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Dynamic landscapes in northwestern North America structured populations of wolverines (*Gulo gulo*)

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Cyclic climatic and glacial fluctuations of the Late Quaternary produced a dynamic biogeographic history for high latitudes. To refine our understanding of this history in northwestern North America, we explored geographic structure in a wide-ranging carnivore, the wolverine (*Gulo gulo*). We examined genetic variation in populations across mainland Alaska, coastal Southeast Alaska, and mainland western Canada using nuclear microsatellite genotypes and sequence data from the mitochondrial DNA (mtDNA) control region and Cytochrome *b* (*Cytb*) gene. Data from maternally inherited mtDNA reflect stable populations in Northwest Alaska, suggesting the region harbored wolverine populations since at least the Last Glacial Maximum (LGM; 21 Kya), consistent with their persistence in the fossil record of Beringia. Populations in Southeast Alaska are characterized by minimal divergence, with no genetic signature of long-term refugial persistence (consistent with the lack of pre-Holocene fossil records there). The Kenai Peninsula population exhibits mixed signatures depending on marker type: mtDNA data indicate stability (i.e., historical persistence) and include a private haplotype, whereas biparentally inherited microsatellites exhibit relatively low variation and a lack of private alleles consistent with a more recent Holocene colonization of the peninsula. Our genetic work is largely consistent with the early 20th century taxonomic hypothesis that wolverines on the Kenai Peninsula belong to a distinct subspecies. Our finding of significant genetic differentiation of wolverines inhabiting the Kenai Peninsula, coupled with the peninsula's burgeoning human population and the wolverine's known sensitivity to anthropogenic impacts, provides valuable foundational data that can be used to inform conservation and management prescriptions for wolverines inhabiting these landscapes.

Key words: colonization, evolutionarily significant unit, genomic comparison, glacial history, *Gulo gulo*, phylogeography

Cold-adapted species are excellent models to study the effects of climate warming, which may threaten their persistence in high-latitude and high-elevation biomes (Scheffers et al. 2016). Population genetic structuring is the result of present and historical processes, reflecting the presence of important barriers to dispersal, bioclimatic restrictions, or past colonization routes that may be shared with other syntopic species (Hewitt 1999). Historically, biomes in Alaska and western Canada were strongly influenced by a dynamic glacial history through the Quaternary (Cook et al. 2006; Hope et al. 2011; Rowe et al. 2014). Glacial ice sheets covered most of northern North America (growing and receding > 24 cycles; 2.6 Mya to 11.7 Kya) and have been implicated in structuring populations of numerous species in these regions (Shafer et al. 2010; Hope et al. 2013; Knowles et al. 2016). The location of larger glacial refugia (e.g., Beringia) is well-documented in fossils, pollen

records, genetic structure of fauna and flora, and bathymetric and stratigraphic evidence (Barrie and Conway 1999; Cook et al. 2017), while the influence or even existence of smaller refugia (e.g., Kodiak Island, Kenai Peninsula) is still debated (Harlin-Cognato et al. 2006; Gentili et al. 2015).

Beringia extended from eastern Siberia to at least the Mackenzie River in northwestern Canada (Hultén 1972; Abbott et al. 2000). Although often portrayed as a single large and continuous refugium, Beringia likely was a heterogeneous landscape (Hoffmann 1981; Guthrie 2001; McLean et al. 2016). In northern Alaska, Beringia was fragmented by a glacial ice sheet along the Brooks Range until 13.5 Kya (Dyke 2004), as reflected in geographic structure of widely distributed species (e.g., Arctic ground squirrel [*Urocitellus parryii*]—Eddingsaas et al. 2004; Galbreath et al. 2011).

Smaller coastal refugia have been hypothesized along the North Pacific Coast, today consisting of a series of archipelagos and a thin strip of mainland in Southeast Alaska and British Columbia (Josenhans et al. 1995; Fleming et al. 1999; Mandryk et al. 2001). Finally, south of the Cordilleran and Laurentide ice sheets, a series of large southern refugia were hypothetically separated by physiographic features such as the southern Rocky Mountains and Mississippi River (Swenson and Howard 2005). Isolation of Beringian and North Pacific Coastal refugia, combined with topographic complexity (e.g., mountain ranges, peninsulas, islands) and variable biomes (e.g., tundra, taiga—Laliberte and Ripple 2004) in this region, potentially created a complex evolutionary history of sequestration and subsequent colonization for species in northwestern North America (Cook et al. 2017).

The wolverine (*Gulo gulo*) is considered one of several sentinel species for biodiversity declines (Rondinini and Visconti 2015). As a species adapted to cold environments of the Holarctic, developing an understanding of how wolverine populations were structured by past climate shifts can provide a basis for monitoring their response to changing environmental conditions in the near future (Hope et al. 2015). Wolverines are highly vagile and have been hypothesized to be largely unaffected by physiographic barriers such as rivers, reservoirs, valleys, or mountain ranges (Hornocker and Hash 1981), their distribution therefore appears to be shaped primarily by climatic conditions and human influence (positive or negative responses to anthropogenic impact vary depending on the timing and nature of the disturbance—Magoun 1985; May et al. 2006; Scrafford et al. 2017). Wolverines show some genetic structuring across portions of their North American distribution in nuclear microsatellite loci (Rico et al. 2015, for Canadian populations only), the mitochondrial DNA (mtDNA) control region (Zigouris et al. 2013, mainland Alaska and Canada), and 365 – 367 base pair (bp) portion of the mtDNA Cytochrome *b* (*Cytb*) gene (Tomasik and Cook 2005; western North America and Eurasia). Previously, this genetic structure has been partially explained by isolation-by-distance (Zigouris et al. 2013, but see Tomasik and Cook 2005). Most genetic analyses of far northwestern North American wolverines, however, either have focused on a subset of populations in Alaska (Dalerum et al. 2007) or treated all populations from Alaska as a single unit (e.g., Kyle and Strobeck 2002; Zigouris et al. 2013). Those approaches failed to leverage multiple marker classes (Tomasik and Cook 2005) or consider the variable impact of the dynamic Quaternary history in structuring Alaska's biota (Knowles et al. 2016; McLean et al. 2016).

Using spatially extensive and population intensive sampling within and adjacent to Beringia, we aim to refine our understanding of the evolutionary history of wolverines in far northwestern North America. Based on fossil evidence (Graham and Lundelius 2010), we predict enduring genetic signatures of glacial refugia will be found in northwestern and northern Alaska populations (formerly Beringia) and Southeast Alaska. Previous genetic analyses have shown that large carnivores on the Kenai Peninsula are divergent from mainland populations (lynx [*Lynx canadensis*]—Bailey 2002; black bear [*Ursus americanus*]—Robinson et al. 2007; wolf [*Canis lupus*]—Weckworth et al. 2011). Similarly, we predict divergent signatures in the Kenai Peninsula wolverine population. Prior analyses of the wolverine mtDNA control region and *Cytb* (Tomasik and Cook 2005) found a genetic signal of discreteness of the Kenai wolverines, corroborating the early 20th century taxonomic hypothesis based on morphology that wolverines of the Kenai Peninsula comprise a distinct subspecies, *G. g. katschemakensis* (Matschie 1918:151, cited in Pasitschniak-Arts and Larivière 1995; see Hall 1981). We expand prior sampling and genetic analyses (Tomasik and Cook 2005) to examine population-level differences, including comparing Kenai to mainland populations, using data from 20 biparentally inherited microsatellite loci and sequencing the entire mtDNA *Cytb* gene. We test for signatures of persistent refugial isolation, polarity in gene flow, and recent expansion to identify potentially distinctive populations of conservation priority (Kawecki 1995).

