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RESEARCH ARTICLE

Lack of sex-biased dispersal promotes fine-scale genetic structure in alpine ungulates

Gretchen H. Roffler · Sandra L. Talbot · Gordon Luikart · George K. Sage · Kristy L. Pilgrim · Layne G. Adams · Michael K. Schwartz

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Abstract Identifying patterns of fine-scale genetic structure in natural populations can advance understanding of critical ecological processes such as dispersal and gene flow across heterogeneous landscapes. Alpine ungulates generally exhibit high levels of genetic structure due to female philopatry and patchy configuration of mountain habitats. We assessed the spatial scale of genetic structure and the amount of gene flow in 301 Dall's sheep (*Ovis dalli dalli*) at the landscape level using 15 nuclear microsatellites and 473 base pairs of the mitochondrial (mtDNA) control region. Dall's sheep exhibited significant genetic structure within contiguous mountain ranges, but mtDNA structure occurred at a broader geographic scale than nuclear DNA within the study area, and mtDNA structure for other North American mountain sheep populations. No evidence of male-mediated gene flow or greater philopatry of females was observed; there was little difference between markers with different modes of inheritance (pairwise nuclear DNA $F_{ST} = 0.004$–0.325; mtDNA $F_{ST} = 0.009$–0.544), and males were no more likely than females to be recent immigrants. Historical patterns based on mtDNA indicate separate northern and southern lineages and a pattern of expansion following regional glacial retreat. Boundaries of genetic clusters aligned geographically with prominent mountain ranges, icefields, and major river valleys based on Bayesian and hierarchical modeling of microsatellite and mtDNA data. Our results suggest that fine-scale genetic structure in Dall's sheep is influenced by limited dispersal, and structure may be weaker in populations occurring near ancestral levels of density and distribution in continuous habitats compared to other alpine ungulates that have experienced declines and marked habitat fragmentation.

Keywords Fine-scale genetic structure · Sex-biased dispersal · Philopatry · Landscape genetics · Population connectivity · Non-invasive sampling · *Ovis dalli dalli*

Introduction

By quantifying genetic structure, it is possible to gain understanding of historical distributions (Shafer et al. 2011) and the influence of landscape features on gene flow (Manel et al. 2003; Holderegger and Wagner 2008; Landguth et al. 2010). Patterns of genetic structuring in many wildlife species are influenced by dispersal characteristics and habitat configuration (Chesser 1991a; Coltman et al. 2003; Storz 1999). Sex-biased dispersal patterns are common and are influenced by evolutionary responses to variable environments and population dynamics, ultimately playing a role in shaping the genetic structure of populations across landscapes (Greenwood 1980; Clutton-Brock 1989; Chesser 1991b). In the majority of mammal species, females are philopatric and remain with their natal group,

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G. H. Roffler (✉) · S. L. Talbot · G. K. Sage · L. G. Adams
US Geological Survey, Alaska Science Center, 4210 University Drive, Suite 201, Anchorage, AK 99508, USA
e-mail: groffler@usgs.gov

G. Luikart
Flathead Lake Biological Station, Fish and Wildlife Genomics Group, Division of Biological Sciences, University of Montana, Polson, MT 59860, USA

K. L. Pilgrim · M. K. Schwartz
US Forest Service Rocky Mountain Research Station, 800 E. Beckwith, Missoula, MT 59801, USA

whereas males have greater rates and distances of dispersal (Greenwood 1980; Pusey 1987; Goudet et al. 2002). However, a number of studies have recently revealed much variation within patterns of dispersal (for a review, see Lawson Handley and Perrin 2007; Clutton-Brock and Lukas 2012) and exceptions to the generally accepted paradigm of the direction of sex bias in mammals have been reported (Pérez-González and Carranza 2009; Pérez-Espona et al. 2010; Kierepka et al. 2012). Furthermore, empirical studies reveal that factors influential to dispersal are complex, sometimes difficult to detect, and not mutually exclusive (Lawson Handley and Perrin 2007).

Traditionally, it has been thought that sex-biased dispersal in polygynous mammals often contributes to genetic patterns wherein maternally-inherited markers exhibit stronger and more finely subdivided genetic structure than paternally or biparentally inherited markers (Goudet et al. 2002; Prugnolle and De Meeüs 2002). Female philopatry and male-mediated dispersal are common in alpine ungulates (Geist 1971; Festa-Bianchet 1991; Loison et al. 1999; Valdez and Krausman 1999; Festa-Bianchet and Côté 2008), and these behavior patterns contribute to pronounced genetic structure at broad spatial scales (Forbes and Hogg 1999; Worley et al. 2004; Shafer et al. 2011). Distinct differences in patterns of genetic structure ascertained from sex-inherited and autosomal markers have been demonstrated in alpine ungulates, indicating high levels of sex-biased dispersal (Ramey 1995; Boyce et al. 1997; Schaschl et al. 2003).

Landscape configuration may also influence dispersal, which in turn affects the degree of genetic structuring. Species with high levels of gene flow attributed to vagility, continuous distribution of habitat, or habitat generalization generally have weak genetic structure (Forbes and Hogg 1999; Kyle and Strobeck 2001; Schwartz et al. 2002). Mountainous landscapes may increase genetic structure in natural populations by limiting seasonal movements and dispersal distances (Lomolino and Davis 1997). Alpine ungulates generally display high levels of genetic structuring influenced by naturally patchy (e.g., mountainous terrain) or anthropogenically fragmented landscape patterns (Bleich et al. 1990; Epps et al. 2005; Crestanello et al. 2009). For example, Worley et al. (2004) identified significant levels of genetic structure ($F_{ST} = 0.160$) among 24 populations across the thinhorn sheep (*Ovis dalli*) species range, defined spatially by disjunct major mountain ranges. Sheep populations in Alaska were more genetically differentiated than populations in other parts of the species range presumably due to large areas of unsuitable habitat between mountain ranges (Worley et al. 2004). Additional genetic substructure within mountain ranges was also identified suggesting a need for further investigation into genetic structure at the scale of contiguous mountain ranges.

Dall's sheep (*Ovis dalli dalli*) are thought to occur at ancestral levels of abundance and distribution compared to fragmented and reintroduced bighorn sheep (*Ovis canadensis*) populations south of the Canadian Rockies (Valdez and Krausman 1999). Thus, assessments of Dall's sheep fine-scale genetic structure and natural patterns of genetic variability provide a useful basis for comparison to southern bighorns as well as other alpine ungulates. Such comparisons can provide insight into the relative influences of habitat configuration and dispersal behavior in shaping population genetic structure. Understanding the ecological factors that contribute to genetic structure is an important consideration in wildlife management and conservation, in particular where populations are becoming more fragmented and attempts are being made to maintain population and habitat connectivity.

We assessed the pattern, degree, and spatial scale of genetic structure and gene flow among Dall's sheep over a contiguous mountain landscape in southern Alaska (Fig. 1). We used both individual-based and population-based approaches and a continuously distributed sampling scheme at a finer scale than has been performed previously in alpine ungulate studies, and therefore expected to obtain higher spatial resolution. We hypothesized that patterns of female philopatry and male-mediated gene flow would result in stronger genetic structure at finer spatial scales for females than males. We tested this hypothesis by comparing measures of genetic differentiation in two ways: (1) by using markers with different modes of inheritance (mtDNA with maternal inheritance, and nuclear microsatellites with biparental inheritance), and (2) by comparing male and female microsatellite genotypes for signatures of sex-biased dispersal. We also tested whether geographic (Euclidian) distance was the most significant influence on genetic spatial patterns among sheep in the study area, or if the partitioning of genetic diversity aligned geographically with prominent mountain ranges, given that naturally patchy habitats inhibited dispersal and thus gene flow between putative sheep populations.

