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Genetic connectivity for two bear species at wildlife crossing structures in Banff National Park

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Roads can fragment and isolate wildlife populations, which will eventually decrease genetic diversity within populations. Wildlife crossing structures may counteract these impacts, but most crossings are relatively new, and there is little evidence that they facilitate gene flow. We conducted a three-year research project in Banff National Park, Alberta, to evaluate the effectiveness of wildlife crossings to provide genetic connectivity. Our main objective was to determine how the Trans-Canada Highway and crossing structures along it affect gene flow in grizzly (*Ursus arctos*) and black bears (*Ursus americanus*). We compared genetic data generated from wildlife crossings with data collected from greater bear populations. We detected a genetic discontinuity at the highway in grizzly bears but not in black bears. We assigned grizzly bears that used crossings to populations north and south of the highway, providing evidence of bidirectional gene flow and genetic admixture. Parentage tests showed that 47% of black bears and 27% of grizzly bears that used crossings successfully bred, including multiple males and females of both species. Differentiating between dispersal and gene flow is difficult, but we documented gene flow by showing migration, reproduction and genetic admixture. We conclude that wildlife crossings allow sufficient gene flow to prevent genetic isolation.

1. Introduction

Roads connect human populations, but fragment wildlife populations [1]. Busy roads such as highways can act as barriers to animal movement through direct mortality of dispersers or behavioural avoidance [2,3]. This can reduce access to habitat and mates [1], and thereby reduce survival and breeding opportunities [1]. In addition, when roads prevent migration between populations, genetic diversity may be reduced and this will happen fastest in smaller, more isolated populations [4,5]. Climate change may be particularly harmful to fragmented populations [6], because animals will be less able to move in response to changing environmental conditions, and because barriers may negatively impact the evolutionary potential of populations, i.e. their ability to genetically adapt to changing environmental conditions. However, the deleterious effects of roads may be minimized by wildlife crossing structures that may allow animals to change movement patterns (i.e. latitude, elevation) in response to rising temperatures and allow the flow of genes so that populations can adapt and evolve to rapidly changing environments.

Fragmentation divides large populations into smaller populations that are more prone to extinction owing to demographic and environmental stochasticity [7]. Small, isolated populations also have less genetic diversity and are more prone to extinction owing to inbreeding depression than larger, connected populations [8–10]. Genetic variation within a population can be lost because of genetic drift following population fragmentation [11]. Small populations that are isolated are more likely to experience inbreeding, because the probability of identity by descent (i.e. identical copies of the same allele from a common ancestor) increases faster than in larger populations. Small populations are also more susceptible to disease epidemics and less able to cope with extreme environmental conditions owing to a lack of genetic variation. Migration plays

an important role in maintaining genetic diversity along with mutation, selection and genetic drift [12], particularly within fragmented populations [13].

Landscape genetics studies have revealed that roads can impact the genetic structure of wildlife populations in a number of ways [14,15]. Several studies have looked at gene flow across major roadways and found that reduction in migration rates led to population differentiation [16–18]. Research on beetles has shown that road-caused fragmentation can lead to loss of genetic variability [19]. Roads can even act as partial filters to migration, resulting in sex-biased dispersal or reduced gene flow. For example, Proctor *et al.* [16] found substantial male-biased dispersal of grizzly bears (*Ursus arctos*) across a major highway in Southern Canada using population assignment tests. Riley *et al.* [18] examined dispersal and genetic differentiation in coyote (*Canis latrans*) and bobcat (*Lynx rufus*) populations separated by a major California freeway. Using radio-telemetry, they found that both species frequently crossed the highway, but coalescent modelling revealed that the level of gene flow was much less than movement rates alone would suggest, leading the authors to conclude that the discrepancy was owing to the lack of breeding opportunities for typically young dispersers encountering occupied adult home ranges piled up on the other side. As stated in a related article in *Nature*, ‘if the discrepancy between dispersal and gene flow that Riley *et al.* have found is widespread, as seems likely, the effectiveness of highway dispersal corridors needs to be tested to see how well they meet their goal of ensuring genetic connections among populations’ ([20], p. 876).

Wildlife crossing structures are designed to serve as small-scale corridors to increase permeability across highways, but few studies have examined their effectiveness beyond simply documenting animal movements. Often, highways have been fenced for the purpose of decreasing wildlife–vehicle collisions and research has shown that exclusion fencing reduces highway accidents and decreases wildlife mortality [21,22]. Numerous species use a variety of crossing structure types (e.g. overpasses, culverts) to traverse highways [23–26]. Most studies that have assessed the effectiveness of crossing structures can only document the species and the direction of movement with track pads or remote cameras. These methods are relatively inexpensive, but cannot accurately identify individuals, determine genders nor infer relationships among crossers of many species [27]. Recent studies have demonstrated that crossing structures provide demographic connectivity [28]; however, their ability to provide genetic connectivity and counteract the effects of genetic isolation remains almost completely unknown [29,30].

