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HOT SPOTS OF GENETIC DIVERSITY DESCENDED FROM MULTIPLE PLEISTOCENE REFUGIA IN AN ALPINE UNGULATE

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Species that inhabit naturally fragmented environments are expected to be spatially structured and exhibit reduced genetic diversity at the periphery of their range. Patterns of differentiation may also reflect historical processes such as recolonization from glacial refugia. We examined the relative importance of these factors in shaping the spatial patterns of genetic differentiation across the range of an alpine specialist, the North American mountain goat (*Oreamnos americanus*). Contrary to fossil evidence that suggests a single southern refugium, we detected evidence for additional refugia in northern British Columbia and the Alaskan coast using both mitochondrial and microsatellite DNA. A core area of elevated genetic diversity characterized both regions, and molecular dating suggested a recent Pleistocene split was followed by demographic expansion. Across their range, mountain goats were highly genetically structured and displayed the expected pattern of declining diversity toward the periphery. Gene flow was high within contiguous mountain ranges, but cross-assignments paradoxically suggest that long-distance contemporary dispersal movements are not uncommon. These results improve our understanding of how historical vicariance and contemporary fragmentation influence population differentiation, and have implications for conserving the adaptive potential of alpine populations and habitat.

KEY WORDS: **Biogeography, center-marginal hypothesis, isolation-by-distance, mountain goats, population structure, refugia.**

The spatial genetic structure of a population has a profound effect on evolutionary processes and the maintenance of genetic diversity (Whitlock 2004). The extent to which populations are genetically structured is a consequence of both historical vicariance and contemporary dispersal, in addition to the basic evolutionary processes that regulate genetic variation (e.g., drift, selection). In particular, past refugia and natural habitat fragmentation influence the patterns of genetic differentiation that we observe today (e.g., Poissant et al. 2005; Louy et al. 2007; Paun et al. 2008). These processes create genetic disequilibria, the detection of which can be used to infer the relative influence of both refugia and fragmentation in shaping population genetic structure. Elucidating both historical and spatial effects on genetic structure is considered important for identifying evolutionary significant units (sensu Moritz et al. 1994; e.g., Cossíos et al. 2009), understanding the potential for local adaptation (Jorgensen et al. 2006) and speciation (Wagner and McCune 2009), and can be an important predictive tool for modeling the effects of climate change on wildlife (Provan and Bennett 2008).

In western North America, glacial dynamics have had a profound effect on species distributions (Soltis et al. 1997; Brunsfeld et al. 2001). For example, glacial-induced vicariance is thought to have precipitated speciation in North American wild sheep, *Ovis* spp. (Cowan 1940; Pielou 1991; Geist 1999), and the most

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recent glacial oscillations have produced detectable morphological and genetic differentiation between populations of thinhorn sheep, *Ovis dalli* (Worley et al. 2004; Loehr et al. 2006). Phylogeographic studies in western North America have also revealed cryptic refuges during the last glaciation (Golden and Bain 2000; Loehr et al. 2006; Marr et al. 2008), and complex recolonization patterns following the recession of ice (Godbout et al. 2008). These historical patterns are generally inferred from unique ice-age signatures, with genetic heterozygosity being highest in areas that acted as a refuge (Hewitt 1996, 2000), or recently colonized areas phylogenetically nested within a refugial base (Brunsfeld et al. 2001; Carstens et al. 2005).

Within many species, genetic differentiation increases with geographic distance resulting from a drift-gene flow equilibrium (Hutchison and Templeton 1999). But in the mountainous regions of western North America, genetic differentiation among alpine populations situated on "sky islands" is more pronounced among than within contiguous mountain ranges (Worley et al. 2004; Galbreath et al. 2009). This natural habitat fragmentation has important evolutionary consequences, as mammals adapted to alpine environments often have limited dispersal across intervening valleys (Brown 1971; Lomolino and Davis 1997), resulting in isolation and reduced gene flow between mountains. Such spatial heterogeneity may facilitate local adaptation and the maintenance of diversity by producing genetic differentiation (Wegmann et al. 2006), as opposed to highly connected networks where populations remain genetically uniform. Evolutionary theory also predicts that populations at the periphery of the range will show less genetic diversity than those in the center (i.e., center-marginal hypothesis: see Eckert et al. 2008). Thus, refugial history and contemporary connectivity influence genetic differentiation and diversity across the range of alpine populations.

Evolutionary and population genetic studies of western North American alpine mammals are limited to a few species (e.g., thinhorn sheep [Sage and Wolff 1986; Worley et al. 2004], arctic ground squirrel *Spermophilus parryii* [Eddingsaas et al. 2004], yellow-bellied marmot *Marmota flaviventris* [Floyd et al. 2005], and American pikas *Ochotona princeps* [Galbreath et al. 2009]). However, the mountain goat (*Oreamnos americanus*) may be the most exemplar alpine species. Mountain goats are endemic to the mainland mountains of western North America, ranging from 44°N to 63°N (Cowan and McCrory 1970; Côté and Festa-Bianchet 2003) and are renowned for living in some of the most inhospitable alpine environments (Hornaday 1906). They are thought to have arrived via the Bering land bridge during the Pleistocene (Cowan and McCrory 1970; Rideout and Hoffman 1975), and fossil evidence suggests mountain goats survived in a single refugium south of the ice sheets during the last glacial maximum (Cowan and McCrory 1970). Relatively little is known about the evolutionary history and population genetic structure of mountain goats. Mainguy et al. (2005, 2007) and Poissant et al. (2009) reported low genetic variability in a small number of individuals sampled from a few locations, and the limited field data available suggest that there is dispersal, but not necessarily gene flow, between herds (Festa-Bianchet and Côté 2008). Mountain goat herds on the range's periphery appear to be small and isolated, often consisting of fewer than 50 individuals (Smith 1988; Hamel et al. 2006). As a result, these peripheral "sky island" populations may be at risk of becoming genetically impoverished due to the effects of genetic drift and inbreeding (Frankham 1997).

We hypothesized mountain goats would conform to a "southerly refugia model," which is characterized by a leading edge expansion, producing decreased genetic heterozygosity from south to north (Hewitt 1996, 2000) and nested haplotypes (Brunsfeld et al. 2001; Carstens et al. 2005). Relative to other large mammals, we anticipated high genetic differentiation between subpopulations (Forbes and Hogg 1999), especially between mountain blocks (Worley et al. 2004). Finally, we expected mountain goats to exhibit reduced genetic diversity at the periphery of their range (Eckert et al. 2008). Thus, examining the mountain goat across its entire range provides the unique opportunity to assess the effects of historical and contemporary vicariance on population genetic structure, and examine the effects of the periphery, distance, mountains, and isolation on gene flow and diversity.