Methods

Study area

Wrangell-St. Elias National Park and Preserve (WRST) contains an estimated 11,000–14,500 Dall's sheep (Schmidt and Rattenbury 2013) equivalent to 15 % of the subspecies' population (Festa-Bianchet 2008) and encompasses approximately 28,000 km^2 of contiguous sheep habitat across multiple mountain ranges (Fig. 1). Sheep in WRST typically occupy areas with open vegetation in steep, rugged terrain between 900 and 1,500 m

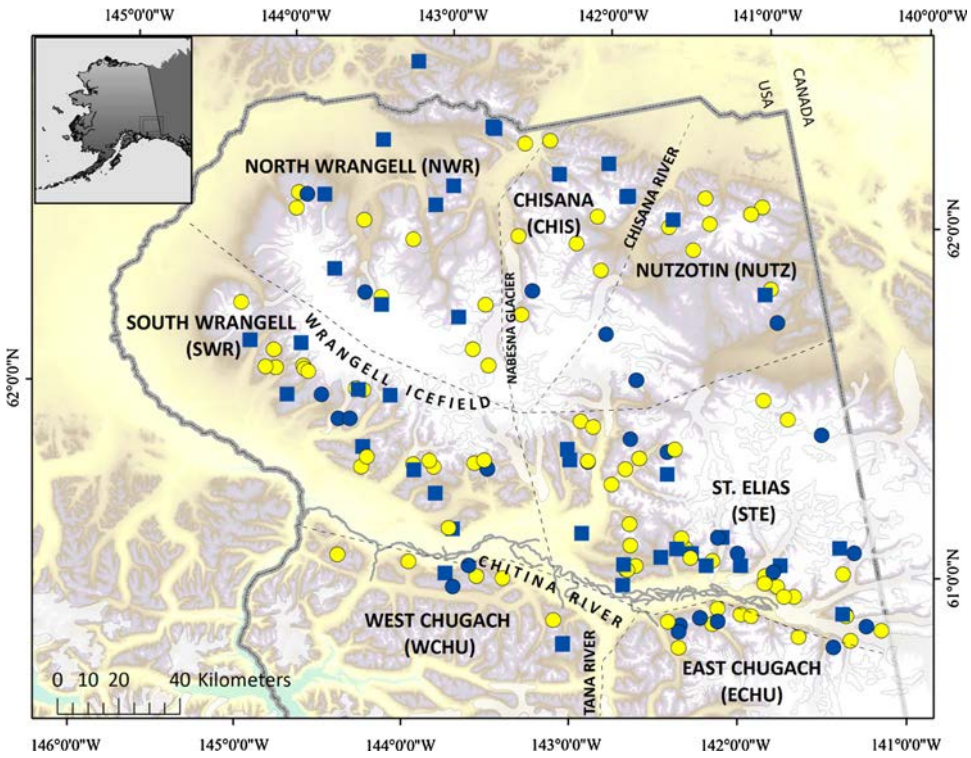

Fig. 1 Locations of Dall's sheep genetic sampling from 7 hypothesized populations defined by mountain range (*dashed lines*), Wrangell-St. Elias National Park and Preserve (*gray border*), Alaska, 2007–2009. *Circles* represent fecal samples collected from ewe-lamb bands (*light*) and ram groups (*dark*), and *dark squares* represent muscle samples collected from harvested males

(Terwilliger 2005). Areas below 900 m, primarily forests and major river valleys between mountain ranges, are generally avoided by Dall's sheep (Nichols and Bunnell 1999; Walker et al. 2007), and thus could serve as potential barriers to dispersal and gene flow.

Sample collection

We collected fresh feces in late summer 2007–2009 ($n = 47$, 69, and 120, respectively) using a fixed-wing aircraft to locate sheep groups and a helicopter to land nearby and collect samples. During 2007–2008 we focused sampling efforts in the southern portion of WRST, and in 2009 expanded the study area to also include sheep habitat throughout the entire National Park and Preserve. Each year fecal samples were collected from randomly selected 50 km^2 grid cells throughout the study area to uniformly distribute samples across the landscape (Schwartz and McKelvey 2008). We sampled from one sheep group per selected grid cell, and collected samples from 1 to 3 adults per group identified as isolated piles of large-sized pellets. Sheep were classified visually into the following sex and age composition categories: rams (≥1/4 curl), ewe-like (ewes and sub-adult males indistinguishable from females), and lambs. Samples were stored in 20 mL plastic vials with 15 mL of 100 % ethyl alcohol. We also obtained muscle samples and harvest locations from mature rams (≥3/4 curl) taken during the annual hunting season (10 August–20 September), 2007–2009 ($n = 29$, 16, and 50, respectively).

DNA extractions

DNA from fecal samples was extracted 1 month after collection using the DNeasyTM Blood Kit (Qiagen, Valencia, CA) modified to include an initial wash of one fecal pellet for 20 min in 900 µL lysis buffer (0.1 M Tris–HCl pH 8.0, 0.1 M ethylene-diamine-tetra-acetic acid pH 8.0, 0.01 M NaCl, 1 % N-lauroyl sarcosine, pH 7.5). We used approximately 300 µL of the lysis buffer directly in the extraction protocol (per Maudet et al. 2004). We extracted DNA from muscle samples using the Epicentre Biotechnologies MasterPure^{TM} Genomic DNA Purification Kit (Epicentre Cat# MG71100). Genomic DNA extractions were quantified using fluorometry and diluted to 50 ng/µL working solutions.

Microsatellite genotyping

We used DNA from the muscle samples to test a set of 15 microsatellite loci previously used in bighorn sheep, 7 of which are linked to functional genes (Luikart et al. 2008; Table 1 in Supplementary material). We initially screened all fecal samples by amplifying 6 microsatellite loci using two separate polymerase chain reaction (PCR) runs. Samples that did not produce identical results at more than 3 loci were excluded from further analysis. We amplified the remaining fecal samples at the 15 microsatellite loci in 5 multiplex PCR amplifications. The reaction volume (10 µL) contained 1.0 µL DNA, 1× reaction buffer (*Applied Biosystems*), 2.0 mM $MgCl_2$, 200µM of each

Table 1 Population (P) and individual (I) based analyses performed in this study using microsatellite (ms) and mitochondrial control region (mtDNA) data from Dall's sheep populations in Wrangell-St. Elias National Park and Preserve, Alaska, 2007–2009

Test	Analysis	Level	Genetic marker	Analysis tool	Reference
Genetic variation					
Genetic diversity	*A, AR, Ho, He*	P	ms	GENEPOP	Raymond and Rousett (1995)
Departures from selective neutrality	F_{ST} outlier approach	P	ms	LOSITAN	Antão et al. (2008)
Genetic diversity	*h*, л	P	mtDNA	ARLEQUIN	Nei (1987), Excoffier and Lischer (2010)
Best evolutionary model	AIC	P	mtDNA	MODELTEST	Posada and Crandall (1998)
Departures from selective neutrality, historical population fluctuations	Fu's F_S, Tajima's D	P	mtDNA	ARLEQUIN	Fu (1997), Tajima (1989)
Phylogenetic relationships	Reduced-median-network	P	mtDNA	Network	Excoffier and Smouse (1994), Bandelt et al. (1999)
Genetic structure					
Population differentiation (allele and haplotype frequencies)	F_{ST} analyses	P	ms, mtDNA	GENEPOP	Weir and Cockerham (1984)
Population differentiation (genetic distance between haplotypes)	Φ_{ST} analyses	P	mtDNA	ARLEQUIN	Excoffier et al. (1992)
Hierarchical genetic structure	Spatial partitioning of variation	P	ms, mtDNA	SAMOVA	Dupanloup et al. (2002)
Sex-specific spatial structure	Spatial autocorrelation of genetic structure	I	ms	GenAlEx	Peakall and Smouse (2006)
Detecting barriers to gene flow	Monmonier's algorithm	I	ms	AIS	Miller (2005)
Most likely number of genetic clusters	Bayesian clustering	I	ms	STRUCTURE	Pritchard et al. (2000)
Most likely number of genetic clusters and spatial discontinuities	Spatially explicit Bayesian clustering	I	ms	GENELAND	Guillot et al. (2005)
Sex-biased dispersal					
Comparison of genetic structure accounting for differences in N_e	Comparison of standardized nu F_{ST} and mtDNA F_{ST}	P	ms, mtDNA	NA	Zink and Barrowclough (2008)
Probability of being an immigrant	Assignment index correction (AIc)	I	ms	GenAlEx	Mossman and Wasser (1999), Peakall and Smouse (2006)
Bayesian methods of likelihood-based assignment	Individual assignment probabilities	P	ms	GeneClass	Cornuet et al. (1999), Paetkau et al. (2004), Piry et al. (2004)
Bayesian methods of likelihood-based assignment	Detecting first generation immigrants	P	ms	GeneClass	Rannala and Mountain (1997), Paetkau et al. (2004), Piry et al. (2004)

