

## FAST-TRACK

# A southern California freeway is a physical and social barrier to gene flow in carnivores

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## Abstract

Roads present formidable barriers to dispersal. We examine movements of two highly mobile carnivores across the Ventura Freeway near Los Angeles, one of the busiest highways in the United States. The two species, bobcats and coyotes, can disappear from habitats isolated and fragmented by roads, and their ability to disperse across the Ventura Freeway tests the limits of vertebrates to overcome anthropogenic obstacles. We combine radio-telemetry data and genetically based assignments to identify individuals that have crossed the freeway. Although the freeway is a significant barrier to dispersal, we find that carnivores can cross the freeway and that 5–32% of sampled carnivores crossed over a 7-year period. However, despite moderate levels of migration, populations on either side of the freeway are genetically differentiated, and coalescent modelling shows their genetic isolation is consistent with a migration fraction less than 0.5% per generation. These results imply that individuals that cross the freeway rarely reproduce. Highways and development impose artificial home range boundaries on territorial and reproductive individuals and hence decrease genetically effective migration. Further, territory pile-up at freeway boundaries may decrease reproductive opportunities for dispersing individuals that do manage to cross. Consequently, freeways are filters favouring dispersing individuals that add to the migration rate but little to gene flow. Our results demonstrate that freeways can restrict gene flow even in wide-ranging species and suggest that for territorial animals, migration levels across anthropogenic barriers need to be an order of magnitude larger than commonly assumed to counteract genetic differentiation.

*Keywords:* carnivores, conservation genetics, gene flow, habitat fragmentation, road and anthropogenic barriers, territoriality

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## Introduction

Roads are the primary threat to dispersal between habitat patches in urban landscapes (Forman & Alexander 1998) and, without dispersal, isolated populations may be doomed to extinction (Brown & Kodric-Brown 1977; Hanski *et al.* 1996). Roads can also isolate populations

previously in genetic communication, and the resulting genetic erosion may contribute to population decline (Saccheri *et al.* 1998; Westemeier *et al.* 1998). Empirical data on the frequency of dispersal across roads and its effect on genetic variation and population persistence are few and limited to small and relatively abundant species with low vagility (e.g. frogs, Reh & Seiz 1990; voles, Gerlach & Musolf 2000; beetles, Keller & Largiadér 2003). In contrast, the ability of large carnivores to disperse long distances could mitigate the effects of genetic isolation. However, for carnivores, population densities are low and home range

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sizes and dispersal distances are large (Sunquist & Sunquist 2001), so their persistence should be strongly influenced by the isolating effects of roads and development. Additionally, carnivores are susceptible to the deleterious genetic effects of inbreeding in small populations (O'Brien *et al.* 1985; Roelke *et al.* 1993; Vila *et al.* 2003; Liberg *et al.* 2005). Although conservation biologists have focused on the importance of connectivity for carnivores, actual demonstrations of the genetic consequences of roads for such long-lived and mobile species have been lacking.

We studied dispersal and genetic differentiation in coyote (*Canis latrans*) and bobcat (*Lynx rufus*) populations separated by a wide and heavily travelled freeway in the highly urbanized landscape of southern California. To obtain a direct estimate of dispersal, we radio-tracked animals on both sides of the freeway over seven years, from 1996 to 2003. To obtain a genetic estimate of dispersal, we genotyped individuals using highly variable microsatellite loci and then used genetic assignment tests based on the composite genotypes to assign animals genetically to each side of the freeway. We then assessed levels of genetic differentiation between populations on the same and different sides of the freeway to measure the effect of the road on gene flow (genetically effective migration). Finally, we determined whether levels of observed migration were consistent with levels of gene flow estimated by a coalescent model based on the number of generations since the freeway was established. The use of both radio-tracking information and genetic data to monitor migration and gene flow allows an assessment of the long-term genetic consequences of roads and other anthropogenic barriers on genetic diversity and can lead to the development of plans to mitigate their effects.

## Materials and methods

We studied coyote and bobcat populations separated by the Ventura Freeway (US101), a congested 10–12 lane road in the San Fernando Valley 40 km from downtown Los Angeles. More than 150 000 vehicles use the road daily and the presence of a meridian fence largely restricts dispersal to underpasses and culverts (Fig. 1A) (Ng *et al.* 2004). The freeway was established in 1949 from a relatively undeveloped secondary road with minimal traffic (B. Marquez, personal communication), and there are no other natural barriers such as rivers or topographic features following the freeway that would have historically restricted gene flow. The study area consisted of 358 km<sup>2</sup> of chaparral, grassland, and oak woodland habitat (Table S1, Supplementary material) interspersed with secondary roads and housing developments. The 28.4-km length of the freeway connects the towns of Agoura Hills and Thousand Oaks that have a combined population of 200 000. The area is considered a critical habitat corridor connecting the Santa Monica Mountains with extensive natural habitat to the north (Penrod *et al.* 2001).

## Animal capture and radiotelemetry

Bobcats and coyotes were captured with padded foothold traps in 1996–1998 and with box traps (bobcats) and neck snares (coyotes) in 2000–2003. We also genotyped a population of bobcats from northern California where bobcats were captured with box traps from 1992 to 1995 (Riley *et al.* 2004). Captured animals were aged, sexed, weighed, measured, ear-tagged, radio-collared, and released at the capture site. Blood, hair, scat, and in some cases tissue samples were taken for genetic analyses.

