Population Genetic Structure Of Thinhorn Sheep From The Yukon And Northwest Territories.

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Abstract: We examined genetic variation in thinhorn sheep (*Ovis dalli*) from management zones in the Mackenzie Mountains of the Northwest Territories and from two Yukon Territory zones using microsatellite genetic markers. DNA was extracted from 394 horn core samples obtained from trophy hunted rams, from a total of ten zones, and typed at five highly variable microsatellite markers. Levels of genetic variation within each zone were high compared to data from other ungulate species. Heterozygote deficits were observed in all zones, with significant results in half of those sampled. We suspected the presence of null alleles at two loci, but suggest that the major cause of non-Hardy Weinberg allele frequencies is the presence of more than one genetic stock in some zones. There was evidence for significant genetic differentiation between eight of the ten zones sampled, suggesting that most zones as designated may constitute distinct genetic stocks. Genetic differentiation of zones was found to fit the isolation by distance model. This suggests that philopatry limits the dispersal of thinhorn sheep, resulting in genetic differentiation between populations. The gradient of the isolation by distance plot was higher than carnivores, but of a similar magnitude to other mountain ungulate species. We found no significant evidence for genetic differentiation based on colour polymorphism within both Yukon zones sampled.

Keywords: *Ovis dalli*, microsatellites, genetic structure, Yukon, Northwest Territories

Thinhorn sheep of the Yukon and Mackenzie Mountains of the Northwest Territories comprise native populations thought to number 22,000 and up to 26,000 respectively (Barichello et al. 1989; Veitch & Simmons 1999). Both thinhorn subspecies, the all white Dall's (Ovis dalli dalli) and the darker Stone's (O. d. stonei) are present in the Yukon, while only the former inhabit the Northwest Territories. The proportion of each subspecies is not uniform throughout the Yukon, with more Stone's found in the southern range than in the north, although in total there are around six times more Dall's sheep in the territory. In addition, where the colour morphs overlap an intermediate can be found. These Fannin sheep are thought to result from interbreeding between subspecies (Barichello et al. 1989). In this paper we are treating all morphs as one species in all our analyses unless otherwise stated.

Currently, little is known of population genetic structure within the species range. This information could be beneficial when managing sheep populations. If genetic stocks could be identified, managers could use the information to maintain genetic variation within a sheep population. An individual harvest target could be given for each genetic stock, thus preventing biased removal of some stocks over others. Maintaining genetic variation may prove to be valuable, especially if traits such as horn size are later found to have a genetic

component. Even if this is not the case, maintaining genetic diversity in a herd is advantageous in that problems associated with inbreeding are avoided. The effects of inbreeding depression on fitness include an increased incidence of mortality due to environmental factors, a decrease in juvenile survival, and increased disease susceptibility (Keller & Waller 2002). A genetic dataset across the species range would also be very useful for forensics cases. With the existence of such a database, cases of suspected illegal hunting and reporting incorrect kill site locations could be investigated immediately.

There are several reasons to suspect genetic differences between animals across the species range. It is known that wild sheep are very philopatric, with individuals utilizing the same home range each year. This lack of dispersal also limits gene flow to within short distances, enabling genetic differences to accumulate between isolated populations. The effects of philopatry on genetic structure in thinhorn sheep have not previously been shown. If there is an effect, the distances needed to allow such population differentiation could be estimated. Previous studies on bighorn sheep (Ovis canadensis) populations have shown the presence of genetic structure. By sequencing a gene from desert bighorn (*O. c. nelsoni*) ewes, Boyce et al. (1999) showed that genetic variation was not randomly distributed, but reflected structure in the population as expected in a philopatric species. Significant genetic distances between Rocky Mountain bighorn (*O. c. canadensis*) populations have also been reported, from both across the species range (Forbes et al. 1995), and between populations transplanted from a common source herd (Fitzsimmons et al. 1997). One previous study on Dall's sheep concluded that little genetic variation was present across locations sampled (Sage & Wolff 1986), although allozymes were used as the genetic markers in this case. If there are high enough levels of genetic variation to allow a population study, then allozyme analysis is not the best way to find it. As translated loci, changes in DNA sequence at these sites may result in an inactive enzyme product, and hence be disadvantageous to the carrier. Mutations will therefore be selected against unless they are beneficial. As a result there are lower levels of variation at allozyme loci than in non-coding regions of the genome, where mutations do not have negative consequences to the individual.

