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Population genetic structure of North American thinhorn sheep (*Ovis dalli*)

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Abstract

The thinhorn sheep (*Ovis dalli* ssp.) provides a rare example of a North American large mammal that occupies most of its native range and maintains close to ancestral population size. There are currently two recognized subspecies, Dall's sheep (*O. d. dalli*) and Stone's sheep (*O. d. stonei*), the validity of which remains uncertain. We investigated the spatial genetic structure of thinhorn sheep populations representing both subspecies by genotyping individuals (n = 919) from across the species range at 12 variable microsatellite loci. We found high levels of genetic diversity within (H_E = 0.722) and significant genetic structure among the 24 sampled areas (F_{ST} = 0.160). Genetic distance measures and Bayesian clustering analyses revealed the presence of at least eight subpopulations that are delineated by mountain range topology. A strong overall pattern of isolation-by-distance is evident across the sampling range (r = 0.75, P < 0.001) suggesting limited dispersal and extensive philopatry. Partial Mantel tests of this relationship showed mountain range distinctions represent significant barriers to gene flow (P = 0.0001), supporting the Bayesian analyses. Genetic structure was more strongly pronounced in southern Yukon and Alaska than elsewhere. We also show evidence for genetic differences between the two currently recognized thinhorn subspecies.

Keywords: assignment test, F_{ST}, isolation-by-distance, microsatellites, *Ovis*, ungulate

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Introduction

Many species show geographical stratification of genetic diversity. At selectively neutral loci, population genetic structure is primarily determined by the interplay between genetic drift and the rate of gene flow between geographically separated subpopulations (Slatkin 1987). In theory, relatively little migration is required for the homogenization of allele frequencies across populations at equilibrium under the island model (Wright 1978). However, as most species have a dispersal range considerably less than that of the species range, population structure often reflects a pattern of isolation-by-distance (Slatkin 1993). Quantifying levels of genetic structure can therefore give an insight into the colonization history of a species (Perez *et al*. 2002), rates of dispersal (Waser & Strobeck 1998) and the effects of environmental barriers on gene flow (Paetkau *et al*. 1999; Carmichael *et al*. 2001; Kyle & Strobeck 2002). In trophy game animals, such as thinhorn sheep (*Ovis dalli* spp.), knowledge of genetic structure may also have practical implications for harvest management and for forensic analyses of suspected illegal hunting (Primmer *et al*. 2000).

Due to the variety of factors that affect population structure, there are differences in scale and pattern of structure across different taxa. Many signatures of structure can be predicted based on life history traits. Large carnivores, such as wolves and bears, generally have larger potential dispersal distances and therefore decreased levels of structure than mountain ungulates (Forbes & Hogg 1999). Forest- or

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plains-dwelling ungulates show low to moderate levels of genetic structure (Broders *et al*. 1999; Wilson & Strobeck 1999; Polziehn *et al*. 2000) as they are able to utilize larger areas of continuous suitable habitat and may conduct long-distance migration. Animals with more stringent habitat preference criteria, such as mountain sheep, may be expected to exhibit more pronounced population structure.

Wild sheep are dependent upon habitat with steep rugged cliffs for use as escape terrain with nearby open grazing areas for feeding (Geist 1971; Valdez & Krausman 1999). For most populations, the overall annual range consists of relatively unlimited summer habitat, restricted only by accessibility to escape terrain and bounded by natural barriers. Topographical features such as forested valleys and rivers present natural boundaries to dispersal. The range shrinks in winter to smaller areas with limited snow accumulation, open grassland and available escape terrain, resulting in higher sheep densities and setting the upper limit to herd size. Mountain sheep are highly philopatric, with individuals utilizing the same seasonal home ranges each year (Geist 1971; Festa-Bianchet 1991). As is the case with most mammals (Greenwood 1980), dispersal is largely male-biased (Hogg 2000) and ewes rarely leave the natal home range (Festa-Bianchet 1991; Valdez & Krausman 1999). The combination of limited gene flow and small population size should cause genetic differences to accumulate rapidly between geographically separated populations.

Perhaps unsurprisingly, previous studies on mountain sheep have demonstrated considerable genetic structure. Mitochondria DNA analyses in desert bighorn (*Ovis canadensis nelsoni*) ewes showed significant differentiation at the level of the home range group (Ramey 1995; Boyce *et al*. 1999). Studies using nuclear markers have found significant genetic distances between Rocky Mountain bighorn (*O. c. canadensis*) populations sampled across the species range (Forbes & Hogg 1999), and on a much finer scale between recently reintroduced populations (Fitzsimmons *et al*. 1997).

Human activity is also likely to have influenced the genetic structure of bighorn sheep and other mountain ungulates. Translocations, population declines due to unregulated hunting and habitat loss and the transmission of novel pathogens from domestic sheep have severely impacted bighorn sheep population structure (Luikart & Allendorf 1996). These factors make it difficult to sample representatively the native range of the bighorn sheep. In contrast, thinhorn occupy most of their historic range in approximately ancestral numbers. Their range encompasses much of mountainous northwestern North America, from Alaska to the Mackenzie River in the east, and to the Pine Pass of the Rocky Mountains of northeastern British Columbia in the southeast. There are an estimated 130 000 individuals present (Barichello *et al*. 1989; Valdez & Krausman 1999; Veitch & Simmons 1999). Cases of domestic livestock introduction in these mostly remote areas are few, having little impact on wild sheep numbers. In addition there have been limited recorded translocations either into or out of populations. Thinhorn sheep are therefore an ideal model in which to study mountain ungulate population structure as it evolved prior to anthropogenic influence.

Thinhorn sheep are classified in two subspecies on the basis of coat colour. The more abundant white Dall's sheep (*O. dalli dalli*) are found across much of the species range, except the far south. The darker Stone's sheep (*O. d. stonei*) are less numerous and are found in the Yukon and northern British Columbia only. The taxonomic validity of the subspecies status in thinhorn and other wild sheep species is debatable. Genetic analyses of desert bighorn have shown that currently recognized subspecies do not provide a full explanation for phylogenetic boundaries (Ramey 1993; Gutierrez-Espeleta 1999). Rather, it could be that differences between individuals are representative of variation in one species over a geographical range. Genetic study may aid our understanding of the unresolved taxonomy of thinhorn.