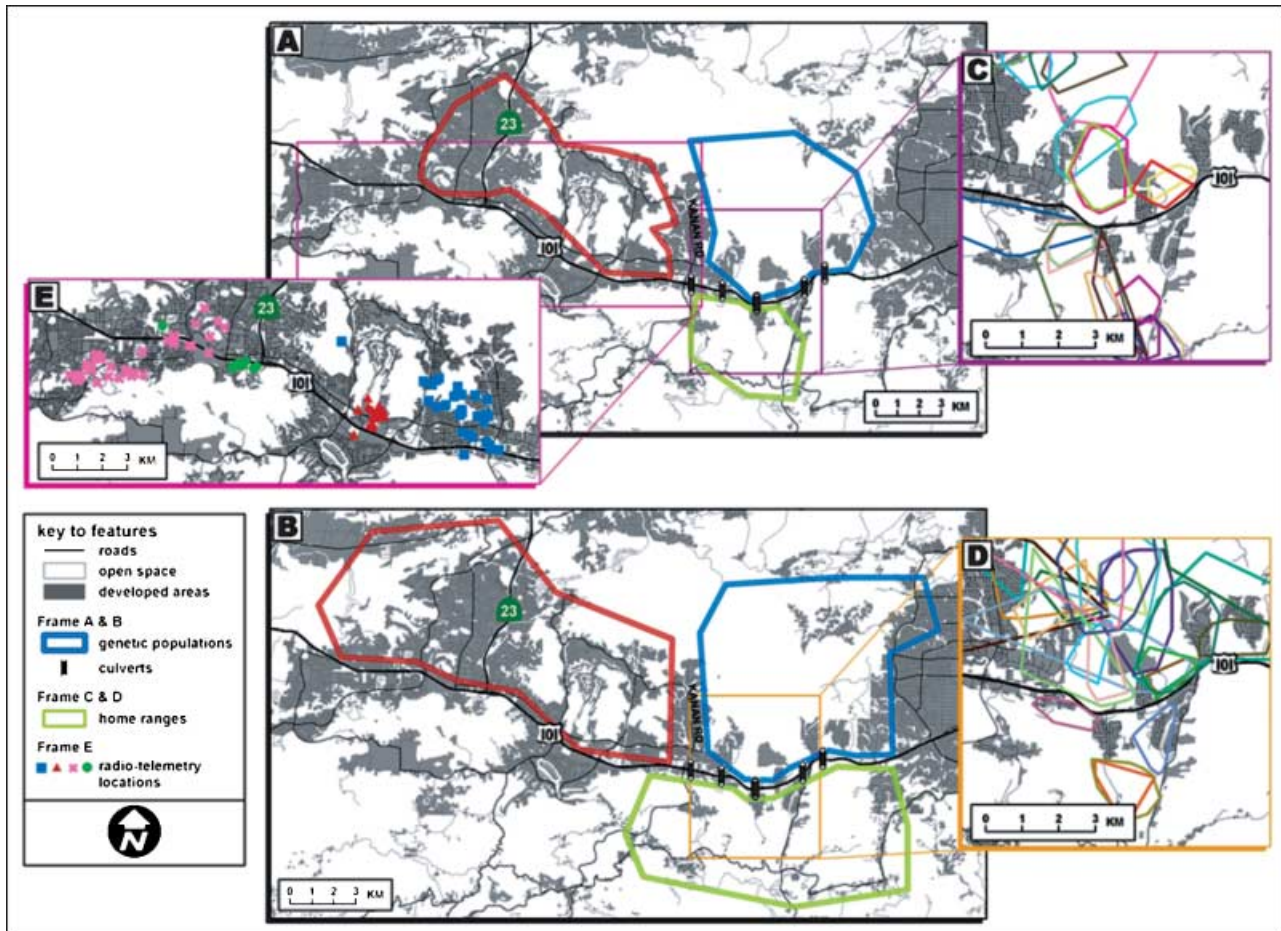
Animals were radio-located by triangulation using three consecutive or two simultaneous compass directions obtained with a peak directional antenna. Locations were obtained throughout the 24-h daily cycle. We computed 95% minimum convex polygon home ranges for animals with at least 20 locations. Home range overlap was computed for animals that were radio-tracked concurrently. We computed overlap as the total percentage of the home range that intersected with any other animals of the same sex.

## Genetic sampling

All bobcat and coyote DNA samples were extracted from blood or tissue (ear punch) samples taken at time of capture. Standard phenol–chloroform extraction methods were utilized. Seven dinucleotide microsatellite loci developed for the domestic cat (Menotti-Raymond *et al.* 1999) and validated for polymorphism in the bobcat (Ernest *et al.* 2000) were used to genotype all bobcat specimens (FCA026, FCA043, FCA045, FCA077, FCA090, FCA096, and FCA132). Seven tetranucleotide microsatellite loci developed for the domestic dog (Breen *et al.* 2001) and validated as polymorphic for the coyote by the authors were used to type all coyote specimens (FH2001, FH2137, FH2422, FH2062, FH2226, FH2293, and PEZ19). Polymerase chain reactions (PCR) utilized either (i) a fluorescent dye-labelled forward primer, or (ii) a hybrid combination of forward primers consisting of the published forward primer with the M13F (–20) sequence (16 bp) added to the 5' end and a fluorescent dye labelled M13F (–20) primer. The unlabelled reverse primer was used in both cases. We used the PCR conditions for the hybrid combination primer (a two-step cycle) (Boutin-Ganache *et al.* 2001). Primer dye labelling utilized BeckmanCoulter dye D4 and PCR products were sized on the BeckmanCoulter CEQ2000XL DNA Analysis System.