A average number of alleles at 15 microsatellite loci, *AR* allelic richness (El Mousadik and Petit 1996), H_o observed heterozygosity, H_e expected heterozygosity, *h* haplotype diversity (Nei 1987; Eq. 8.4), л nucleotide diversity (Nei 1987; Eq. 10.4), F_S Fu's F_S (Fu 1997), *D* Tajima's D (Tajima 1989), AIC Akaike information criterion (Akaike 1974)

dNTP, 1 μM reverse primer, 1 μM dye-labeled forward primer, 1.5 mg/ml BSA, and 1 U *Taq* polymerase (*Applied Biosystems*). The PCR profile was 94 °C/5 min, (94 °C/1 min, 55 °C/1 min, 72 °C/30 s) × 45 cycles. The resultant products were visualized on a LI-COR DNA analyzer (LI-COR Biotechnology). We accepted data from the fecal samples only if the microsatellites produced consistent scores in 2–4 PCR amplifications. We used DROPOUT (McKelvey and Schwartz 2004, 2005) to estimate and efficiently eliminate genotyping errors, and estimated the genotyping error rate as the proportion of the successful PCRs that amplify DNA. We additionally estimated the proportion of all PCRs yielding the correct consensus genotype. Sex-determination for each individual sheep was accomplished by PCR amplification of the Y-chromosome amelogenin gene (Pidancier et al. 2006).

Mitochondrial DNA sequencing

We used DNA extracted from both muscle and feces to screen and develop markers to access sequence data from 3 mtDNA genes (Table 1 in Supplementary material), including 496 base pairs (bp) of the cytochrome oxidase I (COXI), 686 bp of the 5′ portion of the cytochrome b genes (CytB), and ca. 473 bp of the hypervariable I portion of the control region (CR). We used these markers to test the feasibility of using DNA extracted from the fecal samples. The CR had the highest variability, thus the final dataset comprised a 473 bp of the hypervariable I domain, gathered from 220 individuals. PCR products were electrophoresed on a LI-COR (1999) automated sequencer (Sonsthagen et al. 2004).

Genetic variation

Each microsatellite locus was tested for deviation from Hardy–Weinberg proportions (HWP) and linkage disequilibrium (LD) in GENEPOP 4.0 (Raymond and Rousett 1995) within each population, between pairs of loci, and at the global level. In Table 1, we summarize all analyses performed, with additional description below.

We measured the level of genetic diversity and variation within and among putative populations at neutral microsatellite loci by calculating the mean number of alleles per locus (*A*), allelic richness (*AR*) per population, and mean observed and expected heterozygosities (H_o and H_e) using GENEPOP. We tested for departures from selective neutrality using an F_{ST} outlier approach based on simulation methods (Beaumont and Nichols 1996) implemented in LOSITAN (Antão et al. 2008). Loci falling outside the 99 % confidence intervals from a plot of average locus heterozygosity vs. F_{ST} were identified as potentially under selection. We conservatively removed loci from genetic analyses assuming neutrality if they were consistently flagged as outliers in pairwise population comparisons, regardless of initial classification as neutral or potentially adaptive (following Johnson et al. 2011).

We estimated haplotype (h) and nucleotide (π) diversity (Nei 1987; Eq. 8.4 and 10.6, respectively) of the mtDNA in ARLEQUIN 3.5 (Excoffier and Lischer 2010), and used MODELTEST 3.06 (Posada and Crandall 1998) to select the evolutionary model that best described the pattern of nucleotide substitution within our data. Model selection was based on Akaike Information Criterion (AIC; Akaike 1974). We tested for selective neutrality, and for historical fluctuations in population demography (Aris-Brosou and Excoffier 1996), using Fu's F_S (Fu 1997) and Tajima's D (Tajima 1989), implemented in ARLEQUIN. For population pairwise analyses, we coded an indel in haplotype CR44 as a transition (A–G). A network of haplotypes based on polymorphic sites of mtDNA sequences was constructed using reduced-median-network approaches implemented in Network 4.611 (Bandelt et al. 1999; http://www.fluxus-engineering.com).[1] In the median-joining network approach all minimum spanning trees are combined within a single network and inferred intermediate haplotypes added to reduce length of tree using the maximum variance parsimony criterion (Excoffier and Smouse 1994).

Genetic structure

Population-based analyses

To test for patterns of fine-scale genetic structure within a region of continuous habitat, we assigned samples a priori to the seven distinct mountain regions within this area where sheep were sampled (Fig. 1): the Southern (SWR) and Northern (NWR) Wrangell Mountains, the St. Elias (STE), Nutzotin (NUTZ), Chisana (CHIS), and the eastern (ECHU) and western (WCHU) Chugach Mountains. We hypothesized that these different mountain regions would characterize sheep populations because they are separated by potential barriers to dispersal such as large icefields (e.g. up to 100 km long, >30 km wide), glaciers (>20 km long, 1–10 km wide), and major river valleys (Fig. 1). We performed F_{ST} analyses (Weir and Cockerham 1984) to determine variance in microsatellite allele frequencies using GENEPOP. For mtDNA, we calculated F_{ST} (based only on frequencies of haplotypes) and also Φ_{ST} which also accounts for genetic distance between haplotypes (Excoffier et al. 1992) using ARLEQUIN. Confidence intervals were used to determine whether F_{ST} values were significantly greater than zero, and significance of F_{ST} values were based on random permutation tests ($N = 10{,}000$). We used SAMOVA (Dupanloup et al. 2002) to examine spatial partitioning of microsatellite and mtDNA variation within and among populations, and hierarchical groupings of populations defined by geographical regions. This method maximizes genetic variance between groups of populations (F_{CT}) through a simulated annealing process. We performed 1,000 permutations for each run, and tested groupings with K ranging from 2 to 6.

Individual-based analyses

To make inferences about dispersal and levels of gene flow among sheep in the study area, we employed an individual-based approach and compared results to population-based analyses. We tested for a genetic signature of isolation by distance with Mantel tests (Mantel 1967) performing random permutations of comparisons of pairwise genetic and

[1] Fluxus Engineering (2012) Network 4.611

geographical distances using GenAlEx. To assess sex differences in fine-scale spatial genetic structure, we performed spatial autocorrelation analyses for males and females combined and separately using GenAlEx. We identified areas of genetic discontinuity across the study area using Monmonier's maximum difference algorithm (Monmonier 1973), implemented in the program Alleles In Space (Miller 2005), and compared these population boundaries to those determined a priori. This method calculates genetic distances among all pairs of individuals in a spatially explicit Delauney triangulation network, and infers barriers to gene flow by identifying and ranking areas in the network with high levels of genetic distance.