Wildlife crossing structures in Banff National Park (BNP), Alberta, Canada are a unique and well-known study system to evaluate how roads and mitigation measures affect gene flow and population structure. One of the world’s most developed and well-studied highway mitigation systems is located within the heart of BNP. With more than four million visitors per year, BNP is one of the most heavily visited national parks in North America and this high level of human visitation and accompanying traffic act as major stressors on the ecosystem [31]. The Trans-Canada Highway (TCH) is one of Canada’s busiest highways and serves as the nation’s primary East–West transportation route. The TCH has been a barrier to wildlife movement since its completion in 1962, but two overpasses and 23 underpasses were built in the 1980s and the 1990s to reduce

wildlife–vehicle collisions and allow wildlife movement across the TCH [21,23,24].

Bears are good study species to investigate the effectiveness of crossing structures, because they are ecologically important and sensitive to fragmentation. Recent evidence indicates that the loss of carnivores can trigger far-reaching trophic cascades and many of these species are experiencing rapid global declines owing to human causes [32]. Wide-ranging, large-bodied carnivores that inhabit BNP, such as grizzly and black bears (*Ursus americanus*), are easily susceptible to road-caused fragmentation owing to their low densities and reproductive rates combined with large home range requirements [33]. Numerous studies have shown that roads can significantly reduce bear movements [16,34] or influence where bears cross busy highways [35]. Grizzly bears avoid roads with high traffic volumes [36,37], whereas black bears are more tolerant of human disturbance and willing to cross busy highways [38]. Research on grizzlies using radio and global positioning system (GPS) collars has demonstrated that higher traffic volumes are correlated with stronger barrier effects [37,39]. Despite the relatively long generation time in ursids, genetic discontinuities have been detected in a number of grizzly bear populations fragmented by major highways throughout western North America [16,40,41].

In April 2006, we initiated a three-year investigation to evaluate the effectiveness of wildlife crossing structures to allow genetic connectivity for bears in BNP. We sampled crossing structures using a novel hair-snagging method [27] and sampled the surrounding grizzly and black bear populations using a combination of hair traps and bear rubs [42]. Our main objective was to determine how the TCH and the crossing structures along it affect gene flow and population structure in grizzly and black bears in BNP. This is the first study we are aware of to quantify genetic connectivity by examining all three major components of gene flow: migration, successful reproduction and genetic admixture.

2. Material and methods

(a) Study area

Our 2246 km² study area was located in the Bow Valley of BNP in Alberta, Canada (figure 1). BNP was established in 1885 and is the flagship of Canada’s extensive national park system and the centrepiece of UNESCO’s Rocky Mountain World Heritage Site. The Bow Valley is a human-dominated landscape with the TCH, the Banff Townsite, a golf course, three ski areas, a railway and a secondary highway. Between 1982 and 1997, 45 km of the TCH extending west from the eastern park boundary were widened from two to four lanes for public safety reasons and a total of 25 crossing structures—two overpasses and 23 underpasses—were constructed, along with 2.4 m high fencing to reduce wildlife–vehicle collisions and facilitate animal movement across the four-lane section of TCH [43]. Detailed ecological descriptions of the study area can be found in Holroyd & Van Tighem [44] and Holland & Coen [45].

(b) DNA collection

We studied bear crossings at 20 of 25 crossing structures along the TCH (figure 1). Five were not included in this study because they are near Banff Townsite, receive high human use, and are rarely used by bears [24]. We set up our hair-sampling system by stretching two parallel lengths of barbed wire between metal stakes, at 30 and 70 cm height, perpendicular

