Special Section: Wolverine

Inferring Geographic Isolation of Wolverines in California Using Historical DNA

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ABSTRACT Delineating a species' geographic range using the spatial distribution of museum specimens or even contemporary detection–non-detection data can be difficult. This is particularly true at the periphery of a species range where species' distributions are often disjunct. Wolverines (*Gulo gulo*) are wide-ranging mammals with discontinuous and potentially isolated populations at the periphery of their range. One potentially disjunct population occurred in the Sierra Nevada Mountains, California, USA, and appears to have been extirpated by the 1930s. Many early 20th century naturalists believed that this population was connected to other populations occurring in the Cascade Range of northern California, Oregon, and Washington, USA, but a recent analysis of historical records suggests that California wolverines were isolated from other populations in North America. We used DNA extracted from museum specimens to examine whether California wolverines were isolated. Both nuclear and mitochondrial DNA data indicate that California wolverines were genetically distinct from extant populations, suggesting long-term isolation. We identified 2 new control region (mitochondrial DNA) haplotypes located only within California. We used these data and referenced sequences from the Rocky Mountains, USA, to make inferences regarding potential wolverine translocations into California. In addition, we used these genetic data to make inferences about wolverine conservation throughout western North America. (JOURNAL OF WILDLIFE MANAGEMENT 71(7):2170–2179; 2007)

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Understanding a species' historical and contemporary geographic range is fundamental to understanding its ecology, evolutionary history, and conservation status. For most species, however, we have limited information on this critically important parameter. Often, the only data available on historical distributions are from museum or herbarium specimens. These specimens often date to the late 1800s and early 1900s, when collecting expeditions were common. Although museum specimens are sometimes the only way that historical geographic ranges can be studied, information from collections is subject to sampling biases (Ponder et al. 2001, Stockwell and Peterson 2002). Another source of geographic range data are large-scale surveys (e.g., the Breeding Bird Survey). Such surveys have been useful for assessing phenomena such as range shifts after environmental changes (Mehlman 1997) but have been criticized for incomplete data and biased sampling (Link and Sauer 1998, Keller and Scallan 1999). Surprisingly few contemporary studies have been conducted with the primary goal of determining the geographic range of a particular species (e.g., Aubry and Lewis 2003).

Delineation of a species' geographic range is a complex task, which becomes even more difficult if a species is fragmented at the periphery of its geographic range (Sargeant et al. 2005) or is highly vagile. To avoid issues associated with delineating a geographic boundary, many maps portray a species' distribution by plotting all known locations (i.e., a dot map; Johnson and Sargeant 2002, Anderson and Martinez-Meyer 2004). Dot maps, however, leave the exact boundaries of a species' geographic range undefined and are considered overly conservative (Anderson and Martinez-Meyer 2004). Recently, new distributional modeling approaches have attempted to remove biases associated with either dot or outline maps (Peterson and Kluza 2003, Sargeant et al. 2005). Although these models are an improvement, many rely on problematic assumptions about habitat relationships and others are only applicable to spatially continuous distributions.

For wolverines (*Gulo gulo*) in the Pacific Coast mountains of the western United States (Cascade Range and Sierra Nevada), it has been unclear whether the population was continuously distributed from Alaska, USA, to southern California, USA, or whether the California population was disjunct. Joseph Grinnell (1877–1939), the famed naturalist and vertebrate taxonomist from the University of California at Berkeley, noted that by 1893, wolverines were restricted in California to high-elevation (2,500–4,000 m) alpine and subalpine habitats in the southern Sierra Nevada (Grinnell et al. 1937). Grinnell and his colleagues also believed that California wolverines occurred at extremely low densities

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Table 1. California wolverine museum specimens analyzed with mitochondrial and nuclear DNA markers. All samples were collected between 1891 and 1922.

Sample no.	Museum[a]	Date collected (collector)	Sex	Location collected	DNA extracted
032571	NMNH	1891 (J. H. Lowry)	M	Pine City, CA	No
032487	NMNH	Feb 1892 (J. H. Lowry)	F	Chiquito Lake, Fresno County, CA	Yes
051317	NMNH	Jan 1893 (J. H. Lowry)	F	Chiquito Lake, Fresno County, CA	Yes
16373	MVZ	Sep 1911 (J. W. Drouillard)	F	Sierra Nevada Mountains, Tulare County, CA	Yes
22121	MVZ	Jul 1915 (C. L. Camp)	F	Head of Lyell Canyon, Yosemite National Park, CA	Yes
22120	MVZ	Jul 1915 (C. L. Camp)	F	Head of Lyell Canyon, Yosemite National Park, CA	No
30049	MVZ	Aug 1919 (E. W. McDonald)	U[b]	Head of Twin Lake, Tulare County, CA	Yes
32807	MVZ	Dec 1921 (A. J. Gardinsky)	F	Mono Lake, Mono County, CA	Yes
33475	MVZ	Feb 1922 (A. J. Gardinsky)	F	Saddlebag Lake, Mono County, CA	Yes

[a] NMNH = National Museum of Natural History, Washington D.C., USA; MVZ = Museum of Vertebrate Zoology, Berkeley, CA, USA.
[b] Unknown.

within this geographically restricted range at that time (Grinnell et al. 1937).