Materials and Methods

Sampling.—Wolverine specimens were obtained from commercial trappers through cooperative efforts with federal, state, and provincial/territorial, natural resource agencies from 1989 to 2015 (e.g., Jung et al. 2016; Kukka et al. 2017) and deposited at University of Alaska Museum of the North at the University of Alaska at Fairbanks or Museum of Southwestern Biology at the University of New Mexico. Samples (Supplementary Data SD1) with reliable spatial and temporal information were selected to broadly represent the region, with populations identified based on major geographic and topographic features (Fig. 1): Russia (RUS); northwestern Alaska (NWAK); northern Alaska (NAK); central Alaska (CAK); southern Alaska (SAK); Kenai Peninsula (KAK); northwestern Yukon (NWY); southeastern Yukon (SEY); British Columbia (BC); Southeast Alaska (SEAK); Northwest Territories (NWT, used only for mtDNA); and Nunavut (NU). Political boundaries were used to assign regional group names. Genetic diversity and population divergence of groups were analyzed for F_{IS} inbreeding coefficient (Table 1; Wright 1921) in FSTAT v1.2 (Goudet 1995) to identify

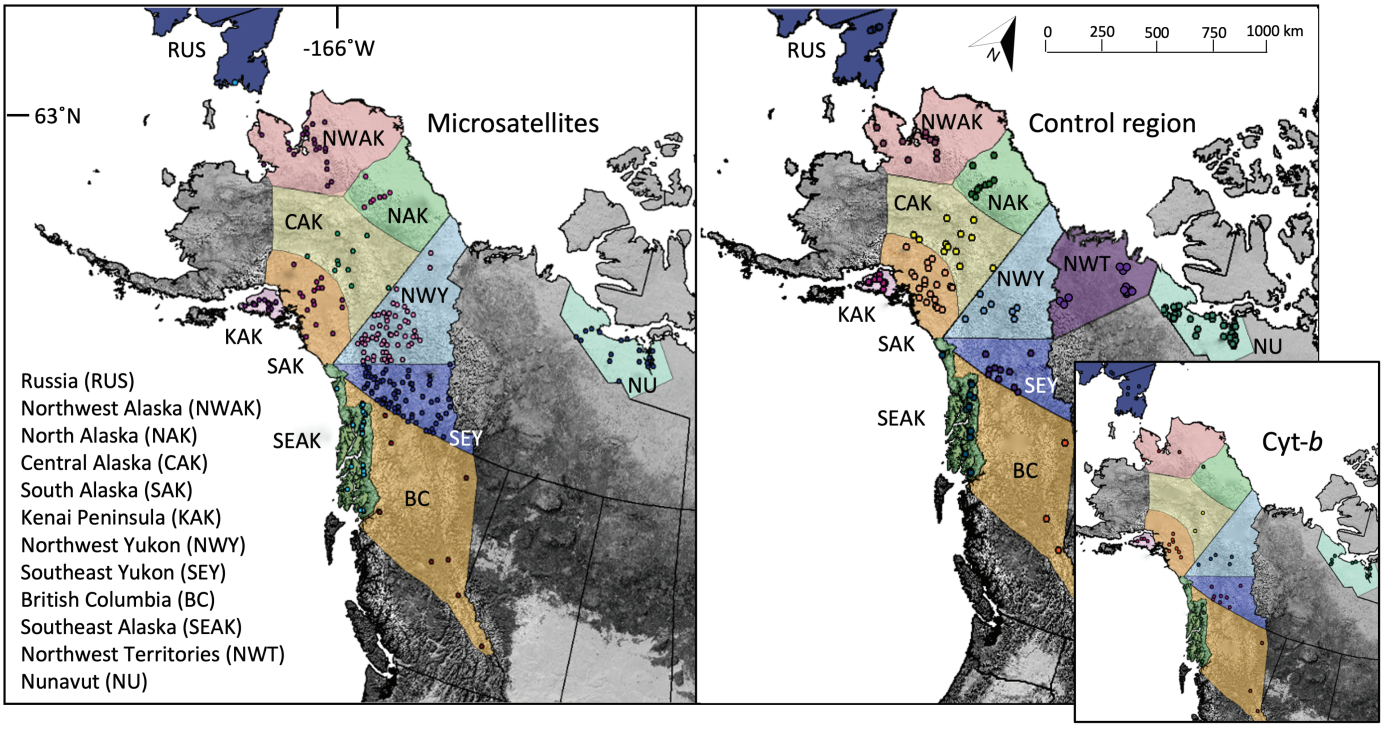

Fig. 1.—*Gulo gulo* populations and individual sampling localities for microsatellites and mitochondrial DNA (control region and Cytochrome *b*) sequences. Populations were identified based on major geographic and topographic features. Political boundaries were used to assign regional group names.

subpopulation structure and prevent lumping of distinct populations (Goudet 1993; Goudet et al. 1994).

Following calibration of genotypes between laboratories, data from previous work (Dalerum et al. 2007) were incorporated to represent sampling from NWAK (n = 117). Those specimens were represented by 10 microsatellite loci, whereas all other sampling regions were genotyped for 20 loci (the original 10 loci reported in Dalerum et al., 2007, and 10 additional loci; Supplementary Data SD4). Comparisons between NWAK and all other populations were carried out using the 10-locus suite common among all specimens. Similarly, analyses of genetic diversity (i.e., H_O/H_E and STRUCTURE output) were carried out with these 10 loci when comparing NWAK with other populations; otherwise, 20 loci were used for intrapopulation analyses or comparisons that excluded NWAK. Two individuals had either 10% (CAK, n = 1) or 5% (SEY, n = 1) missing data. Exploratory analyses demonstrated those missing data had no significant impact on our results (not shown).

Laboratory procedures.—DNA was isolated using a modified salt extraction method (Fleming and Cook 2002). Primer sets L15926 and H16498 (Tomasik and Cook 2005) were used to produce 366 bp control region sequences from mtDNA. Primer sets MSB05 and MSB14 (Hope et al. 2010) were used to sequence 1,140 bp of the *Cytb* region of the mitochondrial genome.

DNA was amplified by polymerase chain reaction (PCR) in a final volume of 25 µL containing 2–50 µg genomic DNA, 1.5–2.5 mM magnesium chloride, 0.2 mM deoxynucleotide triphosphates, 0.2 µM each of forward and reverse primers, 1 × PCR buffer (Perkin Elmer Cetus I), DNA-free water, and 0.65 U/µL AmpliTaq DNA polymerase (Thermo Fisher Scientific, Waltham, Massachusetts). PCRs had an initial denaturation of 94°C for 3 min followed by 35 cycles of 94°C for 15 s, 50–51°C for 30 s, 72°C for 30 s, and a 10-min final extension at 72°C. Negative controls accompanied each set of PCRs. PCR products were visualized on an agarose gel, purified by polyethylene glycol (PEG) precipitation, resuspended in 10 mM Tris, and cycle-sequenced through the UNM Molecular Biology Facility on an ABI 3130-XL.

Geneious v8.0 (http://www.geneious.com—Kearse et al. 2012) was used to reconcile sequences. Sequences were assembled using a consensus sequence generated from data on GenBank (Benson et al. 2009). Sample size was augmented to a total of n = 252 for control region and n = 69 for *Cytb* by including data accessioned on GenBank (Supplementary Data SD2). The two mitochondrial genes were analyzed as a concatenated unit; when analyzed independently (jModeltest v2.1.4—Darriba et al. 2012) they had similar models of evolution (HKY+I control region and concatenated; HKY *Cytb*).

Microsatellite genotyping.—Microsatellite genotypes from 20 loci were determined for 177 individuals (in downstream analyses) from Alaska and western Canada (Supplementary Data SD3). One or both primers (forward or reverse) from each of eight of the loci were redesigned from the published sequence to change the size or improve amplification of targeted loci (Supplementary Data SD4). Forward primers were tailed with universal sequences (Oetting et al. 1995). PCR amplifications were carried out in seven multiplex reactions and one singleplex reaction, each in a final volume of 10 µL and containing 2–50 ng genomic DNA, 0.2 mM deoxynucleoside triphosphates, 1–5 pmols unlabeled

Table 1.—Summary statistics from microsatellite and mitochondrial DNA control region data among populations of *Gulo gulo* in Alaska, western Canada, and eastern Russia. Values with an asterisk indicate significance (Tajima's D and F_{IS} at $P < 0.05$, Fu's F_s at $P < 0.02$). n = sample size for each analysis; F/M/U = count of females, males, and unknown sex in each population; H = no. of haplotypes; H_P = no. of private haplotypes; H_d = haplotype diversity; Tajima's D, Fu's F_s; microsatellite loci no. for each population; H_O = observed heterozygosity; H_E = expected heterozygosity; R_A = rarefied allelic richness, R_P = private allelic richness; F_{IS} = values with an asterisk indicate an inbreeding coefficient significantly greater than zero. Population abbreviations are as follows: RUS (Russia), NWAK (Northwest Alaska), NAK (North Alaska), CAK (Central Alaska), SAK (South Alaska), KAK (Kenai Peninsula), NWY (Northwest Yukon), SEY (Southeast Yukon), BC (British Columbia), SEAK (Southeast Alaska), NWT (Northwest Territories), NU (Nunavut).

