

**From:** [Grizzle, Betty](#)  
**To:** [Stephen Torbit](#)  
**Subject:** Fwd: Research Questions and References  
**Date:** Monday, May 22, 2017 12:53:22 PM  
**Attachments:** [McKelvey et al 2014.pdf](#)  
[Kyle and Strobeck 2001.pdf](#)  
[Kyle and Strobeck 2002.pdf](#)  
[Zigouris et al 2013.pdf](#)  
[Schwartz et al 2009.pdf](#)  
[Tomasik and Cook 2005.pdf](#)  
[Cegelski et al 2006.pdf](#)

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----- Forwarded message -----

From: **Grizzle, Betty** <[betty\\_grizzle@fws.gov](mailto:betty_grizzle@fws.gov)>  
Date: Wed, May 3, 2017 at 3:00 PM  
Subject: Research Questions and References  
To: John Wenburg <[john\\_wenburg@fws.gov](mailto:john_wenburg@fws.gov)>, Jeffrey Olsen <[jeffrey\\_olsen@fws.gov](mailto:jeffrey_olsen@fws.gov)>

Hi John and Jeff - I put together a list of questions (see below) to give you a better idea of what we are trying to sort through for North American wolverines, which includes addressing DPS criteria.

I am also attaching selected published references --- some are relatively recent (e.g., 2014, McKelvey et al.), some are not so recent (two papers by Kyle and Strobeck), and some are in-between --- that have evaluated genetic diversity of wolverines in North America. [There are similar studies from Russia and Scandinavia] I also have a master's thesis (Frances 2008) and a PhD dissertation (Zigouris 2014). Let me know if you are interested in those.

Thank you for your time, and please let me know your thoughts as to how we can move forward.

**(1a) Do genetic studies indicate that there is a loss of connectivity between wolverines in the northern Rocky Mountains (or the Cascades region) and Canada?**

**(1b) Relatedly, how does immigration of wolverines from Canada influence the genetic diversity of wolverines in the contiguous United States?**

**(2a) Based on the genetic information you have, does the contiguous U.S. population of wolverines exhibit a reduction in genetic diversity?**

**(2b) Relatedly, is there evidence of genetic drift in wolverines in the contiguous US population? What might this mean to long term persistence of wolverines in the US?**

**(2c) If either (2a) or (2b) are true, have there been consequences to breeding, reproduction, or fitness of wolverine populations in the contiguous US?**

**(3) What is the "effective population size" of wolverines in contiguous United States, AND across it's entire North American range?**

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# Recovery of Wolverines in the Western United States: Recent Extirpation and Recolonization or Range Retraction and Expansion?

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**ABSTRACT** Wolverines were greatly reduced in number and possibly extirpated from the contiguous United States (U.S.) by the early 1900s. Wolverines currently occupy much of their historical range in Washington, Idaho, Montana, and Wyoming, but are absent from Utah and only single individuals are known to occur in California and Colorado. In response, the translocation of wolverines to California and Colorado is being considered. If wolverines are to be reintroduced, managers must identify appropriate source populations based on the genetic affinities of historical and modern wolverine populations. We amplified the mitochondrial control region of 13 museum specimens dating from the late 1800s to early 1900s and 209 wolverines from modern populations in the contiguous U.S. and Canada and combined results with previously published haplotypes. Collectively, these data indicated that historical wolverine populations in the contiguous U.S. were extirpated by the early 20th century, and that modern populations in the contiguous U.S. are likely the descendants of recent immigrants from the north. The Cali1 haplotype previously identified in California museum specimens was also common in historical samples from the southern Rocky Mountains, and likely evolved in isolation in the southern ice-free refugium that encompassed most of the contiguous U.S. during the last glaciation. However, when southern populations were extirpated, these matrilineages were eliminated. Several of the other haplotypes found in historical specimens from the contiguous U.S. also occur in modern North American populations, and belong to a group of haplotypes that are associated with the rapid expansion of northern wolverine populations after the last glacial retreat. Modern wolverines in the contiguous U.S. are primarily haplotype A, which is the most common and widespread haplotype in Canada and Alaska. For the translocation of wolverines to California, Colorado, and other areas in the western U.S., potential source populations in the Canadian Rocky Mountains may provide the best mix of genetic diversity and appropriate learned behavior. © 2014 The Wildlife Society.

**KEY WORDS** extirpated, *Gulo*, haplotype, historical, introduction, mitochondrial, wolverine.

Wolverine (*Gulo gulo*) populations that occupied portions of the contiguous United States (U.S.) historically were greatly reduced by the early 1900s and may have been extirpated (Newby and Wright 1955, Nowak 1973, Aubry et al. 2007).

Currently, wolverines occupy much of their historical range in Washington, Idaho, Montana, and Wyoming, but are absent from Utah (Aubry et al. 2007) and only single individuals are known to exist in California (McKelvey et al. 2008, Moriarty et al. 2009) and Colorado (Inman et al. 2009). Spatial patterns in historical records from Montana led Newby and Wright (1955) to conclude that wolverines had been extirpated from the state by the early 1900s, but began to recolonize the area from the north in the

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early 1930s; by the early 1960s, wolverines had apparently recolonized most of western Montana (Newby and McDougal 1964). A later assessment of historical and current wolverine records by Nowak (1973) indicated that by the 1970s many areas in the contiguous U.S. from which wolverines had been extirpated were being recolonized. Additionally, during the last several decades, wolverines have recolonized the Cascade Range in northern Washington and southernmost British Columbia, and are continuing to expand their range southward (McKelvey et al. 2011b, Aubry et al. 2012). However, despite the recent long-distance movement of a lone male wolverine into Colorado from Wyoming in 2009 (Inman et al. 2009), and the discovery of a wolverine in California in 2008 that had originated in Idaho (McKelvey et al. 2008, Moriarty et al. 2009), there is no evidence of extant wolverine populations in areas south of the Greater Yellowstone Area in Wyoming, the Sawtooth Range in Idaho, or the northern Cascade Range in Washington (Aubry et al. 2007).

It may appear contradictory that wolverine populations are expanding their distribution southward and reclaiming portions of their former range at a time when global warming is reducing the areal extent and connectivity of wolverine habitat in the western contiguous U.S. (McKelvey et al. 2011a). However, recolonization of the western U.S. by wolverines is occurring at much larger spatial scales and more rapidly than habitat losses from global warming; predictive modeling indicates that many areas in the western mountains, including sizable areas in Colorado, will provide relatively large expanses of wolverine habitat into the next century, even if current trends in global warming continue (see Fig. 13b in McKelvey et al. 2011a).

Although wolverines may eventually recolonize the southern portions of their historical range naturally, the state of Colorado is considering translocating wolverines to accelerate this process (Colorado Division of Wildlife 2013), and less-formal discussions have also been occurring in California (Institute for Wildlife Studies 2013). Because of the potential for reintroduction of wolverines to southern portions of the contiguous U.S. in the near future, managers must identify the most appropriate source populations to use for such efforts. Such decisions will depend on the genetic objectives of reintroduction efforts, which can vary depending on local circumstances. For example, one goal may be to restore populations that are genetically similar to those that were extirpated to improve the likelihood that the introduced population will contain local adaptations (cf. Templeton et al. 1986), whereas another may be to provide a genetically diverse founding population to avoid inbreeding depression and associated increases in extinction risks (Frankham 1998).

The historical wolverine population in California was genetically distinct from modern populations in North America, indicating an extended period of isolation (Schwartz et al. 2007). Thus, this population may have evolved local adaptations that improved its fitness. Elliot (1903) originally considered wolverines in California to represent a distinct species (*Gulo luteus*), but this taxon was

later reduced to subspecific status by Grinnell (1913). No studies have determined whether the unique haplotypes found in California wolverines by Schwartz et al. (2007) were limited to California, nor if unique haplotypes existed in other isolated populations in the western U.S. (Aubry et al. 2007).

The objectives of this study were to 1) investigate the hypothesis that wolverines were extirpated from their historical range in the contiguous U.S. by the early 1900s; and 2) evaluate potential source populations for future translocations to reestablish wolverine populations in California, Colorado, and other portions of the western U.S.

## METHODS

To locate historical wolverine specimens from the contiguous U.S., we contacted curators from 114 museums in the United States and Canada, and searched online museum databases for wolverines collected prior to 1930. To minimize damage to museum specimens, we collected nasal turbinate bone samples from each skull (Wisely et al. 2004). To develop a more complete understanding of the genetic characteristics of modern wolverine populations, we also obtained tissue or hair samples from individual wolverines in North America from various sources. We added the haplotypes we derived to previously published data from both historical and modern populations (Wilson et al. 2000, Tomasik and Cook 2005, Cegelski et al. 2006, Schwartz et al. 2007, Zigouris et al. 2012).

To analyze ancient DNA, we followed the methods and protocols used by Schwartz et al. (2007). Previous extractions from turbinate bones of historical wolverine specimens provided low (0.6–1.0 ng/μl) DNA concentrations and erratic amplification of nuclear DNA (2 successes out of 9 attempts; Schwartz et al. 2007). Consequently, for this study we analyzed only mitochondrial DNA (mtDNA) from the control region. We chose the control region because all published studies of wolverine mtDNA amplified this region, allowing cross-study comparisons. We extracted DNA in an isolated, satellite laboratory equipped and used solely for the processing of ancient and historical DNA, and followed recommended ancient DNA protocols to avoid contamination (Hofreiter et al. 2001, Gilbert et al. 2005). We initially sequenced 344 bp of the left domain of the mtDNA control region from a modern wolverine tissue sample using universal primers, protocols from Shields and Kocher (1991), and the polymerase chain reaction (PCR). Using this sequence, we then designed a set of 3 short, overlapping segments to amplify DNA obtained from museum specimens. These segments ranged in size from 152 bp to 165 bp (see Table 3 in Schwartz et al. 2007 for details). For extractions from museum specimens, controls included an ambient (empty tube) from the sampling museum and a negative control. Additionally, we re-amplified and re-sequenced all historical samples that produced a haplotype to verify previous results.

We amplified modern tissues using primers *Gulo 0F* and *H16498* (Schwartz et al. 2007). Reaction volumes of 50 μl contained 50–100 ng DNA, 1× reaction buffer (Applied

Biosystems, Foster City, CA), 2.5 mM MgCl<sub>2</sub>, 200  $\mu$ M each dNTP, 1  $\mu$ M each primer, and 1 U Taq polymerase (Applied Biosystems). The PCR program for all primer sets was 94° C/5 min, (94° C/1 min, 55° C/1 min, 72° C/1 min 30 s)  $\times$  34 cycles, and 72° C/5 min. We determined the quality and quantity of template DNA using 1.6% agarose gel electrophoresis, and purified PCR products using ExoSap-IT (Affymetrix-USB Corporation, Cleveland, OH) according to manufacturer's instructions. We obtained DNA sequence data using the Big Dye kit and the 3700 DNA Analyzer (ABI; High Throughput Genomics Unit, Seattle, WA). We generated DNA sequence data using the given primers, viewed and aligned sequences using Sequencher (Gene Codes Corp., Ann Arbor, MI), and determined resulting haplotypes using DAMBE (University of Ottawa).

Following Schwartz et al. (2007) and Zigouris et al. (2012), we examined relationships among haplotypes by constructing a minimum spanning network (Posada and Crandall 2001). We input network connections generated from Arlequin version 3.5.1.2 (Excoffier and Lischer 2010) into HapStar version 0.7 (Teacher and Griffiths 2011). To properly place haplotypes into their worldwide

context, we included all North American haplotypes, as well as all published haplotypes from Eurasia, including Scandinavia (Walker et al. 2001), unknown locations in Asia (Tomasik and Cook 2005), and Mongolia (Schwartz et al. 2007).

## RESULTS

### Historical Samples

Schwartz et al. (2007) successfully amplified DNA from 7 wolverine specimens collected in California between 1891 and 1922. To these, we added 13 specimens collected between 1870 and ca. 1900 (4 from Colorado, 4 from Idaho, and 1 each from Washington, Montana, Wyoming, Utah, and Minnesota; Table 1). The most common haplotype among historical wolverine specimens from the contiguous U.S. was Cali1 (11/20, 55%; Schwartz et al. 2007). Haplotype O was the next most common (4/20, 20%; Cegelski et al. 2006), 2 (10%) were haplotype A (Wilson et al. 2000), 1 (5%) was haplotype Cali2 (Schwartz et al. 2007), 1 was haplotype F (Wilson et al. 2000), and 1 was haplotype I (Wilson et al. 2000). Haplotypes Cali1 and O were widely distributed historically; we found Cali1 in

**Table 1.** Historical museum specimens from the contiguous U.S. genotyped during this study, including California specimens genotyped by Schwartz et al. (2007).

| Museum | Catalog number | Date of collection | State        | County      | Location                     | Sex | Specimen type                    | Collector                             | Haplotype |
|--------|----------------|--------------------|--------------|-------------|------------------------------|-----|----------------------------------|---------------------------------------|-----------|
| USNM   | 32571          | 1891               | California   | Mono        | Pine City                    | M   | Skull                            | J. H. Lowry                           | ?         |
| USNM   | 32487          | 1892               | California   | Fresno      | Chiquito Lake                | F   | Skin, Skull                      | J. H. Lowry                           | Cali1     |
| USNM   | 51317          | 1893               | California   | Fresno      | Chiquito Lake                | F   | Skin, Skull                      | J. H. Lowry                           | Cali1     |
| MVZ    | 16373          | 1911               | California   | Tulare      | Monache Meadows              | F   | Skin, Skull                      | J. W. Drouillard                      | Cali1     |
| MVZ    | 22121          | 1915               | California   | Mariposa    | Head of Lyell Canyon         | F   | Skin, Skull,<br>Partial Skeleton | C. L. Camp                            | Cali1     |
| MVZ    | 22120          | 1915               | California   | Mariposa    | Head of Lyell Canyon         | F   | Skin, Skull,<br>Partial Skeleton | C. L. Camp                            | Cali2     |
| MVZ    | 30049          | 1919               | California   | Tulare      | Head of Twin Lake            | ?   | Skin, Skull                      | E. W. McDonald                        | ?         |
| MVZ    | 32807          | 1921               | California   | Mono        | Virginia Lake                | F   | Skin, Skull                      | A. J. Gardinsky                       | Cali1     |
| MVZ    | 33475          | 1922               | California   | Mono        | Saddlebag Lake               | F   | Skin, Skull,<br>Partial Skeleton | A. J. Gardinsky                       | Cali1     |
| YPM    | MAM 6566       | 1870               | Colorado     | Denver      | Denver                       | ?   | Skeleton                         | Yale College<br>Scientific Expedition | O         |
| DMNS   | Mammals 85     | 1876               | Colorado     | Summit      | Pass Creek                   | F   | Skin, Skull                      | E. Carter                             | Cali1     |
| DMNS   | Mammals 2723   | circa 1900         | Colorado     | Clear Creek | Idaho Springs                | M   | Skin, Skull                      | E. Carter                             | O         |
| YPM    | MAM 6567       | ? <sup>a</sup>     | Colorado     | Denver      | Denver                       | ?   | Skull                            | ?                                     | Cali1     |
| USNM   | 188246         | 1875               | Idaho        | ?           | Eastern                      | ?   | Skull                            | ?                                     | Cali1     |
| USNM   | A 30912        | 1890               | Idaho        | ?           | Salmon River Mountains       | F   | Skull                            | Bailey, Dutcher                       | F         |
| USNM   | 26463          | 1891               | Idaho        | ?           | Sawtooth Mountains           | F   | Skin, Skull                      | F. C. Parks                           | Cali1     |
| USNM   | 81799          | 1896 <sup>b</sup>  | Idaho        | ?           | Sawtooth                     | F   | Skull, Skeleton                  | F. C. Parks                           | A         |
| USNM   | 110281         | 1899               | Minnesota    | Itasca      | T61N, R25W, Sec. 7           | M   | Skull                            | H. V. Ogden                           | I         |
| USNM   | 67370          | 1894               | Montana      | ?           | Bear Tooth Mountains         | F   | Skin, Skull                      | B. H. Dutcher                         | O         |
| USNM   | A 21493        | Late 1800s         | North Dakota | ?           | Fort Union                   | ?   | Skull, Skelton                   | H. A. Ward                            | ?         |
| USNM   | 236529         | 1921               | Utah         | ?           | Upton                        | M   | Skin, Skull                      | G. E. Rickins                         | Cali1     |
| USNM   | 64358          | 1894               | Washington   | Chelan      | Chelan                       | ?   | Skull                            | C. D. Woodin                          | ?         |
| USNM   | 64359          | 1894               | Washington   | Chelan      | Chelan                       | ?   | Skull                            | C. D. Woodin                          | A         |
| USNM   | 62614          | 1895 <sup>c</sup>  | Wyoming      | ?           | Yellowstone<br>National Park | F   | Skin, Skull                      | ?                                     | O         |

DMNS, Denver Museum of Nature and Science, Denver, Colorado; MVZ, Museum of Vertebrate Biology, Berkeley, California; USNM, National Museum of Natural History, Washington, D.C.; YPM, Yale Peabody Museum of Natural History, New Haven, Connecticut. Haplotypes A, F, and I were identified by Wilson et al. (2000), O by Cegelski et al. (2006), and Cali1 and Cali2 by Schwartz et al. (2007); haplotype ? means DNA from that sample did not amplify.

<sup>a</sup> Probably collected in 1870.

<sup>b</sup> Received at the museum in 1896, date of collection unknown.

<sup>c</sup> Received at the museum in 1895, date of collection unknown.

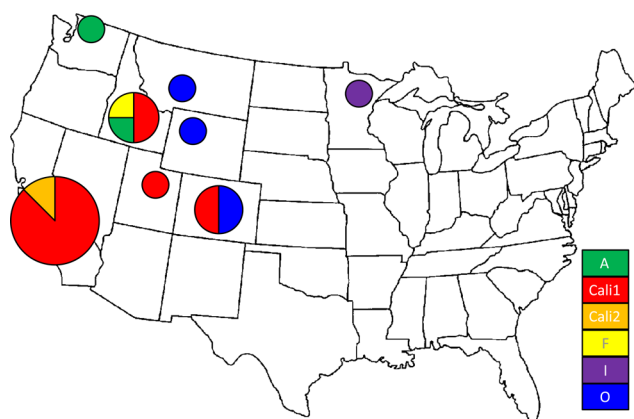


**Figure 1.** Proportions of haplotypes associated with historical museum specimens from the contiguous U.S. by state. A, F, and I refer to haplotypes identified by Wilson et al. (2000), O by Cegelski et al. (2006), and Cali1 and Cali2 by Schwartz et al. (2007). Pie chart sizes approximate the total number of samples from each state.

California, Colorado, Utah, and Idaho and O in Colorado, Wyoming, and Montana (Fig. 1). We found haplotype A in Washington and Idaho, and haplotypes Cali2, F, and I in California, Idaho, and Minnesota, respectively (Fig. 1).

### Modern Samples

We obtained 209 DNA samples from Alaska, Idaho, Montana, Oregon, Washington, and Wyoming in the U.S., and from Alberta, British Columbia, and Ontario in Canada (Appendix). Modern wolverine samples were dominated by haplotype A (150/209, 72%; Fig. 2, Appendix), and exhibited patterns in the geographical distribution of haplotypes that were similar to those reported by Schwartz et al. (2007). Wolverines from the Cascade Range in northern Washington and southernmost British Columbia were a notable exception; all 18 of the samples we analyzed were haplotype C, and this was the only population sampled in the contiguous U.S. that contained this haplotype (Fig. 2, Appendix).

Combined with previously published haplotypes, 741 wolverines from modern populations in North America have now been haplotyped (Appendix). Samples from the contiguous U.S. are plentiful (319; Fig. 2) with A being the dominant haplotype throughout that region (266/319, 83%; Fig. 2, Appendix).

### Phylogenetic Relationships Among Haplotypes

The network we generated is very similar to that produced by Schwartz et al. (2007), but contains additional haplotypes

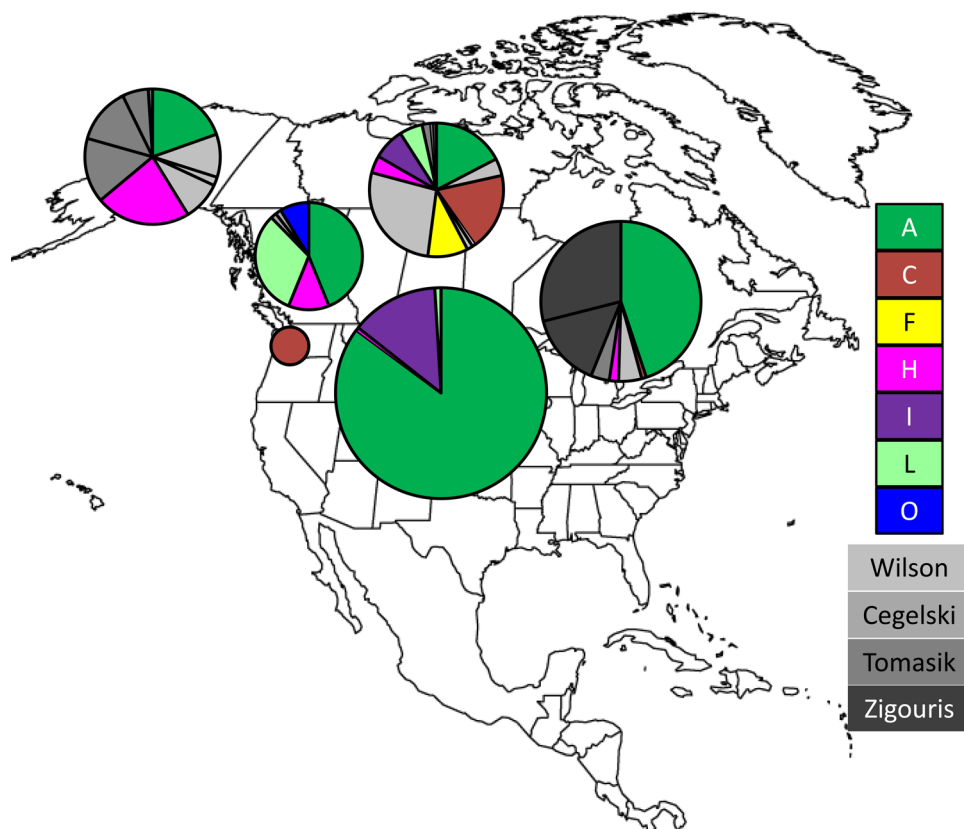


**Figure 2.** A simplified presentation of wolverine population structure among modern wolverine populations in North America. Colored wedges are haplotypes that either occur in modern populations or occurred in historical populations within the contiguous U.S. A, C, F, H, and I refer to haplotypes identified by Wilson et al. (2000), and L and O by Cegelski et al. (2006). Grayed wedges, representing haplotypes only found in Alaska or Canada, are grouped by the paper where they were first published. Wilson refers to Wilson et al. (2000), Cegelski to Cegelski et al. (2006), Tomasik to Tomasik and Cook (2005), and Zigouris to Zigouris et al. (2012). Pie chart sizes approximate the total number of samples from each area.

24 and 25 reported by Zigouris et al. (2012; hereafter, Z\_24 and Z\_25). Thus, our minimum spanning network includes all published haplotypes. Similar to the findings of Schwartz et al. (2007), our minimum spanning network places Cali1 and Cali2 onto a branch that contains mostly Asian haplotypes (Fig. 3). Haplotype Z\_25, which is common in Ontario and Manitoba, Canada (Zigouris et al. 2012), is on this same branch but is removed from Cali1 by an additional 2 substitutions (Fig. 3). As in the network presented by Schwartz et al. (2007), haplotype H—the only haplotype found in both North America and Asia—appears to be the haplotype that links these continents. In North America, we observed a star phylogeny (Gillespie 1984) with haplotype A, the most common and widely distributed haplotype in North America (Fig. 2, Appendix), and with haplotype F, which has been found in modern samples from Alaska and northern Canada, and in 1 historical sample from the western contiguous U.S. Both haplotypes A and F are connected to 7 haplotypes by a single substitution (Fig. 3). A chain of substitutions consisting of North American haplotypes and culminating in haplotype Z\_24, which is also common in eastern Canada (Zigouris et al. 2012), represents a unique branch of this phylogeny (Fig. 3).

## DISCUSSION

### Genetic Characteristics of Historical and Modern Wolverine Populations

Three hypotheses could explain the haplotype shifts we documented between historical and modern wolverine populations in the contiguous U.S. The first is that historical haplotypes Cali1, Cali2, F, and O are still present in the contiguous U.S. but have not yet been found because of insufficient sampling. The second is that wolverines were

nearly extirpated from the contiguous U.S., but some individuals with haplotypes A, C, H, I, and L persisted and subsequently founded modern populations in the contiguous U.S. The third is that wolverines were extirpated from the contiguous U.S., and modern populations resulted from recent colonizations by northern wolverine populations.

In our view, the hypothesis that historical haplotypes have been overlooked because of insufficient sampling is highly unlikely. In large areas of the contiguous U.S. where these historical haplotypes were found (California, Colorado, and Utah), wolverines were extirpated early in the 20th century (Aubry et al. 2007). In the remaining areas (Idaho, Montana, Oregon, Washington, and Wyoming), recent sampling has been intensive, resulting in haplotypes from 319 wolverines. In Idaho, samples were obtained during a radiotelemetry study in the Sawtooth Mountains of central Idaho (Copeland et al. 2007), a similar study in the Teton Range (Inman et al. 2012), and opportunistically from both central and northern Idaho. In western Montana, samples were obtained from the state Department of Fish, Wildlife, and Parks, where trappers are required to submit the skulls of all trapped wolverines (Schwartz et al. 2009), and from 4 scientific studies (Squires et al. 2007, Schwartz et al. 2009, Copeland et al. 2010, Inman et al. 2012). In Oregon, recent surveys detected 3 wolverines in the Wallowa Mountains, one of which was genotyped (Magoun et al. 2013; Appendix). In Washington and Wyoming, samples were obtained both opportunistically and from ongoing radiotelemetry studies in the Cascade Range (Aubry et al. 2012; Appendix) and Greater Yellowstone Area (Inman et al. 2012; Appendix). Thus, within their current range in the contiguous U.S., wolverines have been well sampled everywhere they are known to exist. Additionally, organized sampling has been

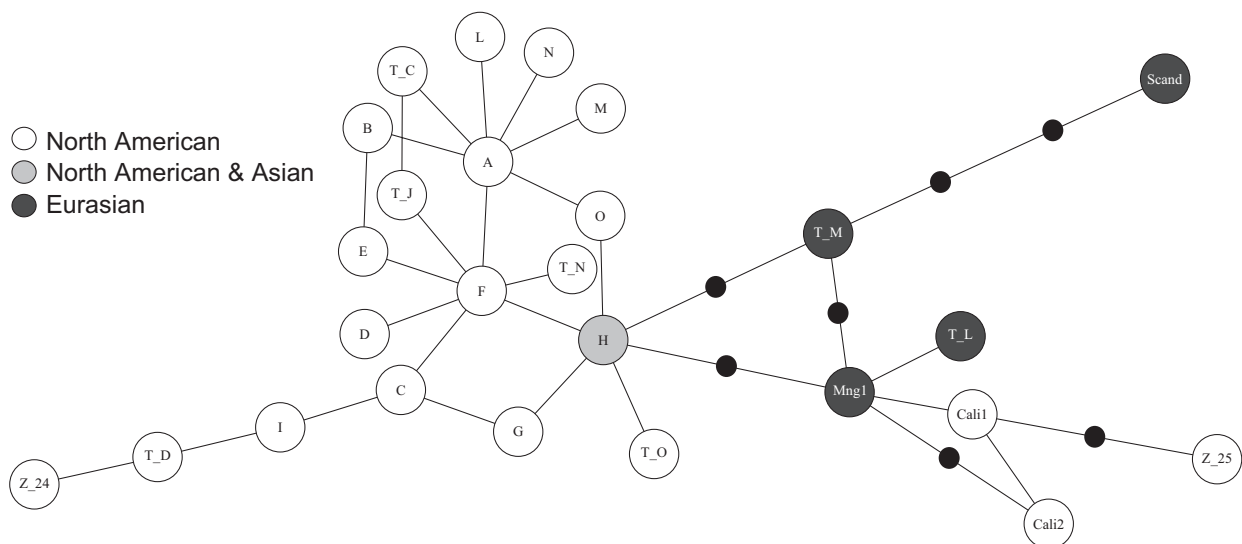


**Figure 3.** Minimum spanning network for all published wolverine haplotypes. Each node indicates a single base-pair substitution. Haplotypes A–I were identified by Wilson et al. (2000), L–O by Cegelski et al. (2006), T\_C–T\_O by Tomasik and Cook (2005), and Z\_24–Z\_25 by Zigouris et al. (2012). Scand was identified by Walker et al. (2001) and Cali1, Cali2, and Mng1 by Schwartz et al. (2007). Scand was found in Scandinavia, Mng1 in Mongolia, and T\_L and T\_M in unspecified Asian locations. Haplotype H is the only haplotype known to occur in both North America and Asia; all other haplotypes were found exclusively in North America.

attempted in other areas where they have been reported (e.g., Hiller and McFadden-Hiller 2013). Thus, there is little chance that entire matrilineages have gone unsampled in the contiguous U.S. In North America, 741 individuals have been genotyped based on samples that encompass the current geographic range of wolverines (Fig. 2, Appendix). The absence of haplotypes Cali1 and Cali2 from this large collection of modern samples makes their continued existence anywhere in North America extremely unlikely. Zigouris et al. (2012) report 2 haplotypes (Z\_24 and Z\_25; Appendix) that were previously unpublished; however, these types are consistent with types J and K reported by Chappell et al. (2004, Zigouris et al. 2012: Table 6), which were based on a shorter sequence of mitochondrial DNA. If we assume that Z\_24 and Z\_25 were previously reported by Chappell et al. (2004), then no new haplotypes from extant populations have been reported since Cegelski et al. (2006), even though 444 individuals have subsequently been examined.

Wolverine populations in the contiguous U.S. were greatly reduced and locally extirpated during the early 20th century (Aubry et al. 2007). For current populations to be derived from resulting relictual populations rather than immigrants, this bottleneck event would need to have eliminated most of the matrilineages present in our historical sample (haplotype A in the Cascades and haplotypes Cali1, F, and O from the northern Rocky Mountains) while allowing the persistence of matrilineages that we did not detect (haplotypes C in the Cascades and H, I, and L in the northern Rocky Mountains). Moreover, because population bottlenecks preferentially remove rare haplotypes (Nei et al. 1975), our historical sample would need to contain mostly rare haplotypes while lacking common ones, which seems highly unlikely. Additionally, the spatial recolonization patterns described by Newby and Wright (1955) in Montana are not consistent with expansion from relictual sources.

We believe, therefore, that the hypothesis that wolverines were extirpated from the contiguous U.S. and modern populations resulted from recolonization from the north provides the most compelling and parsimonious explanation for observed haplotype shifts. Most historical wolverine samples from the western U.S. were haplotypes Cali1, Cali2, F, or O (17/19, 89%; Fig. 1), none of which occur in modern populations from that region (Fig. 2, Appendix). Moreover, neither haplotype Cali1 nor Cali2 has been found among extant wolverines anywhere in North America. As noted above, it is highly unlikely that these haplotypes are present but undetected. Historically, both Cali1 and O were widespread geographically in the western U.S. (Fig. 1). Among modern populations, haplotype O (Cegelski et al. 2006) has been found only near Revelstoke, British Columbia, which is approximately 230 km north of the U.S. border. In this localized area, it was found both by Cegelski et al. (2006) and in more recent surveys (Appendix).

The only haplotype found among historical samples from the western U.S. that also occurs among modern populations in that region is haplotype A (Figs. 1 and 2). However, A is the most common haplotype in Alaska and Canada,

including southern British Columbia and Alberta (Appendix), the most likely source areas for immigration into the western contiguous U.S. Although the historical specimen from Washington is haplotype A, all 18 modern wolverine specimens from the Cascade Range in northern Washington and southernmost British Columbia are haplotype C. Outside that region, C has been found only in Alberta, Saskatchewan, and Nunavut. This provides strong evidence that the recent recovery of wolverines in the Cascades resulted from colonization by northern populations, not the expansion of relictual populations. The implications of finding haplotype I in Minnesota, which was the only historical sample available from the eastern U.S., are unknown because I is a common haplotype in modern populations throughout North America, including the western U.S. (Figs. 1 and 2, Appendix). However, in a recent study of wolverine genetics in central Canada, Zigouris et al. (2012) did not detect haplotype I in Ontario, the closest (approx. 500 km) extant population to Minnesota.

Modern wolverine populations have many control region haplotypes (at least 20; Appendix), and exhibit a high level of matrilineal spatial structure. For example, haplotype L (Cegelski et al. 2006) appears to be a relatively common haplotype in British Columbia and Alberta (27/79, 34%), but is nearly absent in Montana and Idaho (Appendix). Based on the distribution of suitable habitat conditions for wolverines (Copeland et al. 2010), northern populations in Alaska and Canada have the potential for gene flow, yet exhibit a high level of genetic structure and contain uncommon and apparently local haplotypes (Tomasik and Cook 2005). Thus, the presence of unique haplotypes among historical populations in the contiguous U.S. is not unexpected.

The vast majority of North American haplotypes are associated with star phylogenies around common haplotypes and therefore likely represent recent radiation. However, the association between haplotypes Cali1, Cali2, and Z\_25 and exclusively Asian haplotypes is unlikely to be the product of recent radiation. The calculated temporal depth of the genetic split between Cali1, Cali2, and the other North American haplotypes depends strongly on assumed mutation rates and could plausibly vary between approximately 2,000–100,000 years (Table 5 in Schwartz et al. 2007). However, Cali1, Cali2, and Z\_25 are as divergent from the other North American haplotypes as are purely Eurasian types (Fig. 3), and these have been separated at least since the submergence of the Beringian land bridge at the end of the Pleistocene. This would suggest a temporal depth of between approximately 10,000–100,000 years.

### Evolutionary History of the Wolverine in Western North America

Tomasik and Cook (2005) argued that during the Holocene wolverines that had been isolated in Beringia by continental glaciers rapidly spread southeastward into present-day Alaska and Canada; additional haplotypes found by Cegelski et al. (2006) supported this hypothesis. Thus, the diversity of haplotypes found among modern wolverine populations in North America likely represents a relatively recent radiation

that led to many closely related haplotypes (Tomasik and Cook 2005, Cegelski et al. 2006, Schwartz et al. 2007). As noted above, Cali1, Cali2, and Z\_25 were not associated with this recent radiation. Here, we examine the case for haplotypes Cali1 and Cali2 being associated with glacial vicariance.

In the late Pleistocene, wolverine specimens dating to the last glacial period (ca. 100,000 to 10,000 years ago [yBP]) have been found in both Alaska and Yukon, and in the western contiguous U.S. (Bryant 1987, Neomap 2013). At the height of the last glaciation (ca. 18,000 yBP), when continental ice completely separated Beringia from the contiguous U.S. (see Fig. 1B in Davison et al. 2011), wolverine populations in North America were isolated in 2 disjunct ice-free refugia: one in Beringia in the north and another, or perhaps several (Shafer et al. 2010, Zigouris et al. 2012), in the contiguous U.S. south of the continental ice sheets (Kurtén and Anderson 1980, Neomap 2013).

Tundra conditions were prevalent in the southern Rocky Mountains ca. 12,000–10,000 yBP, and were replaced by spruce-fir (*Picea-Abies*) forests in the early Holocene (Anderson et al. 2008). Thus, environmental conditions in the southern Rocky Mountains during the late Pleistocene and early Holocene were consistent with contemporary wolverine habitat (Copeland et al. 2010). The hypothesis that wolverines used the southern Rocky Mountains as a refuge during this period is supported by the fossil record. Wolverine specimens dating from the late Pleistocene to early Holocene have been recovered from sites in Colorado, Idaho, Wyoming (Kurtén and Anderson 1980, Anderson 1998), Nebraska, Utah (Neomap 2013), and eastern Nevada (Mead and Mead 1989, Neomap 2013).

Deserts were much more limited in the American Southwest during the last glaciation than they are today (Thompson and Anderson 2000). Cool moist conditions in the Great Basin were reinforced by lake-effect weather associated with large pluvial lakes (Hostetler et al. 1994) and boreal mammals, such as the pika (*Ochotona princeps*) and yellow-bellied marmot (*Marmota flaviventris*), were present at low-elevation sites that are currently desert (Grayson 1987, 2006; Schmitt and Lupo 2012). Presumably, the climatic and ecological conditions present in the Great Basin during the late Pleistocene were more favorable for wolverines moving between the Sierra Nevada and Rocky Mountains than they are currently. Climatic conditions in the American Southwest changed rather abruptly in the early Holocene (ca. 10,000 to 8,000 yBP), however, and became particularly warm and dry in the Great Basin (Grayson 2000).

During the Holocene, desert vegetation replaced the woodlands that characterized the Great Basin during the last glacial maximum (Thompson and Anderson 2000, Anderson et al. 2008), whereas the Sierra Nevada became progressively wetter; tree species such as true firs (*Abies* spp.) and mountain hemlock (*Tsuga mertensiana*) became increasingly common in high-elevation pollen deposits after ca. 6,000 yBP (Anderson 1990) and particularly within the last 3,000 years (Davis et al. 1985), a period also characterized by the

growth of mountain glaciers (Bowerman and Clark 2011). Thus, during the Holocene, habitat conditions for the wolverine probably improved in the Sierra Nevada as this area became wetter and snowier. Concurrently, the spread of hot, dry desert conditions throughout the Great Basin would have increased the isolation of wolverine populations in the Sierra Nevada from those in the Rocky Mountains.

Genetic evidence supports the isolation of wolverine populations in the Sierra Nevada from those in the Rocky Mountains and Cascade Range during the Holocene. This is indicated both by the presence of Cali2, which is derived from Cali1 (Fig. 3; Schwartz et al. 2007), and which we did not find elsewhere, as well as the lack of Beringian haplotypes (e.g., A, F, and O) in California. Lastly, nuclear markers (microsatellites) from wolverines in California were divergent from those associated with other North American populations (Schwartz et al. 2007), suggesting that the isolation of California wolverine populations was not limited to matriline but occurred in all segments of the population. Consequently, we believe that the unique haplotypes we found among historical wolverine populations in the western U.S. arose during 2 successive isolation events. The Cali1 haplotype evolved during the isolation of southern refugial populations in the contiguous U.S. during the last glaciation, whereas Cali2 evolved during the subsequent isolation of wolverine populations in the Sierra Nevada from those in the Rocky Mountains and Cascade Range as present-day climatic conditions developed during the Holocene. Zigouris et al. (2012) posit a similar scenario to explain the occurrence of haplotype Z\_25 in Ontario.

### Comparative Phylogeography

Available information on the genetic characteristics of historical wolverine populations in the contiguous U.S. is extremely limited but is unlikely to be augmented by the discovery of additional specimens. Because of this paucity of information, comparing phylogeographic patterns of the wolverine with those of other species may help elucidate their evolutionary history (Arbogast and Kenagy 2001). Patterns of glaciation, in particular the isolation of southern North America from Beringia during the last glacial maximum, strongly influenced the phylogeography of many species (reviewed by Shafer et al. 2010). Here, we focus on 2 well-studied North American terrestrial carnivores whose phylogeography has been linked to glacial vicariance: the montane red foxes (*Vulpes vulpes cascadenis*, *V. v. necator*, and *V. v. macroura*) and brown bears (*Ursus arctos*).

In the western mountains of the contiguous U.S., the red fox occupied historical ranges that were largely concordant with that of the wolverine (e.g., Cary 1911, Bailey 1936, Grinnell et al. 1937, Dalquest 1948). Both species occupied the western portion of the southern refugium during the last glaciation in isolation from populations in the northern refugium (Bryant 1987, Neomap 2013). North American red foxes separate into 2 strongly distinct genetic clades: the Holarctic clade found in Alaska, western Canada, and Eurasia, and the Nearctic clade, which occupies montane regions of the western U.S. and portions of southeastern

Canada (see Fig. 1 in Aubry et al. 2009). Red foxes in the Nearctic clade are descended from populations that were isolated in the southern refugium during the last glacial maximum, whereas northern red fox populations are descended from populations that colonized North America from Asia during the last glaciation (Aubry et al. 2009).

A similar pattern occurs in brown bears, whereby those in Alaska and Eurasia form a common northern clade (clade 3a in Davison et al. 2011) that colonized North America from Asia during the last glacial maximum (Davison et al. 2011). As with the montane red foxes and wolverines, brown bears in southern North America represent a distinct clade (clade 4 in Davison et al. 2011) that, interestingly, also occurs in Japan (see Figs. 1 and 3 in Davison et al. 2011). Thus, both montane red foxes and brown bears exhibit phylogeographic patterns that are similar to those of the wolverine, and in all 3 species these patterns can be directly linked to glacial vicariance during the Pleistocene.

## MANAGEMENT IMPLICATIONS

Based on dental characteristics, early naturalists believed that wolverines in the Sierra Nevada in California represented a distinct subspecies, the southern wolverine (Grinnell et al. 1937). These were wolverines with Cali1 or Cali2 haplotypes that were restricted to the western montane regions of the contiguous U.S. Whether these wolverines warranted recognition as a distinct subspecies is unknown (see Newby and Wright 1955) but if, as we argue, these wolverines evolved in isolation due to glacial vicariance, southern wolverines may have evolved unique adaptations to the high-elevation montane ecosystems they occupied. Data indicate, however, that the wolverines described by Joseph Grinnell and others as being taxonomically distinct were extirpated by the early 1900s (Aubry et al. 2007) and their matriline eliminated from North America.