Grinnell et al. (1937) were unsure of the geographic extent of the wolverine's range in California prior to 1893 but found little evidence that wolverines occurred north of the central Sierra Nevada historically. A recent analysis of the historical distribution and broad-scale habitat relations of wolverines in the contiguous United States (Aubry et al. 2007) revealed what appears to be a substantial gap in the wolverine's distribution between the central Sierra Nevada in California and the northern Cascade Range in Washington, USA. Their analyses suggest that the lack or extreme scarcity of historical records in northern California, Oregon, USA, and southern Washington reflects a lack of suitable habitat in those areas (Aubry et al. 2007). In contrast, most current maps of historical wolverine distribution, as well as maps from many early naturalists, depict an extensive and continuous distribution across much of the mountainous western United States, including an area extending from the Canadian border to the southern Sierra Nevada (Seton 1929, Hall and Kelson 1959, Hash 1987). Given that the last verifiable record of wolverine occurrence in the Sierra Nevada was in 1922 (Grinnell et al. 1937, Aubry et al. 2007), validating the results of such work is problematic, and novel approaches and techniques are required to further elucidate the historical distribution of wolverines in California.

We combined recent developments in molecular biology with both phylogenetic and population genetic analyses to investigate the potentially disjunct nature of the wolverine's historical distribution in the Pacific Coast mountains of the western United States. If wolverines were restricted to the southern Sierra Nevada and did not occur in northern California or southern Oregon, we would expect California wolverines to have unique genetic signatures. Conversely, if wolverine populations in the western mountains had a continuous distribution or maintained connectivity by regularly traversing unoccupied regions of the Cascade Range or Great Basin, we would expect California wolverines to share haplotypes with other populations in North America. We used data on both nuclear and mitochondrial DNA (mtDNA) obtained from museum specimens of California wolverines collected in the late 19th and early 20th centuries, and tested the hypothesis that California wolverines were isolated from other populations in North America (vs. the null hypothesis of non-isolation). In addition, we used these genetic data, which included samples from throughout North America, to make inferences about wolverine conservation in California and throughout western North America.

METHODS

Wolverine Samples and DNA Extraction

We attempted to obtain DNA from 9 wolverine skulls collected in California and located at the Smithsonian Institution's National Museum of Natural History and the University of California's Museum of Vertebrate Zoology (Table 1). All specimens were skulls collected between 1891 and 1922 (Table 1). To minimize damage to museum specimens, we sampled the maxilloturbinal bone from each skull (Wisely et al. 2004*b*). In the laboratory, we extracted the DNA in a separate satellite laboratory equipped to process ancient and historical DNA and exclusively used for the extraction and processing of DNA from museum specimens. We followed recommended ancient DNA protocols to avoid contamination (Herrmann and Hummel 1994, Hofreiter et al. 2001, Wisely et al. 2004*b*, Gilbert et al. 2005).

We compared the California wolverine sequences to published sequences from Northwest Territories, Canada (Wilson et al. 2000); Scandinavia (Walker et al. 2001); the Rocky Mountains (Cegelski et al. 2006); Alaska; northwestern Canada; and Eurasia (Tomasik and Cook 2005) and samples collected through our ongoing research efforts in the Rocky Mountains, Alaska, and Mongolia (Table 2, Fig. 1; Ulizio et al. 2006, Copeland et al. 2007, Squires et al. 2007). We did not compare these datasets quantitatively with similar data presented by Chappell et al. (2004) because of the short fragment length (200 base pairs [bp]) presented by these authors.

Mitochondrial DNA Sequencing

We initially sequenced 344 bp of the left domain of the mtDNA control region from a contemporary wolverine tissue sample by using universal primers, protocols from Shields and Kocher (1991), and the polymerase chain reaction (PCR). Using this sequence, we then designed a set

Table 2. Wolverine samples used in this study to compare mitochondrial DNA results. Data from Idaho, USA, reported in Cegelski et al. (2006) are likely from the same individuals we sequenced and we did not present them in the table. We did not report data from Tomasik and Cook (2005) in this table, nor did we use it in the spatial analysis of molecular variance, because haplotype frequencies were not reported in the manuscript and sampled areas were largely covered by our work and that of others. However, we used haplotypes from Tomasik and Cook in the program TCS (version 1.21; Clement at al. 2000) and phylogenetic analyses.

			Haplotype																
Region	Detailed location	*N*	Cali1	Cali2	A	B	F	H	I	Mng 1	C	D	E	G	L	M	N	O	Scand
CA, USA	Sierra Nevada	7	6	1															
AK, USA	Alaska Range	6			1	2	2	1											
Greater MT, USA	West Central MT	3			3														
	South Central MT	25			25														
	Northwest MT	10			10														
	Greater Yellowstone	22			20				2										
Central ID, USA	Sawtooth Mountains	13			13														
Mongolia		5						4		1									
NT, Canada[a]	Site1 (west)	12			7	3	1						1						
	Site2 (west)	3							2			1							
	Site3 (east)	20					4		4		8			4					
	Site4 (east)	3			1									2					
	Site5 (east)	3						1						2					
Scandinavia[b]		169																	169
MT and WY, USA[c]		101			74			1	24						2				
BC, Canada[c]	Williston Lake	19			11										6	1	1		
	Revelstoke	16			4			5							4			3	
AB, Canada[c]	Grande Cache	17			5		2				2				8				

[a] Data from Wilson et al. (2000).
[b] Data from Walker et al. (2001).
[c] Data from Cegelski et al. (2006).

of 3 short, overlapping segments to amplify the DNA collected from the museum specimens (Table 3). These segments ranged in size from 152 bp to 165 bp. We chose the control region because it has proven variable in other wolverine genetic studies (e.g., Wilson et al. 2000, Walker et al. 2001).