		Population comparisons													
		Mitochondrial DNA					Microsatellite DNA								
Region	Pop.	n	H	H_P	H_d	Tajima's D	Fu's F_s	n	F/M/U	Loci	H_O	H_E	R_A	R_P	F_{IS}
Western	RUS	8	3	2	0.589	−0.025	0.343	1	0/0/1						
	NWAK	23	5		0.645	−0.064	0.108	30	10/20/0	10	0.65	0.54	3.22	0.16	−0.059
	NAK	10	4		0.561	−0.044	0.260	9	4/4/1	20	0.59	0.49	2.82	0.05	−0.045
	CAK	20	6		0.597	−0.058	0.136	13	4/7/2	20	0.62	0.56	3.31	0.09	0.017
	SAK	34	8	4	0.638	−0.064	0.072	17	6/10/1	20	0.57	0.54	3.24	0.05	0.095*
Peninsula	KAK	25	3	1	0.591	−0.049	0.094	26	14/12/0	20	0.56	0.45	3.06	0.03	−0.016
Eastern	NWY	10	6	2	0.588	−0.044	0.250	30	7/23/0	20	0.67	0.60	3.41	0.03	−0.011
	SEY	13	4		0.494	−0.027	0.237	30	10/20/0	20	0.64	0.59	3.39	0.10	0.003
	BC	5	3	1	0.618	−0.026	0.377	9	2/7/0	20	0.54	0.49	3.43	0.19	0.069
	SEAK	30	5	1	0.650	−0.064	0.034	26	8/18/0	20	0.53	0.54	3.23	0.01	0.111*
	NWT	15	6	1	0.564	−0.038	0.197								
	NU	63	6	1	0.509	−0.056	−0.016	16	3/13/0	20	0.62	0.53	3.3	0.04	−0.033

primers, 0.15–2.25 pmoles IRD-labeled primer, 1.0 µg bovine serum albumin, 1 × PCR buffer (Perkin Elmer Cetus I), and 0.25–0.5 units of GoTaq Flexi DNA polymerase (Promega, Madison, Wisconsin). PCRs began at 94°C for 2 min followed by 40 cycles each of 94°C for 15–30 s, 50°C for 15–30 s, and 72°C for 30–60 s and a 30-min extension at 72°C concluded each reaction.

Fluorescently labeled PCR products were electrophoresed on a 48-well 6% polyacrylamide gel on a LI-COR 4200 LR or IR^2 DNA automated sequencer (LI-COR, Lincoln, Nebraska). To standardize allele sizes among the 10 loci common between this study and that of Dalerum et al. (2007), several wolverine samples from Dalerum et al. (2007) were extracted to run on every subsequent gel as a standard. Two of those calibration standards then were used on all subsequent gels, occupying six lanes across each 48-well gel. For the remaining loci, size standards were generated for each locus by scoring the same suite of individuals against a fluorescently labeled M13 sequence, and those samples were used in each subsequent gel, again occupying at least six lanes across each 48-well gel. Based on these comparisons, genotypes for each individual were determined using GeneImagIR 4.05 software (Scanalytics, Inc., Billerica, Massachusetts). For quality control, 10% of samples were extracted, amplified, and genotyped in duplicate. Overall error rate was determined to be < 1% for non-NWAK samples (electrophoresis issues causing mis-scores, switched samples, weak lower alleles, and possible allelic dropout, etc.). In all future microsatellite analyses including NWAK samples, loci were reduced to the 10 in common among all sampling regions. MICROCHECKER 2.2.3 (Van Oosterhout et al. 2004) was used to identify genotyping errors and check for null alleles. Positive and negative controls were included throughout. Microsatellite genotypes are provided in Krejsa et al. (2021).

To limit the oversampling of family groups (and correct for family-based structure that might confound population structure—Falush et al. 2003; Bergl and Vigilant 2007; Anderson and Dunham 2008), analyses were run with a data set restricted by relatedness (r). One individual in each of a given pair with relatedness of $r_{xy} > 0.5$ in IDENTIX v1.1.5 (Belkhir et al. 2002) was removed (using the Queller and Goodnight 1989 relatedness estimator after 1,000 per locus bootstraps to achieve a 95% confidence interval for each). Total sample size after parsing for relatedness was 207 individuals (177 genotyped in this study, and 30 from Dalerum et al. 2007).

Descriptive statistics.—Genepop on the Web v4.2 (Raymond and Rousset 1995; Rousset 2008) was used to evaluate Hardy–Weinberg equilibrium (HWE) for each microsatellite locus and linkage disequilibrium (LD) for all pairs of loci. HWE was tested using probability, heterozygosity deficiency, and heterozygosity excess with no enumeration of alleles using Markov Chain parameters: 10,000 dememorizations, 1,000 batches, and 10,000 iterations. LD was tested with log-likelihood and probability tests and the same Markov Chain parameters. Alpha-values ($\alpha = 0.05$) were adjusted by the number of populations, implementing a Bonferroni correction, to achieve a critical

value to test for significance (0.005) across all comparisons (Rice 1989).

The temporal span of sampling exceeded two generations in some populations. Therefore, following testing of populations for conformation to HWE, we tested for differences in the distribution of alleles (Raymond and Rousset 1995) for each of two populations (n = 26–30) that spanned two or more generations (NWY and SEY), assuming 2–3 years per generation for wolverines (Rauset et al. 2015). Although sample sizes were lower for within-population temporal comparisons (n = 9–12), we also tested KAK, for which data spanned 6–10 generations. We calculated the χ^2 distribution of alleles (Raymond and Rousset 1995) between each temporal group using Genepop on the Web.

F-statistics (F_{ST} and F_{IS}—Wright 1949; Weir and Cockerham 1984) were calculated using the software FSTAT 2.1 (Goudet 1995) with significance levels set at α = 0.001 and 10,000 randomizations. We assessed the possibility that microsatellite markers were sex-linked by comparing allele frequencies between males and females (genetic methods of sex determination are summarized in Supplementary Data SD3). Heterozygosity estimates (expected and observed) and number of alleles were estimated in Microsatellite Toolkit (Park 2001). We used the program HP-RARE (Kalinowski 2005) to calculate both allelic richness (R_A) and private allelic richness (R_p). HP-RARE uses rarefaction analyses to account for differences in sample size among populations. To assess degree of genetic structuring among microsatellite loci, we performed an analysis of molecular variance (AMOVA) using Arlequin v3.5 (Excoffier and Lischer 2010).

Inbreeding statistics also were calculated using FSTAT 2.1 (Goudet 1995). Positive values indicated inbred lines, whereas negative values may reflect crossing of differentiated lineages. An $F_{IS} > +0.043$ indicates an excess of contemporary inbreeding (Wright 1965). A strongly negative value indicated that the delineation of populations should be further refined (e.g., individuals placed in the same population when they should be separated).

Haplotype assignment and frequency rates among populations for sequences also were determined in Arlequin v3.5. Summary statistics were generated including haplotype diversity (H_d; DnaSP v5—Rozas et al. 2010), number of haplotypes (H), number of private haplotypes (H_p), AMOVA, and F_{ST} (Arlequin v3.5). F_{ST} values were computed using pairwise difference and 1,000 permutations (Table 1; Supplementary Data SD5).

Population structure.—The Bayesian clustering approach available in STRUCTURE v2.3 (Falush et al. 2007) was used to examine genetic population structure without a priori designation of populations or sampling locations (Pritchard et al. 2000). Optimal number of subpopulations was determined by varying the likely number of clusters or populations (k) from 1 to 10 allowing for genetic admixture and correlated allele frequencies (settings which aid in differentiating genetically similar populations—Falush et al. 2003). Each run used a burn-in of 50,000 and a Markov Chain Monte Carlo (MCMC) of 500,000 steps. This process was replicated eight times for each value of k (Evanno et al. 2005) to quantify the standard deviation among the runs for a particular assumed k. The optimal number of k-clusters was determined by Structure Harvester v0.6.94 (Pritchard et al. 2000), with the method developed by Evanno et al. (2005) to evaluate the rate of change in the log probability of the data (Δk) among eight runs for each assumed k and estimate the highest Ln probability of the data or Ln P(d). Individual membership probabilities of the inferred k-clusters from the eight independent replicates were averaged using CLUMPP v1.1.2 (Jakobsson and Rosenberg 2007). Subsequent STRUCTURE analyses on identified clusters were conducted to test the effect of high cluster assignment on underlying structure (Pritchard and Wen 2003).

As another test of population structure, BAPS 5.3 (Bayesian Analysis of Population Structure—Corander and Marttinen 2006; Corander et al. 2006) was used to describe genetic structure. Unlike STRUCTURE, BAPS infers clusters based on similarities in the variance of data from assumed source populations (i.e., a priori defined groups—Corander et al. 2006; Ball et al. 2010). As a result, the inference of k-clusters was set not to exceed the number of sampling areas (10). We inferred the maximum k to be between 1 and 10, with 20 replications of each inferred k. For the admixture analyses, parameters were set as: minimum population size of five individuals for admixture analyses with 10,000 iterations per population and at least five reference individuals from each population with 10,000 iterations per reference individual. Finally, a Mantel test for isolation-by-distance was run in GenAlEx 6.5 (Peakall and Smouse 2006, 2012) to test for a correlation between microsatellite-based genetic distances and geographic distance.

Demographic changes and bottlenecks.—Graphical displays of demographic change in sequence data were executed using Mismatch Analysis in DnaSP v5 (Librado and Rozas 2009). Tests for selection or deviations from neutrality using Tajima's D (Tajima 1989) and Fu's F_s (Fu 1997) also were carried out in DnaSP v5 (10,000 replicates for each). The calibrated mutation rate for wolverines was set at 0.0428 substitutions/site/Myr (Hope et al. 2014).

