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Population structure and dispersal of wolves in the Canadian Rocky Mountains

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In the Canadian Rocky Mountains, the gray wolf (*Canis lupus*) has experienced range contractions and expansions, which can greatly affect pack stability as well as population structure. In addition, this area has a highly heterogeneous landscape that may form barriers to dispersal. To understand factors affecting pack structure and large-scale gene flow across the Rocky Mountains, we examined wolf genetic structure using 1,981 noninvasive and invasively collected samples. We sampled over 44 packs in Alberta and British Columbia and, from these, identified 540 individuals based on 12 microsatellites. Relatedness of individuals within packs was greater than between packs, and female relatedness was greater than males suggesting strong pack structure and female philopatry. Relatedness within packs was greater near major roads suggesting decreased dispersal from natal packs with proximity to roads. Across the study area, 2 significantly differentiated genetic clusters were identified, corresponding to a north/south split. Landcover distance was a significant correlate for 2 of 4 genetic distance measures, where packs in the north were in areas of dense coniferous forest, while packs in the south were primarily in open coniferous forest. These landcover differences suggest natal associations or could relate to prey distribution. Fine-scale investigation of pack dynamics across this continuous distribution, together with large-scale estimators of population structure, highlights different drivers of gene flow at the pack and population level.

Key words: Canadian Rocky Mountains, *Canis lupus*, genetic structure, gray wolf population structure, landscape genetics, microsatellite

Populations may be genetically structured at different spatial scales, ranging from local (Brouat et al. 2003; Coltman et al. 2003; Bouzat and Johnson 2004) to regional (Cegelski et al. 2003; Eriksson et al. 2004) to continental (Kyle and Strobeck 2001; Geffen et al. 2004; de Barro 2005). Processes that occur at these different scales affecting genetic structure include population dynamics such as colonization (Excoffier and Ray 2008), social organization (Lehman et al. 1992; Pope 1992; Girman et al. 1997; Støen et al. 2005), and/or dispersal limits (Wright 1943; Rousset 1997). As well, other factors may also result in genetic structure, and these can include habitat affinity (Sacks et al. 2004), natural physical barriers (Keyghobadi et al. 1999; Carmichael et al. 2001; Hyung Eo et al. 2002; Walker et al. 2003; Worley et al. 2004), anthropogenic barriers (Epps et al. 2005; Proctor et al. 2005), prey specialization (Carmichael et al. 2001; Pilot et al. 2006; Musiani et al. 2007; Muñoz-Fuentes et al. 2009), and climatic variation (Geffen et al. 2004; Pilot et al. 2006). For continuously distributed species, genetic structure arises at the scale at which these factors influence dispersal and ultimately gene flow.

Gray wolves (*Canis lupus*) have a nearly continuous distribution in North America, representing the largest historical range among terrestrial mammals. However, human persecution and habitat loss has led to a considerable reduction in southern Canada and across the contiguous United States (Musiani and Paquet 2004, and references therein). More recently, attitudes toward wolves have changed, which has allowed the wolf to recolonize areas formerly occupied (Fritts and Carbyn 1995). An example of this is wolves in the Canadian Rocky Mountains. This population has experienced considerable

demographic stochasticity, where multiple extirpation and recolonization events have occurred (Cowan 1947; Gunson 1983). The last recolonization was quite recent when animals from northern Alberta and British Columbia recolonized the southern Canadian Rocky Mountains after extirpation in the 1950s (Hayes and Gunson 1995). Recolonization of this area may result in genetic bottlenecks, potentially leading to genetically structured populations. However, the capacity for wolves to disperse long distances (Ballard et al. 1983; Fritts 1983; Gese and Mech 1991; Boyd and Pletscher 1999; Wabakken et al. 2001; Canigila et al. 2014) may reduce the potential for genetic structure to develop.

Population structure may also be influenced by landscape characteristics. For example, the border region of Alberta and British Columbia is transected by high elevation mountain ranges that may present a dispersal barrier resulting in genetic subpopulations as a result of reduced gene flow (Slatkin 1987; Hewitt 1996; Shafer et al. 2010). Studies on gray wolves have also described genetic structure related to climate (Geffen et al. 2004) and habitat because of prey specialization (Musiani et al. 2007; Muñoz-Fuentes et al. 2009; Stronen et al. 2014). Finally, wolves tend to avoid high traffic roads and areas of human disturbance (Jensen et al. 1986; Mech et al. 1988; Oakleaf et al. 2006), as a result dispersal may be low in association with these features resulting in genetically differentiated subpopulations.

Given this potentially complex system, we used invasive and noninvasive methods to sample genetic material from a large number of wolves in the Canadian Rocky Mountains. Sampling at both the pack and landscape level allowed us the opportunity to address impacts on structure at both scales. Using microsatellite analysis, we examined relatedness among packs to identify extrinsic factors that may influence pack dynamics, where we assume high pack relatedness indicates areas of stable pack structure (Jêdrzejewski et al. 2005; Vonholdt et al. 2008; Rutlidge et al. 2010). We then used an a posteriori method to determine the number of genetic groups in the area and used this to inform a landscape genetic approach to examine whether the same extrinsic factors were related to gene flow at the broad scale.

Materials and Methods

Study area.—The study area covered approximately 145,000 km^2, straddling the continental divide along the Rocky Mountains in British Columbia and Alberta (Fig. 1). The region is dominated by rugged mountain ranges, bisected by 3 major east–west passes and wide, flat valley bottoms aligned south–southeast to north–northwest. Three major east–west highways (Highways 1, 3, and 16) and associated rail lines follow the major passes across the northern, central, and southern parts of the study area. This area includes several national and provincial protected areas.