After designing the primers for the historical DNA samples, we used PCR to amplify contemporary wolverine tissues with primers Gulo 0F and H16498 (Table 3). We conducted the PCR in a reaction volume of 50 µL that contained 50–100 ng DNA, 1× reaction buffer (Applied Biosystems, Foster City, CA), 2.5 mmol $MgCl_2$, 200 µmol each dNTP, 1 µmol each primer, and 1 unit of *Taq* polymerase (Applied Biosystems). The PCR program was 94° C for 5 minutes; 34 cycles of 94° C for 1 minute, 55° C for 1 minute, and 72° C for 1.5 minutes; followed by 72° C for 5 minutes. We ran PCR products (385 bp) in a 2% agarose gel containing ethidium bromide (1.5 µL) and 1× Tris-acetate EDTA buffer.

Next, we amplified DNA from the museum specimens using the 3 overlapping primers listed in Table 3. Reaction conditions were similar to those used for tissues, but we used 5 µL of DNA preparation, along with 2 µg/mL bovine serum albumin and 1 unit of *Taq* Gold DNA polymerase (Applied Biosystems). The PCR profile was similar, except for an increase to 45 cycles and an initial denaturation step of 94° C for 10 minutes.

We directly sequenced PCR products (USB, Cleveland, OH) and ran them on LI-COR DNA analyzer (LI-COR Biotechnology, Lincoln, NE). To avoid sequencing errors, we sequenced all PCR products from historical samples ≥2

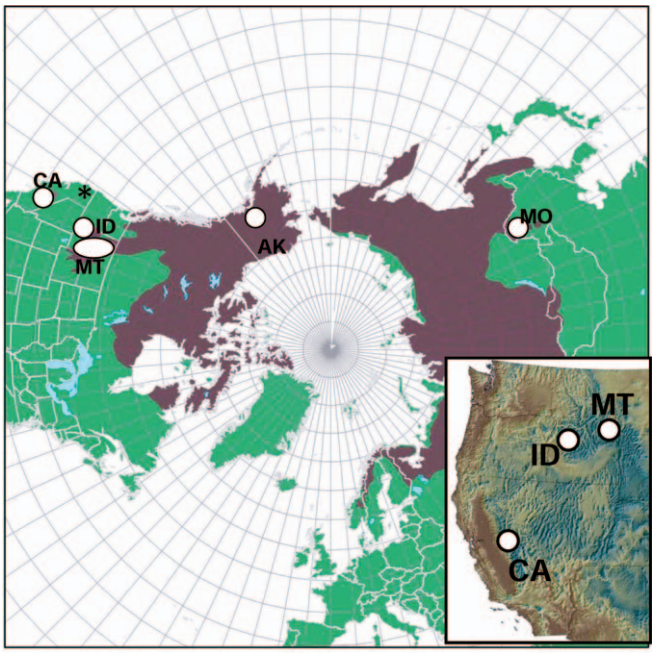

Figure 1. Schematic denoting the sampling locations of wolverine DNA samples analyzed in the laboratory in this study. The samples are from California, USA (CA), greater Montana, USA (MT), central Idaho, USA (ID), Alaska, USA (AK), and Mongolia (MO). Dark shading represents the current range of wolverines (for a discussion of historical vs. current range see Aubry et al., 2007); the black asterisk marks the hypothesized presettlement gap in wolverine distribution in the Pacific Coast mountains. The inset provides a more detailed schematic of the sampling locations within the United States.

Table 3. Primer sequences we used to obtain 344 base pairs of sequence data from the left domain of the mitochondrial DNA control region of the wolverine museum specimens from California, USA. All samples analyzed with these primers were collected between 1891 and 1922.

Primer pairs	Primer name	Primer sequence
1	Gulo0F	GGAGAACACCATCTCCCTAA
	Gulo1R	GGGAGGAAAAGGGTACATAC
2	Gulo2F	AACAACATTTACTGTGCTTCC
	Gulo2R	ATGGTGTTTAAGCTCGTGAT
3	Gulo3F	TTTTACATGCTGCATCTCAC
	H16498[a]	CCTGAACTAGGAACCAGATG

[a] Primer H16498 is from Shields and Kocher (1991).

times with the forward and reverse primers in separate cycle-sequencing reactions. We aligned sequences using Sequencher 4.1 (Gene Codes Corporation Inc., Ann Arbor, MI).

Sequence Analysis

We estimated the genealogical relationship among sequences using program TCS (version 1.21; Clement et al. 2000). This program creates a minimum spanning network based on recommendations by Templeton et al. (1992) and has been used extensively to determine population-level genealogies when divergences between sequences are low, as is often the case in analyses of intraspecific phylogeography (Clement et al. 2000). We used our data, plus data from Wilson et al. (2000), Walker et al. (2001), Tomasik and Cook (2005), and Cegelski et al. (2006) for this analysis.