Materials and Methods

SAMPLE COLLECTION AND DNA EXTRACTION

A total of 876 samples were acquired from across the entire native range of mountain goats mostly collected between 2004 and 2009. Sample localities included the Canadian provinces/territories of Alberta and British Columbia, Yukon and Northwest Territories, and the US American states of Alaska, South Dakota, Washington, Idaho, and Montana (Table 1). Based on range map and accumulated census data (Festa-Bianchet and Côté 2008), goat populations in the extreme northern (Northwest Territories, Kenai Peninsula Alaska) and southern (South Dakota, Washington, Idaho, and Montana) periphery of the range appear to be small and isolated (Table 1). Most tissue samples were acquired from hunters at compulsory inspection or registration, and subsequently stored in 95% ethanol. A subset of the Alberta, British Columbia, Washington and Alaska samples came from ear punches at field studies. Six hair samples from the Northwest Territories were also acquired. When available, each sample had the age, sex, and location (UTMs) of kill/capture recorded. DNA was extracted using the DNeasy™ Blood and Tissue Kit (Qiagen, Inc., Valencia, CA, USA) following the manufacturer's protocol.

Table 1. Estimates of genetic variability for individual mountain goats assigned to a sample area belonging to a particular mountain range. Regional population estimates are from Festa-Bianchet and Côté (2008). Sample areas are identified by their abbreviation (Abbr.) throughout the study. Statistics include observed heterozygosity (H_O), expected heterozygosity (H_E), and allelic richness (A) estimated by rarefaction (South Dakota was not included in calculation), and Wright's inbreeding coefficient (F_{IS}). Also reported are the number of individuals assigned to a sample area.

Region	Estimated regional population	Sample area	Abbr.	Mountain range	N	H_O	H_E	A	F_{IS}
Alaska	24,000–33,500	Boundary range[1]	BouR	Coast mountains	249	0.47	0.54	3.7	0.14
		Kenai peninsula (P)	Ken	Coast mountains	12	0.19	0.18	1.8	−0.03
British Columbia (BC)	39,000–67,000	Cariboo mountains	Car	Columbian	12	0.50	0.49	3.5	0.03
		Purcell mountains	Pur	Columbian	17	0.47	0.52	3.2	0.12
		Selkirk mountains	Sel	Columbian	33	0.42	0.46	3.2	0.11
		Kitimat and Hazelton mountains	KitH	Coast mountains	71	0.42	0.48	3.5	0.12
		Pacific range	PacR	Coast mountains	19	0.49	0.51	3.8	0.06
		Omineca	Omi	Interior mountains	16	0.41	0.46	3.2	0.13
		Skeena	Ske	Interior mountains	25	0.50	0.49	3.6	0.00
		Northern interior	NorI	Interior mountains	43	0.52	0.53	3.7	0.04
		Northern rockies	NorR	Rocky mountains	14	0.45	0.46	3.4	0.08
Alberta and BC	2750 (Alberta)	Continental mountains	Con	Rocky mountains	221	0.46	0.49	3.3	0.08
Idaho	2700	Salmon River mountains (P)	SalR	Salmon River	20	0.31	0.35	2.4	0.15
Montana	2295–3045	Bitterroot/Absaroka (P)	BitA	Rocky mountains	10	0.31	0.36	2.3	0.22
Northwest Territories and Yukon	1000 1400	McKenzie mountains (P)	MckM	McKenzie mountains	13	0.39	0.45	3.2	0.17
South Dakota	80–100	Black Hills (P)	BlaH	Black Hills	2	0.32	0.18	1.4	−0.46
Washington	2000	Cascades (P)	Cas	Coast mountains	26	0.26	0.34	1.6	0.26

[1]Boundary Range includes samples from southwest Yukon.
(P) denotes periphery of range.

GENOTYPING

The initial genomic DNA extraction was used as a template in all polymerase chain reactions (PCRs). Previously optimized microsatellite markers (Mainguy et al. 2005) were used in duplex and triplex PCRs, totaling 19 markers (Table 2). The 10 μl multiplex reactions contained 4.66 to 4.90 μl of double-distilled water, 0.75 to 0.80 μl of $MgCl_2$ (20 mM), 1 μl 10× PCR buffer, 2 μl of dNTPs (0.2 mM each), a 20× primer mix diluted to between 0.24 and 0.34 μM each, 0.08 μl of Taq (0.5 units), and 1 μl of DNA template (25 ng). One primer of the pair was fluorescently labeled (fluorescent tags: 6-FAM, TET, or HEX). The multiplex PCR were hot-started and began with an initial 3-min denaturation at 95°C, followed by 38 cycles of 30 sec denaturation at 94°C, 90 sec annealing at 49°C, and 30 sec extension at 72°C. The run concluded after 30 min at 60°C. The microsatellite amplicons were loaded on an ABI 3730 DNA sequencer (Applied Biosystems, Foster City, CA, USA) with a GS500TAMRA size standard (Applied Biosystems). Microsatellite alleles were detected, scored, and manually verified using GENEMAPPER version 4.0 (Applied Biosystems). To assess the genotyping error rate, we re-extracted DNA and blindly genotyped individuals from the previously genotyped Caw Ridge, Alberta mountain goat herd (Mainguy et al. 2005, 2009a, 2009b).

MICROSATELLITE DATA ANALYSIS

Seventeen sampling areas were defined a priori according to geographic mountain ranges (Table 1, Fig. 1A). We quantified genetic diversity as expected (H_E; Nei 1987) and observed heterozygosity (H_O) calculated using GenAlEx 6.2 (Peakall and Smouse 2006). Allelic richness was estimated using the rarefaction method implemented in HP-RARE 1.0 (Kalinowski 2005). We tested for deviation from Hardy–Weinberg equilibrium (HWE) using the exact test (Guo and Thompson 1992) implemented in Genepop 4.0 (Rousset 2008). FSTAT 2.9.3 (Goudet 1995) was used to test for linkage disequilibrium and significance assessed with 1000 permutations.

POPULATION DIFFERENTIATION

Microsatellite analyzer (MSA) 4.05 (Dieringer and Schlottere 2003) was used to calculate Nei's (1972) genetic distance (D_S) between sampling areas. Wright's fixation indices for genetic differentiation (F_{ST}) and inbreeding (F_{IS}) within areas were also estimated using Weir and Cockerham's (1984) unbiased

Table 2. Number of alleles (A), observed heterozygosity (H_O), and expected heterozygosity (H_E) for each marker used in this study across the entire range of mountain goats. Chromosome locations are based on cow and sheep as described by Mainguy et al. (2005).

