Demographic fragmentation of a protected wolverine population bisected by a major transportation corridor

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ABSTRACT

Roads fragment ecosystems around the globe, but the effects of this fragmentation on biodiversity remain poorly understood. Wolverines (*Gulo gulo*) are snow-dependent carnivores that occur at low densities and they exhibit low genetic diversity at the southern extent of their range where they are snow-limited and fragmented by human development. Therefore, understanding the effect of roads and transportation infrastructure on population connectivity in protected strongholds such as national parks is crucial to effective wolverine management in a changing climate. We assessed whether the Trans-Canada Highway, Canada's largest east-west transportation corridor, affects wolverine gene flow in the Rocky Mountains. We used noninvasive genetic sampling methods (i.e., hair traps, backtracking) to collect DNA samples (i.e. hair, scat) from an 8000 km^2 area of Banff, Kootenay, and Yoho National Parks and provincial lands and then used population and individual-based genetic analyses (e.g., assignment tests, principal coordinates analysis) to examine genetic structure across the highway in the national parks complex. We collected 2586 DNA samples between 2010 and 2013 from which we identified 49 unique individuals (29 males, 20 females). We detected weak population structure in males and relatively strong genetic differentiation in females spanning the highway with complementary nuclear and mitochondrial DNA analyses. Our results demonstrate that sex-biased dispersal across a major highway can lead to genetic isolation and demographic fragmentation in a protected carnivore population, highlighting the urgent need to maintain connectivity for wildlife over an expanding global road network in the face of climate change, landscape degradation, and loss of biodiversity.

1. Introduction

Humans have caused long-lasting environmental changes to the Earth's ecosystems (Hansen et al., 2002; Watts et al., 2007; Haddad et al., 2015); one of the most prominent landscape modifications has been the degradation and fragmentation of wildlife habitat (Fahrig, 2003; Fischer and Lindenmayer, 2007). Fragmented landscapes have smaller habitat patches, greater isolation and more edge effects; therefore, they support less biodiversity (i.e. species richness) than intact systems (Hanski, 2015). Fragmentation creates smaller and more isolated wildlife populations, which have less genetic diversity than larger, connected populations (Frankham, 1996, 1997) and are more vulnerable to the effects of demographic and environmental stochasticity (Mills, 2007) and inbreeding due to the loss of genetic diversity from genetic drift (Frankham, 1998). Landscape connectivity (Taylor et al., 1993) helps to maintain demographic and genetic processes for wide-ranging wildlife species such as wolverines, by allowing the movement of individuals and the flow of genes.

Roads are the "largest human artifact on Earth" and they are one of the biggest threats to biodiversity (Forman et al., 2003). The global road network in 2018 was estimated at > 21 million km and growing fast in undeveloped areas (Meijer et al., 2018). Roads can negatively affect ecosystems (Trombulak and Frissell, 2000) by acting as barriers to animal movements and fragmenting landscapes (Shepard et al., 2008). Highways can block gene flow leading to a loss of genetic diversity from genetic drift (Keller and Largiader, 2003; Epps et al., 2005; Riley et al., 2006; Kuehn et al., 2007; Holderegger and Di Giulio, 2010). Sex-biased dispersal across roads can lead to female population structure and isolation, otherwise known as demographic fragmentation; this lack of demographic connectivity can result in vulnerably small populations (Proctor et al., 2005). The Trans-Canada Highway (TCH) in the Canadian Rocky Mountains has long been recognized as a lethal
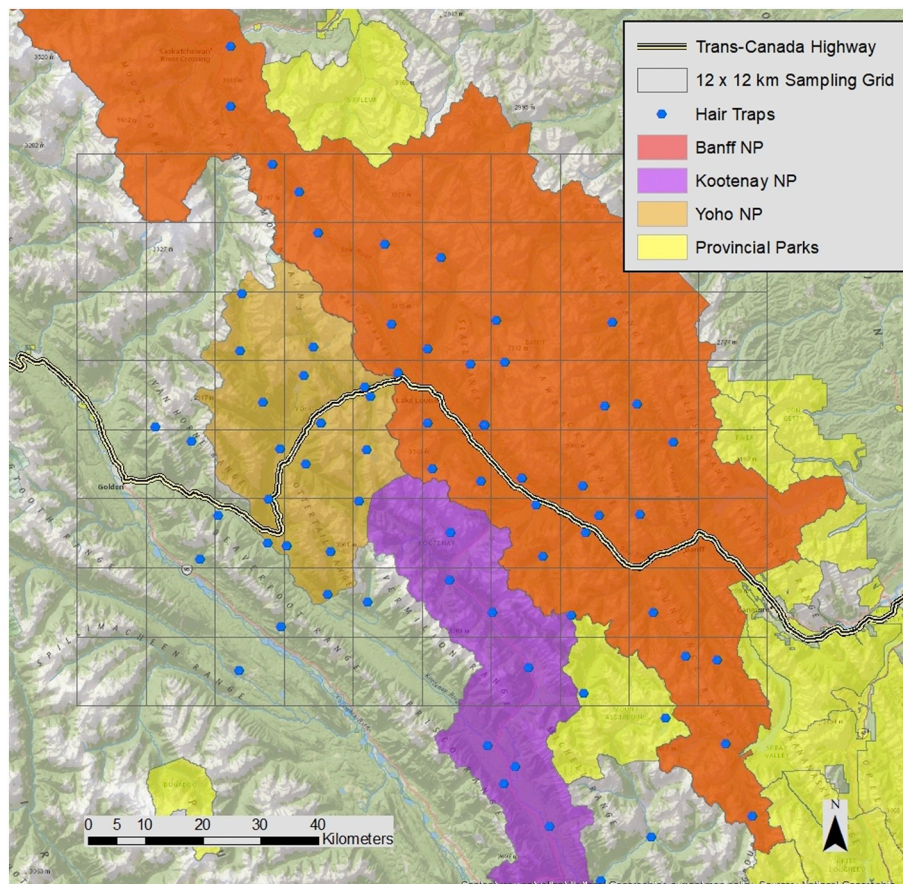
Fig. 1. The 12 × 12 km sampling grid and hair trap locations used to collect genetic data from wolverines in the Banff-Yoho-Kootenay national parks complex in Alberta and British Columbia, Canada between 2010 and 2013.

barrier to wildlife and an acute fracture zone for population connectivity at local and trans-boundary scales (Clevenger et al., 2001; Chruszcz et al., 2003; Sawaya et al., 2014). Given the national importance of the cross-country transportation corridor and popular attraction of Banff National Park (BNP), highway traffic volumes have doubled in the last 10 years (Parks Canada Highway Engineering Services, unpublished data). Continuing expansion of the TCH in BNP's Bow Valley enters high-quality subalpine habitats at the spine of the Continental Divide. This high elevation ecosystem is doubly important given it is acutely impacted by a warming climate and its north-south axis is bisected by a major east-west transportation corridor.