In this study we aimed to quantify levels of genetic variation in thinhorn sheep populations across the species range. A previous thinhorn study using allozyme markers found little variation (Sage & Wolff 1986). Here we used ungulate-derived microsatellites (de Gortari *et al*. 1997; Slate *et al*. 1998) to investigate population structure and differentiation between thinhorn subspecies. We hypothesized that thinhorn sheep are highly genetically structured, with genetic variation partitioned among contiguous mountain ranges and subspecies.

Materials and methods

Sample locations and collection

We sampled 919 thinhorn sheep from 24 sampling areas across the species range, from northwestern Alaska to British Columbia (Fig. 1). Samples for DNA extraction were collected between 1994 and 2002 and comprised flakes of horn produced by drilling horn material to insert metal identification plugs (horn 'corings') of hunted rams taken at kill registration or compulsory inspection. Alaskan samples comprised whole blood taken from both rams and ewes during the period 1999–2002. Sample area boundaries were defined from game management zones or units in the Northwest Territories (NWT) and northern British Columbia (BC) and by groups of adjacent management subzones from the Yukon Territory (YK). Alaskan sample areas comprised individuals collected from the same mountain range, with maximum distance separating samples of between 20 km (in the Central Alaskan Range: CAR) and 140 km (in the Gates of the Arctic Reserve: GAAR). The 24 sample areas represent seven contiguous mountain blocks defined in Table 1. These mountain blocks consist of continuous

Table 1 Genetic variability estimates from populations included in the study (mean number of alleles per locus (A), observed (H_O) and expected heterozygosities (H_E), and mean estimates of F_{IS} (Weir & Cockerham 1984)). Also included are the number of loci showing significant deviation from Hardy–Weinberg Equilibrium at each population (at $P < 0.05$). *Global departure across all loci from HWE; significance of F_{IS} is indicated *$P < 0.05$, **$P < 0.01$

Region	Sample area	Abbr.	Mountain group	N	% Dall's in sample	H_O	H_E	Loci not in HWE	A	F_{IS}
Alaska	Central Alaskan Range	CAR	Alaska range	25	100	0.58	0.58	1	5.17	0.020
	Gates of the Arctic National Park Preserve	GAAR	Brookes range	34	100	0.55	0.60	3*	5.17	0.107**
	Noatak National Preserve	NK	Brookes range	25	100	0.63	0.60	0	3.97	−0.028
	Yukon-Charley Rivers National Preserve	YUCH	White Hills	36	100	0.57	0.59	4*	5.25	0.050*
Northwest Territories	Game management zone D/OT/01	D/OT/01	Mackenzie mountains	40	100	0.62	0.65	0	6.92	0.055**
	Game management zone D/OT/02	D/OT/02	Mackenzie mountains	42	100	0.54	0.57	5*	6.92	0.070**
	Game management zone G/OT/01	G/OT/01	Mackenzie mountains	40	100	0.60	0.62	1	6.92	0.047*
	Game management zone S/OT/01	S/OT/01	Mackenzie mountains	40	100	0.62	0.63	2	7.50	0.040
	Game management zone S/OT/02	S/OT/02	Mackenzie mountains	40	100	0.64	0.65	3	7.33	0.024
	Game management zone S/OT/03	S/OT/03	Mackenzie mountains	40	100	0.60	0.65	1	7.08	0.096**
	Game management zone S/OT/04	S/OT/04	Mackenzie mountains	40	100	0.59	0.66	1	7.25	0.119**
	Game management zone S/OT/05	S/OT/05	Mackenzie mountains	39	100	0.64	0.67	2	7.42	0.061*
Yukon Territories	Game management zone 2 (Ogilvie mountains)	Y2W	Mackenzie mountains	40	93	0.62	0.67	4	6.58	0.087**
	Game management zone 2 (Wernecke mountains)	Y2E	Mackenzie mountains	40	100	0.63	0.66	4	6.58	0.057*
	Game management zone 4	Y4	Pelly mtns	40	36	0.59	0.66	4*	7.17	0.116**
	Game management zone 5	Y5	Southwest range	40	100	0.56	0.58	3	5.67	0.051*
	Game management zone 7	Y7	Southwest range	40	100	0.55	0.59	5	6.17	0.064**
	Game management zone 8	Y8	Pelly mtns	40	38	0.57	0.61	2	6.00	0.070**
	Game management zone 10	Y10	Pelly mtns	40	32	0.60	0.64	0	6.33	0.070**
British Columbia	Game zone 742	BC742	Northern Rockies	40	0	0.48	0.49	2	4.42	0.042
	Game zone 750	BC750	Northern Rockies	40	0	0.51	0.54	2	5.17	0.071**
	Game zone 751	BC751	Northern Rockies	40	0	0.47	0.52	2	5.58	0.104**
	Game zone 752	BC752	Northern Rockies	38	0	0.47	0.48	1	5.00	0.043
	Game zone 754	BC754	Northern Rockies	40	0	0.46	0.51	2	4.83	0.101**

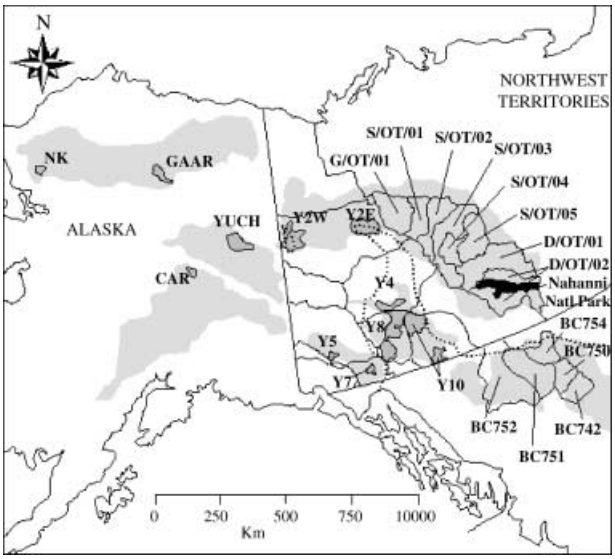

Fig. 1 North American *Ovis dalli* population locations included in this study. Sampling locations in Yukon and Alaska were within the black shaded areas. Populations in the Northwest Territories and British Columbia cover all the area within the labelled region. Abbreviations for sample area names are described in Table 1. Lightly shaded regions represent the full extent of mountain ranges. The dotted line represents the northern limits of Stone's sheep range.

upland regions separated by unsuitable sheep habitat such as major river valleys or lowland forest. Between 25 and 42 individuals were sampled per area (mean of 38). Horn corings were stored dry in sealed paper envelopes, while whole blood samples were stored at –20 °C in EDTA.