Locus	A	H_O	H_E	Chromosome location
MAF36a	3	0.20	0.26	22
OARHH35a	9	0.49	0.66	4
OARJMP29a	2	0.31	0.38	24
TGLA122a	13	0.59	0.76	21
AR028a	11	0.42	0.56	2
BM1225a	8	0.47	0.69	20
RT27a	9	0.51	0.66	unassigned
MCM152a	9	0.38	0.47	13
ILSTS058a	15	0.66	0.86	17
TGLAlOa	8	0.49	0.57	2
OARHH62a	4	0.22	0.30	16
HUJ1177a	4	0.35	0.45	3
RT9a	7	0.60	0.72	unassigned
OARCP26a	12	0.44	0.52	4
MAF64a	10	0.41	0.56	1
Huj616a	11	0.61	0.78	13
BM1818a	12	0.42	0.50	23
BM6444a	5	0.45	0.57	2
McM64a	2	0.35	0.44	2
Average	8.1	0.44	0.56	
(SE)	(0.89)	(0.03)	(0.04)	

estimators in FSTAT. Significance was tested using 10 000 permutations. Neighbor-joining trees of the sampling areas were constructed with the NEIGHBOR program in PHYLIP 3.69 (Felsenstein 1989). Gene frequencies were bootstrapped over loci 100 times with MSA. Consensus trees were then constructed using the CONSENSE program in PHYLIP and displayed using TreeView (Page 1996).

BAYESIAN ANALYSIS OF GENETIC CLUSTERS AND MOVEMENT

We used STRUCTURE 2.2 (Pritchard et al. 2000) to assess genetic structure independent of sampling area. STRUCTURE uses a Markov chain Monte Carlo algorithm to cluster individuals with multilocus genotypes into populations. We assumed an admixed model with correlated allele frequencies (Falush et al. 2003). The admixed model was selected because male goats are known to move between herds during the rut (Mainguy et al. 2008). Five independent runs from $K = 1$ to $K = 20$ were performed using 1,000,000 iterations with the first 25% removed as a burn in. We used the ΔK method of Evanno et al. (2005) to select the most distinct genetic subdivision in the data, thus only clusters that were supported statistically were retained. Individuals were then assigned to each genetic cluster based on their highest percentage membership (q) calculated from the five runs using the full search in CLUMPP 1.1.1 (Jakobsson and Rosenberg 2007). We expected a complex and hierarchical pattern of population structure at the scale of our sampling. Therefore, after individuals were assigned to a primary genetic cluster, we repeated the STRUCTURE analyses on each primary cluster using the same methods until there was no longer an increase in likelihood supported by ΔK. Population differentiation among hierarchical clusters was quantified by Nei's D_S and F_{ST}. Neighbor-joining trees of the final subpopulations were constructed as above.

To identify individuals cross-assigned between subpopulations (i.e., dispersers), polygons were constructed around the core group (sensu Bélichon et al. 1996) of individuals assigned to a genetic cluster and belonging to the same mountain range(s) using the HAWTH'S TOOLS (Beyer 2004) extension in ARCGIS 9.0 (ESRI, Redlands, CA, USA). An individual with a $q > 0.80$ located on a different mountain range not encompassed by its population's polygon was considered cross-assigned. The selection of a 0.80 cutoff is based on the assumption that individuals between 0.20 and 0.80 are admixed (Lecis et al. 2006; Vähä and Primmer 2006; Bergl and Vigilant 2007). Only individuals who moved a sufficient distance (>100 km) from their clusters were scored as cross-assigned, thus increasing our confidence in identifying true dispersers.

ISOLATION-BY-DISTANCE AND IMPACT OF MOUNTAIN RANGES

We estimated isolation-by-distance (IBD) between sampling areas (less South Dakota because of small sample size) and STRUCTURE-inferred subpopulations. We calculated the Euclidean distance between sampling area and STRUCTURE subpopulations using the mean longitude and latitude of all samples assigned to each group and plotted against pairwise D_S. Genetic distance matrices were obtained from MSA, and a Euclidian distance matrix was constructed using the HAWTH'S TOOLS extension in ARCGIS 9.0. Because many of the sampling areas and subpopulations were nested within a larger mountain range, we examined the effect of mountain range on genetic differentiation by controlling for geographic distance using partial Mantel tests (Mantel 1967). Because the mountain goat's distribution is extensive and may be disjunct between north and south populations (figure 1.8 in Festa-Bianchet and Côté 2008), we also tested for a second, northern refugium using a partial Mantel test. Both the mountain range and refugial matrices were binary, consisting of a zero for sampling areas found in the same mountain range or putative refugia, and one for those on different mountain ranges or refugia. Both simple and partial Mantel tests were performed in the program ZT (Bonnet and Van de Peer 2002) using Pearson's correlation coefficient between the matrices. Significance was