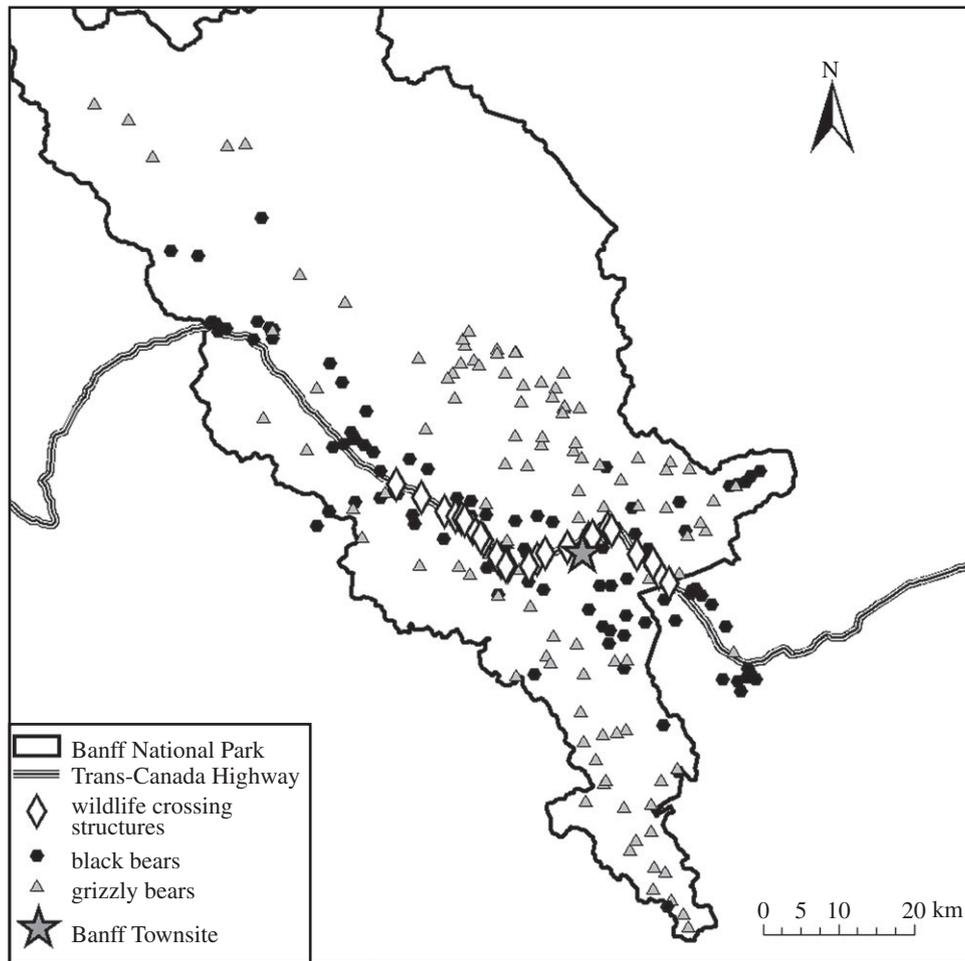


Figure 1. Detection centres for individual black and grizzly bears detected using non-invasive genetic sampling methods in the Bow Valley of BNP, Alberta, Canada between April and October of 2006–2008.

to the line of movement of passing bears [27]. We conducted hair sampling at crossing structures between 15 May and 18 October 2006, 22 April and 29 October 2007, and 22 April and 18 October 2008 (figure 1). Detailed descriptions of crossing structure sampling methods and results can be found in Sawaya *et al.* [28].

We collected hair samples from black and grizzly bear populations in the Bow Valley of BNP using hair traps [46] and bear rubs [40] between May and October in 2006, 2007 and 2008. We monitored 210 hair traps in 2006 and 2008, 330 bear rubs in 2006 and 497 bear rubs in 2007 and 2008 (electronic supplementary material, figure S2). We also obtained additional DNA samples (tissue or hair) from bear management actions (i.e. capture, mortalities). Detailed descriptions of population sampling methods and results can be found in Sawaya *et al.* [42]. Methods and results presented here include all data from Sawaya *et al.* [42] along with data from 176 additional bear rubs.

(c) Genetic analysis

Detailed descriptions of genetic analysis methods and results can be found in Sawaya *et al.* [42]. We used protocols for DNA extraction and microsatellite analysis of non-invasive samples as described by Paetkau [47]. We used seven microsatellite loci for individual identification: G10J, G1A, G10B, G1D, G10H, G10M and G10P [48]. Once we had complete multi-locus genotypes, we analysed one sample per individual for sex using amelogenin [49,50]. We extended genotypes to 20 loci using the following loci: G10C, G10L, CXX110, CXX20, Mu50, Mu51, Mu59, G10U, Mu23, G10X, MSUT-2, REN145PO7 and CPH9 [48,51–53]. We calculated marker variability using data from 113 grizzlies and

101 black bears. We used GENALEX [54] to calculate expected allele frequencies, expected and observed heterozygosity (H_e , H_o), the probability of identity (P_{ID}) that two randomly drawn individuals would share the same multi-locus genotype, and the probability that full siblings would have identical multi-locus genotypes (P_{SIB}). We used GENALEX to evaluate our loci for conformance to Hardy–Weinberg proportions by testing for heterozygote deficiency and linkage disequilibrium.