Here, microsatellites were used to study the genetic structure of thinhorn sheep. Microsatellites are regions of the genome composed of one to five base pair tandem repeat units, the dinucleotide repeat CACACA…… being one of the most common found in animals. Such sites are non-coding, and are therefore not under selection. Microsatellites mutate in a stepwise manner, with sequential addition or loss of repeat units. The mutation rate is high due to the absence of selection, allowing a large number of alleles to accumulate at each locus. This leads to substantial levels of genetic variation between individuals, making microsatellites extremely useful tools for population genetic studies (reviewed in Jarne & Lagoda 1996).

Three main issues were addressed in this study: the magnitude of genetic variation present within each game management zone sampled; the extent of the differentiation between zones and; the probability of correctly assigning an individual to the zone from which it was collected based on genotype data alone (the assignment test) (Waser & Strobeck 1998). We also investigated the presence

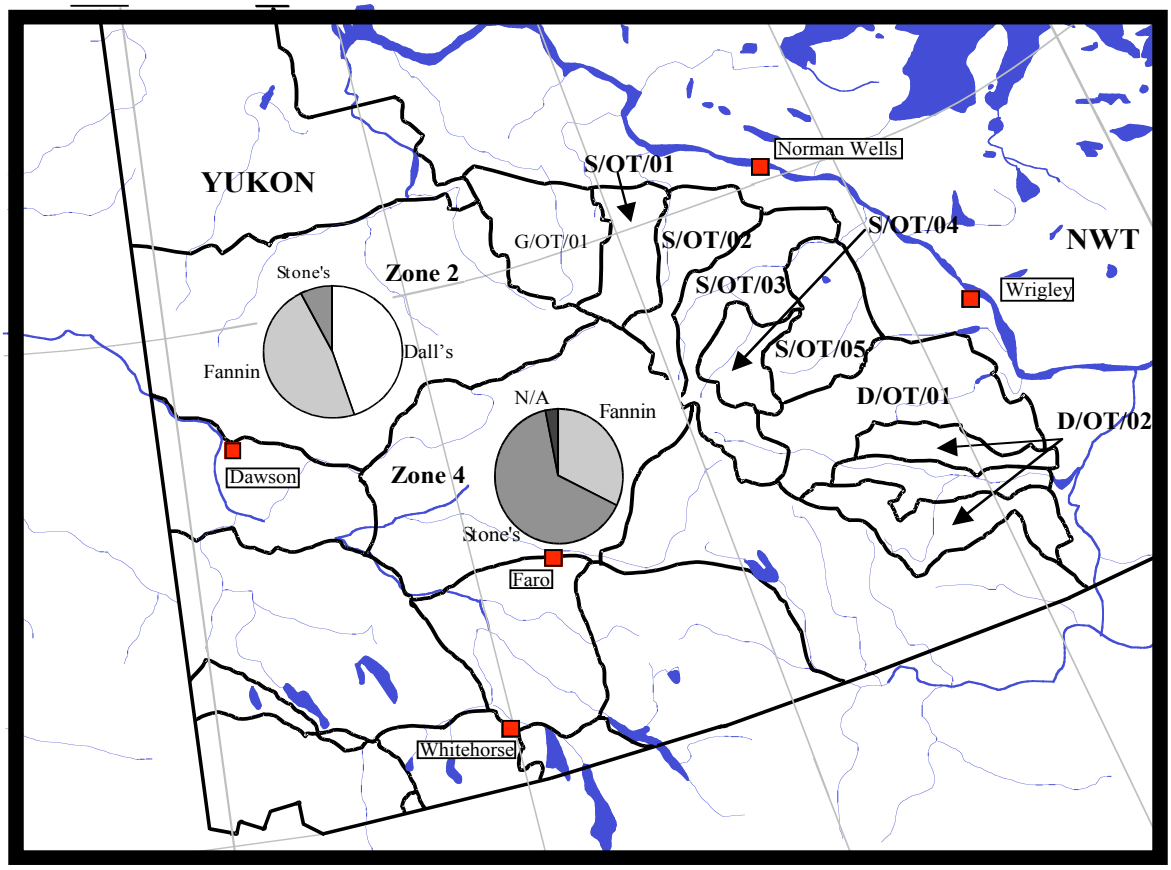

Figure 1. Game management zone locations in the Yukon and Northwest Territories. Pie charts illustrate proportions of each subspecies in samples genotyped from Yukon zones.

of genetic differences between thinhorn subspecies.

