Genetic connectivity detected in two sympatric canids across highways with different usage intensities in California.

ABSTRACT

Roads networks may have profound impacts on the viability of wildlife populations. Highways can be barriers to wildlife movement, leading to genetic diversity loss, inbreeding, and increased extinction risk for small, isolated populations on either side. Differences in highway characteristic, particularly environmental disturbances tied to higher traffic rates, can increase wildlife avoidance of highways. The effects that highways have on wildlife movement can be variable, dependent on the unique disturbance tolerance of individual species. In this study, we examined genetic connectivity relative to traffic rates in coyote populations adjacent to highways with different usage intensities. Additionally, we examine how genetic connectivity across a highway varied between two species, coyote and gray fox. These species are biologically similar but differ in ecological specialization and tolerance for disturbance. We collected canid scat samples for genetic analysis from opposite sides of I-580 and I-680 in the East Bay region of the San Francisco Bay Area and I-80, US 50, SR49 and SR 20 in the Sierra Nevada foothills. East Bay highways have high traffic rates (115,000-260,000 annual average daily traffic) while the Sierra Nevada foothill highways range from high to lower traffic rates (3,000-258,000 annual average daily traffic). Allelic richness and heterozygosity were high for both species at all sampling sites. We found little evidence of contemporary genetic structure based on FST across focal highways for either species, suggesting that these highways may be permeable to dispersal. The low levels of genetic differentiation that we did observe was driven by family structure within sampled populations. Signals of decreased dispersal may take longer to manifest in large, genetically diverse populations. Increased vehicle traffic on highways in the study regions have only recently become high. As human populations in the focal areas are projected to increase, along with associated intensification of traffic rates, maintaining genetic connectivity across highways for species will become increasingly important, especially for species with small population sizes, low diversity, or that are less vagile.

INTRODUCTION

While facilitating connectivity for human activities, road networks have the potential to reduce connectivity of wildlife populations and negatively impact their viability. Habitat loss through direct conversion into road or the alteration of abiotic conditions of the landscape adjacent to a roadway can negatively impact habitat use. Reductions in dispersal through direct mortality or avoidance of roadway environments may serve to further disrupt gene flow between population fragments (Jaeger et al. 2005, Coffin 2007, Taylor and Goldingay 2010). Demographic stochasticity, genetic diversity loss, and inbreeding depression are intensified in isolated population fragments, increasing the risk of local extinction (Lande 1988, Frankham 1996, Epps et al. 2005, Holderegger and Di Giulio 2010).

Overpasses or undercrossings may be installed to restore natural gene flow patterns. However, to effectively plan these and other mitigation activities, transportation agencies must know which roads to target, whether existing structures are providing wildlife passage, and which species are most affected. The impacts of roads on wildlife connectivity are challenging to generalize in space and among taxa, complicating mitigation planning. Physical characteristics of roads (e.g. width, gradient, and traffic volume) can affect their permeability to different species (Marsh et al. 2005, Charry and Jones 2009). Traffic volume in particular has been shown to be an important determinant of wildlife response to roads (Jaeger et al. 2005). In highways through the Canadian Rocky Mountains, including the Trans-Canada Highway, sections of low to moderate traffic were more permeable to carnivore passage than sections of high traffic (Alexander et al. 2005). Annual average daily traffic volumes (AADT) as low as 10,000 vehicles per day can create nearly complete barriers to wildlife movements (Charry and Jones 2009).

The degree to which wildlife move across roads depends upon species-specific dispersal behaviors and life history characteristics. Some taxa, such as reptiles, amphibians, and mid- to large sized mammals, are particularly impacted by roads (Fahrig and Rytwinski 2009, Benítez-López et al. 2010). Even within sensitive species there are differences in behavioral responses to roads. Disturbance tolerance is thought to be one characteristic that determines a species’ sensitivity to roads. For example, the Trans-Canada Highway acts as a barrier for grizzly bear (*Ursus arctos*) but not for black bear (*Ursus americanus*) movement, likely because grizzly bears tend to avoid human activity and therefore approach roads less often (Sawaya et al. 2014). Perception of risks not tied directly to human activities can influence behavior for organisms faced with crossing a road surface. For example, smaller snake species more vulnerable to avian predation were more likely to avoid open habitat created by roads than larger snakes (Andrews and Gibbons 2005).