(d) Genetic connectivity

We used four methods for identifying bears that crossed the TCH. We defined a migrant as any bear that we detected moving across the highway [41] using four criteria: (i) bear detected at crossing structures, (ii) bear detected on both sides of the highway using non-invasive genetic sampling (NGS) methods (i.e. bear rubs, hair traps), (iii) bear cross-assigned to population of origin using frequency-based assignment tests and (iv) bear cross-assigned to population of origin using Bayesian assignment tests. For bears that were detected on both sides of the TCH with NGS, we assigned bears to the north or the south populations based on their last known location in order to maximize our ability to detect migration events.

Geography can restrict dispersal and gene flow, which can lead to spatial patterns of genetic differentiation known as isolation by distance (IBD) [55]; therefore, we examined IBD patterns in bears using Mantel tests to determine the correlation between geographical distances and genetic distances [56,57]. Geographical distance was calculated as the Euclidean distance (in metres) between pairs of individual locations. We determined bear locations from sampling points using GPS and recorded locations in the Universal

Transverse Mercator coordinate system. Many of the bears in this study were located more than once. For these bears, we determined geographical coordinates by calculating centroids for Minimum Convex Polygons for individuals with three or more locations. Otherwise, we used one point or averaged two locations to represent an individual's detection centre on the landscape.

We used Alleles In Space [58] to calculate Nei's [59] genetic distances and to perform Mantel tests. For partial Mantel tests, we used GENEPOP [60,61] to calculate the individual pair-wise genetic distance of Rousset's \hat{a} [62]. We employed a partial Mantel test in the R package ECODIST v. 1.2.3 [63] to determine whether the TCH has a significant effect on genetic distance by assigning a dummy variable to pairs of bears depending on whether they were detected on the same side (=0) or not (=1).

We calculated population differentiation (F_{ST}) between north and south populations using GENALEX [54]. We used individual-based methods to examine population genetic structure and identify recent migrants. We first used multidimensional factorial correspondence analysis (FCA) in GENETIX [64] to examine genetic structure in black and grizzly bear populations. FCA uses allele sharing to cluster individuals in a multidimensional hyper-space based on allele sharing similarities using no *a priori* assumptions about population origin [41]. To identify first-generation migrants, we used Paetkau *et al.* [48] frequency-based assignment tests in GENECLASS2 [65]. We detected GENECLASS2 migrants using cross-population assignments (i.e. assignment to population other than origin) with an assignment threshold of 0.05. We used STRUCTURE to determine the number of populations present in the study area, and to identify admixed individuals or interpopulation migrants [66]. We performed ten independent repetitions of $K = 1-5$ using a 500 000 burn-in period and 500 000 data collection runs. We determined the number of populations by testing different values of K from 1 to 5 and using the *ad hoc* approach described in Evanno *et al.* [67]. We selected the optimal value of K using the posterior probability of the data for K ($\ln P(K)$) and the second-order rate of change of log probability of the data between successive values of K (ΔK) [67]. We identified migrants based on the percentage of membership (q) attributed to a population other than the one in which it was detected (i.e. cross-population assignments) [41] and assuming $K = 2$. We used a threshold value of $q \geq 0.9$ for cross-population assignments of all bears and $q \geq 0.6$ for population assignments of bears that used crossings. We only accepted population and cross-population assignments if results were repeatable across independent runs.

We used PARENTE [68] to conduct parentage analysis using multi-locus genotypes and confirmed the results with CERVUS [69,70]. We identified maternal and paternal relationships simultaneously using the probability of successfully allocating an individual offspring to its parents [68]. Our confidence in relationship assignments was based on high parentage probabilities ($p > 0.95$), few mismatches (less than or equal to 1 MM) and compatibility with other triads (i.e. twins and triplets). We used a sampling rate of 0.7, although altering the rate had no meaningful impact on results. Identifying both parents simultaneously provides more accurate parentage assignments and allows directionality of relationships to be inferred, which would not be possible with hair samples alone (i.e. unknown ages) [68]. We used additional data (e.g. mortalities, remote photos) to confirm age classes and relationships whenever possible. We examined relative reproductive success and parental-offspring relationships of bears that used crossings.

3. Results

(a) DNA collection

We collected 1317 hair samples from crossing structures. We collected 2009 hair samples from hair traps. We collected 6543 hair samples from bear rub trees.

(b) Genetic analysis

Marker power was high for grizzly and black bears using 20 loci (electronic supplementary material, table S1). Heterozygote deficiency and linkage disequilibrium tests indicated that all loci conformed to Hardy-Weinberg proportions. P_{ID} was 5.0×10^{-18} for grizzly bears and 7.0×10^{-24} for black bears. P_{SIB} was 1.2×10^{-7} for grizzly bears and 3.5×10^{-9} for black bears. Mean H_o across all 20 loci was higher for black bears (0.79) than for grizzly bears (0.66). The mean number of alleles per locus was also higher for black bears (9.4) than for grizzlies (6.9).