METHODS

Sample locations

The 394 thinhorn rams included in this study were sampled from 10 game management zones, all eight zones of the Northwest Territories (NWT) and two from the Yukon (zone 2 representing the Ogilvie range and zone 4 the Anvil range) (figure 1), with between 31 and 42 individuals per zone. All NWT sheep were Dall's sheep, while Yukon zones contained Dall's, Stone's and Fannin, with proportions of each depending on zone. Yukon zone 2 was largely Dall's and Fannin, whereas Stone's sheep occurred frequently among the zone 4 samples. Horn samples used were taken from plugs removed from hunted animals for the insertion of identification tags at registration between 1994 and 2000.

Molecular Techniques

Genomic DNA was extracted from approximately 0.5ml of horn material per sample using a tissue extraction kit (Qiagen, Crawley, West Sussex, UK). DNA was amplified by the polymerase chain reaction (PCR) over five highly variable dinucleotide microsatellite loci (AE16, BM1225, BM848, CP26 & FCB266) developed in domestic sheep and cattle. Each 10□l reaction contained 1.5□l DNA template at an annealing temperature

of 54oC. Full details of molecular techniques used can be found in Coltman et al (2002). PCR products were genotyped using an ABI 377 sequencer and analyzed using the software GENESCAN and GENOTYPER (Applied Biosystems, Foster City, California, USA).

Data Analyses

All measurements of genetic variation, both within and among game management zones were made using the computer program GENEPOP 3.1 (Raymond & Rousett 1995). Basic genetic parameters, including allelic diversity (the number of alleles observed at a locus) and allele frequency were calculated. Genetic structure within each zone was examined using exact tests to quantify deviations from Hardy Weinberg expectations (Guo & Thompson 1992), and by the statistic FIS. This is a measure of population wide deviation from random mating, or inbreeding, as calculated via associated reduction in heterozygosity. Significance levels of this measure and all other multiple tests were corrected using Bonferroni methods. Means of observed and expected heterozygosity, and allelic diversity were tested for equality between zones. The presence of linkage disequilibria between loci was tested using the exact test method of GENEPOP.

Genetic differentiation between zones was estimated by exact tests for differences in allele frequency both globally and pairwise between zones. The statistic FST was used to quantify genetic distance between zones. The relationship between genetic and geographic distance was examined to assess isolation by distance, with significance tested via the Mantel methodology employed by GENEPOP. Genetic distance was measured by FST/(1-FST), while geographic distances between zones were calculated by measuring the linear distance between zone central points. For Yukon samples distances were measured from the centre points of all sub-management zones included (only a small area of the total management zone).

Within the two Yukon zones, samples were divided into the groups Dall's, Stone's, or Fannin. Genetic differentiation between these groups was tested by exact tests for differences in allele frequency as well as by the genetic distance measure FST.

Assignment tests calculate the probabilities that a sample originated from all zones in the dataset, before assigning the sample to the zone with the highest likelihood of being the source. Each sample is removed from the dataset in turn and placed in the zone from which it most closely matches the group allele frequency profile. This information can be useful to investigate several aspects of population structure. If individuals have low likelihood of assignment within the source zone, it indicates genetic similarity between zones. Individuals assigning to neighboring zones could represent migrants, and their descendents, so assignment testing is informative for estimating dispersal. Another use of this data is to identify the most likely origin of illegally hunted rams. Genotypic data could for example indicate that a ram was unlikely to originate from where it was reported to have been. The program WHICHRUN (Banks & Eichert, 2000) was used here to assign each sample to the zone that most closely matched its allelic profile.

RESULTS

Variation within zones

Heterozygosity observed in all zones was relatively high, ranging from 0.681 in S/OT/04 and Yukon 2 to 0.783 in S/OT/02

Table 1. Genetic variation at five microsatellite loci in ten thinhorn sheep management zones.