We used phylogeographic reconstruction methods and data from our study and from Wilson et al. (2000), Walker et al. (2001), Tomasik and Cook (2005), and Cegelski et al. (2006) to compare with the results of the TCS analysis. Specifically, we generated maximum parsimony (MP) and maximum likelihood (ML) trees using these data. We assessed the fit of various substitution models using Modeltest v.3.6 (Posada and Crandall 1998). The best supported model based on Akaike Information Criterion (Akaike 1979) was the Hasegawa–Kishino–Yano (HKY) model (Hasegawa et al 1985) with unequal base frequencies and the proportion of invariable sites (I) = 0.921. To ensure that our results were not strongly influenced by the model of evolution chosen by Modeltest, we also examined the results from the top 5 models (Kelchner and Thomas 2007). Subsequent ML and parsimony trees were generated using the HKY + I model with program PAUP* (V4.0b, Swofford 2003) using the European pine marten (*Martes martes*) as an outgroup (Marmi et al. 2004). We estimated support for individual nodes in these trees with 1,000 bootstrap replicates.

We also investigated the geographical structure of our wolverine samples using the mtDNA data with a simulated annealing procedure as implemented in the spatial analysis of molecular variance (SAMOVA) algorithm (Dupanloup et al. 2002). This algorithm defines clusters of populations that are geographically homogeneous but maximally different from one another. We identified the most likely number of groups by running SAMOVA with 2–13 putative groups (K) and selecting the number of groups with a maximum F_{ct} (the proportion of total genetic variance due to differences among groups of populations; Dupanloup et al. 2002). We ran the program for 10,000 iterations for each K value from each of 500 random initial conditions. We used data from our study, Wilson et al. (2000), Walker et al. (2001), and Cegelski et al. (2006) for this analysis.

Microsatellite Analysis

We analyzed all samples that were sequenced successfully at the control region using 9 microsatellite loci used on wolverine samples in previous studies: *Gg4*, *Gg7*, *Ma2*, *Ma8*, *Tt4* (Davis and Strobeck 1998); *Ggu101*, *Ggu234*, *Ggu238* (Duffy et al. 1998), and *Mvis020* (Flemming et al. 1999). We amplified microsatellites using protocols detailed in the respective sources, except for the California samples, which we amplified using 1 unit of *Taq* polymerase and subjected to 45 PCR cycles. The resultant products were visualized on a LI-COR DNA analyzer (LI-COR Biotechnology). We accepted data from museum specimens as error-free only if the microsatellites produced consistent scores in 3 PCR amplifications (Eggert et al. 2003, Schwartz et al. 2004).

To determine similarity between California and Rocky Mountain samples, we estimated pair-wise genetic distances among individuals based on microsatellite data using the proportion of shared alleles algorithm (D_{ps}). D_{ps} is an allele frequency-based formula that relies on differentiation by genetic drift to establish distance (Bowcock et al. 1994, Minch et al. 1997) and is considered the most appropriate model for comparing microsatellite data between individuals (Rosenberg et al. 2001). We constructed phylogenetic trees from D_{ps} distances using program NEIGHBOR (version 3.63; Felsenstein 1989) and drew them using program TREEVIEW (version 1.6.6; Page 1996).

Relationships between wolverine sampling locations (i.e., populations) in the Rocky Mountains, Canada, and Alaska have been examined elsewhere (Kyle and Strobeck 2001, 2002; Cegelski et al 2003). To further compare California wolverines to other North American populations, we grouped samples into 4 geographic areas: California, Alaska, the greater Montana, USA, area (which included samples from MT and the greater Yellowstone ecosystem), and central Idaho and used program GENEPOP (Raymond and Rousset 1995) to estimate F_{st} and R_{st} (indices of population subdivision). Arguably, the central Idaho population and greater Montana area could be lumped into one Rocky Mountain population, but doing so resulted in significant deviations from Hardy–Weinberg proportions suggestive of a Wahlund effect. We found no significant deviations when we analyzed samples from central Idaho and the greater Montana area separately.

RESULTS

MtDNA Data

We obtained sequence data from 7 of the 9 California wolverine samples. These produced 2 distinct haplotypes not previously reported (Cali1 and Cali2; GenBank accession numbers AY880314, and AY880315; Tables 2 and 4). In

Table 4. Polymorphic sites associated with the left domain of the mitochondrial DNA control region for wolverines in our study and other published studies from North America, Europe, and Asia. Site number is based on polymorphic sites described by Wilson et al. (2000; haplotypes A–I).

Haplotype identification	Polymorphic site												
	59	61	69	98	107	108	111	173	196	202	234	249	250
A	G	A	C	T	A	T	C	:	C	C	G	T	C
B	G	A	C	T	A	C	C	:	C	C	G	T	C
C	G	A	C	T	A	T	T	:	T	C	G	T	C
D	G	A	A	T	A	T	C	:	T	C	G	T	C
E	G	A	C	T	A	C	C	:	T	C	G	T	C
F	G	A	C	T	A	T	C	:	T	C	G	T	C
G	G	A	C	T	A	T	T	:	T	C	A	T	C
H	G	A	C	T	A	T	C	:	T	C	A	T	C
I	G	A	C	T	A	T	T	C	T	C	G	T	C
Cali 1[a]	G	A	C	C	A	T	C	:	T	C	A	C	T
Cali 2[a]	G	A	C	C	A	T	C	:	T	T	A	C	T
Mng 1[a]	G	A	C	T	A	T	C	:	T	C	A	C	T

[a] New haplotype discovered in this study.

addition, we detected one new haplotype from Mongolia (GenBank *AY880313*). The other 4 samples from Mongolia were consistent with haplotype H reported by Wilson et al. (2000) in Northwest Territories, suggesting incomplete lineage sorting between North America and Eurasia. All haplotypes from Alaska and the Rocky Mountains have been reported elsewhere (Wilson et al. 2000, Tomasik and Cook 2005).