Sample collection.—Samples were collected between 1990 and 2005 noninvasively (hair, scat, and blood in snow) or by handling live animals (hair and blood) and wolf carcasses (muscle or skin tissue; Fig. 1). Noninvasive samples were collected by snow-tracking in winter to ensure high-quality samples (Lucchini et al. 2002). Tracks from uncollared wolf packs were located while travelling on foot, snowmobile, or truck through wolf habitat. When possible, consecutive days were spent following a single pack to increase the probability of collecting samples from all pack members. Telemetry was used to locate and track several packs that had radiocollared individuals.

Scat samples were stored in sealed plastic bags at −20°C, scat was subject to −80°C for > 48 h to inactivate eggs of *Echinococcus multilocularis* and *E. granulosus* parasites before DNA extraction (Veit et al. 1995; Hildreth et al. 2004). Follicle-bearing hairs (range 1 to > 40) were collected from bed sites, natural snags, and during tracking. Hair samples were stored in paper envelopes at room temperature with low humidity.

Hair was plucked or blood was sampled from live-captured wolves. Blood was stored in ethylenediaminetetraacetic acid tubes at −20°C until DNA extraction. Plucked hair was stored similar to hair collected noninvasively. Information on age, sex, and reproductive status of wolves was collected during capture events. All capture and handling methods complied with the guidelines of the Canadian Council on Animal Care through the Animal Care and Use Committee for Biosciences at the University of Alberta and the American Society of Mammalogists' guidelines for the use of wild mammals in research (Sikes et al. 2011). Tissues from legally harvested wolves and other sources of human-caused mortality were provided by trappers and government agencies and stored at −20°C in sealed plastic bags before DNA extraction.

DNA extraction and genotyping.—Negative controls were used throughout extraction and genotyping to monitor for contamination (Taberlet et al. 1996). Scat samples were extracted using the QIAamp DNA Stool Mini-kit (Qiagen, Toronto, Canada) with slight modifications to manufacturer's recommendations (i.e., incubated at 70°C with Proteinase K for 30 min instead of the recommended 10 min). Blood, hair, and tissue samples were extracted using Qiagen DNeasy extraction kits (Qiagen, Toronto, Canada) following the manufacturer's directions. Samples were typed at 13 microsatellite loci of canine origin (Table 1), along with the Y-chromosome for sex (Sundqvist et al. 2001).

Two methods were used to optimize polymerase chain reaction (PCR) success of different sample types. For noninvasive scat, hair, and blood samples, the microsatellite multiplex combinations were amplified in a 10 µL volume containing 5 µL of Qiagen Multiplex Mix (Qiagen, Toronto, Canada), 1 µL of primer mix (100 mM concentration of fluorescently labeled forward and unlabeled reverse primers), 0.4 µL of 10 mg/ml bovine serum albumin (BSA), and 3.6 µL of DNA. PCR for noninvasive samples had an initial denaturation and activation of the HotStarTaq of 15 min at 95°C, followed by 30 cycles of 30 sec denaturation at 94°C, 90 sec annealing at 59°C, 60 sec extension at 72°C, and a final extension of 30 min at 60°C. Blood and tissue samples were amplified in 10 µL containing 5 µL of Qiagen Multiplex Mix, 2 µL water, 1 µL of primer mix (100 mM concentration of fluorescently labeled forward and unlabeled reverse primers), and 2 µL of DNA. PCR conditions

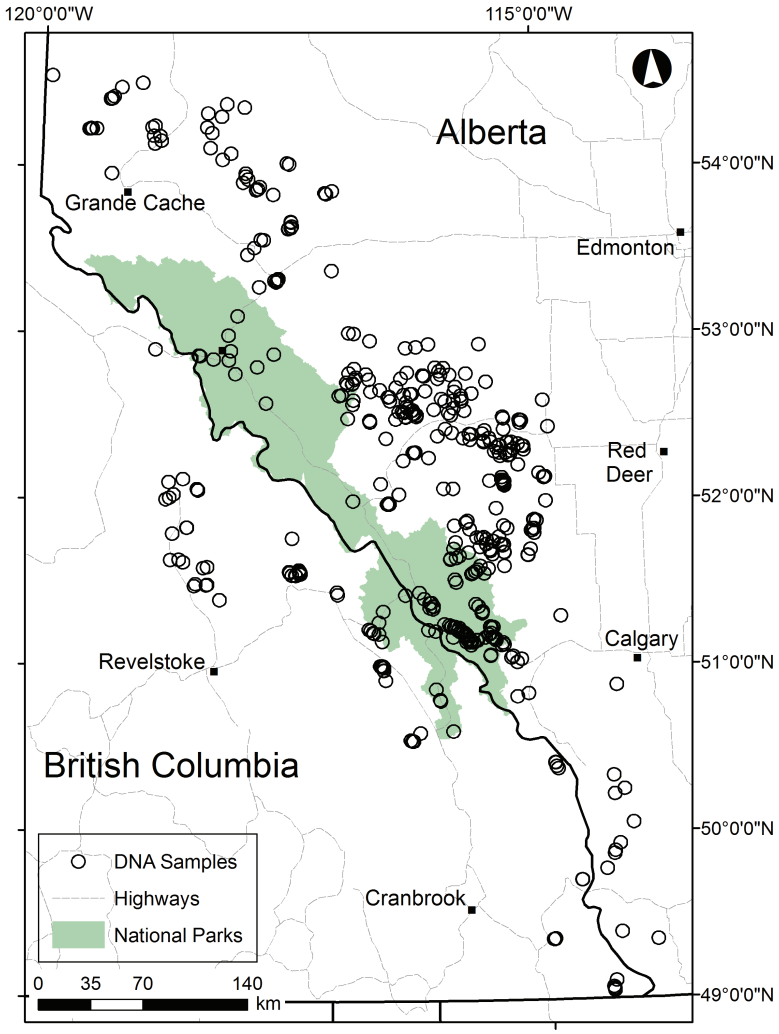

Fig. 1.—Study area in the Canadian Rocky Mountains with wolf DNA sample locations (black circles).