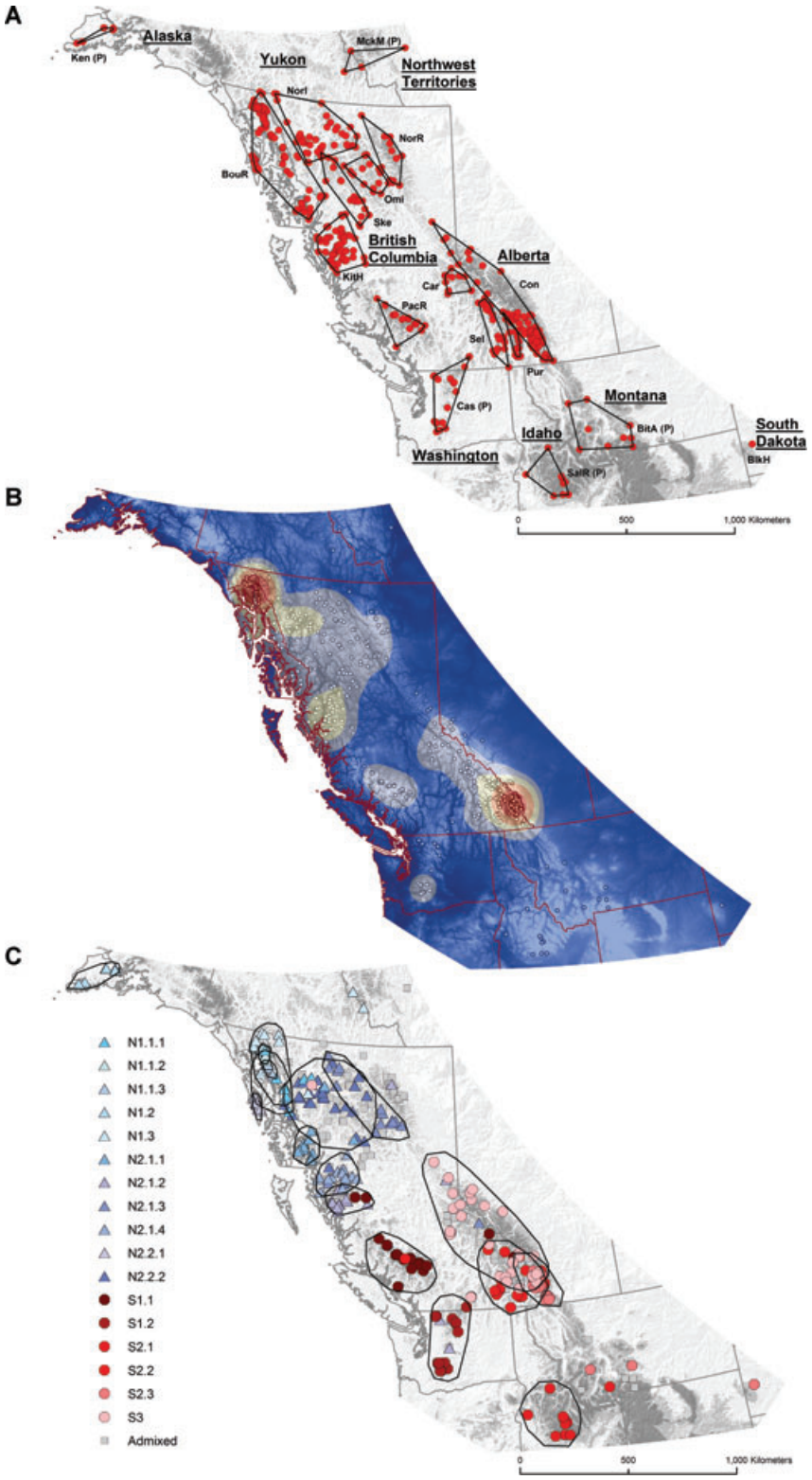

Figure 1. (A) Map of sample localities for North American mountain goats (*Oreamnos americanus*) across their native range. Mountain ranges are delimitated by polygons and abbreviations are listed in Table 1 and peripheral populations are denoted by (P). (B) Map of hot spots of individual genetic heterozygosity for mountain goats across their range. Areas with high heterozygosity are warmer (yellow to red) and were detected using spatial analyst in ARCGIS 9.0. Map (C) Locations of 17 genetic clusters of mountain goats identified by STRUCTURE. Southern (triangles) and northern (circles) samples denote the uppermost hierarchical split supported by STRUCTURE. Individuals assigned to a subpopulation have a $q > 0.80$, whereas those admixed are left unassigned (gray squares). Because of the scale of sampling, a single point may represent multiple individuals.

assessed using 1,000,000 randomizations of the rows, and one column in the matrix.

MITOCHONDRIAL DNA SEQUENCE

We sequenced the mitochondrial control region of a subset of samples from across the range using the primers L15527 and H00438 (Wu et al. 2003). The control region was amplified via PCR in a 25 μl solution containing 2.5 μl of template DNA (25 ng), 2.5 μl dNTPs (0.2 mM each), 2.5 μl 10× buffer, 0.4 μl each primer (10 μM), 1 μl $MgCl_2$ (25 mM), 15.5 μl distilled water, and 0.5 μl Taq DNA polymerase (0.5 U). The PCR profile was as follows: hot-start followed by an initial 2-min denaturation at 94°C, followed by 35 cycles of 45 sec denaturation at 94°C, 45 sec annealing at 63.5°C, and 1-min extension at 72°C. The run concluded after 3 min at 72°C.

Double-stranded PCR amplified products were checked by electrophoresis on a 1% agarose gel. Ten μl of PCR product was then treated with 5 μl of ExoSAP (USB Corporation, OH, USA) and incubated at 37°C for 15 min followed by heating to 80°C for 15 min. A total of 2.5 μl of the ExoSAP-treated PCR product was used in a sequencing reaction. Amplicons were directly sequenced in both directions using a Big Dye Terminator Kit (Applied Biosystems, Foster City, CA). Excess of Big Dye Terminator was removed via ethanol precipitation. Sequences were generated on an ABI 3730.

PHYLOGENETIC ANALYSES, DIVERGENCE ESTIMATES, AND TESTS OF EXPANSION

Sequences were aligned using the ClustalW algorithm (Thompson et al. 1994) and edited with Bioedit 7.0.9 (Hall 1999). We calculated haplotype (h) and nucleotide diversity (π) using the software DnaSP 4.0 (Rozas et al. 2003). Three different phylogenetic methods were used to construct evolutionary trees. Neighbor-joining analysis was conducted using the software package MEGA 3 (Kumar et al. 2004). For Bayesian and maximum-likelihood (ML) approaches, the appropriate model of nucleotide substitution was determined in Modeltest version 3.07 (Posada and Crandall 1998). The ML analysis was run in Garli version 0.95 (Zwickl 2006) with parameters fixed according to Modeltest specifications. MrBayes version 3.1.2 (Huelsenbeck and Ronquist 2001) was run under default priors with two independent runs of four chains (three heated) run for 1,000,000 generations, with the first 25% discarded as a burn-in. Confidence in topologies was evaluated based on 1000 bootstrap replicates or posterior probability.

To test different evolutionary hypotheses, topologies were constrained according to two refugial scenarios (1 vs. 2 refugia). The single refugium constraint consisted of northern areas nested within those more southern (a stepping stone model), whereas the two-refugia scenario enforced distinct northern and southern clades. Under a parsimony framework, a heuristic search with tree-bisection-reconnection and maxtrees set to 100 was run with constraints according to refugial scenarios. A pairwise Templeton test (Templeton 1983), which implements a Wilcoxon signed-rank statistic, was used to determine whether one topology was significantly more parsimonious than the other. We also used the likelihood-based SH test (Shimodaira and Hasegawa 1999). Ten thousand bootstrap replicates of each ML tree under different refugial scenarios were resampled using the re-estimated log likelihoods, and significance assessed using a one-tailed t-test.

Time of divergence between clades was estimated using MDIV (Nielsen and Wakeley 2001). Under a Markov chain Monte Carlo coalescent-based model, MDIV estimates the parameters θ (where $\theta = 4N_e\mu$) and T (where $T = t/2N_e$). MDIV was run for 200,000 generations with the first 10% discarded as a burn-in. The migration parameter (M) ranged from 0 to 10, under the assumption of limited historic gene flow between clades. A μ of 24% per locus/per million years (based on sheep control region: Hiendleder et al. 2002; Loehr et al. 2006) was adjusted to account for generation time (gt) and sequence length (l). The following formula was used to calculate time of divergence (t) in years before present (ybp):

$$t = [T \cdot \theta]/[2 \cdot l \cdot \mu \cdot gt].$$

Two approaches were used to test for demographic expansion. Fu's F_S (Fu 1997), where negative F_S values represent recent population expansion, was calculated in ARLEQUIN 3.1 (Excoffier et al. 2005) using a coalescent simulation algorithm. Significance of F_S values was evaluated by 1000 permutations. The growth parameter (g) was calculated in LAMARC 2.1.3 (Kuhner 2006). Three runs were conducted using 10 short chains and two long chains, with sampling increments of 10 (for 2,000,000 steps) and a burn-in of 1000 samples.