Bottleneck v1.2.02 (Cornuet and Luikart 1996) was used to test recent patterns of fluctuation in effective population size (N_e). Under a mutation-drift equilibrium scenario, more heterozygotes than expected (heterozygosity excess) given the number of alleles would indicate a bottleneck. In contrast, significant heterozygote deficit relative to the number of alleles would indicate an influx of alleles into a population. (Cornuet and Luikart 1996). Heterozygosity excess was tested using a Wilcoxon sign rank test (optimal for 20 or fewer loci—Piry et al. 1999) under a two-phase model of microsatellite evolution (TPM, ideal for testing dinucleotide repeat loci—Di Rienzo et al. 1994) for 10,000 iterations. The infinite allele model (IAM—Kimura and Crow 1964) is a more liberal model but can indicate recent bottlenecks, and the strict stepwise mutation model (SMM—Ohta and Kimura 1973) is more conservative but can indicate more historical bottlenecks. These models also were tested to evaluate the consistency in the identification of

bottlenecks. If SMM and TPM both indicate a bottleneck, it is likely to have occurred historically; if IAM alone is significant for heterozygosity excess it could be a false positive or evidence of a recent bottleneck. Variance for TPM was tested at 9 and 30 while proportion of SMM in TPM was left at 80% (Piry et al. 1999; Garza and Williamson 2001).

Migration and connectivity.—Source–sink dynamics were examined through the program MIGRATE v3.6.11 (Beerli 1998, 2002; Beerli and Felsenstein 1999) to examine number of migrants per generation for mtDNA control region data (N_fm) among sampled sites. MIGRATE incorporates two parameters scaled to the mutation rate (μ): Θ, the effective population size parameter (N_fμ), and M, the rate of gene flow (m/μ). MIGRATE gene flow estimates are averaged over the past n generations, where n equals the number of generations in which the populations have been at mutation-drift equilibrium (going back many generations and estimating historical migration). Gene flow estimates included a full migration model (Θ and M were estimated individually from the data) that was compared to the restricted model (Θ was averaged and M was symmetrical between populations). Gene flow was estimated using maximum-likelihood search parameters; 10 short chains (5,000 trees used out of 1 million sampled), five long chains (10,000 trees used of 2 million sampled), and five adaptively heated chains (start temperatures: 1, 1.5, 3, 6, and 12; swapping interval = 1). Models were carried out three times and parameter estimates converged. The alternative model was evaluated for goodness-of-fit given the data, using a log-likelihood ratio test (Beerli and Felsenstein 2001).

Results

Genetic diversity: microsatellites and mtDNA.—For the microsatellite loci, there were no significant departures from HWE, and LD was not evident. NWAK and NWY had the highest observed heterozygosity at microsatellite loci, while SEAK and BC had the lowest (Table 1). Rarefied allelic richness ranged from 2.8 to 3.4 alleles per region, with NAK and KAK having the lowest and NWY, SEY, and BC having the highest (Table 1). Private allelic richness was highest in BC and NWAK.

We evaluated genetic differentiation at microsatellite markers within three populations between early and late time periods (NWY: 2005–2007 and 2013–2015, n = 26 for both temporal periods; SEY: 2005–2007 and 2013–2015, n = 30 and 27, respectively; KAK: 1989–1992 and 2007–2011, n = 12 and 9, respectively). We found no significant levels of genic differentiation (Bonferroni corrections applied, $\alpha_{adjusted} = 0.0025$) between early and late time periods within all three populations (χ^2 ranged from 39.474 to 57.179, P ranged from 0.033 to 0.494, $d.f.$ = 40 for all comparisons), suggesting that pooling samples collected across temporal periods within populations likely did not influence the broader cross-population phylogeographic signals. Similar to analyses that pooled across temporal periods for KAK (see below), we detected significant differences in allele frequencies when each of the two temporal periods assayed for KAK were compared with each of two temporal periods assayed in NWY and SEY (χ^2 ranged from 86.72 to 141.716, P ranged from $< 2.63^{-5}$ to 1.04^{-14}, $d.f.$ = 40 for all comparisons). This provided evidence that significant shifts in allele frequencies would likely have been detected between temporal periods within KAK, despite their representation by small sample sizes (n = 9–12). We used these analyses to justify pooling of all data within each population for further analyses.

For mtDNA, haplotype diversity was lowest in SEY and NU. Highest haplotype diversity was found in NWAK and SEAK. All values for Tajima's D were negative, indicating the presence of more low frequency polymorphisms than expected, but no values were significant (Table 1). We constructed mismatch distribution plots (Fig. 2) that demonstrated demographic stability (or long-term occupation) in RUS, NWAK/NAK, and SEAK. SAK and KAK may also share this signature, but to a lesser degree as they are bimodal instead of multimodal. CAK, YT, BC, and NU, in contrast, had signals of expansion or more recent colonization.

Based on F_{IS} results, our populations have been appropriately assigned and no groups are excessively outbred (Table 1). SEAK had the highest inbreeding coefficient and was significantly different from zero at +0.111.

Microsatellite data showed limited evidence of a recent ($2N_e$–$4N_e$ generations) bottleneck in SAK, KAK, NWY, SEY, and SEAK, in at least one test; that is, they each exhibited heterozygosity excess. All signatures of a bottleneck were detected under the IAM (Kimura and Crow 1964). For KAK, the standardized differences test (Cornuet and Luikart 1996) strongly supports that all loci fit the IAM (α = 0.00139), which is consistent with the positive Wilcoxon sign test result for KAK (α = 0.00060) and suggests a recent bottleneck.

Population differentiation: microsatellites.—Delta k was maximized with a k of 2 (Δk = 50, Ln|P = −18,500), but k = 2, 3, and 4 are shown for identification of patterns at higher ks. In all inferred k, KAK remained distinct from other sampling groups (k = 2, 3, 4; Fig. 3A). Although genetic clusters were not exclusively represented by individuals collected at the same geographic location, individuals from the Kenai Peninsula were predominately assigned to a single cluster. All individuals in KAK on average had a membership coefficient of 75% to the Kenai-dominated cluster, and one-third of the group had > 90% membership coefficient to that cluster. This group was removed for a subsequent analysis of STRUCTURE which resulted in more overall mixing.

In BAPS a priori-based analysis, the most likely k was four groups roughly distributed among mainland Alaska (NWAK, NAK, CAK, SAK), Kenai, western mainland Canada (NWY, NU, parts of SEY), and Southeast Alaska (SEAK, BC, parts of SEY; Fig. 3B). No correlation was found between pairwise genetic distance and geographic distance based on a Mantel test (r = 0.00003, P = 0.1700), thereby the data fail to support an isolation-by-distance model.

Population differentiation: mtDNA.—The number of mitochondrial haplotypes represented in a population (Fig. 4; Supplementary Data SD6) was between 3 and 8 with an average of 4.7. Although