Populations also varied with regard to the subspecies of the sheep sampled. NWT and Alaskan populations comprised all Dall's sheep, while Yukon populations contained herds of both Dall's and Stone's sheep (see Table 1). British Columbia individuals were exclusively Stone's.

Molecular techniques

Genomic DNA was extracted from approximately 0.5 mL of horn material per sample using a tissue extraction kit (Qiagen, Crawley, West Sussex, UK). Blood samples were extracted using a phenol–chloroform technique (Sambrook *et al*. 1989) from 200 μL of blood. Twelve dinucleotide microsatellite loci developed in domestic sheep and cattle (Table 2) were amplified for each individual. Loci were chosen based on levels of variability and previous successful use in bighorn sheep (Coltman *et al*. 2002; Coltman *et al*. 2003a).

Each polymerase chain reaction (PCR) was carried out in 10 μL reactions. Reactions contained 2 μL of DNA template, 80 μmol of each primer, 0.16 mM dNTPs, 2 mM $MgCl_2$ and 0.5 units *Taq* polymerase (Sigma, Gillingham, Dorset, or Bioline, London, UK). Additionally, the quality of some products was improved by the addition of 25 mg/mL bovine serum albumin (BSA). The PCR profile consisted of 35 cycles of 30 s each at 94 °C and 54 °C followed by 40 s at 72 °C. Cycles were preceded by 5 min at 94 °C and terminated with 10 min at 72 °C. PCR products were genotyped using an ABI 377 sequencer and analysed using the software GENESCAN and GENOTYPER (Applied Biosystems, Foster City, CA, USA).

Data analyses

Genetic diversity. We quantified genetic variability within each sample area by the number of alleles per locus (A) and expected heterozygosity (H_E) as calculated by GENETIX version 4.01 (Belkhir 1996). GENETIX was also used to determine these values for each locus. Homogeneity of genetic

Table 2 Comparison of the genetic diversity found at the 12 microsatellite markers used (number of alleles over all populations (A), size range of alleles and mean observed (H_O) and expected heterozygosities (H_E) of all sample areas). Also included are the number of populations out of 24 that show significant deviation from HWE ($P < 0.05$). *Global departure from HWE

Locus	A	Allele size range (bp)	H_O	H_E	Populations deviating from HWE	Reference
AE16	9	82–98	0.6250	0.6628	6*	(Penty *et al*. 1993)
BM1225	13	225–265	0.6284	0.6813	6*	(Bishop *et al*. 1994)
BM4505	11	239–275	0.4775	0.5472	9*	(Bishop *et al*. 1994)
BM4513	16	131–161	0.7122	0.7316	1	(Bishop *et al*. 1994)
BM848	22	201–243	0.7525	0.8305	10*	(Bishop *et al*. 1994)
BMC1222	4	288–294	0.1937	0.2254	1	(de Gortari *et al*. 1997)
CP26	19	123–165	0.6939	0.7168	3	(Ede *et al*. 1995)
FCB266	13	82–106	0.6262	0.6498	4	(Buchanan & Crawford 1993)
MAF209	5	109–119	0.2020	0.2067	1	(Buchanan & Crawford 1992)
MAF36	13	84–116	0.7227	0.7353	5	(Swarbrick *et al*. 1991)
TGLA126	11	112–144	0.5379	0.5361	5	(Georges & Massey 1992)
TGLA387	12	129–153	0.6771	0.7031	3	(Georges & Massey 1992)

variation between the 24 sample areas was tested using Wilcoxon's signed-rank tests blocks (Table 1). Departures from Hardy–Weinberg equilibrium (HWE) were examined using exact tests (Guo & Thompson 1992) using a Markov chain as implemented by GENEPOP version 3.3 (Raymond & Rousset 1995). Loci were combined using Fisher's method to examine departure from equilibrium for each area, with the significance interpreted after sequential Bonferroni correction for multiple comparisons (Rice 1989). The presence of linkage disequilibrium between loci over all areas was also tested using exact tests with GENEPOP software.

Genetic differentiation. Exact tests were performed for allele frequency differences between all pairs of areas. Genetic divergences between sample areas were quantified using the distance statistics F_{ST}, Nei's unbiased genetic distance, D_s (Nei 1978) and the distance $(\delta\mu)^2$ based on allele size (Goldstein *et al*. 1995). D_S has been shown to fare better at fine-scale population differentiation, while $(\delta\mu)^2$ has proved more useful for examining relationships between more distinct populations and for estimating evolutionary times (Paetkau *et al*. 1997). F_{ST} is insensitive when migration rates are low. We therefore used D_S rather than F_{ST} when constructing isolation-by-distance plots and trees. F_{ST} was calculated with GENEPOP, other distance measures were calculated using the software SPAGEDI version 1.0 (Hardy & Vekemans 2002). Significance of F_{ST} was tested with 10 000 permutations using GENETIX. PHYLIP 3.5 (Felsenstein 1993) was used together with the program TREEVIEW 1.5 (Page 1996) to construct an unrooted tree from the D_S distance matrix.

The Bayesian methodology of STRUCTURE version 2.0 (Pritchard *et al*. 2000) was implemented to determine the level of genetic substructure in the data set independently of sampling areas. To estimate the number of subpopulations (K), five independent runs of $K = 1–20$ were carried out at 100 000 Markov chain Monte Carlo (MCMC) repetitions. The most probable number of populations was taken using the log-likelihood of K. Individuals were then assigned to each subpopulation, based on the highest percentage membership (q). Sample locations of these individuals were then plotted on a map of the species range to examine the relative geographical position of subpopulations.