Results

MICROSATELLITE GENETIC DIVERSITY STATISTICS

A total of 876 samples (permanently archived at the University of Alberta) were genotyped at 19 loci yielding a >98% complete dataset (Dryad Digital Repository, doi:10.5061/dryad.1558). We estimated a genotyping error rate of less than 0.01% by blindly regenotyping 100 individuals from the Caw Ridge mountain goat herd (Mainguy et al. 2005, 2009a). The total number of alleles per locus ranged from 2 (OarJMP29 and McM64a) to 15 (ILSTS058), and the allelic richness per area ranged from 1.42 to 3.84 (Tables 1 and 2). Fourteen of the 17 areas showed positive F_{IS} values (average $F_{IS} = 0.10$, excluding South Dakota), and observed heterozygosities per area ranged from 0.19 to 0.52 (Table 1). Linkage disequilibrium (LD) was not detected after Bonferroni correction (Rice 1989).

POPULATION STRUCTURE

Significant population subdivision was observed between the sampling areas (Fig. 1A), with D_S values ranging from 0.03 to 0.66 (Appendix S1) and a global D_S, which is a measure of central tendency for the distribution of D_S across loci, of 0.20. Neighbor-joining tree topologies suggested that the southern and northern halves of the range were divergent (Fig. 2A). We observed two distinct areas of elevated heterozygosity, one in the northern region in southeast Alaska, and one in the south surrounding the Continental mountains (Fig. 1B). Peripheral populations had visibly lower heterozygosity (Fig. 1B) and significantly less genetic diversity as measured by H_E ($t = -3.4$, df $= 4.6$, $P = 0.02$) and number of rarified alleles ($t = -4.9$, df $= 5.6$, $P < 0.01$).

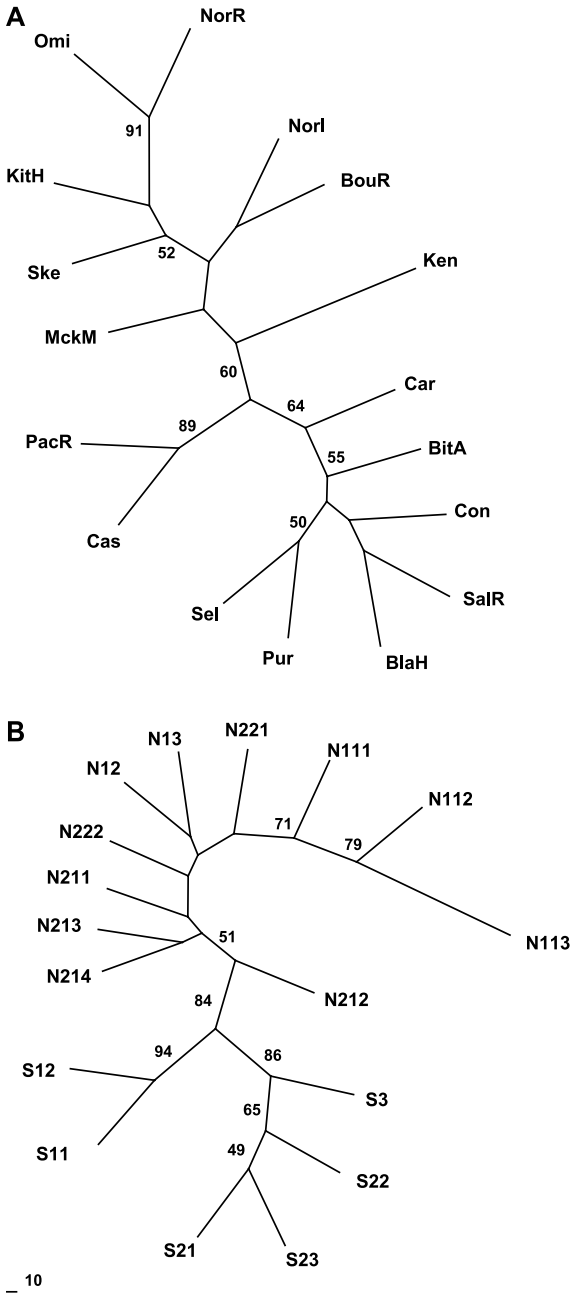

Figure 2. Neighbor-joining trees for: (A) sampling areas specified a priori, and (B) STRUCTURE designated subpopulations. Bootstrapped values are from 100 replicates with those >50% presented. Abbreviations are listed in Table 1.

BAYESIAN ANALYSIS OF POPULATIONS AND MOVEMENT

Bayesian cluster analysis resolved a primary subdivision at $K = 2$ based on ΔK, corresponding to north and south clusters (Figs. 1C and 2B). The north and south clusters were then broken down further into two and three subclusters, respectively, arranged east to west. When these subclusters were broken down as far as possible, we resolved a total of 17 clusters or subpopulations that were in HWE. All clusters were observed in all five replicate STRUCTURE runs and supported by ΔK and CLUMPP with H′ (a measure of similarity between runs) of >0.98. Genetic clusters are presented in Figure 1C for individuals assigned to their respective cluster with $q > 0.80$. Individuals with $q < 0.80$ were considered admixed.

The degree of differentiation among clusters ranged from D_S 0.04 to 0.61 (Appendix S2) with a global D_S of 0.28. Sixteen of the 17 subpopulations had positive F_{IS} values with an overall average of 0.08 (Table 3). A split between north and south clusters was apparent in the neighbor-joining tree topology (Fig. 2B). Dispersal between areas was assessed through cross-assignment where we observed 30 (17 males, 9 females, 4 unknown) of 466 individuals

Table 3. Number of samples (*N*), Wright's inbreeding coefficient (F_{IS}), and mean percent membership for the 17 subpopulations designated by STRUCTURE 2.2.

Subpopulation	*N*	F_{IS}	Mean percent membership (q)
N111	47	0.06	0.73
N112	53	0.01	0.71
N113	29	0.03	0.77
N12	12	−0.03	0.99
N13	71	0.07	0.91
N211	29	0.05	0.78
N212	34	0.17	0.76
N213	48	0.08	0.71
N214	51	0.08	0.70
N221	28	0.08	0.94
N222	55	0.04	0.94
S11	32	0.08	0.92
S12	21	0.17	0.93
S21	35	0.17	0.84
S22	93	0.09	0.73
S23	96	0.06	0.74
S3	142	0.08	0.86

Table 4. Results from simple and partial mantel tests examining the effects of distance, refugia, and shared mountain ranges on population differentiation. Variable following the period is controlled for (i.e., Refugia.Distance).

Model	r^2	*P* value
Isolation-by-distance		
D_S~Distance	0.63	<0.001
D_s~Distance[1]	0.66	<0.001
Historical		
D_s~Refugia.Distance	0.05	0.09
D_s~Refugia.Distance[1]	0.12	0.04
Contemporary		
D_s~Mountains.Distance	0.02	0.06
D_s~Mountains.Distance[1]	0.10	0.04

[1]STRUCTURE 2.2 inferred subpopulations.