## Genetic analysis

Observed and expected heterozygosities, allelic diversity, and deviation from Hardy–Weinberg equilibrium were assessed with GENEPOP (Raymond & Rousset 1995; Table S2, Supplementary material). Bonferroni corrections to significance levels were applied to account for multiple



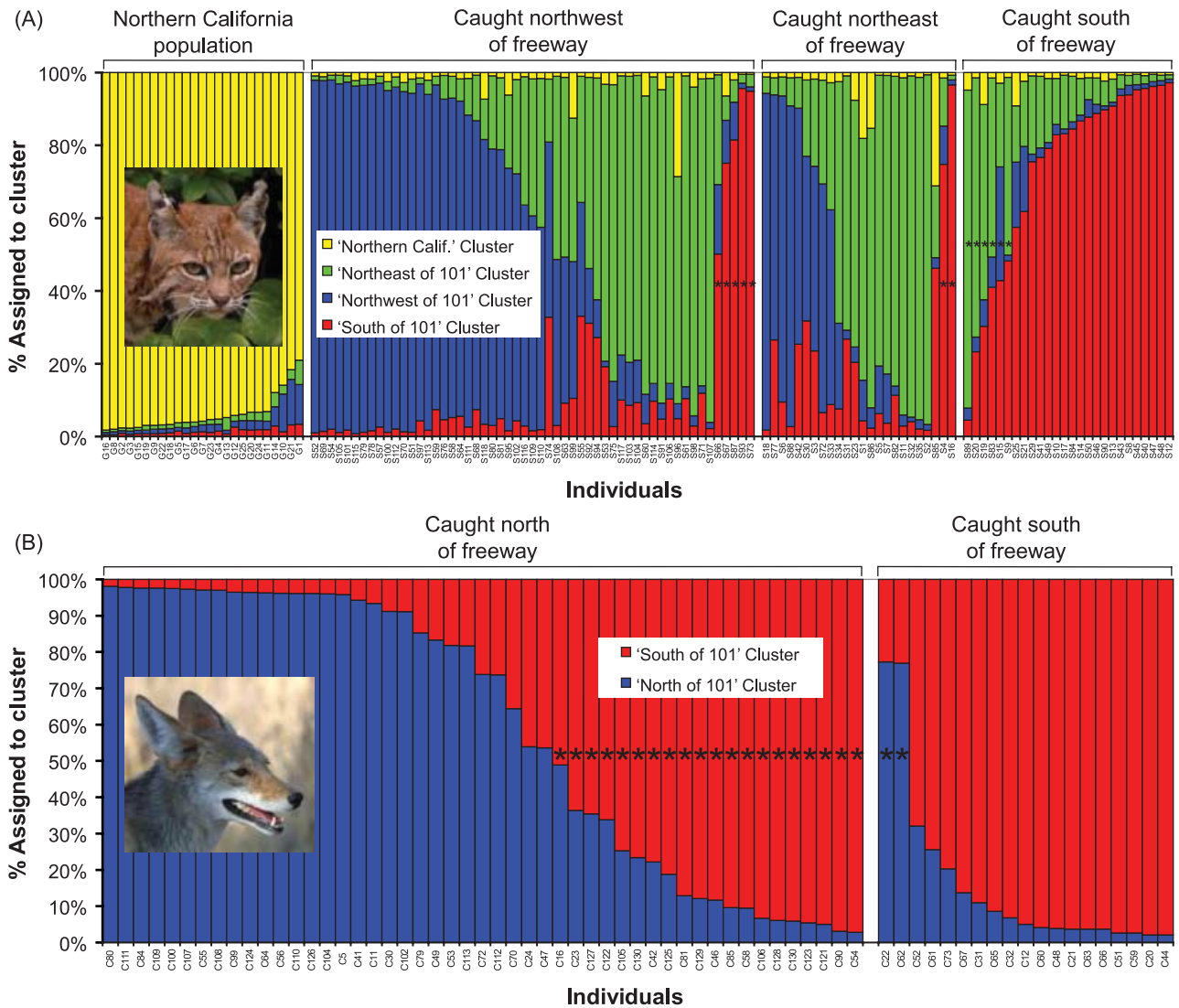
**Fig. 1** Study populations, home ranges and radio-locations of bobcats and coyotes near the Ventura Freeway in southern California. Bobcat (A) and coyote (B) study populations are located north and south of the Ventura Freeway. The north side populations are further divided by Kanan road into northeast and northwest populations. 95% minimum convex polygon (MCP) home ranges are shown for all radio-tracked bobcats (C) and coyotes (D) near the freeway between the south and northeast populations. In (E), radio-telemetry locations are shown for four bobcats with locations north and south of the freeway that were captured north of the freeway but assigned genetically to the population south of it (see Fig. 2).

tests in the determination of heterozygote deficiency/excess (Rice 1989). Coyote samples were grouped into two populations north and south of the freeway, whereas bobcats were divided into three populations, two north and one south of the freeway according to *STRUCTURE* results (see Results and discussion).

To obtain a genetic estimate of dispersal, we used assignment tests based on the composite microsatellite genotypes. Assignment tests identify the population of origin for each individual, and thus individuals assigned to a population other than the one in which they were caught can be considered migrants (Berry *et al.* 2004). We used the program *STRUCTURE* (Pritchard *et al.* 2000) to assign individuals to populations. *STRUCTURE* is a Bayesian clustering algorithm that uses multilocus genotypes to infer population structure and assign individuals to populations. All individuals were combined into one data set for analysis,

without any a priori population assignments. We utilized a burn-in of 50 000 iterations, followed by 500 000 iterations of the Gibbs sampler. Admixture was allowed. We evaluated  $K$  values, the number of assumed populations, from 1 to 6. Each value of  $K$  was run a minimum of three times to evaluate stability. For the highest likelihood  $K$  value, we then evaluated the cluster assignment results for each individual with respect to its capture location.

For potential migrants identified in the initial *STRUCTURE* analysis, we modified the *STRUCTURE* input data file to reassign them to their putative population of genetic origin based on the cluster assignment results. We then calculated the posterior probability of correct population assignment with this revised data set in *STRUCTURE* using the ancestry model with admixture, incorporating population information with the migration parameter set to  $v = 0.1$  (Table S5, Supplementary material).