To estimate the most likely number of genetic clusters (K), we used an individual-based Bayesian algorithm implemented in GENELAND 3.3.0 (Guillot et al. 2005), and compared results to those derived from STRUCTURE 2.3.2 (Pritchard et al. 2000). Both methods assign individuals to clusters by minimizing HWP and LD between loci within populations, but GENELAND incorporates spatial information from sample locations. We tested all values of K from 1 to 15, using 500,000 iterations with a burn-in period of 20,000 for GENELAND and 500,000 for STRUCTURE. Using GENELAND, we conducted analyses defining allele frequencies as uncorrelated based on Dirichlet distributions (Pritchard et al. 2000) and assessed the influence of different values of spatial uncertainty (between 50 and 10,000 m) on population structure, replicating each model 20 times and ranking them by the mean logarithm of posterior probability. We used an admixture model in STRUCTURE, defined allele frequencies as correlated, and excluded location priors representing the mountain range where samples were collected. We determined K first by calculating the maximum likelihood value ($\ln[Pr(X|k)]$), and second with ΔK (Evanno et al. 2005) using the program Structure Harvester v0.6.7 (Earl Earl and VonHoldt 2012) and compared the results. We assessed population structure of males and females combined and separately.

Sex-biased dispersal and recent migration

Typically mtDNA produces higher F_{ST} values than nuclear DNA data because the N_e of mitochondrial genes is approximately 0.25 that of nuclear genes (Birky et al. 1983), although there is some variability in this estimate based on deme N_e, migration rate and substructure (Whitlock and McCauley 1999). To account for differences in effective population size (N_e) between mtDNA and nuclear DNA genomes, we calculated the expected mtDNA F_{ST} values using nuclear DNA F_{ST} assuming an island model of migration and equal dispersal rates among sexes. Dall's sheep exhibit a polygynous mating system (Bowyer and Leslie 1992), and therefore variance in reproductive success is higher in males than females, similar to other polygynous species (Clutton-Brock 1988). As a result, N_e for males is likely smaller than what would be expected in a system of random breeding success among individuals. Thus, we substituted 0.5 for the value of mitochondrial N_e using the following formula: $F_{ST}\,[\mathrm{nu}] = 1 - e^{0.5\ln[1-F_{ST}(\mathrm{mtDNA})]}$, and then compared observed and standardized F_{ST} values of mtDNA and nuclear markers (Zink and Barrowclough 2008).

We tested for sex-biased dispersal using microsatellites and a modified population assignment test which produces an assignment index correction (AIc) value for each sex following the method of Mossman and Wasser (1999), implemented in GenAlEx. Negative AIc values identify individuals with a higher probability of being immigrants, whereas individuals with positive values have a lower probability. We compared mean AIc values for each sex with a non-parametric Mann–Whitney U test.

To estimate recent migration, we used the male and female multi-locus genotypes and performed two tests using Bayesian methods of likelihood-based assignment (Rannala and Mountain 1997; Cornuet et al. 1999) coded in GeneClass 2.0 (Piry et al. 2004). Using populations defined by the Bayesian clustering algorithms, we first determined individual assignment probabilities by testing the null hypothesis that an individual originated from the population where it was sampled (Rannala and Mountain 1997). We then identified first generation migrants with the ratio of L_h/L_{max}, wherein L_h is the likelihood of finding a given individual in the population in which it was sampled, and L_{max} is the likelihood of finding that individual among all sampled populations (Paetkau et al. 2004). For both tests we employed the Bayesian criterion of Rannala and Mountain (1997) in combination with the Monte Carlo resampling algorithm for probability computation with 100,000 simulated individuals (Paetkau et al. 2004). We employed a conservative probability threshold of $\alpha = 0.05$, below which any individual was assigned as a migrant.

Results

Genetic variation

Microsatellites

During the initial screening phase, 16 fecal samples did not produce consistent genotypes at more than 3 loci and were dropped from analysis. Genotyping error rate (defined as the proportion of multi-locus genotypes with an error for >1 locus) in the fecal samples was 7.7 %, and controlled for by repeated genotyping 3–4 times to obtain consensus genotypes. Of the 236 fecal samples analyzed at all loci, we obtained high quality, consensus genotypes for 206 unique individuals (87.3 %). The probability that 2 randomly

Table 2 Genetic variation at 15 microsatellite loci and the mtDNA control region among putative populations of Dall's sheep defined by geographic regions, 2007–2009, Wrangell-St. Elias National Park and Preserve, Alaska

Populations	Microsatellite DNA							Mitochondrial DNA					
	ID	*n*	*A*	*AR*	H_o	H_e	F_{IS}	*n*	*K*	*h*	л	F_S	*D*
Chisana	CHIS	26	5.13	3.90	0.588	0.590	0.027	17	9	0.91	0.012	−0.25	−0.53
East Chugach	ECHU	28	3.60	3.00	0.472	0.467	0.005	17	1	0.00	0.000	[a]	0.00
Nutzotin	NUTZ	23	4.47	3.69	0.562	0.597	0.082	17	7	0.85	0.010	1.20	0.37
North Wrangell	NWR	50	5.73	4.01	0.578	0.605	0.053	26	6	0.80	0.030	11.57	2.89
St. Elias	STE	102	5.27	3.41	0.482	0.555	0.138	65	15	0.75	0.007	−2.69	−1.11
South Wrangell	SWR	56	4.67	3.43	0.518	0.534	0.039	39	10	0.66	0.007	−0.33	**−1.79**
West Chugach	WCHU	16	3.47	3.08	0.564	0.511	−0.07	15	2	0.13	0.001	1.32	**−1.81**

Significant values are in boldface type

A average number of alleles at 15 microsatellite loci, *AR* allelic richness (El Mousadik and Petit 1996), H_o observed heterozygosity, H_e expected heterozygosity, F_{IS} inbreeding coefficient (Wright 1951), *K* number of haplotypes observed, *h* haplotype diversity (Nei 1987; Eq. 8.4), л nucleotide diversity (Nei 1987; Eq. 10.4), F_S Fu's F_S (Fu 1997), *D* Tajima's D (Tajima 1989)

[a] Fu's F_S cannot be calculated with only 1 haplotype present in sample

selected unrelated individuals would have identical genotypes by chance (PID) was 1.70×10^{-8} for 15 loci, providing adequate power to discern individuals from the fecal samples.

After Bonferroni correction, 10 of 105 tests for HWP were significant, but there was no pattern across loci or populations (Table 2 in Supplementary material). Population SWR ($\chi^2 = 51.47$, $df = 28$, $P = 0.004$) deviated from HWP overall. We did not detect any LD among loci across or within populations. Given the deviations from HWP were relatively small and loci were independent, all loci were retained in our analyses.

Using fecal and muscle samples we obtained complete multilocus genotypes of 301 sheep (Table 2). Sex was determined for 202 (of 206) individuals genotyped from fecal samples. Including the muscle samples, which all originated from rams, we obtained genotypes from 120 females and 177 males. Sheep from nursery groups and genetically identified as males ($n = 24$) were excluded from the analyses of sex-biased dispersal.

F_{ST} outlier tests indicated that KERA could be under directional selection in 2 population pairwise comparisons, and MAF48 under balancing selection for 2 population pairwise comparisons. Loci that have a signature of balancing selection are generally not removed from population genetic analyses (Allendorf et al. 2013). Although KERA is believed to be linked to a functional gene and demonstrated as under selection in some bighorn populations (Luikart et al. 2008), neither this locus nor MAF48 were consistently flagged as outliers in pairwise population comparisons in WRST Dall's sheep. Furthermore, because results did not change when including and excluding these loci, we retained them in analyses. The number of alleles per locus and per population ranged from 3.23 to 5.85 (mean = 4.66, SE = 0.373), and *AR* was similar across populations, although lowest for the southern populations (CHU, STE, and SWR; Table 2). Averages for H_o and H_e were 0.56 and 0.57, respectively.