Most modern wolverine populations in North America are closely related; those in the contiguous U.S., whether of local or Canadian origin, are closely related to populations to the north and are unlikely to contain specialized adaptations to southern climates. Thus, for any proposed reintroduction of wolverines in the western contiguous U.S., translocating genetically diverse individuals is likely to be more important than choosing a particular source population. The large contiguous populations in the north are more diverse than those in the south (Fig. 2, Appendix) and would provide an appropriate genetic stock for reintroductions. However, many of these populations occur in relatively flat terrain in tundra or taiga habitat conditions. In montane regions of southern North America, wolverines occupy more heterogeneous habitats and may have learned behaviors that contribute to survival and reproduction; these characteristics may be particularly important during the period immediately following release. The need to consider both genetic diversity and appropriate behavioral repertoires in future reintroductions suggests that the optimal source location for wolverine translocations to California or Colorado may be the Canadian Rocky Mountains, where genetic diversity is relatively high

(Fig. 2, Appendix), and environmental conditions are similar to those in the western mountains of the contiguous U.S.

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**Appendix 1.** Haplotypes associated with recent (1989–2012) wolverine specimens in Alaska, Canada, and the contiguous U.S. Previously published samples are from Wilson et al. (2000), Tomasik and Cook (2005), Cegelski et al. (2006), and Schwartz et al. (2007). Data from Tomasik and Cook (2005) were extracted from their Figure 1. Haplotypes A–I were identified by Wilson et al. (2000), L–O by Cegelski et al. (2006), T\_C–T\_O by Tomasik and Cook (2005), and Z\_24–Z\_25 by Zigouris et al. (2012).

| Study area                           | Haplotype |   |    |   |   |   |    |   |    |   |   |   |   |     |     |     |     |     |                   |                   |
|--------------------------------------|-----------|---|----|---|---|---|----|---|----|---|---|---|---|-----|-----|-----|-----|-----|-------------------|-------------------|
|                                      | A         | B | C  | D | E | F | G  | H | I  | L | M | N | O | T_C | T_D | T_J | T_N | T_O | Z_24 <sup>a</sup> | Z_25 <sup>a</sup> |
| Wilson et al. (2000)                 |           |   |    |   |   |   |    |   |    |   |   |   |   |     |     |     |     |     |                   |                   |
| Site1 (West), NWT                    | 7         | 3 |    |   | 1 | 1 |    |   |    |   |   |   |   |     |     |     |     |     |                   |                   |
| Site2 (West), NWT                    |           |   |    | 1 |   |   |    |   | 2  |   |   |   |   |     |     |     |     |     |                   |                   |
| Site3 (East), NWT                    |           |   | 8  |   |   | 4 | 4  |   | 4  |   |   |   |   |     |     |     |     |     |                   |                   |
| Site4 (East), NWT                    | 1         |   |    |   |   |   | 2  |   |    |   |   |   |   |     |     |     |     |     |                   |                   |
| Site5 (East), NWT                    |           |   |    |   |   |   | 2  | 1 |    |   |   |   |   |     |     |     |     |     |                   |                   |
| Tomasik and Cook (2005) <sup>b</sup> |           |   |    |   |   |   |    |   |    |   |   |   |   |     |     |     |     |     |                   |                   |
| Northwestern AK                      | 7         |   |    |   | 1 |   |    | 8 |    |   |   |   |   |     | 6   |     |     |     |                   |                   |
| Northern AK                          |           | 5 |    |   | 1 | 3 |    | 1 |    |   |   |   |   |     |     |     |     |     |                   |                   |
| Seward Peninsula, AK                 |           |   |    |   |   | 1 |    |   |    |   |   |   |   |     |     |     |     |     |                   |                   |
| Kenai, AK                            |           |   |    |   |   | 1 |    | 9 |    |   |   |   |   | 6   |     | 6   |     |     |                   |                   |
| Southern AK                          | 5         | 1 |    |   |   |   |    | 2 |    |   |   |   |   | 9   |     |     |     |     |                   |                   |
| Southeastern AK                      | 4         |   |    |   |   |   |    |   |    |   |   |   |   |     | 7   |     | 1   |     |                   |                   |
| Nunavut                              | 1         |   | 4  |   |   | 2 | 5  | 3 | 2  |   |   |   |   |     | 1   |     |     | 1   |                   |                   |
| Cegelski et al. (2006)               |           |   |    |   |   |   |    |   |    |   |   |   |   |     |     |     |     |     |                   |                   |
| Williston Lake, BC                   | 11        |   |    |   |   |   |    |   |    | 6 | 1 | 1 |   |     |     |     |     |     |                   |                   |
| Revelstoke, BC                       | 4         |   |    |   |   |   |    | 5 |    | 4 |   |   |   | 3   |     |     |     |     |                   |                   |
| Grande Cache, AB                     | 5         |   | 2  |   |   | 2 |    |   |    | 8 |   |   |   |     |     |     |     |     |                   |                   |
| MT and WY                            | 74        |   |    |   |   |   |    | 1 | 24 | 2 |   |   |   |     |     |     |     |     |                   |                   |
| Schwartz et al. (2007)               |           |   |    |   |   |   |    |   |    |   |   |   |   |     |     |     |     |     |                   |                   |
| Alaska Range, AK                     | 1         | 2 |    |   |   | 2 |    | 1 |    |   |   |   |   |     |     |     |     |     |                   |                   |
| West Central MT                      | 3         |   |    |   |   |   |    |   |    |   |   |   |   |     |     |     |     |     |                   |                   |
| South Central MT                     | 25        |   |    |   |   |   |    |   |    |   |   |   |   |     |     |     |     |     |                   |                   |
| Northwestern MT                      | 10        |   |    |   |   |   |    |   |    |   |   |   |   |     |     |     |     |     |                   |                   |
| Greater Yellowstone                  | 20        |   |    |   |   |   |    |   | 2  |   |   |   |   |     |     |     |     |     |                   |                   |
| Sawtooth Mts., ID                    | 13        |   |    |   |   |   |    |   |    |   |   |   |   |     |     |     |     |     |                   |                   |
| Zigouris et al. (2012)               |           |   |    |   |   |   |    |   |    |   |   |   |   |     |     |     |     |     |                   |                   |
| NWT                                  | 2         |   | 5  |   |   |   | 17 | 1 |    |   |   |   |   |     | 2   |     |     |     | 1                 |                   |
| Nunavut                              | 2         |   | 8  |   |   | 4 | 11 | 2 | 1  |   |   |   |   |     |     |     |     |     |                   |                   |
| Saskatchewan                         | 3         |   | 1  |   |   |   | 5  | 2 |    |   |   |   |   |     | 4   |     |     |     | 1                 |                   |
| Manitoba                             | 6         |   |    |   |   |   |    |   |    |   |   |   |   |     |     |     |     |     | 9                 | 15                |
| Ontario                              | 35        |   |    |   |   |   |    |   |    |   |   |   |   |     |     |     |     |     | 5                 | 14                |
| Previously unpublished               |           |   |    |   |   |   |    |   |    |   |   |   |   |     |     |     |     |     |                   |                   |
| Washington                           |           |   | 18 |   |   |   |    |   |    |   |   |   |   |     |     |     |     |     |                   |                   |
| Banff/Kananaskis, BC                 | 14        |   |    |   |   |   |    | 2 |    | 8 |   |   |   |     |     |     |     |     |                   |                   |
| Revelstoke/GNP, BC                   |           |   |    |   |   |   |    |   |    | 1 |   |   | 2 |     |     |     |     |     |                   |                   |
| Ontario                              | 4         |   |    |   |   |   |    |   |    |   |   |   |   |     |     |     |     |     | 1                 | 2                 |
| Alaska                               | 11        | 2 |    |   |   | 2 |    | 1 |    |   |   |   |   | 5   | 8   |     | 1   |     |                   |                   |
| Oregon                               | 1         |   |    |   |   |   |    |   |    |   |   |   |   |     |     |     |     |     |                   |                   |
| Montana                              | 60        |   |    |   |   |   |    |   | 5  |   |   |   |   |     |     |     |     |     |                   |                   |
| Idaho                                | 35        |   |    |   |   |   |    |   |    |   |   |   |   |     |     |     |     |     |                   |                   |
| Wyoming                              | 25        |   |    |   |   |   |    |   | 1  |   |   |   |   |     |     |     |     |     |                   |                   |

<sup>a</sup> Haplotypes Z\_24 and Z\_25, reported by Zigouris et al. (2012), are consistent with haplotypes reported by Chappell et al. (2004). However, Chappell et al. (2004) amplified a shorter region of approximately 200 base pairs (bp) compared to the 360-bp region amplified by Zigouris et al. (2012). Because of ambiguities associated with the shorter region in Chappell et al. (2004), results from that study are not reported here.

<sup>b</sup> Tomasik and Cook (2005) included samples from Wilson et al. (2000) in their populations for Nunavut and the Northwest Territories. The numbers presented here are for samples that were unique to Tomasik and Cook (2005).

# Genetic structure of North American wolverine (*Gulo gulo*) populations

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## Abstract

Wolverines (*Gulo gulo*) are found in low densities throughout their circumpolar distribution. They are also potentially susceptible to human-caused population fragmentation (development, recreation and fur harvesting). The combination of these factors has contributed to this species being listed as having either vulnerable or endangered status across much of its current range. The effects of inherently low densities and anthropogenic pressures on the genetic structure and variation of wolverine populations are, as yet, unknown. In this study, 461 individuals were typed at 12 microsatellite loci to investigate the population genetic structure of wolverines from north-western Alaska to eastern Manitoba. Levels of gene flow and population differentiation among the sampled regions were estimated via a genotype assignment test, pairwise  $F_{ST}$ , and two genetic distance measures. Our results suggest that wolverine populations from southernmost regions, in which anthropogenic factors are strongest, revealed more genetic structuring than did northern populations. Furthermore, these results suggest that reductions in this species' range may have led to population fragmentation in the extreme reaches of its southern distribution. The continued reduction of suitable habitat for this species may lead to more populations becoming isolated remnants of a larger distribution of northern wolverines, as documented in other North American carnivore species.

**Keywords:** forest carnivore, *Gulo gulo*, microsatellite, Mustelidae, population structure, wolverine

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## Introduction

Wolverines (*Gulo gulo*) are the largest terrestrial member of the family Mustelidae (the weasel family). This species has a circumpolar distribution and is found in tundra, taiga and forest zones of North America and Eurasia (Wilson 1982). In North America, prior to human settlement, wolverines were distributed across Canada and Alaska with projections southward into the conterminous US through montane regions as far as New Mexico and Arizona (Hash 1987). Currently, wolverines in Canada west of Hudson's Bay are found in the northern regions of the prairie provinces, in Alberta's western national parks, and throughout most of British Columbia and the Canadian Territories (Banci 1994). East of Hudson's Bay, wolverines are exceedingly rare and are listed as endangered by COSEWIC (1998). In the conterminous US

the range of wolverines has steadily retracted since the 1840s, with animals persisting in isolated regions of Montana, Idaho, Wyoming, Colorado, Oregon and California (Hash 1987). The contracting range of wolverines in North America is the result of habitat loss, overharvest and other anthropogenic factors, which together have led to a decline in the numbers of these animals (Wilson 1982; Banci 1994).

Wolverines are found at low density across their Holarctic distribution with estimates of one animal per 40–800 km<sup>2</sup> (see review by Banci 1994). This species also establishes large home-ranges (100–900 km<sup>2</sup>) which seem to be maintained between years (Magoun 1985; Banci 1987) and which vary in size in relation to food abundance (Banci 1994). Males typically maintain larger home-ranges than females and may overlap the home-ranges of several females (Banci 1994).

Wolverines are reproductive from 2 years of age onwards, although the fecundity of females is thought to decrease after the age of 6 (Banci & Harestad 1988). Not all females become pregnant in a given year (53–92% in the Yukon;

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Banci & Harestad 1988) depending on food availability. Even when females do give birth, kit mortality is thought to be high with estimates of  $\approx 36\%$ . These figures are considered to underestimate the true mortality rate (Banci 1994).

Most juvenile females exhibit natal area fidelity and establish home-ranges adjacent to their mothers (Magoun 1985), although some females have been observed to disperse far beyond their natal range. Subadult males typically disperse 30–100 km from their natal home-range (Gardner 1985; Magoun 1985), although several movements further than this have been reported. Gardner *et al.* (1986) reported a 378-km straight-line movement of a 2-year-old from southcentral Alaska to the Yukon Territory in a 7-month period. A yearling female was reported by Magoun (1985) to have dispersed 300 km in a 5-month period. It should be noted, however, that some rare long-distance movements in this species do not seem to be linked to dispersal, but are simply temporary forays from their home-range (Banci 1994). It is believed that half to one-third of all dispersing individuals die, either by starvation, harvest or predation (Krott 1982).

The movement of wolverines does not seem to be influenced by the presence of lakes, rivers, mountain ranges or other topographical features (Hornocker & Hash 1981; Banci 1987). Only human development and major access routes are thought to function as barriers to dispersal for this species. Furthermore, human activity may influence kit survival, thereby limiting the expansion of wolverine populations (Banci 1994).

With increasing concern about the status of this forest carnivore and how it might be managed effectively, it is necessary to determine the genetic distinctiveness of wolverine populations and the levels of gene flow between geographical areas. Population genetic data may also be used to determine whether harvested populations are replaced solely from within or if replacement from other populations plays an important role in maintaining a population's numbers.

Wolverines, where they persist north of the 38th parallel in North America, are currently considered to be a continuous breeding group, however, the extent of movement between the larger Canadian populations and remnant conterminous US populations is unknown (Banci 1994). Recent work by Edelman & Copeland (1999) suggests that movement between Idaho and Oregon populations may be restricted due to a lack of suitable habitat between them. These findings may be reflected in the population genetic structure of wolverine populations in this area. Another recent study by Wilson *et al.* (2000) evaluated the genetic variability of wolverines in the Northwest Territories, Canada using both mitochondrial DNA (mtDNA) and allozyme markers. A high degree of population structure was found using mtDNA ( $\phi_{ST} = 0.536$ ), although much less

structure was observed among the sampled regions using nuclear allozyme markers ( $F_{ST} = 0.076$ ). These results are not surprising given this species' high potential for dispersal, chiefly male-mitigated dispersal, and female fidelity to natal areas. These findings, however, were based on small sample sizes ( $n = 3, 3, 3, 12$  and  $20$  for each of the five studied 'populations', respectively) and limited genetic variation which the authors admit pose considerable constraints on the conclusions which can be drawn from the study.

The first goal of this study was to elucidate the population genetic structure of wolverines across much of their North American distribution, which to date is largely unknown. The second goal was to determine what underlying factors influence the population genetic structure of this species. We hypothesize that the genetic structuring of wolverine populations will be higher in the southern regions of this species' range, where anthropogenic factors potentially act as barriers to dispersal, than those populations found in undisturbed northern habitats.

To detect genetic differentiation among wolverine populations, high resolution (fast evolving) neutral genetic markers are preferred. Previous studies have found little or no genetic variation in wolverines using other markers (haemoglobin, Seal 1969; lactate dehydrogenase, LeDoux & Kenyon 1973; 337 bp of mtDNA sequence and five allozyme markers, Wilson *et al.* 2000). For this reason, we have chosen to use hypervariable microsatellite loci. These polymorphic, tandem repeats of DNA have proven useful in other studies of mammalian species with high vagility, such as the polar bear (*Ursus maritimus*; Paetkau *et al.* 1995, 1999) and the North American brown bear (*Ursus arctos*; Paetkau *et al.* 1998). Microsatellites identified clear genetic differentiation between populations of these animals despite the long-range movements known to occur in these species, and the fact that little variation was detected using other methods.

## Materials and methods

### Sample collection

Twelve polymorphic loci were used to examine the population genetic structure of wolverines from 12 geographical regions. Bone samples were acquired from six Alaskan regions (all samples were provided by the University of Alaska, Fairbanks Museum and are sorted according to their quadrat system): Anchorage ( $n = 36$ ); Arctic region, including samples from Arctic, Fort Yukon and Black River ( $n = 47$ ); Nabesna ( $n = 38$ ); Russian Mission region, including samples from Russian Mission and Holy Cross ( $n = 34$ ); Noatak region, including samples from Noatak, Point Hope, Point Lay, Meade River, Survey Pass and Howard Pass ( $n = 38$ ); and the Nome region, including samples from Solomon and Nome ( $n = 38$ ). All samples

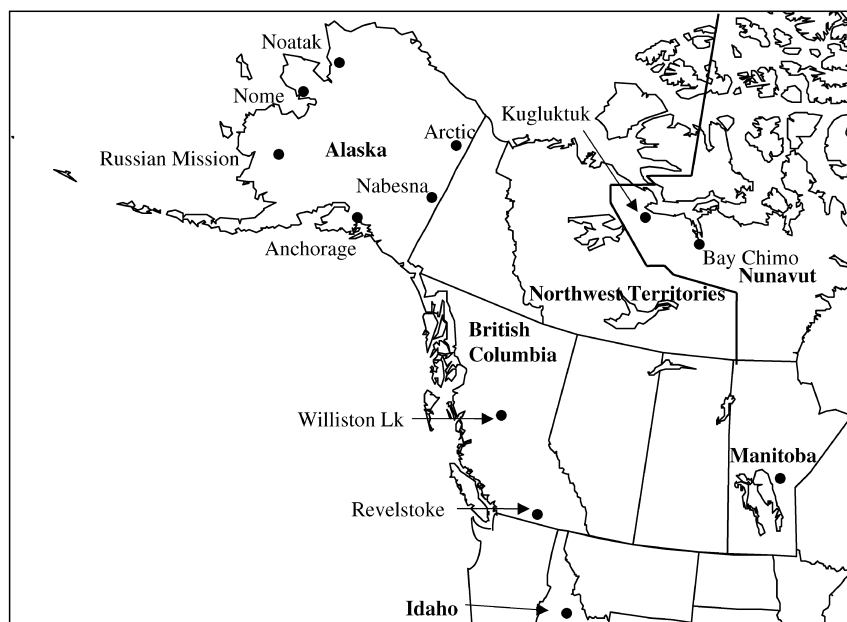


Fig. 1 Map of sampled regions.

from Alaska date from the late 1950s through to the mid-1960s. Tissue samples were obtained from two Nunavut territory regions (then part of the Northwest Territories): Kugluktuk ( $n = 67$ ) and Bay Chimo ( $n = 40$ ). Ear plug and hair samples were obtained from the Revelstoke ( $n = 47$ ) and Williston Lake ( $n = 37$ ) regions in British Columbia, eastern Manitoba ( $n = 28$ ) and central Idaho ( $n = 14$ ) (Fig. 1). All samples, other than those collected from Alaska, are of recent origin (early 1990s).

#### Laboratory methods

DNA was extracted using a QIAamp® Tissue Extraction Kit (QIAGEN). The primers used to amplify the microsatellites were developed by: Davis & Strobeck (1998) in badgers (BA-1 and BA-4), martens (MA-3) and wolverines (GG-3, GG-4, GG-7, GG-14); by Duffy *et al.* (1998) in wolverines (Ggu 101, Ggu 216 and Ggu 234); by Flemming *et al.* (1999) in mink (Mvis-75); and by Dallas & Pierny (1998) in Eurasian otters (L-604). Amplification of DNA was performed as in Davis & Strobeck (1998). The DNA fragments were visualized using an ABI Prism™ 377 DNA sequencer. Analysis of DNA fragments was done using the programs GENESCAN™ ANALYSIS 2.02 and GENOTYPER® 2.0.

#### Data analysis

A G-test for heterogeneity (Sokal & Rohlf 1995) was performed for each of the sampled areas by making pairwise comparisons of allele distributions. Departures from Hardy–Weinberg equilibrium (HWE) were tested for each of the 12 loci as assessed by GENEPOP version 3.1d

(Raymond & Rousset 1995) which uses a Markov chain method following the algorithm of Guo & Thompson (1992). This software was also used to evaluate genotypic disequilibrium among the loci used.

The relative genetic variation in each population was first assessed using allele frequency data from which the mean number of alleles, unbiased expected heterozygosity,  $H_E$  (formula as per Nei & Roychoudhury 1974), and unbiased overall probability of identity,  $P_{ID}$  (Paetkau *et al.* 1998) were determined. The genetic distances between the populations were estimated using two measurements: Nei's standard genetic distance,  $D_S$  (Nei 1972) which is calculated from genotype frequencies, and the genotype likelihood ratio distance,  $D_{LR}$  (Paetkau *et al.* 1997) which is calculated from genotype probabilities. These measures were both identified by Paetkau *et al.* (1997) to perform better than other measures of genetic distance that rely on the stepwise mutation model. Both sets of genetic distance values were calculated by programs from the website, <http://www.biology.ualberta.ca/jbrzusto/Doh/html>, designed by John Brzustowski.

An unrooted neighbour-joining tree of the  $D_S$  values was created using PHYLIP 3.572 (Felsenstein 1995). The geographical and genetic distance values were also entered into a two-way Mantel test (Mantel 1967) within the set of programs called the 'R' Package for multivariate analysis designed by Alain Vaudor (software package can be found on Pierre Legendre's website: <http://www.fas.umontreal.ca/BIOL/legendre/>) to determine the correlation between genetic distance and geographical proximity. This software package was also used to run a three-way Mantel test (as per Smouse *et al.* 1986). We used this method to determine

whether the curves on the scatter plot of genetic distance against geographical distance had significantly different intercepts. Populations which had significantly different curves from the expectation of isolation by distance were taken to have some barrier to gene flow other than geographical distance present. Each population was tested by creating a matrix of ones and zeros for the presence or absence of a barrier, while controlling for the geographical distance. All geographical distances were calculated using the DISTGEO program, also found within the 'R' package, from approximate latitudes and longitudes for each region.

Pairwise  $F_{ST}$  estimates were obtained from the software package GENEPOP 3.1d (Raymond & Rousset 1995; as per Weir & Cockerham 1984).

The assignment test (Paetkau *et al.* 1995), also found on the aforementioned web site, was run for all populations. This program determines both the probability of a genotype occurring in the region from which it was sampled and the probability of it occurring in the regions with which it is being compared. It then assigns each individual to the population in which that individual's genotype has the highest probability of occurring (see Waser & Strobeck 1998). Unlike other pairwise population statistics, the assignment test uses the information from each individual's genotype to determine how similar are the gene pools of the two populations. The test was always run with the option of replacing allele frequencies of 0 with 0.01.

The significance of the assignment test results was obtained using two types of randomization tests. In the first randomization test, for each replicate, the same number of individual genotypes as found in a population is drawn using the allele frequencies at each locus in the combined gene pool (all populations) assuming HWE. This randomization notes the proportion of replicates in which the number of individuals in population A assigned to population B is less than or equal to the observed number of individuals assigned from population A to population B. When the proportion is  $< 0.05$ , there is evidence that significant heterogeneity exists between the genotype frequencies in populations A and B.

In the second randomization test, for each replicate, the same number of individual genotypes as found in a population is drawn using the allele frequencies at each locus found in that population, assuming HWE. This randomization notes the proportion of replicates in which the number of individuals in population A assigned to population B is less than or equal to the observed number of individuals in population A assigned to population B. When this proportion is  $> 0.95$  then there is a significant number of cross-assignments from A to B. This implies that some of the individuals in A must be in genotypic disequilibrium with population A and are therefore presumably immigrants into A from a population with allele frequencies different

for those found in A. Ten thousand replicates were used to obtain the significance values for both randomization tests.

## Results

### *Tests of disequilibrium*

All 12 loci used in this study were tested for departures from HWE. After accounting for sample-wise error (as per the Dunn-Sidak method; Sokal & Rohlf 1995), four departures from HWE were found: locus GG-3 in the Williston Lake British Columbia (BC) region, locus BA-4 in the Manitoba region, and locus Ggu 234 in the Anchorage and Nabesna regions. All departures from HWE were found to be heterozygote deficits which may imply the presence of null alleles in these populations. Low copy number may also be responsible for the heterozygote deficit in the two Alaskan populations as the DNA was extracted from bone samples. As deviations from HWE were not found in other populations for these loci, they were retained for analyses. Genotypic disequilibrium was suggested for the following pairs of loci: GG-7 with GG-14 in the Manitoba population, GG-3 with BA-4 in the Bay Chimo region, and GG-4 with GG-14 in the Revelstoke region. As these genotypic disequilibria were not found in more than one population it is unlikely that any loci are physically linked. Hence, all loci were retained for analyses. It is possible that these disequilibria are the result of linkage disequilibrium in the founding population that has not yet dissipated or that a recent admixture of populations with differing gametic frequencies has occurred (Hartl & Clark 1989).

### *Heterogeneity of sampled regions*

Twelve geographical areas were sampled in this study (Fig. 1). All sampled regions were found to have significantly ( $P < 0.05$ ) different allele frequencies by a G-test for heterogeneity. The comparison of the allele frequencies in the Russian Mission and Arctic regions and the comparison of the Russian Mission and Nabesna regions were both significant at the 5% level. These differences, however, were found not to be significant when the experiment-wise error rate was applied (as per the Dunn-Sidak method; Sokal & Rohlf 1995). Despite these findings we treated these three regions as populations because of their geographical separation from one another ( $\approx 1000$  km).

### *Genetic variation*

The population with the highest mean number of alleles was in Arctic, Alaska (AK) with a value of 5.83, whereas Idaho had the lowest value of 2.83 (Table 1).  $H_E$  values ranged from 68% in Nabesna, AK to 42% in Idaho (Table 1).

|                     | Abbreviation | <i>n</i> | A    | <i>H<sub>E</sub></i> (%) | <i>P<sub>ID</sub></i> |
|---------------------|--------------|----------|------|--------------------------|-----------------------|
| Anchorage, AK       | Anch         | 36       | 5.17 | 65.45                    | 6 450 000 000         |
| Arctic, AK          | Arct         | 47       | 5.83 | 66.90                    | 10 190 000 000        |
| Nabesna, AK         | Nabe         | 38       | 5.25 | 68.48                    | 36 100 000 000        |
| Russian Mission, AK | Russ         | 34       | 5.00 | 63.53                    | 1 647 000 000         |
| Noatak, AK          | Noat         | 38       | 5.00 | 65.31                    | 5 090 000 000         |
| Nome, AK            | Nome         | 35       | 4.67 | 62.31                    | 1 004 000 000         |
| Bay Chimo, NU       | BayC         | 40       | 4.83 | 63.61                    | 1 278 000 000         |
| Kugluktuk, NU       | Kugl         | 67       | 5.00 | 64.68                    | 2 540 000 000         |
| Revelstoke, BC      | Reve         | 47       | 5.17 | 60.98                    | 377 000 000           |
| Williston Lake, BC  | Will         | 37       | 4.92 | 61.18                    | 1 231 000 000         |
| Idaho               | Idah         | 14       | 2.83 | 42.09                    | 452 000               |
| Manitoba            | Mani         | 28       | 4.50 | 66.99                    | 1 865 000 000         |

**Table 1** Genetic variation estimates from sampled regions including the mean number of alleles (A), the mean unbiased expected heterozygosity (*H<sub>E</sub>*), and the unbiased probability of identity (*P<sub>ID</sub>*)

**Table 2** Population assignments from the genotype assignment test

|      | <i>N</i> | Anch | Arcti | Nabe | Russ | Noat | Nom | Bay  | Kugl | Reve | Willi | Idah | Mani |
|------|----------|------|-------|------|------|------|-----|------|------|------|-------|------|------|
| Anch | 36       | 8    | 7**   | 1    | 7    | 2    | 2   | 0    | 5*   | 1    | 1     | 0    | 1    |
| Arct | 47       | 4    | 10    | 4    | 4    | 9*   | 3   | 6*   | 1    | 1    | 4     | 0    | 1    |
| Nabe | 38       | 5    | 4     | 11   | 2    | 9**  | 2   | 1    | 3    | 0    | 1     | 0    | 0    |
| Russ | 34       | 3    | 7**   | 3    | 6    | 4    | 6** | 2    | 2    | 1    | 0     | 0    | 0    |
| Noat | 38       | 2    | 5     | 3    | 7*   | 13   | 2   | 1    | 2    | 1    | 2     | 0    | 0    |
| Nome | 35       | 2    | 3     | 2    | 2    | 1    | 21  | 2    | 2    | 0    | 0     | 0    | 0    |
| BayC | 40       | 0    | 2     | 1    | 7**  | 2    | 0   | 19   | 7    | 0    | 0     | 0    | 2    |
| Kugl | 67       | 2    | 1     | 2    | 3    | 3    | 3   | 20** | 23   | 0    | 5     | 0    | 5*   |
| Reve | 47       | 1    | 2*    | 0    | 1    | 0    | 0   | 2    | 1    | 35   | 4     | 0    | 1    |
| Will | 37       | 3    | 0     | 2    | 3    | 3    | 3   | 1    | 1    | 5    | 15    | 1*   | 0    |
| Idah | 14       | 0    | 0     | 0    | 0    | 0    | 0   | 0    | 0    | 0    | 0     | 14   | 0    |
| Mani | 28       | 0    | 0     | 0    | 0    | 1    | 1   | 0    | 3*   | 0    | 0     | 0    | 23   |

\*Significant at the 5% level for randomizations within each gene pool. \*\*Significant at the 1% level for randomizations within each gene pool.

*P<sub>ID</sub>* values ranged from 1/36 000 000 000 in Nabesna, AK to 1/452 000 in Idaho (Table 1). The Revelstoke population also had a low *P<sub>ID</sub>* value (1/377 000 000) relative to the other populations. The low estimates of the mean number of alleles in Idaho may be explained, in part, by the low sample size (*n* = 14) relative to the other populations (*n* > 30), although measures of heterozygosity are less affected by sample size.

#### Assignment test

The results from the genotype assignment test revealed little genotypic distinctiveness between the Alaskan regions sampled, as evidenced by the relatively low number of correctly assigned individuals (1/4 to 1/6). The one exception was Nome, in which 21/35 individuals were assigned to the region from which they were sampled (Table 2). Most cross-assignments in Alaskan regions went to other Alaskan regions. A relatively high number of cross-assignments

also went to the Nunavut regions in which 5/36 Anchorage individuals were assigned to Kugluktuk and 6/47 Arctic individuals were assigned to Bay Chimo.

Slightly more structure was observed in the Nunavut populations, with 19/40 and 23/67 individuals assigned correctly to Bay Chimo and Kugluktuk, respectively. The majority of the cross-assignments in Kugluktuk went to Bay Chimo (20/67). In Bay Chimo, 7/40 individuals were cross-assigned to both Kugluktuk and Russian Mission, AK.

Of the two British Columbian populations, Revelstoke demonstrated more population genetic structure than did Williston Lake. In Revelstoke, 35/47 individuals were assigned correctly, whereas the Williston Lake population had only 15/37 individuals assigned correctly to itself. In both British Columbian populations the cross-assignments were most likely to go to the other British Columbian region with 5/37 Williston Lake individuals assigned to Revelstoke and 4/47 individuals from Revelstoke assigned to Williston Lake.

**Table 3** Upper diagonal represents pairwise  $F_{ST}$  values (Weir & Cockerham 1984); lower diagonal as calculated by the GENEPOP 3.1d software package. Lower diagonal is the approximate geographical distances (km) between populations as calculated by the program DISTGEO using approximate latitudes and longitudes of from the centre of each sampled region

|       | Anch | Arct  | Nabe  | Rus   | Noat  | Nom   | Bay   | Kug   | Reve  | Will  | Idah  | Mani  |
|-------|------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| Anch  |      | .0088 | .0102 | .0017 | .0092 | .0178 | .0346 | .0201 | .0720 | .0344 | .2087 | .0897 |
| Arcti | 730  |       | .0117 | .0066 | .0044 | .0251 | .0255 | .0182 | .0611 | .0301 | .1961 | .0820 |
| Nabe  | 390  | 560   |       | .0134 | .0093 | .0232 | .0432 | .0290 | .0680 | .0301 | .1787 | .0816 |
| RusM  | 590  | 980   | 940   |       | .0088 | .0165 | .0267 | .0135 | .0626 | .0278 | .2157 | .0915 |
| Noat  | 950  | 865   | 1140  | 575   |       | .0247 | .0293 | .0166 | .0651 | .0213 | .1948 | .0943 |
| Nome  | 875  | 970   | 1135  | 390   | 230   |       | .0408 | .0242 | .0692 | .0412 | .2079 | .1016 |
| BayC  | 2235 | 1665  | 1870  | 2640  | 2470  | 2615  |       | .0070 | .0597 | .0469 | .2067 | .0915 |
| Kugl  | 1760 | 1205  | 1395  | 2170  | 2035  | 2170  | 480   |       | .0534 | .0272 | .1916 | .0963 |
| Revel | 2245 | 2285  | 1940  | 2840  | 3070  | 3075  | 1920  | 1780  |       | .0359 | .1686 | .1205 |
| Will  | 1605 | 1670  | 1295  | 2200  | 2430  | 2430  | 1700  | 1415  | 645   |       | .1670 | .1109 |
| Idaho | 2885 | 2980  | 2610  | 3475  | 3745  | 3735  | 2515  | 2445  | 700   | 1320  |       | .1838 |
| Manit | 3210 | 2860  | 2820  | 3735  | 3720  | 3820  | 1435  | 1730  | 1595  | 1900  | 1800  |       |

Both Idaho and Manitoba were found to be highly structured populations with 14/14 individuals correctly assigned to Idaho and 23/28 individuals correctly assigned to Manitoba.

#### Pairwise $F_{ST}$ , and genetic distance measures

Pairwise  $F_{ST}$  values ranged from 0.0017 to 0.2157 (Table 3). The smallest  $F_{ST}$  values were found within Alaska (0.0017–0.0251) and Nunavut (0.0070). There was also little structure between the Alaskan and Nunavut regions (pairwise  $F_{ST}$  ranged from 0.0135 to 0.0432). The highest pairwise  $F_{ST}$  estimates were found for the Revelstoke (0.0359–0.0720), Manitoba (0.0816–0.1205) and Idaho (0.1670–0.2157) populations.

Estimates of the likelihood ratio ( $D_{LR}$ ) and Nei's standard ( $D_S$ ) genetic distances paralleled the results from both the assignment test and pairwise  $F_{ST}$  estimates.  $D_{LR}$  values ranged from 0.24 to 8.87 and  $D_S$  values ranged from 0.03 to 0.36. The Alaskan populations were all found to be genetically similar using these measures with values of  $D_{LR}$  ranging from 0.24 to 0.68 and  $D_S$  values ranging from 0.03 to 0.05 with the exception of Nome. Nome was more genetically distinct from other Alaskan populations, with larger genetic distances relative to between the other regions. The two Nunavut populations were also genetically similar ( $D_{LR} = 0.25$ ;  $D_S = 0.03$ ). Pairwise comparisons of the Alaskan populations to the Nunavut populations yielded  $D_{LR}$  values ranging from 0.65 to 1.39 and  $D_S$  values ranging from 0.05 to 0.10, again suggesting little distinction between these regions. Similar to the findings of the assignment test and pairwise  $F_{ST}$  values, these genetic distance measures identified Idaho, Revelstoke and Manitoba as the most genetically distinct regions relative to all other regions sampled.

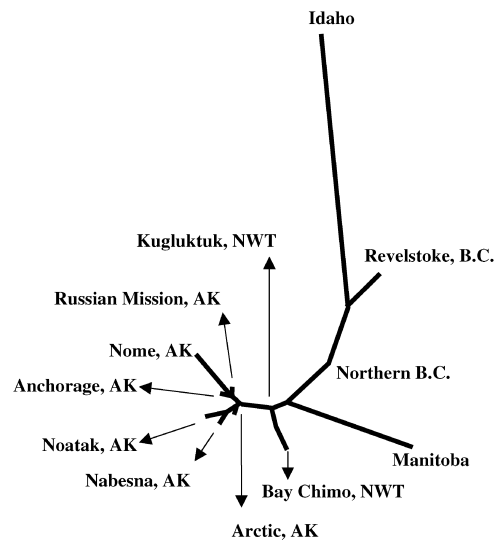


**Fig. 2** Unrooted neighbour-joining trees of the  $D_S$  genetic distance values (Nei's standard). The length of the tree branches is relative to the genetic distances.

An unrooted neighbour-joining tree of the  $D_S$  values is illustrated in Fig. 2 which reveals the relationships of genetic distances to all populations. The length of the tree branches is relative to the genetic distances. The tree shows that the Alaskan populations cluster, the Nunavut populations cluster, the British Columbia populations do not cluster, but were closely associated, and the Manitoba and Idaho populations are each on their own branches.

The results from both genetic distance measures were entered into a two-way Mantel test with geographical distance. The results from these two tests suggest that both genetic distance measures are correlated to geographical

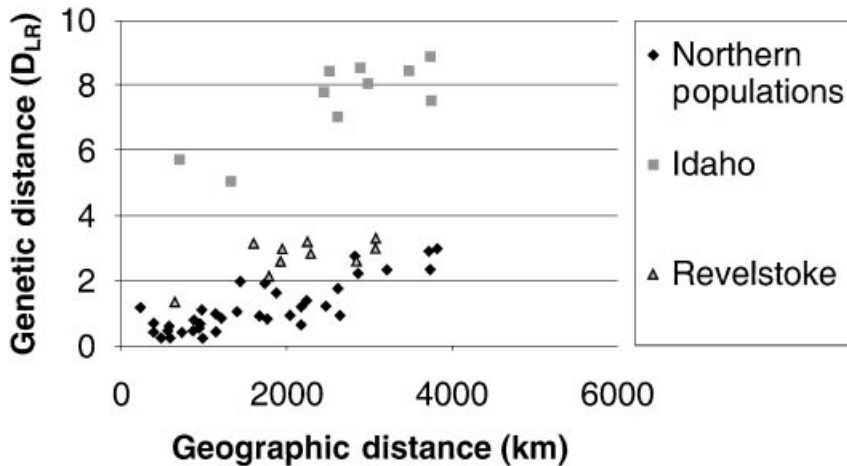


Fig. 3 Relationship of likelihood ratio genetic distance,  $D_{LR}$ , to geographical distance.

distance ( $D_{LR}$  vs. geographical distance  $r = 0.63$   $P = 0.00004$ ;  $D_S$  vs. geographical distance  $r = 0.63$   $P = 0.00003$ ).  $D_{LR}$  and  $D_S$  were also found to be highly correlated to one another ( $r = 0.99$   $P = 0.00009$ ). The  $D_{LR}$  values were also entered into a three-way Mantel test to test for the presence or absence of a barrier to gene flow while controlling for geographical distance. The results revealed that the Idaho population is highly correlated to a barrier to gene flow other than distance ( $r = 0.90$   $P = 0.0002$ ). The Revelstoke population was not as highly associated with a barrier to gene flow other than distance, yet the result was still significant ( $r = 0.46$   $P = 0.01$ ) (Fig. 3).

## Discussion

This study has elucidated the population genetic structure of wolverine populations across a large part of their North American distribution. Our results suggest that there is little genetic structuring of populations in the northern regions of this species' range, as reflected by an assignment test, pairwise  $F_{ST}$  estimates, and two genetic distance measures,  $D_S$  and  $D_{LR}$ . Furthermore, we found the more southerly populations, in which anthropogenic factors are most pronounced, to be more genetically structured than those found in the north. These results reflect similar findings to another recent study of wolverines using allozymes (Wilson *et al.* 2000) which revealed little genetic structure between regions in the Northwest Territories using nuclear markers ( $F_{ST} = 0.076$ ). A study by Paetkau *et al.* (1998) of North American brown bears (*Ursus arctos*) also reflects findings similar to those presented here. This is significant as both brown bears and wolverines have a similar distribution, density and potential for dispersal. In both these species, populations in the southern reaches of their range are more genetically distinct, and have less within-population variation, than those found in less

disturbed northern habitats. Our results are also consistent with those found for another mustelid species, the North American pine marten (*Martes americana*). Marten populations sampled from the Yukon and Northwest Territories revealed little population genetic structure and much gene flow was evident across the entire northern sampling range (Kyle *et al.*, 2000). We suggest that the level of genetic structuring in the southern reaches of this species' distribution is the result of low effective population sizes, restricted gene flow and potentially population fragmentation, whereas the low level of structure in the northern regions is consistent with high levels of gene flow and few barriers to dispersal.

## Sampling in time and space

It is important to mention, as noted in Materials and methods, that all Alaskan samples collected in the late 1950s to mid-1960s were treated as a contemporary to samples collected in the 1990s. We made the assumption that 30–40 years difference ( $\approx 6$ –8 generations) between the collection times of the samples has had little effect on the genotype frequencies in these regions. There is little evidence to suggest that large demographic changes have taken place during the time separating the collection of these samples. We are unaware of any drastic population bottlenecks or large range expansions that may have greatly affected the genotype frequencies in these Alaskan regions during this time. We have also presented results suggesting that extensive gene flow exists among northern regions. With an extensive mixing of populations genotype frequencies would be less likely to change significantly over a period of six to eight generations. Despite our assumptions, we have no direct evidence that genotype frequencies have not changed in this time, therefore our findings may not reflect current levels of genetic variation and gene flow in these regions.