Table 1.—Diversity statistics for the 13 microsatellite loci based on 526 samples collected from wolves in the Canadian Rocky Mountains. Includes the number of alleles (N_a), observed (H_O) and expected (H_E) heterozygosities, Weir and Cockerham's F_{IS} (Weir and Cockerham 1984), estimated null allele frequency, allele sizes (in base pairs) and multilocus probability of identity (PID) for the population (Unbiased) and for siblings (SIB). Cumulative probability of identity is an estimate of the probability that 2 wolves will share the same genotype. Loci sourced from Breen et al. (2001), Hapke et al. (2001), and Neff et al. (1999).

Locus	N_a	H_o	H_e	F	Null allele frequency	Allele size range (bp)	Cumulative PID	
							Unbiased	SIB
FH2834	2	0.164	0.167	0.014	0.004	263–265	7.10E-01	8.40E-01
FH2096	3	0.495	0.530	0.064	0.052	96–104	2.31E-01	4.76E-01
PEZ19	5	0.592	0.633	0.064	0.051	184–204	4.65E-02	2.30E-01
FH2010	5	0.663	0.654	–0.016	–0.030	219–235	8.60E-03	1.08E-01
FH2088	8	0.639	0.668	0.044	–0.012	89–128	1.32E-03	4.92E-02
PEZ12	11	0.758	0.764	0.007	0.023	256–300	1.13E-04	1.92E-02
FH2001	7	0.792	0.804	0.014	0.010	125–150	7.64E-06	7.01E-03
PEZ6[a]	9	0.737	0.797	0.074	0.034	232–323	5.15E-07	2.58E-03
FH2422	15	0.801	0.800	–0.002	0.036	206–245	3.26E-08	9.45E-04
FH2054	9	0.808	0.831	0.028	0.034	167–197	1.66E-09	3.28E-04
FH2004	15	0.817	0.840	0.027	0.005	139–172	7.35E-11	1.12E-04
FH3313[a]	20	0.738	0.887	0.168	0.111	343–410	1.59E-12	3.50E-05
$\bar{X}$	8.091	0.661	0.681	0.029				

[a] Loci that deviate significantly from Hardy–Weinberg equilibrium (adjusted nominal level for $P < 0.05$).

for blood and tissue samples were as above over 35 cycles with annealing at 58°C. PCR amplification was completed using a Mastercycler thermocycler (Eppendorf, Hamburg, Germany). Amplification products were diluted and profiled on an ABI Prism 3100 Avant capillary DNA sequencer (Perkin-Elmer, Waltham, Massachusetts). Scans were scored using the GeneMapper 3.0 software package (Applied Biosystems 2002) and manually checked for errors. Genotyping success (n = 1,981) was measured as the mean number of loci amplified for each sample type.

A modified version of the multiple tubes approach developed by Taberlet et al. (1996) incorporating a matching protocol (Frantz et al. 2003) was used to determine genotypes with a low probability of error. Scat samples were amplified and genotyped in a minimum of 7 replicates, hair in 5 replicates, and blood and tissue samples in duplicate. Genotypes for a given sample were pooled to create a single provisional consensus genotype. To determine the provisional consensus genotype, each allele had to be present at least 2 times for a heterozygote and 3 times for a homozygote. Because we were using the Y-chromosome as a sex marker, we required the allele to be seen a minimum of 3 times across replicates. We assumed nonamplification across all replicates to represent a female. Based on the proportion of missing data across genotypes (see "Results"), we were confident that the majority of individuals were sexed correctly. We considered more than 2 alleles at a locus erroneous and were not recorded. Provisional consensus genotypes were compared to all other genotypes for matches with other samples (Frantz et al. 2003).

With noninvasive sampling, there is the potential for multiple samples from the same individual; therefore, multiple matching genotypes from 1 wolf could exist in the data set (Taberlet and Luikart 1999). Additionally, it was possible that more than 1 wolf, and therefore sample, might share the exact same genotype with another wolf by chance. We used probability of identity statistics (PID—Paetkau and Strobeck 1994) to form criteria for minimum number of matching loci necessary to ensure a low probability of a random match (Waits et al. 2001). We calculated PID_{random} (Paetkau et al. 1998) and PID_{sib} (Evett and Weir 1998) for the data using GIMLET v1.3.2 (Valière 2002). Using CERVUS (Marshall et al. 1998), we assigned all samples to unique individuals; based on PID_{sib} (see "Results"), samples that matched at 6 loci were considered to be the same individual.

Complete unique genotypes were used to calculate expected (H_E) and observed (H_O) heterozygosities and null allele frequencies in CERVUS 2.0 (Marshall et al. 1998). Exact tests for Hardy–Weinberg equilibrium (HWE) and linkage disequilibria (LD) were implemented in FSTAT v2.9.3 (Goudet 1995). Using all consensus genotypes, we checked for null alleles and genotyping error using Micro-Checker (van Oosterhout et al. 2004).

Relatedness.—The geographic coordinates for packs were set as the centroid of the minimum convex polygon based on sample locations of pack members. Geographic distance between individuals within the same pack was assumed to be zero. Euclidean distances between pack locations were used to create a distance matrix. We completed fine-scale analyses of relatedness using packs consisting of at least 3 sampled individuals (n = 47), whereas individuals without pack information were excluded from all analyses. We used COANCESTRY (Wang 2010) to test for relatedness patterns. For this analysis, we calculated allele frequencies from the data and accounted for inbreeding using 100 reference individuals and 1,000 bootstrap permutations. All relatedness values were highly correlated across the data set; therefore we used the estimate of Lynch and Li (Lynch 1988; Li et al. 1993) to test whether relatedness among individuals was greater within packs than between packs, and whether females were more related than males within packs; significance for all tests was estimated by 1,000 bootstraps.