**Fig. 2** Genetic assignment results from the program STRUCTURE for bobcat (top) and coyote (bottom) populations. Individuals are grouped according to capture location. Genetic population clusters are coded with different colours and the fraction of colour for each individual represents the probability of assignment to the cluster with that colour. Four clusters were found in bobcats, corresponding to a northern California group (yellow), two groups on the north side of the freeway (blue and green) and one group on the south side of the freeway (red). In coyotes, two clusters are supported by STRUCTURE, corresponding to a north (blue) and south (red) cluster. Asterisks denote misassigned individuals (individuals with greater than 50% assignment to a cluster different from the capture location) that are potential migrants.

We measured population differentiation by calculating population pairwise  $F_{ST}$  values and significance ( $g$ -test) using the program FSTAT (Goudet 2002). We estimated gene flow as the number of genetic migrants ( $Nm$ ) between populations from  $F_{ST}$  values [ $F_{ST} \approx 1/(1 + 4Nm)$ ; Wright 1921]. Population membership was based on the capture location north or south of the Ventura Freeway. The northern population was additionally split into northwest and northeast populations to measure differentiation between populations on the same side of the freeway. Computing  $F_{ST}$  between these two populations north of the

freeway allows us to compare the amount of genetic differentiation across the freeway with that over the same or greater distance without the barrier. The east and west populations were divided by Kanan Road, a secondary road, and surrounding development (Fig. 1A,B).

We also computed the pairwise relatedness coefficient,  $R$  between individuals, (Queller & Goodnight 1989) using the program KINSHIP (Goodnight 2005). We identified pairs of individuals significantly related ( $\alpha = 0.05$ ) at the  $R \geq 0.25$  level by first performing a simulation in KINSHIP using the complete population data set at  $R = 0.25$ , followed



by log-likelihood calculations for all possible pairs. Based on the three defined populations, two north and one south of the freeway, for both bobcats and coyotes, we then determined the percentage of number of close relatives ( $R \geq 0.25$ ) across the freeway, across a similar distance on the same side of the freeway, and within each population (Fig. S1, Supplementary material).

We used coalescent simulation to generate predicted levels of genetic differentiation based on a population isolation event with the program SIMCOAL (version 1.0) (Excoffier 2004). SIMCOAL is a computer program for the simulation of molecular genetic diversity in an arbitrary number of diploid populations based on a retrospective coalescent approach. SIMCOAL was utilized to estimate expected population pairwise  $F_{ST}$  values for both bobcat and coyote microsatellite results, based on the time since the freeway was built. A sensitivity analysis was performed on the parameter space of inference for the simulations by varying population effective sizes, sample sizes, migration rates, number of generations since a historical event (population separation), and microsatellite mutation rate. The microsatellite mutation rate was varied from  $10^{-3}$  to  $10^{-2}$  based on observed mutation rates for dinucleotide and tetranucleotide microsatellites for the domestic dog (Francisco *et al.* 1996). Effective population sizes ( $N_e$ ) were estimated as the number of territorial adults in the study area based on telemetry observations. This calculation assumes that all territorial individuals reproduce equivalently; however, sensitivity analysis showed that results were not sensitive to small changes in  $N_e$  or sample size. One thousand analysis iterations were performed for each combination of input parameters. SIMCOAL output results were then analysed using ARLEQUIN (Schneider *et al.* 2000) for pairwise population  $F_{ST}$  estimation.  $F_{ST}$  values were determined by averaging the  $F_{ST}$  values of all 1000 iterations. Because sensitivity analysis showed that population effective size and sample size had minimal effect on  $F_{ST}$  within the range of our study values, they were held constant at 200/50 (population effective size/sample size) for bobcats and 75/25 for coyotes. The number of generations since separation was varied from 25 to 100, and migration rate was varied from 0 to 10% of source population. We assume a 2-year generation time for coyotes and bobcats (Knick *et al.* 1985; Bekoff & Wells 1986).

## Results and discussion

We captured bobcats and coyotes on the north and south sides of the freeway and placed radio-telemetry collars on 110 (92 north side, 18 south side) coyotes and 87 (64 north side, 23 south side) bobcats. Using radio-telemetry, from 1996 to 2003 five (4.5%) radio-collared coyotes and 10 (11.5%) radio-collared bobcats were observed to cross the

freeway, whereas 58 (52%) coyotes and 40 (45%) bobcats crossed major secondary roads. Because a principal study objective was to understand the effects of roads, and in particular the freeway, on carnivore movement, all radio-collared animals were captured within dispersal distance, and many within an average home range diameter, of both the freeway and secondary roads. However, only 213 (2.3%) of 9311 bobcat locations and 19 (0.4%) of 4565 coyote locations were on the opposite side of the freeway from the capture location of the individual.

Home range perimeters followed but did not cross roads such as the freeway, implying that they functioned as artificial territorial boundaries (Fig. 1C, D). For both male and female bobcats, home ranges that bordered on hard boundaries such as the freeway or development were significantly smaller than those that did not (males:  $t = -1.919$ , one-sided  $P = 0.033$ ; females:  $t = -2.186$ , one-sided  $P = 0.018$ ). Also, for adult female bobcats, the age class most important for reproduction, home range overlap was three times higher among individuals with home ranges adjacent to development and the freeway than in those with nonadjacent home ranges (38.8% vs. 12.4% overlap;  $t = -1.77$ , one-sided  $P = 0.045$ ). For coyotes, we did not radio-track a sufficient proportion of territorial individuals throughout the study area to reliably measure territory overlap. Overall, the freeway was a significant barrier to movement as only about 5–10% of individuals crossed in the 7 years of observation, and home range boundaries, overlap, and size were affected by its proximity.