**Table 4** Genetic distances: likelihood ratio genetic distance ( $D_{LR}$ , Paetkau *et al.* 1997) in the lower diagonal and Nei's standard genetic distance ( $D_S$ , Nei 1972) in the upper diagonal

|      | Anch | Arcti | Nabe | Russ | Noat | Nom  | Bay  | Kugl | Reve | Willi | Idah | Mani |
|------|------|-------|------|------|------|------|------|------|------|-------|------|------|
| Anch |      | 0.04  | 0.05 | 0.03 | 0.04 | 0.06 | 0.09 | 0.06 | 0.16 | 0.08  | 0.36 | 0.15 |
| Arct | 0.42 |       | 0.04 | 0.04 | 0.03 | 0.07 | 0.07 | 0.05 | 0.14 | 0.08  | 0.32 | 0.12 |
| Nabe | 0.43 | 0.47  |      | 0.05 | 0.04 | 0.06 | 0.10 | 0.07 | 0.14 | 0.06  | 0.27 | 0.14 |
| Russ | 0.25 | 0.24  | 0.55 |      | 0.04 | 0.06 | 0.07 | 0.05 | 0.14 | 0.07  | 0.35 | 0.13 |
| Noat | 0.68 | 0.47  | 0.44 | 0.61 |      | 0.07 | 0.08 | 0.05 | 0.14 | 0.06  | 0.30 | 0.15 |
| Nome | 0.8  | 1.11  | 0.99 | 0.7  | 1.18 |      | 0.10 | 0.07 | 0.15 | 0.09  | 0.33 | 0.16 |
| BayC | 1.39 | 0.92  | 1.63 | 0.93 | 1.22 | 1.76 |      | 0.03 | 0.13 | 0.11  | 0.33 | 0.12 |
| Kugl | 0.83 | 0.85  | 1.05 | 0.65 | 0.94 | 1.2  | 0.25 |      | 0.12 | 0.07  | 0.31 | 0.13 |
| Reve | 3.2  | 2.83  | 2.98 | 2.6  | 2.99 | 3.3  | 2.59 | 2.14 |      | 0.08  | 0.21 | 0.18 |
| Will | 1.12 | 1.22  | 1.08 | 1.09 | 1.14 | 1.71 | 1.59 | 1.02 | 1.35 |       | 0.20 | 0.15 |
| Idah | 8.53 | 8.04  | 7.01 | 8.44 | 7.51 | 8.87 | 8.42 | 7.78 | 5.71 | 5.05  |      | 0.35 |
| Mani | 2.33 | 2.22  | 2.75 | 2.34 | 2.9  | 2.98 | 1.98 | 1.92 | 3.15 | 2.59  | 8.14 |      |

### Lack of genetic structure in northern regions

Little structure was observed among the northern regions sampled in Alaska and Nunavut. Measures of  $F_{ST}$ ,  $D_S$  and  $D_{LR}$  among the Alaskan regions revealed the smallest pairwise differences, supporting the conclusions from the assignment test that little structure exists between these regions despite large geographical distances separating them. To put these values into some perspective, although they are not directly comparable as different microsatellite markers were used, the smallest  $D_S$  value reported in brown bears by Paetkau *et al.* (1998) was between two adjacent Northwest Territory populations with a value of 0.053. The range of  $D_S$  genetic distances among wolverine populations in Alaska and Nunavut vary between 0.03 and 0.07 (Table 4).

The one Alaskan exception, which did reveal a slightly higher level of genetic structure relative to the other Alaskan regions, was Nome. This population had almost 2/3 of the animals sampled assigned to itself compared to only 1/6 to 1/4 of the individuals being correctly assigned in the other Alaskan regions (Table 2). It is unclear why this region would be more structured than other Alaskan regions, although the fact that Nome is found on a peninsular projection of Alaska may have somehow isolated this region.

The two Nunavut regions, Bay Chimo and Kugluktuk, are separated from each other by 400–500 km and reveal a similar pattern to that found in Alaska. There seems to be little genetic structuring between these northern regions, as reflected by the relatively high number of cross-assignments shared between these two regions and the estimates of  $F_{ST}$ ,  $D_{LR}$  and  $D_S$  (Tables 3 and 4).

There also seems to be little genetic structure between the Alaska and Nunavut populations. Of the two populations sampled in Nunavut many of the cross-assigned individuals went to Alaskan populations (Table 2). The

genetic distance measures (Table 3) between Nunavut and Alaska are also relatively low, with Kugluktuk having slightly smaller pairwise estimates of genetic distance to the Alaskan populations than Bay Chimo, as follows from their geographical proximity to these regions (Fig. 1).

The lack of genetic structuring in the northern reaches of this species' range is most likely the result of extensive gene flow among these regions. This conclusion is supported by several wolverine life history characteristics: topographical features such as rivers, lakes and mountain ranges do not limit dispersal (Hornocker & Hash 1981; Banci 1987), subadult males are known to disperse large distances from their natal range to establish their own home ranges (Magoun 1985; Copeland, personal communication), and wolverines are capable of moving long distances with relative ease as documented by Gardner (1985), Gardner *et al.* (1986), and Magoun (1985). The combination of these life history characteristics has most likely had the effect of genetically homogenizing wolverine populations in undisturbed habitats in which few barriers to dispersal exist. Only a few migrants per generation are capable of genetically homogenizing populations over time (Slatkin 1985).

An alternative to the aforementioned hypothesis is that the lack of structure observed in the northern reaches of this species' range may not represent extensive gene flow, but rather a relatively recent postglacial colonization of the north. However, wolverines were thought to exist in several refugia during the Wisconsin glaciation, both south of the ice sheets and in Beringia as revealed by fossil evidence (Bryant 1987). The current pattern of genetic structure therefore has most likely not been influenced by a rapid radiation of wolverines northward from a southern glacial refugium.

Without samples from the Yukon or Northwest Territories it is unclear whether the lack of genetic differentiation observed between northern regions in this study is true of

all northern regions. Given that no large barriers to dispersal are present in these areas, and the life history traits of wolverines, we would expect a similar pattern of genetic structure to persist across all of these northern regions east of Hudson's Bay.

Despite the lack of genetic differentiation in the northern reaches of this species distribution we cannot say that all the animals are similar in adaptive traits. Adaptive traits can occur between populations showing little genetic structure at neutral genetic loci (Karhu *et al.* 1996).

#### *Genetic structuring of western Canadian populations*

Only two regions were sampled from western Canada, both from British Columbia. These regions, however, revealed very different levels of genetic structure. The Williston Lake population had a relatively high level of cross-assignments to all regions (only 15/37 individuals correctly assigned) and pairwise measures of  $F_{ST}$ ,  $D_S$  and  $D_{LR}$  were comparable with those found for the Bay Chimo region in Nunavut (Tables 3 and 4). This central British Columbia region also seemed to be more closely associated with populations sampled in Nunavut and Alaska than the southern British Columbia population of Revelstoke with respect to these estimates (Tables 3 and 4).

The Revelstoke population was found to be relatively distinct from all other regions sampled, with 35/47 individuals correctly assigned to itself (Table 2). A plot of geographical vs. genetic distance (Fig. 3) also revealed that the  $D_{LR}$  measures in Revelstoke were higher than expected from the values in the northern regions. This difference was found to be significantly correlated to some barrier to gene flow by a three-way Mantel test ( $r = 0.46$   $P = 0.01$ ).

The difference in genetic structuring between these two regions may potentially be attributed to differences in anthropogenic pressures. Both regions are intensely forested, yet the Revelstoke region also has major transportation corridors present (Canadian Pacific Railway and the Trans Canada Highway) which are known to account for a high percentage of the mortality of wolverines in this area (J. Krebs, personal communication). The Revelstoke region also has more human inhabitation around it than the Williston Lake region. These and other anthropogenic factors may contribute to decreased gene flow to and from the more southerly population. Our results may also reflect that the Revelstoke population simply has a smaller effective population size than more northern populations.

#### *Potential isolation of the Idaho population*

The decreased level of  $H_E$  and the increased levels of genetic structure based on the assignment test,  $F_{ST}$ ,  $D_S$  and  $D_{LR}$  in Idaho are consistent with other studies which have identified insular populations. In a study of black bears

(*Ursus americanus*) Paetkau & Strobeck (1994) found  $H_E$  to be  $\approx 80\%$  in all regions with one exception. Bears found in Terra Nova National Park, which are isolated on the island of Newfoundland, were found to have an  $H_E$  of 49%. A study of brown bears by Paetkau *et al.* (1998) found  $H_E$  to range from  $\approx 60$  to 80% with the exception of two insular populations, Kodiak Island, AK and Yellowstone National Park which had  $H_E$  values of 26.5 and 55.4%, respectively. The decreased genetic variability of the Yellowstone brown bears was attributed to population fragmentation in the early 1900s from the larger continuous population in the north. Here, the lack of genetic variation in the Idaho population, relative to the homogenous levels of variation in most other regions sampled suggests that this population may have become fragmented from the larger distribution of wolverines in the north (Table 1). An alternative to this suggestion would be that a historical bottleneck is responsible for the lack of genetic variation found in this population. Although wolverine numbers are thought to have been declining in the southern reaches of their distribution since the 1840s (Hash 1987), there is no evidence that a historical population bottleneck has caused this decrease in genetic variation.

The results from the assignment test (Table 2) also support our suggestion that the Idaho population has restricted gene flow and may be fragmented from the other sampled regions. The Idaho population is only 700 km from the Revelstoke population, yet it reveals no cross-assignments to Revelstoke, or any other regions. This would suggest that there is a potential barrier to gene flow between these two areas other than isolation by distance.

The genetic measures of  $F_{ST}$ ,  $D_S$  and  $D_{LR}$  (Tables 3 and 4) all revealed that the Idaho population was the most genetically distinct region sampled. The genetic distance estimates from Idaho to all other regions are not proportional to the geographical proximity of this population to others (Fig. 3). When values of  $D_{LR}$  were entered into a three-way Mantel test, controlling for distance, it was found that these values were strongly and significantly associated with a barrier to gene flow ( $r = 0.932$   $P = 0.0012$ ).

In the conterminous US wolverines are only thought to persist in isolated regions of Montana, Idaho, Wyoming, Colorado, Oregon and California from a once continuous population ranging as far south as Arizona and New Mexico (Hash 1987), supporting the suggestion that this population may be fragmented. The Idaho population also presents a similar example to that of insular Yellowstone brown bears (Paetkau *et al.* 1998). A recent study by Edelman & Copeland (1999) also suggests that the central Idaho population may be isolated from other north-west US wolverine populations. As wolverine dispersal is not thought to be limited by topographical features (Banci 1994), the fragmentation of southern populations may be attributed to habitat loss, overharvest and other anthropogenic factors as suggested by Banci (1994) and Wilson (1982).

In the American north-west, more populations will be needed to determine if these regions all represent genetically insular groups or if gene flow does occur among them and the more northern populations. It is not clear whether other remaining north-western US populations are presently self-sustaining populations or if they are dependent on emigration from Canadian populations (Montana, Newby & McDougal 1964; Idaho, Groves 1988; Oregon, Johnson 1977). It would appear that the surveyed Idaho population is not maintained by Canadian immigrants, as they are genetically distinct from all other sampled populations. It should be noted, however, that the most likely dispersal corridors for wolverines in the American north-west run north/south in the mountain ranges, as most valleys are developed with transportation corridors and human habitation, potentially restricting wolverine movements. The north/south corridor from Idaho to Canada would run into Glacier National Park in southern Alberta. Samples were not available from this region and so we were unable to determine whether animals from central Idaho are genetically connected to them.

The conclusions from the analyses of the Idaho populations must be tempered, however, due to the fact that only 14 individuals were sampled. A small sample size may potentially skew results by providing genotype frequencies that do not reflect those actually present in the population and making the population appear more distinct than it is. A further bias may also have been introduced into our results by including two known relationships in this sample. A potential father was also identified for these relationships. Despite the inclusion of these relationships we still believe that the Idaho population is genetically depauperate as all other potential fathers for the identified mother/offspring relationships matched at 13–15 of 16 loci (data not shown), reflecting the level of genetic relatedness in this region. Furthermore, running the data with and without the known relationships had little effect on our presented results (data not shown).

### Eastern populations

In the east only one population from Manitoba was sampled. This population was found to be genetically distinct from all other regions in this study. The two-way Mantel tests suggests that the genetic distances observed for this population are consistent with isolation by distance. However, with no immediately adjacent populations sampled in the east we are unable to say that Manitoba is not genetically isolated from other wolverine populations.

### Conclusions

Although this study elucidated the levels of genetic structure between wolverine populations, we are unable to

fully describe the likely complicated local social structure of wolverines. Local wolverine groups, which may exist with juvenile females establishing home ranges adjacent to their mothers, will not be revealed by sampling over a broad scale, as in this study.

This study has shown that populations of wolverines that are exposed to fewer anthropogenic factors potentially have much gene flow among them. In direct contrast, the southern populations of Idaho and to a certain degree Revelstoke, which are exposed to greater pressure from humans (settlement, road systems, recreation in remote areas, and so on), seem to be more genetically isolated. The viability of these populations seems to be dependent, to a great extent, on large areas of undisturbed habitat and corridors among them.

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This project is one of several ongoing population genetic studies of mustelid species by Christopher Kyle as part of his PhD thesis. Various other projects on worldwide mammalian species are currently taking place in Dr Curtis Strobeck's laboratory.

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## CONNECTIVITY OF PERIPHERAL AND CORE POPULATIONS OF NORTH AMERICAN WOLVERINES

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Wolverines are highly vagile carnivores, with long-distance dispersal documented for males and females. Consequently, the species was thought to represent 1 large, panmictic unit in North America. In this study, we examined the connectivity of populations on the edge of their historical distribution to the larger, continuous, northern distribution of wolverines. Twenty-two regions were sampled, and 671 individuals were genotyped at 12 microsatellite loci. Our results confirmed that high levels of gene flow do occur among all the northern wolverine populations sampled. We also observed progressively increasing genetic structure at the periphery of their southern and eastern distributions, suggesting that these populations may have been partially fragmented from what was once a panmictic unit. Peripheral populations may be more susceptible to extirpation and, therefore, may be the most appropriate targets for concerted conservation efforts to prevent the elimination of wolverines from yet more of their historical range.

Key words: genetic structure, *Gulo gulo*, mustelid, population fragmentation, wolverine

Wolverines are an enigmatic and rarely observed mustelid, inhabiting the tundra, taiga, and forest zones of portions of North America and Eurasia (Wilson 1982). In North America, the abundance and distribution of the species have declined since the advent of European settlement, most notably in eastern Canada, Vancouver Island, southern Rocky Mountains, and California (Wilson 1982; V. A. Banci, in litt.). Historical persecution (trapping and poisoning) and displacement from native habitat may be responsible for these declines. As a result, wolverines have been granted special conservation status across much of their current range. In Canada, the eastern population is listed as endangered (although potentially extirpated), whereas the western population is granted special concern status (COSEWIC 2001). In the contiguous United States, petitions have been put forth to

list the wolverine as endangered; to date these petitions have been unsuccessful due to the lack of adequate information supporting this designation (<http://www.wolverinefoundation.org>).

Wolverines are highly vagile creatures, with males and females able to disperse vast distances in a relatively short period of time (Copeland 1996; Gardner 1985; Magoun 1985; Vangen et al. 2001). This characteristic has prompted the theory that wolverines may exist as a single panmictic unit in North America. In northern North America, this is true (Kyle and Strobeck 2001) because high levels of gene flow were observed among populations from western Alaska to central Nunavut. In contrast, relatively high levels of genetic structure were found between southern and northern populations (Kyle and Strobeck 2001). It was proposed that increased anthropogenic pressures (e.g., fur harvest, habitat destruction,

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heavily traveled transportation corridors) on the southern populations could be reducing the level of gene flow between populations of this species.

A study of Scandinavian wolverines from northern and southern Norway and Sweden by Walker et al. (2001) reported findings similar to those found for southern North American populations (Kyle and Strobeck 2001). Using microsatellites, Walker et al. (2001) found significant levels of genetic structuring between wolverine populations ( $F_{ST} = 0.045$ ) and low estimates of genetic variation ( $H_E = 39\%$ ). They attributed their results to long-term anthropogenic influences, such as predator-removal programs and hunting, that reduced the number and range of wolverines in Scandinavia before 1970 (Landa and Skogland 1995). However, subsequent protective legislation has resulted in increased wolverine densities in these regions (Landa 1997) and in the recent recolonization of southern Norway.

This study expands upon previous work by investigating the genetic structure of additional northern wolverine populations, populations on the edges of their current North American distribution, and 1 population in eastern Russia. Our goal was to determine if populations on the periphery of the current distribution of wolverines, where their historic ranges and numbers have been reduced in the last century, are genetically isolated from the larger continuous core of populations. In such regions, the influences of genetic drift could lead to genetically distinct populations, especially for this midsized carnivore existing at a low population density (V. A. Banci, in litt.). If these peripheral populations are found to be small fragments of a larger, continuous core of northern wolverine populations, they may be more susceptible to stochastic events leading to local extirpation (Hanski 1999). As such, these regions would be appropriate for concerted conservation efforts to reestablish connectivity between frag-



FIG. 1.—Map depicting 22 sampled localities of wolverines. Squares represent sampled localities from Kyle and Strobeck (2001), circles represent localities sampled in this study.

mented populations and the larger continuous distribution of animals.

#### MATERIALS AND METHODS

This study combines the data of Kyle and Strobeck (2001) from Alaska, Nunavut, Williston Lake (British Columbia), Revelstoke (British Columbia), Manitoba, and Idaho, with samples from 10 additional geographic regions. Hair, pelt, and tissue samples were obtained from eastern Russia, the Whitehorse region in the Yukon, the Fort Rae and Rennie Lake regions of the Northwest Territories, the Smithers and Prince George regions of British Columbia, the Grande Cache region of Alberta, northwestern Saskatchewan, northwestern Ontario, and the Yellowstone region of Wyoming (Fig. 1).

DNA was extracted using a DNAeasy® Tissue Extraction Kit (QIAGEN Inc., Mississauga, Ontario). Twelve polymorphic microsatellites were amplified using primers developed in badgers (BA-1, BA-4—Davis and Strobeck 1998), martens (MA-3—Davis and Strobeck 1998), wolverines (GG-3, GG-4, GG-7, GG-14—Davis and Strobeck 1998; and Ggu 101, Ggu 216, Ggu 234—Duffy et al. 1998), mink (Mvis-75—Flemming et al. 1999), and Eurasian otters (L-604—Dallas and Piernney 1998). Amplification conditions are given in Davis and Strobeck (1998). DNA fragments were visualized using an ABI Prism® 377 DNA sequencer (PE Applied Bio-

systems Inc., Foster City, California). DNA fragments were analyzed using the programs GeneScan<sup>™</sup> Analysis 2.02 and Genotyper<sup>®</sup> 2.0 (PE Applied Biosystems Inc.).

A *G*-test for heterogeneity of allele distributions, averaged across all loci (Sokal and Rohlf 1995), was performed for each pair of the sampled areas. Departures from Hardy–Weinberg equilibrium were tested for each of the 12 loci using Genepop 3.1d (Raymond and Rousset 1995) that uses a Markov chain method following the algorithm of Guo and Thompson (1992). Genepop also was used to evaluate genotypic disequilibria among loci.

Relative genetic variation in each population was assessed using mean number of alleles and unbiased expected heterozygosity,  $H_E$  (Nei and Roychoudhury 1974). Pairwise genetic distances were estimated using Nei's standard genetic distance,  $D_S$  (Nei 1972), calculated from genotype frequencies, and the genotype likelihood-ratio distance,  $D_{LR}$  (Paetkau et al. 1997), calculated from genotype probabilities. Both pairwise distances were calculated using programs designed by John Brzustowski (<http://www.biology.ualberta.ca/jbrzusto/Doh/php>).

Pairwise  $F_{ST}$  estimates were obtained from the software package Genepop 3.1d (Raymond and Rousset 1995, as per Weir and Cockerham 1984).

An unrooted neighbor-joining tree of  $D_S$  values was created using PHYLIP 3.573 (J. Felsenstein, in litt.). The programs Seqboot (1,000 bootstraps) and Consense, also within the software package PHYLIP, were used to obtain statistical support for the neighbor-joining tree. The association between geographic and genetic distance values was tested with a 2-way Mantel test (Mantel 1967—<http://www.fas.umontreal.ca/BIOL/legendre/>), and regressions of the data were performed to obtain comparisons of genetic structure per unit geographic distance.

The assignment test (Paetkau et al. 1995—<http://www.biology.ualberta.ca/jbrzusto/Doh/php>) was conducted on all populations. This program determines the probability of a genotype occurring in the region from which it was sampled and the probability of it occurring in each of the other regions included in the test. Individuals are then assigned to the population where their genotype has the highest probability of occurrence (see Waser and Strobeck 1998). Randomizations (10,000) of the data were per-

formed within each gene pool assuming Hardy–Weinberg equilibrium. This test identifies cross-assignments that are unlikely to have occurred due to chance alone, thus identifying individuals that may be migrants.

## RESULTS

*Tests of disequilibrium.*—After accounting for samplewise error (Dunn–Sidak method—Sokal and Rohlf 1995), 1 departure from Hardy–Weinberg equilibrium was found at locus L-604 in the Yukon population. Heterozygote deficiencies were responsible for this result, suggesting the presence of null alleles in this population. Because L-604 conformed to Hardy–Weinberg equilibrium in all other populations, it was retained for further analyses.

Genotypic disequilibrium was observed for 2 pairs of loci in the Alberta population: GG3 with GG4, and BA1 with L604. Because genotypic disequilibria were not found for these loci in any other population, it is unlikely that they are physically linked and were therefore retained for further analyses. This result may reflect the presence of a number of related individuals in the Alberta population.

*Heterogeneity of sampled regions.*—Due to the broad sampling scheme, large number of regions sampled, low pairwise genetic distances and  $F_{ST}$  values, and for ease of comparison, all adjacent regions that did not differ significantly in their genotypic frequencies ( $\alpha = 0.005$ ) were pooled (cf. Kyle and Strobeck 2001, where  $\alpha = 0.05$ ). Hence, the Williston Lake, Prince George, and Smithers regions were combined into 1 population and referred to as central British Columbia; the Rennie Lake and Fort Rae regions of the Northwest Territories were combined into 1 population; all Alaskan regions were pooled, and the Kugluktuk and Bay Chimo regions of Nunavut were pooled. Additional details on the genetic variation within and among the Alaskan and Nunavut populations can be found in Kyle and Strobeck (2001).

*Genetic variation.*—The mean number of

TABLE 1.—Genetic variation of wolverines as revealed by microsatellite data ( $n$  = sample size,  $A$  = mean number of alleles, and  $H_E$  = average expected heterozygosity).

| Population               | $n$ | $A$ | % $H_E$ |
|--------------------------|-----|-----|---------|
| Russia                   | 64  | 5.7 | 63      |
| Alaska                   | 228 | 5.2 | 65      |
| Yukon                    | 23  | 4.7 | 65      |
| Northwest Territories    | 40  | 4.7 | 65      |
| Nunavut                  | 107 | 4.9 | 64      |
| Central British Columbia | 68  | 5.0 | 61      |
| Revelstoke               | 47  | 5.2 | 61      |
| Alberta                  | 17  | 5.0 | 61      |
| Saskatchewan             | 15  | 4.3 | 64      |
| Idaho                    | 14  | 2.8 | 42      |
| Wyoming                  | 8   | 3.6 | 56      |
| Manitoba                 | 28  | 4.5 | 67      |
| Ontario                  | 12  | 3.8 | 63      |
| Total                    | 671 |     |         |
| Average                  |     | 4.6 | 61      |

alleles ranged from 2.8 (Idaho) to 5.7 (Russia). The average level of heterozygosity ( $H_E$ ) among all sampled regions was 61% (Table 1). At 42%, Idaho was the only population with a significantly lower level of genetic variation (pairwise comparisons; Wilcoxon’s signed-ranks test,  $\alpha = 0.05$ —Sokal and Rohlf 1995).

*Pairwise  $F_{st}$  and genetic distance measures.*—Pairwise  $F_{ST}$  values ranged essen-

tially from 0 to 0.235 (Table 2). Among the northern regions (Alaska, Yukon, Northwest Territories, Nunavut, central British Columbia, and northern Saskatchewan), pairwise  $F_{ST}$  values ranged essentially from 0 to 0.029.  $F_{ST}$  values were relatively low (0.012) among eastern (Manitoba and Ontario) populations and were moderate between eastern and northern populations (0.032–0.057). Among the southern regions (Revelstoke, Idaho, and Wyoming) the pairwise values were relatively high (0.047–0.147). These values were even higher when comparing southern regions with northern regions (0.034–0.200), with the exception of comparisons between southern and Alaskan populations where the  $F_{ST}$  values were relatively low (0.004–0.015). The lowest values observed between Russian and North American populations were to Alaska (0.038). All other comparisons between Russia and North America yielded relatively high values (0.116–0.235).

The genetic distances,  $D_{LR}$  (data not shown) and  $D_S$  (Table 2), were found to correlate with one another by a 2-way Mantel test ( $r = 0.72$ ,  $P = 0.0004$ ).  $D_S$  values ranged from about 0.021 to 0.490. The  $D_S$  values paralleled and were correlated with the findings from the  $F_{ST}$  estimates ( $r = 0.77$ ,  $P = 0.0005$ ). The main exceptions

TABLE 2.—Pairwise genetic distances for wolverine populations using microsatellite data: Nei’s standard genetic distance,  $D_S$  (below diagonal) and  $F_{ST}$  (above diagonal); see Fig. 2 for abbreviations.

|    | Population |       |       |       |       |       |       |       |       |       |       |       |       |
|----|------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
|    | RU         | AK    | YK    | NT    | NU    | BC    | RV    | AB    | SK    | ID    | WY    | MB    | ON    |
| RU | —          | 0.038 | 0.116 | 0.127 | 0.128 | 0.138 | 0.170 | 0.126 | 0.124 | 0.235 | 0.165 | 0.145 | 0.156 |
| AK | 0.287      | —     | 0.003 | 0.004 | 0.007 | 0.007 | 0.015 | 0.005 | 0.001 | 0.016 | 0.004 | 0.008 | 0.003 |
| YK | 0.298      | 0.068 | —     | 0.018 | 0.028 | 0.028 | 0.055 | 0.026 | 0.019 | 0.159 | 0.063 | 0.032 | 0.034 |
| NT | 0.323      | 0.053 | 0.068 | —     | 0.002 | 0.020 | 0.035 | 0.16  | 0.000 | 0.176 | 0.047 | 0.037 | 0.045 |
| NU | 0.317      | 0.041 | 0.084 | 0.021 | —     | 0.029 | 0.054 | 0.036 | 0.000 | 0.169 | 0.060 | 0.046 | 0.057 |
| BC | 0.323      | 0.44  | 0.076 | 0.053 | 0.065 | —     | 0.034 | 0.034 | 0.026 | 0.127 | 0.049 | 0.057 | 0.055 |
| RV | 0.433      | 0.125 | 0.131 | 0.081 | 0.116 | 0.071 | —     | 0.051 | 0.055 | 0.141 | 0.047 | 0.078 | 0.076 |
| AB | 0.313      | 0.124 | 0.097 | 0.065 | 0.101 | 0.087 | 0.117 | —     | 0.026 | 0.201 | 0.057 | 0.041 | 0.043 |
| SK | 0.327      | 0.059 | 0.086 | 0.034 | 0.034 | 0.081 | 0.135 | 0.103 | —     | 0.200 | 0.076 | 0.031 | 0.045 |
| ID | 0.490      | 0.290 | 0.257 | 0.303 | 0.297 | 0.188 | 0.208 | 0.311 | 0.322 | —     | 0.147 | 0.185 | 0.234 |
| WY | 0.433      | 0.181 | 0.181 | 0.138 | 0.165 | 0.139 | 0.126 | 0.168 | 0.218 | 0.210 | —     | 0.094 | 0.102 |
| MB | 0.405      | 0.119 | 0.109 | 0.101 | 0.116 | 0.127 | 0.181 | 0.129 | 0.273 | 0.330 | 0.273 | —     | 0.012 |
| ON | 0.437      | 0.132 | 0.126 | 0.138 | 0.158 | 0.144 | 0.185 | 0.146 | 0.149 | 0.395 | 0.264 | 0.084 | —     |

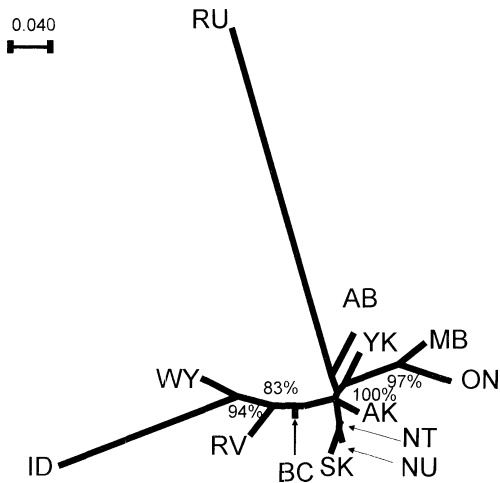


FIG. 2.—Unrooted neighbor-joining tree of Nei's standard genetic distance,  $D_S$ . The length of tree branches is relative to genetic distances. Only branch support (from 10,000 bootstraps) of greater than 60% is shown. Localities include Russia (RU); Idaho (ID); Wyoming (WY); Revelstoke (RV); central British Columbia (BC); Alberta (AB); Saskatchewan (SK); Manitoba (MB); Ontario (ON); Kugluktuk and Bay Chimo, Nunavut (NU); Fort Rae and Rennie Lake, Northwest Territories (NT); Whitehorse, Yukon (YK); Arctic, Anchorage, Nome, Nabesna, Natak, and Russian Mission, Alaska (AK).

were the pairwise  $D_S$  values between Russia and North America; unlike the  $F_{ST}$  values,  $D_S$  values were relatively consistent, ranging from 0.287 to 0.490. An unrooted neighbor-joining tree, based on pairwise  $D_S$  values (Fig. 2), summarizes relationships between populations. In this tree the Idaho and Russian populations appear nearly equally distinct from the northern North American populations.

Pairwise geographic and genetic distance ( $D_S$ ) were plotted against each other (Fig. 3). Using a 2-way Mantel test, geographic and genetic distance,  $D_S$ , were correlated with each other ( $r = 0.52$ ,  $P = 0.00001$ ). Fig. 4 illustrates the genetic structure of several carnivores relative to that of wolverines, in relation to geographic distance. The steepest slopes were found in brown

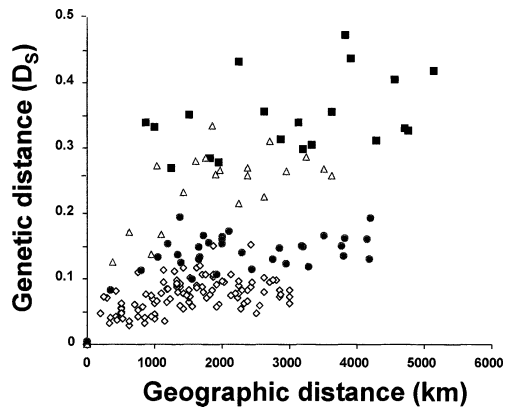


FIG. 3.—Plot of geographic distance relative to Nei's standard genetic distance,  $D_S$ , for all sampled wolverine localities. Closed squares represent pairwise values to Russia, open triangles are pairwise values to Idaho, open diamonds are northern population pairwise values, and closed circles are pairwise comparisons to eastern populations (Ontario and Manitoba).

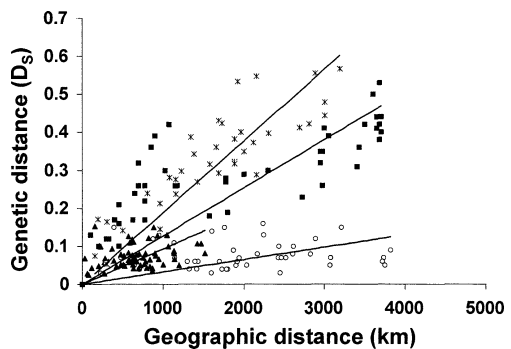


FIG. 4.—Plot of geographic distance relative to Nei's standard genetic distance,  $D_S$ , for wolverines (open circles), martens (closed triangles—Kyle et al. 2000), fishers (closed squares—Kyle et al. 2001), and brown bears (asterisks—Paetkau et al. 1998), with trendlines for each species. Peripheral populations and reintroduced populations with disproportionate genetic distances per unit geographic distance are excluded (brown bears, Wyoming and Kodiak Island bears excluded; fishers, reintroduced populations excluded; martens, all populations included; wolverines, Russia, Idaho, Wyoming, and Revelstoke populations excluded).

TABLE 3.—Genotype assignment test for wolverine populations. Left column represents sampling locality and row headings represent populations wolverines were assigned to. For example, 96.9% of the samples obtained from Russia were assigned to Russia, whereas 1.56% of the Russian animals were assigned to the Yukon and 1.56% to Alberta. Note that rounding of percentages may result in values not summing to 100%. Asterisk represents cross-assignments that were found to be significant ( $\alpha = 0.05$ ) by randomizations of individual gene pools assuming Hardy–Weinberg equilibrium. See Fig. 2 for abbreviations.

|    | % of individuals assigned to each region |       |       |      |      |       |      |      |       |      |      |       |       |
|----|------------------------------------------|-------|-------|------|------|-------|------|------|-------|------|------|-------|-------|
|    | RU                                       | AK    | YK    | NT   | NU   | BC    | RV   | AB   | SK    | ID   | WY   | MB    | ON    |
| RU | 96.9                                     |       | 1.56  |      |      |       |      | 1.56 |       |      |      |       |       |
| AK | 0.4                                      | 56.1  | 6.1   | 3.1  | 10.1 | 9.6   | 1.8  | 1.3  | 7.5   |      | 1.3  | 1.3   | 1.3   |
| YK |                                          | 4.3   | 43.5  | 13.0 |      | 17.4* |      | 4.3  |       |      |      | 13.0* | 4.3   |
| NT |                                          | 10.0  | 2.5   | 20.0 | 20.0 | 2.5   | 5.0  | 10.0 | 20.0* |      |      | 10.0  |       |
| NU |                                          | 6.5   | 2.8   | 15.9 | 49.5 | 3.7   | 0.9  | 3.7  | 10.3  |      |      | 4.7   | 1.9   |
| BC | 1.5                                      | 8.8   | 5.9   | 8.8  | 4.4  | 42.6  | 10.3 | 4.4  | 2.9   | 2.9* | 2.9  | 2.9   | 1.5   |
| RV |                                          | 2.1   | 6.4*  | 6.4  |      | 12.8  | 61.7 |      | 4.3   | 2.1  | 2.1  |       | 2.1   |
| AB |                                          | 5.9   | 5.9   | 23.5 |      |       |      | 41.2 | 11.8  |      | 5.9  | 5.9   |       |
| SK |                                          | 13.3  | 7.7   | 13.3 | 26.7 |       |      |      | 26.7  |      |      | 13.3  |       |
| ID |                                          |       |       |      | 7.1* |       |      |      |       | 92.9 |      |       |       |
| WY |                                          |       |       | 12.5 | 12.5 |       |      |      | 25.0* |      | 50.0 |       |       |
| MB |                                          | 14.3* | 3.6   | 7.1  | 3.6  |       |      | 3.6  |       |      |      | 75.0  | 17.9* |
| ON |                                          |       | 25.0* |      |      |       |      |      |       |      |      | 16.7  | 58.3  |

bears (0.137/1,000 km, *SE* 0.013—Paetkau et al. 1998) and fishers (0.092/1,000 km, *SE* 0.008—Kyle et al. 2001), whereas the lowest values were obtained for wolverines (0.0183/1,000 km, *SE* 0.005) and northern martens (0.057/1,000 km, *SE* 0.009—Kyle et al. 2000).

*Assignment test.*—Of the Russian samples processed, 97% of the individuals were assigned to Russia (Table 3). For the 2 cross-assignments observed from Russia to North America, assignment probabilities suggest that the individuals were nearly as likely to occur in Russia as in North America (Fig. 5A).

In the northern regions, the vast majority of cross-assignments went to other northern regions. In eastern populations, most cross-assignments were exchanged between the 2 populations in Ontario and Manitoba, with the remainder being assigned to northern regions.

The southern populations, compared with northern regions, produced fewer cross-assignments, with the exception of Wyoming. In this population, 50% of the assignments

were assigned to northern populations (Figs. 5B and 5C).

#### DISCUSSION

Wolverine populations in northern North America experience high levels of gene flow. This is consistent with the impressive dispersal abilities of both male and female wolverines (Copeland 1996; Gardner 1985; Magoun 1985; Vangen et al. 2001) and with a previous study by Kyle and Strobeck (2001). But genetic structure in the southern and eastern extremes of the distribution of wolverines is relatively high, possibly reflecting the fragmented nature of these populations at the periphery of their historical range.

The Russian wolverine population was found to be distinct from all other North American populations (Tables 2 and 3), with the few cross-assignments observed from Russia to North America likely occurring due to chance (Fig. 5A). But rare transcontinental migration events may not be impossible for this species. Arctic foxes are known to cross the Bering Sea via St.

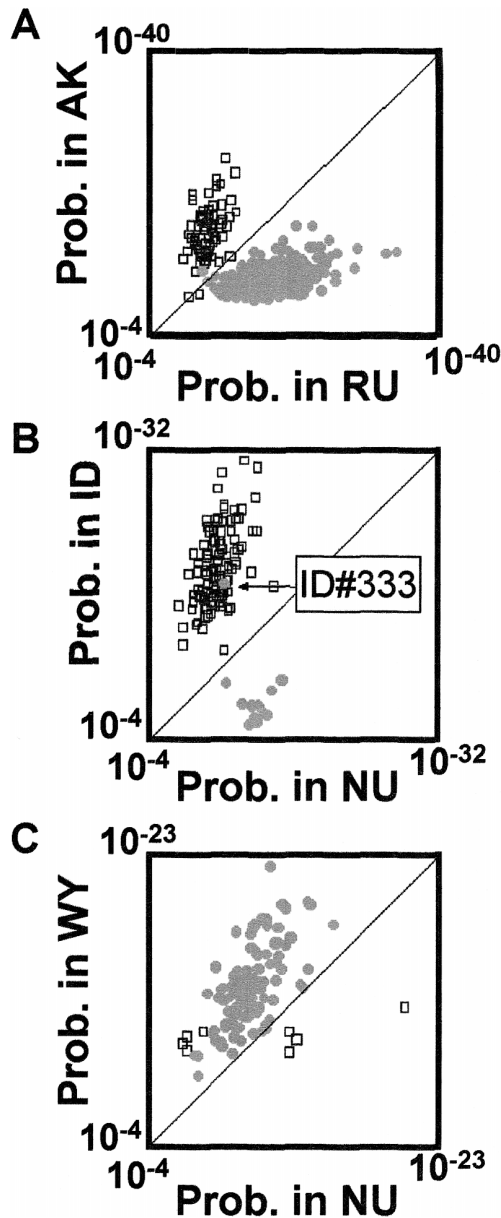


FIG. 5.—Pairwise plots of genotypic probabilities. Individuals found on the opposite side of the diagonal represent cross-assignments. A) Russia versus Alaska, B) Idaho versus Nunavut, C) Wyoming versus Nunavut.

Lawrence Island on the pack ice (Fay and Stephenson 1987), so it may also be possible for wolverines to do the same. In general, however, Russia and Alaska have been considered isolated for >10,000 years, and observed genetic distance/ $F_{ST}$  results may provide a baseline for evaluating isolation among North American populations.

Little genetic structure exists among northern regions (Tables 2 and 3), suggesting the occurrence of extensive gene flow. These findings are expected, given the life history characteristics of wolverines, including their ability to move long distances with relative ease, as documented by Copeland (1996), Gardner (1985), Gardner et al. (1986), and Magoun (1985), and the presence of relatively continuous habitat. It appears that these characteristics have had the effect of genetically homogenizing wolverine populations in habitats with relatively few anthropogenic influences.

An alternative to the aforementioned hypothesis is that the lack of structure in northern regions may reflect a relatively recent postglacial colonization of this area. But wolverines were thought to exist in several refugia during the Wisconsin glaciation, both in regions south of the ice sheets and in Beringia (fossil evidence—Bryant 1987). Genetic trends currently observed have most likely not been influenced by a rapid radiation of wolverines northward from a southern glacial refugium, but reflect recent and consistent migration events.

Our results conflict slightly with a previous study by Wilson et al. (2000) who investigated the genetic structure of wolverines from the Northwest Territories and found a moderate level of genetic structure with enzymes ( $F_{ST} = 0.076$ ) and relatively strong structure with mtDNA ( $\Phi_{ST} = 0.536$ ). The mtDNA results, however, likely reflect male-biased dispersal that is thought to occur in this species (V. A. Banci, in litt.).

A high level of gene flow was found to occur between most western Canadian populations and regions further north suggest-

ing that they are part of the nearly continuous northern distribution of wolverines. But as suggested by Kyle and Strobeck (2001), the Revelstoke population in British Columbia shows signs of genetic isolation from more northern populations. The apparent lack of gene flow to and from Revelstoke may reflect heightened anthropogenic pressures in this region. Studies by Alexander and Waters (2000) and Austin (1998) indicate that the Trans Canada Highway (running through Revelstoke) acts as an impediment to movement for wolverines (among other species). Furthermore, historical effects from predator control programs (L. Hancock, in litt.) and trapping from the early to mid-1900s, have reduced, and thus to some extent isolated, populations in this region. This and the effects of changes as a result of transportation development may, in part, be responsible for the current levels of genetic structure observed.

All results suggest that the 2 eastern populations, Manitoba and Ontario, are quite similar to each other, but relatively distinct from the other regions. Due to the their proximity to the prairies (vast expanse of unsuitable habitat) and Hudson's Bay, and the fact that these populations are on the periphery of the current distribution of wolverines, gene flow between these eastern populations and northern sources may be limited. This is reinforced partially by the genetic distances and number of cross-assignments to northern regions.