Mitochondrial DNA

Thirty-eight haplotypes were observed among 196 individuals obtained from muscle ($n = 79$) and feces ($n = 117$). We observed no indication of PCR or sequencing error based on 50 % quality control reruns. The complete sequences for the haplotypes are deposited in GenBank (accession numbers pending). The evolutionary model that best fit via MODELTEST was the TrN + I distance model (−Ln L = 1,083.64; AIC = 2,177.28; proportion of variable sites = 0.79; tv/ts ratio = 56.847). Haplotype diversity was high in the 3 northern populations (CHIS, NUTZ, NWR) and low in the Chugach Mountains (ECHU and WCHU; Table 2). Based on the frequencies of segregating nucleotide sites using Tajima's D, 2 of the southern populations had genetic signatures of population expansion (SWR and WCHU).

The haplotype network reflected high levels of diversity and 18 private haplotypes (Fig. 2a). A star-like pattern was present with 1 high-frequency haplotype located centrally (Fig. 2a; haplotype CR4) including haplotypes observed in all populations except CHIS. Rare haplotypes radiating from CR4 were found in the southern region (STE, 12 haplotypes; SWR, 6 haplotypes). Geographic patterns of haplotype frequencies indicate high levels of diversity within all populations except ECHU (CR4), and WCHU (CR4 and CR27; Fig. 2b). No haplotypes were shared between all populations.

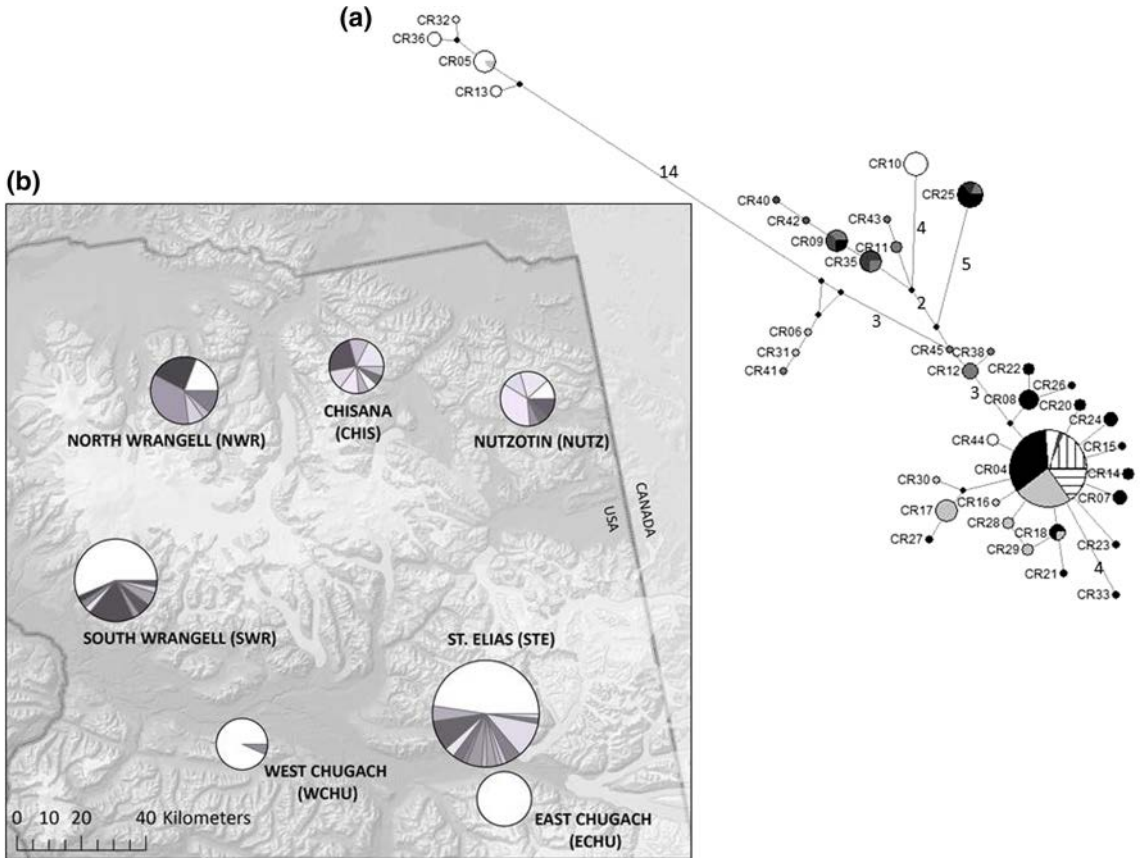

Fig. 2 **a** A median-joining network of 38 mtDNA control region haplotypes from Dall's sheep during 2007–2009, Wrangell-St. Elias National Park and Preserve, Alaska. The size of the each circle represents the frequencies of the haplotype, with each color showing the population of origin by mountain range (CHIS: *grey*, ECHU: *horizontal lines*, NUTZ: *dark grey*, NWR: *white*, STE: *black*, SWR: *light grey*, WCHU: *vertical lines*). Branch size represents the number of mutations between haplotypes with the number of substitution differences (<1) summarized on each branch. **b** The frequency of observed haplotypes within each mountain range. The size of each pie chart represents the total number of individuals, and each pie slice is proportional to haplotype frequency

Population structure

Population-based analyses

Analyses of variance in microsatellite allele frequencies uncovered significant substructuring overall ($F_{ST} = 0.056$, $P < 0.001$). Pairwise comparisons of F_{ST} results indicated that all 7 a priori populations are significantly differentiated from each other (Table 3). Similarly, analyses of variance in mtDNA haplotype frequency uncovered significant substructuring overall ($F_{ST} = 0.188$, $P < 0.001$). Pairwise comparisons were also significant with the exception of 2 of the northern populations (CHIS and NUTZ), and the Chugach populations (ECHU and WCHU; Table 3). Applying a model of evolution, all sheep populations were significantly differentiated ($\Phi_{ST} = 0.004$–0.594) except for CHIS and NUTZ, and WCHU from the other 3 southern populations (ECHU, STE, and SWR).

The SAMOVA model that maximized microsatellite variation among population groupings was based $K = 4$ ($F_{CT} = 0.014$), whereas $K = 2$ ($F_{CT} = 0.433$) explained the greatest amount of among-population variation for mtDNA. Significant population differentiation for both marker types occurred between the southern groups (ECHU, STE, SWR, and WCHU) and the northern groups (CHIS, NUTZ, and NWR), although microsatellite hierarchical groupings indicated more fine-scale genetic structure of the northern populations at nuclear DNA.