Within California, highways have been documented as significant barriers to gene flow for numerous taxa (Riley et al. 2006, Delaney et al. 2010, Riley et al. 2014b). These studies have focused on the Southern California region, which hosts highly urbanized counties with heavily trafficked highway networks. Few studies have investigated whether highways in Northern California, where urban centers are more discrete, have a similar impact on wildlife gene flow. Urbanization approaches levels observed in Southern California primarily in the greater Bay Area and Sacramento regions.

The ability to maintain population connectivity across California highways is related to a species’ willingness to utilize edge habitats, such as those alongside roadways. Habitat generalists, like coyotes (*Canis latrans*) and deer (*Odocoileus* spp) may be less affected by roads than habitat specialists, such as gray foxes (*Urocyon cinereoargenteus*), which in California tend to be tied to large patches of mid-elevation scrub-lands (Harrison 1997, Fedriani et al. 2000, Farias et al. 2005, Sacks et al. 2005, 2008, Kowalski et al. 2015). We examined the genetic diversity present in populations of coyote alongside major highways in the East Bay and Sierra Nevada foothill regions, and gray fox populations adjacent to two highways in the Sierra Nevada foothills to examine how highways affect genetic connectivity. These two species have been documented inhabiting urban settings and have similar reproductive phenology, dispersal timing, territoriality, and diet, differing mainly in the degree of habitat specialization (Harrison 1997, Riley 2006, Kapfer and Kirk 2012, Lombardi et al. 2017). Additionally, both species are abundant and leave conspicuous scats that can be collected for genetic analysis.

In this study, we …We predicted that 1) highways pose a barrier to gene flow for both species, 2) disruption in genetic connectivity increases with increasing traffic volumes, and 3) genetic connectivity across highways is reduced more in gray fox than in coyote.

METHODS

Study Highways and Regions

We studied coyotes separated by Interstates 680 (I-680) and 580 (I-580) in the inland valleys of the East Bay region of the Bay area (Figure 1), and Interstate 80 (I-80) and U.S Route 50 (US 50) in the lower Sierra Nevada foothills (Figure 2). These are 6-10 lane highways, with central median barriers, and are heavily trafficked. East Bay highways were travelled by >180,000 vehicles daily within a heavily populated urban and suburban matrix. The Sierra Nevada foothills traffic volumes ranged from >140,000 vehicles/day in the southern section to 65,000 vehicles/day in the northern, more rural region of our study area (Caltrans 2015). The southern portion of the study area is comprised of an urban matrix surrounding Sacramento with human population densities decreasing as the highways travel east and north from the city.

Additionally, we studied both coyote and gray fox populations separated by State Route 49 (SR 49) and 20 (SR 20) between the cities of Auburn and Grass Valley in the Sierra Nevada Foothills (Figure 3). These are 2 lane, undivided highways, traveled by 2000-40,000 vehicles daily (Caltrans 2015). The landscape flanking these state routes was comprised of a mixture of urban, suburban, suburban-rural, and rural land. Urban centers are concentrated around the cities of Auburn, Grass Valley and Nevada City. The density of human habitation decreases with distance from the city centers. Rural land use ranges from agriculture (vineyards and grazing), to commercial timber and managed forest operated by land trusts and the US Forest Service.

Although both the East Bay and Sacramento regions are highly developed, gray fox and coyotes have been shown to inhabit urban and suburban habitats and therefore development alone is not likely to act as a barrier to dispersal (Atkinson and Shackleton 1991, Harrison 1997, Grinder and Krausman 2001, Crooks 2002, Rountree III 2004, Riley 2006, Grubbs and Krausman 2009). Therefore, the highways are the only major human generated landscape feature likely to disrupt gene flow. In addition to the presence of the study highways in the Sierra Nevada foothills, the American River mainstem and the North Fork American River run through the center of the study region and may serve as dispersal barriers.

Molecular Methods

Sample collection and DNA Extraction

Sampling was conducted in open space and parkland in regions within 10 km of the study highways or along road transects within 13 km of the SR 20 and SR 49. We collected mesocarnivoremesocarnivore fecal samples (scat) along road transects in the study area from November 2014 to August 2015 and February to November 2016. A fraction of each scat was preserved in 95% ethanol in the field for later DNA extraction. In addition, we obtained tissue samples from road-killed coyote and gray fox observed along road transects. GPS points recorded the exact location where each sample was collected. Fecal samples were stored at 4 ⁰C upon return to the lab. DNA was extracted using the QIAamp Mini Stool Kit (QIAGEN). To minimize opportunities for contamination, all extractions were done in a laboratory isolated from post-PCR products and lab benchtops were bleached before and after fecal samples were handled.