Mammalian carnivores are particularly vulnerable to the effects of habitat fragmentation and reduced connectivity from roads due to their biological traits, such as large body sizes, large home range requirements, low densities and low reproductive rates (Crooks et al., 2011, Ceia-Hasse et al., 2017). Canada's Rocky Mountain front harbors the richest diversity of large mammalian carnivores remaining in North America (Weaver et al., 1996). This landscape is among the continent's least disturbed natural areas and provides a critical trans-boundary linkage with the United States. Maintaining landscape connectivity throughout the ecoregion could be an effective conservation strategy to mitigate effects of climate change and habitat fragmentation (Krosby et al., 2010).

Wolverines are wide-ranging, rare, sensitive to human disturbance, and associated with high elevation alpine and avalanche environments in the Rocky Mountains (Copeland et al., 2007; Krebs et al., 2007; Fisher et al., 2013); therefore, they are a good species for studying the combined effects of habitat fragmentation and environmental change. Current projections are for wolverine habitat, particularly in the southern extent of their range, to decrease in area and become more

fragmented within the foreseeable future as a result of a warming climate (Brodie and Post, 2010; Peacock, 2011; McKelvey et al., 2011). Wolverine males and females display different dispersal patterns with females having shorter dispersal distances and stronger philopatric tendencies (Vangen et al., 2001). Despite frequent male-mediated gene flow, substantial genetic structuring has been observed in wolverine populations at the southern extent of their range where genetic diversity is low, and disturbance is high (Cegelski et al., 2006).

Wolverines need connectivity for metapopulation dynamics (Inman et al., 2013) so they may be particularly vulnerable to fragmentation effects by linear barriers to movement such as transportation infrastructure. A localized study in Kicking Horse Pass, British Columbia (Yoho National Park) found wolverines rarely crossed the TCH (Austin, 1998). A recent study in Northern Alberta showed wolverines were averse to selecting habitats and crossing roads with relatively low traffic volumes compared to the TCH (Scrafford et al., 2018). There is a lack of information regarding how wolverines respond to major transportation corridors, such as the US Interstate highway system or the east-west TCH corridor in the Canadian Rocky Mountains. Knowing how wildlife movements and metapopulations are affected by high speed, high traffic volume highways will be critical for informing decision making and designing effective landscape conservation strategies in the face of climate change.

The goal of this study was to assess demographic and genetic connectivity for wolverines in a key protected area complex in the Canadian Rocky Mountains. Genetic approaches can be useful for understanding many aspects of population connectivity (Lowe and Allendorf, 2010). Landscape genetics approaches (Manel et al., 2003), that combine population genetics and landscape ecology disciplines, may be particularly well-suited to examining the effects of roads on

wildlife populations (Balkenhol and Waits, 2009; Simmons et al., 2010). Between 2010 and 2013, we used noninvasive genetic sampling methods to systematically collect genetic data in Banff, Yoho and Kootenay national parks. We examined the hypothesis that a heavily traveled 4-lane highway affected gene flow and population structure in male and female wolverines within the multi-park complex. This research is the first attempt we are aware of to assess the effects of a major transportation corridor on carnivore connectivity at fine and continental scales. We discuss the implications of our findings in the context of conserving wolverine populations and maintaining connectivity for wildlife amidst an expanding global transportation network.

2. Materials and methods

2.1. Study area

Our study area (Fig. 1) was located in the Canadian Rocky Mountains, encompassed approximately 8000 km² and included parts of Banff, Yoho and Kootenay National Parks, Mt. Assiniboine Provincial Park, the Columbia Valley and adjacent provincial lands in British Columbia (hereafter referred to as the park complex). The study was focused on the Bow River Valley of Banff National Park, situated within the front and main ranges of the Canadian Rocky Mountains. The topography is steep and mountainous with elevations from 1300 to 3400 m, and a valley floor width from 2 to 5 km. The climate is continental and characterized by relatively long winters and short summers (Holland and Coen, 1983). Vegetation in the park encompasses montane, subalpine and alpine ecoregions. Montane habitats are found in low elevation valley bottoms. The lower Bow River Valley is a human-dominated landscape with the TCH, the Banff Townsite (10,000 residents), a golf course, 3 ski areas, Canadian Pacific Railway, and a secondary highway. Neighboring Yoho National Park is situated on the west side of the Continental Divide and is characterized by steep rugged terrain with narrow valleys and continental climate consisting of short, cool summers and long winters with high snowfall. The Kicking Horse River is the main east-west aligned watershed in Yoho National Park (YNP), which parallels the TCH and CPR mainline. Differing from the Bow River Valley, the Kicking Horse Valley is sparsely populated, as Field (300 residents) is the only townsite within the national park.