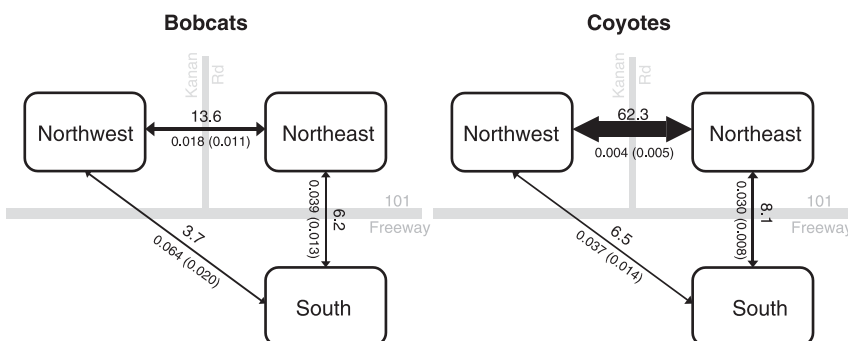
We genotyped 68 (49 north side, 19 south side) coyotes and 108 (82 north side, 26 south side) bobcats for variation in seven microsatellite loci. Microsatellite loci were highly polymorphic for both species (Table S2, Supplementary material). We also typed a population of 25 bobcats from Golden Gate National Recreation Area in the San Francisco Bay Area, a geographically remote and genetically distinct population (Riley *et al.* 2004). For bobcats, the STRUCTURE assignment test results determined that four populations best fit the data (Table S3, Supplementary material), with the northern California population clearly separated from those in southern California and two populations located north and one south of the freeway (Fig. 2A). All bobcats from northern California were assigned correctly to that population. However, seven bobcats caught north of the freeway, five to the west and two to the east, were assigned genetically to the south side population with assignment probabilities and posterior probabilities of greater than 50% (Fig. 2A; Table S5, Supplementary material). We had radio-telemetry observations on six of these seven bobcats, and four of the six were located south of the freeway at least once (Fig. 1E), corroborating their status as migrants. Six bobcats assigned north of the freeway were captured to the south (Fig. 2A). None of these bobcats were found north of the freeway based on

radio-telemetry observations, although three were located within 500 m of it, and two within 100 m. Therefore, based on genetic assignment and in part supported by telemetry observations, 9% (7 of 80) of the bobcats sampled from the north side of the freeway and 23% (6 of 26) of the bobcats sampled from the south side are putative migrants (see Fig. 2A). Considering the limited sampling of activity provided by radio-telemetry, the genetically derived estimate of migration (13 of 106 individuals or 12.3%) and that based on telemetry (10 of 87 individuals or 11.5%) are remarkably similar and suggest that about 12% of the sampled bobcat population crossed the freeway over the 7-year study period, or 3.4% per generation.

For coyotes, the assignment test determined that two populations best fit the observed data, one north and one south of the freeway (Fig. 2B; Table S3, Supplementary material). Two of 19 individuals (11%) captured south of the freeway were assigned to the north side population with assignment probabilities and posterior probabilities of greater than 50% (Fig. 2B; Table S5). Radio-telemetry observations were available for one of these two coyotes, and none of the 107 locations were north of the freeway, although six of the first seven locations were within 1.5 km of it, indicating that this coyote may have dispersed from the north. Twenty of the 49 coyotes (41%) captured on the north side of the freeway were assigned to the south side. We had radio-telemetry observation on 18 of these coyotes, and none showed evidence of crossing the freeway, although 11 of 18 were observed within 1 km of it. Consequently, the genetic results suggest that 10% of the South side population and as much as 41% of the north side population may be migrants. This implied migration fraction of 32% (22 of 68 individuals) or 9.1% per generation is much larger than the value of 4.5% or 1.3% per generation based on telemetry observations. Considering higher assignment thresholds of 80% and 90%, 21% (14 of 68) or 5.9% per generation and 10% (7 of 68) or 2.9% per generation, respectively, are still misassigned and likely to be migrants. The discrepancy between genetic- and telemetry-based estimates may reflect the unlikely probability of observing dispersal with limited radio-telemetry

observations or the difficulty of classifying migrants in genetically similar populations. The use of assignment tests to measure dispersal requires a modest degree of genetic differentiation between populations, otherwise historical gene flow will confound the assessment of recent dispersal events (Berry *et al.* 2004). However, two observations argue for a higher dispersal rate than suggested by telemetry observations. First, coyotes generally disperse farther than bobcats (Van Vuren 1998), which had higher observed and implied rates of migration (see above). Second, of the 22 coyotes that were genetically misclassified, 14, or 64%, were juveniles or yearlings, the ages when coyotes typically disperse (Gese *et al.* 1989). This compares to 39% juveniles or yearlings in the population of coyotes that were not misclassified.