(7%) cross-assigned to different populations with a $q > 0.80$. Most notably, five individuals between the Pacific range (S1.1) and the Rocky and Columbian mountain range populations (S3) were cross-assigned, which represent a distance >250 km.

IBD AND MOUNTAIN RANGE EFFECT

A total of 803 samples were sufficiently geo-referenced to test for IBD according to sampling area and STRUCTURE-inferred subpopulations. D_S increased significantly with linear distance (Table 4, Fig. 3), and the fit was better explained by linear distance than by the natural logarithm of distance (data not shown). When controlling for distance, both mountain ranges and multiple refugia explained additional variance (Table 4, Fig. 3). All patterns were stronger (i.e., higher r^2) in the STRUCTURE-inferred subpopulations.

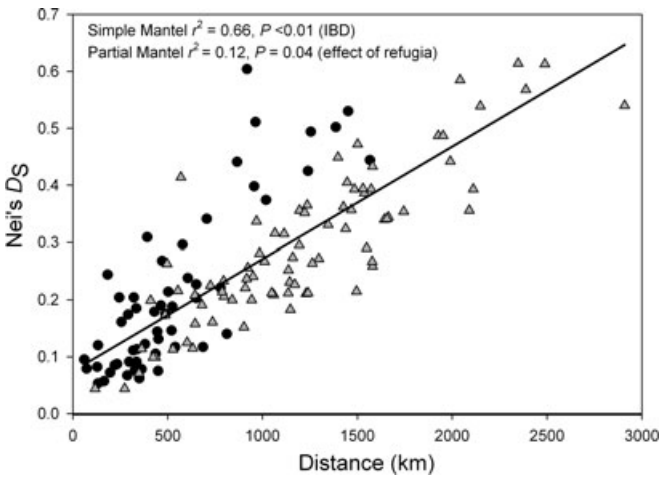

Figure 3. Isolation-by-distance relationships between populations of mountain goats defined by subpopulation. Gray triangles indicate population pairs from different refugial origin, and black circles denote a common refugium. The effects of distance and refugia on genetic differentiation are shown.

MITOCHONDRIAL PHYLOGENETIC PATTERNS, DIVERGENCE DATES, AND DEMOGRAPHIC EXPANSION

A total of 200 samples were sequenced from across the range of mountain goats (Fig. 4). All 17 subpopulations were represented and sequences are deposited in GenBank (HM230898-HM231097). The three phylogenetic methods used all had 100% bootstrap support or posterior probability for the north–south split (Fig. 4). Support for phylogenetic structure within the north and south clades was limited, as they were essentially polytomies (Fig. 4). Haplotype and nucleotide diversity measures showed that major clades were equally diverse (Table 5). The Templeton test found the optimal tree of a north–south split (no. of steps = 562) to be significantly more parsimonious ($Z = -6.46$, $P < 0.01$) than a single refugium model represented by a nested south to north topology (no. of steps = 732). The likelihood-based SH also rejected the single refugium model as a viable phylogeny ($P < 0.01$).

MDIV parameters T and θ both had bell-shaped curves with peaks in likelihood at 0.32 and 109, respectively. Using a generation time of 4 years and sequence length of 811 bp, we estimated the date of divergence between north and south clades to be 224,003 ybp. Both north and south clades showed evidence of recent demographic expansion (North clade: $F_S = -24.03$ ($P < 0.01$), $g = 217$ with 95% CI: 96–358; South clade: $F_S = -23.92$ ($P < 0.01$), $g = 358$ with 95% CI: 172–577).

Discussion

HISTORICAL PATTERNS OF GENETIC DIFFERENTIATION

Our results support the possibility of a second, northerly refugium for mountain goats. In several North American mammals, glacial-induced vicariance produced distinct north and south refugia (Fleming and Cook 2002; Loehr et al. 2006; Aubry et al. 2009); however, fossil evidence suggested only a southern refugium existed for mountain goats during the last glacial maximum (Cowan and McCrory 1970; Rideout and Hoffman 1975). Recent findings have uncovered a now extinct coastal refugium (Nagorsen and Keddie 2000) raising the possibility of additional coastal refugia existing for mountain goats. These data do not support the "southerly refugia model" for mountain goats, and suggest Beringia, or northern British Columbia (the latter suggested by Loehr et al. 2006), was a major refuge during the last glacial maximum. Given that the current range of mountain goats falls almost entirely within the proposed extent of ice-sheets during the last glacial maxima (see Dyke et al. 2003), a northern British