The genealogical relationship among mtDNA sequences from the 91 samples we analyzed, 169 Scandinavian samples (Walker et al. 2001), 168 samples from the Rocky Mountains (Cegelski et al. 2006), 159 samples from northwestern North America and Eurasia (Tomasik and Cook 2005), and 41 samples from northwestern Canada (Wilson et al. 2000) indicate that wolverines from California are genetically more similar to Eurasian wolverines than to other North American populations (Fig. 2). Although the difference between California haplotypes is ≥1 mutational step away from 2 different haplotypes found in Eurasia, it is 3 steps away from a haplotype found in Eurasia, Alaska, and parts of Northern Canada (haplotype H; Wilson et al. 2000, Tomasik and Cook 2005) and 5 steps from the most common haplotype found in the Rocky Mountains of the United States (haplotype A; Wilson et al. 2000).

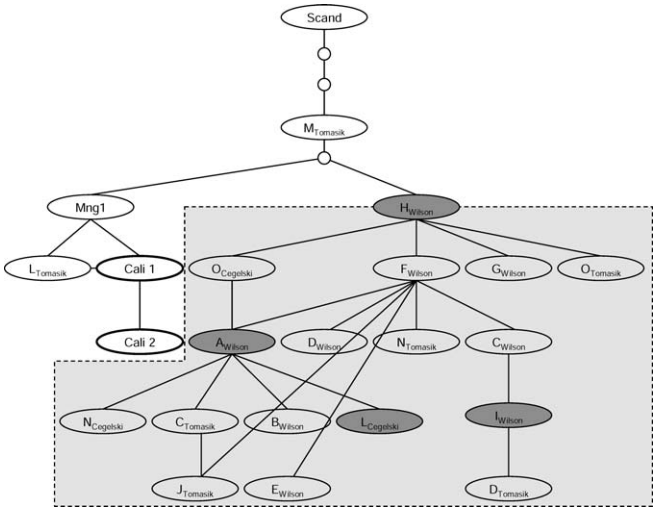

Figure 2. Diagram of the genealogical relationship among wolverine sequences as estimated using program TCS. The gray area enclosed by the dotted-line box denotes haplotypes found in North America, with the exception of California, USA. The dark gray circles signify the haplotypes found in the Rocky Mountains of the United States. The circles with heavier lines are California haplotypes obtained from museum specimens that were ≥82 years old. The samples from Mongolia are of haplotype H (also found in the United States Rocky Mountains) and Mng 1. Haplotypes are described in the resulting tree by using the lead author's last name followed by their designated name of the haplotype to avoid confusion. If 2 publications used different names for the same haplotypes (e.g., Wilson et al. 2000 and Tomasik and Cook 2005), we used the last name of the first author to publish the haplotype.

There was strong congruence between the MP and ML trees (trees not shown) and the haplotype network; the haplotype network had shallow branching and the phylogenetic reconstruction provided virtually no support for most branches. This lack of phylogenetic support was true regardless of the models of evolution used. Using the HKY + I model of evolution, a branch that separated the Scandinavia, Mongolia, and California haplotypes only had bootstrap support between 56% (ML) and 60% (MP), whereas the branch that supported California from Mongolia was supported with a bootstrap of 62% (ML and MP). There was no support (bootstrap support <50%) for any branches associated with any of the haplotypes found in the remainder of North America. This is similar to results from the haplotype network where all haplotypes found in North America (excluding CA) are <1 mutation away from another existing haplotype (see gray box in Fig. 2).

Using the observed average mtDNA sequence divergence between the California and Mongolia samples and the fastest published mutation rates for mitochondrial DNA (Lambert et al. 2002), we calculated that California wolverines diverged ≥2,200 years ago (Table 5). More conservative estimates place the divergence event closer to 10,500 years ago (Quinn 1992; Table 5). Given the paucity of variable sites found, however, these data should be interpreted cautiously.

Table 5. Time to most recent common ancestors (TMRCA) between 2 California, USA, haplotypes found among wolverine samples collected between 1891 and 1922 and a Mongolian haplotype based on published mutation rates.

Proportion of sites different	Mutation rate	TMCRA (yr)	Citation
1.5	2.00×10^{-8} mutations/base pair/yr	109,011	Shields and Wilson 1987
1.5	2.08×10^{-7} mutations/base pair/yr	10,482	Quinn 1992
1.5	9.60×10^{-7} mutations/base pair/yr	2,271	Lambert et al. 2002