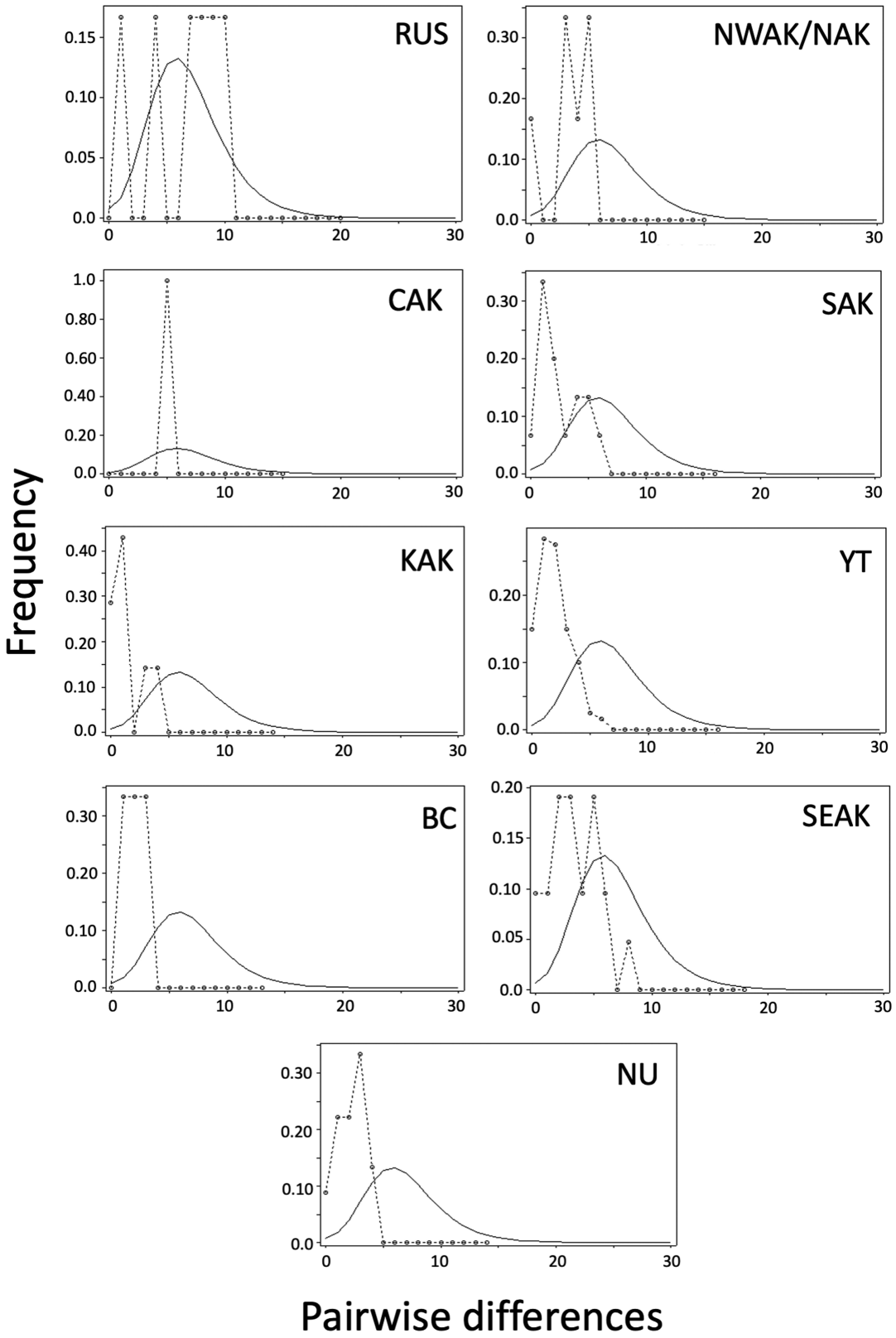

Fig. 2.—Population expansion graphs for concatenated mitochondrial genes Cytochrome *b* and control region (1,507 bp) for *Gulo gulo*. Solid lines illustrate expected frequencies under a model of population expansion and dotted lines depict observed frequencies of pairwise haplotype differences. Bimodal or multimodal patterns indicate stable populations. Sample size for each population: RUS (Russia) 4, NWAK/NAK (Northwest Alaska/North Alaska) 4, CAK (Central Alaska) 2, SAK (South Alaska) 6, KAK (Kenai Peninsula) 7, YT (all of the Yukon Territory) 16, BC (British Columbia) 3, SEAK (Southeast Alaska) 7, NU (Nunavut) 10.

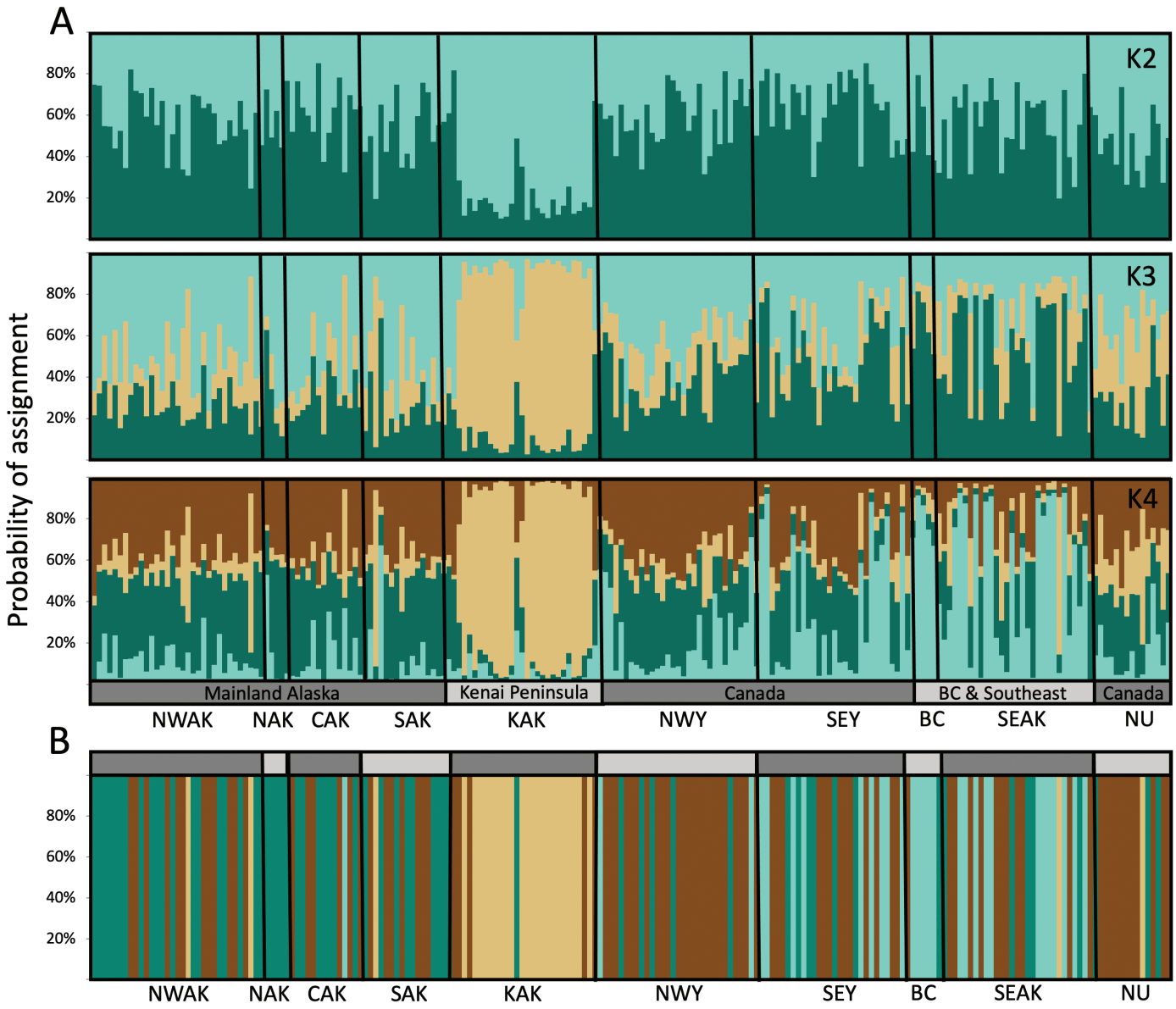

Fig. 3.—Patterns of microsatellite genetic variation in *Gulo gulo* across the sampled regions: (A) STRUCTURE barplots of population membership coefficients for an inferred *k* of 2 through 4 genetic clusters, the mostly likely *k* given the data is 2; (B) BAPS (Bayesian Analysis of Population Structure) barplot for population membership with $k = 4$ as the mostly likely *k* given the data. Abbreviations are as follows: NWAK (Northwest Alaska), NAK (North Alaska), CAK (Central Alaska), SAK (South Alaska), KAK (Kenai Peninsula), NWY (Northwest Yukon), SEY (Southeast Yukon), BC (British Columbia), SEAK (Southeast Alaska), NU (Nunavut).

sampling may impact these metrics, sufficient sampling in KAK ($n = 25$) yielded few haplotypes (3), while SAK ($n = 33$) yielded more (8). Haplotype 5 was most common, appearing in nine out of 12 populations (23% of individuals overall), although absent from KAK, RUS, and NAK, all of which generally were low in haplotype richness. Private haplotypes were found in RUS (two haplotypes; 50% of the sampling group); SAK (four haplotypes; 18%); KAK (one haplotype; 24%); NWY (two haplotypes; 20%); BC (one haplotype; 20%); SEAK (one haplotype; 3%); NWT (one haplotype; 7%); and NU (one haplotype; 29%).

Haplotype 1 is found primarily in RUS (50%) and the interior Alaskan populations (NWAK 35%; NAK 10%; CAK 11%; SAK 6%; KAK 48%) while being absent in Canadian groups except NU (6% prevalence). Haplotype 9 is unique to CAK, SAK, KAK, and SEAK (CAK 47%; SAK 36%; KAK 28%; SEAK 10%; Supplementary Data SD6).