Isolation-by-distance. The relationship between genetic and geographical distances was examined to assess isolation-by-distance (Slatkin 1993). Geographic distances between pairs of areas were calculated from linear distances between mean latitude and longitude positions of samples from each area and plotted against the genetic distances D_S and $(\delta\mu)^2$. We also investigated the effects of mountain block and colour polymorphism on genetic distance controlling for the effects of geographical distance by partial Mantel tests (10 000 permutations) as calculated by the R-PACKAGE version 4.0 (Casgrain & Legendre 2001). To assess the effects of subspecies and mountain block on genetic distance independently of geographical distance, we used a general linear model of the residuals from linear regressions of genetic on geographical distances.

Results

Genetic diversity and tests of disequilibrium

Twelve locus microsatellite profiles were recorded for 919 thinhorn sheep. To assess the accuracy of profiles obtained from horn material, a subset of 50 individuals were retyped. In all cases identical genotypes were returned (total of 600 genotypes from 12 loci), showing the profiles to be repeatable. Overall, our rate of missing data was 1.9%.

Between four (*BMC1222*) and 22 (*BM848*) alleles were found at the 12 loci (mean 12.33, SE 0.43). Expected heterozygosities per locus ranged from 0.229 (*MAF209*) to 0.922 (*BM848*) (Table 2). At less diverse loci several alleles were present in Mackenzie and southern YK populations, while one allele was fixed in Alaska and BC. Populations had a mean number of alleles per locus (A) between 3.97 and 7.50 (Table 1). Measures of genetic variation did not differ between areas (A: $Z = 0.1065$, $P = 0.9152$; H_O: $Z = -0.0152$, $P = 0.9879$), although the highest measures of genetic diversity were observed in areas from the Mackenzie and Yukon (Pelly and southwest) mountain ranges. F_{IS} for all but one area was positive, ranging from −0.028 to 0.119. Several significant departures from HWE due to heterozygote deficit were found, with every locus showing deviation in at least one area (see Tables 1 and 2 for distribution). The repeatability of our microsatellite genotypes, low rate of missing data and consistent levels of HWE disequilibrium across loci suggest that heterozygote deficiencies did not arise from PCR artefacts.

Tests for genotypic disequilibrium revealed no significant linkage after correcting for multiple comparisons. When uncorrected, linkage was suggested between *BM1225* and *FCB266* ($\chi^2 = 71.35$, d.f. = 48, $P = 0.016$). Physical linkage between these markers is unlikely as they are located on separate chromosomes in domestic sheep (Maddox *et al*. 2001) (16 and 25, respectively).

Genetic differentiation

Allele frequencies between pairs of sample areas differed significantly at up to all 12 loci (Table 3). Significant genic differentiation (all with $P < 0.0001$) was recorded between all but two pairs of areas (BC751 and BC754; $\chi^2 = 20.867$, d.f. = 24, and G/OT/01 and S/OT/03: $\chi^2 = 35.288$, d.f. = 24, $P = 0.0642$) when tests were combined across loci. Global F_{ST} was 0.160 with pairwise F_{ST} ranging from −0.0034 to 0.3575 (Table 3). The greatest levels of differentiation were

Table 3 Genetic distance matrix of pairwise F_{ST} (Weir & Cockerham 1984) as calculated by GENEPOP (Raymond & Rousset 1995) below the diagonal. All F_{ST} values are significant at < 0.01 except those marked *. Values above the diagonal are the number of significantly differentiated loci (from the 12 used) between pairwise sample areas

	CAR	GAAR	NK	YUCH	D/OT/01	D/OT/02	G/OT/01	S/OT/01	S/OT/02	S/OT/03	S/OT/04	S/OT/05	Y2W	Y2E	Y4	Y5	Y7	Y8	Y10	BC742	BC750	BC751	BC752	BC754
CAR		11	11	11	10	11	11	11	12	10	10	12	10	9	11	11	12	11	11	11	11	11	11	11
GAAR	0.140		8	11	12	12	12	12	12	12	12	12	11	11	12	12	11	11	11	12	12	12	12	12
NK	0.201	0.099		11	12	12	12	12	12	12	12	12	12	11	12	12	12	12	12	12	12	12	12	12
YUCH	0.112	0.148	0.191		12	12	12	11	11	12	12	12	12	10	11	12	12	11	11	12	12	12	12	12
D/OT/01	0.157	0.138	0.216	0.141		10	8	9	9	7	6	7	11	11	10	11	12	11	11	11	11	11	11	11
D/OT/02	0.164	0.167	0.240	0.150	0.065		11	11	10	10	10	11	12	11	10	11	12	12	12	11	10	10	10	11
G/OT/01	0.150	0.160	0.215	0.115	0.036	0.083		4	5	3	7	10	12	10	11	12	12	11	11	12	11	11	11	12
S/OT/01	0.140	0.130	0.207	0.124	0.037	0.066	0.023		3	4	5	11	12	9	12	12	12	12	12	12	12	12	12	12
S/OT/02	0.135	0.127	0.193	0.124	0.043	0.048	0.030	0.013		5	5	8	11	11	11	12	12	12	12	12	12	12	12	12
S/OT/03	0.144	0.145	0.210	0.121	0.018	0.064	0.003*	0.023	0.025		4	8	11	10	11	12	12	11	11	12	11	11	11	12
S/OT/04	0.128	0.119	0.188	0.115	0.017	0.056	0.026	0.008	0.019	0.013		8	11	9	11	12	12	11	11	12	12	11	11	12
S/OT/05	0.114	0.138	0.198	0.134	0.036	0.058	0.061	0.044	0.026	0.043	0.029		12	12	9	12	12	11	11	12	11	11	11	12
Y2W	0.119	0.155	0.186	0.108	0.096	0.113	0.081	0.066	0.052	0.083	0.069	0.082		10	12	12	12	12	12	12	12	12	12	12
Y2E	0.104	0.108	0.166	0.068	0.057	0.095	0.031	0.035	0.043	0.033	0.033	0.068	0.059		12	11	12	12	12	11	11	11	11	11
Y4	0.125	0.152	0.206	0.137	0.104	0.114	0.117	0.094	0.089	0.107	0.083	0.094	0.106	0.104		11	11	10	8	11	11	11	11	11
Y5	0.168	0.196	0.234	0.180	0.210	0.253	0.217	0.201	0.203	0.207	0.182	0.204	0.178	0.173	0.101		10	11	12	11	10	11	11	11
Y7	0.197	0.203	0.245	0.243	0.220	0.259	0.239	0.225	0.207	0.215	0.203	0.200	0.199	0.219	0.138	0.123		9	10	12	12	12	11	12
Y8	0.182	0.167	0.232	0.160	0.132	0.187	0.155	0.129	0.144	0.138	0.120	0.136	0.156	0.133	0.086	0.107	0.153		8	12	12	11	11	12
Y10	0.179	0.183	0.220	0.169	0.132	0.165	0.152	0.136	0.150	0.136	0.116	0.137	0.147	0.134	0.054	0.121	0.192	0.082		12	11	11	11	12
BC742	0.289	0.281	0.342	0.276	0.200	0.251	0.231	0.222	0.244	0.209	0.202	0.219	0.250	0.217	0.215	0.283	0.301	0.170	0.172		6	8	7	10
BC750	0.266	0.261	0.324	0.261	0.171	0.225	0.205	0.196	0.214	0.181	0.179	0.195	0.219	0.194	0.182	0.259	0.272	0.154	0.146	0.025		5	5	4
BC751	0.264	0.266	0.338	0.259	0.176	0.221	0.211	0.204	0.219	0.190	0.182	0.197	0.230	0.205	0.192	0.263	0.278	0.156	0.147	0.047	0.022		6	0
BC752	0.298	0.297	0.358	0.283	0.210	0.254	0.242	0.227	0.246	0.217	0.210	0.224	0.250	0.229	0.206	0.282	0.310	0.152	0.151	0.044	0.029	0.026		5
BC754	0.265	0.273	0.345	0.260	0.182	0.229	0.215	0.207	0.225	0.196	0.185	0.198	0.235	0.209	0.196	0.266	0.291	0.162	0.152	0.047	0.020	−0.003*	0.034	