Species Identification and Genotyping

Samples were identified to the species level by sequencing a 354-bp locus of the cytochrome b region of the mitochondrial DNA in the forward direction using previously published primers and protocols (RF14724, RF15149; Perrine et al. 2007). Cytochrome b is a region of mitochondrial DNA commonly used to distinguish between mammal species (Bidlack et al. 2007, Statham et al. 2012). All samples identified as non-target species (e.g. bobcat, skunk) were archived for future study. Samples confirmed to have originated from coyote were genotyped using 13 microsatellite loci optimized for use with coyote fecal DNA: AHT137, AHT142, AHTh171, CPH11, CPH18, CXX279, CXX374, CXX468, CXX602, INU055, REN54P11, REN162C04, and REN169O18 (Quinn and Sacks 2014). Those samples identified as originating from gray fox were genotyped using 13 microsatellite loci optimized for use with gray fox fecal DNA: AHT142, AHTh171, CPH18, CPH8, FH2004, FH2010, FH2088, INU055, REN105L03, REN162C04, REN54P11, RF2001Fam, and RFCPH2 (Moore et al. 2010). Microsatellite loci were multiplexed using the QIAGEN Multiplex PCR Kit (QIAGEN) with two multiplexes containing 7 loci each. Two microliters of PCR product were combined with 9.5 μl of highly deionized formamide and 0.5μl of Genescan 500 LIZ size standard (Thermo Fisher Scientific; Thermo). Fragment analysis was performed on an ABI PRISM 3730 DNA Analyzer (Thermo) and alleles were scored with STRand 2.4.110 software (Toonen and Hughes 2001). Negative PCR controls were included with each PCR to detect contamination. Samples were genotyped three times at each locus to detect and correct for allelic dropout and other genotyping errors commonly encountered when working with degraded samples (Waits and Paetkau 2005). Only samples with >85% complete genotypes were used for genetic analysis. The R package Allelematch (Galpern et al. 2012) was used with these samples to identify unique genotypes and remove duplicates.

Data Analysis

Before any analyses were conducted, microsatellite loci were tested for conformance to Hardy-Weinberg equilibrium and linkage equilibrium using GenAlEx version 6.502 (Peakall and Smouse 2006, 2012) using sequential Holms-Bonferroni corrections to account for multiple comparisons (Rice 1989). We used side of focal highway as locations for samples collected for these and later analyses (Figures 1-3). We then examined genetic diversity within and among canid populations in our study areas by calculating the number of alleles, allelic richness, expected and observed heterozygosity (He, Ho) in GenAlEx. Because small sample sizes can negatively bias genetic diversity estimates, we did a rarefaction analysis in HP-Rare (Kalinowski 2005) to develop estimates of allelic richness rarefied to the smallest sample sizes. Additionally, we estimated pairwise relatedness (r) among coyotes in GenAlEx to identify close relatives (first and second-order) in our dataset and compared these relationships within and among sampling locations.

We used STRUCTURE version 2.3.4 (Pritchard et al. 2000) to examine how genetic diversity was partitioned across our sampling locations. STRUCTURE, a Bayesian clustering algorithm, inferred the most likely number of populations of coyote and gray fox in the study areas. Since our sampling was conducted on a relatively fine scale for wide-ranging species, we expected population structuring to be weak, even if the focal highways were significant barriers to gene flow. Therefore, we used the Hubisz et al. (2009) LOCPRIOR model that improves STRUCTURE’s ability to detect weak population structure by using geographic sampling location as a prior. For this purpose, we divided samples into units separated by focal highways (Figures 1-3). We used the population admixture model with correlated allele frequencies (Falush et al. 2003). The correlated allele frequency model assumes some level of background correlation in allele frequencies between populations due to recent common ancestry and is therefore better at detecting subtle changes that occur in recently diverged populations. Each run consisted of 100,000 Markov chain Monte Carlo iterations following a burn-in period of 1,000,000 iterations. We tested the likelihood of K=1 through K=5 for the East Bay and K=1 through K=6 for the Sierra Nevada foothills datasets, where K is the number of discrete genetic clusters assumed. Ten replicates were conducted for each K. We selected the best-supported K by examining plots of the mean likelihood value ln Pr(X|K) and calculating ∆K (Evanno et al. 2005) in STRUCTURE HARVESTER (Earl and vonHoldt 2011). The program CLUMPAK (Kopelman et al. 2015) was used to compile individual assignments across replicates and we used custom R code implemented in the ggplot2 package to create bar plots to visualize results (Wickham 2016).