Source–sink dynamics and connectivity.—Gene flow as estimated in MIGRATE was moderate. There were few cases of extreme asymmetry in gene flow between population pairs, with the exception that KAK appears to have historically served as a source population for several other regions (NAK, SAK, NWY, SEAK), SEY apparently serves as a source population for other regions (RUS, NAK, SAK, BC), and SEAK appears to have historically received more immigrants (RUS, KAK, BC) than has provided emigrants, thus serving as a sink population (Table 2). Number of migrants per generation (N_fm) ranged from 7.1

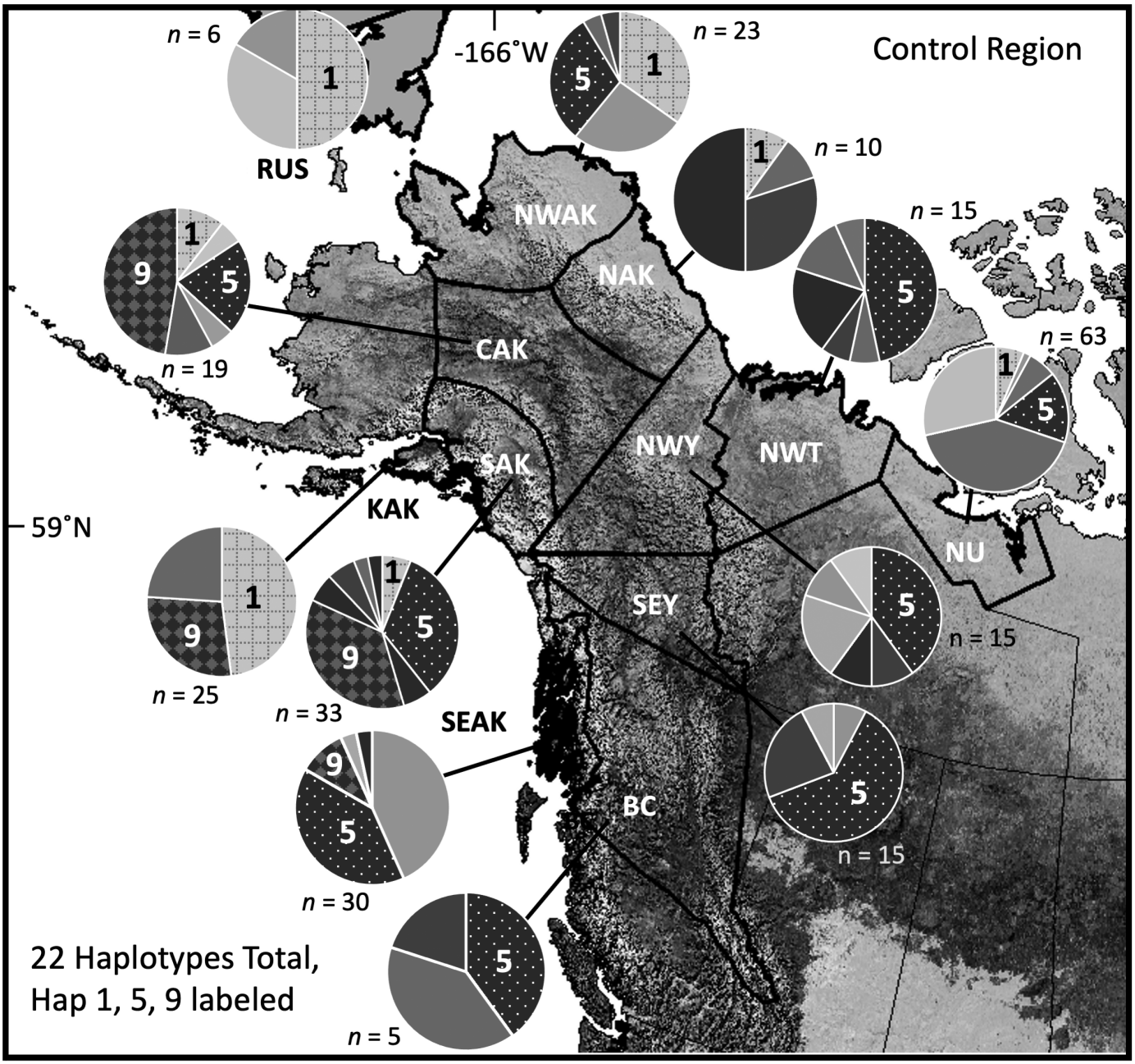

Fig. 4.—Frequency distribution plots of *Gulo gulo* control region (mitochondrial DNA) sequences for each sampled region in Alaska and western Canada. Abbreviations are as follows: RUS (Russia), NWAK (Northwest Alaska), NAK (North Alaska), CAK (Central Alaska), SAK (South Alaska), KAK (Kenai Peninsula), NWY (Northwest Yukon), SEY (Southeast Yukon), BC (British Columbia), SEAK (Southeast Alaska), NWT (Northwest Territories), NU (Nunavut).

to 33.6 N_fm. Estimates of lowest emigration (dispersing immigrants) was from Southeast Alaska into Southeast Yukon, and estimates of highest immigration (receiving immigrants) was from Kenai into South Alaska (Table 2).

Genome structure comparison.—Pairwise F_{ST} values were higher for mitochondrial data, ranging from –0.500 to 0.524, than for microsatellites, where F_{ST} ranged from –0.006 to 0.265 (Table 3). Significant F_{ST} values represented 30% of the mitochondrial pairwise comparisons while 53% of the pairwise microsatellite values were significant. For both microsatellite and mitochondrial data, comparisons that included either RUS or KAK had notably high (though not consistently significant) F_{ST} values. The mitochondrial AMOVA showed a higher proportion of genetic variance explained among populations (23.78%) relative to microsatellites (2.91%).

Discussion

Over the past several decades, surprising levels of phylogeographic structure in highly vagile carnivores in northern North America have been detected, including refugial

Table 2.—Gene flow estimates[a] based on the coalescent N_{fm} (number of migrants per generation) in MIGRATE among *Gulo gulo* populations in Alaska and western Canada, calculated from the mitochondrial DNA control region. Directionality (Dir) of gene flow between population pairs (source, sink, and symmetrical [-]) assigned on the basis of 95% confidence intervals. Directions with an asterisk highlight population pairs with strong asymmetry (> 2× N_fm difference).

	Russia			Northwest Alaska			North Alaska			Central Alaska		
	Imm	Em	Dir	Imm	Em	Dir	Imm	Em	Dir	Imm	Em	Dir
RUS				12.5	10.5	-	21.68	8.9	Sink	11.13	11.5	-
NWAK	10.5	12.5	-				11.24	9.65	-	9.36	21.92	Source*
NAK	8.9	21.68	Source	9.65	11.24	-				10.42	7.88	-
CAK	11.5	11.13	-	21.92	9.36	Sink*	7.88	10.42	-			
SAK	8.8	13.88	Source	13.23	8.22	Sink	11.39	8.63	-	11.36	11.58	-
KAK	9.2	6.81	-	13.39	10.86	-	23.45	12.55	Sink*	10.11	13.93	-
NWY	13.2	13.97	-	14.87	10.59	-	10.55	11.81	-	7.78	9.81	-
SEY	21.2	13.81	Sink	10.65	14.45	-	15.91	7.44	Sink*	14.05	13.48	-
BC	12.2	8.41	-	9.36	14.71	Source	8.21	13.85	Source	12.74	12.07	-
SEAK	8.3	13.48	Source	11.59	10.95	-	12.3	10.64	-	9.16	11.66	-
NU	17.6	10.41	Sink	11.01	11.4	-	12.51	16.28	-	15.66	9.07	Sink

	South Alaska			Kenai Peninsula			Northwest Yukon			Southeast Yukon		
	Imm	Em	Dir	Imm	Em	Dir	Imm	Em	Dir	Imm	Em	Dir
RUS	13.88	8.8	Sink	6.81	9.2	-	13.97	13.2	-	13.81	21.2	Source
NWAK	8.22	13.23	Source	10.86	13.39	-	10.59	14.87	-	14.45	10.65	-
NAK	8.63	11.39	-	12.55	23.45	Source	11.81	10.55	-	7.44	15.91	Source
CAK	11.58	11.36	-	13.93	10.11	-	9.81	7.78	-	13.48	14.05	-
SAK				10.62	33.61	Source*	10.15	11.76	-	9.42	14.63	Source
KAK	33.61	10.62	Sink*				14.08	8.06		12.86	17.28	-
NWY	11.76	10.15	-	8.06	14.08	Source				9.88	14.8	-
SEY	14.63	9.42	Sink	17.28	12.86	-	14.8	9.88	Sink			
BC	12.08	9.57	-	13.34	15.47	-	13.1	11.55	-	8.73	17.92	Source
SEAK	9.59	8.92	-	10.94	19.52	Source	7.43	9.43	-	7.13	8.17	-
NU	10.14	11.77	-	11.15	14.95	-	9.27	9.62	-	10.37	14.03	-

	British Columbia			Southeast Alaska			Nunavut		
	Imm	Em	Dir	Imm	Em	Dir	Imm	Em	Dir
RUS	8.41	12.2	-	13.48	8.3	Sink	10.41	17.6	Source
NWAK	14.71	9.36	Sink	10.95	11.59	-	11.4	11.01	-
NAK	13.85	8.21	Sink	10.64	12.3	-	16.28	12.51	-
CAK	12.07	12.74	-	11.66	9.16	-	9.07	15.66	Source
SAK	9.57	12.08	Source	8.92	9.59	-	11.77	10.14	-
KAK	15.47	13.34	-	19.52	10.94	Sink	14.95	11.15	-
NWY	11.55	13.1	-	9.43	7.43	-	9.62	9.27	-
SEY	17.92	8.73	Sink*	8.17	7.13	-	14.03	10.37	-
BC				16.52	9.88	Sink	13.24	16.53	-
SEAK	9.88	16.52	Source				12.33	7.98	-
NU	16.53	13.24	-	7.98	12.33	-			

[a] Gene flow estimates listed as immigration (Imm) into population A from population B and emigration (Em) from population A into population B. For example, gene flow between Russia and North Alaska is 21.68 N_fm out of Russia into North Alaska; therefore, Russia is listed as the source. Population abbreviations are as follows: RUS (Russia), NWAK (Northwest Alaska), NAK (North Alaska), CAK (Central Alaska), SAK (South Alaska), KAK (Kenai Peninsula), NWY (Northwest Yukon), SEY (Southeast Yukon), BC (British Columbia), SEAK (Southeast Alaska), NU (Nunavut).