Zone	N	H_e	H_o	H_{diff}	A	F_{IS}
NWT						
D/OT/01	40	0.783	0.735	-0.048	8.4	0.0618
D/OT/02	42	0.711	0.705	-0.006**	10.0	0.0042
G/OT/01	40	0.747	0.714	-0.033	8.2	0.0445
S/OT/01	40	0.790	0.764	-0.026	9.4	0.0313
S/OT/02	40	0.788	0.783	-0.005	9.2	0.0081
S/OT/03	40	0.780	0.698	-0.082*	8.6	0.1056
S/OT/04	40	0.803	0.681	-0.122**	8.8	0.1523
S/OT/05	41	0.779	0.686	-0.093	7.4	0.0714
Yukon						
Zone 2	40	0.758	0.681	-0.077**	8.2	0.1105
Zone 4	31	0.768	0.743	-0.025**	7.8	0.0189
Mean	39.4	0.771	0.719	-0.052	8.59	0.0609

N, sample size, H_e expected heterozygosity, H_o, observed heterozygosity, H_{diff}, H_o-H_e (measure of deviation from HWE), A, allelic diversity, F_{IS}, measure of deviation form random mating. Levels of population deviation from Hardy Weinberg equilibrium (H_{diff}) that are statistically significant are indicated. *P<0.05, **P<0.01.

(Table 1). Mean allelic diversity ranged from 7.4 to 10.0, reflecting the high levels of variation present at microsatellite loci. Measures of heterozygosity and the number of alleles per locus did not differ significantly across zones (paired Wilcoxon signed rank tests, all with P>0.2), indicating similar levels of genetic diversity are present across the sampled range.

Values of expected heterozygosity differed significantly from those observed in five of the ten zones, while all ten zones exhibited some degree of heterozygote deficit, indicating violation of random mating assumed in Hardy Weinberg equilibrium. Deviation from Hardy Weinberg expectations (HWE) was most pronounced in the NWT zones D/OT/02 and S/OT/04 and in both Yukon zones. Global tests of HWE by locus revealed that BM848 and FCB266 deviated significantly (P<0.001 in both cases). The deviation found in zones S/OT/04, Yukon 2 and Yukon 4 appear to have been the result of these two loci, especially BM848, which was not at equilibrium in any of these zones. The locus BM848 was then removed from the data and tests for significant heterozygote deficit repeated. Results showed that Yukon zones and S/OT/04 remained significant for deviation from HWE. In zone D/OT/02 three out of the five loci showed deviation from equilibrium. The marginal significance seen in S/OT/03 was found to be the result of the locus AE16.

Table 1 also shows values of FIS for each zone. All values were positive, ranging from 0.0042 in D/OT/02 to 0.1523

in S/OT/04. Significant positive values of FIS can indicate population substructure and inbreeding. The high value found in S/OT/04 corresponds to the highly significant deviation from HWE. FIS by locus ranged from 0.0157 for CP26 to 0.1232 for BM848. Again all values were positive. There was no evidence of genotypic disequilibria between the loci, with only one from all individual comparisons showing significance at $P<0.05$ after Bonferroni correction.

Population differentiation

Allele frequencies exhibited highly significant differentiation at all five loci ($P<0.001$ in all cases by exact tests), indicating the presence of more than one genetic stock. 141 out of 225 pairwise comparisons of allele frequency differences were significant. Those comparisons with Yukon zones were the most differentiated, the highest was seen as 42 significant differences out of 45 comparisons with Yukon zone 4. All zonal pairwise comparisons for differentiation over all loci were highly significant ($P<0.001$) except for those of G/OT/01 with S/OT/03 and S/OT/01 with S/OT/02 ($0.02<P<0.05$; this is non significant when Bonferroni corrected for multiple comparisons with □ at 0.001). This apparent lack of differentiation between these two zonal pairs is reflected in the corresponding low incidence of individual allele frequency differences (both have no significantly differentiated loci from the five tested). Population specific rare alleles were seen in zones D/OT/02 (AE16), S/OT/02 (FCB266) and Yukon zone 2 (BM1225).

Over all zones FST was 0.0656. Pairwise values ranged from 0.0055 between G/OT/01 and S/OT/03; reflecting the lack of population differentiation here, to 0.1721 between G/OT/01 and Yukon zone 4 (Table 2). Analyses indicated that Yukon zones were more genetically distant from NWT zones than NWT zones were from other NWT zones. The highest value seen between NWT comparisons was observed between D/OT/02 and G/OT/01, reflecting the largest geographic distance between zones. FST by locus ranged from 0.0504 for FCB266 to 0.0816 for AE16.