To determine whether there were extrinsic factors that affect pack relatedness, we examined the relationship between within-pack relatedness and terrain ruggedness (slope and elevation), landcover composition (19 variables described in Supporting Information S1), distance to major roads, distance to rivers, pack sample size, sex ratio, and location (easting and northing) using regression and Akaike's Information Criterion (AIC) model selection in R (R Development Core Team 2011). Slope was estimated using the spatial analyst in ArcMap 10.2 (ESRI 2014). To calculate landcover composition, we created 200-km^2 buffers around each pack centroid and estimated percent landcover for each pack using Spatial Analyst in ArcMap. Before modeling, we estimated the correlation among variables, and individual variables were removed in a stepwise fashion if they were highly correlated ($r > |0.70|$). As a result of having a large number of variables, we used a stepwise AIC model selection first (using the stepAIC command in R), and then based on the significant terms in the model, we generated competing models and used AIC to again choose the best model. Significance of the terms in the final model was estimated using analysis of variance (ANOVA). Human density was not considered because it is relatively homogenous across the study area (1–3.5 people/km^2—Natural Resources Canada 2015).

Population structure.—Individuals were assigned to genetic subpopulations using the Bayesian clustering method of STRUCTURE 2.3.4 (Pritchard et al. 2000; Falush et al. 2003), which assigns individual genotypes into groups independent of sampling location. We used the Evanno method to estimate the most likely number of groups (K—Falush et al. 2003; Evanno et al. 2005). Five independent analyses of K = 1–10 were completed with burn-in and Markov chain Monte Carlo repetitions of 100,000 and 750,000, respectively. Individuals were assigned to groups based on the resulting q-values ($q \geq 0.7$). We completed an analysis of molecular variance (AMOVA—Excoffier et al. 1992) based on the number of clusters identified to quantify the amount of genetic variation partitioned between the groups, as well as within packs among groups.

To investigate whether there was any signature of expansion from the north to the south as a result of recolonization, we examined the relationship of heterozygosity, allelic richness,

and number of alleles at the pack level, with northing using regression in R.

Landscape effects.—To understand the influence of landscape on genetic structure, we developed competing landscape resistance models in ArcMap 10.2. The selection of different resistance models was based on a literature search of wolf habitat studies (Table 2). We developed 4 landscape resistance models: 1) null, represents isolation by distance where only geographic distance was included; 2) human disturbance, included both towns and roads as dispersal barriers; 3) habitat resistance, where dense and open coniferous, deciduous and mixed wood were considered high quality, and sparse forest as low quality; and 4) elevation. Resistance values for each of these models are indicated in Table 2. We calculated the distances between pack centroids based on these resistance surfaces using CIRCUITSCAPE (McRae and Beier 2007). CIRCUITSCAPE is based on circuit theory where organisms are treated as electrical currents and landscape resistances reduce the amount of gene flow. We then used the resistance values for each of our landscape models and tested their fit to genetic distance. We used 4 different genetic distance measures: the Bray–Curtis dissimilarity matrix for pack allele frequencies, pack relatedness, F_{ST}, and pack distance using the STRUCTURE q-values (Murphy et al. 2008). To estimate the Bray–Curtis dissimilarity matrix, we used the "distance" function in the ecodist package in R (Goslee and Urban 2007). For pack relatedness, we used Lynch (1988) and Li et al. (1993) to be consistent with the fine-scale relatedness analysis. Recently, q-values have been used to assess physical barriers to gene flow and are a better measure for individual-based analyses when dealing with continuously distributed species (Balkenhol et al. 2014; Anderson et al. 2015). Distance measures for STRUCTURE q-values were based on the 2 clusters identified (see "Results"); therefore, we estimated an average q-value for each pack using their assignment to cluster 1. We then created a pairwise distance matrix between packs by subtracting their respective q-values, taking the absolute value. We included F_{ST} to allow for comparisons across our measures as this is most typically used for landscape genetic analyses, knowing that the majority of our pack sizes were not large enough to estimate this parameter well. For our 4 genetic distance measures, we only included packs with 4 or more sampled individuals ($n = 44$).

The resistance maps created in CIRCUITSCAPE are influenced by the number of nodes and their distribution, in our case, each pack represented a node. Because all packs were not sampled across the region, we wanted to examine the influence of the landscape without the potential bias caused by the node locations, where we have better sampling in the north than the south (Fig. 2) and this can underestimate connectivity in the south. As an alternate approach to look at habitat effects, we created a landcover distance between packs using the landcover composition from the relatedness analysis. To create a single distance between packs, we used a Hellinger transformation to correct for the large proportion of zero data (Legendre

Table 2.—Details of the resistance models developed for wolf gene flow including the variables considered, the size of the buffer developed around linear and point features, and their resistance score (1 = nonresistant). Model fit and significance values are given for each of the model fits to 3 different genetic distance measures (relatedness, F_{ST}, and the average q-distance), values in bold are significant ($\alpha = 0.05$). References relevant to the corresponding models are included.

Model	Habitat	Buffer	Resistance score	Allele frequencies		Relatedness		Average q-distance		F_{ST} matrix		References
				R^2	P	R^2	P	R^2	P	R^2	P	
Null	Geographic distance		1	0.002	0.573	**0.055**	**0.002**	**0.100**	**0.001**	0.023	0.080	Forbes and Boyd (1997); Vilà et al. (1999); Carmichael et al. (2001)
Human disturbance	TransCanada	5,000 m	50	0.002	0.655	0.016	0.196	0.020	0.543	0.001	0.459	Thiel (1985); Jensen et al. (1986); Mech et al. (1988); Mladenoff et al. (1995); Oakleaf et al. (2006)
	Secondary highways	2,500 m	25									
	Towns	5,000 m	50									
	Intervening matrix		1									
Habitat resistance	Dense forest		1	**0.092**	**0.01**	0.003	0.582	0.008	0.252	0.002	0.751	Oakleaf et al. (2006); Musiani et al. (2007); Muñoz-Fuentes et al. (2009)
	Open forest		1									
	Water		10									
	Sparse forest		40									
	Herbacious		50									
	Wetland		75									
Elevation	> 2,500 m		50	0.004	0.577	0.017	0.170	0.001	0.667	0.002	0.774	Boyd (1997); Forbes and Boyd (1997); Carroll et al. (2003)
	Intervening matrix		1									
Slope	0–19.72%		1	0.002	0.713	0.008	0.367	0.000	0.991	0.006	0.340	
	19.80–31.08%		50									
	31.10–85.00%		100									
Landcover distance			NA	**0.035**	**0.014**	0.014	0.064	**0.189**	**0.0001**	0.001	0.767	
q-distance combined	Null							**0.23**	**0.0007**			
	Landcover								**0.0001**			