We identified a total of 113 unique grizzly bears and 101 black bears from multi-locus genotypes across all methods and years. Genetic analysis of samples collected at crossings identified 15 grizzlies (eight males and seven females) and 17 black bears (nine males and eight females) using underpasses and overpasses to traverse the TCH (table 1).

(c) Genetic connectivity

We observed IBD patterns for black and grizzly bears. Mantel test results confirmed a positive correlation for black bears ($r = 0.202$, p -value = 0.001) and grizzlies ($r = 0.104$, p -value = 0.002). The correlation between genetic distance and geographical distance was greater for female ($r = 0.298$, p -value = 0.001) than for male black bears ($r = 0.137$, p -value = 0.007). There was significant correlation for female grizzly bears ($r = 0.246$, p -value = 0.001), but no statistically significant correlation for male grizzly bears ($r = 0.004$, p -value = 0.444). The partial Mantel test showed an effect of the highway on genetic distance for grizzly bears ($r = 0.098$, p -value < 0.0001) but not for black bears ($r = -0.008$, p -value = 0.725). When males and females were analysed separately, the effect of the highway on genetic distance was not detected in female ($r = 0.014$, p -value = 0.265) or male black bears ($r = -0.024$, p -value = 0.826) and was stronger for female ($r = 0.127$, p -value < 0.0001) than for male grizzly bears ($r = 0.068$, p -value = 0.008) (electronic supplementary material, figure S2).

We found more genetic differentiation for grizzly bears than for black bears across the TCH. F_{ST} was low for both species, but higher for grizzly bears ($F_{ST} = 0.02$) than for black bears ($F_{ST} = 0.007$). Tests for genic differentiation in GenePop [60,61] indicated that F_{ST} was significantly different from 0 for black (p -value = 0.009) and grizzly bears (p -value = 0.012). Visual inspection of FCA plots also indicated little genetic structuring in black bears (figure 2*a*), but revealed a genetic discontinuity for grizzly bears across the TCH with clustering of individuals from the north and south mostly separated across the primary multidimensional axis (figure 2*b*). GENECLASS2 identified 45 first generation black bear migrants out of 101 bears, a self-assignment probability of 55.4% that provides further evidence of little genetic structure in black bears. GENECLASS2 identified 12 grizzly bear migrants (two females and 10 males) out of 113 bears, a self-assignment probability of 89.4% (table 1). As there was little evidence for genetic differentiation in black bears, and because the Evanno method cannot find the best K if $K = 1$ [67], we did not report STRUCTURE results for black bears here as we would be unable to distinguish between $K = 1$ or $K = 2$. Conversely, STRUCTURE identified two populations of grizzly bears (electronic supplementary material, figure S3) that, with few exceptions, corresponded to bears north and south of the TCH (electronic supplementary material,

Table 1. The number of black and grizzly bear migrants detected across the TCH in BNP, Alberta, Canada using non-invasive genetic sampling methods between April and October of 2006–2008. Four different criteria were used to identify migrants: detections at wildlife crossings, DNA detections spanning the highway, repeated cross-population assignments with $q \geq 0.9$ in program STRUCTURE [66], and first generation migrants detected with frequency-based assignment probabilities of Paetkau *et al.* [48] in GENECLASS2 [65]; we did not calculate black bear assignment probabilities. M, male; F, female.

	black bears			grizzly bears		
	total	M	F	total	M	F
total						
detected with DNA sampling	101	46	55	113	66	47
detected to north of highway with DNA sampling	57	24	33	73	41	32
detected to south of highway with DNA sampling	52	28	24	54	33	21
migrants						
detected at wildlife crossings	17	9	8	15	8	7
detected both sides of highway with DNA sampling	6	4	2	11	6	5
first generation migrants from STRUCTURE	n.a.	n.a.	n.a.	5	5	0
first generation migrants from GENECLASS2	n.a.	n.a.	n.a.	12	10	2
% DNA migrants detected at crossings	83	75	100	91	83	100
% STRUCTURE migrants detected at crossings	n.a.	n.a.	n.a.	40	40	0
% GENECLASS2 migrants detected at crossings	n.a.	n.a.	n.a.	17	20	0