Table 3.—Pairwise F_{ST} values based on microsatellite genotypes (msats; above the diagonal) and concatenated Cytochrome *b* and control region sequences (below the diagonal) among 11 sampling regions for *Gulo gulo*. Single asterisks (*) indicate statistical significance ($\alpha = 0.05$) after 1,000 permutations. Weir and Cockerham's (1984) theta (F_{ST}) calibrated with the maximum global value of F_{ST} was used to generate the scales. Population abbreviations are as follows: RUS (Russia), NWAK (Northwest Alaska), NAK (North Alaska), CAK (Central Alaska), SAK (South Alaska), KAK (Kenai Peninsula), NWY (Northwest Yukon), SEY (Southeast Yukon), BC (British Columbia), SEAK (Southeast Alaska), NU (Nunavut).

	RUS	NWAK	NAK	CAK	SAK	KAK	NWY	SEY	BC	SEAK	NU	Pop Ave msats	Microsatellite scale	
RUS		0.154*	0.265	0.179	0.170	0.161	0.149*	0.178*	0.186	0.207*	0.130	0.178		
NWAK	0.200		0.010	0.006	0.008	0.057*	0.006	0.020*	0.016*	0.042*	0.025*	0.035	$F'_{ST} < 0.05$	low
NAK	−0.368	−0.500		0.003	0.013	0.073*	0.023*	0.035*	0.020	0.045*	0.037*	0.052	F'_{ST} 0.05–0.15	moderate
CAK	0.270	−0.258	−0.111		−0.006	0.042*	−0.001	0.008	0.007	0.014	0.008	0.026	F'_{ST} 0.15–0.25	great
SAK	0.460*	0.065	0.367	0.067		0.046*	0.005	0.012	0.013	0.035*	0.021*	0.032	F'_{ST} 0.25–0.35	very great
KAK	0.509*	0.241	0.524	0.374	0.142*		0.043*	0.056*	0.065*	0.094*	0.033*	0.067	$F'_{ST} > 0.35$	exceptionally great
NWY	0.490*	0.086	0.485	0.177	0.058	0.315*		0.001	−0.004	0.028*	0.022*	0.027	Hartl and Clark (1997)	
SEY	0.492*	0.059	0.364	0.031	0.021	0.290*	0.016		0.010	0.013*	0.018*	0.035	Microsatellite scale	
BC	0.342	−0.125	0.400	−0.125	−0.042	0.302*	−0.033	−0.083		0.023	0.046*	0.038	$F_{ST} < 0.02$	low
SEAK	0.485*	0.120	0.376	0.066	0.040	0.245*	0.148*	−0.048	0.023		0.037*	0.054	F_{ST} 0.02–0.06	moderate
NU	0.428*	−0.015	0.185	0.144	0.304*	0.373*	0.256*	0.255*	0.100	0.321*		0.038	F_{ST} 0.06–0.10	great
Pop Ave	0.331	−0.013	0.172	0.064	0.148	0.332	0.200	0.140	0.076	0.178	0.235		F_{ST} 0.10–0.14	very great
Mitochondrial													$F_{ST} > 0.14$	exceptionally great
													Weir and Cockerham (1984)	

signatures (Talbot and Shields 1996; Colella et al. 2018b) and structure between mainland and Kenai Peninsula populations (wolves—Weckworth et al. 2005, 2011; brown bears—Morton et al. 2015). We provide a detailed examination of geographic structure in wolverines and the data are consistent with the isolation of the Kenai Peninsula wolverine population based on signals inferred from both the mitochondrial and nuclear genomes. This finding largely is consistent with the hypothesis that Kenai Peninsula wolverines are distinctive and potentially an endemic subspecies (*G. g. katschemakensis*—Hall 1981), although the data do not support species level differentiation as originally proposed by Matschie (1918). The presence of a private haplotype for the peninsular population expands preliminary findings of prior research based on mtDNA (Tomasik and Cook 2005).

Augmenting prior genetic analyses (Tomasik and Cook 2005), our analyses showed that wolverines on the Kenai Peninsula also demonstrated significant differences in the variance of microsatellite allele frequencies from other regions in Alaska and possessed minimal allelic variation, but unlike mtDNA, did not harbor private alleles. Because male wolverines typically disperse farther than females (Wilson et al. 2000; Tomasik and Cook 2005), the contrast in signatures between marker types may reflect limited female dispersal (Aronsson and Persson 2018), while males are maintaining higher levels of gene flow with mainland populations that is reflected in the biparentally inherited nuclear loci. Another explanation may be the different temporal signals present in mtDNA and microsatellites, because the former likely is detecting older population processes. Our analyses suggested that wolverines on the Kenai Peninsula display asymmetry in gene flow, acting as a source population in several pairwise comparisons, but those data should be interpreted cautiously due to variation in sample sizes.

Expansion statistics for the microsatellite DNA showed a severe reduction in effective population size, potentially reflecting a possible founder event and subsequent isolation on the peninsula, consistent with patterns in other large, mobile carnivores demonstrating reduced genetic diversity on the Kenai Peninsula (lynx—Schwartz et al. 2003; wolf—Weckworth et al. 2005; brown bear—Jackson et al. 2008). Holocene glacial advance of the Portage Glacier (Bartsch-Winkler et al. 1983) at the base of the Kenai Peninsula may have influenced the exchange of individuals between the Kenai population and adjacent populations.

Genetic flow at the Alaska–Yukon border was mixed among the markers and analyses. BAPS (with $k = 4$) results supported genetic discontinuity, while STRUCTURE showed the sampling areas were more of a mixing zone for wolverines. F_{ST} values illustrate moderate divergence, gene flow estimates in MIGRATE neither are strongly skewed nor entirely absent between sampling regions within Alaska and Yukon, and many haplotypes and alleles were shared across the region. Results from STRUCTURE are consistent with incidental observations of long-distance movements of wolverines between Alaska and Yukon (e.g., Gardner et al. 1986).

Some islands in Southeast Alaska's Alexander Archipelago and the Haida Gwaii Archipelago off the coast of British Columbia have been hypothesized to have been a refugium for various flora and fauna during glacial periods (Swenson and Howard 2005; Colella et al. 2018a). A multimodal mismatch distribution plot suggests wolverine populations have remained stable within Southeast Alaska. A unique haplotype made up 3.3% of the Southeast Alaska subsampled population, and mitochondrial F_{ST} values (0.178) for Southeast indicate high genetic differentiation from other populations (Hartl and Clark 1997). Although possessing a private haplotype, pairwise comparisons with other sampling regions in gene flow analyses suggest the region is a sink with regard to directionality of gene flow. In contrast, British Columbia has been hypothesized as a source for lower-latitude wolverine populations in the continental United States and southern Canada (Krebs et al. 2004).

Although Southeast Alaska is a hypothesized refugium for some species, wolverines have not been detected in the fossil record with the exception of a single fossil from Prince of Wales Island in Southeast Alaska that dates from the postglacial Holocene (based on substrate recovery—Heaton et al. 1996). Instead, the signature of demographic stability, unique cluster assignment for microsatellite genotypes from this region, and presence (albeit at low frequencies) of a haplotype unique to the Southeast Alaska coastal area suggest the extant population of wolverines inhabiting the region may have originated from one or more early post-Pleistocene colonizing populations. The lack of historical records of wolverines on Haida Gwaii (Slough 2007) provides further support that the population in Southeast Alaska is postglacial. Other species in Southeast Alaska demonstrating similar patterns of genetic diversity that have been interpreted as signaling post-Pleistocene colonization (Cook et al. 2006) include another highly mobile carnivore (gray wolf—Weckworth et al. 2005, 2011). Genetic analyses of wolverines inhabiting this region thus contribute to our understanding of the processes of Pleistocene and post-Pleistocene population dynamics acting on genetic structure of mobile carnivores in these complex, fragmented landscapes.