The Idaho population appears to be genetically distinct from all other regions. Interestingly, the Idaho and Russian populations are nearly equally genetically distant from northern regions (Fig. 2). But the genetic distances to the northern North American populations from Russia are the result of isolation since the last glaciation. In contrast, the genetic distances between Idaho and other North American populations may represent more recent (early to mid-1900s) population fragmentation or founder events. Historical persecution and displacement from native habitat were thought to have

led to the extirpation of wolverines from much of the lower 48 states by the 1920s, including Idaho (Davis 1939), Montana (Newby and McDougal 1963; Newby and Wright 1955), and Washington (Johnson 1977). Thus, current populations in Montana and Idaho are a result of a relatively recent expansion from the north or an expansion of small populations that may have been reduced to minimum levels during the early to mid-1900s. In either case, the likely small effective size of the Idaho population has led to rapid genetic drift and the current distinctiveness of this population.

The 1 cross-assignment from the Idaho population went to Nunavut (Table 3). Although we are not suggesting that an individual migrated from Nunavut to Idaho, the individual genotype was much more likely ( $\alpha < 0.05$ ) to be from a more northern population than from Idaho (probability of  $10^{-19}$  in Idaho compared with  $10^{-12}$ – $10^{-14}$  for northern populations, individual probability data not shown; Fig. 5B). Given the potential for dispersal in this species, it is not unreasonable to suggest that there is some amount of gene flow between central Alberta or British Columbia and Idaho. Although less likely, it should be mentioned that reintroductions to the lower 48 states have taken place with wolverines originally obtained from northern Canada (e.g., John Denver and Stouffer Productions of Aspen, Colorado, released 2 Canadian wolverines into the Maroon Bells-Snowmass Wilderness area of Colorado in 1978—Murray 1987).

The results for Wyoming were mixed, with several individual genotypes resembling northern genotypes, whereas others appeared distinct from all other regions sampled. The cross-assignments from Wyoming to the northern regions were highly significant ( $\alpha < 0.05$ ; Fig. 5C). The lack of samples from intermediate populations in Montana makes it unclear if there is some amount of gene flow between Wyoming and Canadian populations.

The genetic structure observed in wol-

verines is similar to that of other carnivores; American pine martens (*Martes americana*) sampled from the Canadian north show little population genetic structure between populations (Kyle et al. 2000). A similar result was also obtained in lynx (*Lynx canadensis*) sampled from across their North American distribution. In lynx, like wolverines, a high potential for dispersal is thought to be responsible for the lack of genetic structure across vast distances (Schwartz et al. 2002). Although more structure exists between brown bear (*Ursus arctos*) populations than between wolverine populations, Paetkau et al. (1998) found that populations in the southern reaches of the bear range were more genetically distinct and showed less genetic variation than did bears occupying less disturbed northern habitats. The parallels between the genetic structure of wolverines and brown bears at the southern periphery of their range may be correlated with low densities of humans and roads (Carroll et al. 2001).

#### CONCLUSIONS

Our results confirm that high levels of gene flow do occur among northern wolverine populations. We also observe progressively increasing genetic structure at the southern and eastern peripheries of their current distribution. Historical persecution and displacement from native habitat may have resulted in smaller populations, partially fragmented from what was once, likely, a panmictic unit. Small, isolated populations are more susceptible to extirpation (Hanski 1999) and, therefore, should be targets of concerted conservation efforts. To reverse these trends, consideration should be given to reestablishing gene flow between peripheral and core populations to ensure the persistence of this species in regions that are under increasing anthropogenic pressure.

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# Phylogeography and Post-Glacial Recolonization in Wolverines (*Gulo gulo*) from across Their Circumpolar Distribution

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## Abstract

Interglacial-glacial cycles of the Quaternary are widely recognized in shaping phylogeographic structure. Patterns from cold adapted species can be especially informative - in particular, uncovering additional glacial refugia, identifying likely recolonization patterns, and increasing our understanding of species' responses to climate change. We investigated phylogenetic structure of the wolverine, a wide-ranging cold adapted carnivore, using a 318 bp of the mitochondrial DNA control region for 983 wolverines ( $n=209$  this study,  $n=774$  from GenBank) from across their full Holarctic distribution. Bayesian phylogenetic tree reconstruction and the distribution of observed pairwise haplotype differences (mismatch distribution) provided evidence of a single rapid population expansion across the wolverine's Holarctic range. Even though molecular evidence corroborated a single refugium, significant subdivisions of population genetic structure ( $0.01 < \Phi_{ST} < 0.99$ ,  $P < 0.05$ ) were detected. Pairwise  $\Phi_{ST}$  estimates separated Scandinavia from Russia and Mongolia, and identified five main divisions within North America - the Central Arctic, a western region, an eastern region consisting of Ontario and Quebec/Labrador, Manitoba, and California. These data are in contrast to the nearly panmictic structure observed in northwestern North America using nuclear microsatellites, but largely support the nuclear DNA separation of contemporary Manitoba and Ontario wolverines from northern populations. Historic samples (c. 1900) from the functionally extirpated eastern population of Quebec/Labrador displayed genetic similarities to contemporary Ontario wolverines. To understand these divergence patterns, four hypotheses were tested using Approximate Bayesian Computation (ABC). The most supported hypothesis was a single Beringia incursion during the last glacial maximum that established the northwestern population, followed by a west-to-east colonization during the Holocene. This pattern is suggestive of colonization occurring in accordance with glacial retreat, and supports expansion from a single refugium. These data are significant relative to current discussions on the conservation status of this species across its range.

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## Introduction

Understanding phylogeographic differentiation and patterns of genetic diversity for many extant species in the Northern Hemisphere hinges on understanding the interglacial-glacial cycles of the Quaternary [1,2]. Cold adapted species can be particularly informative as these species typically experience population increases and range expansions during glacial, and range contractions during interglacials [1,3]. These species may also undergo range contractions into refugia when continental ice-sheets attain their greatest extent (e.g. [4]), further shaping present-day patterns of biodiversity in the arctic [5].

Refugial history has focused predominately on temperate taxa [6], revealing major refugia - like Beringia [7]. However, progressively more investigations are proposing 'cryptic' glacial refugia beyond the limit of glaciations (see review by Shafer et al. [2]). Such refugia are usually limited and sporadic in geographic extent, and often overlooked in glacial biogeographic reconstructions

based on fossil records [8,9]. However, identification of the same regions by multiple studies (see Beatty and Provan [10]) signifies these refugia are not so much cryptic as additional to conventional Pleistocene refugia [11]. For many taxa, isolation of populations in separate refugia resulted in the formation of distinct genetic lineages [6,12]. Patterns of genetic differentiation are also reflective of stochastic processes like genetic drift and species specific dispersal abilities [13]. Wide-ranging species generally display a lack of population structuring across their range, increasing the likelihood of similar mitochondrial haplotypes being observed across very distant geographical locations [14,15]. Overall, these data are relevant to understanding how historic processes influence contemporary genetic patterns and how these data should be interpreted in context of management actions for species of conservation concern.

Most phylogeographic studies of cold adapted taxa have focused on vegetation [16], birds [17], and small mammals [18]. Investigations of large mammals remain limited [19,20], even

though large body size ( $>5.5$  kg) is a strong predictor of extinction risk for mammals [21]. Furthermore, relic populations may contain biogeographic traits associated with glacial refugia [22], particularly at range peripheries. This has conservation implications where peripheral populations – faced with increased environmental fluctuations [23], reduced gene flow and low densities [24] – may maintain unique genetic variability necessary in responding to climate change [25].

Wolverines (*Gulo gulo*) are a cold adapted carnivore with a circumboreal distribution (Figure 1, [26]). Fossil evidence is minimal for this species [27], and from those records that do exist, fossils in Europe extend from the Iberian Peninsula [28] eastwards to the Czech Republic [29]. In North America, late Pleistocene fossil remains have been found in Alaska [30] and the Yukon Territory [31]. Given the gaps in the fossil record, genetic evidence could provide a clearer picture of how glacial refugia have shaped postglacial recolonization of wolverines and other cold adapted species.

In the past half-century, wolverines have experienced substantial range reductions along the southern edge of their circumpolar distribution due to habitat alterations, persecution, and other anthropogenic influences such as indirect poisoning campaigns targeted at wolves (Figure 1, [26,32,33]). In North America, this animal has lost 37% of its historic range [34]. Although globally a species of least concern [35], its regional status ranges from stable (Alaska, [36]) to endangered (Norway, [37]), and in some areas, functionally extirpated (Quebec-Labrador, [38]). Wolverines occur at low densities [39] and have very large home range sizes (188–2 563 km<sup>2</sup> for males, [40,41]), increasing their vulnerability to habitat fragmentation and external anthropogenic threats such as logging, fur harvesting and direct persecution [42–44].

Wolverines are highly vagile [40,41], largely explaining the high levels of gene flow observed among populations based on nuclear DNA [45–49]. Genetic structure, however, tends to increase towards range peripheries in North America [46,47,49–51], suggesting irregular distributions of populations [36] due to range contractions [44]. Additionally, spring snow cover is positively

correlated with wolverine distribution [52,53] and genetic differentiation [54], underlining the sensitivity of wolverines to climate change. Mitochondrial DNA (mtDNA) studies of this species have revealed strong genetic structure over small geographic scales, reflecting female philopatry (e.g. [49,55]). In North America, haplotypes from the eastern periphery drastically differed from those in the west, suggesting a longstanding subdivision reflective of historical processes [51].

Here, we investigated the phylogeographic patterns of wolverines across the full breadth of their Holarctic range ( $>5$  million km<sup>2</sup>) using conventional Bayesian approaches. We obtained samples from 209 individuals together with existing data from 774 wolverines to assess the genetic variation of the mtDNA control region. In addition, we apply a coalescent-based approximate Bayesian computation (ABC) method to test among competing hypotheses of postulated recolonization pathways influencing population divergence. We propose four alternative hypotheses that may explain observed patterns of haplotype distribution and resulting groupings that include: (1) present-day populations diverged from a single ancestral population during the last glacial maximum (LGM); (2) a single incursion across Beringia resulted in the divergence between Eastern and Western Hemisphere wolverines, with divergence of North American populations occurring during glacial retreat; (3) a single incursion across Beringia during the LGM, followed by a west-to-east stepping-stone divergence pattern across North America; and (4) two incursions from Beringia during the LGM, with the second incursion being followed by a west-to-east stepping-stone divergence. Understanding the response of cold adapted species to past climatic fluctuations could help identify evolutionary significant units [56] and improve predictions of the effects of climate change on arctic wildlife [57].

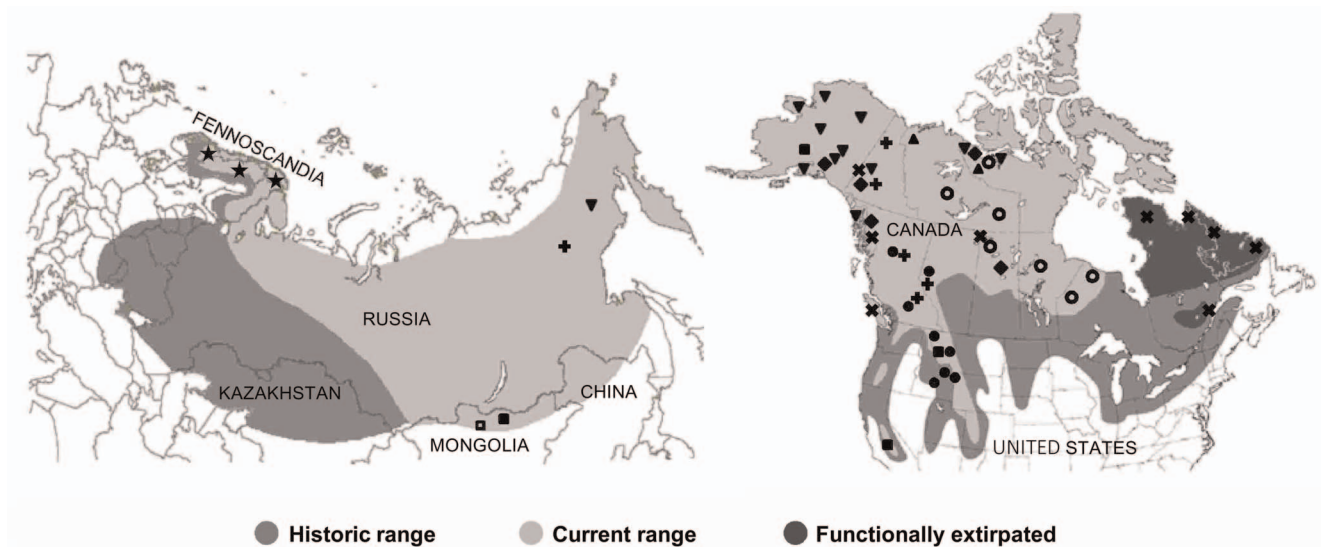


**Figure 1. Historic and contemporary Holarctic distribution of wolverines and sampling localities.** Historic and current distribution of wolverines in Eurasia (adapted from National Geographic Society [116]) and North America (adapted from COSEWIC [115]), and sampled localities from Wilson et al. [45] - triangle; Walker et al. [15] - star; Tomasik and Cook [55] - inverted triangle; Cegelski et al. [49] - solid circle; Schwartz et al. [60] - solid square; Frances [59] - diamond; Zigoris et al. [51] - open circle; Rochnov and Meschersky unpub. - open square; New contemporary samples - plus sign; and New historic samples - x symbol.  
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## Methods

### Samples

We processed contemporary samples collected from: Russia (RUS,  $n = 49$ ), Yukon (YK,  $n = 26$ ), British Columbia (BC,  $n = 81$ ), Alberta (AB,  $n = 26$ ) and Manitoba (MB,  $n = 1$ ), and historical samples (Figure 1, Table S1) collected between 1889–1944 as identified by the Global Diversity Information Facility (<http://www.gbif.org/>) from the Yukon ( $n = 9$ , c. 1923–1932), Nunavut (NU,  $n = 1$ , c. 1944), British Columbia ( $n = 11$ , c. 1910–1927; with one sample representing *G. gulo vancouverensis* from Vancouver Island, Canada), Saskatchewan (SK,  $n = 1$ , c. 1920), Ontario (ON,  $n = 1$ , c. 1920) and Quebec-Labrador (QC/NL,  $n = 15$ , c. 1883–1900). Contemporary samples for Russia, Yukon, Alberta, and Manitoba were collected from pelts through fur auction houses and pelt dealers. British Columbia samples were obtained from pelt samples through fur auction houses, and tissue samples (ear plug and hair) collected by the Columbia Basin Fish and Wildlife Compensation Program. Permission to acquire tissue samples from archived specimens was obtained from all museums and institutions, and all tissue samples from historic specimens were donated. Our data were combined with mtDNA control region data from previous studies (Figure 1, [15,45,49,51,55,58,59], Rochnov and Meschersky unpublished data) – including samples from: Sweden (SWE,  $n = 62$ ), Norway (NOR,  $n = 108$ ) Mongolia (MNG,  $n = 6$ ), Russia ( $n = 5$ ), Alaska (AK,  $n = 148$ ), Yukon ( $n = 23$ ), Nunavut ( $n = 81$ ), Northwest Territories (NT,  $n = 53$ ), British Columbia ( $n = 5$ ), Saskatchewan ( $n = 16$ ), Manitoba ( $n = 30$ ), Ontario ( $n = 54$ ), Montana (MT,  $n = 148$ ), Wyoming (WY,  $n = 13$ ), Idaho (ID,  $n = 15$ ), and California (CA,  $n = 7$ ). We did not include data reported in Cegelski et al. [49] for BC and AB, and for ON reported by Frances [59] as samples were obtained from C.J. Kyle and likely the same individuals. We also excluded sequence data from Chappell et al. [48] as the mtDNA control region was 200 bp and did not encompass five known variable sites in the larger fragment.

### Preparation of Bone Samples and DNA Extraction

Bone dust (~100  $\mu$ L) was collected from museum specimens by drilling mandibles or from turbinate bones frozen with liquid nitrogen and crushed into bone powder using a mortar and pestle. Mandibles were initially washed with Decon solution (1:49), rinsed with DNAase-free ddH<sub>2</sub>O (Gibco), and the outer surface removed using a Dremel tool. Strict laboratory protocols were followed to minimize risk of cross-contamination from contemporary sources. Specifically, equipment was sterilized with Decon solution (1:9) and rinsed with DNAase-free water between handling each sample, and filter tips and disposable pipettes were used. Extraction blanks were included at the beginning and end of extractions, and after every fifth sample to assess sample cross-contamination. Historic samples were processed in rooms not exposed to contemporary samples.

Extraction procedures followed the manufacturer's protocol for Qiagen's DNeasy tissue extraction kit (Qiagen) for all contemporary samples, with the following modifications for historic samples: (1) historic samples were rotated overnight at room temperature in 1.5 mL of ethylenediaminetetraacetic acid (EDTA; 0.5 M, pH 7.5) to decalcify the bone powder, and after 24 h samples were centrifuged at 9 000 rpm for 60 s, EDTA was poured off and a second treatment applied; (2) used 1.5 mL of Buffer ATL and treated samples with a second dose of 20  $\mu$ L Proteinase K (600 mAU/mL) (Qiagen, Mississauga), followed by a 2 h incubation at 56°C; (3) incubated for 10 min at 56°C after adding buffer AL; (4) performed two elution steps with heated buffer at 70°C;

and (5) transferred both elutions to an Amicon Ultra-0.5 centrifugal unit that yielded approximately 20  $\mu$ L of DNA.

### Mitochondrial DNA Sequencing

A 360 bp fragment of the control region was independently amplified twice using primers Gulo0F [60] and H16498 [61]. Amplification of a shorter sequence length allowed for data from previous studies to be compared with our data set. Sequencing procedures followed Zigouris et al. [51], however, for the historic samples Taq was increased from 0.05 to 0.1 U/ $\mu$ L and PCR cycles increased from 30 to 50. Additionally, PCR products that showed extra banding were run on a gel, and the target band was cut out. Excised bands were frozen overnight at  $-80^{\circ}\text{C}$ , vortexed and centrifuged. Amplified DNA expelled from excised gel bands and from samples with low DNA concentrations were re-amplified a second time to confirm sequence data.

### Data Analyses

Sequences were edited with MEGA 4.0.2 [62], aligned with Clustal W [63], and verified visually. We identified variable nucleotide positions and compiled unique sequences using FA-BOX 1.35 [64]. New haplotypes were only confirmed when independent PCR reactions generated the same sequence. Historic and contemporary samples were both treated as separate entries (historic SK omitted due to  $n = 1$ ), as well as combined by sampling region. In this study, historic and contemporary samples are reflective of sampling occurring pre- and post-20th century population declines [65]. Thus, frequency differences between pre- and post-population declines may provide insight of the underlining processes influencing present-day patterns of haplotype distribution and frequency. Nucleotide ( $\pi$ ) and haplotype ( $h$ ) diversity values were estimated with ARLEQUIN 3.5 [66]. Haplotype richness and number of private haplotypes were calculated manually and with a rarefaction test (ADZE 1.0, [67]) based on the smallest sample size. We tested for departures from neutrality (Tajima's  $D$ , [68]) and population growth (Fu's  $F_s$ ,  $P < 0.02$ , [69]) using ARLEQUIN with 10 000 bootstrap replicates.

Differentiation among sampling sites were estimated with pairwise  $\Phi_{ST}$  values using ARLEQUIN (10 000 permutations,  $P < 0.05$ ). An analysis of molecular variance (AMOVA) was used to quantify genetic variability among groups of populations, as well as within and among populations [70], in ARLEQUIN for 10 100 permutations. Groupings reflected the sharing of haplotypes among sampled regions irrespective of geographic location. We evaluated optimal grouping of sites by defining groups of samples maximally differentiated, but geographically proximate to each other. We performed a spatial analysis of molecular variance (SAMOVA) with SAMOVA 1.0 [71],  $2 \leq K \leq 10$ , using 100 random initial conditions and 10 000 iterations. Historic samples from YK, BC and SK were omitted from SAMOVA because they represented some of the main haplotypes observed in contemporary samples. SAMOVA analyses were performed with and without historic samples from CA and QC/NL for all contemporary data and for North American samples only. Historic samples from CA and QC/NL were included, as contemporary data was absent from these two peripheral regions. We also conducted Mantel tests [72] using Isolation by Distance (IBD) Web Service 3.23 [73] to test for correlations between genetic and geographic distances. To minimize error in characterizing IBD [74], we excluded sample regions with  $n < 10$  individuals. Correlations were tested between the natural logarithm of geographical distances and the regression of paired  $\Phi_{ST}/(1 - \Phi_{ST})$  estimates as proposed by Rousset [75]. Mantel tests were performed on North American regions and the data as a whole.

For Eurasia, there were too few distance classes for IBD to be detected [76].

We performed Bayesian analyses in BEAST 1.7.1 [77] to investigate phylogenetic composition of lineages and estimate divergence times (e.g. [78]). The Hasegawa-Kishino-Yano (HKY, [79]) model with a gamma shape distribution and invariant sites best fit our data based on Akaike Information Criterion (AIC) values (jModelTest 0.1.1, [80]). Base frequencies were estimated and rate variation among sites was modeled using four gamma rate categories. Divergence estimates were calculated using two independent calibration points [81], averaged across two phylogenetic studies ([82,83]: divergence between the *Gulo/Martes* clade and *Mustela* 11.9 (9.9–14.1, 95% CI) million years ago (Mya); and divergence of *Gulo* and *Martes* 6.1 (4.3–8.1, 95% CI) Mya). Control region sequences of mtDNA for *Mustela putorius*, *M. frenata* and *M. nivalis* represented the Mustelidae outgroup (GenBank Accession Numbers: AY962032; HM106321; HM106319), and *Martes martes* was used for the *Martes* outgroup (Accession Number: AJ585357). We used an uncorrelated lognormal relaxed clock model with a Coalescent constant size tree and normal distribution priors. Analyses were run for 40 million generations, with a burn-in of the first 4 million, and a sampling frequency of 500 steps. Tracer 1.5 [84] was used to evaluate estimated values and effective sample size (ESS) for each model parameter. For all parameters, ESS >450 suggested sufficient sampling and acceptable mixing. Bayesian analyses were computed multiple times to check for convergence. The phylogenetic tree was constructed using TreeAnnotator with a 20% burn-in of total trees generated, and viewed in FigTree 1.3.1 [85]. A median-joining network was created to visualize haplotype relationships using Network 4.5 [86].

To test for population expansion, we used ARLEQUIN with 10 000 bootstrap replicates to estimate the distribution of observed pairwise haplotype differences (mismatch distribution, [87]) among individuals. Populations that have undergone recent expansion display a unimodal distribution, whereas populations at demographic equilibrium are multimodal [87]. The validity of the estimated expansion model was evaluated using the sum of squared deviations (SSD) between observed and expected mismatch distribution values [88], and the raggedness index ( $r$ , [89]) that measures the smoothness of the mismatch distribution. Given the low power of  $r$  to detect population expansions, we also calculated Fu's  $F_s$  statistic on the whole dataset.

Based on the results of the preceding analyses, we assumed that all populations had the same colonization origin, and likely located near Beringia. We compared four alternative colonization pathways (Figure 2) using the coalescent-based approximate Bayesian computation (ABC, [90]) in DIYABC v2.0 (Cornuet et al. [91]). The first two hypotheses take into account the wolverine's vagility and adaptation to cold environments, and postulate that colonization included regions that were not entirely ice-free. Under this assumption, individuals from the ancestral population could have dispersed in multiple directions, colonizing several regions simultaneously (e.g. [92]). The first scenario tests if all populations diverged from a single ancestral Holarctic population during the LGM, where the incursion across Beringia and multiple colonization events occurred concurrently. The second scenario includes a time lag between the single incursion event across Beringia during the LGM, and multiple colonization events. The third and fourth hypotheses assume that colonization was constrained by glaciers, and dispersing individuals followed retreating ice-sheet fronts. The third hypothesis tests a single incursion from Beringia during the LGM giving rise to a northwestern AK-YK-BC-AB-MT-WY-ID population (Pop1),

from which colonization occurs in a west-to-east stepping-stone direction during the Holocene. This would result in a 'leading edge' pattern of colonization, with decreasing genetic diversity further away from the putative refugium [93]. Given the likelihood of multiple incursions across Beringia (e.g. red fox, *Vulpes vulpes*, [94]), our fourth hypothesis tests the occurrence of two incursions across Beringia during the LGM, resulting in the divergence between RUS-MNG (Pop5) and AK-YK-BC-AB-MT-WY-ID (Pop1) and between Pop 5 and NU-NT-SK (Pop2). This second incursion was then followed by a sequential, eastward stepping-stone dispersal scenario during the Holocene. We conducted ABC analyses for all regions except for SWE, NOR, and CA<sub>H</sub>. These localities were excluded based on either a large geographic gap in information between sampled regions (e.g. SWE-NOR and RUS-MNG) or small sample size (e.g. CA<sub>H</sub>,  $n < 10$ ). Although wolverine populations in North America experienced declines during the 20th century due to predator control programs and fur trading [65], we assumed that only Pop3 (MB-ON) experienced a genetic bottleneck given the low abundance of wolverines in this region. We applied a 75% reduction in the effective population size ( $N_e$ ), thus simulating an extreme bottleneck [95]. Simulations for all scenarios were performed with and without this bottleneck. For all scenarios, the sex ratio was equal and generation time was four years [36]. Range limits and associated conditions of parameters used in DIYABC analyses are listed in Table S2. We simulated  $10^6$  data sets for each scenario to build a reference table. The closest 1% of the simulated datasets to the observed data was used to estimate relative posterior probabilities per scenario. Type I and type II errors were calculated from 500 simulated data sets per scenario using the same prior distribution parameter values as the scenarios. Because Bayesian posterior probabilities were used, generated type I and type II errors are not reflective of a classical frequentist hypothesis framework, where the null is never accepted but rejected when data are incompatible with it [96]. When type II errors are small under the ABC approach there is good confidence in the results, even though type I errors can be large [97].

## Results

### Haplotype Diversity

We sequenced 360 bp of the control region for 183 contemporary samples and 26 out of 38 extracts for museum specimens (Table S1). Analyses were performed on 318 bp to include data from Walker et al. [15]. Three variable sites were lost when the 360 bp fragment was shortened to 318 bp. However, the inclusion of Walker et al. [15] data added two different variable sites, resulting in the overall loss of a single variable site. Haplotypes 'C' and 'J' from Tomasik and Cook [55] were amalgamated with haplotypes 'A' and 'F' from Wilson et al. [45], respectively (Table S3). In addition, haplotype 'Mong1' from Schwartz et al. [60] grouped with haplotype 'L' from Tomasik and Cook [55]. All three amalgamated haplotype pairs differed by one base pair. We compared all haplotypes to reports in the literature [45,48,49,51,55,59]. Although multiple studies identified identical haplotypes, naming was inconsistent among publications. All haplotypes identified for the control region of *G. gulo* were catalogued by publication date (Table S3).

A total of 39 haplotypes and 28 variable sites were identified from 983 individuals (Table S4), with 34 haplotypes observed in previous studies. All five new haplotypes identified in this study were sequenced from pelt samples, with the majority of the samples tested having high molecular weight DNA, and confirmed with independent PCRs. Voucher sequences of new haplotypes were submitted to GenBank (Accession Numbers: KC182788 -

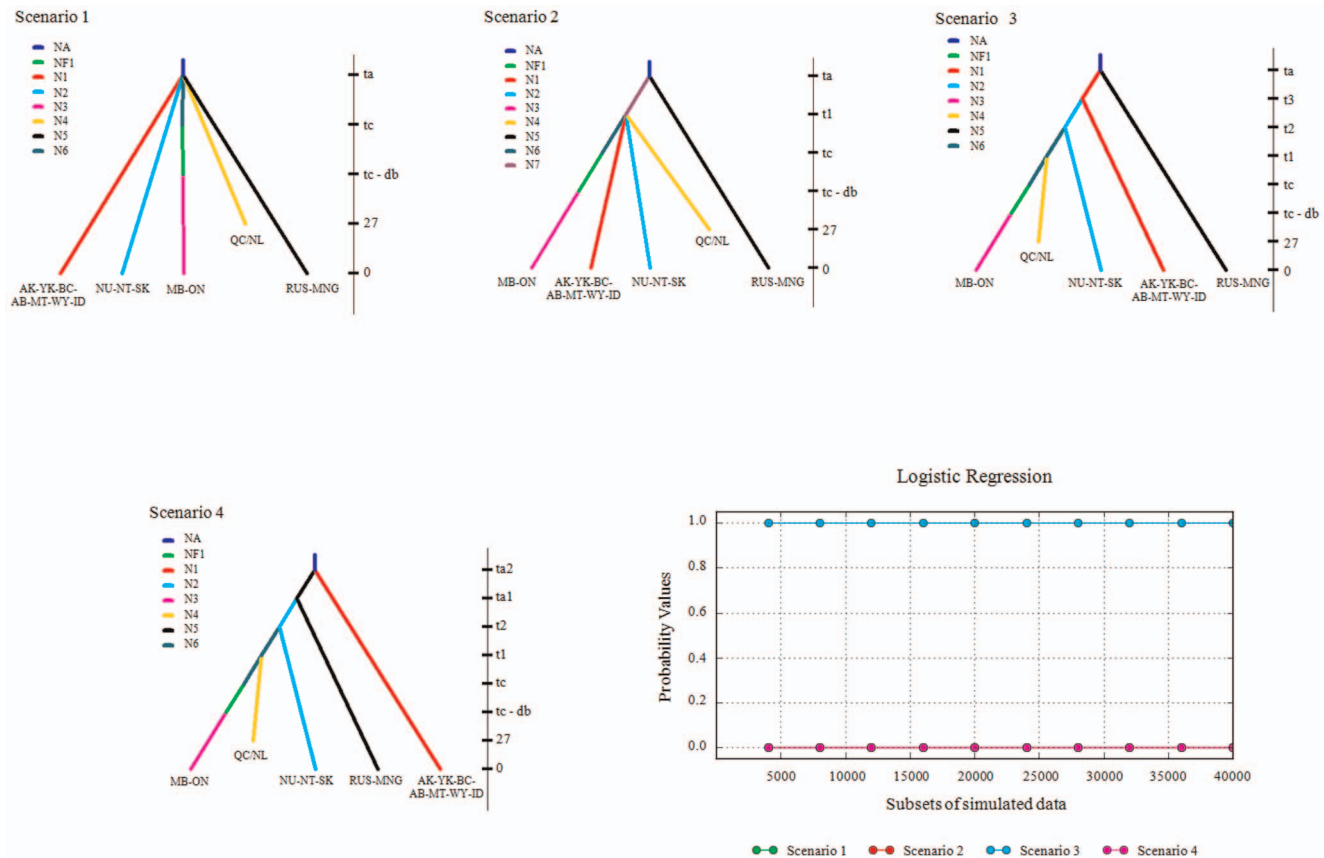


**Figure 2. Scenarios used in DIYABC and logistic regression.** Graphic representation of the four scenarios used in DIYABC and logistic regression of posterior probabilities with a recent bottleneck (20th century) for MB-ON. Please note that time is not to scale. doi:10.1371/journal.pone.0083837.g002

KC182792). The most common haplotype was Hap1, found within 36% of all individuals. Hap1 was only observed in the Western Hemisphere, comprising 46% of North America samples and even occurring on Vancouver Island. The next most frequent haplotypes were Hap10 and Hap8, comprising 17% and 9% of all samples, respectively (Figure 3, Table S5). Fourteen of the remaining haplotypes occurred at frequencies between 1–5% and represented 36% of the data. The remaining 22 haplotypes were considered rare, each <1% and overall comprising 2% of the data (Figure 3, Table S5).

Population genetic parameters were consistent among groupings of contemporary and historic samples, but we present only results where contemporary and historic samples were treated as separate entries. Nucleotide diversity ( $\pi$ ) was low across all regions, but highest in MB and ON (Table 1). Haplotype diversity ( $h$ ) ranged from 0 (NOR and ID) to 0.83 (NT and SK), and was highest in northwestern North America and Russia (Table 1). Accounting for sample size (standardized to  $g_{\text{CONTEMPORARY}} = 6$ , and  $g_{\text{HISTORIC}} = 5$ ), private haplotypes were highest in YK and RUS, and absent from NOR, WY, ID, BC<sub>H</sub>, and QC/NL<sub>H</sub> (0.00 to 1.16, Table 1). All tests, with the exception of two, produced non-significant values for both Tajima's  $D$  and Fu's  $F_s$  (Table 1). Significant values were observed for Tajima's  $D$  ( $-1.44$ ,  $P=0.04$ ) for Sweden and for Fu's  $F_s$  ( $-5.46$ ,  $P=0.04$ ) for Alaska.

### Genetic Structure

Pairwise  $\Phi_{\text{ST}}$  estimates (Table 2) revealed several differentiated groups. In Eurasia, wolverines were not genetically differentiated

between SWE and NOR ( $\Phi_{\text{ST}}=0.01$ ,  $P=0.36$ ), nor between MNG and RUS ( $\Phi_{\text{ST}}=-0.06$ ,  $P=0.79$ ). However, wolverines from SWE and NOR had high levels of divergence with MNG and RUS ( $\Phi_{\text{ST}} \geq 0.89$ ), and all four were differentiated from North America ( $\Phi_{\text{ST}} \geq 0.21$ ; Table 2). In North America, five main divisions were observed based on the pairwise  $\Phi_{\text{ST}}$  estimates that largely pooled the Arctic regions (NT, NU, northern SK), western regions (AK, YK, BC, AB, MT, WY, ID), and eastern regions (ON, QC/NL<sub>H</sub>). The additional two divisions included MB and CA<sub>H</sub> that were differentiated from the other North American regions (Table 2), with divisions being more pronounced for CA<sub>H</sub>.

SAMOVA identified negligible differences between  $\phi_{\text{CT}}$  values as  $K$  increased for all analyses, with the exception of  $K=2$  for North American samples with CA<sub>H</sub> and QC/NL<sub>H</sub>. This inflection point separated CA<sub>H</sub> from the remaining samples. An inflection point of diminished values was also observed for among population within groups variation across all data combinations. The  $K$  value for which  $\phi_{\text{ST}}$  values greatly decreased varied among data groups, however, a similar hierarchical pattern of genetic divisions was observed for all analyses (Table 3). The overall regional genetic groups delineated by  $\phi_{\text{ST}}$  results were SWE-NOR, MNG, RUS, CA<sub>H</sub>, QC/NL<sub>H</sub>, MB, NT-NU-SK, and the remaining samples. Weaker relationships were found when populations were grouped according to geographic distribution in ANOVA.

Mantel tests revealed a significant correlation between genetic and geographic matrices among sites ( $r=0.190$ ,  $P=0.001$ ), indicating that genetic differentiation was partially explained by

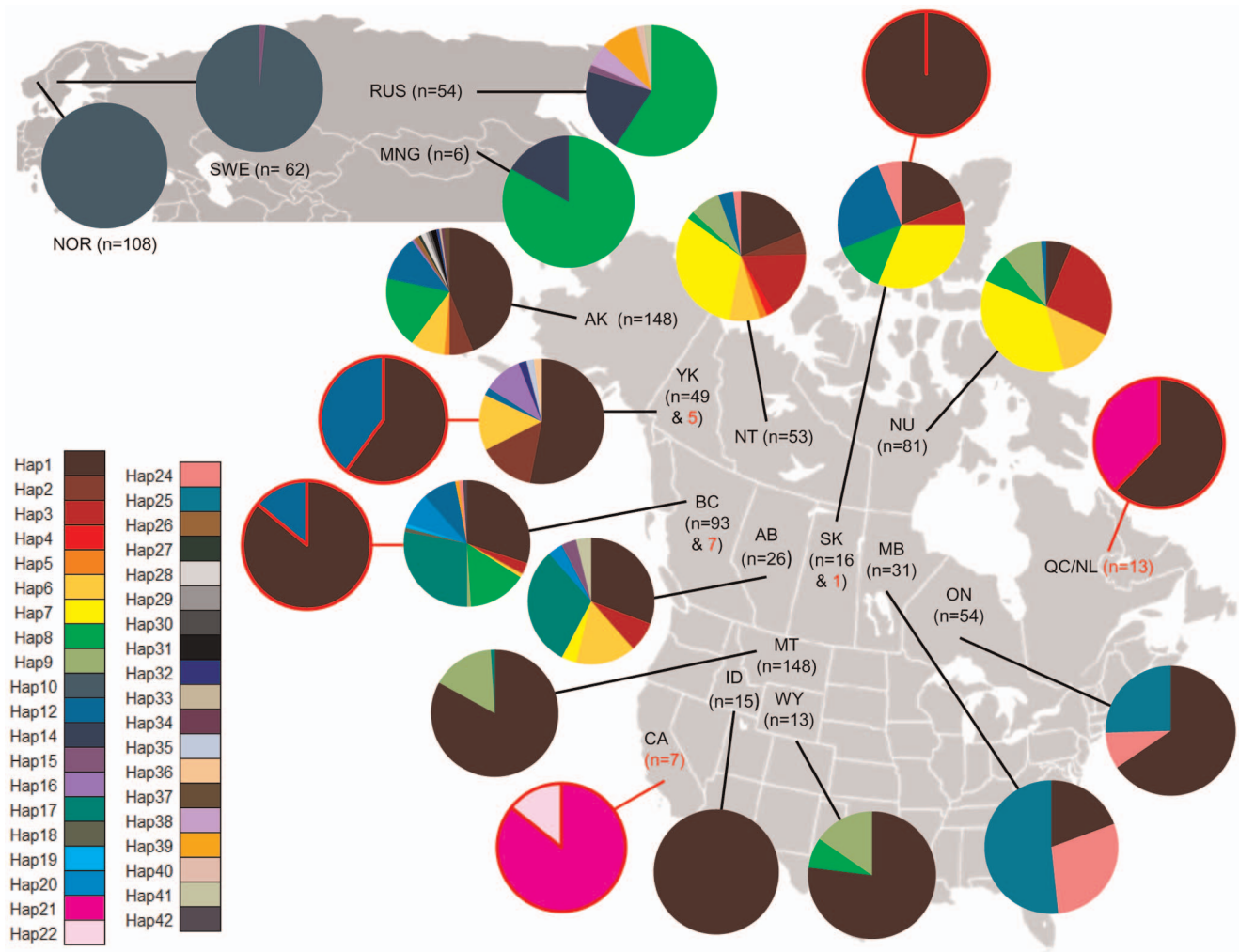


**Figure 3. Geographical distribution of mtDNA haplotypes.** The geographic distribution of mtDNA control region haplotypes based on frequencies observed for each region. Historic samples are presented separately and identified with a red border and red sample size number. doi:10.1371/journal.pone.0083837.g003

isolation by distance. Similarly, isolation by distance was also found to be significant among North American localities ( $r = 0.254$ ,  $P = 0.033$ ).

### Phylogenetic Analysis

The Bayesian phylogenetic tree (not shown) revealed low support (posterior probability  $< 0.75$ ) for all but one node in the derived wolverine phylogeny. The supported node (1.0 posterior probability) was found near the base of the tree, suggesting a single phylogenetic haplogroup across this carnivore's Holarctic range. The predominance of low bootstrap values is likely the result of haplotypes being separated by single point mutations as revealed by the median-joining network (Figure 4). Although the mismatch distribution did not display the pattern of a typical Poisson distribution, it was unimodal (Figure S1), and consistent with a rapid range expansion. The variance ( $SSD = 0.0099$ ;  $P = 0.636$ ) and Harpending's raggedness index ( $r = 0.026$ ;  $P = 0.799$ ) suggested that the observed mismatch distribution did not differ from the expected distribution of a population expansion model. Similarly, Fu's  $F_s$  test showed a signal of population expansion across all wolverine samples ( $F_s = -10.688$ ,  $P = 0.031$ ). The median-joining network revealed a star shaped topology associated with Hap1.

The main body of the network was characterized by several reticulations that included Hap3, Hap6, Hap7, Hap8, which primarily occurred in the northwestern North America and Russia (Figure 4). Additionally, the network identified eight mutational steps separating the two haplotypes predominately found in MB and ON, Hap24 and Hap25 (Figure 4).

### ABC Analysis

The selection of the most optimal model did not change whether or not a bottleneck was modeled within the MB-ON population. Results for both sets of analyses were comparable, thus we only present findings for the reference table with the population bottleneck for MB-ON. The DIYABC analysis revealed the third hypothesis of a single Beringia incursion during the LGM established the northwestern Pop1 and then followed by a west-to-east Holocene colonization was the most supported. The posterior probability of the logistic regression for scenario 3 was 1.0, with a type I error of 0.750 and a type II error of 0.147.