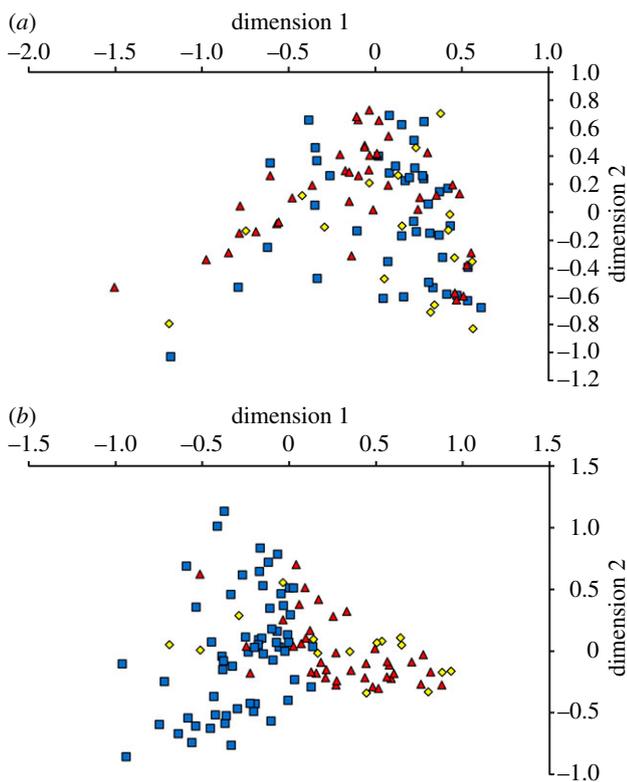


Figure 2. FCA plot from program GENETIX (Belkhir [64]) showing multidimensional clustering of individual black bears (a) and grizzly bears (b) detected north (squares) and south (triangles) of the TCH in BNP, Alberta, Canada using non-invasive genetic sampling methods. Individuals detected at wildlife crossing structures are displayed as 'crossers' (diamonds). (Online version in colour.)

figure S4). STRUCTURE identified three male grizzly migrants from the north and two migrants from the south (table 1). Many of the migrants identified with NGS were also detected

at crossing structures (table 1). One of the two male grizzly migrants that STRUCTURE identified was also detected at crossing structures (table 1).

Parentage analysis revealed 27 parent–offspring triads for black bears and 48 for grizzly bears. We used 136 grizzly bears and 105 black bears in our parentage analysis, including 23 grizzly bears and four black bears that were identified from captures, mortalities or during the pilot study at the crossing structures [27]. Of the 75 triads, 70 had 0 mismatches and 73 had probabilities greater than 0.98 (two triads with probabilities less than 0.95 were accepted based on other field data). We identified four sets of black bear triplets and two sets of twins, whereas we identified four sets of grizzly bear triplets and eight sets of twins. Many of the male and female bears using crossings are successful breeders, including eight out of 17 black bears and four out of 15 grizzlies (table 2). We identified 27 black bear offspring–parent pair triads and many of the offspring were assigned to parents that used crossing structures to traverse the TCH (18 paternal, seven maternal relationships). Many grizzly bear offspring were also assigned to parents that used crossing structures to cross the TCH (six paternal, six maternal relationships). More grizzly bear crossers than black bear crossers were assigned to mothers and fathers who also used the crossings (table 2).

The parentage analysis showed that some of the bears that frequently crossed the highway had offspring on both sides of the road. For example, the male black bear that was detected most frequently at crossings had the highest relative reproductive success of any bear, being assigned paternity in 11 out of 27 triads. This bear mated with five different females and fathered numerous offspring that were detected on both sides of the TCH (table 2). One male grizzly was assigned paternity in five out of 48 triads, mated with a female who frequently used crossings and fathered three offspring who were all subsequently detected using crossings (table 2). Another male grizzly that assigned

Table 2. Summary information for black bears and grizzly bears detected at wildlife crossing structures in BNP, Alberta, Canada between May 2006 and October 2008. Bear ID (F, female; M, male), number of crossings, number of offspring, number of mates, ID of mother or father if they were detected at the crossings, number of detections to north or south of TCH and population assignment (Pop Assign) determined using program STRUCTURE [66]; bears were ranked according to their number of crossings.

	crossings	offspring	mates	mother	father	detections		Pop Assign
						N	S	
black bear								
F1	21	0	0			1	2	n.a.
F2	11	3	1	F7	M1	3	1	n.a.
F3	8	1	1			3	0	n.a.
F4	3	0	0			0	6	n.a.
F5	2	0	0			1	0	n.a.
F6	1	0	0			3	0	n.a.
F7	1	3	1			3	0	n.a.
F8	1	0	0			0	0	n.a.
M1	24	11	5			3	2	n.a.
M2	11	2	1			1	1	n.a.
M3	5	2	1			1	0	n.a.
M4	5	0	0			0	0	n.a.
M5	4	2	2			0	0	n.a.
M6	2	0	0			0	2	n.a.
M7	2	0	0			1	1	n.a.
M8	1	1	1			3	0	n.a.
M9	1	0	0			1	0	n.a.
grizzly bear								
F1	18	3	1			1	2	south
F2	9	0	0			22	1	north
F3	7	0	0	F1	M1	1	17	south
F4	4	0	0	F1	M1	1	8	south
F5	4	0	0			4	1	north
F6	3	0	0	F1	M1	0	1	south
F7	2	3	2			0	6	south
M1	34	5	2			16	58	south
M2	29	0	0			3	36	south
M3	15	0	0			7	26	south
M4	7	0	0	F7	M6	12	8	south
M5	6	0	0			3	2	north
M6	4	1	1			0	34	south
M7	1	0	0			0	25	north
M8	1	0	0	F7		0	0	south