Although we were primarily interested in the relationship of the California haplotypes to the remainder of the haplotypes surveyed, we also examined questions of structure using SAMOVA. Dupanloup et al. (2002) suggest that groups of populations that are geographically homogeneous and maximally differentiated from each other can be inferred when F_{ct} is maximized. In this study, F_{ct} was at its maximum at $K = 11$, but F_{ct} values differed by only 1% between $K = 6$ and $K = 13$ (Fig. 3). Thus, we also examined the point on the curve where the plateau was reached ($K = 6$; Fig. 3; similar to criteria suggested by Heuertz et al. 2004). At the maximum F_{ct} point, the following 11 groups are supported: 1) Scandinavia, 2) California, 3) Mongolia, 4) Alaska, 5) eastern region of Northwest Territories (sites 3–5 in Wilson et al. 2000), 6) western region of Northwest Territories (sites 1–2 in Wilson et al. 2000), 7) Williston Lake, British Columbia, Canada, 8) Revelstoke, British Columbia, 9) Grande Cache, Alberta, Canada, 10) Crazybelts, Montana, and 11) the aggregation of Idaho, Rocky Mountain Front, Gallatin, and Wyoming—the greater Yellowstone ecosystem. Using the point at which F_{ct} plateaus, the following 6 groupings are supported: 1) Scandinavia, 2) California, 3) Mongolia, 4) eastern region of Northwest Territories (sites 3–5 in Wilson et al. 2000), 5) the Crazybelts, Montana, and 6) the remainder of the groups. California separated into its own group for all $K > 2$ (at $K = 2$, only Scandinavia split from the rest of the sampling locations).

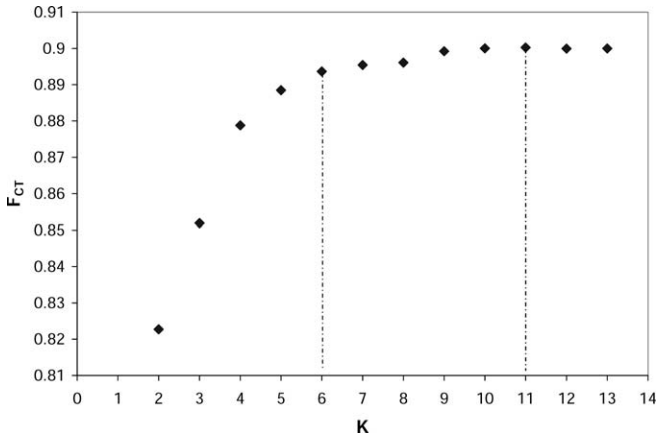

Figure 3. Spatial analysis of molecular variance results using the wolverine haplotype data. F_{ct} is the proportion of total genetic variance due to differences among groups of populations and K is the number of groups of sampling locales (populations). The number of groups supported occurs where F_{ct} is maximized (Dupanloup et al. 2002) or where the function reaches an inflection (Heuertz et al. 2004). The pregrouped sampling locales were as follows: Scandinavia (Walker et al. 2001); Mongolia (this study); California, USA (this study); Alaska, USA (this study); Northwest Territories, Canada, eastern group and western group (Wilson et al. 2000); Williston Lake, British Columbia, Canada (Cegelski et al. 2006); Grande Cache, Alberta, Canada (Cegelski et al. 2006); Revelstoke, British Columbia (Cegelski et al. 2006); Rocky Mountain Front, USA (Cegelski et al 2006, this study); Crazybelts, USA (Cegelski et al. 2006); Gallatin, USA (Cegelski et al. 2006); Yellowstone, USA, and Wyoming, USA (Cegelski et al. 2006, this study); and Idaho, USA (this study).

Microsatellite Data

We used microsatellite data to confirm the previous relationships with independent nuclear markers; given the small sample sizes used in this analysis, results should be considered with caution. Two historical California samples provided consistent scores across 3 runs at each of the microsatellites and we used them in subsequent analyses. We compared those samples to 7 samples from Alaska, 13 from central Idaho, and 45 from greater Montana to confirm our mtDNA trees with nuclear markers (Fig. 4). The tree based on microsatellites was similar to the mtDNA tree; California samples were not closely related to samples from the greater Montana area, Alaska, or central Idaho.

In all areas, all loci met Hardy–Weinberg expectations (when adequate sample sizes allowed testing) except for locus *Ggu238* in central Idaho, which showed a deficit of heterozygotes ($F_{is} = 0.688$; Table 6). F_{st} between the 4 areas was 0.16 (95% CI = 0.11–0.22) and R_{st} was 0.11. Pair-wise

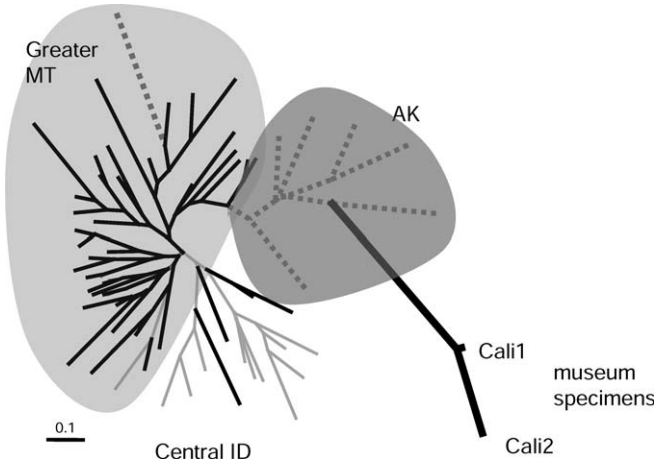

Figure 4. Tree created using wolverine microsatellite data and the proportion of shared alleles algorithm. The dashed line represents Alaska, USA, samples, black line represents greater Montana, USA, samples, the light gray lines represent central Idaho, USA, samples, and the heavy black lines represent California, USA, samples. The background shading is to aid in the visual identification of groups. The California samples were collected between 1891 and 1922, whereas the other samples were collected between 1992 and 2004.