**Table 1.** MtDNA nucleotide diversity ( $\pi$ ), haplotype diversity ( $h$ ), haplotype richness ( $H_R$ ), private haplotype ( $H_P$ ) counts, their standard deviations ( $SD \pi$ ,  $SD h$ ,  $H_{Rstd}$ , and  $H_{Pstd}$ ) and standardized to the smallest sample size for both contemporary ( $g = 6$ ) and historic ( $g = 5$ ) samples using ADZE rarefaction, Tajima's  $D$ , and Fu's  $F_s$ .

| Region           | $n$ | $\pi$  | $SD \pi$ | $h$  | $SD h$ | $H_R$ | $H_{Rstd}$ | $H_P$ | $H_{Pstd}$ | Tajima's $D$ | $P$  | Fu's $F_s$ | $P$  |
|------------------|-----|--------|----------|------|--------|-------|------------|-------|------------|--------------|------|------------|------|
| Contemporary SWE | 62  | <0.001 | 0.001    | 0.03 | 0.03   | 2     | 1.10       | 0     | 0.07       | -1.44        | 0.04 | -0.57      | 0.14 |
| NOR              | 108 | 0      | 0        | 0    | 0      | 1     | 1          | 0     | 0          | 0            | 1    | -          | -    |
| MNG              | 6   | 0.002  | 0.002    | 0.33 | 0.22   | 2     | 2          | 0     | 0.24       | -1.13        | 0.15 | 0.95       | 0.61 |
| RUS              | 54  | 0.003  | 0.003    | 0.61 | 0.06   | 7     | 2.86       | 2     | 1.00       | -0.08        | 0.52 | -1.56      | 0.21 |
| AK               | 148 | 0.006  | 0.004    | 0.75 | 0.03   | 17    | 3.64       | 9     | 0.77       | -0.59        | 0.31 | -5.46      | 0.04 |
| YK               | 49  | 0.003  | 0.003    | 0.68 | 0.06   | 8     | 3.23       | 2     | 1.16       | -0.74        | 0.27 | -2.72      | 0.07 |
| NT               | 53  | 0.006  | 0.004    | 0.83 | 0.03   | 11    | 4.10       | 1     | 0.42       | 0.53         | 0.74 | -2.97      | 0.09 |
| NU               | 81  | 0.004  | 0.003    | 0.78 | 0.03   | 7     | 3.63       | 0     | 0.20       | 0.60         | 0.75 | -0.69      | 0.40 |
| BC               | 86  | 0.006  | 0.004    | 0.80 | 0.02   | 13    | 3.84       | 3     | 0.65       | -0.08        | 0.53 | -3.39      | 0.09 |
| AB               | 26  | 0.005  | 0.004    | 0.81 | 0.05   | 8     | 3.85       | 0     | 0.68       | 0.14         | 0.60 | -2.10      | 0.10 |
| SK               | 16  | 0.007  | 0.005    | 0.83 | 0.06   | 6     | 3.99       | 0     | 0.19       | 1.46         | 0.93 | -0.26      | 0.45 |
| MB               | 31  | 0.013  | 0.007    | 0.63 | 0.05   | 3     | 2.65       | 0     | 0.40       | 2.49         | 0.99 | 8.07       | 0.99 |
| ON               | 54  | 0.009  | 0.005    | 0.51 | 0.06   | 3     | 2.31       | 0     | 0.03       | 1.42         | 0.92 | 6.83       | 0.98 |
| MT               | 148 | 0.003  | 0.002    | 0.30 | 0.04   | 3     | 1.74       | 0     | 0.20       | 0.11         | 0.62 | 2.47       | 0.87 |
| WY               | 13  | 0.003  | 0.003    | 0.41 | 0.15   | 3     | 1          | 0     | 0          | -0.48        | 0.30 | 0.98       | 0.71 |
| ID               | 15  | 0      | 0        | 0    | 0      | 1     | 1          | 0     | 0          | 0            | 1    | -          | -    |
| Historic YK      | 5   | 0.008  | 0.006    | 0.60 | 0.18   | 2     | 2.00       | 0     | 0.29       | 1.57         | 0.96 | 3.02       | 0.91 |
| BC               | 7   | 0.004  | 0.003    | 0.29 | 0.20   | 2     | 1.71       | 0     | 0          | -1.36        | 0.08 | 2.05       | 0.83 |
| QC/NL            | 13  | 0.008  | 0.005    | 0.51 | 0.08   | 2     | 1.96       | 0     | 0          | 2.11         | 0.99 | 5.45       | 0.99 |
| CA               | 7   | 0.001  | 0.001    | 0.29 | 0.20   | 2     | 1.71       | 1     | 0.76       | -1.01        | 0.23 | -0.09      | 0.23 |

SWE = Sweden; NOR = Norway; MNG = Mongolia; RUS = Russia; AK = Alaska; YK = Yukon; NT = Northwest Territories; NU = Nunavut; BC = British Columbia; AB = Alberta; SK = Saskatchewan; MB = Manitoba; ON = Ontario; QC/NL = Quebec-Labrador; MT = Montana; WY = Wyoming; ID = Idaho; CA = California.  
doi:10.1371/journal.pone.0083837.t001

## Discussion

### Pleistocene Influence

Species with widespread contemporary distributions and/or highly mobile species are said to more likely to have occupied multiple glacial refugia during glaciations [98]. The star phylogeny and unimodal mismatch distribution, with non-significant sum of square deviations between observed and expected values, indicate a rapid range expansion of wolverines occurred from a single glacial refugium. The mismatch graph (Figure S1) did not display a standard Poisson distribution, but was similar to the mismatch distribution produced by Excoffier and Schneider [99] when an early population expansion also experienced a historic (400 generations ago) bottleneck. The further back in time bottlenecks take place, the longer the time period for increased genetic drift, resulting in increased variance of the mismatch distribution and higher frequencies of low difference classes (e.g. 0 and 1, [99]). This expansion event was also supported by a significantly negative Fu's  $F_s$  value for the whole data set. Although Fu's  $F_s$  statistic was not significant for individual regions, except for AK, the occurrence of negative values implies that there may be some deviation from neutrality for the northwestern region.

This expansion event from a single glacial refugium is in contrast to the phylogeographical structure of other cold adapted species such as woodland caribou (*Rangifer tarandus caribou*), a widely distributed and highly vagile ungulate. For woodland caribou a multimodal mismatch distribution pattern of mtDNA sequences [3] reflected postglacial expansions from three putative regions in North America [20]. Although data presented in this study were

collected throughout the Holarctic range of wolverines, certain areas like the region between Scandinavia and the Russian Far East remained unsampled. In this study, Scandinavia represented a geographic outlier. Implementing a more systematic sampling scheme may identify new haplotypes or missing branching lineages, or change the frequency and distribution of known haplotypes. This may help resolve some of the reticulated haplotypes in the network and provide a more comprehensive assessment of the relationship between Hap10 (SWE-NOR) and adjacent haplotypes. Furthermore, severe population declines experienced by Scandinavian populations [26], with drift likely undermining our ability to resolve historic processes and links with other populations.

Our observation of low nucleotide diversity among sequences may be attributed to the amplification of a 318 bp fragment of the mtDNA control region. This sequence length is likely not sufficient to provide the necessary resolution needed for making phylogenetic inferences as observed with the unsupported phylogenetic tree. This small fragment size was used to allow us to expand our study and compare samples from existing databanks and get the largest distribution possible, and is clearly a compromise that does put some limits on our interpretations. The presence of low nucleotide diversity has also been observed in other mustelid species like pine marten (*Martes martes*, [13]) and fisher (*Martes pennanti*, [100]). In the case of pine marten, similarly truncated mtDNA control region sequences were also used (320 bp), with results suggesting European colonization from a single refugium following a recent glaciation [13]. Although a slightly longer sequence of the mtDNA control region was used for woodland

**Table 2.** Pairwise estimates of population genetic distance for mtDNA among sampling localities ( $\Phi_{ST}$ , below diagonal), and associated  $P$  values (above diagonal).

|                        | SWE  | NOR   | MNG  | RUS   | AK    | YT <sub>C</sub> | YT <sub>H</sub> | NT   | NU    | BC <sub>C</sub> | BC <sub>H</sub> | AB   | SK   | MB   | ON   | QC/NL <sub>H</sub> | MT    | WY   | ID   | CA <sub>H</sub> |      |
|------------------------|------|-------|------|-------|-------|-----------------|-----------------|------|-------|-----------------|-----------------|------|------|------|------|--------------------|-------|------|------|-----------------|------|
| SWE •                  | 0.36 |       | 0.00 | 0.00  | 0.00  | 0.00            | 0.00            | 0.00 | 0.00  | 0.00            | 0.00            | 0.00 | 0.00 | 0.00 | 0.00 | 0.00               | 0.00  | 0.00 | 0.00 | 0.00            |      |
| NOR 0.01               | •    |       | 0.00 | 0.00  | 0.00  | 0.00            | 0.00            | 0.00 | 0.00  | 0.00            | 0.00            | 0.00 | 0.00 | 0.00 | 0.00 | 0.00               | 0.00  | 0.00 | 0.00 | 0.00            |      |
| MNG 0.97               | 0.99 | •     |      | 0.79  | 0.00  | 0.00            | 0.00            | 0.00 | 0.00  | 0.00            | 0.00            | 0.00 | 0.01 | 0.03 | 0.01 | 0.03               | 0.00  | 0.00 | 0.00 | 0.00            |      |
| RUS 0.89               | 0.93 | −0.06 | •    |       | 0.00  | 0.00            | 0.00            | 0.00 | 0.00  | 0.00            | 0.00            | 0.00 | 0.00 | 0.00 | 0.00 | 0.00               | 0.00  | 0.00 | 0.00 | 0.00            |      |
| AK 0.81                | 0.84 | 0.33  | 0.40 | •     |       | 0.00            | 0.33            | 0.00 | 0.00  | 0.00            | 0.40            | 0.01 | 0.00 | 0.00 | 0.00 | 0.00               | 0.00  | 0.30 | 0.00 | 0.00            |      |
| YT <sub>C</sub> 0.92   | 0.95 | 0.60  | 0.61 | 0.06  | •     |                 | 0.02            | 0.00 | 0.00  | 0.00            | 0.40            | 0.00 | 0.00 | 0.00 | 0.00 | 0.00               | 0.00  | 0.21 | 0.03 | 0.00            |      |
| YT <sub>H</sub> 0.67   | 0.99 | 0.53  | 0.61 | 0.01  | 0.23  | •               |                 | 0.06 | 0.00  | 0.09            | 0.53            | 0.05 | 0.25 | 0.06 | 0.14 | 0.05               | 0.04  | 0.29 | 0.06 | 0.00            |      |
| NT 0.86                | 0.91 | 0.31  | 0.41 | 0.14  | 0.33  | 0.13            | •               |      | 0.04  | 0.00            | 0.00            | 0.00 | 0.18 | 0.00 | 0.00 | 0.00               | 0.00  | 0.00 | 0.00 | 0.00            |      |
| NU 0.89                | 0.92 | 0.40  | 0.46 | 0.27  | 0.49  | 0.34            | 0.03            | •    |       | 0.00            | 0.00            | 0.00 | 0.03 | 0.00 | 0.00 | 0.00               | 0.00  | 0.00 | 0.00 | 0.00            |      |
| BC <sub>C</sub> 0.84   | 0.88 | 0.33  | 0.40 | 0.05  | 0.10  | 0.09            | 0.21            | 0.35 | •     |                 | 0.28            | 0.52 | 0.00 | 0.00 | 0.00 | 0.01               | 0.00  | 0.16 | 0.01 | 0.00            |      |
| BC <sub>H</sub> 0.97   | 0.99 | 0.65  | 0.53 | −0.01 | <0.01 | −0.01           | 0.25            | 0.47 | 0.02  | •               |                 | 0.18 | 0.01 | 0.01 | 0.16 | 0.11               | 0.62  | 0.80 | 0.32 | 0.00            |      |
| AB 0.91                | 0.95 | 0.40  | 0.47 | 0.07  | 0.11  | 0.15            | 0.23            | 0.40 | −0.01 | 0.05            | •               |      | 0.00 | 0.00 | 0.01 | 0.05               | 0.00  | 0.12 | 0.00 | 0.00            |      |
| SK 0.92                | 0.95 | 0.30  | 0.45 | 0.19  | 0.44  | 0.06            | 0.02            | 0.07 | 0.25  | 0.28            | 0.30            | •    |      | 0.00 | 0.00 | 0.00               | 0.00  | 0.00 | 0.00 | 0.00            |      |
| MB 0.79                | 0.86 | 0.21  | 0.36 | 0.33  | 0.42  | 0.19            | 0.28            | 0.37 | 0.34  | 0.27            | 0.31            | 0.19 | •    |      | 0.00 | 0.01               | 0.00  | 0.00 | 0.00 | 0.00            |      |
| ON 0.81                | 0.86 | 0.27  | 0.39 | 0.09  | 0.13  | 0.09            | 0.21            | 0.35 | 0.10  | 0.05            | 0.10            | 0.23 | 0.15 | •    |      | 0.09               | 0.00  | 0.08 | 0.01 | 0.00            |      |
| QC/<br>NL <sub>H</sub> | 0.91 | 0.95  | 0.27 | 0.39  | 0.19  | 0.29            | 0.22            | 0.31 | 0.47  | 0.12            | 0.19            | 0.09 | 0.32 | 0.18 | 0.06 | •                  |       | 0.00 | 0.04 | 0.01            | 0.01 |
| MT 0.91                | 0.93 | 0.67  | 0.67 | 0.09  | 0.07  | 0.21            | 0.38            | 0.53 | 0.12  | −0.05           | 0.15            | 0.50 | 0.54 | 0.19 | 0.40 |                    | •     | 0.71 | 0.12 | 0.00            |      |
| WY 0.96                | 0.98 | 0.59  | 0.59 | 0.01  | 0.02  | 0.08            | 0.22            | 0.43 | 0.03  | −0.09           | 0.05            | 0.30 | 0.29 | 0.06 | 0.20 |                    | −0.03 | •    | 0.09 | 0.00            |      |
| ID 0.99                | 1.00 | 0.92  | 0.71 | 0.12  | 0.07  | 0.52            | 0.41            | 0.60 | 0.11  | 0.12            | 0.17            | 0.54 | 0.41 | 0.14 | 0.36 |                    | 0.06  | 0.12 | •    | 0.00            |      |
| CA <sub>H</sub> 0.98   | 1.00 | 0.84  | 0.66 | 0.67  | 0.82  | 0.80            | 0.69            | 0.76 | 0.65  | 0.87            | 0.70            | 0.69 | 0.37 | 0.55 | 0.49 |                    | 0.85  | 0.84 | 0.98 | •               |      |

SWE = Sweden; NOR = Norway; MNG = Mongolia; RUS = Russia; AK = Alaska; YT = Yukon; NT = Northwest Territories; NU = Nunavut; BC = British Columbia; AB = Alberta; SK = Saskatchewan; MB = Manitoba; ON = Ontario; QC/NL = Quebec-Labrador; MT = Montana; WY = Wyoming; ID = Idaho; CA = California; <sub>C</sub> = Contemporary; <sub>H</sub> = Historic.  
doi:10.1371/journal.pone.0083837.t002

**Table 3.** Results of delineated genetic groupings identified by SAMOVA for different population configurations.

| Data Assemblage                                                | Delineated Genetic Grouping                                                                               | Variance Components | Percentage of Variance | $\phi$ -statistics    | $P$    |
|----------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------|---------------------|------------------------|-----------------------|--------|
| Eurasia, North America, CA <sub>H</sub> , & QC/NL <sub>H</sub> | $K = 8$ (SWE-NOR vs. MNG vs. RUS vs. CA <sub>H</sub> vs. QC/NL <sub>H</sub> vs. MB vs. NT-NU-SK vs. Rest) |                     |                        |                       |        |
|                                                                | Among groups                                                                                              | 1.38                | 68.55                  | $\phi_{CT} = 0.68549$ | <0.001 |
|                                                                | Among populations within groups                                                                           | 0.06                | 2.90                   | $\phi_{SC} = 0.09226$ | <0.001 |
|                                                                | Within populations                                                                                        | 0.57                | 28.55                  | $\phi_{ST} = 0.71451$ | <0.001 |
| Eurasia & North America                                        | $K = 7$ (SWE-NOR vs. MNG vs. RUS vs. MB vs. NT-NU vs. SK vs. Rest)                                        |                     |                        |                       |        |
|                                                                | Among groups                                                                                              | 1.39                | 68.88                  | $\phi_{CT} = 0.68876$ | <0.001 |
|                                                                | Among populations within groups                                                                           | 0.06                | 2.95                   | $\phi_{SC} = 0.09490$ | <0.001 |
|                                                                | Within populations                                                                                        | 0.57                | 28.17                  | $\phi_{ST} = 0.71829$ | <0.001 |
| North America, CA <sub>H</sub> & QC/NL <sub>H</sub>            | $K = 5$ (CA <sub>H</sub> vs. QC/NL <sub>H</sub> vs. MB vs. NT-NU-SK vs. Rest)                             |                     |                        |                       |        |
|                                                                | Among groups                                                                                              | 0.43                | 35.54                  | $\phi_{CT} = 0.35540$ | <0.001 |
|                                                                | Among populations within groups                                                                           | 0.07                | 5.49                   | $\phi_{SC} = 0.08510$ | <0.001 |
|                                                                | Within populations                                                                                        | 0.71                | 58.97                  | $\phi_{ST} = 0.41026$ | <0.001 |
| North America                                                  | $K = 3$ (MB vs. NT-NU-SK vs. Rest)                                                                        |                     |                        |                       |        |
|                                                                | Among groups                                                                                              | 0.37                | 32.62                  | $\phi_{CT} = 0.32624$ | <0.001 |
|                                                                | Among populations within groups                                                                           | 0.07                | 5.78                   | $\phi_{SC} = 0.08575$ | <0.001 |
|                                                                | Within populations                                                                                        | 0.70                | 61.60                  | $\phi_{ST} = 0.38401$ | 0.001  |

SWE = Sweden; NOR = Norway; MNG = Mongolia; RUS = Russia; NT = Northwest Territories; NU = Nunavut; SK = Saskatchewan; MB = Manitoba; QC/NL<sub>H</sub> = Historic Quebec-Labrador; CA<sub>H</sub> = Historic California.  
doi:10.1371/journal.pone.0083837.t003

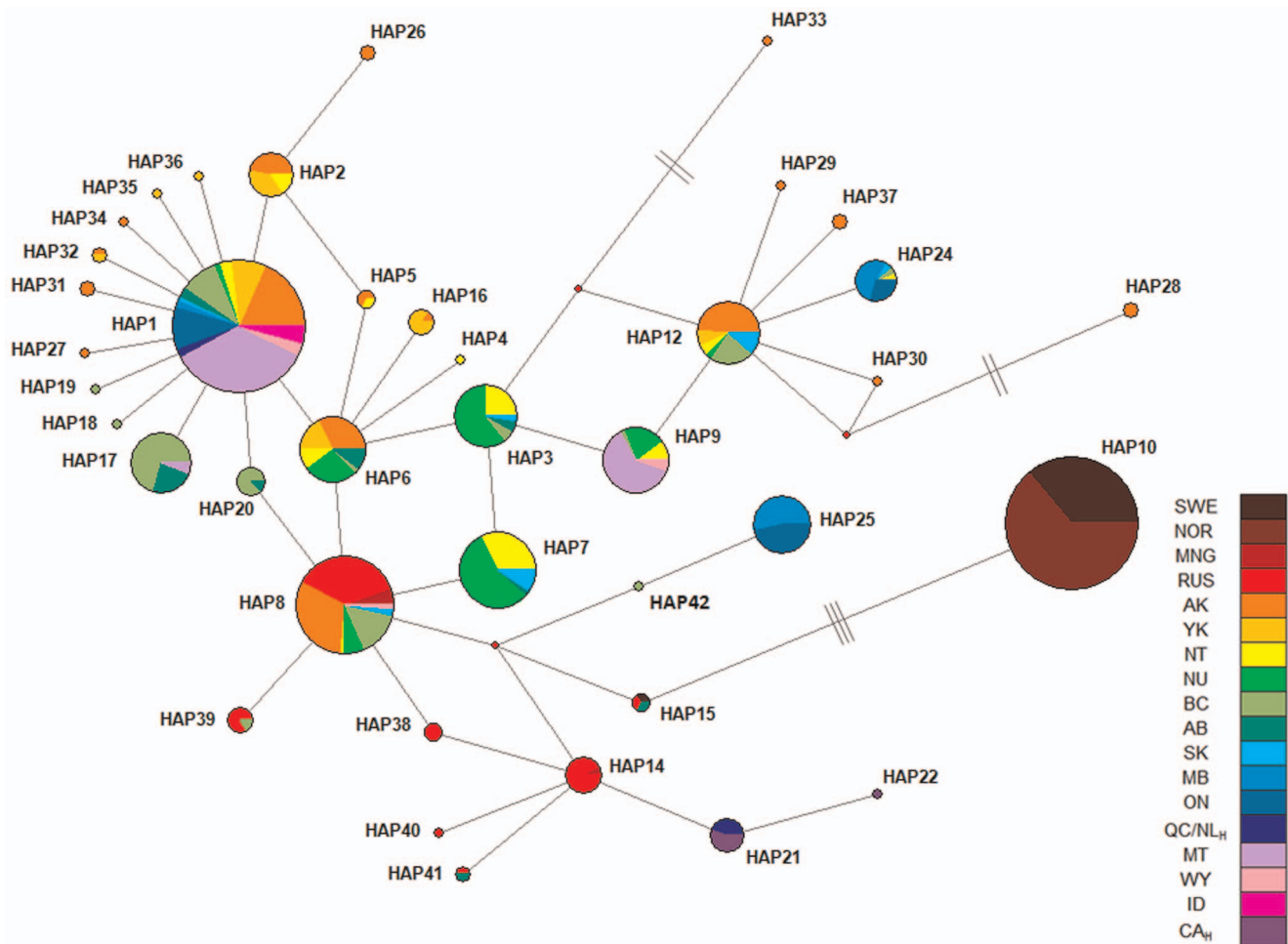


**Figure 4. Median-joining network.** Median-joining network of the mtDNA control region haplotypes for all samples combined. Haplotype size reflects relative frequency. Each branch represents one mutational step, unless otherwise noted. Black circles represent missing intermediate haplotypes.

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caribou (429 bp, [20]), the finding of three highly supported phylogroups may be reflective of woodland caribou's classification into different ecotypes. This shows that such a small mtDNA fragment can provide insight into phylogeographic processes.

If multiple refugial lineages persisted in present-day wolverine populations, we would expect to find clearly delineated subclades for each glacial refugium. Our network would also have numerous substitutions along branches connecting each of the subclades, as observed in the Nearctic clade d-loop network of the red fox [101]. The most differentiated haplotype was Hap10 found in SWE and NOR, but this haplotype was separated by only three mutations from Hap15 found in RU and AB. This step-wise mutation pattern does not provide evidence of longstanding genetic differences of animals isolated by multiple glacial refugia, but is suggestive of long range dispersal movements characteristic of wolverines. Single mutational step differences among control region haplotypes were also observed for the arctic fox (*Alopex lagopus*), with several haplotypes having a Holarctic distribution indicative of the long-distance dispersal capabilities of this carnivore [1]. Although our data suggests wolverines likely underwent a postglacial expansion from a single glacial refugium, the colonization pattern of this expansion and its effect on contemporary genetic structure of mtDNA remains unknown. A hypothesis

testing approach could provide insights into different recolonization scenarios, and distinguish among these alternative hypotheses the most likely one.

#### Hypothesis Testing of Population Divergence Scenarios

The most supported ABC model was a single incursion from Beringia during the LGM giving rise to a northwestern AK-YK-BC-AB-MT-WY-ID population, followed by a west-to-east stepping-stone divergence scenario during the Holocene. This pattern is suggestive of recolonization occurring in accordance with glacial retreat, where initial recolonization along the western coast of North America was followed by an inland recolonization pattern [102]. The inclusion of a recent bottleneck for the MB-ON populations did not change which scenario was best supported. Although scenario 3 had the highest posterior probability and a low type II error, summary statistics for all simulated data sets did not surround the observed data set, reflecting a poor fit by all four scenarios. This does not necessary mean that scenario 3 was erroneously selected, but that additional events may need to be incorporated in the proposed colonization patterns. One likely explanation for the poor fit may be the omission of a historic bottleneck across multiple populations as indicated by the mismatch distribution. While the mismatch analysis identified a

historic bottleneck, additional information such as duration, time period, and geographic extent were not provided, but required for DIYABC simulations when modeling a bottleneck event. In addition, the DIYABC limitation that dispersal is absent among populations once they have diverged [103] may contribute to our poor fit between simulated and observed data sets. Both male and female wolverines have demonstrated extensive dispersal distances [51,104], resulting in gene flow among diverged populations. Finally, our use of a short mtDNA fragment may not provide enough resolution to establish a good fit between simulated and observed data sets despite ABC suggesting some models are highly probable. We suggest that a cautionary approach be taken regarding ABC model choice, and this method be viewed as an exploratory tool [105].

### Historic and Contemporary Influences

The Bering Strait represents a barrier to wolverine movement across Eastern and Western Hemispheres. However, excluding the Eurasian samples from Mantel tests still produced a low  $r$  value ( $r=0.293$ ) for North America. Tomasik and Cook [55], and Cegelski et al. [49] found no support for IBD and suggested barriers may be influencing differentiation patterns. Extrinsic factors that appear to influence wolverine genetic structure include summer temperatures, spring snow cover and ecological changes associated with anthropogenic land use activities [36,43,52–54]. Additionally, our use of a maternal marker for a carnivore with male biased dispersal [50] would result in a stronger isolation by distance value particularly for the philopatric sex [106], but also be more apparent across all individuals. This absence of a stronger IBD further supports the influence of external factors on population differentiation.

Negligible differences between  $\Phi_{CT}$  statistics of SAMOVA for  $K$  values before and after inflection points were also observed by Schwartz et al. [60], while Frances [59] found an overall lack of geographic structure. Our SAMOVA analyses revealed lower  $K$  values being representative of SWE-NOR, CA<sub>H</sub>, MNG, QC/NL<sub>H</sub>, and MB. Schwartz et al. [60] also observed CA<sub>H</sub> as a separate group for  $K>2$ , and that SWE-NOR separated from all other locations at  $K=2$ . These lower  $K$  groupings represent peripheral regions of the wolverine's contemporary distribution. Range peripheries are generally characterized by decreased density, ecologically marginal habitats and isolation [24,107] that may bring about more frequent extinction-recolonization events, and likely result in a distinct diversity gradient as the species range edge is approached (see Peterman et al. [108]). Alternatively, more pronounced selection pressures in conjunction with reduced gene flow at range peripheries may lead to increased genetic distinctiveness of edge populations [23,24].

Event though we observed spatial genetic structuring of mtDNA over small geographic scales, reflecting female philopatry [49,55], we also found haplotypes displaying disjointed distributions. Hap15 was found in SWE and RUS, while Hap21 occurred in CA<sub>H</sub> and QC/NL<sub>H</sub> - the two most peripheral populations in North America. The presence of Hap21 and Hap15 in QC/NL<sub>H</sub> and RUS samples in this study, and their independent observation in CA<sub>H</sub> [60] and SWE [58] supports that our observation of these haplotypes are not the result of sequencing errors. The patchy distribution of Hap15 and Hap21 may reflect leptokurtic dispersal [109] following post-glacial colonization. Alternatively, both haplotypes may have been more widely distributed in the past but have since decreased in frequency or become lost from adjacent regions due to direct human persecution in both hemispheres [26,65], extensive range contractions in North America [52], and random effects of genetic drift. To distinguish

between long-distance dispersal and fragmentation, additional sampling is needed between Scandinavia and the Russian Far East, along with the inclusion of supplementary history samples from adjacent regions of CA<sub>H</sub> and QC/NL<sub>H</sub>.

Manitoba and Ontario were characterized by Hap1, Hap24, and Hap25, with the latter two haplotypes occurring almost exclusively in these two regions (Figure 3). The separation of MB and ON by both SAMOVA and pairwise  $\Phi_{ST}$  estimates was unexpected based on the very different composition of MB and ON in comparison to the other regions, and that microsatellite data group MB and ON as a separate genetic cluster from the panmictic northwest population [51]. Based on this, MB and ON should be pooled together as a separate genetic group. Contrasting haplotype frequencies among the three regions likely explains why MB and ON were separated into different genetic clusters [51]. In particular, the higher frequency of Hap1 in ON (65%) compared to MB (19%) is presumed to have influenced the grouping of ON with other regions also having high Hap1 frequencies. This high Hap1 frequency in ON may have occurred as a result of samples consisting of closely related individuals than expected by chance. The majority of samples from ON were collected using baited hair snares encompassing 2 000 km<sup>2</sup> [110], increasing the likelihood of close relatives. Alternatively, substantial population declines during the early 1900s [65], where densities have historically been considered low, likely increased the effects of genetic drift and strong female philopatry continuing to maintain altered haplotype frequencies [45,55,60].

### Conservation Implications

Pairwise  $\Phi_{ST}$  estimates pooled QC/NL<sub>H</sub> with contemporary ON. This grouping is likely due to Hap21 (QC/NL<sub>H</sub>) and Hap25 (ON) being derived from the same missing intermediate haplotype (Figure 4), and the high frequency of Hap1 in both regions. This genetic clustering of QC/NL<sub>H</sub> with the extant peripheral cluster of MB-ON brings into question the classification of wolverines from Quebec-Labrador as the 'eastern' population. This potential grouping will also influence the selection of a source population for the proposed translocation of wild animals as outlined in the national recovery plan for the eastern wolverine population in Quebec-Labrador [38]. However, this grouping does not preclude the possibility of QC/NL<sub>H</sub> with MB-ON being considered a designatable unit. For a population to be identified as a designatable unit (DU), the population needs to be recognized as being discrete and evolutionary significant [111]. Using both mtDNA and microsatellites, Zigouris et al. [51] found MB-ON formed a separate genetic cluster from core regions (NT-NU-SK). In addition, genetic analysis of multilocus genes found in the major histocompatibility complex (MHC) for wolverines across their Canadian range revealed a duplicated DRB exon 2 with the peptide binding region having a significant excess of non-synonymous substitutions - indicative of positive selection acting on MHC of wolverines [112]. Further research is needed on wolverines along their eastern range edge in North America, including combining ecological data with genetic markers under natural selection [113,114], in order to appropriately define a population as a DU [111].

### Conclusions

In contrast to previous glacial refugia studies of arctic species (e.g. [18–20]), we found no molecular evidence of wolverines inhabiting multiple glacial refugia during the last glacial maximum. In addition, approximate Bayesian computations supported a wolverine colonization of North American where

individuals followed retreating glaciers. Even with molecular evidence of a single expansion event, significant subdivisions of population genetic structure over small spatial scales were observed. This genetic structure reflected historic population declines throughout the wolverine's Holarctic range, as indicated by the mismatch distribution, along with the influence of subsequent genetic drift and strong female philopatry (e.g. [45,55,60]). We acknowledge that the mtDNA analyses in this study were based on a short sequence fragment of the control region, restricting the resolution needed for us to make conclusive inferences. However, these data provide some insights into the post-glacial colonization and phylogeographic relationships among contemporary wolverine populations. In particular, our observation of low genetic differentiation between QC/NL<sub>H</sub> and ON, which puts into question the designation of Quebec-Labrador as the 'eastern' population [115]. These underlining genetic associations among regions highlight key areas and questions where future research should focus on, including using longer mtDNA fragment or alternative markers like single nucleotide polymorphisms (SNPs) to obtain deeper resolution of phylogeographic history. We also suggest further research be undertaken with functional markers to investigate the possibility of local adaptation among the different genetic clusters. This information will have strong implications for the identification of designatable units [111], and future conservation and recovery activities of wolverines, particularly at the eastern periphery of their North American range.

## Supporting Information

**Figure S1 Mismatch distribution.** Pairwise mismatch distribution performed on mtDNA haplotypes among individuals. Bars represent observed values, a solid line represents the expected distribution according to the sudden expansion model, and dotted lines show  $\pm 95\%$  confidence intervals. (TIF)

**Table S1 Catalogue information of all wolverine samples obtained from collections and if samples were sequenced.**

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(DOC)

**Table S2 Minimum and maximum limits and associated conditions of parameters used in DIYABC analyses.** (DOC)

**Table S3 Control region haplotype labels discussed in this study and their corresponding designation(s) as identified in previous studies. Sequential numbering of each haplotype is reflective of when it was first published in the literature.** (DOC)

**Table S4 Variable nucleotide positions of identified haplotypes are denoted according to their location within the 318 bp portion of the D-loop mtDNA control region.** (DOC)

**Table S5 Percent frequencies of wolverine mtDNA control region haplotypes within and across regions.** (DOC)

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## Author Contributions

Conceived and designed the experiments: JZ JAS CJK. Performed the experiments: JZ. Analyzed the data: JZ. Contributed reagents/materials/analysis tools: JZ CF CJK. Wrote the paper: JZ. Revised MS critically for important intellectual content: JAS CJK.

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## Wolverine gene flow across a narrow climatic niche

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**Abstract.** Wolverines (*Gulo gulo*) are one of the rarest carnivores in the contiguous United States. Effective population sizes in Montana, Idaho, and Wyoming, where most of the wolverines in the contiguous United States exist, were calculated to be 35 (credible limits, 28–52) suggesting low abundance. Landscape features that influence wolverine population substructure and gene flow are largely unknown. Recent work has identified strong associations between areas with persistent spring snow and wolverine presence and range. We tested whether a dispersal model in which wolverines prefer to disperse through areas characterized by persistent spring snow cover produced least-cost paths among all individuals that correlated with genetic distance among individuals. Models simulating large preferences for dispersing within areas characterized by persistent spring snow explained the data better than a model based on Euclidean distance. Partial Mantel tests separating Euclidean distance from spring snow-cover-based effects indicated that Euclidean distance was not significant in describing patterns of genetic distance. Because these models indicated that successful dispersal paths followed areas characterized by spring snow cover, we used these understandings to derive empirically based least-cost corridor maps in the U.S. Rocky Mountains. These corridor maps largely explain previously published population subdivision patterns based on mitochondrial DNA and indicate that natural colonization of the southern Rocky Mountains by wolverines will be difficult but not impossible.

**Key words:** corridors; effective population size; geographic information system; *Gulo gulo*; landscape resistance; least-cost paths; population genetics; wolverines.

### INTRODUCTION

Habitat fragmentation has been shown to decrease dispersal, increase mortality (Fahrig et al. 1995), and reduce genetic diversity of wildlife populations (Saccheri et al. 2001, Goossens et al. 2006), thereby increasing extinction risks (Allendorf and Luikart 2006). Despite the clear importance of habitat connectivity for population persistence, specific factors mediating connectivity are largely unknown for most species (Bowne and Bowers 2004). Thus, managers and conservationists face a unique challenge in trying to enhance connectivity without species-specific information on movement, dispersal, and gene flow.

One such species where this challenge exists is the wolverine (*Gulo gulo*), a member of the mustelid family known for large home ranges (between 73 km<sup>2</sup> and >950 km<sup>2</sup>), exceedingly low densities (0.3–6.2 wolverine/1000 km<sup>2</sup>) and long distance movements (Hornocker and Hash 1981, Lofroth and Krebs 2007). In fact, movements exceeding 150 km are not uncommon for

both sexes of wolverines (Copeland 1996, Inman et al. 2004) with reports of dispersal events as far as 378 km (Gardner et al. 1986, Moriarty et al. 2009). The wolverine is also a species of management interest and has recently been considered for listing under the U.S. Endangered Species Act (USFWS 2008).

Initial beliefs by researchers were that wolverines could persist in a broad array of habitat types (Pasitschniak-Arts and Lariviere 1995). More recently, this view has been challenged as fine-scale wolverine occurrence, documented via radiotelemetry, has been associated with high elevation alpine and avalanche environments (Copeland et al. 2007, Krebs et al. 2007, Lofroth and Krebs 2007). At a broader scale, the only habitat feature that has been correlated with the historical distribution of wolverines in North America is spring snow cover (Aubry et al. 2007; J. P. Copeland et al., *unpublished manuscript*). J. P. Copeland et al. (*unpublished manuscript*) suggest that wolverines are obligate habitat specialists that require spring snow cover. This is partially due to female wolverine's preference for denning in snow caves (Magoun and Copeland 1998). However, this ecological association with spring snow cover also extends to year-round locations throughout the species worldwide distribution (J. P. Copeland et al., *unpublished manuscript*). What

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remains unknown is the degree to which this ecological association affects dispersal, colonization, and gene flow.

One way to investigate the influence of landscape features on gene flow is to transform landscape features into putative costs to movement, derive matrices of least-cost paths among individuals across these hypothetical landscapes, and then correlate the least-cost paths with matrices of genetic relatedness among individuals (Manel et al. 2003, Coulon et al. 2006, Cushman et al. 2006, Cushman et al. 2009). For example, Cushman et al. (2006) used least-cost path modeling with black bears (*Ursus americanus*) to show that patterns of genetic structure were primarily related to landscape gradients of land cover and elevation. Using a similar approach, McRae and Beier (2007) modeled landscapes as electrical circuits with movement resistance modeled as resistors in an interconnected web. While McRae and Beier (2007) investigated wolverine population substructure in relation to the physical shape of the wolverine's distribution, with all nodes within the range having equal resistance, their method is compatible with Cushman et al.'s (2006) multiple-model approach.

When using genetic patterns to infer connectivity, it is often unclear if the genetic pattern has arisen recently or if there is a significant time lag associated with the pattern. The longer the time lag between changes in connectivity and resultant genetic structure, the less pertinent the results are for directing current management and the more difficult the interpretation becomes. The time lag between a change in connectivity and its genetic detectability is directly related to effective population size (Nei and Chakravarti 1977), with small effective population sizes being associated with rapid divergence. Historically, estimation of effective population size required two temporally separated samples (reviewed in Schwartz et al. 1998), precluding its calculation in many cases. Recently, estimates of effective population size based on single samples have been developed (Tallmon et al. 2008, Waples and Do 2008), allowing estimates to be based on the same samples used to determine spatial patterns.

The bioclimatic envelope hypothesis provided by J. P. Copeland et al. (*unpublished manuscript*) defines wolverine distribution as a discrete space within which we can investigate questions of wolverine gene flow. Here, using wolverine samples from across its distribution in the U.S. Rocky Mountains, we ask the following questions: (1) Does the arrangement of areas of persistent spring snow cover explain the observed genetic distance among individuals after controlling for the effects of Euclidean distance and (2), if so, what major movement pathways (corridors) are suggested by our models that link areas of spring snow? We then evaluate effective population size, to provide inference for the time lag associated with these genetic patterns. Last, we use these findings to evaluate previously published subpopulation divisions and assess colonization potential between extant popu-

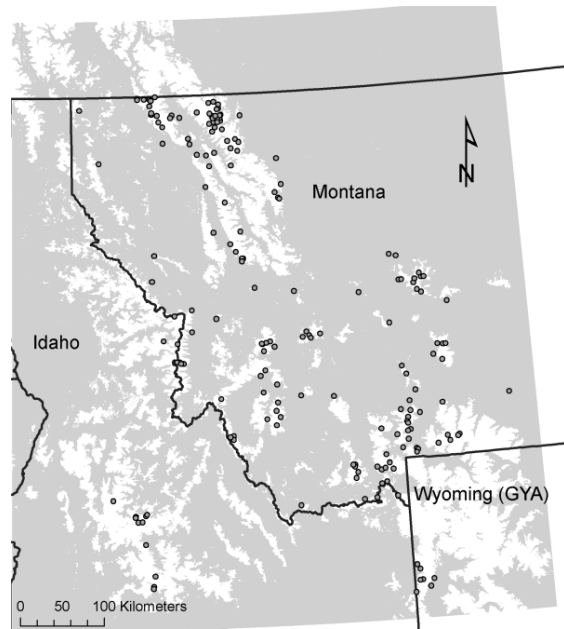


FIG. 1. Map of the northern U.S. Rockies. The white areas are locations of the spring snow cover bioclimatic envelope (J. P. Copeland et al., *unpublished manuscript*), whereas the gray areas do not have spring snow cover. Circles are locations of samples collected between 1989 and 2006. GYA stands for the Greater Yellowstone Area.

lations and areas of historical extirpation in the southern Rocky Mountains.

## METHODS

### Study area

The study area consists of a region covering northern and central Idaho, western Montana, and northwest Wyoming (Fig. 1). The area is composed of discrete mountain ranges each characterized by high alpine areas (maximum elevation 3900 m) and surrounded by wide areas of semiarid grasslands and irrigated agriculture (elevation ~1400 m). Within this landscape, areas characterized by persistent spring snow cover are fairly contiguous in the northwest portion of the study area, but become increasingly fragmented to the south and east, with the exception of the Greater Yellowstone Area (GYA), providing another large, contiguous area of persistent spring snow cover at the southern edge of the study area.

### Genetic samples and laboratory analyses

Two-hundred ten wolverine tissue samples were examined in this analysis (Fig. 1). One hundred forty-two samples were harvest samples collected by the Montana Department of Fish Wildlife and Parks between 1989 and 2006 while the remainder were collected between 1992 and 2007 as part of ongoing research efforts in Idaho, Montana, and Wyoming

(Copeland 1996, Ulizio et al. 2006, Copeland et al. 2007, Inman et al. 2007, Squires et al. 2007).

All samples were analyzed at 16 microsatellite loci used in previous mustelid studies (Kyle and Strobeck 2001, 2002, Cegelski et al. 2003, 2006, Zielinski et al. 2006, Schwartz et al. 2007): *Gg3*, *Gg4*, *Gg7*, *Ma2*, *Ma8*, *Ma9*, *Tt1*, *Tt4* (Davis and Strobeck 1998); *Ggu101*, *Ggu216*, *Ggu234*, *Ggu238* (Duffy et al. 1998); *Mvis020*, *Mvis72*, *Mvis075* (Flemming et al. 1999); and *Lut604* (Dallas and Piertney 1998). We amplified microsatellites using protocols detailed in the respective sources. The resultant products were visualized on a LI-COR DNA analyzer (LI-COR Biotechnology, Lincoln, Nebraska, USA) and independently scored by two technicians before being accepted as reliable.

Mitochondrial DNA (mtDNA) patterns among wolverine sampling locations have been examined extensively (Wilson et al. 2000, Walker et al. 2001, Tomasik and Cook 2005, Cegelski et al. 2006, Schwartz et al. 2007). In this study we reexamined 17 samples taken from wolverines in the areas of persistent spring snow cover in the Crazy and Little Belt Mountains using the 344 bp segment of the left domain of the mtDNA control region (see Shields and Kocher 1991 for protocol details). We use these mtDNA data as an independent test of our least-cost path corridor analysis, which was developed based on microsatellite data.