Isolation by distance

Significant correlation was found between measures of genetic and geographic distance (Figure 2, $R2=0.310$, $F1, 44=19.34$, $P<0.0001$), indicating the presence of isolation by distance. This points to limited dispersal being the causative factor for genetic differentiation across the sampling sites. There could have been some indication that comparisons with the zones containing a higher frequency of Stone's sheep (Yukon zone 4) showed greater genetic distance measures than expected, as all points in Figure 2 comparing between subspecies fall above the regression line. At present there is no statistical evidence that this is the case, as distances are not significantly higher than expected. When taken alone, comparisons between subspecies show no statistical evidence of isolation by distance ($P=0.838$), although the associated sample size is low ($n=9$).

Colour polymorphism in thinhorn sheep

Due to genetic differentiation found between the two Yukon zones, data could not be pooled, so zones were considered individually. All exact tests for genetic differentiation within zones 2 and 4 based on colour were non significant ($P>0.189$ in all cases, with the only comparison between Dall's and Stone's with $P=0.479$), and pairwise FST values were at the lower

Table 2. Genetic distance matrix

Zone	D1	D2	G1	S1	S2	S3	S4	S5	Y2
D2	0.0454								
G1	0.0579	0.1084							
S1	0.0433	0.0871	0.0475						
S2	0.0258	0.0491	0.0445	0.0101					
S3	0.0257	0.0606	0.0055*	0.0322	0.0236				
S4	0.0169	0.0522	0.0491	0.0111	0.0094	0.0243			
S5	0.0390	0.0341	0.1073	0.0600	0.0362	0.0658	0.0318		
Y2	0.0886	0.0902	0.1288	0.0782	0.0645	0.0987	0.0780	0.0941	
Y4	0.1145	0.1141	0.1721#	0.1170	0.1102	0.1485	0.0975	0.1387	0.1227

Pairwise F_{ST} is given to measure genetic distance. The lowest (*) and greatest (#) values are indicated.

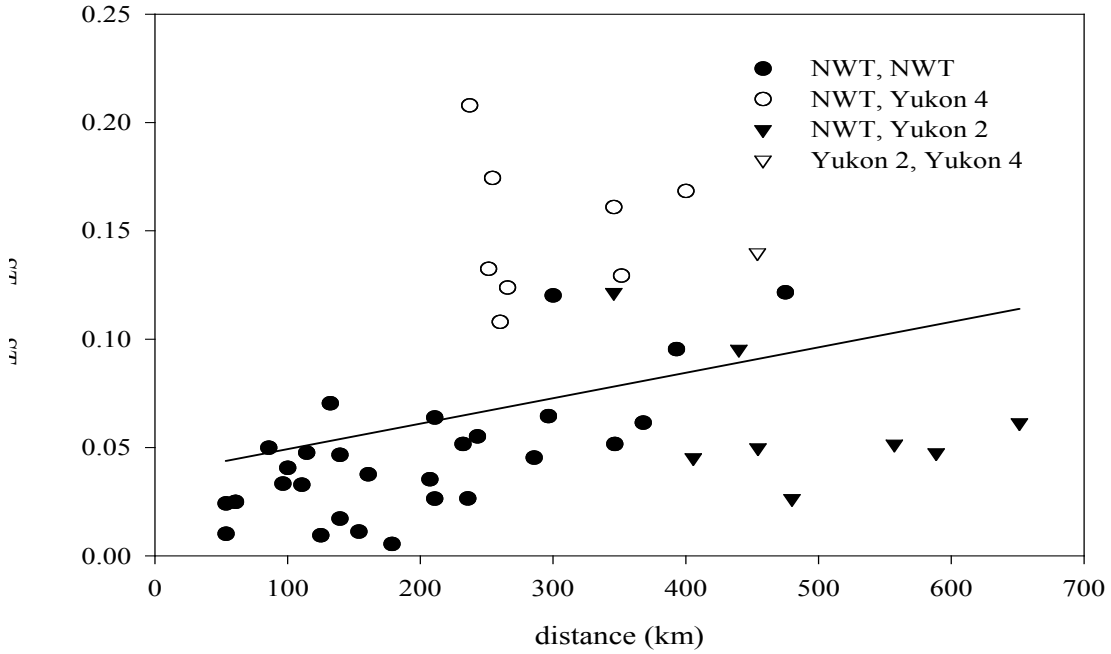

Figure 2. Plot of genetic distance, given by $F_{ST}/(1-F_{ST})$ by geographical distance for all thinhorn sheep zones. The regression line corresponds to all points on the plot. Within Dall's sheep comparisons (filled symbols) show a significant correlation, whereas the regression slope between subspecies comparisons (open symbols) was not significant.