Table 6. Genetic diversity measures from microsatellite data by area sampled for the contemporary wolverine samples analyzed in this study.

Sampling area	*n*	$\bar{x}$ no. of alleles/locus	Obs heterozygosity	SE	Exp heterozygosity	SE
Historical CA, USA	2	1.44	0.22	0.19	0.22	0.06
AK, USA	7	3.33	0.60	0.08	0.61	0.03
Greater MT, USA	45	3.78	0.57	0.02	0.56	0.06
Central ID, USA	13	3.33	0.54	0.07	0.55	0.07

comparisons between California and each region produced F_{st} values between 0.31 and 0.45 (Table 7).

DISCUSSION

Our primary objective was to investigate the relationships of extinct California wolverines to a subset of extant populations in North America. Thus far, all North American wolverine samples analyzed at greater than 340 bp of the mtDNA control region ($n = 335$) have shown limited differences. All North American haplotypes were one mutational step away from an existing state, with the maximum difference between haplotypes being 5 mutations away, representing a divergence of <1% between haplotypes. This lack of differentiation is supported by the polytomies found while constructing ML and MP trees.

Our sequence data from the Rocky Mountains are consistent with the published literature (Wilson et al. 2000, Cegelski et al. 2006); our mtDNA sequences from California, however, appear unique and separated from the Rocky Mountains by 3 and 4 mutational steps. At a minimum, this suggests that wolverines were not one large, genetically homogenous population in the western United States. The genealogical relationship among mtDNA sequences from central Idaho, the greater Montana area, Alaska, western Canada, Mongolia, California, and Scandinavia indicate that wolverines from California were genetically more similar to Eurasian wolverines than to other North American populations. This similarity may be because of a split upon North American colonization such that California wolverines proceeded along a different evolutionary path or because of homoplastic convergence. There is some support for homoplasy, because haplotype J from Chappell et al. (2004), the dominant haplotype in the Canadian prairie region of Manitoba and Nunavut, matches the Cali1 haplotype. Chappell et al. (2004), however, only sequenced 200 bp of the mitochondrial control region, excluding 5 known variable sites among wolverine haplotypes. If we assume that wolverines from California were descendants of Eurasian wolverines, as supported by the haplotype network, a conservative estimate of the time to most recent common ancestor places this split to ≥2,000 before present and likely much longer (Table 5). Given this relatively recent split, it is not surprising to see incomplete lineage sorting with haplotype H appearing in both Eurasian and North American samples.

Table 7. F_{st} results from the microsatellite data generated on wolverine samples collected in the greater Montana area, USA; Alaska, USA; historical California, USA, samples; and central Idaho, USA. The California samples were collected between 1891 and 1922, whereas the other samples were collected between 1992 and 2004.

Location	Historical CA	AK	Greater MT	Central ID
Historical CA		0.31	0.40	0.45
AK	0.31		0.16	0.24
Greater MT	0.40	0.16		0.07
Central ID	0.45	0.24	0.07	

Although it was not our primary intent to make inferences regarding wolverines in the Rocky Mountains of the United States, our genetic analyses have provided some new insights into their relationships. Our SAMOVA results suggest that with the exception of the isolated mountain ranges in north-central Montana (called the Crazybelts in Cegelski et al. 2003, 2006), the United States Rocky Mountain sampling locations group together based on mtDNA analyses. This includes the grouping of Idaho with sampling locations in Montana, a result that contradicts the finding of Cegelski et al. (2006). This grouping of the United States Rocky Mountain samples is not surprising; all United States Rocky Mountain sampling locations, except the Crazybelts, are dominated by haplotype A (71 samples in our study), with an occasional haplotype I (2 samples in our study), and haplotype H (not found in our study but reported once in Cegelski et al. 2006). Differences in haplotype frequency between areas in the United States Rocky Mountains can be easily explained by sampling error or stochastic variance in the reproductive success of females with differing haplotypes.

Mitochondrial DNA is only one genetic marker and only reflects patterns of maternal inheritance. We sought support for our mtDNA results using microsatellite (nuclear) analyses with 9 microsatellites, even though we only could obtain reliable results from 2 California samples. These analyses show the same pattern as the mtDNA results, with California substantially deviating from all North America samples (Fig. 4). Furthermore, microsatellite-based pair-wise F_{st} estimates between California and other populations are >0.31. Although this result could be a function of sampling variance, it is higher than any pair-wise F_{st} values reported by Kyle and Strobeck (2001, 2002), Chappell et al. (2004), and Cegelski et al. (2003, 2006). Unfortunately, we did not have samples from Canada to add to the microsatellite analysis, but Kyle and Strobeck (2002) demonstrated with microsatellite data that high gene flow exists among northern populations (AK and Canada).