#### *Descriptive statistical genetic analyses*

Descriptive statistics such as the number of alleles per locus, Hardy Weinberg proportion tests (Bonferonni corrected), and expected and observed heterozygosity were calculated using GENALEX (Peakall and Smouse 2006) and GENEPOP (Raymond and Rousset 1996). Spatial autocorrelation between samples was estimated using GENALEX (Peakall and Smouse 2006) using 25 bins of 25 km, although the influence of various binning classes was investigated. Variance estimates for spatial autocorrelation were based on 9999 permutations and 999 bootstraps.

We used two measures of genetic distance in these analyses, the proportion of shared alleles ( $D_{PS}$ ; Bowcock et al. 1994) and the pairwise individual-by-individual squared genetic distances of Smouse and Peakall (1999). Except for autocorrelation analyses, we only present the  $D_{PS}$  results as the two were highly correlated (Mantel test;  $P = 0.01$ ,  $r = 0.942$ ).

#### *GIS modeling of the spring snow cover niche*

Aubry et al. (2007) found that the historical distribution of wolverine were consistent with the distribution of persistent spring snow; J. P. Copeland et al. (*unpublished manuscript*) used seven years of classified moderate resolution imaging spectroradiometer (MODIS) satellite images (Hall et al. 2006) from 2000 to 2006 at a 0.5-km<sup>2</sup> resolution to define a more detailed area of persistent snow. Specifically, Copeland et al. characterized persistent spring snow cover in the

entire northern hemisphere based on a 21-day composite (24 April–15 May) of images. They found that >99% of wolverine den sites and >89% year-round telemetry locations were located within areas that were classified as having persistent spring snow in at least one of the seven years for which data were available. We used Copeland et al.'s area to test if this bioclimatic niche facilitated wolverine gene flow. While the seven-year window used by Copeland et al. is limited by available data, it was important to identify an area which encloses virtually all den sites. Therefore, we can say that effectively all wolverines that mated and transferred genes within this landscape were born within the areas identified as having persistent spring snow, and thus we can be reasonably certain that we are not missing any potential sources of wolverine genes when analyzing these landscapes.

We built hypothetical resistance surfaces by setting the value of areas not snow covered to resistances of 5, 10, 15, 20, 50, and 100 per raster while keeping snow-covered raster resistance costs at 1. We used the ArcView (Environmental Systems Research Institute, Redlands, California, USA) extension PATHMATRIX (Ray 2005) and our own ARC Macro Language scripts (Cushman et al. 2006) to compute pairwise least-cost paths between all wolverine locations for each resistance surface. Additionally, we computed the pairwise straight-line (Euclidean) distances between wolverine locations (equivalent to least-cost paths with all rasters set to 1) in ArcView. Following Cushman et al. (2006), we used Mantel tests to determine correlation significance between matrices of pairwise movement costs and genetic distances, and partial Mantel tests to separate the effects of remaining in snow areas from those due to Euclidean distance. All Mantel and partial Mantel tests were conducted using the Ecodist library (Goslee and Urban 2007) in program R (*available online*).<sup>6</sup> There has been debate regarding the use of partial Mantel tests (see Raufaste and Rousset 2001, Castellano and Balletto 2002); however, this approach is still the most commonly used method (e.g., Walker et al. 2008) for examining the association among distance matrices in the genetics literature.

#### *Corridor analysis and population subdivision*

We overlaid a 15 × 15 km grid of locations across the study area, eliminating those grid nodes that were not in areas characterized by spring snow to model corridors connecting spring snow areas large enough to support wolverine reproduction. This density of nodes was based on minimum female home range size (Banci 1994) and guaranteed that at least one grid node would occur in each snow-covered area large enough to support at least one resident female wolverine (Krebs et al. 2007). We then computed pairwise least-cost paths between all

<sup>6</sup> [r-project.org/](http://r-project.org/)

nodes. We applied a 2500 m radius parabolic kernel around each one-raster wide path (Cushman et al. 2009) on the assumption that the least-cost paths represented the central tendency of wolverines whose actual paths imperfectly followed least-cost routes due to small-scale behavioral choices by individual animals. We then summed kernels associated with each least-cost path at each raster such that the highest values were associated with those areas having the highest density of least-cost wolverine paths. These computer-generated corridors simulated dispersals associated with a well-distributed population of wolverines. Our rationale was that the more paths that crossed an area, the more important it would be for dispersal, assuming that wolverines were well distributed throughout the study area. Additionally, we computed average pairwise cost distances associated with moving from each start location to all other start locations to estimate the relative isolation of each location given a well-distributed population.

Subsequently, we repeated this corridor analysis for areas extending from the Greater Yellowstone Area to the southern extent of the wolverine's historical distribution in the Rocky Mountains (Aubry et al. 2007) to evaluate the relative resistances and locations of possible colonization pathways and to infer historical movement paths.

#### *Isolation-by-resistance*

McRae and Beier (2007) suggested that isolation-by-resistance as estimated by electrical circuit theory could better describe gene flow than least-cost path modeling. We therefore generated cost path models using McRae's (2006) CIRCUITSCAPE software. However, computational limits of their program required aggregation of rasters in our base map to one-tenth the number of rasters using the majority rule for raster reclassification. To compare the electrical circuit theory approach to least-cost paths, we re-ran the least-cost path models on these aggregated maps and conducted Mantel tests as for cost distance matrices derived from both circuit and least-cost path approaches.

#### *Effective population size*

Using only those samples from the main part of the Rocky Mountains (i.e., eliminating samples from the Crazy and Belt Mountains, as Cegelski et al. 2003 suggested they form a different subpopulation), we estimated the effective population size and 95% credible limits of the estimate via summary statistics and approximate Bayesian computation methods using ONE-SAMP (Tallmon et al. 2008). To determine the temporal stability of the wolverine population, we subdivided the samples into three time frames and used ONE-SAMP to estimate effective population size in each frame.

### RESULTS

#### *Descriptive statistical genetic analyses*

Despite the broad scale of sampling, the majority of loci were in Hardy-Weinberg proportions; loci *Gg7*,

*Ggu216*, and *Mvis020* were out of Hardy-Weinberg proportions. The tests for heterozygote excess using all loci showed no significant heterozygote excess, while the tests for heterozygote deficits produced highly significant results ( $P < 0.001$ ;  $F_{IS} = 0.075$ ). Overall, 65 alleles were detected, mean number of alleles per locus was 4.06 (SE = 0.28), expected heterozygosity was 0.504 (SE = 0.049), and observed heterozygosity was 0.473 (SE = 0.052). The first six bins (25 km per bin) in the spatial autocorrelogram were positively autocorrelated (using criteria of Peakall and Smouse 2006), suggesting that wolverine are spatially more related than by chance at distances up to 150 km (Fig. 2). Altering the number and width of bins did not significantly change these results (*data not shown*). Additionally, Mantel tests suggested a significant positive correlation between genetic distance and Euclidean distance ( $r_M = 0.215$ ,  $P = 0.001$ ).

Analysis of the mtDNA control region showed that 100% (5/5) of the Crazy Mountains samples in the areas with spring snow were haplotype I and 70% (7/10) of the Little Belt Mountains samples found in the areas with spring snow were haplotype I, with the remainder being haplotype A.

#### *Corridor analysis and population subdivision*

Significant positive correlations between genetic distance and cost distance were detected for all cost models based on spring snow cover with correlations increasing as we increased resistance for movement across areas not covered by spring snow (Fig. 3), although the relationship was asymptotic and correlations did not appreciably improve for models with non-snow costs above 20 (Fig. 3). In all cases, cost models based on snow cover had significantly stronger correlations with observed genetic distances than Euclidean models.

Given that both Euclidean distance and cost distance were significantly related to genetic distance, we used partial Mantel tests to remove the influence of each variable while correlating the other with genetic distance. For all cost models based on snow cover, there was no significant relationship between genetic distance and Euclidean distance once the spring snow-based correlation was removed (Table 1). However, the relationship between genetic distance and cost distance was always significant when Euclidean distance was partialled out of the relationship (Table 1).

The least-cost path analysis among all pairs of systematically gridded points suggested several important wolverine corridors connecting areas of spring snow cover (orange paths in Fig. 4). Plotting the average cost distance between all pairs of individuals in the northern U.S. Rocky Mountains suggested a bimodal distribution of cost distances, with a few isolated locations (Fig. 4). Fig. 4 showed smaller average costs between individuals in the Bitterroot Mountains on the Idaho-Montana border and within the Bob Marshall Wilderness complex than between the southern GYA and the Gallatin Mountains. The largest average costs were associated

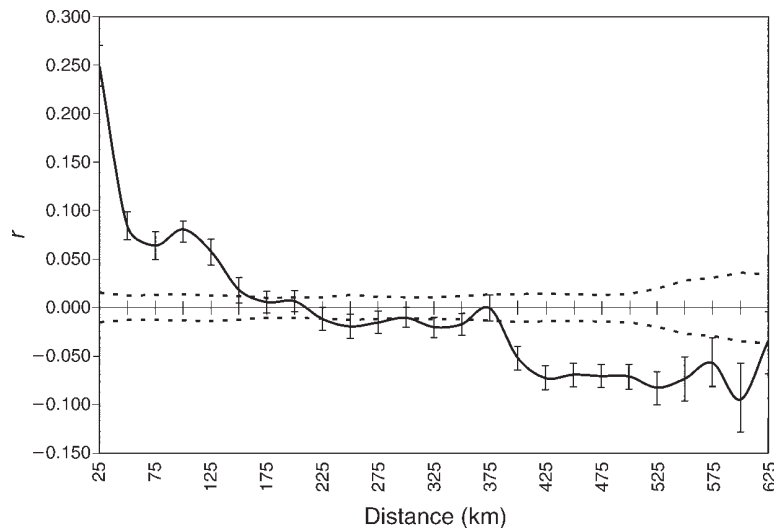


FIG. 2. Decline in the genetic correlation of wolverines ( $r$ ; Peakall and Smouse 2006) with geographic distance. Dashed lines indicate permuted 95% confidence bounds and the error bars are the 95% confidence intervals generated by bootstrapping.

with movements between the Little Belt and Crazy Mountains and all other points. Extrapolating the same spring snow relationships from the northern Rocky Mountains to Colorado and Utah (where wolverines were persistent until the early 20th century; Aubry et al. 2007) showed average cost distances between the GYA and Colorado via the spring snow cover layer were much greater than distances within the northern Rocky Mountains (i.e., the distributions did not overlap; Figs. 4 and 5).

#### Isolation-by-resistance

CIRCUITSCAPE results were similar to the least-cost path results in that genetic distance was correlated with Euclidean distance ( $r_M = 0.180$ ,  $P = 0.001$ ; Table 2). Genetic distance was also correlated with spring snow-based cost distances derived via circuit theory ( $r_M = 0.224$ ,  $P = 0.001$ ), and with spring snow-based cost distances derived via circuit theory after controlling for Euclidean distance ( $r_M = 0.150$ ,  $P = 0.002$ ). However, in all cases less variance was explained using circuit theory than through least-cost-path derived models. Resistance models associated with circuit theory were less effective at finding the effects of gene flow through snow areas than were models based on least-cost paths. In addition, genetic distance was significantly correlated with Euclidean distance after controlling for cost distances derived via circuit theory ( $r_M = 0.074$ ,  $P = 0.001$ ; Table 2). However, this result is likely due to the aggregation of rasters required to meet computational demands of the CIRCUITSCAPE program. Using a least-cost path approach with the aggregated spring snow layer also failed to partial Euclidean distance from resistance effects (Table 2).

#### Effective population size

Effective population size, calculated using ONESAMP (Tallmon et al. 2008), was 35 (credible limits = 28–52) and was found not to change over time ( $N_{e89-94} = 33$ , credible limits 27–43;  $N_{e95-00} = 35$ , credible limits 28–57;  $N_{e01-06} = 38$ , credible limits 33–59).

#### DISCUSSION

##### Spring snow bioclimatic niche

The spring snow bioclimatic niche, which has been identified as important for predicting wolverine denning, range, and year-around telemetry locations (J. P. Copeland et al., *unpublished manuscript*), also provides a spatial explanation for wolverine gene flow. While Euclidean distance was significantly correlated with genetic distance, partial Mantel tests suggested that it

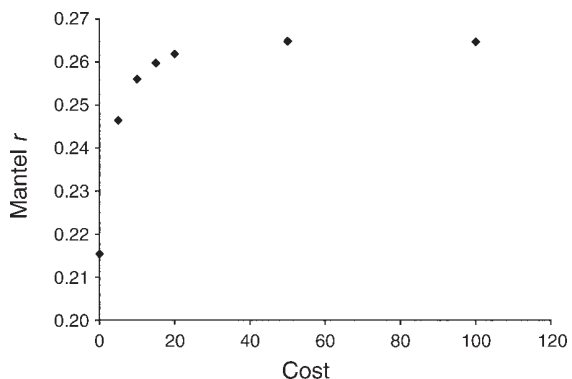


FIG. 3. Plot of  $r$  value (Mantel tests) vs. cost (ecological cost distance, unitless) used for non-spring snow cover cells in the least-cost path modeling. The relationship asymptotes at a resistance of 20.

TABLE 1. Partial Mantel results comparing matrices of genetic distance among all pairs of individuals, Euclidean distances among all pairs of individuals, and least-cost paths through the ecological cost layer, where higher costs (from a resistance of 5–50) were assigned to non-spring snow cover cells.

| Correlation                               | Partialled out            | Mantel $r$ | $P$   |
|-------------------------------------------|---------------------------|------------|-------|
| $D_{PS} \times$ Euclidean distance        | non-spring snow cost = 5  | -0.046     | 0.902 |
| $D_{PS} \times$ Euclidean distance        | non-spring snow cost = 10 | -0.018     | 0.685 |
| $D_{PS} \times$ Euclidean distance        | non-spring snow cost = 15 | -0.004     | 0.530 |
| $D_{PS} \times$ Euclidean distance        | non-spring snow cost = 20 | 0.005      | 0.436 |
| $D_{PS} \times$ Euclidean distance        | non-spring snow cost = 50 | 0.024      | 0.266 |
| $D_{PS} \times$ non-spring snow cost = 5  | Euclidean distance        | 0.131      | 0.001 |
| $D_{PS} \times$ non-spring snow cost = 10 | Euclidean distance        | 0.143      | 0.001 |
| $D_{PS} \times$ non-spring snow cost = 15 | Euclidean distance        | 0.150      | 0.001 |
| $D_{PS} \times$ non-spring snow cost = 20 | Euclidean distance        | 0.153      | 0.001 |
| $D_{PS} \times$ non-spring snow cost = 50 | Euclidean distance        | 0.160      | 0.001 |

Note: The genetic distance measure used was the proportion of shared alleles ( $D_{PS}$ ).

was a nuisance variable and not the main factor that strongly influenced gene flow: persistent spring snow cover had a significant independent effect on genetic distance when we controlled for Euclidean distance, but the converse was not true. MtDNA results suggested some degree of isolation of the GYA and greater isolation of the Little Belt and Crazy Mountains (see Appendix) acting as independent support for our corridor modeling derived from microsatellite data.

#### *Wolverine gene flow and substructure in light of spring snow*

There have been several analyses that have evaluated wolverine gene flow and substructure in North America (Kyle and Strobeck 2001, 2002, Cegelski et al. 2003, 2006, Guillot et al. 2005). All of these analyses, however, were based exclusively on patterns of genetic clustering; explanations for the observed patterns were post hoc. Here we initiated an analysis with a definition of the breeding range for wolverines. The areas of persistent spring snow defined by J. P. Copeland et al. (*unpublished manuscript*) enclosed >99% of wolverine dens in North America and Scandinavia. The locations of the 210 samples used in this study indicate that many of the identified areas contain wolverine (Fig. 1). Importantly, these data also show that the areas exterior to Copeland's area of spring snow do not contain reproductive populations.

Further, we know that there are strong patterns of spatial correlation that extend over 100 km within this population, suggesting gene flow but not panmixia across the population and within subunits. The presence of a strong correlation gradient likely invalidates clustering approaches, particularly when sampling is uneven (Schwartz and McKelvey 2009). In these situations an intrinsically gradient-based approach such as the resistance modeling we applied is more appropriate.

#### *Landscape connectivity, corridors, and climate change*

Increasing human presence in the western United States has led to increasing development of open spaces

(Gude et al. 2007) that historically served to connect isolated habitats. This trend has led both nongovernment organizations and government agencies to consider the importance of biological corridors for conservation planning (*available online*).<sup>7</sup> Maps of corridors are commonly used by land management groups, but unfortunately are most often the product of expert opinion rather than empirical data. The approach used in this study: collecting a spatially distributed genetic sample, hypothesizing likely landscape resistance factors, correlating genetic patterns with putative resistances to determine their validity, and finally using the derived models to infer likely corridors provides a rapid, relatively low cost, empirical approach to determining corridors for many species. We have used these methods to develop corridors maps for bears (Cushman et al. 2009) and are currently analyzing genetic patterns for fisher (*Martes pennantii*), marten (*Martes americana*), and several species of small mammals. Developing empirically based corridors models on the same landscape allows us to test the concept of "umbrella corridors," or multispecies corridors, where it is inherently assumed that a corridor for one species is appropriate for another.

By linking genetic relatedness with an ecologically derived understanding of wolverine distribution, we offer a data-derived understanding of a landscape feature that facilitates movement, which may become useful for land-use planning such as conservation easement targets. In addition, we present likely paths for the natural recolonization of wolverine into Colorado, and show why, due to high average costs associated with those movements, natural recolonization is challenging. Additionally, we propose a central "artery" for wolverine gene flow in the Rocky Mountains, centered on the Bitterroot Mountain chain bordering western Montana and eastern Idaho. Based on least-cost path rules, this putative corridor links wolverines in the north (Bob Marshall Wilderness, Glacier National Park, northern Idaho) and wolverines

<sup>7</sup> (<http://www.westgov.org/wga/publicat/wildlife08.pdf>)

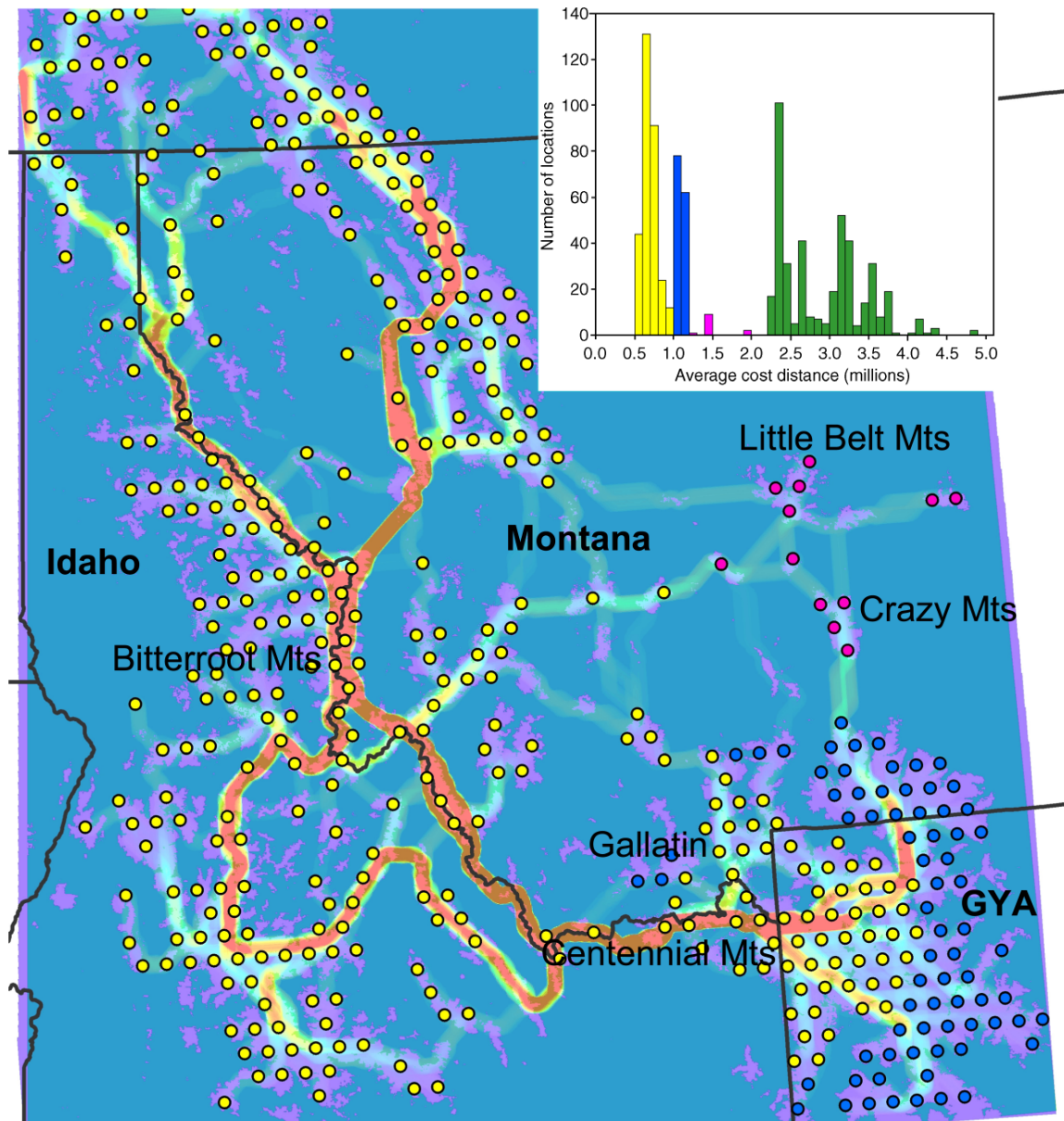


FIG. 4. Map of the northern U.S. Rockies with cumulative least-cost paths between systematically placed locations (circles) in spring snow cover cells. Paths in orange are predicted to be used more often than those in cooler colors. The color of the circle corresponds to the average cost distance between that location and all other locations, based on our models. The inset graph in the upper right is the cumulative plot of average cost distance (in millions of cost units) between each systematically placed location and all other locations. The graph was divided into four modes (three within the northern U.S. Rockies, and one between the Greater Yellowstone Area and Colorado). The yellow mode has the lowest average cost distances (within the northern U.S. Rockies), the blue bars the next lowest, the pink bars (Crazy and Little Belt Mountains) have the greatest average cost distances in the northern U.S. Rockies, and the green bars show the distances between all points from Colorado to the Greater Yellowstone Area.

in the south (GYA, Central Idaho). Furthermore, an often proposed corridor through the Centennial Mountains (on the border between Montana and Idaho) is supported by these analyses. These proposed corridors, while empirically supported, still should be evaluated with independent empirical testing (e.g., through the use

of GPS collars on wolverines that may disperse or through extensive noninvasive genetic monitoring).

#### *Limits of our analysis*

Whereas least-cost path analyses are powerful landscape genetic tools that allow the evaluation of

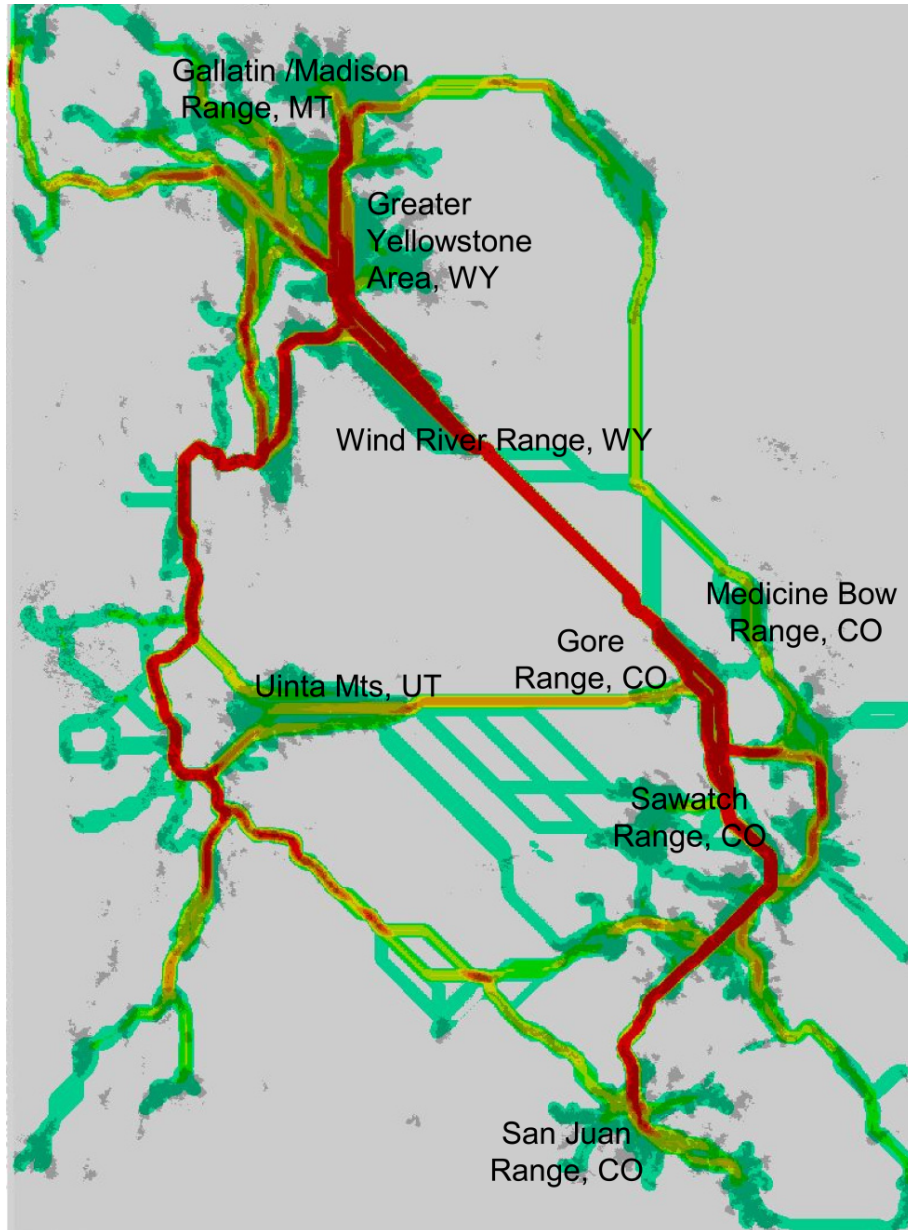


FIG. 5. Map of least-cost paths between Colorado (CO) to the Greater Yellowstone Area (in Wyoming [WY] and Montana [MT]). Areas in red were used more often than those in cooler colors.

biological factors that influence substructure, they are not a panacea. One particular problem, associated with many genetic analyses, is that the inferred time frame can be unclear. This is especially true for species with large effective population sizes, as drift is a function of effective population size and time (Nei and Chakravarti 1977). In the Rocky Mountains of the United States, the effective population size for wolverine is extremely small (35 individuals), and thus observed substructure would be caused by processes that occur on the order of only a

few generations. However, single-sample estimators for effective population size are recent developments, thus their sensitivity to factors such as population structure remain largely unknown. Migration from other populations, however, is likely not an issue as gene flow from the main part of Canada appears minimal (Kyle and Strobeck 2002, Cegelski et al. 2006). Only three haplotypes have been discovered in the Rocky Mountains compared to the 13 haplotypes detected in Canada, suggesting marked separation between these samples.

TABLE 2. Mantel and partial Mantel tests from circuit theory (distance<sub>Circuit</sub>) and least-cost path modeling with aggregated cells (distance<sub>Aggregated</sub>).

| Correlation                                               | Matrix partialled out                     | Mantel <i>r</i> | <i>P</i> |
|-----------------------------------------------------------|-------------------------------------------|-----------------|----------|
| $D_{PS} \times$ Euclidean distance                        |                                           | 0.180           | 0.001    |
| $D_{PS} \times$ ecological distance <sub>Circuit</sub>    |                                           | 0.224           | 0.001    |
| $D_{PS} \times$ Euclidean distance                        | ecological distance <sub>Circuit</sub>    | 0.074           | 0.001    |
| $D_{PS} \times$ ecological distance <sub>Circuit</sub>    | Euclidean distance                        | 0.150           | 0.002    |
| $D_{PS} \times$ ecological distance <sub>Aggregated</sub> |                                           | 0.242           | 0.001    |
| $D_{PS} \times$ Euclidean distance                        | ecological distance <sub>Aggregated</sub> | 0.109           | 0.001    |
| $D_{PS} \times$ ecological distance <sub>Aggregated</sub> | Euclidean distance                        | 0.156           | 0.001    |

Notes: We used an ecological resistance of 20 in non-spring snow cover cells. The base map was the same as Fig. 1, except every 10 cells were aggregated into one cell due to computing limitations. The genetic distance measure used is the proportion of shared alleles ( $D_{PS}$ ).

Given that there are no other potential source populations, gene flow would not be strongly influencing our effective population size estimates, and therefore the likelihood that detected patterns are based on recent phenomena.

Recently, circuit theory has been suggested as a more powerful approach to elucidate gene flow compared to least-cost path models (McRae and Beier 2007). For example, McRae and Beier (2007) used a population approach (as compared to our individual-based approach) and found circuit theory to be an improvement for explaining genetic distance among 12 wolverine populations. However, resistance modeling using circuit theory did not lead to improved correlations in this study. Theoretically, circuit theory should provide better fit to gene flow by generating different gene flow rates across areas where populations are extensive compared to areas where populations are narrowly distributed along linear corridors (McRae and Beier 2007). The two models, however, converge in narrow areas. Wolverine living at the southern extent of their North American range occur in bands of habitat that are often linear and follow major mountain chains (Fig. 1), supporting the use of least-cost path analysis in our study. More comparison between these techniques will be necessary to determine those situations in which one approach will outperform the other.

An additional limitation of our analysis is that our samples were collected over multiple (three) generations, although there is evidence of population stability over time. Furthermore, these samples were from trapped animals which, while coming from both sexes, may have had a social or age bias. Another limitation of this analysis is that the landscape was defined in terms of a single variable, persistent spring snow cover. Other least-cost path analyses have worked with several factors (four in Cushman et al. 2006) and varying levels of each factor allowing comparison of many models. Our goal here was specifically to test one bioclimatic association shown to be predictive of wolverine dens and year-round habitat use. Future work that incorporates other important landscape features, such as human development, could refine our understanding of wolverine gene flow.

### Conclusion

Overall, this research suggests that areas characterized by persistent spring snow cover, which in previous studies have been shown to strongly correlate with wolverine denning locations and year-round movement, also is correlated with gene flow. This spring snow-cover niche has the biologically important elements of snow during the winter and spring, and acts as a surrogate for wolverine's within-home range movements and dispersals year-round. In addition, we identified potential movement corridors that may be critical for the persistence of wolverines. Unfortunately, spring snow cover, and the bioclimatic niche that it indicates, is likely to continue to be strongly impacted by global climate change (Mote et al. 2005), threatening wolverine throughout their geographic distribution.

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## APPENDIX

Distribution of wolverine haplotypes in the U.S. Rocky Mountains (*Ecological Archives* E090-229-A1).

# MITOCHONDRIAL PHYLOGEOGRAPHY AND CONSERVATION GENETICS OF WOLVERINE (*GULO GULO*) OF NORTHWESTERN NORTH AMERICA

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Anthropogenic impacts such as habitat conversion and fragmentation, in combination with predator control and fur trapping, are responsible for substantial reductions in the ranges of many carnivores worldwide. The wolverine (*Gulo gulo*) is classified as vulnerable throughout the Holarctic Region by the International Union for Conservation of Nature and Natural Resources, is designated as endangered in eastern Canada, and has been petitioned twice for listing with the United States Fish and Wildlife Service. We examined genetic structure across populations in northwestern North America by using mtDNA sequences of the left domain of control region and the complete cytochrome-*b* gene (*Cytb*). Nucleotide diversity ( $\pi$ ) and sequence divergence among haplotypes were low for both markers, whereas haplotype diversity ( $h$ ) was generally high. Genetic divergence ( $F_{st}$ ) values were significant, and high for control region (0.21), and moderate to high for *Cytb* (0.16). Globally, Eurasian and Scandinavian wolverines were distinguishable from North American. Within North America, the Kenai Peninsula, southeastern Alaska, and Nunavut populations were distinctive. Comparisons with studies based on nuclear markers reveal greater geographic structure in these maternally inherited mitochondrial markers, a finding consistent with male-biased dispersal in wolverines. Conservation plans for these medium-sized carnivores should emphasize maintenance of genetic diversity and recognize that successful dispersal of females between populations may be limited.

Key words: control region, cytochrome *b*, *Gulo gulo*, mitochondrial DNA, phylogeography, wolverine

Habitat loss and human-caused mortality are the most important factors affecting the conservation of carnivores worldwide (Gittleman et al. 2001; Hornocker and Hash 1981). In addition, climate change in the form of global warming is impacting environments, especially those inhabited by northern-latitude carnivores. Naturally low densities for many carnivores, even in areas not impacted by anthropogenic activities, increase the risk that local extirpations will isolate populations. Hence, there is a need to document geographic variation and assess connectivity among populations for effective management of these species and their biomes. Carnivores such as the wolverine (*Gulo gulo*) have been suggested as potential focal species for large-scale conservation monitoring (Carroll et al. 2001).

Historically, the wolverine ranged throughout boreal, mountain, and tundra regions of Eurasia and North America. In Eurasia, current range includes portions of Estonia, Finland, Mongolia, Norway, Russia, and Sweden (International Union

for the Conservation of Nature and Natural Resources 2002). In North America, the range once included the vast majority of Alaska and Canada, the northern tier of the conterminous United States, and mountains as far south as the Sierra Nevadas in California, and the Rocky Mountains of northern New Mexico (Hall 1981). As a result of habitat loss and human-caused mortality, the current range in North America has been reduced to Alaska, northern and western Canada, and limited areas of Idaho, Montana, Washington, and Wyoming (Banci 1994). The range of the wolverine has also contracted in Europe and Asia (International Union for Conservation of Nature and Natural Resources 2002).

The International Union for Conservation of Nature and Natural Resources ([www.redlist.org](http://www.redlist.org)) classifies the wolverine as vulnerable throughout its Holarctic range. The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) classifies the wolverine east of Hudson Bay as endangered (COSEWIC 2003). The wolverine has been petitioned twice for listing under the federal endangered species act in the conterminous United States. The most recent petition was denied citing lack of information on distribution, habitat requirements, and threats (United States Fish and Wildlife Service 2003). Alaska and Montana remain the only states where wolverines are legally

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harvested, but little information exists on density in Montana, and the wolverine may be declining in parts of its range in Alaska ([www.wolverinefoundation.org](http://www.wolverinefoundation.org)).

The wolverine's affinity for remote wilderness and rugged terrain, low densities, large home range sizes, and sensitivity to human disturbance all contribute to the challenge of managing and conserving this solitary and secretive species (Ruggiero et al. 1994). A general characteristic of areas occupied by wolverines is remoteness from human developments (Banci 1994). Wolverines have low reproductive potential and are more similar ecologically to larger carnivores (Weaver et al. 1996) than to species of similar size. Life history characteristics of the wolverine result in low population resiliency (Banci and Proulx 1999; Weaver et al. 1996). Individual home ranges are relatively large and vary from less than 100 km<sup>2</sup> to greater than 1,500 km<sup>2</sup> in Alaska, the Yukon, Montana, and Idaho (Banci 1987; Copeland 1996; Gardner 1985; Magoun 1985). Males have larger home ranges than females (Copeland 1996; Hornocker and Hash 1981).

Despite the wolverine's capacity for long-range dispersal, studies investigating the phylogeography and conservation genetics of wolverines have found varying levels of geographic partitioning. Occasionally different perspectives have been developed based on mitochondrial and nuclear genomes (Walker et al. 2001; Wilson et al. 2000). The majority of genetic studies on the wolverine have been based on markers from the nuclear genome (Cegelski et al. 2003; Kyle and Strobeck 2001, 2002; Walker et al. 2001; Wilson et al. 2000), and these covered a greater geographic scope than previous mitochondrial-based studies. Nuclear studies revealed moderate to high levels of population differentiation (Kyle and Strobeck 2001, 2002; Wilson et al. 2000). These studies found that northern North American wolverine populations experienced higher levels of gene flow and less genetic structure than more southerly populations. These southern populations had higher levels of genetic structuring, likely associated with more limited gene flow (Kyle and Strobeck 2001, 2002). Published mtDNA studies are limited in geographic scope; one focused on Scandinavia (Walker et al. 2001), and the other on 5 populations in northern Canada (Wilson et al. 2000).

We studied the complete cytochrome-*b* gene (*Cytb*) and the left domain of the control region to provide a female-mediated perspective on geographic structure in North American wolverine. There are limitations when considering mtDNA evidence alone (Ballard and Whitlock 2004). Initial analysis of a spatially extensive *Cytb* data set uncovered relatively low variation and divergence. The control-region data set then was developed for a larger number of individuals and additional populations.

The study area encompassed a considerable portion of the wolverine's range in North America and also included limited samples from Eurasia. North American populations were primarily from the northwestern region of the continent, which is a naturally heterogeneous landscape comprised of a diversity of habitats such as taiga, tundra, temperate rain forests, and large glaciated expanses. This high-latitude area and associated biota were intensely impacted during the Pleistocene glacial cycles, including the most recent glacial maximum 15,000–18,000

years ago (Hewitt 1996; Pielou 1991) when portions of the Cordilleran and Laurentide ice sheets coalesced east of the coastal mountains. During full glacial advances a Beringian glacial refugium connected Asia and North America, with the rising of the Bering Strait separating the continents 10,000–12,000 years ago (Hoffmann 1981, 1984; Hopkins 1967, 1982; Hultén 1937; Macpherson 1965; Pielou 1991). These climatic events likely influenced genetic diversity in modern wolverines.

Populations of particular interest include those on the Kenai Peninsula and from southeastern Alaska. The Kenai Peninsula is isolated as a result of a relatively narrow connection to south-central Alaska, and this population was described as a distinct subspecies (Matschie 1918). Southeastern Alaska has been recognized as a unique biogeographical province by some authors (Klein 1965; Swarth 1936) and is isolated from the remainder of North America (MacDonald and Cook 1996). These peripheral populations may contain genetic signatures of physiographic isolation, but neither has been included in previous molecular studies.

Although mammals are generally regarded as well understood taxonomically, much of this framework has not been tested with molecular approaches and should be reviewed (Engstrom et al. 1994; Nagorsen et al. 2000), particularly for cases such as the wolverine, where taxonomy has been dynamic for both specific and subspecific designations. Wolverines from Eurasia and North America were considered separate species (*G. gulo* and *G. luscus*) by some authors (Cowan 1930; Hall 1981; Miller 1912; but see Bryant 1987). Others have recognized Eurasian and North American populations as distinct subspecies (Kurtén and Rausch 1959). Within North America, Hall (1981) listed 4 subspecies, *Gulo luscus katschemakensis* on the Kenai Peninsula of Alaska, *G. l. vancouverensis* on Vancouver Island, British Columbia, Canada, *G. l. luteus* from western North America, and *G. l. luscus* from the Rocky Mountains eastward across higher latitudes. The Vancouver Island wolverine may have been extirpated (COSEWIC 2003). Currently, the 2 most common taxonomic views either split the wolverine into 2 subspecies with *G. gulo gulo* in Eurasia and *G. gulo luscus* in North America, or recognize *G. gulo* as a single Holarctic taxon.

Specifically, this investigation compares mitochondrial and nuclear perspectives on genetic structure across several high-latitude populations of wolverine, explores the effects of Pleistocene climatic changes on historical biogeography, tests if suspected isolated or peripheral populations are distinctive, and investigates whether phylogeographic patterns in this large mustelid are consistent with taxonomic hypotheses.

## MATERIALS AND METHODS

**Samples.**—Samples were primarily frozen or ethanol-preserved tissues, and 6 samples were from museum study skins. Voucher specimens are deposited at the University of Alaska Museum or the Museum of Southwestern Biology and were salvage specimens obtained largely from hunters, trappers, and natural resource agencies (Appendix I). Samples of this relatively uncommon carnivore are in short supply across much of its range. Many of the samples that do exist have poor location information. The limited availability and accessi-

bility of good-quality samples prohibited our use of equal or larger sample sizes, or inclusion of samples from a greater portion of the wolverine's range. The sampling scheme was designed to cover the greatest geographic area possible given sample availability, and included 2 geographically isolated populations. Sample accessibility was particularly limited for the southern reaches of the wolverine's range.

**Markers.**—The mitochondrial genome is phylogenetically informative as a result of maternal inheritance, lack of recombination, and relatively high variability (Hartl and Clark 1997). The hypervariable left domain of the mtDNA control region is commonly used for intra-specific studies given its relatively high mutation rate, and is particularly useful for detecting genetic variation within and among populations of mammals (Awise 1994; Taberlet 1996; Waits et al. 1998). This region is thought to evolve more rapidly than the protein-coding *Cytb* (Awise 2000). Control-region sequences were used in previous investigations that included mitochondrial markers (Walker 2001; Wilson et al. 2000). *Cytb* has been used extensively in phylogeographic studies of other mammals (Irwin et al. 1991; Ledje and Arnason 1996; Lucid and Cook 2004). Populations were represented by 6–47 individuals for control region (Table 1; excluding populations from the Seward Peninsula, Scandinavia, and Eurasia) and 5–7 individuals for *Cytb* (Tomasik 2003).