2.2. DNA collection and genotyping

We delineated our study area by creating a 30-km buffer in all directions around the TCH from the HWY 93 junction in Banff to the west boundary of Yoho; we used a 30-km buffer to distribute sampling effort ≥2.5 grid cells (12 × 12 km) away from the highway. We collected DNA samples using a systematic sampling design consistent with past research (Fisher et al., 2013) that consists of a hair trap created by wrapping barbed wire around a tree baited with a skinned beaver carcass. Beaver carcasses were obtained from registered fur trappers

selling their pelts and county agricultural staff charged with trapping nuisance beavers flooding farmlands. When the wolverine climbs the tree to investigate the bait, the hair from the passing animal gets caught on the barbed wire. To reduce the possibility of sampling parent-offspring pairs at the same hair traps together, we conducted all sampling in winter when newborn kits were still in the den and unavailable for capture; photographs from remote cameras confirmed that no young kits were present at hair traps. To distribute sampling effort, we overlaid a 12 × 12 km grid over the study area that straddled the TCH transportation corridor and placed one hair trap within each grid cell to maximize probability of detection (Fig. 1). However, to increase probability of detecting cross-highway movements an additional sampling site was placed in select grid cells near the TCH. We placed additional hair traps outside the grid in 2012 to increase sampling coverage for unique individuals. We deployed 75 hair traps and checked them for hair during 3, 30-day sessions between January and April from 2011 to 2013. We also collected hair and scat samples opportunistically on the way to hair traps by following putative wolverine tracks in snow (Ulizio et al., 2006).

We collected hair samples in pre-labeled paper envelopes and treated each barb as a discrete sample. We stored hair samples at room temperature on silica desiccant and analyzed them at the USDA Forest Service Conservation Genetics Lab (Missoula, Montana). The protocols used by the laboratory for DNA extraction and microsatellite analysis of noninvasive samples are described in McKelvey and Schwartz (2005) and Schwartz et al. (2006). We sub-sampled hair samples using a targeted approach to obtain ≥1 genotypes per wolverine visit based upon available funds and remote photographic evidence. To aid with maximizing the number of individual identifications with sub-sampling, we used remote cameras (Reconyx Hyperfire, Holmen, WI) to target hair samples for analysis from sites with the presence of ≥1 wolverine and determined the number of hair samples to analyze per site based on the number of wolverines and visits (i.e. more visits = more samples analyzed). We categorized hair samples by the number of hairs available. We chose the samples with the most hairs for first attempts at obtaining a genotype. If the initial hair sample selected from each site/session failed, we extracted DNA from several additional samples from the site/session (if available) until we obtained positive wolverine genotype/s or we exhausted samples.

Genotyping errors, such as allelic dropout or false alleles, are associated with low quantity DNA samples from hairs, scats, or other noninvasive genetic sampling. We used at least two independent approaches to catch and remove errors. First, DNA from hair samples were run and analyzed at least two times at each microsatellite locus (Taberlet et al., 1996). Allele scores were kept if there was concordance between runs. Samples that failed or were inconsistent were culled from the dataset. The dataset was screened using program DROPOUT 2.3.1.1 (McKelvey and Schwartz, 2005; following the protocol outlined in Schwartz et al., 2006), MICROCHECKER 2.2.3 (Van Oosterhout et al., 2004), and deviations due to Hardy-Weinberg (HW) proportions. Using our panel of 15 microsatellite loci (Schwartz et al., 2009), 49 unique

Table 1
DNA sample collection and genotyping success for wolverine hair samples collected[a] in national parks complex with barbed wire hair traps and snow tracking between 2010 and 2013.

Sampling period	# samples collected	# samples extracted	# genotypes	# individuals	# females	# males
Pilot year	43	27	11	4	2	2
Wtr 2010–2011	849	256	88	21	9	12
Wtr 2011–2012	295	54	22	9	7	6
Wtr 2012–2013	1176	365	132	24	11	13
Peripherals	114	21	13	5	1	4
Incidentals	94	79	48	17	7	10
Hawk Cr Killsite	15	10	0	0	0	0
Totals	**2586**	**812**	**314**	**49**	**20**	**29**

[a] 20 scats, 1 tissue sample, 1 urine sample, and 1 vomit sample were also analyzed.

individuals were identified from the final dataset (Table 1). We evaluated the genotypes by examining bimodality and difference in capture history tests in DROPOUT 2.3.1.1 (McKelvey and Schwartz, 2005). As an extra precaution, final genotypes were also checked for errors using the methods of Paetkau (2003). We determined sex of each individual using the Amelogenin sex marker (Pilgrim et al., 2005).

2.3. Gene flow and population structure

We examined genetic diversity and distribution across the transportation corridor using mitochondrial DNA haplotype analysis. We obtained mitochondrial DNA haplotypes for each unique wolverine individual by sequencing 367 bp of the control region and then examined the spatial distribution of haplotypes for males and females in relation to the highway.

We examined genetic structure across the transportation corridor using population-based and individual-based nuclear DNA analyses. We calculated the population-based metric for genetic differentiation, Fst, (Wright, 1965; Weir and Cockerham, 1984) between the north and south TCH populations using Genalex 6.503 (Peakall and Smouse, 2006). We examined isolation by distance patterns between wolverines using Mantel tests (Mantel, 1967) to calculate the individual pair-wise genetic distance (Smouse et al., 1986, Rousset, 1997). We determined wolverine locations from sampling points using GPS and recorded geographic locations in the UTM coordinate system. Some wolverines in the study were located more than once and their locations were averaged to obtain a detection center. We performed Mantel tests using Genalex 6.503 (Peakall and Smouse, 2006) and examined our data for a correlation between genetic and geographic distance.

We used Genalex 6.503 (Peakall and Smouse, 2006) to perform a principle components analysis (PCA) to visually examine our data for patterns of clustering related to the TCH. This method makes no assumptions about population origin. We then used three independent methods to identify male and female wolverines that dispersed across the TCH. We define migrants as any wolverine detected moving across the fracture zone using any one of 3 criteria (Proctor et al., 2012; Sawaya et al., 2014): 1) individuals detected on both sides of the highway with hair traps or opportunistic DNA sample collections, 2) individuals cross-assigned to population of origin on other side of highway using frequency-based assignment tests (Paetkau et al., 1995), and 3) wolverines cross-assigned to population of origin using Bayesian clustering to detect migrants in program STRUCTURE 2.3.4 (Pritchard et al., 2000; Kalinowski et al., 2007). We performed assignment tests in GenAlex 6.503 (Peakall and Smouse, 2006) using the leave-one-out setting and graphing individual assignment scores color-coded by population origin.