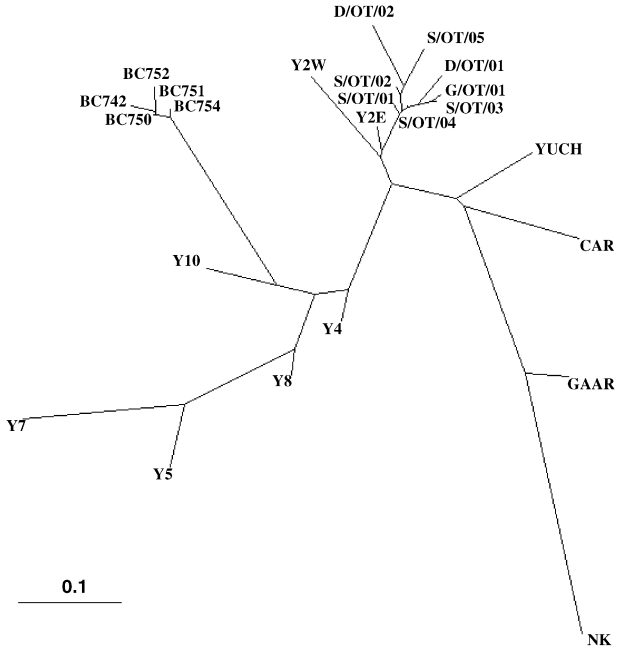

Fig. 2 Neighbour-joining tree of genetic distances (D_S) between sample areas of thinhorn sheep (*O. dalli*) constructed using TREEVIEW (Page 1996).

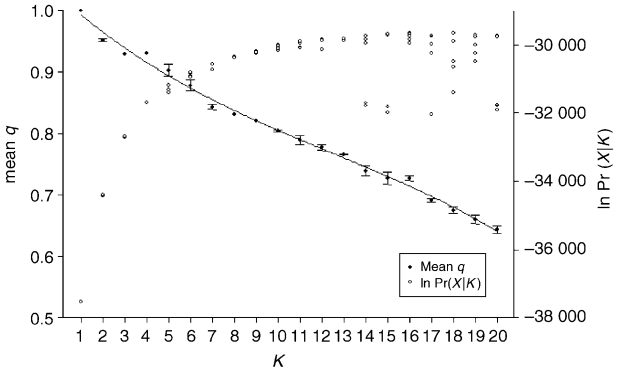

Fig. 3 Likelihood plot of structure results (MCMC with 100 000 repetitions) (Pritchard *et al*. 2000). Mean q is the mean confidence assignment of all individuals to their most probable cluster, indicating robustness of assignment (shown with standard error). ln Pr($X \mid K$) is the log likelihood for each value of K, the number of simulated clusters. The most likely K is that where ln Pr($X \mid K$) is maximized. The plot illustrates the difficulty in deciding on the most likely number of subpopulations in the data set due to isolation-by-distance.

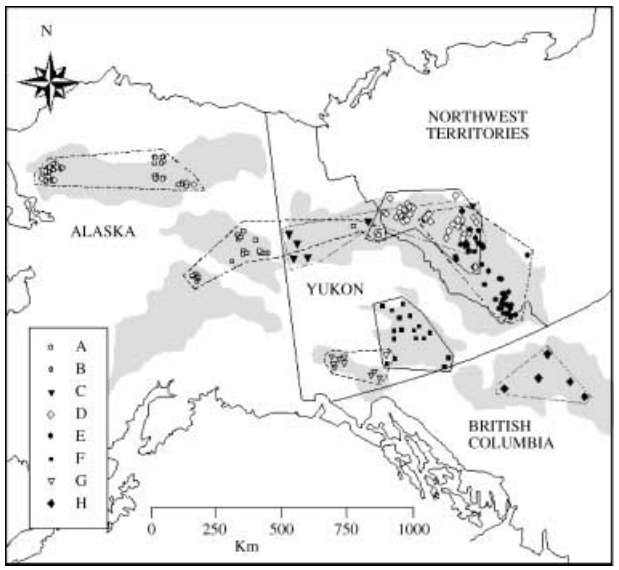

Fig. 4 Geographic locations of genetically similar clusters individuals as assigned by STRUCTURE at $K = 8$ (individuals with $q > 0.9$ only). Boundaries of each cluster are defined by lines surrounding all individuals within each. Due to the close sampling sites of some animals, one point on the map does not equal one sheep, but a variable number and is intended only as a guide to the geographical limitations of the cluster. Mountain range limitations are included in lighter shading to indicate obvious barriers to gene flow.