Individual-based analyses

We found a significant, but weak signature of isolation by distance ($r = 0.201$, $P = 0.001$) in the Mantel test of geographic versus genetic distance and the value of the correlation coefficient r declined with increasing geographic distance throughout WRST. Pairwise genetic distances for males and females were also weakly significant and had similar patterns of spatial autocorrelation (males: $r = 0.213$, $P = 0.001$; females: $r = 0.195$, $P = 0.001$; Fig. 1 in Supplementary material). The Monmonier

Table 3 Pairwise comparison of F_{ST} values from analyses of microsatellite loci and the mitochondrial DNA control region among Dall's sheep populations in Wrangell-St. Elias National Park and Preserve, Alaska, 2007–2009

Population comparisons		Microsatellite DNA F_{ST}	Mitochrondrial DNA		
			Expected F_{ST}	F_{ST}	Φ_{ST}
CHIS	ECHU	0.109	0.325	0.544	0.594
CHIS	NUTZ	0.015	0.019	0.038^	0.057^
ECHU	NUTZ	0.117	0.308	0.521	0.588
CHIS	NWR	0.027	0.076	0.147	0.269
ECHU	NWR	0.105	0.258	0.450	0.394
NUTZ	NWR	0.051	0.083	0.159	0.304
CHIS	STE	0.063	0.085	0.163	0.425
ECHU	STE	0.032	0.093	0.177	0.050
NUTZ	STE	0.070	0.074	0.143	0.400
NWR	STE	0.072	0.076	0.147	0.443
CHIS	SWR	0.049	0.123	0.231	0.472
ECHU	SWR	0.098	0.090	0.172	0.038
NUTZ	SWR	0.057	0.109	0.206	0.457
NWR	SWR	0.026	0.095	0.181	0.393
STE	SWR	0.068	0.016	0.032	0.066
CHIS	WCHU	0.080	0.268	0.464	0.556
ECHU	WCHU	0.058	0.004	0.009^	0.009^
NUTZ	WCHU	0.091	0.249	0.437	0.549
NWR	WCHU	0.062	0.213	0.380	0.371
STE	WCHU	0.028	0.067	0.129	0.043^
SWR	WCHU	0.056	0.059	0.115	0.004^

F_{ST} values from analyses of microsatellite loci are observed values (Weir and Cockerham 1984, Bonferroni correction applied). For mtDNA, expected F_{ST} values were computed by standardizing for the lower effective population size of the mtDNA genome. Pairwise tests for differentiation of mtDNA are based on frequencies of haplotypes only (F_{ST}) and from values derived from analyses employing the TrN + I model of evolution (Φ_{ST}; Excoffier et al. 1992). All F_{ST} values are significant at $P < 0.05$ except those marked ^

algorithm identified genetic discontinuities between the northeastern populations (CHIS and NUTZ) and the other populations in the study area, indicating potential barriers to gene flow (Fig. 3a).

The best-supported GENELAND model produced 3 clusters (Fig. 3c–e), grouping the southern populations (WCHU, ECHU and STE), the northwestern populations (SWR and NWR) and the northeastern populations (CHIS and NUTZ). The posterior distribution of the modal value consistently resulted in 3 populations when the sexes were pooled or considered separately (Fig. 3b; resulting pairwise F_{ST} values 0.040–0.076). The most likely model resulting from the STRUCTURE analysis included 2 populations (Fig. 4b) at the highest hierarchical level based on ΔK (Evanno et al. 2005); however, it is important to note that this method is a second order statistic, and in the presence of an isolation by distance genetic pattern, interpreting K may be challenging (Pritchard et al. 2007; Schwartz and McKelvey 2008). Based on the maximum likelihood of K method (Ln $P(X|K)$, STRUCTURE identified 6 populations (Fig. 4c). The individual assignment histogram distinguishes between the Southern (WCHU, ECHU and STE) and northern (NWR, CHIS and NUTZ) populations, with SWR displaying signs of admixture and clustering with the northern populations at $K = 2$, but distinguished as a separate group at $K = 3$ (Fig. 4a).

Sex-biased dispersal and recent migration

We detected only limited differences between microsatellite and mtDNA F_{ST} values after accounting for differences in nuclear and mitochondrial N_e (Table 3). If male and female dispersal rates are similar, mtDNA F_{ST} values should be approximately 2-4 times larger than nuclear DNA (Birky et al. 1983; Larsson et al. 2009; Allendorf Allendorf et al. 2013), and larger values would suggest male-biased dispersal. After standardization, pairwise F_{ST} estimates for mtDNA data were 1.7–2 times higher than F_{ST} estimates for microsatellites, therefore providing no evidence for sex-biased dispersal. Analysis of sex-biased dispersal using populations defined a priori suggested that males are as likely to be immigrants as females (male mean AIc = 0.208 ± 0.189 SE; female mean AIc = 0.250 ± 0.183 SE; Mann–Whitney U test, female U = 6647, male U = 5134, $P = 0.103$). Using the 3 clusters identified with Bayesian algorithms (South, Northwest, Northeast) in the assignment tests, and the proportions of males and females that assigned to other populations were not significantly different ($\chi^2 = 1.96$; $df = 1$; $P = 0.162$). In total, 33 individuals (12 % of the sampled population) were identified as recent immigrants (18 males, 15 females), and there was no evidence of sex bias ($\chi^2 = 0.027$; $df = 1$; $P = 0.870$). Between 1 and 9 % of WRST sheep from each population of origin were assigned to another population, with sheep from the South cluster displaying high assignment rates to the neighboring Northwest population (7 %).

Discussion

The spatial scale of population structure

Using an intensive sampling design, we observed genetic structure in mountain sheep at a finer geographic scale than has been previously reported. Genetic structuring within the contiguous mountain ranges of WRST was significant overall and among a priori populations (Table 3). The magnitude of nuclear genetic structure of Dall's sheep in WRST was comparable to other mountain sheep