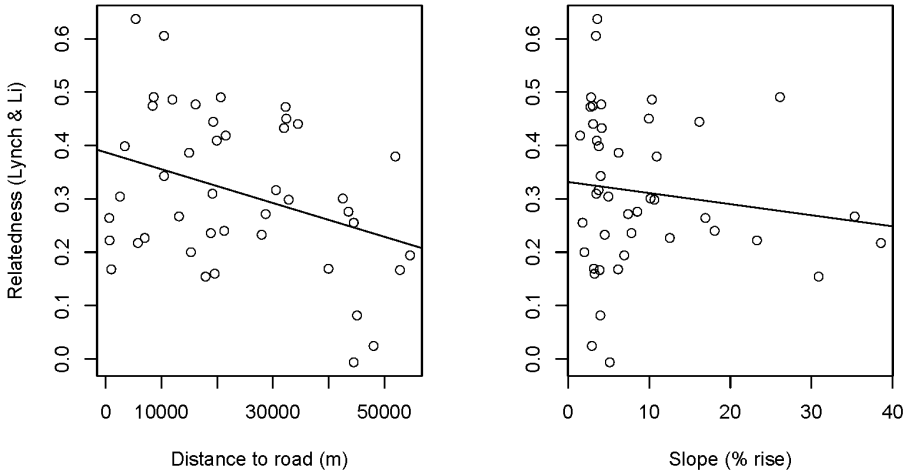

Fig. 2.—Plots of relatedness (Lynch and Li) with regression lines against the 2 most important predictors, distance to roads (m) and slope (percent rise).

and Gallagher 2001; Legendre and Legendre 2012), and used the "distance" function in the ecodist package to estimate the Euclidean landcover distance among packs. Further, to identify which landcover variables affected the distances among the packs, we used principle components analysis (PCA), calculated using the ecodist package.

To assess how well the distance matrices (resistance models and landcover distance) explained the 4 genetic distances, we used multiple regression on distance matrices (MRM) implemented in the ecodist package. This method is more robust at resolving between isolation by distance and isolation by resistance than Mantel and partial Mantel tests (Balkenhol et al. 2009). We used this approach in combination with partial Mantel tests as it provided an opportunity to model the contribution of each landscape distance and provides a measure of significance.

Results

Genotypes were called for 2,081 samples. However, we removed 789 samples, and 1 locus (PEZ8) from the analysis due to poor amplification. The remaining samples (n = 1,192) were used to form consensus genotypes for individuals. Most samples had complete 12 locus genotypes (76%) and those genotypes that amplified < 12 loci had 3.2% missing data. Mean success rates varied among sample types with muscle tissue providing 10.7 typed loci/extraction (SE = 0.25, n = 348) and blood 10.5 loci/extraction (SE = 0.27, n = 254). Both muscle and blood samples amplified significantly better than hair ($\bar{X}$ = 7.1 loci/extraction, SE = 0.25, n = 452; Mann–Whitney U: $P < 0.001$), which amplified significantly better than scat ($\bar{X}$ = 4.0, SE = 0.15, n = 927; Mann–Whitney U: $P < 0.001$). However, scats stored in 100% ethanol ($\bar{X}$ =6.5 loci/extraction, SE = 1.00, n = 25) amplified no differently than hair (Mann–Whitney U: P = 0.77), but better than scat stored at −20°C ($\bar{X}$ = 3.9, SE = 0.16, n = 902; Mann–Whitney U: P = 0.004).

Expected heterozygosity per locus ranged from 0.167 to 0.887, with a range of 2 (FH2834) to 20 (FH3313) alleles per locus (Table 1). Locus FH3313 was removed from the analysis as it was significantly out of HWE with high F_{IS}, and was identified in Micro-Checker as having null alleles. Only 1 other locus (FEZ6) was significantly out of HWE based on the adjusted nominal level (for $\alpha = 0.05$). Linkage disequilibrium was detected in 45 out of the 66 locus pairs at the adjusted nominal level (5%), but none at the 1% adjusted nominal level. Eleven loci were used for the remaining analyses.

The 6 least variable loci provided a PID_{random} of 1.13×10^{-4} and a PID_{sib} of 1.92×10^{-2}. We excluded samples with a $PID_{sib} > 1 \times 10^{-3}$ in subsequent analyses. From 1,192 samples, we identified 526 individual wolves (n = 96 from noninvasive samples). The sex ratio was 247 females: 279 males (0.89:1).