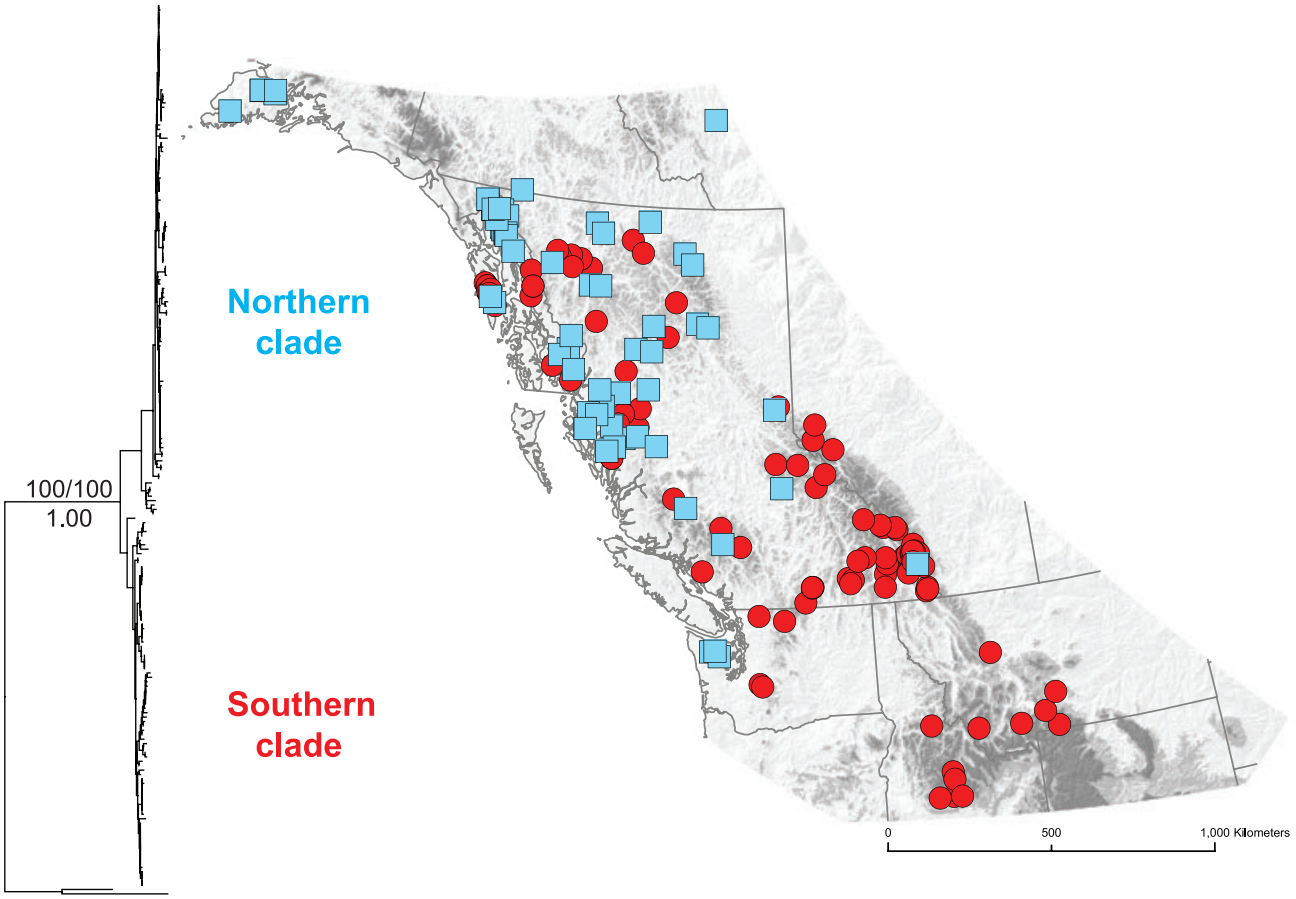

Figure 4. Neighbor-joining tree of 200 mountain goat samples with their individual clade designations plotted on the adjacent map. Support values are based on 1000 bootstrapped datasets or posterior probability. Support values on the main branch correspond to neighbor-joining/maximum likelihood above, and Bayesian below.

Columbia or southeast Alaska refugial site, rather than Beringia proper, appears most likely.

Multiple lines of evidence from these data support a distinct northern refugium. Phylogenetic analyses of both microsatellite and mitochondrial data support a north–south split (Figs. 2 and 4). The date of this split is ~224,000 ybp, which predates the onset of the last glaciation and is consistent with estimates from mountain sheep from the same area (Loehr et al. 2006). Patterns of genetic differentiation support this hypothesis, as partial Mantel tests that incorporated multiple refugia (Table 4) explained an additional 12% of the variance in genetic differentiation. If recolonization had emanated from only a southern refugium following the retreat of the last ice sheet, we would have expected a cline of decreasing genetic diversity from south to north (Hewitt 1996, 2000) or a nested phylogeny (Brunsfeld et al. 2001; Carstens et al. 2005). We do not see this; instead, there are distinct northern and southern hot spots of diversity (Fig. 1B) that are separated by a strongly supported bifurcation (Fig. 4) and the southern refugium stepping stone model is rejected as a viable scenario. Moreover, these hot spots overlay predicted "hot spot clusters" (Fig. 3 in Swenson and Howard 2005) that reflect Pleistocene glacial refugia and/or expansion and common phylogeographic breaks. In mountain goats, the northern and southern hot spots (Fig. 1B) may represent their refugial locations as well where divergent lineages mixed postglaciation. In addition, the lack of phylogenetic resolution (Fig. 4) and signal of demographic expansion (avg. $g = 288$ and $F_S = -24$) suggest mountain goats have gone through a recent, rapid expansion from these refugia when the glaciers receded. Similar patterns of postglacial expansion from refugia

Table 5. Mitochondrial control region diversity statistics for the major mountain goat clades. Included are the number of individuals in each group (*N*), observed haplotypes (*Nh*), haplotype diversity (*h*), and nucleotide diversity (π).

Clade	*N*	*Nh*	*h*	π
North	85	46	0.97	0.013
South	115	54	0.96	0.011
All	200	99	0.98	0.037

have been detected in other northern mammals from the same area (Fedorov et al. 2003; Lessa et al. 2003). Overall, these data suggest that mountain goats were isolated in at least two major refugia during the last glacial maximum, and underwent a rapid demographic expansion following the retreat of the Laurentide and Cordilleran ice-sheets.

A noteworthy anomaly is Alaska's Baranof Island population, which originated from a small number of founders (Paul 2009). Two distinct subpopulations were detected on the island: N2.2.1 which was restricted to the island, and N1.1.1 that was primarily found on the mainland (Fig. 1C). The island also had a mixture of mitochondrial haplotypes, but was predominantly from the southern clade (Fig. 4). Historically, no goats were believed to inhabit Baranof Island, prompting authorities to translocate goats from the adjacent mainland in the early 20th century (Paul 2009). The source population for this translocation is encompassed by the N1.1.1 polygon. Interestingly, N2.2.1 is differentiated from all Alaska populations (minimum $D_s = 0.10$), and is relatively diverse ($H_E = 0.45$). Given the history of the island, it seems unlikely that drift alone could produce two genetically divergent and diverse subpopulations that are not spatially segregated. A possible explanation for this pattern is that N2.2.1 is a glacial relict. There is geological evidence that parts of Baranof Island were ice free during the last glaciation (Carrara et al. 2007), and Cook et al. (2001, 2006) have compelling phylogeographic data that support such a refuge. Moreover, Heaton and Grady (2003) discovered a horn core from a hypothesized *Saiga tatarica* dating 32,000 ybp from the closely situated Prince of Wales Island. Given our data, and because *S. tatarica* is currently restricted to central Asia (Sokolov 1974), it is conceivable that this horn core may actually be that of *Oreamnos*. We envision a scenario in which at the beginning stages of the glacial advance, mountain goats were split into their major clades (Fig. 4) somewhere in southeast Alaska or northern British Columbia, with the Baranof Island population retaining the southern haplotype. During the subsequent isolation, the Baranof Island became population differentiated, yielding the N2.2.1 cluster. The recent translocation from the mainland (Paul 2009) introduced the N1.1.1 genotype, and possibly the northern haplotype. Overall, the detection of a second, northerly refugia and the possibility of a cryptic refugium adds additional insight into the broader biogeographic patterns that have shaped species distributions in western North America (Soltis et al. 1997; Brunsfeld et al. 2001).