observed between Brooks Range and BC populations, whereas comparisons within the BC range and Mackenzie Mountains showed little genetic structure (Table 3). Patterns in the statistics D_S and $(\delta\mu)^2$ paralleled those found in F_{ST}. Correlation between D_S and F_{ST} was high ($r = 0.953$), whereas that of D_S and $(\delta\mu)^2$ ($r = 0.693$) and F_{ST} and $(\delta\mu)^2$ ($r = 0.741$) were moderate. An unrooted neighbour-joining tree constructed based on D_S revealed that sample areas on the same mountain range clustered together (Fig. 2).

The Bayesian structure analysis did not return an unequivocal number of genetic clusters as ln Pr($X \mid K$) appears to gradually reach an asymptote beyond $K = 8$ (Fig. 3). We therefore examined patterns of cluster composition and confidence of cluster assignment at various values of K to determine the most probable number of subpopulations. Up to high values of K the cluster of BC individuals (which first occurred at $K = 2$) remained robust, indicating that this group is the most genetically differentiated within the data set. Other clear clusters were concordant with mountain range boundaries. The clustering at $K = 4$ consisted of Alaskan, Mackenzie, southern Yukon and BC groups. The division of Alaska into a Brooks range and mid-Alaskan cluster occurred at $K = 6$, along with a split of the Mackenzie range into two groups. After $K = 8$, the confidence of assignments fell dramatically, with some clusters having no individuals assigned at $q > 0.9$. Also, after $K = 8$, most new clusters were formed by further divisions within the Mackenzie range. We therefore decided to assign individuals to eight subpopulation clusters (Fig. 4). The split at $K = 2$ also showed evidence of subspecies differentiation. Individuals cross-assigning into the BC cluster were from sample areas consisting of mixed subspecies (Y4, 8 and 10). Stone's sheep from these mixed sample areas cross-assigned to the purely Stone's BC areas significantly more than Dall's ($\chi^2 = 4.81$,

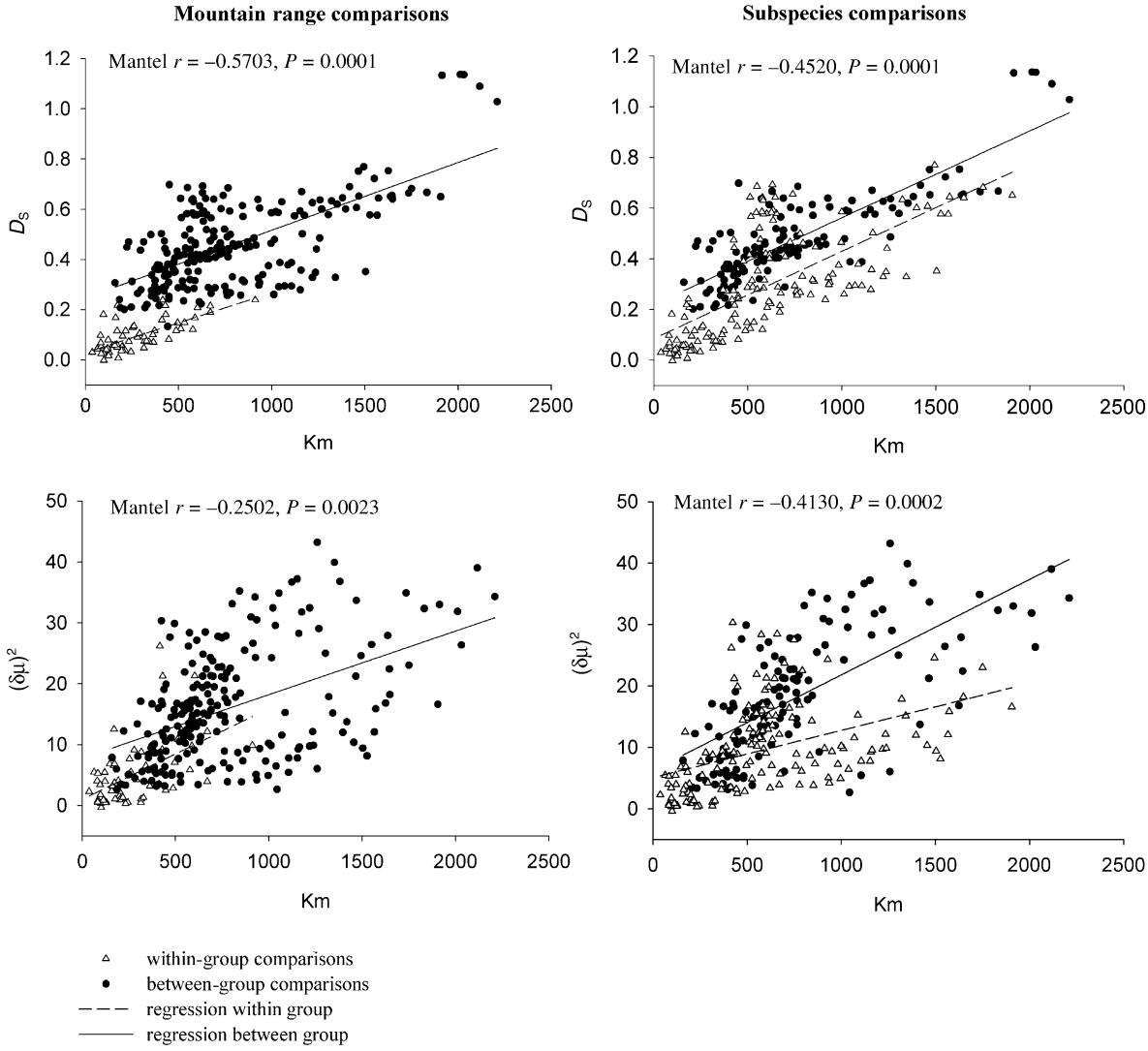

Fig. 5 Isolation-by-distance relationships between population pairs (D_S and $(\delta\mu)^2$) by mountain group and putative subspecies. Two measures of genetic distance are plotted (D_S and $(\delta\mu)^2$). Both show significant isolation-by-distance ($P = 0.0001$). Results of partial Mantel tests for the effect of mountain range and subspecies are shown.

d.f. = 1, $P < 0.05$). Cross-assigned sheep had lower confidences of assignment, q, than sheep originating from BC.