Relatedness.—Average relatedness within packs (0.304) was significantly higher than average relatedness between packs (−0.009; $t_{1,620}$= −45.29, $P < 2.2^{-16}$). As well, average relatedness of females within packs was higher than that among males (0.328, 0.280, respectively; ANOVA P = 0.015). Relatedness among packs was highly heterogeneous; therefore, we looked at extrinsic factors that may help to explain this variation. Across the 27 variables, 7 were highly correlated and removed (easting, elevation, snow, exposed, coniferous dense, coniferous sparse, and broadleaf sparse). The remaining variables were retained for AIC model selection. Following forward and backward model selection, 8 variables were retained in the model (R^2 = 0.314, P = 0.005; Table 3); however, only distance to roads and slope were significant (P = 0.012, P = 0.012, respectively), while water and herbs were near significant (P = 0.090, P = 0.097, respectively). We compared this model (modall), with 4 additional models: mod1: distance to road + slope, mod2: mod1 + interaction, mod3: distance to road + slope + water + herbs, and mod4: mod 3 + all variable interactions. Following AIC, mod2 was the best, with an AIC weight of 0.60, this model explained 19% of the variation in pack relatedness values (P = 0.009; Table 4), the 2nd best model, mod1, had a weight of 0.25, indicating that distance to road and slope were important determinants of pack relatedness (Fig. 2; Supporting Information S2).

Population structure.—The most likely number of clusters was 2, with ΔK = 53.92. Using an assignment q-value threshold of 0.7, 194 individuals assigned to the 1st cluster, 148 individuals assigned to the 2nd cluster (hereafter referred to as

Table 3.—Model results for identifying factors that explain the heterogeneity in within-pack relatedness. Presented is the best model selected by the stepAIC function in R, as well as the final model as compared with competing models. Included are the *P*-values for both the *t*-test and the ANOVA *F*-test. Significant *P*-values are in bold. AIC = Akaike's Information Criterion; ANOVA = analysis of variance.

Variable	Estimate	*SE*	*T*	*P*	*F*	*P*
Modall						
Intercept	3.734	0.0527	7.085	**2.97E-08**		
Slope	−6.33E-03	2.393	−2.646	**0.012**	1.066	0.309
Distance to road	−3.66E-06	1.28E-06	−2.641	**0.012**	10.469	**0.003**
Water	−1.084	0.622	−1.743	0.090	2.523	0.121
Herbs	0.680	0.399	1.705	0.097	0.605	0.442
Coniferous open	0.151	0.104	1.452	0.156	4.831	**0.035**
Mixedwood dense	1.102	0.718	1.536	0.133	1.901	0.177
Mixedwood open	0.873	0.544	1.605	0.117	4.017	0.053
Mixedwood sparse	7.515	4.986	1.507	0.141	2.272	0.141
mod2						
Intercept	0.493	0.054	9.21	**1.99E-11**		
Distance to road	−7.14E-06	2.16E-06	−3.31	**0.002**	6.433	**0.015**
Slope	−0.010	0.004	−2.607	**0.013**	3.386	0.073
Distance to road:Slope	4.65E-07	2.47E-07	1.881	0.067	3.540	0.067

Table 4.—Model selection results using Akaike's Information Criterion (AIC) estimated in R. The top model, mod2, had the majority of support.

Model	AIC	ΔAIC	Weight
modall	−34.337	14.668	0.000
mod4	−38.911	10.094	0.004
mod3	−46.051	2.954	0.138
mod1	−47.274	1.731	0.254
mod2	−49.005	0.000	0.604

the "north" and "south" populations, respectively), and 184 individuals were unassigned. Average pack assignment can be visualized in Fig. 3. The clusters were significantly differentiated (F_{ST} = 0.155, $P < 0.0001$) and the amount of variance explained by the 2 clusters based on AMOVA was 2.69%, while 12.81% was within the packs, and the remainder was among individuals.

We did not find any relationship between northing and heterozygosity ($P = 0.836$), allelic richness ($P = 0.862$), or number of alleles ($P = 0.489$). The pattern of heterozygosity over the landscape was highly heterogeneous (Supporting Information S3).

Landscape effects.—We estimated the Bray–Curtis dissimilarity matrix (D_{BC}), relatedness (R_{xy}), F_{ST}, and *q*-value matrices (Q_{xy}) to test the relationship between landscape and genetic differentiation. We examined 6 models: isolation by distance, landcover distances, and 4 resistance models: human disturbance, elevation, slope, and habitat. The variance for the pack average of Q_{xy} was small and ranged from 0.125×10^{-5} to 5.80×10^{-5}, indicating that individuals within packs assigned to the same cluster for the majority of the packs. None of the models explained any of the variation in the F_{ST} distance matrix (Table 2). For the other genetic distances, the most consistent models were the landcover distance model and the null model (isolation by distance). The landcover distances explained a large portion of variation in the Q_{xy} distances (R^2 = 0.189, P = 0.0001), and a significant proportion for the D_{BC} matrix (R^2 = 0.035, P = 0.014), while the null model explained a significant proportion for R_{xy} and Q_{xy} (R^2 = 0.055, P = 0.002; R^2 = 0.100, P = 0.001, respectively). Of the resistance models from CIRCUITSCAPE, only the habitat resistance model explained a significant proportion of the D_{BC} distance (R^2 = 0.092, P = 0.01). Only for the Q_{xy} matrix was there more than 1 competing matrix (isolation by distance and landcover distance), when combined they both explained a significant proportion of the variance in the genetic distance (R^2 = 0.230, P = 0.001). Using partial Mantels, the correlation of landcover distance with Q_{xy} was r = 0.381 (P = 0.001) after removing the effects of geographic distance and r = 0.226 (P = 0.001) for geographic distance after removing the effects of landcover. The route of gene flow, based on circuit theory, is indicted for the null (isolation by distance) resistance model (Fig. 3). The PCA plot of the packs highlights open coniferous and dense coniferous as the primary variables driving the landcover distances among packs (Supporting Information S4).

Discussion

We found significant relatedness at the pack level, as well as female philopatry suggesting traditional pack structuring. We found that within-pack relatedness varied across the landscape, where distance to major roads and slope were significant influences. Relatedness was greatest close to roads, with less steep terrain (Fig. 2; Supporting Information S2). At the large scale, we identified 2 significantly differentiated genetic clusters that corresponded to a north/south split. This structure was related to landcover differences in the north and south, where packs to the north were in predominantly dense coniferous forests, while the southern packs were found in open coniferous forests (Fig. 3). This landcover association could be the result of prey distribution and/or natal habitat affinity.