GENE FLOW AND DISPERSAL

Alpine specialists often show high levels of differentiation over relatively short distances (Forbes and Hogg 1999; Perez et al. 2002; Worley et al. 2004). Mountain goats display such a pattern (Fig. 3), and show strong differentiation between subpopulations (Global $D_S = 0.28$; Appendices I and II). Similar to thinhorn sheep (Worley et al. 2004), mountain ranges also explained a significant portion of variance across the range (Table 4). Because mountain ungulates are adapted to naturally patchy alpine terrain (Forbes and Hogg 1999), it is not surprising that mountain ranges facilitate gene flow while valleys would impede it—this is in agreement with Brown's (1971) early observations on boreal mammals. Furthermore, in areas such as the Boundary Range of Alaska, subpopulations were visibly separated by fiords and rivers, which goats are unlikely to cross (K. White, unpubl. data; but see Klein 1965). Similar breaks are observed in the phylogeography and population structure of numerous Alaska fauna (Cook et al. 2001, 2006). These analogous patterns are largely attributable to shared Pleistocene climatic events that fragmented boreal and alpine terrain.

During the last glacial maximum, global cooling forced species into temporary refugia. Individuals subsequently recolonized available habitat as the glaciers receded. In the alpine, climatic warming permitted the forests to encroach and naturally fragment the terrain creating "sky islands." This pattern has been used to explain the patterns of diversity and differentiation in wild sheep (Sage and Wolff 1986; Forbes and Hogg 1999; Worley et al. 2004; Loehr et al. 2006), which occupy similar habitat as mountain goats. Because alpine ungulates typically exhibit a small effective population size, high site fidelity, and limited dispersal (Forbes and Hogg 1999; Worley et al. 2004; Festa-Bianchet and Côté 2008), drift is considered the paramount evolutionary factor affecting genetic diversity in these fragmented populations. However, the distribution of genetic diversity during recolonization is also thought to be shaped by long-distance dispersers (Hewitt 1996, 2000).

Long-distance dispersal produces populations with patchily distributed allele frequencies (reviewed by Excoffier et al. 2009). Such a pattern is evident in mountain goats, as many adjacent subpopulations with a common refugial origin are quite divergent (Fig. 3). Moreover, apparent contemporary long-distance dispersal was detected in the dataset, most notably across the British Columbia (BC) interior. For BC mountain goats, any east–west movement would involve crossing hundreds of kilometers of suboptimal habitat. This area is generally considered void of goats—although a handful of observations have been recorded (Mountain Goat Management Team 2010). No recent or direct translocations between these ranges have been conducted (Hatter and Blower 1996), and poaching is unlikely to account for all the cross-assignments. Mountain goats introduced to Oregon moved 71 km from their natal area (Mathews and Heath 2008), and goats in Alberta have been seen 300 km from the nearest known population (Festa-Bianchet and Côté 2008). In addition, mountain goats are known to traverse extensive icefields (Nichols 1985; Hofer 2004) and Mathews and Heath (2008) radio-tracked an individual through agricultural and timber habitat for nearly 250 km.

Clearly, long-distance dispersal and movement across suboptimal habitat has played an important evolutionary role in colonization, and maintenance of gene flow between geographically and temporally distinct subpopulations.

GENETIC DIVERSITY AND EFFECT OF GEOGRAPHY

Mountain goats can be described as having low-to-moderate levels of genetic diversity. Relative to other mountain ungulate populations, mountain goats have similar diversity levels to that of the ibex (*Capra ibex*; Maudet et al. 2002) and chamois (*Rupicapra* spp.; Perez et al. 2002) but lower than that of bighorn (*Ovis canadensis*; Forbes et al. 1995; Forbes and Hogg 1999) and thinhorn sheep (Worley et al. 2004). Based on our scale of sampling, there is likely additional, finer scale hierarchies not resolved in our analyses. For example, the cluster S3 encompasses at least 12 discrete herds in Alberta (Hamel et al. 2006) that cannot be discerned without intensive sampling. This unresolved substructure along with the global patterns across loci and populations (Tables 1 and 2), suggests that the positive F_{IS} values are in part due to the "Wahlund effect." That being said, the southern peripheral mountain goat populations in the United States (Montana, Idaho, and Washington) demonstrate the highest levels of inbreeding in the dataset ($F_{IS} = 0.15$–0.26, less South Dakota), which may not entirely be attributed to undetected substructure. Given the sensitive nature of alpine habitat in response to climate change (Sala et al. 2000), populations on the southern end are likely to be impacted disproportionately. In these southern areas, the effects of any future loss of genetic diversity or inbreeding could be exacerbated by climate change and the highly polygynous mating system of mountain goats (Mainguy et al. 2008) where few males obtain most of the paternities (Mainguy et al. 2009b).

Across the range of mountain goats, genetic diversity was significantly diminished in peripheral populations (H_E from 0.18 to 0.45), whereas populations located near the center of the species range had higher genetic diversity (H_E from 0.36 to 0.54). This can also be visualized in the individual heterozygosity plots (Fig. 1B) where peripheral populations are isolated and less diverse. The low levels of genetic diversity in the peripheral range of the mountain goat are in part the result of small population numbers and isolation, as all these sampling areas have less than 4000 mountain goats (Festa-Bianchet and Côté 2008). Empirical support for the central–marginal hypothesis within vertebrates is accumulating (e.g., Beebee and Rowe, 2000; Hutchison 2003; Howes and Lougheed 2008); but none thus far has examined a species as large and mobile across a naturally fragmented environment as the mountain goat. Peripheral populations are thought to occupy ecologically substandard environments, to have suffered from founder effects, genetic drift, and inbreeding, and thus tend to be smaller, isolated, and less reproductively successful (Brussard 1984; Hoffmann and Blows 1994; Lessica and Allendorf 1995; Hutchison 2003). This could explain why southern mountain goat populations went extinct during the hypsithermal (Rideout and Hoffman 1975). Peripheral populations are of particular evolutionary importance, as they may have unique biographical traits (e.g., climate tolerance), have high levels of genetic differentiation, or be locally adapted, and thus important for the maintenance of biodiversity (Eckert et al. 2008; Bhagwat and Willis 2008; Hampe and Petit 2005). Importantly, these populations may require different conservation practices (see Hampe and Petit [2005] for examples) because of their unique ecological and evolutionary attributes.

Both historical and geographic vicariance has played intricate roles in the distribution and abundance of genetic diversity in species. In western North America, historical climate change and connectivity among mountains have been prominent in shaping genetic differentiation. Across the range of mountain goats, refugial origin and mountain ranges have significantly influenced genetic differentiation, which underscores the need to consider these factors when modeling genetic differentiation in alpine specialists. With alpine ecosystems projected to undergo large changes in biodiversity from small environmental perturbations (Sala et al. 2000), understanding the temporal and geographic effects on the genetic structure of species is required for correctly modeling the effects of climate change (Provan and Bennett 2008), and will be essential for conserving the adaptive and evolutionary potential of both alpine ecosystems and their inhabitants.

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Supporting Information

The following supporting information is available for this article:

Appendix S1. Pairwise D_S and F_{ST} and for designated areas (Table 1: Fig. 1A).
Appendix S2. Pairwise D_S, and F_{ST} for STRUCTURE assigned populations (Fig. 1C).

Supporting Information may be found in the online version of this article.

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