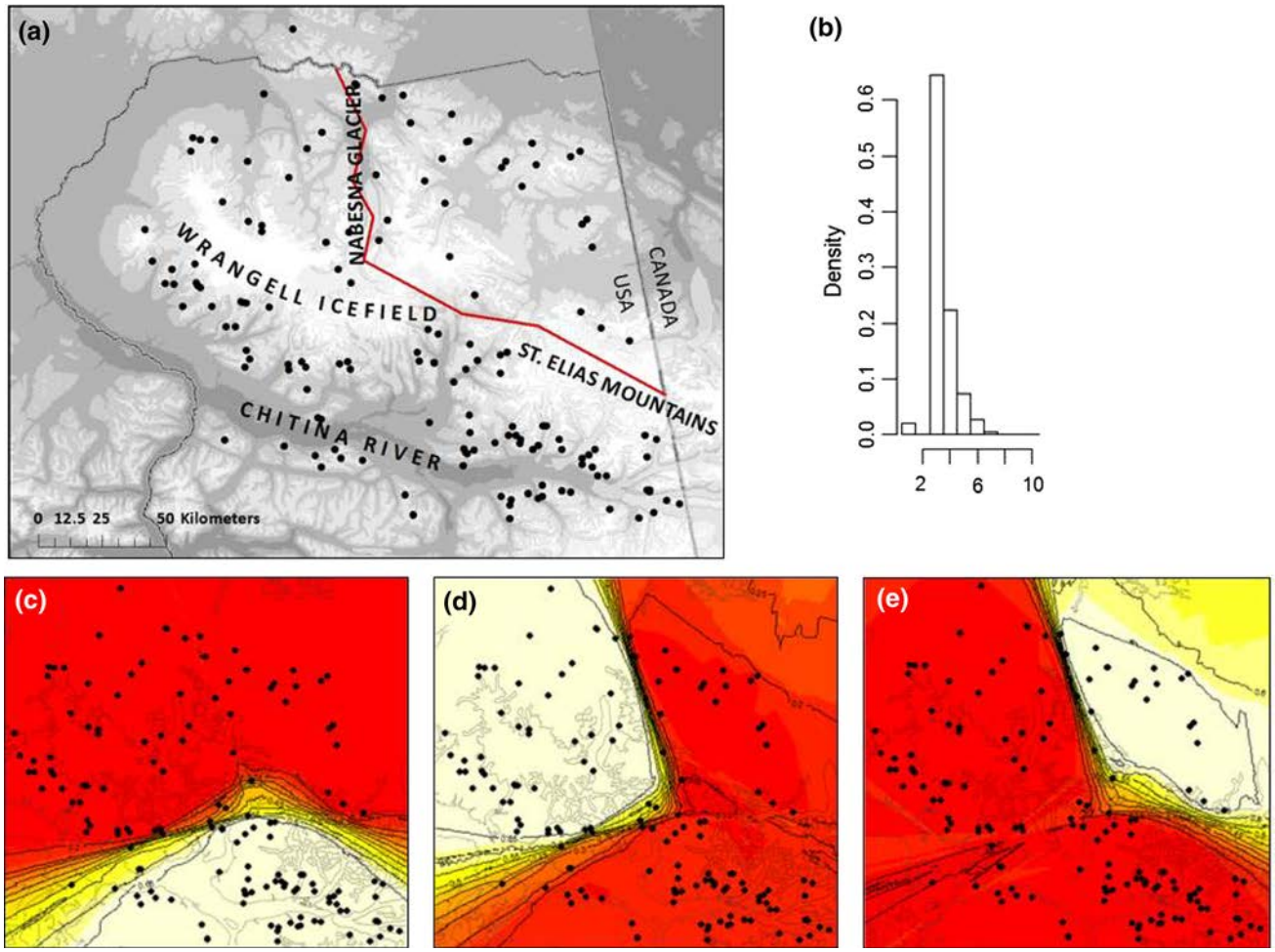

Fig. 3 **a** Potential barrier (in *red*) identified using Monmonier's algorithm to the east of the Nabesna glacier and north of the St. Elias Mountains. **b** Posterior density distribution of the number of genetic clusters estimated from GENELAND. Maps of the individual posterior probability of the 3 genetic clusters identified in GENELAND in the following geographic regions of Wrangell-St. Elias National Park and Preserve, Alaska: **c** South, **d** Northwest, and **e** Northeast. The highest membership values of individuals are identified by *light yellow* areas. (Color figure online)

populations sampled at a similar spatial scale, such as desert bighorn (*O. c. nelsoni*) in Arizona ($F_{ST} = 0.043$; Gutiérrez-Espeleta et al. 2000), and southern California (pairwise $F_{ST} = 0.050$–0.350; Epps et al. 2005), and argali (*Ovis ammon polii*) in Afghanistan ($F_{ST} = 0.035$; Luikart et al. 2011). Although thinhorn sheep exhibit greater levels of genetic structure at the large spatial scale of the entire species range ($\sim$2,000 $\times$ 1,000 km; $F_{ST} = 0.160$; Worley et al. 2004), sheep populations within distinct mountain ranges have similar levels of differentiation as those in WRST (e.g., southern Yukon Territory pairwise $F_{ST} = 0.054$–0.192; Mackenzie mountain range $F_{ST} = 0.003$–0.083; Worley et al. 2004). In contrast, using mtDNA and comparing similar spatial scales, other mountain sheep populations have higher levels of genetic structure than Dall's sheep in WRST. For example, bighorn in southern California ($F_{ST} = 0.330$, Boyce et al. 1999), and throughout the Rockies ($F_{ST} = 0.360$; Luikart and Allendorf 1996) have higher structure among populations than sheep in our study area ($F_{ST} = 0.188$).

A greater degree of population genetic structure across the geographic range of the species may be expected, especially when comparing populations that occupy disjunct habitats, yet examination at a finer spatial scale can reveal behavioral and landscape factors that influence genetic structure within continuous habitats (Pierson et al. 2010). Although alpine ungulate populations tend to display fine-scale genetic structuring, the scale of differentiation is variable between species, and across different landscapes. Individual movement patterns are influential to the genetic structure of populations, and patterns of consistent use of particular ranges contribute to the relatively high levels of genetic structure found in Dall's sheep. For example, Worley et al. (2004) observed genetic differentiation at

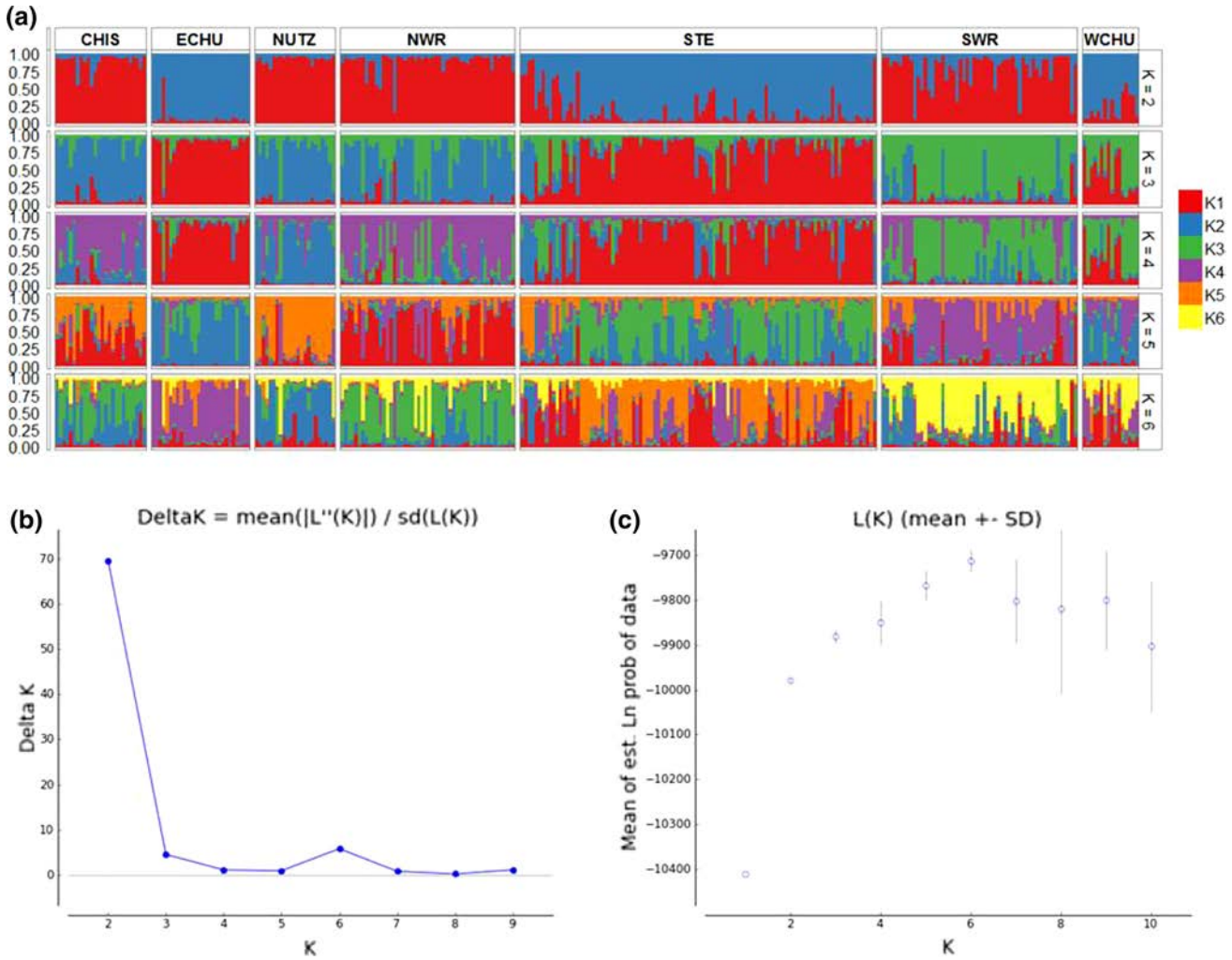

Fig. 4 Bayesian clustering analysis and posterior probability assignment of Dall's sheep ($n = 301$) to genetic clusters analyzed at 15 microsatellite loci using STRUCTURE (Pritchard et al. 2000) without using loc prior. Each individual is represented with the proportion of their genotype that was assigned to each of K clusters. **a** Posterior probability of estimated membership coefficient of each individual to genetic clusters (Q) with each vertical bar representing an individual, assuming $K = 2$–6. **b** Mean variations of data probabilities (Δ(LnPr(X|K)) between values of K (Evanno et al. 2005). **c** Maximum likelihood value (ln[$Pr(X|k)$]) of genetic clusters K (Pritchard et al. 2000)