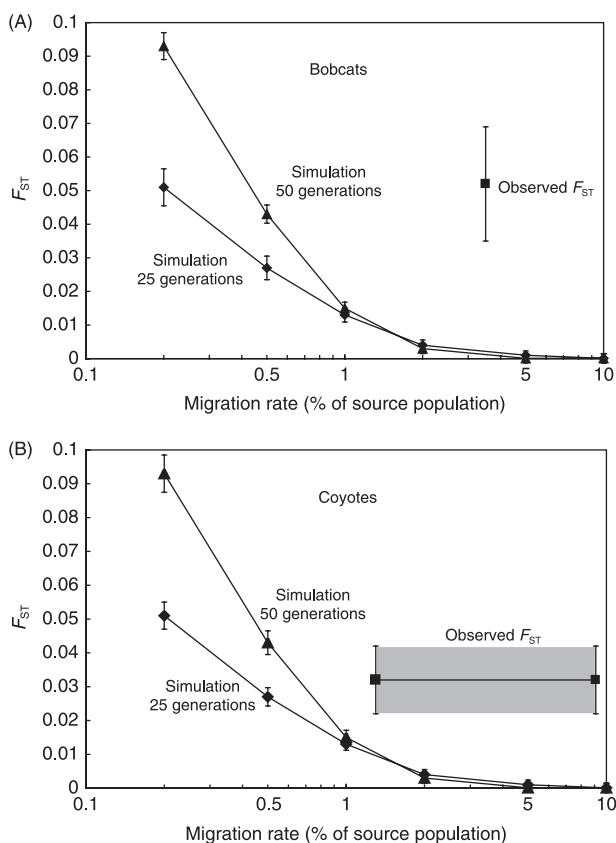
Although the freeway is a barrier to movement, rates of migration of 3.4% per generation for bobcats and from 1.3% to 9.1% per generation for coyotes as suggested by telemetry or genetic data imply high rates of gene flow sufficient to counteract drift (Mills & Allendorf 1996; Vucetich & Waite 2000). To assess levels of differentiation, we measured  $F_{ST}$ , the fixation index, for the seven microsatellite loci. For both species, we found that  $F_{ST}$  values between populations on different sides of the freeway were two to nine times larger (and  $Nm$  2–9 times smaller) than those between populations on the same side, although the latter populations were separated by greater distances (Figs 1A, B and 3). Additionally, 39.2% of alleles for coyotes and 23.6% of alleles for bobcats were unique to one side of the freeway (Table S4, Supplementary material). For coyotes, both pairwise  $F_{ST}$  values across the freeway ( $F_{ST} = 0.030$  northwest-south,  $F_{ST} = 0.037$  northeast-south) were significantly different from zero ( $P < 0.003$ ), whereas the  $F_{ST}$  values between the two subpopulations on the same side of the freeway (0.004, northwest-northeast) was not ( $P = 0.134$ ). For bobcats all three pairwise  $F_{ST}$  values were significantly different from zero ( $F_{ST} = 0.064$  northwest-south,  $F_{ST} = 0.039$  northeast-south,  $F_{ST} = 0.018$  northwest-northeast) ( $P < 0.003$ ), suggesting that Kanan Road, a busy secondary road, and the wide development corridor flanking it, may also be a significant barrier to gene flow for bobcats



**Fig. 3** The number of genetic migrants ( $Nm$ , above line) and levels of genetic differentiation ( $F_{ST}$ , below line, standard error in parentheses) between populations across the freeway (northwest vs. south and northeast vs. south) and on the same side of the freeway (northeast vs. northwest) for bobcats and coyotes. Arrow widths are proportional to  $Nm$  values.

(Fig. 3). Relatedness values also indicated substantially more closely related pairs of animals ( $R \geq 0.25$ ) between populations on the same side of the freeway (northeast vs. northwest) than across it (northeast vs. south) (Fig. S1, Supplementary material). Overall, these results clearly show that the Ventura Freeway is an imposing barrier to gene flow for both species.

To assess if these levels of genetic differentiation were consistent with population history and the observed and implied migration rates, we used coalescent simulation modelling to estimate north side–south side pairwise  $F_{ST}$  indices resulting from the division of a single population by the creation of the freeway in 1949. The simulation results (Fig. 4) showed that a genetically effective migration rate of approximately 0.5% per generation or less is required to explain the observed level of population differentiation for both carnivore species. Consequently, given



**Fig. 4** Comparison of predicted genetic divergence ( $F_{ST}$ ) using coalescence simulations (Y-axis) with observed  $F_{ST}$  values as a function of migration rate (X-axis) between bobcat (A) and coyote (B) populations north and south of the freeway. Migration rates for observed  $F_{ST}$  values are based on radio-tracking observations and genetic assignment tests (see text). Error bars for  $F_{ST}$  values represent one standard error. Observed genetic differentiation is consistent with a migration rate of 0.5% or less for both species given 25 or 50 generations since isolation.

that our observations span a period of about three generations (Knick *et al.* 1985; Bekoff & Wells 1986), the genetically effective migration rates estimated by the coalescent simulation are approximately 3–18 times lower than migration rates between populations separated by the freeway based on direct observation or genetic population assignment.

The disparity between observed migration rates and inferred gene flow likely reflects the lack of reproductive success of migrants. For example, 6 of the 10 bobcats that were found on both sides of the freeway were located across it less than four times, and of the four that stayed across, the two females were known not to have produced kittens the following spring. Of the five coyotes radio-tracked across the freeway, four did not reproduce since they were located six or fewer times across the road and over only a short period of time outside the reproductive season. Further, of the potential migrants observed by telemetry and implied by genetic results, 54% were classified as less than 2 years old. In undisturbed populations, young dispersers enter a matrix of occupied and unoccupied territories, reflecting natural mortality of territorial adults. However, in our study area, home ranges follow the freeway boundary (Fig. 1C, D) and consequently, territorial and reproductive individuals contribute genes to the population on one side of the freeway only. Additionally, in our urban study area, mortality from hunting and trapping is low or nonexistent, and carnivore survival rates were high (Riley *et al.* 2003). High survival rates, small home ranges, high home range overlap, and the barrier effect of roads and development all resulted in territory packing along hard boundaries such as the freeway. This territory pile-up likely produced a formidable social and behavioural barrier to genetically effective dispersal because dispersers are unlikely to obtain and hold territories near the freeway. This unique genetic-isolating effect of roads and other artificial boundaries likely applies to other territorial species.