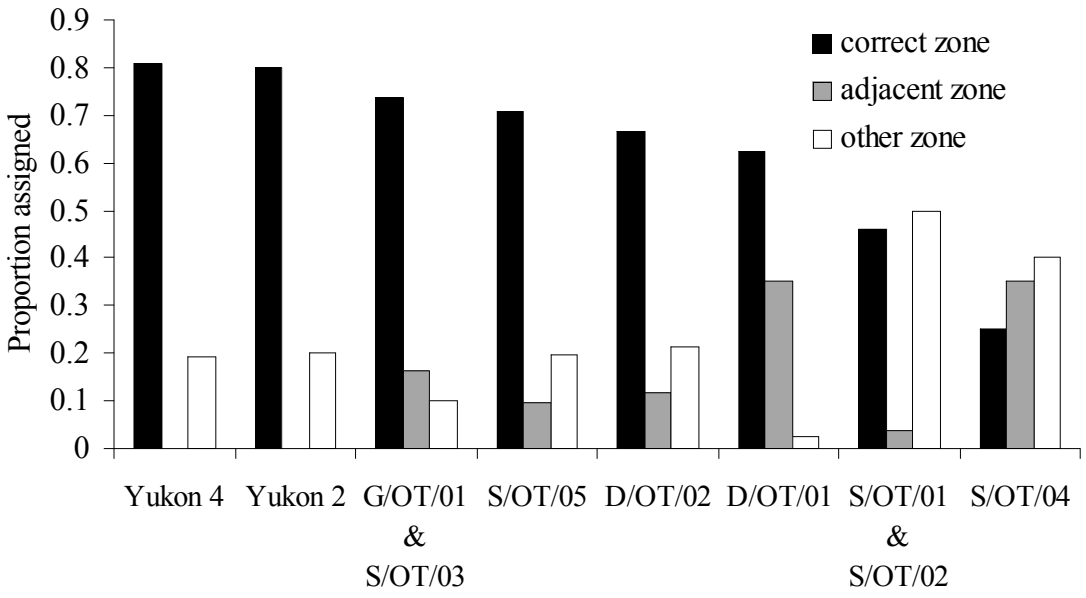

Figure 3. Results of assignment tests with undifferentiated zones combined. Adjacent zone is taken as any zone bordering the zone from which the sample was taken.

levels found in this study, ranging from -0.0012 to 0.0457 (Table 3).

Table 3. Genetic distance between subspecies in Yukon game management zones

Zone 2	Dall's	Fannin
Fannin	-0.0012	
Stone's	0.0203	0.0457

Zone 4	Fannin
Stone's	0.0045

Assignment

We ran our assignment tests with genetically undifferentiated zones combined, and recorded a high mean success rate of 63% (SE 6.7%). Figure 3 showed that Yukon individuals had a very high proportion of correct assignments, at over 80%. The poorest proportion of individuals correctly assigned was found in S/OT/04 at 25%. Those zones separated from others by the smallest genetic distances showed lower success in correct assignment to zones of origin. There was no significant difference in correct assignment between zones (Kruskal-Wallis □2= 7, d.f.=7, P=0.4289). Overall, no difference was seen between the proportions of individuals assigned to neighboring zones to those assigned to other more distant zones (P=0.156, Wilcoxon rank test). When assignments were restricted to those in which the likelihood was ten times that of assignment to any other zone, the proportion of NWT genotypes assigning to a zone fell to low levels (all <20%), and included some samples that assigned to a zone other than the source. However, 43% and 68% of all Yukon genotypes (zones 2 and 4 respectively) still assigned to a zone, and in no case was this zone other than that reported.

DISCUSSION

Genetic variation within management zones

Measures of genetic variation, such as allelic diversity and observed heterozygosity were higher than other mountain ungulate species, including bighorn sheep and Ibex (Capra ibex) (Forbes et al. 1995, Maudet et al. 2002), indicating that a large quantity of genetic variation at microsatellite loci is present within thinhorns. This variation is likely due to the large population size of native thinhorns compared to the smaller fragmented populations of Rocky Mountain bighorns (Valdez & Krausman 1999), many of which have been translocated following extirpations, causing loss of genetic diversity. Low genetic diversity in Alpine Ibex is to be expected as the population decreased to between 90 and 200 individuals at the end of the nineteenth century, most likely due to overhunting (Maudet et al. 2002).