To evaluate whether the highway had an effect on gene flow and resulted in genetically isolating populations north and south of the highway we used an individual clustering method to examine population structure and identify recent genetic migrants (Pritchard et al., 2000; Kalinowski et al., 2007). We first used STRUCTURE 2.3.4 to identify the number of populations in our sample, using the admixture model with a burnin of 500,000 and 500,000 runs with 10 iterations for K = 1–5. The admixture model uses allele sharing to cluster individuals based on proportion of membership (q) using no a priori assumptions about population origin. We used STRUCTURE HARVESTER 0.6.94 (Earl and vonHoldt, 2012) to employ the Evanno method and extract delta K results to determine the most likely number of populations in our sample (Evanno et al., 2005). We then used STRUCTURE 2.3.4 (Pritchard et al., 2000, Kalinowski et al., 2007) to detect cross-highway migrants with a Bayesian clustering model assuming two populations (K = 2) and using knowledge of population origin (i.e. locations) and proportion of membership (q) scores.

To visualize genetic patterns on the landscape, we used Alleles In Space 1.0 (Miller, 2005) to produce landscape shape interpolation plots, three-dimensional graphs showing interpolated genetic and geographic distances from genotypes and UTM coordinates. We explored a range of uniformly spaced grid sizes overlaid on the entire sampled landscape and distance weights (w), settling on a 50×50 grid with $w = 3$ to maximize resolution of residual genetic distance heights. Finally, we examined the location of genetic discontinuities by overlaying geo-referenced genetic distance heights from Alleles In Space 1.0 (Miller, 2005) with landscape features in ArcGIS 10.0 Geographic Information System (GIS) software (ESRI, Redlands, California, USA).

3. Results

3.1. DNA collection and genotyping

Between February 2010 and May 2013, we collected 2563 hair samples, 20 scat samples, 1 tissue sample, 1 urine sample, and 1 vomit sample (Table 1). By far, the majority of samples were collected from hair traps in 2011 and 2013, the years of intensive sampling effort for estimating density. To operate within our budget constraints, we subselected the best samples. We analyzed 812 of the 2586 samples collected (31%) and 314 of those 812 samples (39%) produced multi-locus genotypes (i.e. individual IDs). We had adequate power to differentiate individuals, Power of Identity (P_{ID} = 5.1E-10), and genetic diversity to examine population structure with our suite of 15 loci (Table S1). Across all methods and years, we detected a total of 49 unique individuals (20 females, 29 males). Of the 75 hair trap locations sampled, 60 of them (80%) produced at least one individual ID. More than one individual wolverine was detected at 37 of 75 sites (52%), with 3 hair traps detecting 5 individuals per site over the 3-year study.

3.2. Gene flow and population structure

Using mitochondrial sequencing, we identified four different haplotypes (Wilson A, Wilson H, Wilson I, Cegelski L) within the population of 49 wolverines. We produced haplotype results for 27 of the 29 males (poor sequence = 2, Cegelski L = 14, Wilson A = 9, Wilson H = 2, Wilson I = 2) and 20 of 20 females (Cegelski L = 10, Wilson A = 10). All of these mitochondrial haplotypes had been documented in past studies of populations to the north, south or west of our study area (McKelvey et al., 2014). Three of the four haplotypes (Cegelski L, Wilson A, Wilson H) are common and were previously identified within Alberta, although one haplotype (Wilson I) was only identified in two different males and had never been documented in the Canadian Rocky Mountains before. An examination of the individual spatial locations of haplotypes did not indicate an effect of the TCH on mitochondrial DNA distribution for males; however, it revealed low haplotype diversity and limited distribution for females with only two haplotypes, Cegelski L and Wilson A, present in the population (Fig. 2). We found Wilson A primarily to the south of the TCH (1 female was detected north of highway with this haplotype) and we found females with Cegelski L only to the north of the highway.

We examined population structure and fine-scale movements using 225 unique locations (125 male, 100 female) in space and time (Fig. S1). We detected males less frequently than females, averaging 4.3 detections per male and 5.0 detections per female. Male wolverines were detected throughout the sampling area, whereas female detections were more concentrated towards the center of the mountain park complex (Fig. S1). Of the 49 wolverines, 29 were detected on the north side of the highway (17 males, 12 females) and 28 were detected on the south side of the highway (16 males, 12 females); some individuals (4 males, 4 females) were detected on both sides of the highway so numbers don't sum to 49.