Isolation-by-distance

A significant isolation-by-distance (IBD) relationship was detected with both genetic distance measures (D_S: Mantel $r = 0.747$, $P = 0.0001$; $(\delta\mu)^2$: Mantel $r = 0.593$, $P = 0.0001$), although the measure based on allele identity fitted the data with slightly less scatter. Areas were split into those comprising mostly Dall's (between 90 and 100% of individuals) and those comprising mostly Stone's (between 62 and 100% of individuals) to examine patterns of IBD between and within subspecies (Fig. 5). Partial Mantel tests showed a highly significant effect of subspecies status on D_S (Mantel $r = -0.452$, $P = 0.0001$). When IBD was considered among populations between and within mountain ranges (as defined in Table 1) partial Mantel tests showed a significant within-range effect (Mantel $r = -0.570$, $P = 0.0001$). Analysis of residuals from a regression of genetic on geographical distance showed that D_S was affected by both mountain range ($t = 6.376$, $P < 0.0001$) and subspecies ($t = 4.511$, $P < 0.0001$). However $(\delta\mu)^2$ was affected only by subspecies ($t = 6.179$, $P < 0.0001$) and not by mountain range ($t = 0.418$, $P = 0.676$).

Discussion

This study has revealed for the first time the presence of extensive genetic variation and population substructure in

thinhorn sheep across the species range. Thinhorn sheep show similar or greater levels of microsatellite genetic diversity than that reported in other mountain ungulates including bighorn sheep (Forbes *et al*. 1995; Forbes & Hogg 1999; Gutierrez-Espeleta *et al*. 2000; Coltman *et al*. 2002), ibex (*Capra ibex*) (Maudet *et al*. 2002) and chamois (*Rupicapra* spp.) populations (Perez *et al*. 2002). Similar levels of variation as were found in thinhorn have been detected by microsatellites in other large populations of Holarctic ungulates such as caribou (Wilson *et al*. 1997).

We observed considerable genetic structure at the level of sample areas (overall $F_{ST} = 0.160$). This is likely to be a conservative estimate, considering that we sampled mainly rams, generally the dispersive sex in mammals (Greenwood 1980). We also observed a global heterozygote deficit that suggests additional genetic substructure within sampling areas. When dispersal distance is smaller than sample population area, genetic isolation can occur between subpopulations within the sample area without the presence of any physical barrier to gene flow. In this study, sample areas are probably much larger than migration distances. Furthermore, as with other sheep species, through natal philopatry it is likely that groups consisting of related individuals inhabit adjacent ranges within each sample area (Coltman *et al*. 2003b). The distribution of suitable habitat to specialists such as mountain sheep is also likely to be easily fragmented by forest encroachment or other environmental obstacles to contemporary migration. These factors have probably created a widespread 'Wahlund effect' at the level of our sample area.

Genetic distances between areas were of a similar magnitude to those reported previously in bighorn (Forbes & Hogg 1999) and other mountain ungulates (Maudet *et al*. 2002; Perez *et al*. 2002) but higher than those reported from carnivores (Paetkau *et al*. 1999; Kyle & Strobeck 2002) over similar geographical distances. This is expected due to the lower levels of migration and smaller home ranges characteristic of wild sheep populations. We found genetic differentiation between thinhorn sheep populations sampled less than 40 km apart show, at a similar scale for differentiation as that reported in desert bighorn sheep using mtDNA (Boyce *et al*. 1999) and microsatellites (Gutierrez-Espeleta *et al*. 2000).

Although we have shown fairly strong genetic differentiation and a robust pattern of isolation-by-distance among areas, the delineation of our sampling areas may be partly an artefact of wildlife management and political boundaries. The Bayesian method (Pritchard *et al*. 2000) is robust to bias introduced by sample area boundaries, as populations are not defined a priori. However, the results of the STRUCTURE analyses were not clear to interpret. This may be partly because the algorithm implemented by STRUCTURE is not well-suited for situations where there is isolation-by-distance (Pritchard & Wen 2002). The traditional area-based analysis demonstrated significant differentiation between all but two pairs of areas, whereas STRUCTURE found inconsistent results with $K > 13$ regardless of the length of burn-in. The decision to choose the relatively small number of eight subpopulations based on higher values of q was supported by the clear geographical boundaries of these clusters. Moreover, we observed that after a $K = 8$ further population subdivisions occurred within clearly defined groups of mountain blocks. The lower values of q at higher K reflect mixed membership in multiple groups, which is a probable consequence of finer-scale isolation-by-distance relationships (Pritchard & Wen 2002). The genetic subpopulation boundaries at $K = 8$, which correspond mainly to mountain range topology, were also supported by the traditional measures of distance between predefined populations (Fig. 2).

The magnitude of genetic structure varied greatly across the species range. The Mackenzie Mountains of NWT and the Ogilive and Wernecke Mountains of the Yukon (areas Y2W and Y2E, respectively) form part of the same upland system, hence the grouping of 10 sample areas into the Mackenzie range (Table 1). Genetic distances between areas in the Mackenzie range were relatively low. This is a mainly continuously inhabited area of the species range, and there may be higher rates of migration in this region due to the lack of major habitat breaks. There have been individual documented cases of young ram and nursery group sheep movement within the Mackenzie Mountain range (Simmons 1982), although data on their fate is scarce. While the Mackenzie range was mostly weakly structured, the D/OT/02 sample area was an exception. The Nahanni National Park, where no hunting can take place, divides this area into two subunits. The park includes the large South Nahanni River with a rugged canyon that may present a major barrier to gene flow. This may explain relatively large genetic distances between this area and the rest of the Mackenzie range.