We believe that our nuclear and mitochondrial DNA data indicate that, despite their wide-ranging abilities, California wolverines were not only separated from other populations during Grinnell's time, but that prior to their extirpation they had been isolated for a substantial period of time, consistent with both the hypothesis of Aubry et al. (2007) regarding the disjunct nature of the wolverine's range in the

Pacific Coast mountains and the assertion by Grinnell et al. (1937) of isolation of Sierra Nevada wolverines since at least 1893. Another plausible explanation for our findings is related to female philopatry, whereby relatively recent isolation followed by strong genetic drift (caused by small effective population sizes) could lead to rapid genetic differentiation in microsatellite genotypes. This explanation fails to account for the unique haplotypes we found in California. If we only invoke genetic drift to explain our data, we should have found few haplotypes in California and ones that are shared with the Rocky Mountains. Finding unique haplotypes that are geographically separated requires long-enough isolation for mutations to accumulate. Future genetic research using a Y-linked molecular marker (M specific) could further resolve this issue, as could examining larger areas of the mtDNA genome. Note, however, that we did not simply conduct a survey of wolverine population genetics using multiple molecular markers and report the patterns we found; rather, we used DNA evidence to test the a priori hypothesis that California wolverines were isolated from other populations in North America (Grinnell et al. 1937, Aubry et al. 2007). Not considering a priori hypotheses when interpreting results is a form of the defendant's fallacy (Thompson and Schumann 1987). Therefore, we reject the null hypothesis that wolverines in California were not isolated from other populations and support the alternate hypothesis that wolverines in California were isolated.

Aubry et al. (2007) investigated hypotheses that wolverines may be associated with attributes of alpine vegetation, cold temperatures, or spring snow cover. They found a strong relationship between historical wolverine records and alpine habitat conditions for both the Kuchler and Holdridge land-cover classifications (Kuchler 1964, Holdridge 1967). This was especially true at the periphery of wolverine range in the Pacific Coast mountains and southern Rockies. Furthermore, the habitat layer that best accounted for historical distribution patterns was relatively deep snow cover during the wolverine's spring denning period. Both alpine vegetation and snow-cover maps indicated that the southern Sierra Nevada is a habitat island for wolverines, isolated from similar habitat conditions by hundreds of kilometers in all directions (Aubry et al. 2007). Like Grinnell et al.'s (1937) assertion that the primary range of California wolverines was near timberline, Aubry et al.'s (2007) data and analysis could be artifacts of a declining population that, at the time of Grinnell's recording, had been extirpated from all but the most remote areas. Our findings, however, provide additional support for the broad-scale habitat relations indicated by Aubry et al. (2007).

Delimiting a species' historical and contemporary geographic ranges is a basic component of natural history that is often ignored or understudied. New methods using museum records or current detection–non-detection data (e.g., Sargeant et al. 2005) will advance our ability to delineate geographic ranges. Combining these efforts with molecular genetic approaches will enable researchers to test hypotheses regarding the status and history of peripheral and disjunct populations, rather than simply reporting genetic patterns from available samples, as is commonly done. By combining DNA and habitat-mapping approaches, our results provide empirical evidence that the wolverine population in California was disjunct historically.

A pattern is emerging among North American boreal carnivores (e.g., fisher [*Martes pennanti*] and lynx [*Lynx canadensis*]) that landscape location affects the partitioning of genetic variation (Kyle and Strobeck 2002, Schwartz et al. 2003, Wisely et al. 2004*a*). These species appear to be genetically variable in the northern portions and cores of their ranges but exhibit low levels of genetic variation in southern portions and on the periphery. These biogeographic patterns must be considered when interpreting the mechanisms behind a population's apparent reduced genetic variability (e.g., attributing the result to anthropogenic actions). At the same time, reduced genetic variability in the south and at the periphery may be due to small population sizes in those regions, which may make peripheral populations more vulnerable to anthropogenic influences.

Overall, our findings have important implications for wolverine conservation. Primarily, our results support recent findings about the habitat relationships of wolverines at the southern extent of their range in North America (Aubry et al. 2007). It appears that wolverines, at least in the Pacific Coast mountains, are associated with relatively large expanses of alpine habitat conditions and snow cover that persists through the spring denning season. Although these findings have range-wide implications, they may be most relevant in peripheral portions of the wolverine's range where increasing temperatures associated with global climate change could shift treeline to higher elevations, in effect shrinking the alpine zone (Millar et al. 2004) and decreasing the probability of spring snow cover. If such changes occur, current wolverine habitat at the periphery of the range may lose the ability to support viable populations of wolverines.

Secondarily, range contraction has been significant for wolverines, because they appear to have been extirpated throughout the southernmost portions of their historical range, including California, Utah, and Colorado, USA (Aubry et al. 2007). Several proposals have been developed by nongovernment agencies to reintroduce wolverines into the Sierra Nevada. Our data suggest that although historical wolverines from California had different haplotypes than those occurring in the Rocky Mountains, these differences were at the level of a management unit, not necessarily at the level of a separate evolutionary significant unit (see Moritz 1994, Fraser and Bernatchez 2001). Furthermore, our results show that none of the sampled populations in North America have haplotypes that are identical to those we found in California, thus selection of a source population for reintroduction must be based on data other than population genetics.

MANAGEMENT IMPLICATIONS

Our findings support the wolverine habitat relationships proposed by Aubry et al. (2007) at the southern extent of

their range in North America. Thus, areas without deep snow cover during the wolverine's spring denning period are likely to be in danger of losing extant wolverine populations. Furthermore, our data suggest that the Sierra Nevada wolverine population had been isolated from the main contiguous United States population of wolverines for substantial periods of time prior to extinction. Thus, no genetically identical source populations exist for reintroduction to the Sierra Nevada, although extant wolverine populations are likely from the same evolutionary significant unit as the Sierra Nevada wolverine.

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