Within all ten zones there were fewer heterozygotes than expected, with significant deviations from Hardy Weinberg equilibrium at five of these. This pattern can be explained in several ways. Firstly, null alleles may be present at some loci (Paetkau & Strobeck 1995). A null allele occurs where a mutation in the primer binding site results in non amplification of a specific allele, therefore leading to assignment of the incorrect genotype. Where this is the case deviation from HWE within a zone would be present only at loci containing null alleles. As two of the loci showed deviation from expectations across zones (BM848 and FCB266), we suspect the presence of null alleles at these sites. This explanation could account for the heterozygote deficit seen in three of the five zones deviating from expectations (S/OT/04, Yukon 2 and Yukon 4), as either or both loci suspected of containing null alleles were not at equilibrium there. In the presence of null alleles, we would also expect non-amplifying genotypes, where homozygotes for the null alleles are present. When we compared frequencies of missing genotypes across all five loci, we observed that the two with suspected null alleles did not contain more missing results than the others. Therefore the high frequency of a null allele that is needed to account for the highly significant HW deviation would seem unlikely. In addition to this, when BM848 was removed from the data and the tests re-run, we still found significant deviation from HWE. This evidence does not rule out the presence of null alleles, it still remains likely that they are present, but it would suggest that frequencies of such alleles are too low to cause all the heterozygote deficits. The deviation from HWE found in S/OT/05 could be the result of a rare null allele at AE16 in this zone.

Secondly, the result could have a biological cause. Heterozygote deficit can result from grouping genetically isolated subpopulations for analyses: the “Wahlund effect”. It can be caused when individuals from one genetically isolated population are more likely to breed within than outside this group. The Wahlund effect is therefore a form of inbreeding. Factors limiting gene flow between two divergent populations within a zone include differences in microhabitat. If populations are separated by unfavourable terrain, then differentiated groups are unlikely to meet and breed. In addition to this, the Wahlund effect is also likely to be seen when dispersal ranges are much smaller than the zones of sampling, even without the presence of physical barriers to gene flow. In this case several non-overlapping thinhorn ranges, and therefore isolated populations, can exist within a game management zone. Significant genetic

differences have been reported over relatively small distances in desert bighorn ewes using DNA sequence data (Boyce et al. 1999). Here we have reported probable genetic divergence within zones on a similar geographic scale. The highest FIS statistics were recorded in zones S/OT/04, S/OT/05 and Yukon zone 2. Since this value also indicates inbreeding it appears that these zones are likely to be composed of more than one genetically differentiated breeding stock.

Taking all explanations into consideration, we conclude that although null alleles at low frequencies are likely at BM848 and FCB266, the majority of heterozygote deficiency seen in thinhorn sheep in the Yukon and Northwest Territories is due to genetic substructure within zones. Further analyses including kill site location of each sample within a zone is needed to test this conclusion. It would seem probable that this is the case, as sheep population borders never defined those of management game zones.

The Mackenzie Mountains comprise more than one mountain block. If a zone overlaps two blocks, genetic differences are likely to be found between these populations, as sheep will be unlikely to cross from one to the other. One result that is difficult to explain is the apparent lack of differentiation between zones G/OT/01 and S/OT/03. These two are separated by two other zones, which they show differentiation with.

Genetic differentiation between zones

Both tests for allele frequency differentiation and measures of genetic distance showed significant differentiation between zones. Six zones were genetically distinct from each other, with the remaining four zones containing two isolated stocks, indicating that there were at least eight (more if subpopulations are later found) genetically distinct stocks present. NWT zones showed a greater degree of differentiation from Yukon zones than from other NWT zones, indicating that geographic distance is important in leading to and maintaining differentiation. This was shown statistically by a significant relationship between genetic and geographic distances (Figure 2). We have shown genetic differentiation between zones that are less than 60km apart (S/OT/02 & S/OT/03). This is similar to distances reported in one study of desert bighorn (Boyce et al. 1999), but less than that found in a Rocky Mountain bighorn study (Luikart & Allendorf 1996).