Conceivably, genetic differences may also correspond to changes in habitat composition across the freeway. For example, continent-wide climate and habitat changes have been associated with genetic structure in grey wolves (*Canis lupus*; Geffen *et al.* 2004), and regional-scale biome changes, e.g. between mountainous regions and flat valley expanses, were associated with genetic structure in coyotes (Sacks *et al.* 2004). However, both the geographic scale and diversity in habitats sampled by these studies far exceed the habitat differences observed throughout the area that we studied. Both sides of the freeway consist of a similar mix of the three dominant types of vegetation in the Santa Monica Mountains and Simi Hills including mixed scrub (64–85%), grassland (8–26%) and riparian and oak woodland (2–11%; Table S1, Supplementary material). There is variation in the amounts of specific

types of scrub communities (e.g. coastal sage scrub, dominated by *Salvia* spp. and *Artemisia californica*, and chaparral, dominated by *Adenostoma fasciculatum* or *Ceanothus* spp.) across the entire study area, but coyotes and bobcats on both sides of the freeway used both of these scrub communities, indicating a lack of ecological specialization. Coyotes and bobcats are generalist carnivores, and we feel such small-scale ecological differences are an unlikely explanation of our results. Unfortunately, there are too few historical specimens from our study area in museum collections to directly assess genetic differentiation prior to the construction of the freeway.

Roads are a primary threat to the persistence of animal populations in urban landscapes. This threat is caused directly through vehicle mortality or indirectly by increasing the probability that isolated populations will become extinct through random demographic processes (Forman 2003). Our results show that the genetic effects of roads can also be substantial even for large, highly mobile species. Levels of differentiation between coyote and bobcat populations isolated for about 50 years are as large as those between coyote populations separated by several hundred kilometres (Roy *et al.* 1994). These levels of differentiation were found even between high-density populations of bobcats and coyotes. The isolating effects of roads would likely be even more severe for very small populations and rare species (Spielman *et al.* 2004). An additional insidious factor is the effect of roads in decreasing gene flow well below that expected from migration rates or existing between similarly spaced populations on the same side of the freeway. We suggest this decrease may be caused by: (i) roads acting as home range boundaries for terrestrial and reproductive individuals, and thus decreasing the migration rate of genes, and (ii) home range pile-up near roads that make it less likely that dispersing individuals can find territories. To counteract such genetic isolation, corridors across freeways could conceivably include more natural habitat so that home ranges could extend across the freeway and rates of genetic exchange might be increased. Five of the six potential crossing points in our study (Fig. 1A, B) were unvegetated culverts or paved underpasses. Finally, our results imply that observed migration rates across anthropogenic barriers may be poor surrogates for gene flow, and that molecular genetic studies of even recently isolated populations may provide new insights for conservation.

### Acknowledgements

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### Supplementary material

The supplementary material is available from <http://www.blackwellpublishing.com/products/journals/suppmat/MEC/MEC2907/MEC2907sm.htm>

**Table S1** Percentage of different habitat types in the sampled areas, determined by overlaying the polygons for each population (Fig. 1) on a vegetation map of the Santa Monica Mountains and Simi Hills (Franklin 1997). Chaparral consists of scrub habitat dominated by chamise (*Adenostoma fasciculatum*) and ceanothus (*Ceanothus* spp.); coastal sage scrub consists of scrub habitat dominated by sage (*Salvia* spp.) and California sagebrush (*Artemisia tridentata*); grassland is mostly introduced annual grasses; woodland consists of oak and riparian woodlands and some walnut woodlands.

**Table S2** Observed and expected heterozygosities, allelic diversity and deviations from Hardy–Weinberg equilibrium (significant deviations indicated by  $\pm$ ).

**Table S3** Likelihood values for inferred number of genetic clusters ( $K$ ) from STRUCTURE (3 iterations per value of  $K$ ).

**Table S4** Private alleles as percentage of total alleles per locus in each population.

**Table S5** Cluster assignment and posterior probability of correct genetic population assignment of potential migrants.

**Fig. S1** Comparison of the percentage bobcat and coyote pairs that were closely related genetically, as defined by having an  $R$  value of 0.25 or greater between populations across the freeway (south vs. northwest and northeast) and on the same side of the freeway (across Kanan road, northwest vs. northeast). Bar widths are proportional to percentage values.

**Fig. S2** Sensitivity of the SIMCOAL simulation algorithm for  $F_{ST}$  as a function of microsatellite mutation rate ( $\mu$ ), migration rate, and number of generations since population separation (since construction of the freeway).

### References

- Bekoff M, Wells MC (1986) The social ecology and behavior of coyotes (*Canis latrans*). *Advances in the Study of Behavior*, **16**, 251–338.
- Berry O, Tocher MD, Sarre SD (2004) Can assignment tests measure dispersal? *Molecular Ecology*, **13**, 551–561.
- Boutin-Ganache I, Raposo M, Raymond M *et al.* (2001) M13-tailed primers improve the readability and usability of microsatellite analyses performed with two different allele-sizing methods. *BioTechniques*, **31**, 24–26.
- Breen M, Jouquand S, Renier C *et al.* (2001) Chromosome-specific single-locus FISH probes allow anchorage of an 1800-marker integrated radiation-hybrid/linkage map of the domestic dog genome to all chromosomes. *Genome Research*, **11**, 1784–1795.
- Brown JH, Kodric-Brown A (1977) Turnover rates in insular biogeography: Effect of immigration on extinction. *Ecology*, **58**, 445–449.
- Ernest HB, Penedo MCT, May BP *et al.* (2000) Molecular tracking of mountain lions in the Yosemite Valley region in California: genetic analysis using microsatellites and faecal DNA. *Molecular Ecology*, **9**, 433–441.