to the north of the TCH ($q = 0.95$) mated with a female from the south ($q = 0.99$) and produced a female offspring who had almost equal assignment probabilities to both populations ($q = 0.42$ to north and $q = 0.58$ to south). All three bears were recovered as mortalities, which provided age information verifying that the two putative parents were adults and the offspring was conceived in 2005, born in 2006 and died as a yearling in 2007. The mother was detected using crossings during our pilot study [27] in 2005, the year of conception.

4. Discussion

Population fragmentation from roads can reduce migration rates and genetic diversity, yet there is little evidence that highway mitigation measures such as wildlife crossings allow genetic interchange [29,30]. We provide the first empirical evidence of gene flow occurring at crossing structures by documenting migration, reproduction and genetic admixture in two wide-ranging fragmentation-sensitive species, black and grizzly bears. We determined that there are measurable

differences between the effects of the TCH on black bears versus grizzly bears, but the highway did not completely isolate populations of either species. We conclude that crossing structures provide genetic connectivity for two large carnivore species across a major national transportation corridor that bisects one of the world's preeminent protected areas.

Although distinguishing gene flow from migration between fragmented populations is difficult, our study was able to examine three main components of gene flow separately: migration, successful reproduction and genetic admixture. Individual-based genetic analyses are more sensitive in detecting recent gene flow than population-based methods (e.g. F_{ST}), which offer better historical perspectives [71]. Significant time lags can exist for genetic discontinuities to develop after barrier formation [72] and these time lags make it especially difficult to detect changes in gene flow for species with long-generation times such as grizzly and black bears [38,41]. Individual-based methods can detect recent migration events, but many studies that use them infer functional connectivity from detection of interpopulation migrants, even though there may be huge discrepancies between migration and gene flow [20]. Our study was able to demonstrate gene flow for two bear species at wildlife crossing structures by combining individual-based genetic analyses with parentage analysis to document migration, reproduction and admixture.

We observed IBD patterns in black and grizzly bears consistent with other studies on bears in the region [41]. IBD was stronger for females than for males, which we predicted given that average male dispersal distances are typically greater than female dispersal distances for black and grizzly bears. Proctor *et al.* [73] estimated sex-specific dispersal distances using genetic analysis and found that females disperse 14.3 km, whereas males disperse 41.9 km on average in the Canadian Rocky Mountains. The long dispersal distances for male grizzly bears, along with our relatively small study area, probably explain the lack of statistically significant correlation we found between genetic and geographical distances in male grizzly bears. The results from our Partial Mantel test indicate that the TCH does not affect the genetic structure of black bears, but does affect the structure of male and female grizzlies.

Results of F_{ST} , FCA, frequency-based assignment tests and model-based clustering in STRUCTURE concurred that there was structuring in the grizzly bear population and no detectable genetic structure in black bears. The FCA plot for black bears shows no visible genetic structure associated with the TCH and indicates that there is only one population, with almost complete admixture (figure 2a). The FCA plot for grizzly bears shows genetic differentiation along the primary axis and shows clustering of individuals detected to the north and south of the TCH (figure 2b). Output from STRUCTURE agreed that there were two discrete populations that clustered north and south of the highway (electronic supplementary material, figures S3 and S4), providing multiple lines of evidence of genetic structure in the grizzly population. Black and grizzly bear detection centres were distributed unevenly across the landscape, with black bears closer to roadways and developed areas than grizzly bears (figure 1). This pattern, along with the disparity in the effect of the TCH on genetic structure between black and grizzly bear populations, is consistent with grizzly bears showing far more willingness to cross roads and far less tolerance to humans [36,37] than black bears [38].

We documented extensive interpopulation movements of black and grizzly bears across the TCH using four different methods for migrant detection. The number of male and female black bear migrants that we detected (table 1) most probably explains the lack of genetic differentiation associated with the TCH (figure 2a). The numbers of male and female grizzly bear migrants detected at wildlife crossings and with NGS suggest high levels of interpopulation movements (table 1). Out of the bears that we detected on both sides of the TCH with NGS methods, very high percentages used crossings, which indicated that bears making interpopulation movements are highly likely to traverse the TCH at crossing structures as opposed to climbing over the fence or circumnavigating its end points (table 1). All of the female black and grizzly migrants detected with NGS were also detected using crossings, suggesting that the crossings play a particularly important role in highway permeability for females.

