Our genetic results support van Oort *et al.* (2011) in concluding that deep-snow mountain caribou are in a non-equilibrium metapopulation and narrows the range of management options to transplants and addressing the proximate cause of unsustainable predation (Wittmer *et al.* 2005b). A risk with transplants is that any undetected adaptation to deep-snow conditions would be jeopardized; however, this argument is academic when faced with the imminent extinction of many subpopulations (Serrouya & Wittmer 2010; Wittmer *et al.* 2010). While the specific fitness consequences are unknown, a previous transplant to the deep-snow ecotype (South Selkirks) appeared to stabilize a population decline (Warren *et al.* 1996), with alleles from the shallow-snow ecotype apparently persisting in subsequent generations (Fig. 2, Appendix S3, Supporting information).

In conclusion, neutral genetic data from microsatellite markers suggest that two of three subspecies, and all three ecotypes of the woodland subspecies, lack the cohesion and uniqueness of 'evolutionarily significant' groups, suggesting that current classifications are inappropriate. By contrast, we find support in the genetic data for the demographic independence of many of the deep-snow mountain subpopulations that were originally defined using radio-telemetry data, confirming that it is appropriate to treat these groups as demographically independent for management purposes. Finally, moving beyond the caribou perspective, our samples covered a large range of population sizes, revealing the importance of population size in explaining the degree of differentiation among subpopulations. This finding may be of broad interest to landscape genetic studies seeking to understand population structure of endangered species in particular.

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R.S.'s main research focus has been to test recovery options for caribou and other species by implementing broad-scale adaptive management experiments. D.P. is a population geneticist with a particular interest in using genetic tools to estimate movement using 'real time' approaches. B.M. is most interested in the conservation of challenging species such as grizzly bears and mountain caribou across multiple-use landscapes. S.B.'s interests include population dynamics of boreal species and science that helps people make ecologically-informed land use decisions. M.C. has been studying caribou and muskox in the field for 25 years and his main interest is to integrate demographic and ecological studies to gain a more holistic understanding of the species. D.J. is interested in the conservation and recovery of biological diversity and runs a field-based research program focused on arctic ungulates.

Data accessibility

Sampling locations and microsatellite data: DRYAD entry doi: 10.5061/dryad.250c3s47.

Supporting information

Appendix S1 Matrix of geographically adjusted genetic distance corresponding to populations shown in Fig. 1.

Appendix S2 F_{ST} values for pairs of subpopulations examined within woodland caribou.

Appendix S3 Genetic clusters of individual woodland caribou samples from British Columbia and Alberta using program STRUCTURE, but showing the alternate clustering pattern that occurred in <15% of the STRUCTURE runs. Dominant runs are shown in Fig. 2. Cluster membership is shown by colour, and classification uncertainty is shown by symbols. Also shown are a priori defined ecotypes (colour-shaded polygons), and subpopulation names of woodland caribou. Sample sizes are shown in brackets.

Appendix S4 Factorial Correspondence Analysis using the program GENETIX (Belkhir *et al.* 1996) representing the results from program STRUCTURE at the scale of woodland caribou in British Columbia and Alberta, Canada.

Appendix S5 Expected heterozygosity and mean alleles per locus for subpopulations used in analysis of population size (Figs 3 and 4).