Thinhorn sheep population structure will also be influenced by patterns of range expansion following the recession of the Laurentide and Cordilleran ice sheets after the last Pleistocene glaciation (Sage & Wolff 1986). At the last glacial maximum the southern Yukon and BC were under ice, whereas the Mackenzie Mountains, west-central Yukon and much of Alaska were largely unglaciated (Dyke *et al*. 2002). The current BC populations may have therefore been founded by colonization of a small number of Stone's sheep migrating southward from refugia in south-central Yukon. Alternately, BC thinhorn populations could be relicts from small ice-free refugia that existed in the northern Rocky Mountains in the seam between the Laurentide and Cordilleran ice sheets (Catto *et al*. 1996). Either scenario is consistent with the lower levels of observed genetic variation (Table 1) and genetic homogeneity (Fig. 2) of BC thinhorn populations.

Higher levels of genetic variation and structure were evident in the southern Yukon sample areas. If these areas were mainly ice-free during the last period of glaciation, then higher levels of variation would be consistent with the relative age of these populations. Pronounced population structure and long branch lengths separating these populations (Fig. 2) may therefore reflect bottleneck-like effects of recolonization from multiple refugia in this region. However, it may also be a consequence of reduced contemporary gene flow due to post-Pleistocene forest encroachment and more recent anthropogenic habitat fragmentation.

Alaskan areas were highly genetically differentiated. Genetic distance measures between Alaskan areas were greater than expected from geographical distance alone, and we found very high q-values for individuals in the two clusters based in the region (Fig. 4). This is consistent with topographical features of the region that may limit gene flow. Distances through suitable sheep habitat in Alaska are larger than the linear distance between sampling locations due to large areas of unsuitable habitat, which create semi-insular populations. The Alaskan interior lowlands and the Yukon River valley separate the Central Alaska and Brooks ranges, leading to large genetic distances between the GAAR/NK and YUCH sample areas in Alaska (Table 2, Fig. 2) and discrimination of these groups by STRUCTURE (Fig. 4). The range covered by YUCH is relatively continuous with that of the West Ogilvie Mountains (Y2W), however, providing easier route of gene flow between YUCH and Y2W. This is also reflected in the STRUCTURE analyses, with spread of the mid-Alaskan-based cluster into northern Yukon. We also note that genetic distances may be upwards biased between Alaskan populations, and genetic diversity decreased as a result of the sampling procedure in these regions. Ewes collected close together are likely to be more related than the hunted ram samples from elsewhere. However, we suggest that even with this factor, genetic distances between Alaskan ranges are higher than those between other mountain groups.

After accounting for subspecies and mountain range effects, thinhorn sheep population structure is well described by an isolation-by-distance pattern (Fig. 3) which reflects hypothetical regional equilibrium between gene flow and drift (Hutchison & Templeton 1999). The gradient of the overall isolation-by-distance plot is greater than many carnivore populations, but of a similar magnitude to bighorn sheep (Forbes & Hogg 1999). This positive relationship is caused typically by limited dispersal. Wild sheep are highly philopatric with little migration from the natal region, and a strong association with winter range. Low rates of gene flow among thinhorn sheep populations are suggested by high F_{ST} values, large degree of population differentiation and the relatively steep gradient of the isolation-by-distance plot.

Differences in pelage colour and skull measurements delineate the classification of *O. d. dalli* and *O d. stonei* subspecies (Ramey 1993). Our data also provide evidence of genetic differentiation between subspecies (Fig. 5). Genetic distances between areas consisting of the same subspecies were smaller than distances between areas of different subspecies after correcting for geographical distance. However, genetic distances are quite variable and there is considerable overlap between the within- and between-subspecies comparisons, a trend also reported in bighorn sheep (Forbes & Hogg 1999). Additional evidence of genetically differentiated subspecies comes from STRUCTURE analyses. Stone's sheep from mixed sample areas clustered more with BC samples than with areas comprising pure Dall's. In these mixed areas sheep had lower assignment confidences at $K = 2$ than in areas of one subspecies, reflecting their mixed ancestry. This provides evidence of a zone of introgression whereby 'Stone's' alleles have been introduced from the South. Genetic evidence is supported by pelage patterns with many sheep from southern Yukon showing an intermediate colour. These so-called fannin sheep are presumed to be the result of crossbreeding between the two subspecies. In thinhorn therefore genetic evidence of subspecies status is concordant with pelage colour. This is not often seen in wild sheep.

Figure 5 shows a greater effect of subspecies than mountain range on $(\delta\mu)^2$ than on D_S. As measures of allele size variance may better reveal distinctions between more deeply divergent populations (Forbes *et al*. 1995; Paetkau *et al*. 1997), this suggests that the genetic difference between subspecies is more ancient than the division resulting from mountain block.

In summary, microsatellite analyses of thinhorn sheep populations have demonstrated considerable genetic structure across the species range. The pattern of genetic differentiation is broadly consistent with isolation-by-distance. However, subspecies differentiation, colonization from multiple glacial refugia and the effects of mountain range topology complicate this pattern. Our microsatellite data provide some support for the subspecies status of Dall's and Stone's sheep.

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This study forms part of Kirsty Worley's PhD thesis, supervised by Dave Coltman, on evolutionary genetics of thinhorn sheep. Dave Coltman is now an associate professor at the University of Alberta who is interested in the evolutionary genetics of mountain ungulates and other handsome critters. This project began in Curtis Strobeck's wildlife genetics laboratory. Jean Carey is the Yukon's sheep and goat management biologist. Helen Schwantje is the Wildlife Veterinarian for British Columbia's Ministry of Water, Land and Air Protection and has a special interest in wild sheep and their health. Alasdair Veitch is a senior wildlife biologist with the Government of the Northwest Territories and is responsible for research and management on a wide range of species—primarily ungulates, carnivores and birds. Steve Arthur is an ungulate specialist with the Alaskan Department of Fish and Game.