The results of our Mantel tests for all 49 individuals (Rxy = 0.344, P = 0.01) indicated a correlation between geographic distance and genetic distance in our dataset. This isolation by distance pattern was slightly stronger for females (Rxy = 0.383, P = 0.01) than males (Rxy = 0.342, P = 0.01). We calculated Fst for the total population of
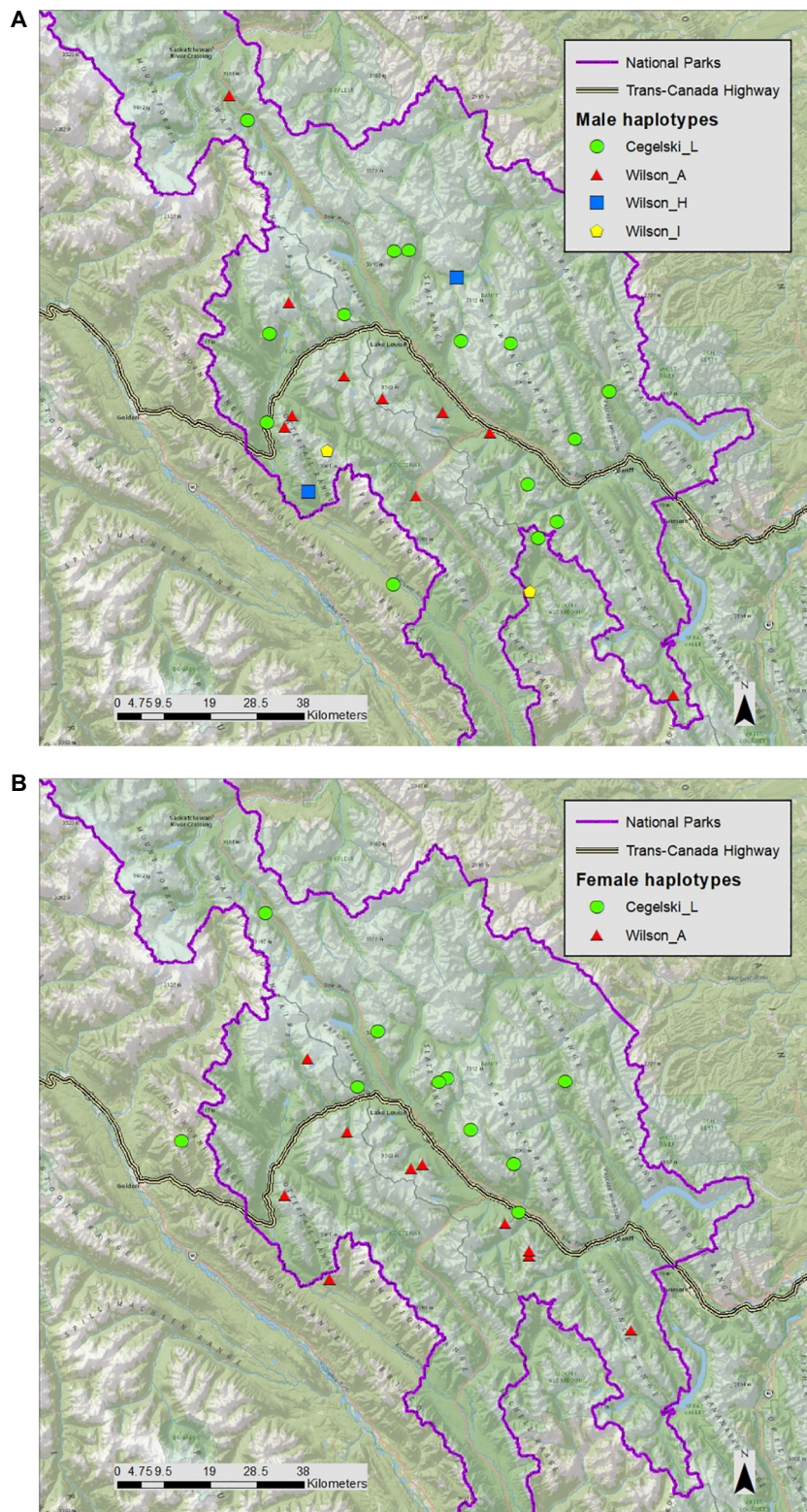
Fig. 2. Individual detection centers of A) 29 male and B) 20 female wolverines color-coded by their mtDNA haplotype to examine the effect of the Trans-Canada Highway on genetic differentiation in the national parks complex between 2010 and 2013. The detection center for one female was taken from an average of 3 locations on the south side of TCH; 1 outlier location from this female north of TCH was dropped for visualization purposes as it placed this individual's location north of highway when it was assigned to the south.
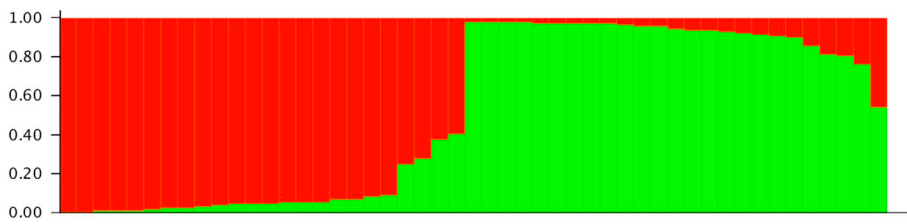
Fig. 3. Bar plot from STRUCTURE (Pritchard et al., 2000; Kalinowski et al., 2007) showing proportion of membership (*q*) for 49 wolverines (29 males, 20 females) sorted by *q* score when K = 2; STRUCTURE identified 2 population clusters straddling the Trans-Canada Highway to the north (green) and south (red) in the national parks complex. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

49 individuals (Fst = 0.035) and for each sex separately (male Fst = 0.029, female Fst = 0.084).

Results from implementing the Evanno method (Evanno et al., 2005) to evaluate the delta K output from the admixture model in program STRUCTURE (Pritchard et al., 2000; Kalinowski et al., 2007) indicated that there were two populations of wolverines (K = 2) in the parks complex (Fig. S2). A spatial examination of the population clusters from STRUCTURE indicates an effect of the TCH on genetic isolation. When the individual proportion of population membership scores (*q*) from the pooled analysis were examined (Fig. 3), clustering was associated with the TCH for females, but unrelated to the highway for males.

Our PCA plot with all 49 individuals (29 males, 20 females) showed some genetic clustering, but not in obvious relation to the highway; however, PCA plots broken out by sex revealed no population structuring of males (Fig. 4A) and clear structuring of females in relation to the highway along the primary axis that explains most genetic variation (Fig. 4B).

We detected 8 wolverines (4 males, 4 females) that crossed the TCH with DNA detections that spanned the roadway. Self-assignment probabilities (total = 0.80, males = 0.62, females = 0.95) and population

assignment graphs from sex-specific frequency-based assignment tests indicated stronger clustering of females (Fig. 5B) than males (Fig. 5A). One female and nine males were identified as cross-population migrants from frequency-based population assignments tests (north = 4, south = 6) in GenAlex (Peakall and Smouse, 2006). No females and two males (north = 1, south = 1) were identified as cross-population migrants from Bayesian population assignments tests in STRUCTURE (Pritchard et al., 2000); these 2 males were also identified as migrants with frequency-based population assignment tests.