Kin relationship within wolf packs is important as members cooperate to raise young, hunt prey, and defend their territory (Mech 1970; Mech and Boitani 2003; Silk 2007). In areas of high

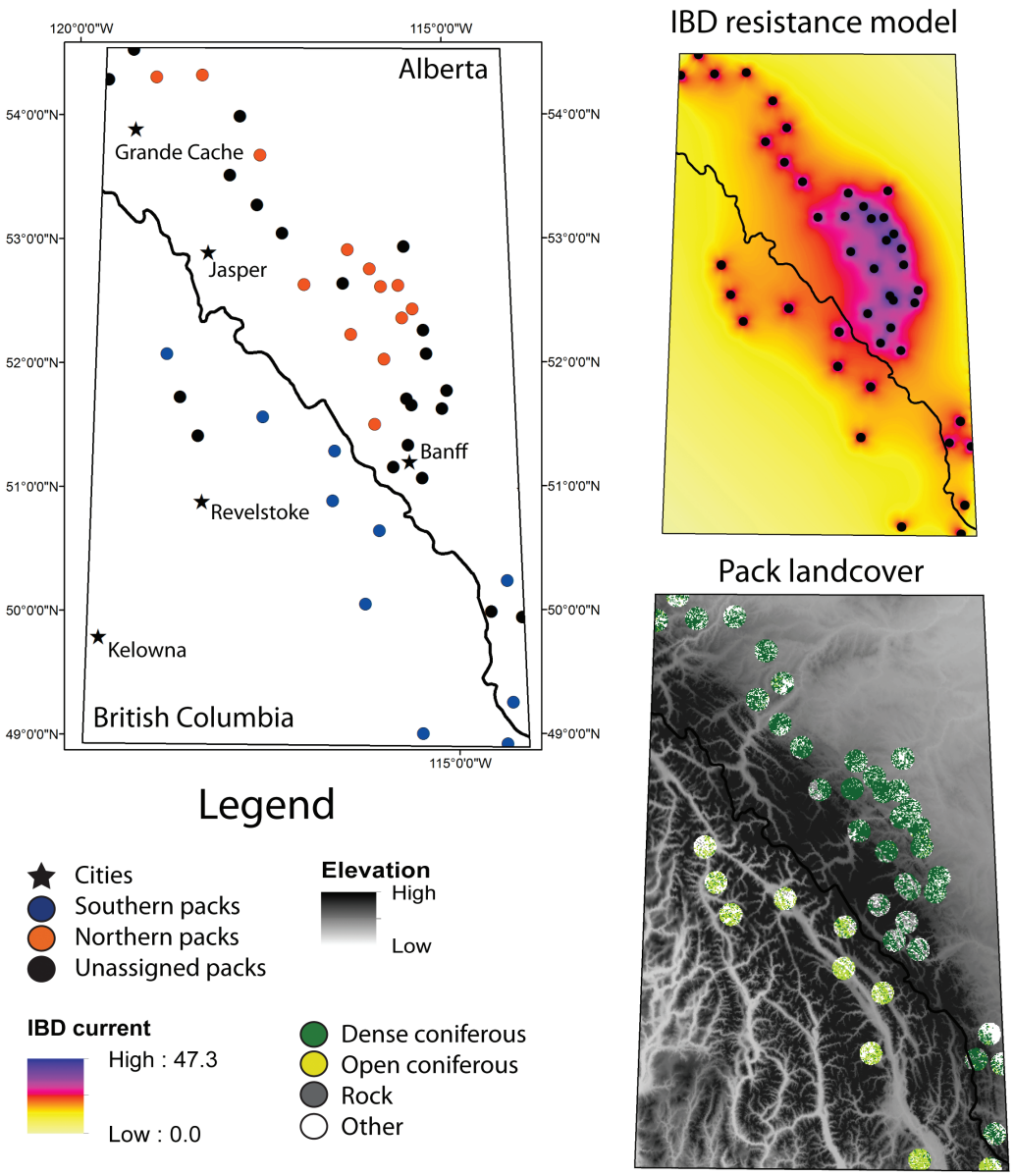

Fig. 3.—The large panel indicates the assignment of packs based on the STRUCTURE *q*-values across the area of sampled wolf packs. Packs included in the CIRCUITSCAPE analysis ($n \geq 4$) are indicated on all map panels. The current map for null surface of isolation by distance (IBD) indicates the likely routes of dispersal, while the landcover map identifies the composition differences between the northern and southern packs.

mortality, pack structure is often disrupted and can result in low relatedness within packs (Grewal et al. 2004; Jêdrzejewski et al. 2005; Rutlidge et al. 2010). Despite the demographic history of wolves in our study area and spatially heterogeneous management scenarios (wolves are protected in parks, but hunted or trapped elsewhere), we found evidence of stable pack structure, where relatedness within packs was greater than relatedness between packs (Mech and Boitani 2003; Jêdrzejewski et al. 2005). While this was the general trend across the study area, there was variation in pack relatedness that ranged from 0.00 to 0.64. Therefore, we wanted to identify factors that may influence pack structure. Studies exploring factors affecting pack structure have identified management and prey biomass to be important drivers (Jêdrzejewski et al. 2005), but we also wanted to identify whether landscape characteristics influenced pack dynamics. Examining landcover, terrain, and human features, we found that the most important explanatory factor for within-pack relatedness was distance to major roads, where relatedness increased with proximity to roads. This was an unexpected finding because studies have found wolves tend to avoid areas with high road densities (Mladenoff et al. 1995, 1999; Gurarie et al. 2011; Kaartinen et al. 2015). We propose 2 possible explanations for our finding. First, if prey use roads as dispersal corridors, then packs located near roads may have better access to prey and therefore maintain more kin (Jêdrzejewski et al. 2005). Unfortunately, we do not have prey biomass information for our area and we could not test that hypothesis. Alternatively, higher relatedness close to roads may be associated with the perceived mortality risk of dispersal by wolves, which in turn could encourage offspring to remain as helpers. We also found

that slope was a factor, where pack relatedness increased with decreasing slope however; this relationship was primarily driven by a few packs in steep areas, with the majority of packs sampled in areas with little or no slope (Fig. 2).