microsatellite loci between thinhorn sheep populations <40 km apart. However, the amount of genetic structure varied across the species range and was apparently influenced by landscape continuity, as areas with more continuous habitat and fewer barriers to movement had lower levels of genetic differentiation (Worley et al. 2004). In our study area, populations defined a priori by mountain ranges (25–160 km apart) were all significantly differentiated based on microsatellite data. However, Bayesian clustering produced fewer populations than hypothesized, indicating that although fine-scale genetic structure is present at the landscape scale, there are areas of genetic continuity that extend beyond mountain ranges. In WRST, individuals 70–100 km apart assigned to different Bayesian-defined genetic clusters, and spatial autocorrelation analyses indicate that Dall's sheep separated by <80 km are more genetically similar than expected by random chance.

For mtDNA haplotype frequencies, some populations were not differentiated between adjacent mountain ranges (ECHU and WCHU, CHIS and NUTZ; median centroids 55–85 km apart) indicating genetic structure at a broader geographic scale than found for nuclear DNA. In comparison, Luikart and Allendorf (1996) found significant differences in haplotype frequencies between populations 5–15 km in northwestern Montana, and attributed this high level of genetic structure to female philopatry, habitat fragmentation, genetic drift resulting from small N_e, and limited gene flow among populations. In sharp contrast to bighorn sheep throughout the southern Rockies, thinhorn sheep population sizes are near historical levels and are

distributed across continuous mountain habitats with little anthropogenic fragmentation. Thus the lack of habitat and population fragmentation provides an explanation for the relatively lower levels of mtDNA structuring, and has implications for the spatial scale at which populations are genetically clustered, a useful measure for identifying units of management and conservation.

Historical influences of landscape on fine-scale genetic structure

Population expansion is well-documented across taxa following the Pleistocene glacial period (Hewitt 2004). Our study area is bounded to the south and east by portions of the largest non-polar icefields in the world and has experienced multiple fluctuations in glacial extent since the late Tertiary period 2.6 MYA (Rampton 1981). The southern regions of WRST contain the most rugged and heavily glaciated terrain in the study area, and glaciers have persisted here longer than on the northern side, which in contrast has a drier climate and contains more continuous and gentle mountain habitat. As glaciers receded at the end of the Wisconsin glaciation period ($\sim$10,000 years ago), sheep likely expanded into the southern portion of our study area from the north.

The genetic signature of shifting glacial extent is apparent in WRST Dall's sheep. Mitochondrial analyses suggested mtDNA lineages north and south of the Wrangell Icefield that have recently (i.e., Holocene) diverged, coupled with a population expansion in the southern region of WRST (Fig. 2). Haplotypes CR5, 13, 32, and 36 formed the most distant branch of the haplotype network and were present mainly in the NWR population, and in combination with mtDNA AMOVA, F_{ST}, and Φ_{ST} results support the presence of a separate historical lineage. An expanding ancestral population in the southern part of the study area that gave rise to many rare haplotypes was revealed in the haplotype network, and in negative Tajima's D test values in southern populations. There was a distinct trend of higher haplotype diversity in the north relative to the southern populations, consistent with a pattern of north-to-south colonization. This genetic signature was discernible considering the lower levels of haplotype diversity found in all southern populations and especially in ECHU and WCHU represented largely by 1 haplotype (CR4). As glaciers recede, sheep populations will likely continue to expand resulting in higher levels of genetic connectivity and variability over time.

Contemporary influences of landscape on fine-scale genetic structure

The spatial configuration of the landscape appeared to be more influential to sheep genetic structure than distances alone. Both population- and individual-based analyses identified the partitioning of genetic diversity at a fine spatial scale, and determined that the boundaries of genetic clusters aligned geographically with prominent mountain ranges, icefields, and major river valleys. The northeastern populations (CHIS and NUTZ) formed a distinct cluster in the GENELAND results, and this region was also delineated by a potential barrier to gene flow with the Monmonier's algorithm. This region is separated from the other populations in the study area by the St. Elias Icefield to the south, and the high rugged mountains to the east of the Nabesna glacier (>2,200 m; Fig. 3a). Sheep dispersal abilities may be bounded by an upper elevation range at which they are not likely to travel. Bayesian clustering of microsatellite data and hierarchical modeling of mtDNA data divided WRST sheep to the north and south of the Wrangell Icefield and mountain range (>3,000 m; Fig. 1). However, there is evidence of contemporary gene flow around the northwestern end of the Wrangell Mountains demonstrated by NWR and SWR grouping as one cluster in GENELAND, and admixture in SWR region in the STRUCTURE results. Finally, GENELAND clustered sheep to the north and south of the lower Chitina river valley (Fig. 3c–e) where the valley is 20 km wide, indicating this landscape feature could serve as a barrier to dispersal.

Sex-biased dispersal

We found little support for sex-biased dispersal based on microsatellite and mtDNA F_{ST} comparisons and male and female assignment tests. Although Dall's sheep are highly mobile, both males and females show strong fidelity to seasonal ranges (Geist 1971) and permanent dispersal of mountain sheep is rare (Festa-Bianchet 1991; Forbes and Hogg 1999; Geist 1971). It is possible that neither sex is dispersing substantial distances, as dispersal in mountainous terrain is fraught with risks (Geist 1971). Sex-biased dispersal is a common behavioral paradigm, but levels can vary and are influenced by a number of factors including habitat configuration. Our results suggest that in this large area of contiguous habitat, movements leading to gene flow are similar for male and female sheep. It is not surprising that female sheep may disperse, as in most species some dispersal also occurs in the typically philopatric sex (Lawson Handley and Perrin 2007), and if the levels of dispersal are high enough any obvious genetic signal of sex-biased dispersal would be obfuscated. Our results contrast with strong female philopatry and sex-biased dispersal documented in alpine ungulates elsewhere, possibly due to the advantages of reducing dispersal in patchy habitats these populations inhabit.

Conservation implications

This study documents genetic substructure within contiguous mountain ranges, thus at a finer spatial scale than previously reported. Application of an intensive sampling plan and non-invasive techniques facilitated greater understanding of the scale and pattern of dispersal across heterogeneous landscapes. Quantifying the amount and spatial distribution of genetic diversity in a continuously distributed population can help guide the geographic scale for monitoring and harvest management, and inform long-term conservation strategies (Palsbøll et al. 2007). Wildlife populations are generally managed at a local level within political boundaries, which do not necessarily correspond to their genetic configuration (Coulon et al. 2006; Coltman 2008). Because Dall's sheep occur throughout relatively continuous habitats at ancestral levels of population density and distribution, increased understanding of their fine-scale genetic structure provides useful assessments of natural patterns of genetic variability in alpine ungulates, especially in comparison to those that have experienced population declines and inhabit fragmented landscapes (Valdez and Krausman 1999). Furthermore, understanding patterns of population connectivity can facilitate management and mitigation of the spread of disease (Archie et al. 2009), which is expected to present an increasing threat to northern ungulates as a result of changing climates (Kutz et al. 2005), and in particular to wild sheep which are believed to be immunologically naïve and therefore more susceptible to infectious diseases (Garde et al. 2005). The results of this research underscores the need to evaluate commonly assumed evolutionary behaviors (e.g. sex-biased dispersal) and their influences in shaping genetic structure of populations.

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