The landscape shape interpolation plots from Alleles In Space (Miller, 2005) showed a similar pattern as other methods of genetic structure in females but not males (Fig. S3); however, these plots also revealed a pattern of decreasing genetic diversity from northwest to southeast for both sexes, particularly females. A closer examination of the landscape shape interpolation output for females in GIS suggests a strong correlation between the location of the TCH and high genetic distances (i.e. genetic discontinuity); genetic distances were greatest between individuals on opposite sides of the TCH and other roadways (Fig. 6).
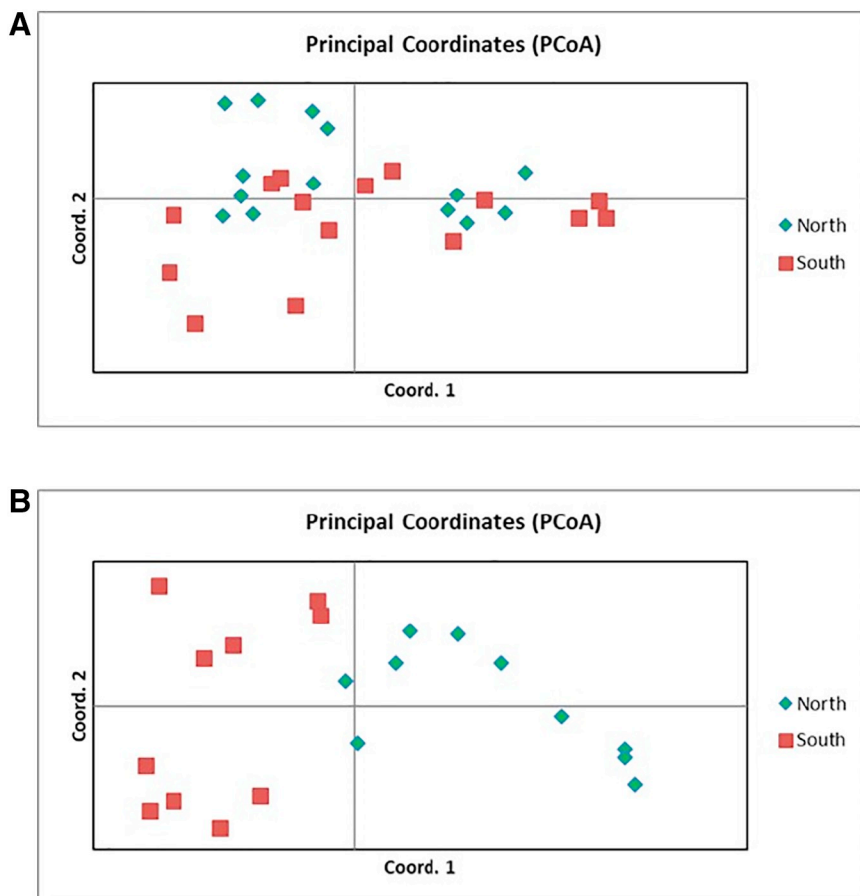

Fig. 4. Graphical plots showing Principle Coordinates Analysis [A) 29 male and B) 20 female] for wolverines detected to the north (green) or south (red) of the Trans-Canada Highway in the national parks complex between 2010 and 2013. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
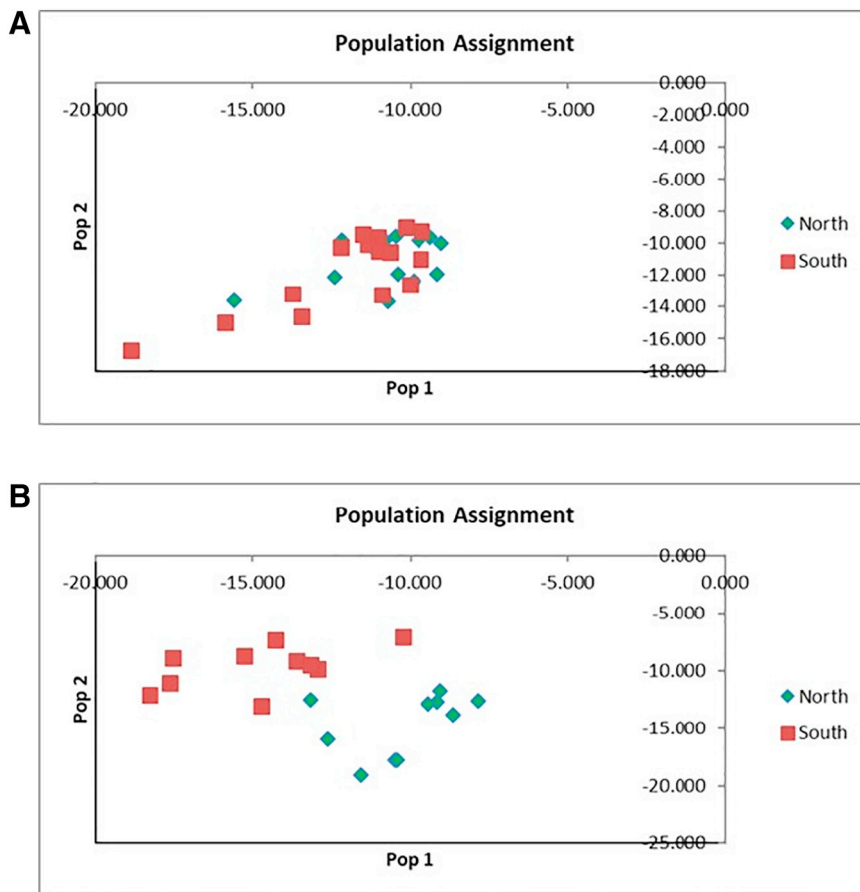
A

B

Fig. 5. Graphical plots showing population assignment tests [A) 29 male and B) 20 female] for wolverines detected to the north (green) or south (red) of the Trans-Canada Highway in the national parks complex between 2010 and 2013. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4. Discussion

Wolverines are well-adapted to snow, exceptionally vagile, and vulnerable to human disturbance; therefore, they are often used as biodiversity indicators for northern montane ecosystems (Clevenger et al., 2016). In the face of climate change, it's increasingly important to understand what landscape features influence movements and population structure of snow-dependent species so that mitigation strategies can be optimized to ensure their survival (McKelvey et al., 2011). With little empirical evidence, the park complex is already considered a haven for wolverine populations in the Central Canadian Rocky Mountains (Fisher et al., 2013) and now we have produced the first evidence-based insight into the effects of transportation infrastructure on population structure in this ecologically important area. Here, we present the first fine-scale examination of wolverine genetic structure and provide results that suggest the transportation corridor has fragmented this protected population by restricted female movements leading to sex-biased dispersal and gene flow.