**Sequences.**—Genomic DNA was isolated from tissue samples by using either a Qiagen DNeasy tissue kit (Qiagen Inc., Valencia, California) or a modified NaCl extraction protocol (Fleming and Cook 2002) with the 2nd desalting wash omitted. Sequencing generally followed protocols outlined in Fleming and Cook (2002), except products were analyzed on an Applied Biosystems 3100 Genetic Analyzer (Applied Biosystems, Inc., Foster City, California). Sequences were aligned by eye by using Sequence Navigator (Applied Biosystems) and compared to reference sequences.

The left domain of control region was sequenced by using the primers L15926 and H16498 (Shields and Kocher 1991; Taberlet 1996). These primers produced reliable sequences of 365–367 base pairs (bp) for both DNA strands including 28 bp of the gene coding for t-RNA<sup>thr</sup>, 66 bp of the gene coding for t-RNA<sup>pro</sup>, and 270–272 bp of the left domain of the control region (these 3 segments are hereafter referred to collectively as “control region”). Total sample size was increased to 159 individuals by using reference sequences obtained from GenBank, including 8 individuals from Scandinavia (GenBank AF245496—Walker et al. 2001), and 43 individuals from Northwest Territories and Nunavut, Canada (GenBank AF210090–AF210132—Wilson et al. 2000). See Wilson et al. (2000) for more detailed location information. Many reference haplotypes were identical to wolverine samples we sequenced. *Cytb* was sequenced by using the primers MVZ04, 05, 16, 14, and 23 (Smith and Patton 1993) and Marten 37 (Demboski et al. 1999). These primers produced reliable sequences of 1,140 bp for both DNA strands of all samples ( $n = 27$ ). Reference sequences obtained from GenBank represented Scandinavia (GenBank X94921—Ledje and Arnason 1996) and Sakhalin Island (GenBank AB051245—Hosada et al. 2000) and increased the total *Cytb* data set to 29. These reference haplotypes were identical to haplotypes that we sequenced.

**Analyses.**—The software package Arlequin 2.000 (Schneider et al. 2000) was applied to calculate measures of genetic diversity and subdivision including nucleotide diversity ( $\pi$ ), haplotype diversity ( $h$ ), corrected average pairwise differences, genetic divergence ( $F_{st}$ ), number of female migrants per generation ( $N_m$ ), and an analysis of molecular variance (AMOVA). The single individual from the Seward Peninsula and the monotypic Scandinavian population were excluded from those calculations.

**TABLE 1.**—Nucleotide diversity ( $\pi$ ), haplotype diversity ( $h$ ), number of samples ( $n$ ), and number of haplotypes for each population (control region).

|                       | $n$ | No.<br>haplotypes | $\pi$ (%) |          | $h$    |        |
|-----------------------|-----|-------------------|-----------|----------|--------|--------|
|                       |     |                   | Mean      | SE       | Mean   | SE     |
| Eurasia               | 5   | 3                 |           |          |        |        |
| Kenai Peninsula       | 22  | 4                 | 0.003956  | 0.002789 | 0.7143 | 0.0480 |
| Northern Alaska       | 10  | 4                 | 0.003535  | 0.002724 | 0.7111 | 0.1175 |
| Northwestern Alaska   | 22  | 4                 | 0.005102  | 0.003383 | 0.7229 | 0.0416 |
| Northwest Territories | 15  | 6                 | 0.003495  | 0.002602 | 0.7619 | 0.0961 |
| Nunavut               | 47  | 8                 | 0.002730  | 0.002078 | 0.8205 | 0.0266 |
| Scandinavia           | 8   | 1                 |           |          |        |        |
| Seward Peninsula      | 1   | 1                 |           |          |        |        |
| Southeastern Alaska   | 12  | 3                 | 0.004691  | 0.003305 | 0.5909 | 0.1079 |
| Southern Alaska       | 17  | 4                 | 0.002985  | 0.002304 | 0.6544 | 0.0891 |
| Total                 | 159 | 17                |           |          |        |        |

Nucleotide diversity ( $\pi$ ) is the probability that a sample of a particular nucleotide site drawn from 2 individuals will differ (Hartl and Clark 1997), and is a weighted sequence divergence between individuals in a population, regardless of the number of different haplotypes (Awise 2000). Haplotype diversity ( $h$ ) is the probability that 2 haplotypes drawn from a population will differ (Hartl and Clark 1997), and condenses information on the numbers and frequencies of different alleles at a locus, regardless of their evolutionary relationships (Awise 2000). These 2 measures of diversity were compared to draw inferences on demographic history (Awise 2000).

Corrected average pairwise differences are the average percentage of nucleotide sites for which 2 populations are different, and  $F_{st}$  values are a measure of population differentiation. AMOVA was used to examine partitioning of genetic variation among and within populations (Excoffier et al. 1992). North American populations were tested for genetic subdivision by calculating pairwise  $F_{st}$  and  $N_m$  values for control-region sequences.

The number of female migrants per generation ( $N_m$ ) has an inverse relationship with  $F_{st}$ , and estimates the effective number of female migrants between populations per generation.  $N_m$  values  $< 1$  indicate that the homogenizing influences of gene flow have not overridden the diversifying effects of genetic drift (Awise 2000; Birky et al. 1983). The mathematical model used to calculate  $N_m$  values makes assumptions that may be violated by real populations. Hence, quantitative information regarding dispersal obtained from gene frequency data may have limited applications (Whitlock and McCauley 1999).  $F_{st}$  represents the primary indicator of population structure in the current study.

Isolation by distance analysis for control region used Mantel tests (Mantel 1967; Sokal and Rohlf 1995) conducted with MANTEL V2.0 (Liedloff 1999) to estimate the association (1,000 iterations) between the average geographic distance matrix for the 7 North American populations, and their population pairwise  $F_{st}$  values and corrected average pairwise differences matrices.

Evolutionary relationships were analyzed with unrooted neighbor-joining trees generated with PAUP\* (V4.0b8—Swofford 2000) based on the Jukes–Cantor distances (Jukes and Cantor 1969) because sequence divergence was low (Nei and Kumar 2000). Support for relationships was estimated with 1,000 bootstrap replicates. Minimum spanning networks were constructed to assess relatedness of haplotypes. Population frequencies were included in the minimum spanning networks and illustrated where unique and rare haplotypes occurred.

Demographic history of North American populations was inferred by using FLUCTUATE V1.3 (Kuhner et al. 1998) for control-region

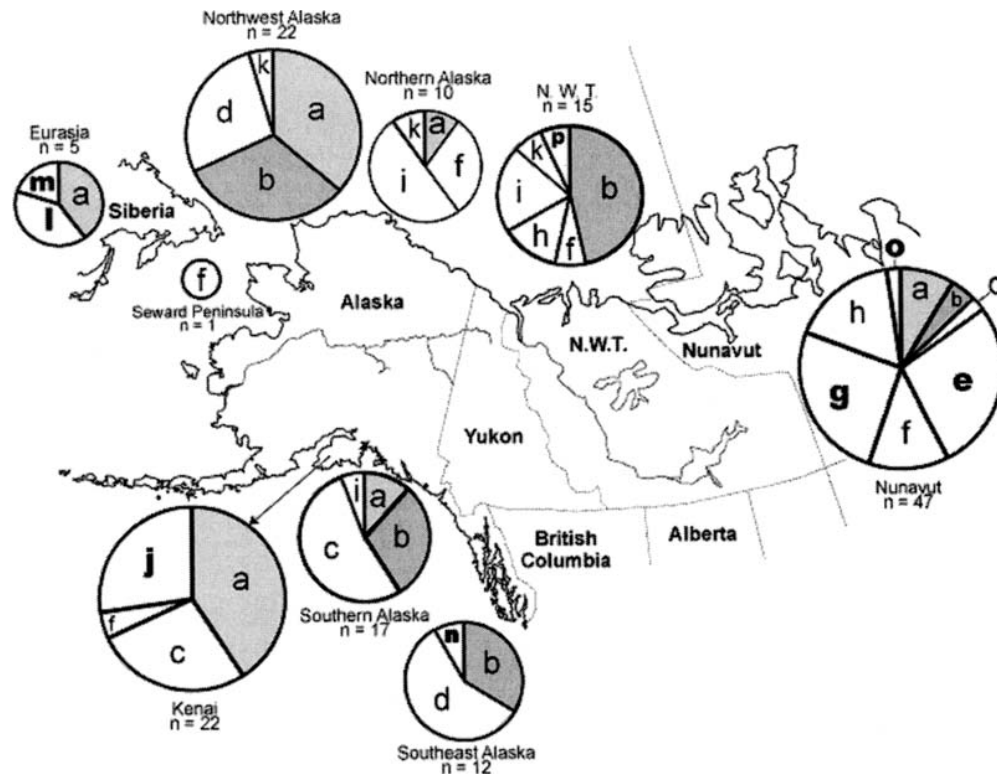


**FIG. 1.**—Geographic distribution of control-region haplotypes of *Gulo gulo* among sampling locales. Pie diagrams indicate frequencies of haplotypes. Shading indicates most common and widespread haplotypes (haplotypes a and b). Bold indicates haplotypes unique to a single population. The control region haplotype labels in this work correspond to those in Wilson et al. (2000) as follows: a = H, b = A, e = G, f = F, g = C, h = I, i = B, k = E, and p = D.

sequences. This program tests the significance of population expansion or decline using likelihood estimation by the Metropolis–Hasting sampling algorithm (see Kuhner et al. 1998; violating the assumption of no migration). The method estimates the goodness of fit of a model of exponential growth or decline, and generates maximum-likelihood estimates of the growth parameter ( $g$ ) and its standard deviation. Positive values of  $g$  indicate demographic growth, negative values indicate decline. Conservative confidence limits of 99% were used to test significance of difference from 0.

Fu's  $F_s$  test of neutrality (Fu 1997) was performed on the control-region sequences by using 1,000 replicates in ARLEQUIN (Schneider et al. 2000).  $F_s$  values tend to be negative when there is an excess of recent mutations (therefore an excess of rare alleles). Large negative values of  $F_s$  are evidence against the neutrality of mutations and may reflect population demographic expansion (Fu 1997; Schneider et al. 2000). To be significant, probability values should be below 0.02, rather than 0.05 (Fu 1997; Schneider et al. 2000).

## RESULTS

**Control region.**—Control-region sequences were composed of 28% adenine, 30% thymine, 28% cytosine, and 14% guanine. Seventeen haplotypes resulted from 17 polymorphic sites (11 transitions, 2 transversions, and 4 insertion–deletions). Nine of the 17 haplotypes were unique to 1 sampling locale (Fig. 1).

Haplotype diversity values ranged from 0.59 to 0.82, and nucleotide diversity values ranged from 0.0027 to 0.0051 (Table 1). The high number of haplotypes relative to sample

size for some populations ( $n = 5$  with 3 haplotypes in Eurasia,  $n = 15$  with 6 haplotypes in the Northwest Territories, and  $n = 10$  in northern Alaska with 4 haplotypes) implies that all haplotype diversity was not captured. Overall  $F_{st}$  value was 0.20579 (1,023 permutations; total  $d.f. = 149$ ,  $P = 0.000 \pm 0.00$ ). AMOVA revealed 21% of variation partitioned among populations and 79% within populations.

Corrected average pairwise difference values ranged from 0.079 to 0.349 (Tomasik 2003), with 7 values above 0.25 and the 4 highest values associated with southeastern Alaska. Population pairwise  $F_{st}$  values ranged from 0.100 to 0.352 (Table 2), with 9 values above 0.25, and the 4 highest  $F_{st}$  values associated with southeastern Alaska.  $N_m$  values ranged from 0.921 to 4.227 (Table 2).

Isolation by distance was not supported by Mantel tests ( $P = 0.05$ ; critical value =  $-1.645$ ) using the average geographic distance versus the population pairwise  $F_{st}$  values ( $g = -0.382$ ;  $Z = 9768.84$ ;  $r = -0.098$ ), or corrected average pairwise differences ( $g = 0.003$ ;  $Z = 9364.41$ ;  $r = -0.001$ ). Isolation by distance remained unsupported when southeastern Alaska and the Kenai Peninsula were removed from analysis (population pairwise  $F_{st}$  values;  $g = -0.565$ ;  $Z = 3983.02$ ;  $r = -0.223$ , corrected average pairwise differences;  $g = -0.895$ ;  $Z = 2659.77$ ;  $r = -0.500$ ).

Estimates of exponential growth rates significantly exceeded 0 in 2 populations ( $g = 644$  and 99% confidence interval [CI]

**TABLE 2.**—Population pairwise comparisons of number of female migrants per generation ( $N_m$ ; upper) and genetic divergence ( $F_{st}$ ; lower) for populations of *Gulo gulo* based on control-region sequences. Asterisks indicate  $F_{st}$  values insignificant at the 0.05 level.<sup>a</sup>

| Population            | Eurasia        | Kenai Peninsula | Southeastern Alaska | Southern Alaska | Northern Alaska | Northwestern Alaska | Northwest Territories | Nunavut        |
|-----------------------|----------------|-----------------|---------------------|-----------------|-----------------|---------------------|-----------------------|----------------|
| Eurasia               |                | 4.22720         | <b>1.01819</b>      | <b>1.44494</b>  | 1.76471         | 3.70354             | 1.73719               | 2.66190        |
| Kenai Peninsula       | 0.10577*       |                 | <b>0.97375</b>      | 2.80458         | <b>1.53295</b>  | 2.70920             | <b>1.41355</b>        | 2.06443        |
| Southeastern Alaska   | <b>0.32934</b> | <b>0.33927</b>  |                     | <b>1.12847</b>  | <b>0.92132</b>  | 4.49646             | 2.06137               | <b>1.64183</b> |
| Southern Alaska       | <b>0.25708</b> | 0.15131         | <b>0.30704</b>      |                 | <b>1.21688</b>  | 1.98669             | 2.45912               | <b>1.64183</b> |
| Northern Alaska       | 0.22078        | <b>0.24595</b>  | <b>0.35179</b>      | <b>0.29123</b>  |                 | <b>1.48801</b>      | 2.72921               | 2.19319        |
| Northwestern Alaska   | 0.11895*       | 0.15580         | 0.10007*            | 0.20107         | <b>0.25151</b>  |                     | 3.46774               | 2.23436        |
| Northwest Territories | 0.22349        | <b>0.26129</b>  | 0.19521             | 0.16897         | 0.15484         | 0.12602             |                       | 2.58008        |
| Nunavut               | 0.15813        | 0.19498         | <b>0.25122</b>      | 0.23345         | 0.18565         | 0.18286             | 0.16233               |                |

<sup>a</sup> Highest 10  $F_{st}$  values and lowest 10  $N_m$  values are indicated in bold.

325–963 for the Northwest Territories, and  $g = 1,009$  and 99% CI 528–1,490 for Nunavut). No significant  $F_s$  values were detected (Tomasik 2003), indicating a neutral model of evolution (Fu 1997; Schneider et al. 2000).

**Cytochrome b.**—Twenty-nine complete sequences had base compositions typical of genuine mammalian mitochondria (28% adenine, 27% thymine, 30% cytosine, and 15% guanine), indicating that a nuclear pseudogene was not amplified. Sequences also exhibited expected codon biases reported for other mammalian taxa (Irwin et al. 1991). Five of the 7 amino acid changes were at sites described as highly variable in mammals (Irwin et al. 1991). One of the remaining amino acid changes occurred in a haplotype (c) unique to the southeastern Alaska population, and the other in a haplotype (f) unique to the Nunavut population. Ten haplotypes resulted from nucleotide substitutions at 15 (1.3%) of 1,140 sites. All 15 nucleotide substitutions were transitions, with substitutions at 6 first, 1 second, and 8 third positions. Eight of the 10 haplotypes were unique to 1 sampling locale (Tomasik 2003). As a result of the relatively small sample sizes used for *Cytb*, inferences based on the distribution of unique alleles are limited.

For *Cytb*, haplotype diversity values ranged from 0.29 to 0.80, and nucleotide diversity values ranged from 0.00025 to 0.00123 (Tomasik 2003). The overall  $F_{st}$  value was 0.15802 (1,023 permutations; total  $df = 28$ ,  $P = 0.01075 \pm 0.0030$ ), indicating great genetic differentiation (Hartl and Clark 1997; Wright 1978). AMOVA revealed that approximately 16% of genetic variation was partitioned among populations and 84% within populations. Frequencies of common and unique haplotypes varied across populations for both markers (Tomasik 2003).

**Trees and networks.**—A minimum spanning network for control-region sequences (Fig. 2) indicated that North American haplotypes were minimally diverged. A single haplotype (a) was shared between Eurasia and North America. One to 5 mutational steps separated Eurasian haplotypes, whereas all North American haplotypes were separated by a single mutational step from other North American haplotypes. Unique haplotypes were found primarily in the Eurasian, Nunavut, southeastern Alaska, and Kenai Peninsula populations. A minimum spanning network for *Cytb* sequences revealed similar geographic patterns (Tomasik 2003).

A neighbor-joining tree of control-region haplotypes (Fig. 3) indicated strong bootstrap support for a monophyletic clade of Eurasian haplotypes. Both *Cytb* and control-region neighbor-joining trees reflected a star phylogeny for North American haplotypes.

## DISCUSSION

**Genetic structure.**—Overall  $F_{st}$  values reflected significant differentiation between populations (Hartl and Clark 1997; Wright 1978) and contrast with nuclear perspectives of relatively homogenous populations of wolverines in the Northwest. Unique haplotypes were prevalent in Kenai, Nunavut, and southeastern Alaska populations. High to moderate gene flow has been documented with nuclear DNA studies of wolverine populations in northwestern North America (Kyle and Strobeck 2001, 2002; Wilson et al. 2000). Given the higher levels of population differentiation exhibited in this mitochondrial study, gene flow appears to be predominantly male mediated, a finding likely associated with increased female philopatry to natal territory, less long-distance dispersal, and lower reproductive success subsequent to long-distance dispersal by females. Lower population differentiation exhibited with nuclear microsatellites may also result from their faster rate of mutation and increased homoplasy. Lack of observed isolation by distance further suggests that geographic barriers may have played a role in differentiation of some populations (Britten et al. 1995; Hartl and Clark 1997; Wright 1943; but see Ballard and Whitlock 2004).

Gene flow among wolverine populations is primarily accomplished by long-range dispersal between low-density populations, which requires large areas of continuous habitat and extensive travel corridors. Human settlement and high-traffic roadways may function as more effective barriers to dispersal than natural features such as rivers and mountains (Banci 1987; Hornocker and Hash 1981). Although wolverines are highly vagile carnivores, we uncovered genetic variation that was spatially partitioned. Diversity among populations should be reduced when dispersal capability is high and barriers do not impede gene flow (Mills and Allendorf 1996; Wright 1969). Long distance dispersal has been observed for both male and female wolverines (Gardner 1985; Gardner et al. 1986; Magoun 1985). Juvenile wolverines can disperse great distances before

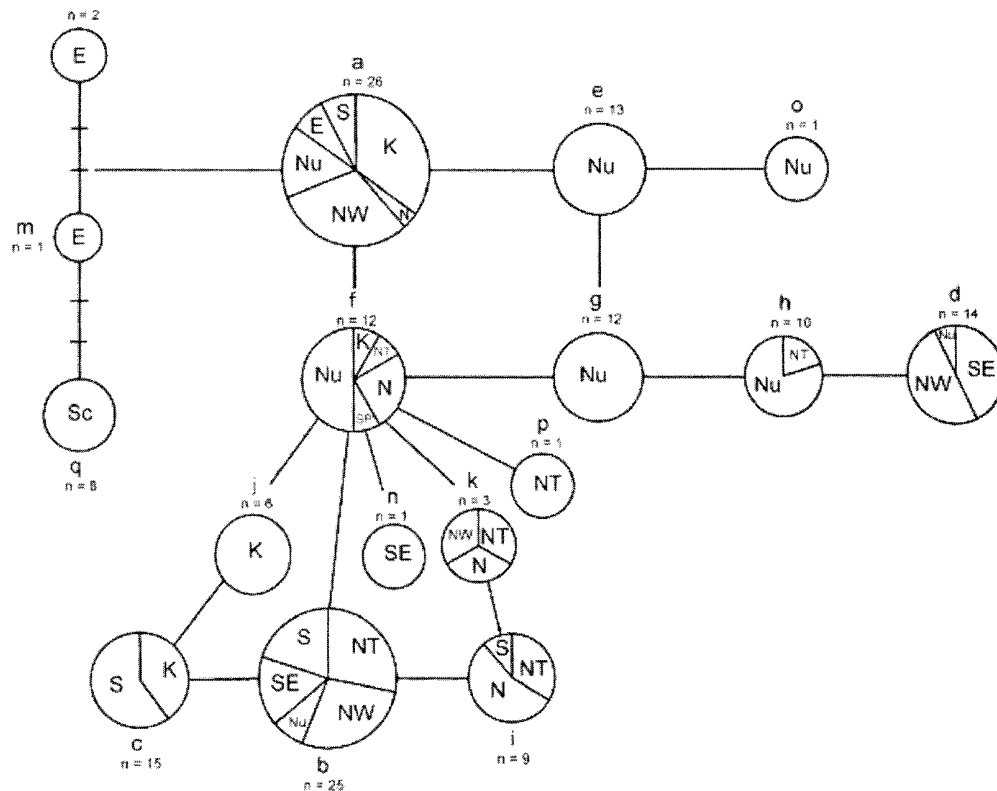


FIG. 2.—Minimum spanning network for 17 control-region haplotypes of *Gulo gulo*. Haplotype and population labels are provided. Bars indicate the minimum numbers of inferred mutational steps along a pathway. Pie diagrams indicate frequencies of populations within haplotypes. E = Eurasia, K = Kenai Peninsula, N = northern Alaska (AK), NT = Northwest Territories, Nu = Nunavut, NW = northwestern AK, S = southern AK, Sc = Scandinavia, SE = southeastern AK, SP = Seward Peninsula.

establishing their own territories. One male moved 378 km over 8 months from south-central Alaska to the Yukon (Gardner et al. 1986); however, dispersal and establishment of territories of juvenile wolverines apparently differs between sexes. Long-distance dispersal and successful reproduction is male biased (Flagstad et al. 2004), with juvenile females frequently establishing territories adjacent to or overlapping their natal territory (Banci 1987; Gardner 1985; Magoun 1985; Persson 2003; Vangen et al. 2001). Hence, maternally inherited markers should provide a distinctive signature of genetic structure.

**Historical biogeography.**—Events such as Pleistocene glacial cycles and sea level fluctuations result in complex scenarios regarding timing, plurality, and specifics of vicariance and dispersal events. The fossil record indicates a long and approximately contemporaneous history of modern *Gulo* on both continents, although the North American fossils may be older (Kurtén and Anderson 1980). Fossils of *G. gulo* occurred in deposits of Irvingtonian age or later in North America (Kurtén and Anderson 1980). Remains of *G. gulo* of possible Sangamonian to Wisconsin age have been recovered in the Yukon and had minor differences in dental morphology (see Bryant 1987 for specifics). Based on fossil evidence, *G. gulo* was thought to exist in both Beringia and refugia south of the ice sheets during the Wisconsin glaciation (Bryant 1987). Modern populations potentially may be descended from these distinctive refugia, or other hypothesized refugia such as the

High Arctic (Rand 1954), or along the North Pacific Coast (Rogers et al. 1991).

Given this history at high latitudes, we explored the magnitude of evolutionary divergence associated with the Bering Strait and whether contemporary populations reflect persistence in multiple glacial refugia. Reciprocal monophyly has not been reached between Eurasia and North America populations. Relatively low evolutionary divergence between Eurasian and North American haplotypes does not support the hypothesis that the Bering Strait divided 2 distinct species of wolverine. It is possible that occasional dispersal across the frozen Bering Strait in winter occurs. Matrilines within North America generally formed a monophyletic group nested within the broader matrilineal variation of Eurasia. Greater genetic variation and divergence in Eurasia is consistent with colonization of North America from Eurasia by modern wolverines, but more extensive geographic sampling in Eurasia is needed to test this hypothesis.

For North American wolverines, the star phylogeny, low sequence divergence, lack of distinct phylogroups, and generally high haplotype and low nucleotide diversity for both markers are all consistent with rapid population growth from an ancestral population with relatively small effective population size ( $N_e$ ) (Avice 2000; Grant and Bowen 1998; Slatkin and Hudson 1991). Although the fossil record suggests that modern *Gulo* persisted south of the ice sheets in North America, there is

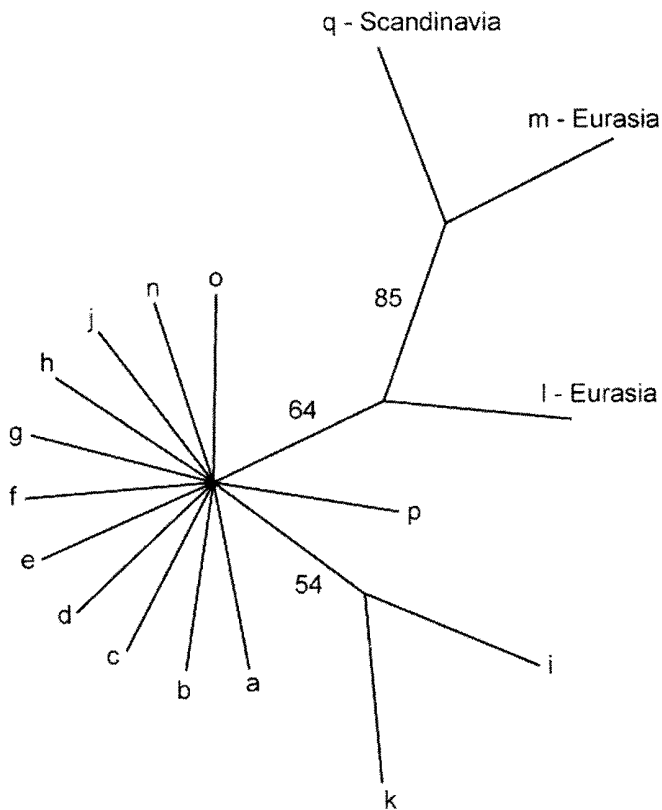


FIG. 3.—Neighbor-joining tree based on Jukes-Cantor distances calculated for control-region haplotypes of *Gulo gulo*. Haplotype labels and Eurasian populations are indicated (remaining haplotype label and population associations as in Fig. 2). Numbers represent bootstrap values > 50 based on 1,000 iterations.

no genetic signature of more than a single source for North American wolverines. Instead, the likely origin of extant North American wolverines is Beringia, with a rapid expansion south and east after deglaciation. Our inability to sample southern populations, most of which have been extirpated, precluded a rigorous assessment of this finding. A single control-region haplotype not found in this study or that of Wilson et al. (2000) was revealed with samples from the wolverine's southerly range in Idaho, Montana, and Wyoming (Cegelski 2001). A Beringian origin for a few other North American boreal mammals also has been suggested by molecular studies, including moose (*Alces alces*—Hundertmark et al. 2001) and tundra voles (*Microtus oeconomus*—Galbreath and Cook 2004). Rausch (1994) hypothesized a similar pattern for bison (*Bison*), wolves (*Canis lupus*), brown bears (*Ursus arctos*), and many coevolved parasites of transberingial host species. In contrast, marten (*Martes americana*—Small et al. 2003; Stone and Cook 2002) and black bear (*Ursus americanus*—Stone and Cook 2000; Wooding and Ward 1997) apparently diversified in southern refugia in North America during Pleistocene glacial cycles.

*Isolated or peripheral populations.*—Peripheral populations generally are isolated to varying degrees and may experience

different selective pressures that enhance genetic divergence (Lesica and Allendorf 1995; Mayr 1970; Safriel et al. 1994).

Southeastern Alaska is relatively isolated, and has been considered a distinct biogeographical province (Klein 1965; Swarth 1936), although the wolverine's ability to traverse rugged terrain may allow for genetic exchange with populations in British Columbia. The southeastern Alaska population is distinctive, as evidenced by high  $F_{st}$  values and pairwise distances, and low  $N_m$  values. Four of the 5 individuals sequenced for *Cytb* had haplotypes unique to this population (Tomasik 2003); however, limited sample size and lack of samples from adjacent British Columbia result in an incomplete view of genetic diversity. Additional research is needed to explore potential connectivity of wolverine populations across the coastal mountains, particularly given the high rate of habitat modification occurring in this region.

The Nunavut population also was distinctive, but variation is consistent with a recent and rapidly expanded population. The Nunavut population was 1 of 2 populations where estimates of the exponential growth rates significantly exceeded 0 (program FLUCTUATE V1.3—Kuhner et al. 1998). There were unique haplotypes for both markers, and rapid population growth can enhance the retention of new mutations (Avise et al. 1984).

In contrast, on the Kenai Peninsula, common and widespread haplotypes dominated, except for a single unique haplotype (control region; 6 of 22 individuals). The low haplotype and nucleotide diversity values (*Cytb*) on the Kenai (Tomasik 2003) may reflect lineage sorting, or a recent bottleneck, founder event, or selective sweep relative to other North American populations. The occurrence of common and widespread haplotypes on the Kenai Peninsula is not consistent with subspecies status for this population.

*Conclusions.*—The emerging mitochondrial view suggests low to modest levels of contemporary female-mediated gene flow between populations, but high levels of historic connectivity (Avise 2000). Matrilineal structure identified for wolverines is similar to that of gray wolves (*C. lupus*). Wolves show high population variability and several widely distributed and common mtDNA haplotypes distributed with frequencies differing between regions of North America, and greater mtDNA subdivision evident in Eurasian gray wolves (Wayne 1996; Wayne et al. 1992). The situation with wolverine contrasts with the high levels of genetic differentiation found in control-region sequences among North American brown bears (*U. arctos*—Waits et al. 1998).

Southeastern Alaska, Nunavut, and perhaps Kenai Peninsula populations harbor a disproportionate amount of the North American mitochondrial diversity. Wolverines may be more vulnerable to environmental stochasticity and anthropogenic disturbances because of limited long-distance dispersal by females, a consideration particularly relevant to those relatively isolated portions of the wolverine's range such as southeastern Alaska. Given our broad geographic coverage, additional finer-scale studies are needed for management and conservation purposes.

Human actions likely will be a controlling factor in the success and persistence of wolverine populations. The cumu-

lative effects of habitat alteration, timber harvest, increased road building, and traffic are not fully understood. Increased road access usually results in greater hunting and trapping pressure, which is a primary mortality factor for wolverines (Banci 1987; Hornocker and Hash 1981; Inman et al. 2002; Magoun 1985). This range contraction has occurred concurrently with human settlement and development. As a result of natural resource extraction, associated road building, and other human activities, wolverines on the Kenai Peninsula and southeastern Alaska may necessitate particular conservation emphasis.

Unique haplotypes are concentrated in areas of physiographic isolation such as the Kenai Peninsula and southeastern Alaska, as well as Nunavut. Appropriate management plans and conservation strategies should include provisions for conserving connectivity among populations, and conserving remaining genetic diversity of this species.

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## APPENDIX I

Specimens examined. Population, marker sequenced, and individual Alaska Frozen Tissue Collection (AF) numbers or GenBank (\*) numbers for individual *Gulo gulo*. Tissues with AF numbers are deposited at the University of Alaska, Fairbanks.

| Population            | Control region | Cytochrome <i>b</i> | Individual identification number                                                                                                                              |
|-----------------------|----------------|---------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Eurasia               |                | X                   | *AB051245, *X94921                                                                                                                                            |
| Eurasia               | X              |                     | *AF245496–245498, AF52360, AF52361                                                                                                                            |
| Eurasia               | X              | X                   | AF52357, AF52358, AF52388                                                                                                                                     |
| Kenai Peninsula       | X              |                     | AF55409, AF56832, AF56905, AF56910–56917, AF56919, AF56922–56924                                                                                              |
| Kenai Peninsula       | X              | X                   | AF56901, AF56902, AF56904, AF56906–56909                                                                                                                      |
| Northern Alaska       | X              |                     | AF4033, AF4036, AF4042, AF4048, AF17989, AF24882, AF27134, AF27137, AF34266                                                                                   |
| Northern Alaska       | X              | X                   | AF354                                                                                                                                                         |
| Northwestern Alaska   | X              | X                   | AF45909, AF45948, AF45974                                                                                                                                     |
| Northwestern Alaska   | X              |                     | AF32004, AF45911, AF45914, AF45915, AF45917, AF45919, AF45921, AF45944, AF45950, AF45953, AF45955, AF45961, AF45966, AF45967, AF45970, AF45986–45988, AF47755 |
| Northwestern Alaska   | X              |                     | AF32004                                                                                                                                                       |
| Nunavut               | X              |                     | *AF210105–210132, AF55314–55316, AF55322, AF55324, AF55393, AF55395, AF55397–55401, AF55403, AF55408                                                          |
| Nunavut               | X              | X                   | AF55320, AF55325, AF55394, AF55396, AF55402                                                                                                                   |
| Nunavut               |                | X                   | AF55323                                                                                                                                                       |
| Northwest Territories | X              |                     | *AF210090–210104                                                                                                                                              |
| Seward Peninsula      | X              |                     | AF45482                                                                                                                                                       |
| Southeastern Alaska   | X              | X                   | AF15901, AF51817, AF51819, AF51874, AF51875                                                                                                                   |
| Southeastern Alaska   | X              |                     | AF15902, AF16071, AF20010, AF51818, AF51820–51822                                                                                                             |
| Southern Alaska       | X              |                     | AF5208, AF11783, AF11784, AF11801, AF24800–24802, AF27577, AF30412, AF30422, AF39937, AF39938, AF39944, AF39946, AF39949, AF39951                             |
| Southern Alaska       | X              | X                   | AF1355                                                                                                                                                        |

## Genetic diversity and population structure of wolverine (*Gulo gulo*) populations at the southern edge of their current distribution in North America with implications for genetic viability

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### Abstract

The current range of wolverines (*Gulo gulo*) within the lower 48 states includes small, remnant populations in Idaho, Washington, Wyoming and Montana. The size and trend of each of these populations and connectivity to adjacent populations in the contiguous United States and Canada are poorly understood. In this study, levels of genetic diversity and population genetic structure were examined in three states (Idaho, Wyoming, and Montana) and two Canadian provinces (Alberta and British Columbia) using both mitochondrial (mtDNA) and nuclear microsatellite DNA. Restricted levels of gene flow were detected among these populations with mitochondrial and nuclear DNA and our observations suggest a pattern of male-mediated gene flow. Populations in the United States appear to be receiving migrants from Canada, however, substantial genetic differentiation suggests that gene flow may not be high enough to prevent genetic drift. Our analyses suggest that at least 400 breeding pairs or 1–2 effective migrants per generation would be needed to ensure genetic viability in the long-term for each of the populations in the United States. Significant matrilineal structuring and restricted female gene flow indicates that demographic viability will depend upon the movement of female wolverines into new territories. Results from this study provide guidelines for conservation and management and indicate the need for more ecological data.

### Introduction

The wolverine (*Gulo gulo*) is one of the least studied species among large terrestrial carnivores due to its elusive nature, low density and large dispersal ability. The species is currently listed as “vulnerable” by the IUCN (Taylor 2000) and is especially sensitive to human disturbance and habitat destruction (Banci 1994). Wolverines have a circumpolar distribution, which historically included the tundra and taiga zones of Scandinavia

and Germany to Northeastern Siberia, as well as North America (Hall 1981). In the past 150 years, the worldwide range of the species has been considerably reduced due to anthropogenic factors such as habitat destruction, exploitation for fur, and lethal predator control programs (Schreiber et al. 1989). Wolverines in the Canadian provinces and the state of Alaska may be the most stable and largest contiguous population of wolverines worldwide (Banci 1994). Currently, wolverines can be found in low numbers in the boreal forests of

Norway, Sweden, Finland, China, Russia, and the contiguous United States (Schreiber et al. 1989). In the contiguous United States, wolverine populations were once distributed across the northernmost tier of states and southward to New Mexico and Arizona (Hash 1987), but are currently patchily distributed in Idaho, Washington, Wyoming and Montana (Hash 1987; Banci 1994; Figure 1).

As a consequence of range contraction, a petition was filed in 1995 with the United States Fish and Wildlife Service (USFWS) to list the wolverine as threatened within the contiguous United States. The USFWS did not find sufficient evidence to list the wolverine as threatened (USFWS 1995). A second petition, filed in 2000, was also denied due to insufficient evidence of a change in status (USFWS 2003). Currently, each population in the contiguous United States is managed independently under state jurisdiction. Effective

management and conservation for wolverines requires an understanding of the demographics and connectivity of these remnant populations to each other and to the larger core population of wolverines in Canada.

Field data regarding dispersal and connectivity of wolverine populations within the contiguous United States is limited to three published studies (Hornocker and Hash 1981; Copeland 1998; Edelmann and Copeland 1999). Difficulties in assessing ecological and demographic requirements of wolverines can be attributed to life history characteristics of the wolverine. It is a solitary species with a propensity to occupy large, remote, high elevation and high latitude habitats at low densities. Home ranges can extend from 100 to 900 km<sup>2</sup> (Magoun 1985; Banci 1987) and densities vary from one individual per 40–800 km<sup>2</sup> (Banci 1994). Daily movements average 30–40 km (Krott 1960; Haglund 1966; Pulliainen 1968; Banci 1994),



Figure 1. Sampling localities for eight wolverine populations in Southern Canada and Northwestern United States.

and although the propensity for long distance movement exists (Hornacker and Hash 1987), this does not necessarily imply gene flow as wolverines frequently make long exploratory movements prior to dispersal (Vangen et al. 2001).

Genetic analyses can provide an alternative and complimentary method for evaluating population health and demographic connectivity. Genetic diversity may be a useful indicator for identifying populations vulnerable to inbreeding, demographic stochasticity, and reduced viability. A loss in genetic diversity may lead to a decrease in fitness (Coltman et al. 1998; Westemeier et al. 1998; Madsen et al. 1999; Reed and Frankham 2003) although this relationship is frequently debated (Hedrick and Miller 1992; Amos and Balmford 2001; Reed and Frankham 2001). Gene flow between populations is also an important consideration because in small populations, it counters the forces of random genetic drift by increasing effective population size and minimizing possible stochastic (Frankham 1995; Storfer 1999) and inbreeding effects (Westemeier et al. 1998; Madsen et al. 1999). Evidence of reduced gene flow among populations can further identify populations of conservation and management concern.

Population genetic structure of wolverines has previously been evaluated in parts of North America, revealing varying levels of connectivity among adjacent populations. A mitochondrial DNA (mtDNA) study by Wilson et al. (2000) in the Northwest Territories of Canada found significant population differentiation among regions separated by 100 km. Female philopatry was proposed as a potential mechanism, although several females were observed to have moved significant distances. Kyle and Strobeck (2001, 2002) examined genetic diversity and population structure of wolverines from regions across much of their North American distribution using 12 microsatellite loci (nDNA). Genetic distance measures and diversity estimates suggested that the northern populations, from Alaska to Nunavut, Canada are connected through relatively high levels of gene flow and that the Idaho, Wyoming, and Revelstoke populations had become fragmented from northern populations. The differences in genetic structure proposed by Kyle and Strobeck (2001, 2002) and Wilson et al. (2000) prompted a re-evaluation of the genetic structure of wolverines in northern Canada by Chappell

et al. (2004). This investigation suggested that there were differences in dispersal patterns between the two sexes (Chappell et al. 2004). In a nDNA evaluation of genetic structure of Montana wolverines, Cegelski et al. (2003) found a high degree of population subdivision and defined three subpopulations; while no such fragmentation had been observed across a similar spatial scale in Northern Canada (Kyle and Strobeck 2001, 2002).

This study provides an examination of genetic diversity and population genetic structure of wolverine populations at the southern extent of their North American range using 217 samples collected from populations in Montana, Idaho, Wyoming, central Alberta and southern and central British Columbia. Unlike previous studies which included these populations, we have combined both nuclear microsatellite and mitochondrial DNA data, enabling a finer-scale investigation of the degree of differentiation among adjacent populations. By combining datasets from previously published studies and adding mtDNA sequence data, we address: (1) the level of gene flow and connectivity among populations in southern Canada and adjacent wolverine populations in the contiguous United States, (2) the potential for sex-biased dispersal among populations and (3) the management implications for long-term persistence of these vulnerable populations at the southern edge of the wolverine's range.

## Materials and methods

### *Sampling*

Tissue and/or extracted DNA samples were obtained from the following localities: central Idaho (n = 15), Yellowstone and Grand Tetons National Parks, Wyoming (n = 13), Grande Cache, Alberta (n = 17), Williston Lake, British Columbia (n = 37), Revelstoke, British Columbia (n = 47) and three populations defined in Montana (n = 89, Cegelski et al. 2003): Rocky Mountain Front (n = 44), Gallatin (n = 26), Crazybelts (n = 19; Figure 1, Table 1). Microsatellite data for these populations were previously reported by Kyle and Strobeck (2001, 2002) and Cegelski et al. (2003) and we have sequenced the mtDNA control region of these samples for the current study.