Phylogeographic studies of Arctic ground squirrels (Eddingsaas et al. 2004; Galbreath et al. 2011) suggest that northwestern and northern mainland Alaska represented a glacial refugium north of the Brooks Range glacier—disjunct from the rest of Beringia—at least for that species. Deglaciation models also support a hypothesis of glacial refugia located in northern Alaska (Dyke 2004), and a phylogeographic break in this region has been observed in other taxa (e.g., Fedorov and Stenseth 2002; Abbott and Comes 2003). Mismatch analysis of mtDNA sequence data from wolverines sampled from northern Alaska returned multimodal mismatch distribution peaks, suggesting a stable population that, based on our analyses, is genetically discrete from other groups (F_{ST}). Mitochondrial data also are consistent with the signature of a refugial population, with a novel haplotype dominant in North Alaska (50% of the sampled population), while Northwestern Alaska shares a larger percent of its subsampled population with a haplotype common to Russia (35% shared with Russia, 4% with North Alaska). Russia also supports signals consistent with retention in a glacial refugium with multimodal peaks in its mismatch distribution plots (n = 6). These data are consistent with the presence of a high-latitude Beringia (Russia, Northwestern Alaska, Northern Alaska) refugium north of the Brooks Range.

In some cases, the spatial pattern of structure differed between biparentally inherited microsatellite loci and maternally inherited mtDNA: nearly all (n = 45/55) of the pairwise F_{ST} comparisons are greater for mtDNA than microsatellite loci. Elevated levels of structure for mtDNA may be attributable to female philopatry coupled with higher nuclear gene flow driven by vagile males (Zink and Barrowclough 2008). Previous studies (Chappell et al. 2004; Tomasik and Cook 2005; Schwartz et al. 2007) also showed a higher proportion of variance explained by mtDNA sequence data than biparentally inherited microsatellites, suggestive of female philopatry, or of the inherent temporal differences of the two marker types—mtDNA is not as affected by contemporary changes like habitat fragmentation on genomic variance. In contrast, wolverines occupying the western Brooks Range do not appear to exhibit sex-bias in dispersal (Dalerum et al. 2007). Although sex-biased behavioral tendencies may be influencing the observed pattern in other regions, landscape features (whether historical or contemporary) instead may be restricting dispersal within wolverines (Sawaya et al. 2019).

Conservation implications.—Identifying the natural and anthropogenic processes that promote population genetic structure provides foundational information for use in conservation and management efforts, including recognition of distinctive populations or evolutionarily significant units (ESUs) and detection of barriers to dispersal that could impact longer-term evolutionary trajectories across species (Fogelqvist et al. 2010; Palsbøll et al. 2010; Haasl and Payseur 2011). With attention to connectivity (Kleven et al. 2019), as well as flexible management (Aronsson and Persson 2017), successful conservation programs for wolverines are emerging in Fennoscandia (Lansink et al. 2020) that are grounded within a solid understanding of geographic variation. Genetic diversity in Old World wolverine populations (Walker et al. 2001; Ekblom et al. 2018; Lansink et al. 2020) is lower than the North American wolverine populations within our study, though our data cover a larger geographic area. Our analyses begin to provide the spatial and temporal framework for understanding how variation is apportioned across North American wolverine populations and starts to lay a foundation for successful adaptive management of wolverines in northwestern North America (Arbogast et al. 2017; Malaney et al. 2017).

Most contemporary populations of wolverines of Alaska and northwestern Canada appear to be relatively well-connected by ongoing gene flow; however, Kenai is distinct. This population appears to be significantly differentiated from populations elsewhere in Alaska and western Canada, and biases in gene flow estimates associated with the Kenai are asymmetrical—evolutionary dispersal from the Kenai occurs more often than into the Kenai. Thus, our study extends the results of Tomasik and Cook (2005), providing independent

genetic support for distinction of the Kenai Peninsula wolverines and thus further supporting the early 20th century hypothesis by Matschie (1918) that the wolverines of the Kenai Peninsula are distinctive. Over three decades ago, Schreiber et al. (1989) listed threats and recommended actions to ensure the persistence of wolverine populations; these included 1) a better understanding of factors that limit population densities, movement patterns, and habitat requirements; and 2) integration of human interests with wolverine protection, given ongoing and likely irreversible fragmentation of wolverine habitat. Schreiber et al. (1989) explicitly recommended clarification of levels of distinctiveness of Kenai Peninsula wolverines and referenced a need to better understand levels of gene flow between the peninsula and mainland populations. Our genetic research begins to populate those data gaps, but further work with larger sample sizes and using both morphological and genome-based approaches is needed to more fully characterize variation and understand demographic dynamics in wolverines of southcentral Alaska (Ekblom et al. 2018; Lansink et al. 2020).

Because this genetically isolated population is characterized by relatively low levels of genetic variation, it may be more vulnerable to the impacts of habitat perturbation, pathogens, or competition for a limited resource base (Bangs et al. 1982; Crowl et al. 2008). Moreover, wolverines in general are difficult to census and monitor at spatial and temporal scales useful for management (Kukka et al. 2017, but see Golden et al. 2007a) and may be susceptible to high harvest pressure on some portions of the Kenai Peninsula because they are adjacent to the region's largest concentration of people (Golden et al. 2007b). Populations of carnivores occupying peninsulas often show reduced standing genetic variability (Jackson et al. 2008; Montana et al. 2017), such as that observed for the wolverines on the Kenai Peninsula. This observation is consistent with a genetic signature of isolation, decreased genetic diversity relative to the closest mainland population, and asymmetrical gene flow where emigration exceeds immigration.

Given the wolverine's relatively large home range requirements (Whitman et al. 1986; Banci and Harestad 1990; Dawson et al. 2010), this species requires substantial areas of suitable habitat to ensure long-term population survival. Habitat fragmentation, whether due to localized anthropogenic activities or broader climatic processes, thus is considered by conservation biologists to represent a risk to the long-term persistence of both Palearctic and Nearctic populations (Schreiber et al. 1989). Wolverines are adapted to cold, snowy environments; the warming conditions such as those currently impacting high-latitude landscapes are hypothesized to reduce and fragment their distribution (Copeland et al. 2010; Hope et al. 2015), potentially reducing effective population sizes and levels of genetic diversity. Given the extremely low levels of genetic variability in some wolverine populations (Ekblom et al. 2018), it is critical to characterize existing genetic diversity to identify populations and regions of potential conservation concern. A facet of that characterization is the assessment of levels and polarity of gene flow among adjacent populations. Processes of landscape fragmentation can synergistically impede gene flow (Putman and Carbone 2014), reducing effective population sizes, decreasing genetic diversity, and increasing population divergence, as reflected in the wolverines of Southeastern Alaska. We failed to uncover a strong signature of a historically persistent refugial population of wolverines in Southeast Alaska, as seen in some terrestrial mammals (e.g., Sawyer et al. 2017; Colella et al. 2021). Still, the wolverine, likely a post-Pleistocene colonizer, appears to comprise a discrete population in Southeast Alaska with limited gene flow between these coastal wolverines and populations in adjacent Canada. Thus, the wolverines of far northwestern North America show relatively high levels of historical connectivity with the possible exceptions of the Southeast Alaska and Kenai Peninsula populations.

Acknowledgments

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Supplementary Data

Supplementary data are available at *Journal of Mammalogy* online.

Supplementary Data SD1.—All samples of *Gulo gulo* analyzed listed by museum identification and sorted by microsatellite and mitochondrial data generated. Microsatellite genotypes were generated in this study (n = 177) except NWAK (n = 30—Dalerum et al. 2007). Control region sequence data were from GenBank (n = 252). Cytochrome b sequence data were generated here (n = 40) and from GenBank (n = 69).

Supplementary Data SD2.—GenBank accession numbers for all *Gulo gulo* sequence data analyzed, listed by museum identification and sorted by region sequenced. Source Key: 1 = Tomasik and Cook (2005) (n = 134), 2 = Frances et al. unpublished (n = 101), 3 = Wilson et al. (2000) (n = 42), 4 = Hosoda et al. 2000 (n = 1), 5 = Rozhnov and Meschersky

unpublished (n = 1), 6 = sequences generated in this study (n = 40).

Supplementary Data SD3.—Sample information and microsatellite data for each *Gulo gulo* genotyped: Krejsa et al. (2021). Genetic data from wolverine (*Gulo gulo*) of North America (ver. 1.0, April 2021): U.S. Geological Survey data release. https://doi.org/10.5066/P908DV9.

Supplementary Data SD4.—Number of samples (n), allele counts (a), repeat motif, length of calls (size range), multiplex assignment (M), forward and reverse primer sequence, citation, and GenBank accession number. Primers in bold text were redesigned from the original published sequence for this study.

Supplementary Data SD5.—Site characteristics, sample size (n), and genetic diversity measures: linkage disequilibrium r^2, haplotype diversity H_d, Tajima's D, Fu's F_s, average number of pairwise differences k, number of polymorphic (segregating) sites S for *Gulo gulo* populations analyzed for mitochondrial sequences control region and Cytochrome b. *P-value > 0.05, **P-value > 0.02, N/A lacks polymorphism or possesses insufficient data/sample size.

Supplementary Data SD6.—Frequency of each mitochondrial DNA control region haplotype among sampling groups of *Gulo gulo*, number of samples (n), and analogous labels for haplotypes identified in previous studies.

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