4.1. DNA collection and genotyping

We were able to detect wolverines and obtain useable DNA using our noninvasive genetic sampling methods (Table 1). We had reasonable sampling coverage on both sides of the TCH (Fig. 1) so we were able to get relatively equal samples sizes of males and females to the north and south of the highway. Our high success at hair traps allowed us to obtain genetic information from an adequate sample size of wolverines to examine genetic structure. Female detections were more concentrated towards the center of the mountain parks, whereas male detections were more dispersed throughout the study area (Fig. S1). However, the greater number of detections and more widespread distribution were not surprising considering the well-documented mobility

of the male wolverine (Vangen et al., 2001).

4.2. Gene flow and population structure

Using a combination of mitochondrial and nuclear DNA measures of genetic structure with and without prior assumptions of population origin, we examined the hypothesis that the TCH affected wolverine population structure in the parks complex. We detected ample male movement across the TCH and lack of genetic differentiation to infer that the highway has not genetically isolated male wolverines, whereas we found that females were highly structured by the TCH. Although we also found direct evidence that at least four females made it safely across the highway, possibly at one of the wildlife crossing structures, movement across highways didn't translate to the flow of genes (Riley et al., 2006). Our results show that male and female wolverines occur throughout the parks complex, but the sexes are affected very differently by transportation infrastructure.

Our sampling design allowed us to collect enough evidence to examine our hypothesis and overcome some of the limitations of drawing conclusions from genetic data. Time lags can make barriers difficult to detect with genetic methods (Landguth et al., 2010), but the TCH has been the busiest East-West transportation corridor in Canada since the 1950s making it a barrier to wildlife movement in the parks complex for many generations (Sawaya et al., 2014). We had good sampling coverage and representation of individuals both north and south of the highway and our sample sizes and methods had adequate power to detect structure at such a fine spatial scale even in a species with home ranges that can exceed the size of many protected areas. Results of examining genetic structure were surprisingly congruent across mitochondrial haplotype analysis, population-based microsatellite analysis (i.e. Fst) and individual-based microsatellite analyses (PCA, assignment tests) which allows for powerful inference that there was a
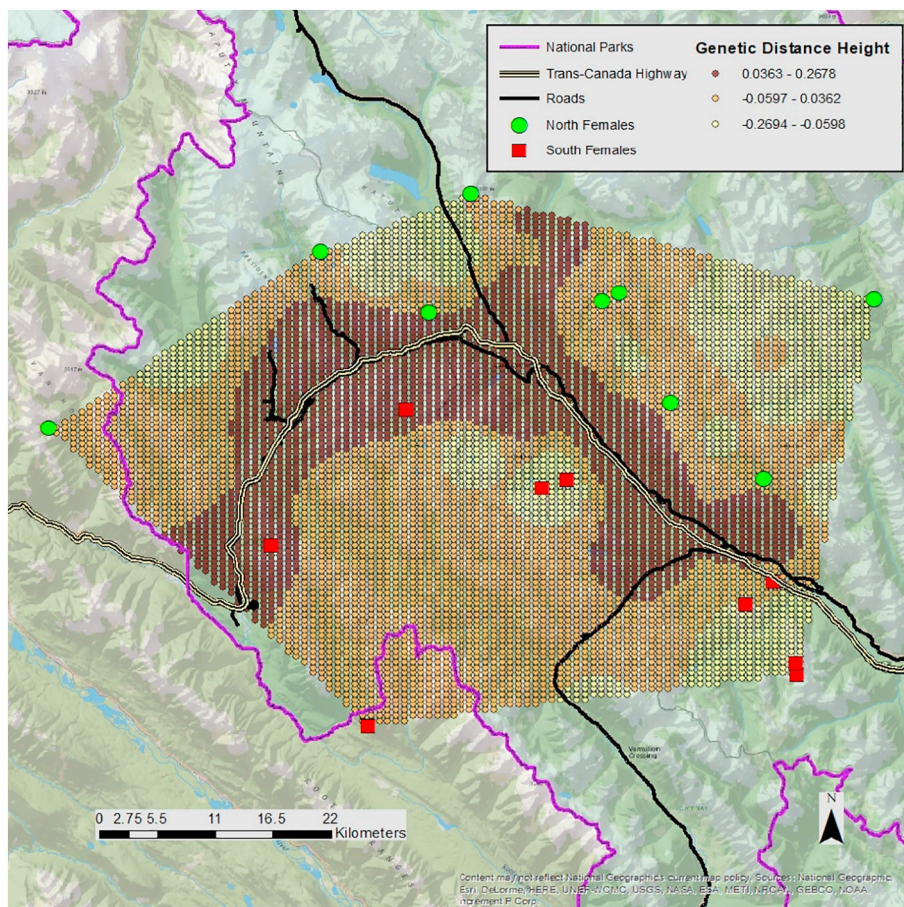
Fig. 6. Interindividual genetic distance heights from a landscape shape interpolation (100 × 100 grid, w = 3) by program Alleles in Space (Miller, 2005) displayed with individual detection centers of 18 female wolverines color-coded by their location either north (green) or south (red) of the highway to examine the effect of the Trans-Canada Highway on genetic differentiation in the national parks complex; only the area within minimum polygon of detection centers are displayed, not the entire 100 × 100 grid. Two individuals with outlying average detection centers were removed for this analysis. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

difference in how the highway affects male and female wolverines. The results of the Mantel tests to look for isolation-by-distance patterns showed that geographic distance accounted for only a little of the genetic variation observed. STRUCTURE indicated the protected population was split into two populations north and south of the TCH, suggesting a strong effect of the highway on genetic isolation. Our landscape shape interpolation analysis confirmed the location of this genetic discontinuity on the landscape and its close association with the transportation corridor (Fig. 6). The weight of multiple lines of evidence suggests that there is an effect of the highway on wolverine population structure in the parks complex and that it's greater for females than for males, though individual-based methods are extremely sensitive so the relative magnitude of the transportation effect is unknown without examining data from surrounding areas for context.