Sex-biased dispersal across major highways has been well documented in grizzly bears [36,41]. We obtained comparable estimates of grizzly male migration rates with all four migrant detection methods (table 1). The number of female migrants that we detected using direct methods (i.e. detections with NGS, detections at wildlife crossings), however, was considerably higher than when using indirect, statistical methods (i.e. STRUCTURE). This discrepancy reveals differences between direct and indirect methods for identifying migrants and shows the importance of using multiple, independent methods to examine interpopulation movements. One to 10 migrants per generation has been proposed as the amount necessary to overcome genetic isolation while allowing for local adaptation [74]. The amount of migration across the TCH that we have documented far exceeds the levels of movement that would be necessary to prevent genetic isolation, but only if migrants also successfully reproduce on the other side.

Research suggests that many individuals that use crossings to traverse busy highways are juveniles and thus less likely to be successful breeders owing to their social status [18,30]. Contrary to these findings, our results show that relatively high percentages of black (47%) and grizzly bears (27%) that used crossing structures were successful breeders (table 2). Interestingly, males detected using crossings most frequently also had the highest relative reproductive success of male crossers. This suggests a positive correlation between the use of crossings and the number of breeding opportunities for males, although further study is warranted to determine whether crossing use actually increases reproductive success. Because we only considered triads in our paternity and maternity assignments in order to infer directionality, we believe that our estimates of relative reproductive success are quite conservative; therefore, breeding success of bears using crossings is probably much higher than we have been able to document in our study.

A higher percentage of grizzly bears than black bears detected at wildlife crossings are related (table 2). This is not surprising as Sawaya *et al.* [28] documented a steady increase in grizzly bear use between 1996 and 2008, and we speculate that some of the increase in use is owing to offspring learning to use crossings with their mothers. Remote photographs revealed long ago that cubs and yearlings of both bear species use crossings with their mothers, but now we have independent proof from maternity assignments. We also know now that a single family group accounted for a high percentage of female grizzly bear crossings (table 2), therefore many of the most important crossings that we documented were from

dependent bears unable to contribute to the gene pool. The benefit to female offspring of using crossings would be that they in turn use crossings as independent adults after learning the behaviour from their mothers; however, none of the three daughters in this family group survived long enough to reach adulthood.

Genetic admixture was detected in grizzly bears using FCA and population assignment in STRUCTURE. Research has shown that gene flow can be unidirectional [75], but our results suggest that gene flow at crossings occurs, although unevenly, in both directions across the TCH. Visual inspection of FCA plots indicates that black bear and grizzly bear individuals using crossings are freely mixing with individuals from the north and south, supporting our contention that crossing structures allow gene flow and genetic admixture between populations (figure 2). Although we were unable to reliably assign black bear individuals to populations of origin, we found evidence of genetic admixture in black bears by detecting offspring of black bear parents that use crossings on both sides of the TCH (table 2). Interestingly, many of the grizzly bears that we detected at crossings were assigned to the south (table 2). In most cases, population assignments were congruent with the frequency of detections to the north or the south of the TCH. Given the habitat differences between the two sides of the valley, we speculate that bears from the south move north of the highway to access seasonally available food resources on south-facing slopes, with earlier emergent vegetation than the north-facing slopes on the south side of the TCH. Nonetheless, male and female grizzly bears from both the north and south populations used crossings to traverse the TCH, providing evidence of bidirectional gene flow and genetic admixture between fragmented populations.

Although the TCH appears to affect black and grizzly bear gene flow differently, it is clear that male and female individuals using crossings structures are successfully migrating, breeding and moving genes across the roadway. Our results are encouraging given that Proctor *et al.* [41] found that genetic discontinuities in grizzly bears were most often associated with high traffic volume highways and human settlement. The authors suggested that the relatively weak genetic structure in grizzly bears across the TCH in BNP as compared with a section of TCH approximately 100 km to the west could be attributed to the presence of crossing structures. We concur that wildlife crossing structures may be helping to counteract the effects of fragmentation on grizzly bears across the TCH. Our documentation of a female grizzly bear from the south that used crossing structures to traverse the TCH and successfully mate with a male grizzly from the north provides compelling, though anecdotal, evidence that migration, reproduction and admixture simultaneously occur at crossing structures. In conclusion, we have demonstrated the ability of crossing structures to provide genetic connectivity for two fragmentation-sensitive carnivore species across a major transcontinental highway.

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