In species where dispersal distances are equal to or larger than the size of the area sampled, distinct subpopulations are more likely to arise due to barriers to dispersal rather than geographic distance. Our study area encompassed the upper limits of dispersal distances for wolves, yet we still identified 2 subpopulations. This observed structure was not likely the result of range expansion in the 1950s (Hayes and Gunson 1995), as we did not find the characteristic relationship between genetic diversity and geography that occurs following colonization (Supporting Information S3; Bernatchez and Wilson 1998; Excoffier and Ray 2008). Rather, the structure suggests some combination of landscape factors may affect population structure. Using available literature, we developed a number of models that could explain the observed population genetic structure.

With landscape genetics, there can be uncertainty in identifying the important factors that limit dispersal. We found limited agreement across our different models and genetic distances to understand population-level movements. To start, none of the models explained the variance in F_{ST}. We included this measure as it is often used in landscape genetics (e.g., Balkenhol et al. 2009; Emaresi et al. 2011; Paulson and Martin 2014; Emel and Storfer 2015), but it is a summary statistic that is influenced by many factors and therefore may not highlight the effects of gene flow (Pearce and Crandall 2004; Epperson 2007). As well, it is influenced by the accurate estimation of allele frequencies, and in our case, some packs may not have been sufficiently sampled to obtain accurate estimates. Only 1 resistance model was a significant predictor for genetic distance (relatedness ~ habitat resistance), while the null model (isolation by distance) and the landcover distance were important predictors for 2 of the 4 genetic distances. Landcover distance was also the best predictor across all models and suggests that wolf movements are not restricted by certain habitats; rather, habitat selection helps to drive population structure (Haddad and Tewksbury 2005).

Isolation by distance was expected, and it has been observed for a number of wolf studies in different areas (Forbes and Boyd 1997; Vilà et al. 1999; Carmichael et al. 2001). The importance of landcover has also been highlighted (Oakleaf et al. 2006; Musiani et al. 2007; Muñoz-Fuentes et al. 2009; Stronen et al. 2014). In our area, we found packs to the north were in dense coniferous forests, while those in the south were associated with open coniferous forest. An association with habitat may exist if wolves direct their movements to ensure access to prey species, resulting in genetic structure related to primary prey species habitat (Musiani et al. 2007; Muñoz-Fuentes et al. 2009; Stronen et al. 2014). Alternatively, the association with habitat may arise from natal fidelity, where individuals are less likely to disperse into unfamiliar habitat (Sacks et al. 2004; Stamps and Swaisgood 2007). Interestingly, neither elevation nor slope appeared to have an effect on structure, despite our expectation of an effect given that the rugged topography is unsuitable habitat and challenging to navigate. Therefore, dispersing wolves may be taking advantage of mountain passes countering possible effects of elevation.

Genetic sampling of wolves frequently occurs at a small scale (e.g., Lehman et al. 1992; Lucchini et al. 2002; Creel et al. 2003) where data on pack size and demography exist or at a larger scale where widely spaced sample locations are considered populations without knowledge of the genetic relationships of the wolves (e.g., Forbes and Boyd 1997; Geffen et al. 2004). Our study shows the value of collecting samples across the range of continuously distributed individuals rather than sampling from widely separated, discrete locations. For example, wolf genetic structure was examined in Montana and the Rocky Mountains from 4 locations (including 3 covered in our study—Boyd 1997; Forbes and Boyd 1997). They found significant genetic distance between all populations over a range of 4,500 km, whereas we found isolation by distance across the area and evidence of weak genetic structure. On a larger scale, no effect of distance on genetic differentiation was found for wolves from across North America (Roy et al. 1994). Both of these studies used samples collected from restricted geographic areas, which may have represented family groups. If the "populations" they sampled comprised highly related individuals then genetic differentiation between their populations would be erroneously high. Our sampling of contiguous packs across a large geographic distance allowed the examination of the effects of isolation by distance and habitat effects across the study area, as well as information regarding factors influencing pack stability.

Given the demographic stochasticity that the wolf population has experienced in this area and the high heterogeneity of the landscape, it is interesting to note that the main pattern observed was isolation by distance and an association with landcover. This suggests that these wolf populations were quite resilient to major demographic fluctuations. This was further evidenced by our finding of stable pack structure and a lack of genetic signature of recent expansion. Also, the pattern of gene flow was not detectably affected by human linear features or the rugged topography of the Canadian Rocky Mountain range. These together indicate a healthy population of this important predatory carnivore, where there is evidence of stable pack structure, connectivity, and high genetic diversity.

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Supporting Information

The Supporting Information documents are linked to this manuscript and are available at Journal of Mammalogy online (jmammal.oxfordjournals.org). The materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supporting data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Supporting Information S1.—Table of a description of the habitat variables whose composition was estimated in buffers around each pack centroid used in the relatedness and landscape genetic analyses.

Supporting Information S2.—Map of slope (percent rise) and major roads that were identified as significant factors influencing pack relatedness, which is visualized as a gradient of colors for each pack location.

Supporting Information S3.—Interpolated surface of observed heterozygosity of wolf packs estimated from 12 microsatellite loci across the Canadian Rocky Mountains.

Supporting Information S4.—Principal components analysis of proportion of landcover types in buffers surrounding each pack location. The landcover types driving the differences among packs are indicated by the arrows, and pack assignment based on structure is indicated by color.

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