Multiple linear barriers to movement in addition to the TCH exist in the Bow Valley, including a secondary highway, a railway, and a river; this overlap makes it difficult to separate the effects of the different landscape features on gene flow. A full compliment of carnivores (e.g., *Ursus arctos*, *Ursus americanus*, *Puma concolor*) in the low broad valley could further influence movements as wolverines encounter competitors or avoid interspecific interactions. The Bow River is likely a natural barrier to dispersal, though our detection data show that wolverines can easily get across it. We suspect that the TCH and adjacent transportation features exacerbate strong natural tendencies for home range distribution and philopatry in this major river valley at the southern extent of wolverine range where population density and genetic diversity are relatively low.

Our results showing an effect of transportation systems on female dispersal in a southern wolverine population are strongly supported by previous research. The pattern of decreasing genetic diversity from north to south revealed by our landscape shape interpolation plots has

also been found in mtDNA haplotypes (McKelvey et al., 2014). Kyle and Strobeck (2001, 2002) used 12 microsatellites to examine population structure of wolverines in North America and they also found structure to be greatest at the southern extent of their study area, which is more fragmented. Cegelski et al. (2003) found significant structuring in wolverine populations of Montana using 10 microsatellites and Cegelski et al. (2006) found significant structuring in wolverine populations in Alberta, British Columbia, Montana, Idaho, and Wyoming using mtDNA and the same suite of microsatellites. Chappell et al. (2004) used 9 microsatellites and mtDNA to examine population structure of wolverines in northern Canada and found little structure but some evidence with mtDNA for female philopatry driving dispersal patterns; their results emphasize the importance of using mtDNA and nDNA methods together. Tomasik and Cook (2005) used mtDNA to examine phylogeography in northwest North America and determined that successful female dispersal between populations was limited. Vangen et al. (2001) found sex-biased dispersal in wolverines using radiotelemetry and home range analysis. A recent study by Scrafford et al. (2018) demonstrated that roads restrict wolverine movements (i.e. avoidance) and decrease habitat quality, particularly roads with higher traffic volumes. The Wilson A haplotype was commonly found in the United States; whereas the Cegelski L haplotype was more localized in British Columbia (Cegelski et al., 2006; McKelvey et al., 2014). The striking haplotype pattern that we observed with Wilson A to south and Cegelski L to north fits with past research indicating southward gene flow may be restricted in the Rocky Mountains (Schwartz et al., 2009) and suggests that the TCH could be a continental barrier to female wolverine movement with potential implications for wolverine conservation in southern Canada and the United States.

Wildlife crossing structures can help to restore demographic connectivity (Sawaya et al., 2013) and genetic connectivity (Sawaya et al.,

2014; Soanes et al., 2017) across busy roadways. After 17 years of monitoring two dozen crossing structures only 10 wolverine crossings were detected (Clevenger, 2013). These 24 crossing structures were not located in high elevation wolverine habitat. However, newly built crossing structures in BNP and YNP are situated at the Continental Divide and can play an important role in keeping wolverine populations connected and reducing risks of road mortality. Although some species may take years to learn to use crossing structures to traverse the highway (Sawaya et al., 2013), evidence suggests that female wolverines may be starting to use wildlife crossings to cross the TCH. For example, a female wolverine (F15) may have been the wolverine photo-detected traveling northward at Castle Underpass on 16 February 2011 (Clevenger, 2013) as she was detected by hair sampling just two days prior at a nearby hair trap south of the underpass, while on 25 April 2011, she was detected at a hair trap north of the TCH.

In 2017, Parks Canada began expanding and mitigating the TCH through YNP, therefore the entire stretch of highway through the parks complex will eventually have wildlife fencing and crossing structures (Clevenger et al., 2016). Interestingly, snow tracking surveys (Austin, 1998) and connectivity models (Clevenger et al., 2016) used to guide crossing structure placement for highway mitigation agreed that Kicking Horse Pass is a regionally important north-south and cross-highway corridor for wolverines. This was the one area we found female haplotype dispersal across the TCH (Fig. 2) and where 2 of 4 females may have traversed the road based on cross-highway DNA detections. The lone female cross-highway migrant we identified by nDNA assignment tests was also detected along this high elevation wildlife corridor, located on the spine of the continental divide near where a wildlife overpass and series of underpasses were recently built (Clevenger et al., 2016).

Future analyses that involve a larger transborder geographic extent and include more individuals from nearby regions in Alberta, British Colombia, and the United States would provide better context for the effects of transportation systems and other anthropogenic activities on wolverine population structure and gene flow in this region. A wider meta-analysis could also help to identify key connections for maintaining gene flow at the southern end of wolverine range for transportation departments and wildlife managers to proactively secure cross-highway linkages and adjacent habitat. Wolverine populations near busy roads such as Highway 3 in Canada and Interstate 90 in the United States are not in protected areas; therefore, they may have lower population density and even less female dispersal than the parks complex where there is no harvest and considerable investments have been made in connectivity restoration measures (i.e. wildlife fencing, crossing structures).

Demographic fragmentation from restricted female movements and sex-biased dispersal across highways can reduce population viability (Proctor et al., 2005). The persistence of many wildlife populations in a changing world will depend on climate connectivity and the ability to identify landscape features that impede dispersal to new habitats with similar environmental conditions (Carroll et al., 2018). Only one-migrant-per-generation is needed to maintain genetic connectivity (Mills and Allendorf, 1996) and that migration rate can easily be achieved with males alone; however, wolverine and other carnivore metapopulations depend on female movements for population re-colonization and range expansion (Inman et al., 2013; Proctor et al., 2005). Although areas dedicated to nature protection are needed to adequately conserve biodiversity (Wilson, 2016), our results demonstrate that protected areas can be susceptible to human disturbance and in some cases the front line for measures to mitigate fragmentation effects. Our results are first to measure the impacts of a major transportation corridor (> 2 lanes of traffic) on wolverine gene flow. In short, we detected a strong sex-biased effect of a major transcontinental highway on wolverine gene flow and population structure, highlighting the urgent need to maintain demographic and genetic connectivity across an expanding global road network with transportation planning, highway

mitigation measures, and wildlife corridor protection.

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