When compared to other mammalian studies, the gradient of the isolation by distance plot is higher than carnivores, but of a similar magnitude to bighorn sheep (Forbes & Hogg 1999). This positive relationship is typically caused by limited dispersal. Wild sheep are highly philopatric with little migration from the natal region, and strong association with winter range. Low dispersal is evident in this study through high FIS values, large degree of zonal differentiation, and the large positive gradient on the isolation by distance plot.

Values of the genetic distance statistic FST were similar to those found in other mountain ungulates (Maudet et al. 2002), but were higher than distances associated in carnivores (Paetkau et al. 1999, Kyle & Strobeck 2001). This again indicates high levels of population structure in Dall's sheep.

There was no evidence that sheep of differing colour polymorphism within the same zone showed genetic differentiation. In fact, the FST of 0.02 observed between Dall's and Stone's sheep in Yukon zone 2 was just as expected given the isolation by distance relationship (intercept on Figure

2). It is only when comparing between Yukon zone 4 (mostly Stone's) and NWT zones (Dall's) that we see trends towards a different isolating mechanism. This could be the result of historical separation of these zones, possibly during past glaciation events. Such separation during evolution could lead to the trend seen in figure 2, where sampling across these areas resulted in higher than expected genetic distances. A similar result was reported by Forbes & Hogg (1999) when sampling over subspecies of bighorn. If there are genetic differences present between thinhorn subspecies, they may be subtle. It could be that interbreeding of animals in zones where both subspecies are present has obscured any subspecies level differentiation. It is only when subspecies are geographically isolated that we are able to see levels of differentiation greater than expected (as we see in comparisons with Yukon zone 4). More data is needed to examine these differences before definite conclusions can be made. Greater power required to detect significant results will be provided when numbers of genetic markers are increased. Therefore, at present this issue remains unresolved.

The high proportion of correct assignments reflects the genetic distances separating zones. Probability of assignment has previously been shown to be related to FST (Maudet et al, 2002). We would expect that when more Yukon zones are sampled, filling in the geographic gaps, the proportions assigned would fall to similar levels to those of NWT zones. Levels of correct assignment show that we would have a good chance of identifying geographical origins of unknown samples using the set of microsatellites in this study, especially for geographically isolated populations. With the use of more loci, finer details of structure could be determined by this method, with possible migrants and their descendents being identified within each zone.

All measures of genetic differentiation presented here are likely to be conservative when considering the species as a whole, as only rams are included in the sample. In mammals it is generally the males that are the dispersive sex (Greenwood 1980). The same is true for wild sheep, although in a lesser degree as even rams have shown very low incidences of permanent dispersal (Festa-Bianchet 1991).

Management implications

We have shown that thinhorn populations of the Yukon and Northwest Territories contain high levels of genetic variation, greater than those seen in some other mountain ungulate species. Already with only five loci we have identified eight differentiated genetic stocks of thinhorn sheep in the Yukon and Northwest Territories. We would suggest that these stocks should be managed separately. It is important to identify genetically distinct populations so that one such group is not selectively targeted over all others by hunting. Although the hunting pressure on the species as a whole is very small and has no substantial impact on the population (Veitch & Simmons 1999), it could have effects on specific genetic stocks, if hunter pressure is not equally distributed across zones. Maintaining a sustainable harvest in the future means attention to the rate of harvest at each genetic unit. This would prove especially important if horn size is later found to have a genetic component, in addition to wishing to avoid problems associated with inbreeding. Bias towards harvesting those rams with the largest horns may also be targeting some genetic stocks over others,

decreasing genetic variation for horn growth, and thereby reducing horn size in future generations. This is especially relevant in wild sheep species, as no senescence is seen (Coltman et al, 2002) and old rams that are more likely to be hunted are still siring offspring. Although environmental factors such as forage quality in the year of birth; whereby rams born in good forage years can attain greater horn size that those born in poor years, play a part in determining horn size, the genetic component would remain important. Rams still pass on the genetic component of their horn size to their sons, who may be born in a differing quality year.

We recorded high probabilities of assignment to source management zone based on genotype data alone. This makes these tests useful tools for thinhorn forensics cases. With the use of more markers, success of this method will increase. In the future we may be able to assign suspected illegal rams to likely kill sites of a reasonably small area, using this expanded dataset as a reference. This will be possible due to the relatively small distances dividing genetically differentiated populations in this species.

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