Table 1. Number of mitochondrial DNA control region haplotypes in each wolverine population sampled in Canada and the United States along with total frequency of the haplotype in the dataset

| Haplotype |     |     |     |     |     |     |     | Frequency in each population |       |       |     |       |       |       |       | Total |
|-----------|-----|-----|-----|-----|-----|-----|-----|------------------------------|-------|-------|-----|-------|-------|-------|-------|-------|
| 111       | 133 | 173 | 197 | 228 | 235 | 251 |     | RMF                          | CB    | GA    | ID  | WY    | WL    | REV   | GC    |       |
| C         | A   | –   | C   | A   | G   | C   | A   | 40                           | 4     | 20    | 15  | 10    | 11    | 4     | 5     | 65%   |
| T         | A   | –   | T   | A   | G   | C   | C   | 0                            | 0     | 0     | 0   | 0     | 0     | 0     | 2     | 1%    |
| C         | A   | –   | T   | A   | G   | C   | F   | 0                            | 0     | 0     | 0   | 0     | 0     | 0     | 2     | 1%    |
| C         | A   | –   | T   | A   | A   | C   | H   | 0                            | 0     | 0     | 0   | 1     | 0     | 5     | 0     | 4%    |
| T         | A   | C   | T   | A   | G   | C   | I   | 4                            | 13    | 5     | 0   | 2     | 0     | 0     | 0     | 14%   |
| C         | A   | –   | C   | A   | G   | T   | L*  | 0                            | 2     | 0     | 0   | 0     | 6     | 4     | 8     | 12%   |
| C         | A   | –   | C   | G   | G   | C   | M*  | 0                            | 0     | 0     | 0   | 0     | 1     | 0     | 0     | <1%   |
| C         | G   | –   | C   | A   | G   | C   | N** | 0                            | 0     | 0     | 0   | 0     | 1     | 0     | 0     | <1%   |
| C         | A   | –   | C   | A   | A   | C   | O*  | 0                            | 0     | 0     | 0   | 0     | 0     | 3     | 0     | 2%    |
| Total     |     |     |     |     |     |     |     | 44                           | 19    | 25    | 15  | 13    | 19    | 16    | 17    | 168   |
| <i>h</i>  |     |     |     |     |     |     |     | 0.17                         | 0.50  | 0.33  | N/a | 0.41  | 0.59  | 0.79  | 0.71  |       |
| $\pi$     |     |     |     |     |     |     |     | 0.002                        | 0.006 | 0.004 | 0.0 | 0.004 | 0.002 | 0.006 | 0.004 |       |

Abbreviations refer to the following populations: RMF=Rocky Mountain Front; CB=Crazybelts; GA=Gallatin, ID=Idaho; WY=Wyoming; WL=Williston Lake; GC=Grande Cache. \*indicates haplotypes that were not previously identified; – denotes a deletion; \*\*N=Tomasik and Cook 2005 (Haplotype B); *h*=haplotype diversity;  $\pi$ = nucleotide diversity.

#### DNA extraction and microsatellite analysis

DNA was extracted from a 1-mm piece of tissue using a modified Qiagen tissue protocol (Qiagen Co., USA). Buffer ATL was substituted with 1× Nucleic Acid Purified Lysis Buffer (Applied Biosystems, USA), which aids in the lysis of older tissue samples (Guglich et al. 1996). DNA was re-suspended in 400  $\mu$ l of Buffer AE (Qiagen Co., USA).

Ten microsatellite loci were amplified: Lut604 (Dallas and Piertney 1998), Ggu101B, Ggu216, Ggu234 (Duffy et al. 1998) Gg3, Gg4, Gg7, Gg14 (Davis and Strobeck 1998), Tt-4 and Ma-3 (Davis and Strobeck 1998). Polymerase chain reaction (PCR) conditions are presented in Kyle and Strobeck (2001) and Cegelski et al. (2003).

#### Mitochondrial DNA sequencing

A short segment (300 bp) of the mitochondrial DNA (mtDNA) control region was amplified for a subset of samples in all of the populations, using the primers, L15926 and H16498 as described in Wilson et al. (2000). PCR products were purified using ExoSap (USB Co., USA) and Sephadex (Sigma Co., USA). Both heavy and light strands were cycle-sequenced using the PRISM DyeDeoxy Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems Inc., USA) and loaded on an

ABI 377. Sequences were analyzed using Sequencher 3.1 and manually aligned with PAUP 3.0 (Swafford 1998). Haplotypes observed only once were re-amplified and sequenced to verify accuracy.

#### Statistical analysis

##### Hardy–Weinberg and linkage disequilibrium

Each population was tested for Hardy–Weinberg equilibrium and linkage equilibrium using GENEPOP (available at <http://www.wbiomed.curtin.edu.au/genepop/>; Raymond and Rousset 1995). A sequential Bonferroni correction ( $\alpha = 0.05$ ) was used to correct for multiple comparisons (Rice 1989).

##### Genetic diversity

Mitochondrial DNA diversity was evaluated using nucleotide diversity ( $\pi$ ), and haplotype diversity values (*h*) for each population using ARLEQUIN 2.0 (Schneider et al. 2000). Nuclear diversity was measured by both mean number of alleles per locus (*A*) and Nei's unbiased expected heterozygosity ( $H_e$ ; Nei 1978). A rarefaction option performed by FSTAT 2.9.3 (available at <http://www.unil.ch/izea/software/fstat.html>) was used to correct *A*

for unequal sample sizes. Corrected estimates of allelic diversity ( $R_i$ ) were obtained based upon the smallest sample size of this study ( $n = 12$ ). Statistical differences in  $H_e$  were evaluated using an arcsine log transformation and paired  $t$ -test (Archie 1985; Nei 1987).

Simulations were carried out using the program GENELOSS (England and Osler 2001) to determine the minimum number of breeders needed to maintain genetic variation (heterozygosity) in the United States populations. Genetic drift was simulated over 100 generations for each population using observed allele frequencies, no migration, and no mutation. The number of migrants ( $N_m$ ) needed to maintain genetic diversity was also calculated using the following equation (Miller and Waits 2003):

$$H_e(\text{source}) = H_e(\text{sampled}) \times [4N_m / (4N_m + 1)]$$

#### *Population genetic structure*

Female population structure was evaluated using mtDNA sequence data. Analysis of Molecular Variance (AMOVA) and pairwise  $F_{st}$  estimates were calculated using ARLEQUIN 2.0 (Schneider et al. 2000). A hierarchical AMOVA analysis (Excoffier et al. 1992) was performed to reflect how mtDNA is partitioned at different levels of a hierarchy. Two groups were *a priori* designated following geographic location (Canada, United States) to determine how genetic variation was partitioned between these two groups, among populations within these groups, and within populations.

A nested clade analysis (NCA) was used to gain a more complete depiction of the patterns and processes responsible for the observed mtDNA population structure (Templeton et al. 1992, 1995; Templeton and Sing 1993). NCA uses statistical tests based upon a minimum spanning network to explain the genetic structure of a population. Hypotheses such as fragmentation, isolation by distance effects, and range expansion can be simultaneously evaluated (Templeton et al. 1998). TCS 1.13 (Clement et al. 2000) was used to estimate the minimum spanning network using the algorithm of Templeton et al. (1992). The network and geographic coordinates were then entered into

the program GeoDIS (Posada et al. 2000) to perform the cladistic analysis described in Templeton et al. (1992). Templeton's updated key (2004) was used to infer population processes (available at <http://www.darwin.uvigo.es/software/geodis.html>).

Nuclear genetic differentiation was measured by pairwise  $F_{st}$  estimates (Weir and Cockerham 1984) and an AMOVA analysis. The AMOVA analysis was conducted for the same hierarchical relationships as the mtDNA. The relationship between geographic distance and genetic distance ( $F_{st}$ ) was further investigated with a Mantel test (Mantel 1967). Geographic distance was measured in kilometers for each pair of sampling locations. A regression of  $F_{st}/(1-F_{st})$  on the logarithm of geographic distance for all population pairs was conducted with Genepop on the Web (Raymond and Rousset 1995). Genetic structure was also visualized using principle component analysis (PCA) of GENETIX (available at <http://www.univ-montp2.fr/~genetix/genetix.htm>) and a dendrogram generated with Cavalli-Sforza and Edward's (1967) chord distance using the program POPULATIONS (Langella et al. 2001).

Two Bayesian assignment tests (STRUCTURE; Pritchard et al. 2000 and GENECLASS; Paetkau et al. 2004) were also utilized to detect migration and the degree of reproductive discreteness among sampling locations. The Bayesian assignment test of STRUCTURE was used to assign individuals to clusters based upon multi-locus genotypes. Five independent runs for each starting number of populations ( $K = 1-10$ ) were performed at 200,000 MCMC repetitions following 200,000 burn-in period using no prior information and assuming correlated allele frequencies and admixture. The posterior probability was then calculated for each value of  $K$  using the estimated log likelihood of  $K$  to choose the optimal  $K$ . Individuals were assigned to respective populations based upon percentage of membership ( $q$ ). The Bayesian assignment test of GENECLASS was used to test each individual for its status as resident or recent immigrant using a Monte Carlo re-sampling method (Paetkau et al. 2004) of 10,000 simulated individuals. The likelihood ratio of drawing a genotype from the population of sampling origin ( $L_h$ ) over the likelihood of observing the genotype in any of the sampled populations ( $L_{max}$ ) was computed using the program GENECLASS and an alpha level of 0.01 was used for significance.

## Results

### *Hardy-Weinberg and linkage equilibrium*

Eighty exact tests for H-W equilibrium were performed, of which, nineteen were significant at the 0.05 level prior to a Bonferroni correction. None of the significant tests clustered around a specific population or locus and only one of the tests was rejected at the 0.05 level following a sequential Bonferroni correction. None of the tests for linkage equilibrium were significant at the 0.05 level with a sequential Bonferroni correction.

### *Mitochondrial DNA diversity*

A 300 bp fragment of the mtDNA control region was sequenced for 168 individuals representing geographic areas in Montana, Idaho, Wyoming, British Columbia and Alberta. Seven variable sites including six transitions and one insertion-deletion identified nine haplotypes (A, C, F, H, I, L, M, N, O); Figure 2). All haplotypes were compared against those previously reported (Wilson et al. 2000; Chappell et al. 2004; Tomasik and Cook 2005) and haplotypes L, M, and O were not previously identified. Our haplotype N matched haplotype B reported by Tomasik and Cook (2005). Haplotype A was the most widespread haplotype, occurring in 53% of the samples. The next most frequent haplotypes, I and L, were found in 14% and 13% of the samples, respectively. Haplotypes C, F, H, M, N, and O accounted for 20% of the samples (Table 1). Haplotype diversity values ( $h$ ) ranged from 0.00 in

Idaho where only one haplotype was recorded to 0.79 in Revelstoke. Five haplotypes (C, F, M, N, and O) were identified in the Canadian populations only, and one haplotype (I) was seen only in the United States populations. Nucleotide diversity values ( $\pi$ ) ranged from 0.001 to 0.006, indicating a close relationship among haplotypes (Table 1, Figure 2).

### *MtDNA population structure*

A hierarchical AMOVA analysis was performed to detect population genetic structure in females. For the two defined groups (Canada, USA): 8% of the variation was detected among groups, 22.21% of the variation was partitioned among populations within groups, and 69.8% of the variation was partitioned within populations. Significant  $\Phi_{st}$  values were found for United States populations paired with Alberta and for all populations paired with the Crazybelts population (Table 2). Furthermore, the Rocky Mountain Front population was significantly differentiated from the Williston Lake, BC population and the Wyoming population was differentiated from the Idaho population. The Idaho population was fixed for one haplotype, and was not significantly differentiated from the Rocky Mountain Front or Gallatin populations due to the prevalence of that haplotype in those populations.

The NCA revealed a network with three distinct clades. A significant geographical association of haplotypes was found for clade 1-1 ( $P < 0.01$ ), clade 1-2 ( $P = 0.035$ ) clade 1-3 ( $P < 0.01$ ) and clade 2-1 ( $P < 0.01$ ). The ancestral and most widespread haplotype found was haplotype A (Figure 2). Clade 1-1 included haplotype A which was found in high frequency in all of the populations; haplotype L which was found in high frequency in the Canadian populations and one population in Montana, and haplotypes N and O which were found only in Canada. Clade 1-1 revealed restricted gene flow with isolation by distance. Clade 1-2 provided an inconclusive outcome due to sampling two haplotypes in only eight individuals but the limited distribution of each haplotype may also indicate limited gene flow among the sampled populations within and between regions. Clade 1-3 provided evidence for restricted gene flow and dispersal with some dispersal among the populations in the contiguous United States.

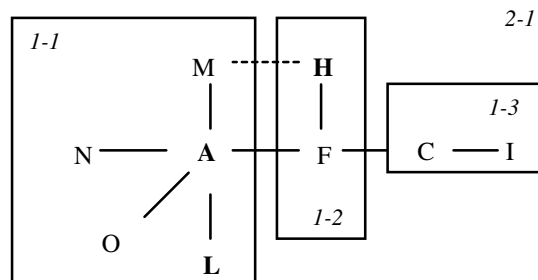


Figure 2. Haplotype network of the wolverine mitochondrial DNA haplotypes with TCS 1.13 (Clement et al. 2000). Each line represents a mutational change. Boxes are drawn around clades.

Table 2. Levels of genetic differentiation measured by  $\Phi_{st}$  for mitochondrial DNA data (below) and  $F_{st}$  for microsatellite data (above) calculated using ARLEQUIN (Schneider et al. 2000)

|     | RMF    | WY     | CB     | GA     | ID     | GC     | WL     | RE     |
|-----|--------|--------|--------|--------|--------|--------|--------|--------|
| RMF |        | 0.008  | 0.105* | 0.084* | 0.124* | 0.090* | 0.064* | 0.055* |
| WY  | 0.002  |        | 0.036* | 0.067* | 0.136* | 0.075* | 0.053* | 0.034* |
| CB  | 0.558* | 0.334* |        | 0.095* | 0.213* | 0.126* | 0.120* | 0.078* |
| GA  | 0.020  | -0.050 | 0.345* |        | 0.192* | 0.156* | 0.173* | 0.120* |
| ID  | 0.019  | 0.121  | 0.607* | 0.120  |        | 0.200* | 0.112* | 0.142* |
| GC  | 0.248* | 0.136* | 0.434* | 0.171* | 0.295* |        | 0.044* | 0.053* |
| WL  | 0.164* | 0.151* | 0.555* | 0.177* | 0.181* | 0.052  |        | 0.037* |
| RE  | 0.220* | 0.072  | 0.415* | 0.152* | 0.219* | 0.078  | 0.114* |        |

Population abbreviations follow Table 1.

\*Indicates significance at the 0.05 level.

Haplotype I was common to the populations in the contiguous U.S. (in unequal frequencies) but not Canada and haplotype C was only found in Canada. Clade 2-1 revealed restricted gene flow/dispersal with occasional long distance dispersal.

#### Nuclear DNA diversity

Average heterozygosity levels ranged from 41.1% in Idaho to 62.8% in the Williston Lake population (Table 3). Allelic richness ( $R_t$ ) measured 2.9 alleles/locus in Crazybelts to 4.3 alleles/locus in Grande Cache, Alberta (Table 3). A Wilcoxon-sign rank test revealed that both the Gallatin and Crazybelt populations in Montana had significantly lower allelic richness than all other populations sampled in the United States and Canada ( $P < 0.05$ ). Heterozygosity levels for the Rocky

Mountain Front population were not significantly different than the Wyoming population, Revelstoke population, or Canadian populations (Revelstoke, Williston Lake, Grande Cache;  $P > 0.16-0.84$ ). Heterozygosity levels of the Gallatin, Crazybelts, and Idaho populations also were not statistically different from each other ( $P > 0.80$ ). However, the Rocky Mountain Front population, Wyoming population, and populations in Canada exhibited significantly higher heterozygosities than the other populations in Montana and Idaho ( $P < 0.05$ ).

#### Nuclear DNA population structure

$F_{st}$  values were significantly greater than zero for all pairwise comparisons except those involving Wyoming (Table 2), which had the lowest  $F_{st}$  values for each comparison (Table 2). The Idaho population had the highest  $F_{st}$  values for all pairwise comparisons. The Mantel test indicated that there was no relationship between genetic distance and geographic distance ( $r^2 = 0.06$ ;  $P = 0.11$ ; Figure 3). A distinct break was identified between the U.S. populations and Canadian populations by both the dendrogram (Figure 4) and PCA analyses (Figure 5). Population scores for the PCA were plotted on two principle axes (PC1, PC2), which cumulatively explained 52% of the genetic variation. The PCA plot and dendrogram both showed that all of the Canadian populations clustered together; the Rocky Mountain Front population clustered with the Wyoming population, the Crazybelts population clustered with the Gallatin population, and the Idaho population was highly differentiated. The AMOVA analysis partitioned

Table 3. Estimates of genetic diversity for wolverine populations at 10 microsatellite loci based upon mean number of alleles per locus ( $A$ ), allelic richness ( $R_t$ ), mean expected heterozygosity ( $H_e$ ; Nei 1978) and mean observed heterozygosity ( $H_o$ ) per population

|                       | n  | A   | $R_t$ | $H_e$ (%) | $H_o$ (%) |
|-----------------------|----|-----|-------|-----------|-----------|
| United States         |    |     |       |           |           |
| Rocky Mountain Front  | 44 | 4.6 | 3.6   | 56.7      | 50.2      |
| Crazybelts            | 19 | 3.4 | 2.9   | 48.8      | 41.5      |
| Gallatin              | 26 | 3.7 | 3.0   | 42.2      | 40.0      |
| Wyoming               | 12 | 3.6 | 3.6   | 56.3      | 55.4      |
| Idaho                 | 15 | 3.2 | 3.2   | 41.1      | 43.6      |
| Canada                |    |     |       |           |           |
| Revelstoke, BC        | 47 | 5.1 | 4.0   | 54.7      | 60.2      |
| Williston Lake, BC    | 37 | 4.9 | 4.3   | 62.8      | 56.6      |
| Grande Cache, Alberta | 17 | 4.7 | 4.1   | 59.8      | 52.9      |

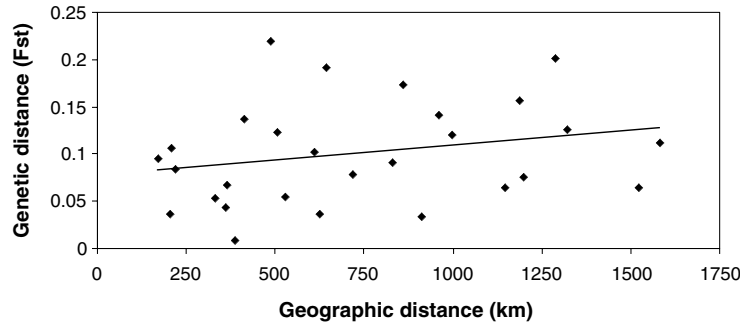


Figure 3. Relationship between genetic distance ( $F_{st}$ ) and geographic distance (km) in southern wolverine populations in North America.

3% of the variance among groups, 7% among populations within groups and 90% within populations.

The Bayesian assignment test of STRUC-TURE was used to identify the number of population clusters and simultaneously assign individuals to each of the identified clusters. Seven population clusters were identified without any

prior population information (Table 4). The highest proportion of membership for the Revelstoke population was found in cluster 1 (Table 5). Cluster 2 mainly consisted of individuals from the Alberta population. Cluster 3 mainly consisted of Idaho samples. In cluster 4, the highest proportion of membership was from Williston Lake. Clusters 5, 6, and 7 represent the Crazybelts, Gallatin, and

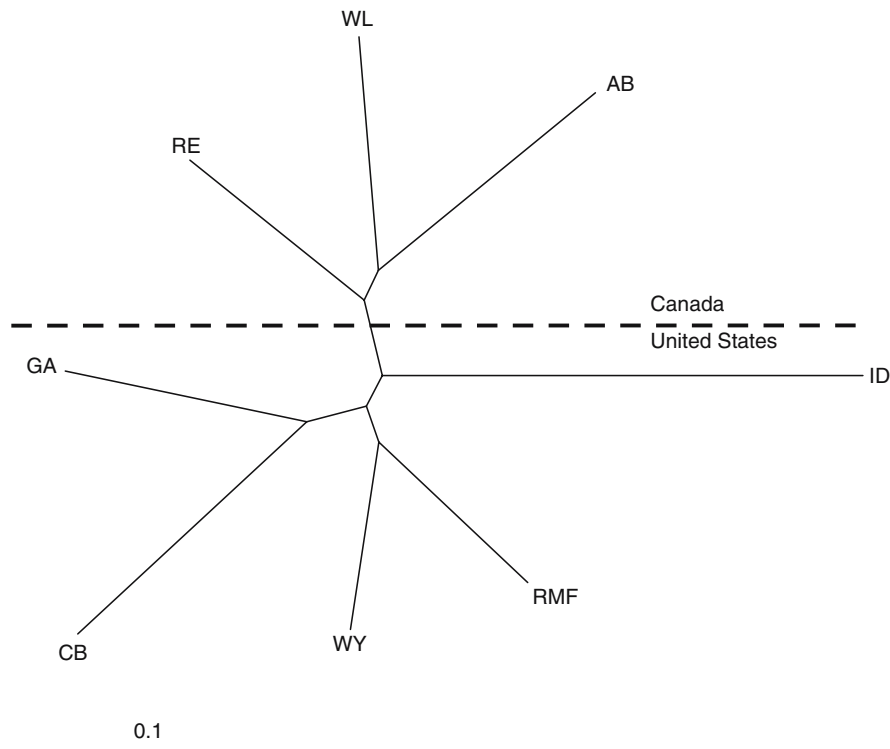


Figure 4. Neighbor-joining dendrogram of the genetic relationships among eight wolverine populations inferred from a matrix of Cavalli-Sforza and Edwards chord distance using 10 microsatellite loci.

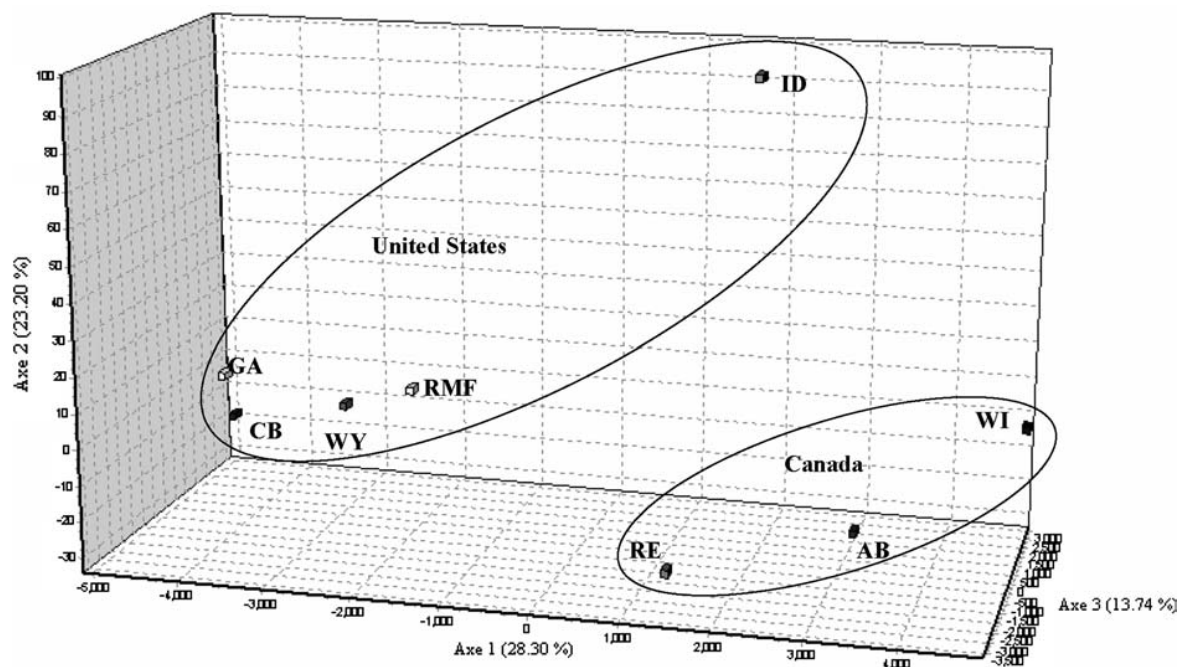


Figure 5. Principle components analysis (PCA) scores of wolverine microsatellite genotypes plotted on the two first axes (PC1, PC2) of a PCA using GENETIX.

Rocky Mountain Front populations, respectively. There was no cluster assigned to the population in Wyoming. The proportion of individuals that were self-assigned to a cluster appeared to be correlated with levels of genetic variability. A regression analysis indicated that self-assignment increased by decreasing levels of expected heterozygosity ( $R^2=0.73$ ;  $P=0.014$ ).

The Bayesian analysis of STRUCTURE was not used to detect migrants since the posterior probabilities for all of the individuals in the sampled populations was low ( $q < 0.90$ ). Therefore the Bayesian assignment test of GENECLASS was used to identify recent migrants. The Bayesian analysis of GeneClass identified nine migrants in total with high stringency ( $P=0.01$ ; Table 5b). For the United States populations, three males immigrated to the Rocky Mountain Front population; two females immigrated to the Crazybelts population; one male and one female immigrated to the Gallatin population; one male immigrated to the Wyoming population; and one male immigrated to the Idaho population. Two individuals (1 male, 1 female) were migrants from Canada to the US, but no migration was detected from US to Canada or between the Canadian populations.

## Discussion

### *Population genetic structure*

Wolverines are believed to have expanded southward following the last glaciation event from a single refugia in Beringia (Bryant 1987; Tomasik and Cook 2005). Our mitochondrial data are consistent with this hypothesis. Haplotype A was the center of the star phylogeny and most widespread haplotype while the limited distribution of recently derived haplotypes suggest that these haplotypes arose more recently and have not become widespread due to restricted gene flow. Haplotype A was also the most widely distributed haplotype in previous studies of Alaska and northern Canada (Chappell et al. 2004; Tomasik and Cook 2005). Five of the haplotypes were only present in the Canadian populations while there was some overlap in the geographic distribution of haplotypes H and L among populations in both the United States and Canada. Substantial frequency differences and lack of geographic overlap among the majority of the haplotypes within populations in each region and between regions support limited contemporary gene flow. The

NCA provided additional support that the association of haplotype distribution with geography was due to restricted gene flow with some long distance dispersal.

Nested clade analysis has been criticized in recent literature for its inability to distinguish the correct history of a species using simulation data (Knowles and Maddison 2002). While a new key has been developed to resolve most of the previous criticism (Templeton 2004), the main criticism of the approach was errors with range expansion inferences. Since the NCA was able to accurately infer fragmentation events with simulation data (Templeton 2004), there is increased support that NCA is accurately depicting population processes for our dataset. Our  $F_{st}$  estimates using mtDNA data also substantiated the NCA results, with high degrees of population differentiation observed among the sampled populations.

The amount of genetic differentiation was larger for mitochondrial DNA compared to the nuclear DNA analyses. In the AMOVA analyses, the nDNA  $\Phi_{st}$  value was 0.09 compared to the mtDNA  $\Phi_{st}$  value of 0.22. Population pairwise  $F_{st}$  estimates were also higher for mtDNA compared to nDNA. This could be a reflection of historical founding events and sex-biased dispersal. While the nDNA is representative of both female and male-mediated gene flow, the mtDNA only reveals female-mediated gene flow. Female wolverines are generally philopatric while males are the predominant dispersers (Banci 1994; Vangen et al. 2001), but occasional long-distance dispersal has been documented for female wolverines in ecological

studies (Vangen et al. 2001). The NCA revealed restricted gene flow and dispersal with occasional long-distance dispersal. These data supports the findings of Chappell et al. (2004), which detected a similar pattern of mtDNA and nDNA structuring in Canada and concluded that male-mediated gene flow was predominant. The microsatellite data also indicated that while female migration was occurring, males are the predominant dispersers. Sixty-six percent of the migrants detected by the Bayesian assignment test were males and a previous analysis of Montana wolverines documented differences in male and female gene flow using  $F_{st}$  and  $q$  values (Cegelski et al. 2003). Therefore, our data indicate that there is limited contemporary female-mediated gene flow among the sampled populations and significant matrilineal structuring.

Although the level of genetic differentiation is much lower for microsatellite DNA, there is still strong evidence for restricted gene flow among the sampled populations. Earlier nDNA studies of gene flow among populations of wolverines in northern North America have documented high gene flow and connectivity of populations across thousands of kilometers (Kyle and Strobeck 2001, 2002; Chappell et al. 2004). In an evaluation of wolverine population structure in northern Canada across over 4000 km from the Northwest Territories to Manitoba, Chappell et al. (2004) documented a global  $F_{st}$  of 0.0004 and only a single population unit using STRUCTURE. In contrast, we observed an optimal  $K$  value of 7 and a global  $F_{st}$  of 0.08 across a much smaller spatial scale. Earlier research has documented this increase in population structure at the southern extent of the range (Kyle and Strobeck 2001, 2002; Cegelski et al. 2003); however, these studies were not able to address the connectivity of wolverines in Idaho or Montana to each other or to adjacent populations in Canada.

This study places all of the sampled populations into context by analyzing them with adjacent populations in the region to determine the extent of gene flow. Our findings suggest that the Idaho population is isolated from neighboring populations in Montana as well as all other populations. The Idaho population had the lowest diversity levels and largest proportion of membership in a single cluster (92%), indicating significant isolation. Only one individual was detected as a disperser from the Rocky Mountain Front

Table 4. Bayesian clustering results of STRUCTURE, without using any prior population information

| $K$      | Log $P(k/x)$ | Variance Log $P(k/x)$ |
|----------|--------------|-----------------------|
| 1        | -5326        | 27.8                  |
| 2        | -5135        | 173.3                 |
| 3        | -4998        | 228.3                 |
| 4        | -4891        | 286.7                 |
| 5        | -4843        | 366.5                 |
| 6        | -4750        | 408.8                 |
| <b>7</b> | <b>-4714</b> | <b>493.8</b>          |
| 8        | -4821        | 754.8                 |
| 9        | -4879        | 871.2                 |
| 10       | -4859        | 978.2                 |

$K$  represents the number of subpopulations. The value in bold indicates the most likely value for  $K$ .

population into Idaho and this individual was killed while crossing a highway (Copeland, personal communication). In Montana, the greatest concentration of wolverines resides within the Rocky Mountain Front population. This population is also the most geographically proximate population to Canada. The Rocky Mountain Front population had diversity levels comparable to Canada and the assignment test of GENE-CLASS suggested that this population had received one recent migrant from the Canadian populations. However, STRUCTURE did not detect a signal of admixture among any of the Canadian populations and the Rocky Mountain Front population that would result from ongoing migration. Other immigrants, from the Gallatin population, were detected in this population. Therefore, this population appears to be receiving migrants from the other sampled populations. The

Gallatin and Crazybelt populations also appear to be exchanging some migrants, although they are more genetically differentiated and isolated from the other sampled populations compared to the Rocky Mountain Front population (Cegelski et al. 2003).

The population in Wyoming is also geographically proximate to those in Idaho and Montana. The Wyoming samples were collected in Yellowstone and Grand Teton National Parks which comprise over 900,000 ha of protected land. Minimal differentiation detected between the Rocky Mountain Front and Wyoming populations compared to the Gallatin population suggests that wolverines may be moving through the Gallatin range without establishing residency or finding alternate routes into these protected areas. The lack of differentiation and assignment of all individuals from Wyoming to other population clusters

Table 5. Assignment test results using 10 microsatellite loci. (a) Bayesian assignment test for  $K=7$  without using any prior population information (Pritchard et al. 2000). Columns indicate the clusters and each row indicates the proportion of individuals from each population assigned to a cluster. The highest proportion of membership is indicated in bold. Population abbreviations follow Table 1. (b) Bayesian assignment test (Paetkau et al. 2004). Number of migrants identified in each population ( $P=0.01$ ) and population of origin followed by the sex of each identified migrant

| N                         |    | Clusters    |             |             |                     |             |                   |             |
|---------------------------|----|-------------|-------------|-------------|---------------------|-------------|-------------------|-------------|
|                           |    | 1           | 2           | 3           | 4                   | 5           | 6                 | 7           |
| (a)                       |    |             |             |             |                     |             |                   |             |
| RMF                       | 44 | 0.09        | 0.09        | 0.09        | 0.05                | 0.06        | 0.12              | <b>0.47</b> |
| CB                        | 19 | 0.02        | 0.02        | 0.02        | 0.05                | <b>0.68</b> | 0.17              | 0.03        |
| GA                        | 26 | 0.02        | 0.05        | 0.05        | 0.02                | 0.10        | <b>0.70</b>       | 0.05        |
| ID                        | 15 | 0.02        | 0.01        | <b>0.85</b> | 0.02                | 0.01        | 0.03              | 0.06        |
| WY                        | 12 | 0.04        | 0.04        | 0.09        | 0.03                | 0.25        | 0.19              | 0.34        |
| RE                        | 47 | <b>0.42</b> | 0.14        | 0.04        | 0.22                | 0.03        | 0.06              | 0.06        |
| WL                        | 37 | 0.12        | 0.23        | 0.08        | <b>0.47</b>         | 0.02        | 0.03              | 0.04        |
| GC                        | 17 | 0.08        | <b>0.56</b> | 0.03        | 0.25                | 0.02        | 0.03              | 0.03        |
| (b)                       |    |             |             |             |                     |             |                   |             |
| Populations               |    |             |             |             | # Immigrants (0.01) |             | Sex of immigrants |             |
| Rocky Mountain Front      |    |             |             |             |                     |             |                   |             |
| From Grande Cache         |    |             |             |             | 1                   |             | Male              |             |
| From Gallatin             |    |             |             |             | 2                   |             | Male, male        |             |
| Crazybelts                |    |             |             |             |                     |             |                   |             |
| From Gallatin             |    |             |             |             | 2                   |             | Female, female    |             |
| Gallatin                  |    |             |             |             |                     |             |                   |             |
| From Wyoming              |    |             |             |             | 1                   |             | Male              |             |
| From Grande Cache         |    |             |             |             | 1                   |             | Female            |             |
| Idaho                     |    |             |             |             |                     |             |                   |             |
| From Rocky Mountain Front |    |             |             |             | 1                   |             | Male              |             |
| Wyoming                   |    |             |             |             |                     |             |                   |             |
| From Crazybelts           |    |             |             |             | 1                   |             | Male              |             |

may be indicative of a recent colonization of Wyoming from the Rocky Mountain Front, while the Crazybelts and Gallatin populations are not as recently established and more differentiated. This result is puzzling and additional field research is underway to understand this pattern (Inman et al. 2003). Although pairwise  $F_{st}$  estimates among the populations in Canada and Wyoming were equivalent or lower than comparisons between U.S. populations and Wyoming, the assignment test did not detect any migrants or signatures of admixture between Canada and Wyoming. The lack of a genetic signature could also be an artifact of a prior introduction of wolverines or accidental release of wolverines into Wyoming as hypothesized by Murray (1987) and Kyle and Strobeck (2001), although there is no documentation to support this idea. Additional samples from Wyoming are needed to address this anomaly.

The United States populations do not appear to be sources for dispersing individuals into Canada and the Canadian populations sampled in this study were also highly differentiated from one another. Our data indicates that significant differentiation has resulted between most of the populations in Canada and the United States despite evidence of some migration. The  $F_{st}$  estimates, dendrogram, and PCA results indicated a distinct break between populations in Canada and the United States and significant differentiation between the Crazybelts, Gallatin and Idaho population compared to the Wyoming and Rocky Mountain Front populations. The assignment test can detect dispersers but cannot detect "effective migrants", therefore, some caution should be used when using assignment tests to detect migrants. Furthermore, the detection of dispersers is dependent upon sample size and may not be easily converted into an effective migration rate in our case, since wolverines are difficult to capture and our samples from Montana were collected across 20 years. With these cautions in mind, our data indicated that some migration is occurring between populations in Canada and the Rocky Mountain Front and among populations in the United States (excluding Idaho). However, substantial allele frequency differences suggest that the number of migrants may not be large enough to counter genetic drift and indicates that migration may be rare and/or not result in successful reproduction.

While the mechanisms that promote and impede gene flow are not well understood, both topographical features and anthropogenic factors likely play a role in the current genetic structure of wolverines at the southern extent of its North American range. The matrix of high mountainous areas surrounded by lowland valleys contrasts the continuously forested areas in the northern parts of the range and may support fewer numbers of wolverines with historically less movement. Anthropogenic effects on wolverine habitat, such as development, recreational use, agriculture and mining activities, are also greater at the southern end of their North American distribution (Banci 1994) and likely limit migration. Major highways including the Trans Canada Highway, Interstate 90, and Interstate 15 bisect all of the sampled populations and approximately 5% of wolverines studying these study areas were found dead alongside highways (Kyle, unpublished data). Wolverines have low tolerance towards disturbance and may also be influenced by habitat changes and fragmentation in this region (Carroll et al. 2001).

#### *Conservation and management implications*

Our data suggest that while some migration is occurring among populations, it may not be large enough to counter the effects of isolation and genetic drift. The long-term persistence of wolverines in the contiguous United States will require maintaining an adequate effective population size to buffer against genetic risks associated with inbreeding and genetic drift. Given generally low population densities and elusive nature of wolverines, estimates of population abundance are not currently available for our sampled populations. Using density estimates of one individual per 105 sq. km (Krebs and Lewis 1999) and the minimum convex polygons created in Cegelski et al. (2003), the estimated habitat available in Montana would support a population size of ~300 individuals in the Crazybelts populations, ~500 individuals in the Gallatin population (independent of Wyoming) and ~1000–2000 individuals in the Rocky Mountain Front population. The software GENELOSS was used to simulate the number of effective breeders necessary to maintain genetic variation in the sampled populations in the lower 48 states in the absence of gene flow. Results indicated that 300 and 200 breeding pairs are needed in the

Rocky Mountain Front and Wyoming populations, respectively, to maintain at least 95% of the variation in the next 100 generations and 200 breeding pairs are needed in the Gallatin, Crazybelts, and Idaho populations to maintain variation (Figure 6). Given a  $N/N_e$  ratio of 0.33 used for wolverines in Scandinavia, (Flagstad et al. 2004), a minimum census size of 1200 adults would be needed for each of the populations.

Gene flow is also an important component to the maintenance of genetic diversity because small amounts of gene flow can prevent genetic drift in the long-term, especially when the effective population size cannot be attained due to habitat constraints or low population densities. Two effective migrants from Canada or Wyoming into the Rocky Mountain Front population will maintain current levels of genetic diversity while one effective migrant from the Canadian, RMF or Wyoming populations is needed to maintain current levels of diversity in the Gallatin, Crazybelts or Idaho populations. Low population densities indicate that the required effective population size may never be realized, therefore, migration is critical for maintaining diversity in these populations. This information provides management guidelines regarding population status and persistence, when ecological information becomes available.

Demographic effects such as vulnerability to stochastic disturbances can also be detrimental in isolated populations and result in localized extinctions (Holsinger 2000). Re-colonization of extirpated areas requires that both sexes migrate (Hanski and Gilpin 1997). While male-mediated gene flow may maintain genetic variation in the long-term, female-mediated gene flow is needed for demographic security. Restricted female-mediated gene flow among these populations suggests that re-colonization of female wolverines into extirpated territories may be rare. The populations sampled in this study were more isolated than those in northern Canada and Alaska. Efforts should be targeted at these populations to ensure that they maintain adequate available habitat to avoid demographic risks as well as genetic risks.

Genetics can provide a basis for understanding population dynamics and for prioritizing populations of management concern. However, additional demographic and ecological studies are greatly needed to better understand the appropriate needs and actions for elusive species such as the

wolverine. In particular, population size and density studies are greatly needed to provide better evaluations of population status. Our understanding of wolverine habitat use is also limited, thus additional research is needed. The results of this genetic analysis should complement field

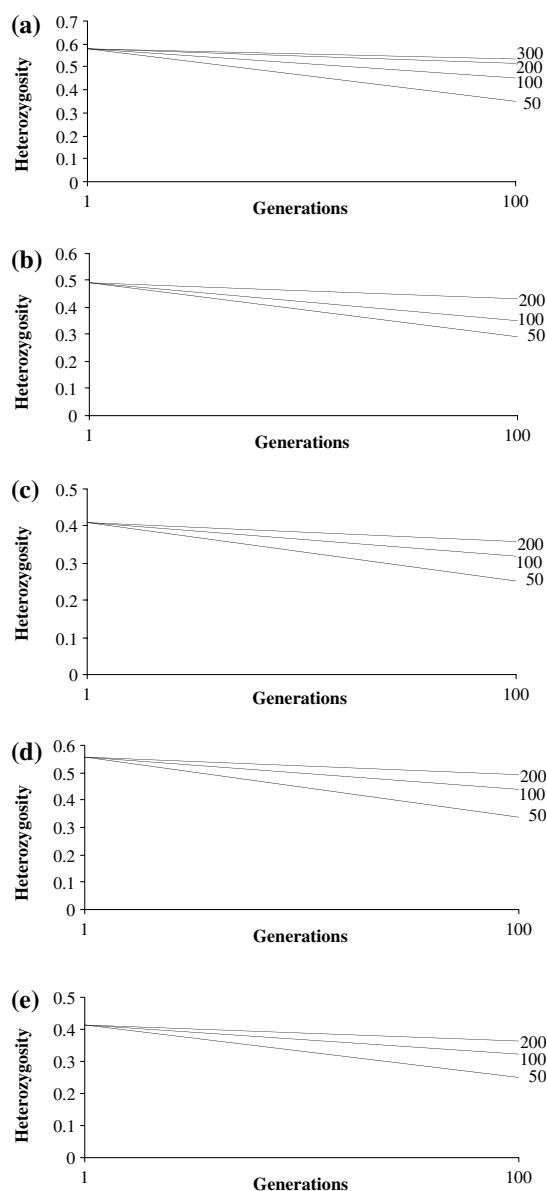


Figure 6. Simulations of the number of breeders needed to maintain heterozygosity in each of the wolverine populations in the United States over 100 generations assuming no migration. (a) Rocky Mountain Front (b) Crazybelts (c) Gallatin (d) Wyoming and (e) Idaho.

studies to determine the type of management actions most appropriate for each of the isolated populations in the contiguous United States.

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