- Excoffier L (2004) *SIMCOAL* (version 2). Available at <http://cmpg.unibe.ch/software/simcoal2> via the internet.
- Forman RTT (2003) *Road Ecology: Science and Solutions*. Island Press, Washington, DC.
- Forman RTT, Alexander LE (1998) Roads and their major ecological effects. *Annual Review of Ecology and Systematics*, **29**, 207–231.
- Francisco LV, Langston AA, Mellersh CS *et al.* (1996) A class of highly polymorphic tetranucleotide repeats for canine genetic mapping. *Mammalian Genome*, **7**, 359–362.
- Franklin J (1997) Santa Monica Mountains National Recreation Area Vegetation Communities. National Park Service, Santa Monica Mountains NRA, Thousand Oaks, CA.
- Geffen E, Anderson MJ, Wayne RK (2004) Climate and habitat barriers to dispersal in the highly mobile grey wolf. *Molecular Ecology*, **13**, 2481–2490.
- Gerlach G, Musolf K (2000) Fragmentation of landscape as a cause for genetic subdivision in voles. *Conservation Biology*, **14**, 1066–1074.
- Gese EM, Rongstad EJ, Mytton WR (1989) Population dynamics of coyotes in southeastern Colorado. *Journal of Wildlife Management*, **53**, 174–181.
- Goodnight KF (2005) *KINSHIP* (version 1.3.1) Available at [www.gsoftnet.us/GSoft.html](http://www.gsoftnet.us/GSoft.html).
- Goudet J (2002) *FSTAT* version 2.9.3.2. Available from Jerome.goudet@ie.zea.unil.ch, via email. Institute of Ecology, UNIL, CH-1015, Lausanne, Switzerland.
- Hanski I, Moilenen A, Gyllenberg M (1996) Minimum viable metapopulation size. *American Naturalist*, **147**, 527–541.
- Keller I, Largiadér CR (2003) Recent habitat fragmentation caused by major roads leads to reduction of gene flow and loss of genetic variability in ground beetles. *Proceedings of the Royal Society of London, Series B, Biological Sciences*, **270**, 417–423.
- Knick ST, Britnell JD, Sweeney SJ (1985) Population characteristics of bobcats (*Felis rufus*) in Washington State, USA. *Journal of Wildlife Management*, **49**, 721–728.
- Liberg O, Andrén H, Pedersen H *et al.* (2005) Severe inbreeding depression in a wild wolf (*Canis lupus*) population. *Biology Letters*, **1**, 17–20.
- Menotti-Raymond M, David VA, Lyons LA *et al.* (1999) A genetic linkage map of microsatellites in the domestic cat (*Felis catus*). *Genomics*, **57**, 9–23.
- Mills LS, Allendorf FW (1996) The one-migrant-per-generation rule in conservation and management. *Conservation Biology*, **10**, 1509–1518.
- Ng SJ, Dole JW, Sauvajot RM *et al.* (2004) Use of freeway undercrossings by wildlife in a fragmented urban landscape in southern California. *Biological Conservation*, **115**, 499–507.
- O'Brien SJ, Roelke ME, Marker L *et al.* (1985) Genetic basis for species vulnerability in the cheetah. *Science*, **227**, 1428–1434.
- Penrod K, Hunter R, Merrifield M (2001) *Missing Linkages: Restoring Connectivity to the California Landscape*. South Coast Wildlands Project, Los Angeles.
- Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics*, **155**, 945–959.
- Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. *Evolution*, **43**, 258–275.
- Raymond M, Rousset F (1995) *GENEPOP* (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity*, **86**, 248–249.
- Reh W, Seiz A (1990) The influence of land use on the genetic structure of populations of the common frog *Rana temporaria*. *Biological Conservation*, **54**, 239–249.
- Rice WR (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.
- Riley SPD, Sauvajot RM, Kamradt D *et al.* (2003) Effects of urbanization and fragmentation on bobcats and coyotes in southern California. *Conservation Biology*, **17**, 566–576.
- Riley SPD, Foley JE, Chomel BB (2004) Exposure to feline and canine pathogens in bobcats and gray foxes in urban and rural zones of a national park in California. *Journal of Wildlife Diseases*, **40**, 11–22.
- Roelke ME, Martensen JS, O'Brien SJ (1993) The consequences of demographic reduction and genetic depletion in the endangered Florida Panther. *Current Biology*, **3**, 340–349.
- Roy MS, Geffen E, Smith D *et al.* (1994) Patterns of differentiation and hybridization in North American wolflike canids, revealed by analysis of microsatellite loci. *Molecular Biology and Evolution*, **11**, 553–570.
- Saccheri I, Kuussaari M, Kankare M *et al.* (1998) Inbreeding and extinction in a butterfly population. *Nature*, **392**, 491–494.
- Sacks BN, Brown SK, Ernest HB (2004) Population structure of California coyotes corresponds to habitat-specific breaks and illuminates species history. *Molecular Ecology*, **13**, 1265–1275.
- Schneider S, Roessli D, Excoffier L (2000). *ARLEQUIN. A software for population genetics data analysis*. Genetics and Biometry Laboratory, Department of Anthropology, University of Geneva, Geneva, Switzerland.
- Spielman D, Brook BW, Frankham R (2004) Most species are not driven to extinction before genetics factors impact them. *Proceedings of the National Academy of Sciences, USA*, **101**, 15261–15264.
- Sunquist ME, Sunquist F (2001) Changing landscapes: consequences for carnivores. In: *Carnivore Conservation* (ed. Gittleman JL, Funk SM, MacDonald D, Wayne RK), pp. 399–418. Cambridge University Press, Cambridge.
- Van Vuren DH (1998) Mammalian dispersal and reserve design. In: *Behavioral Ecology and Conservation Biology* (ed. Caro T), pp. 369–393. Oxford University Press, Oxford.
- Vila C, Sundqvist A-K, Flagstad O *et al.* (2003) Rescue of a severely bottlenecked wolf (*Canis lupus*) population by a single immigrant. *Proceedings of the Royal Society of London, Series B, Biological Sciences*, **270**, 91–97.
- Vucetich JA, Waite TA (2000) Is one migrant per generation sufficient for genetic management of fluctuating populations? *Animal Conservation*, **3**, 261–266.
- Westemeier RL, Brawn JD, Simpson SA *et al.* (1998) Tracking the long-term decline and recovery of an isolated population. *Science*, **282**, 1695–1698.
- Wright S (1921) Systems of mating. *Genetics*, **6**, 111–178.

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