We examined population genetic structure by estimating pairwise FST values (a measure of genetic differentiation) among sampling locations in an AMOVA framework in GenAlEx. Significance of pairwise FST values was determined through 9,999 permutations. We calculated Nei’s genetic distance (Nei 1972, 1978) among sampling locations in GenAlEx. Nei’s genetic distance matrix was paired with a geographic distance matrix to test for isolation by distance (IBD), which occurs when genetic distance between sampling locations increases with geographic distance. Geographical distance was calculated as the Euclidean distance between locations where pairs of individuals were sampled, recorded as GPS points (decimal latitude and longitude). For individuals that were detected twice, we used two averaged locations to represent their detection center. The relationship between genetic and geographic distance in our study areas was assessed with Mantel tests in the R package VEGAN (Dixon 2003).

To determine whether the study highways or traffic rates had a significant effect on genetic distance between sampling locations, we measured the resistance distance between sampling locations using CIRCUITSCAPE v4.0.5 (McRae 2006). In order to test whether highway generally formed barriers to gene flow, all highway cells were coded as high resistance with all other landscape cells treated as low resistance (Resistance = 1). To test whether the strength of highway as barriers were related to traffic volumes, we generated a resistance surface based on traffic rates, sections of highway were binned into ten different traffic intensities based on Caltrans Annual Average Daily Traffic (AADT) counts from below 10,000 to over 202,000 (Caltrans). Highway cells within each section were then coded based on which traffic rate bin they fell within. Connectivity between two points are assessed along all possible pathways based on an eight-neighbor connection method to generate an average resistance between points. Support for each resistance surface was then evaluated by fitting linear mixed-effects models using the maximum-likelihood population effects (MLPE) parameterization in the R package ResistanceGA to account for the nonindependence of values within pairwise distance matrices (Clarke et al. 2002, van Strien et al. 2012, Peterman et al. 2014, Peterman 2018).

RESULTS

Sample collection and species identification

We collected a total of 251 and 327 mesocarnivore scats from our hiking and road transects respectively. The species identification test revealed that 190 of these samples originated from coyote and 213 of these samples were from gray fox. We were able to obtain high quality genotypes (data at >85% of loci) for 102 coyote and 90 gray fox samples (Table 1). Of these, 97 and 60 were unique coyote and gray fox genotypes, respectively. Coyote samples were distributed equally on either side of study highways SR 49, I-80, and I-680. For I-580 in the East Bay region, individuals were largely located north of the highway, while in the Sierra Nevada foothills region, few samples were located south of US 50, in both cases due to poor access for sampling. In gray fox, there were 37 individuals on the east side of SR 49 (East SR 49) and 20 individuals on the west side of SR 49 (West SR 49).

Genetic Diversity

We observed no deviation from linkage equilibrium at any pair of loci after implementing the sequential Bonferroni correction (alpha = 0.0039). However, eight loci each were out of Hardy Weinberg equilibrium (HWE) for coyote in at least one population (AHT137, CXX374, CXX468, CPH11, CPH18, REN54P11, CXX279, and REN162C04). For gray fox eight loci were also out of HWE, four of which occurred in both populations (CHP8, RFCHP2, FH2088, FH2004, AHTh171, FH2010, CXX402 and RF2001). For both species this was likely due to family structure in our samples (see below).

All sampling regions showed high levels of genetic diversity. The total number of alleles observed for coyotes within sampling locations ranged from 54-99 (East Bay), 37-108 (Sierra Nevada foothills), 91 (East SR 49), and 75 (West SR 49). For gray fox, the number of alleles observed ranged from 96 (East SR 49) to 85 (West SR 49). When rarefaction was conducted for each of the sampling regions, coyote allelic richness ranged from 3.5-3.9 in the East Bay and 2.8-4.8 in the Sierra Nevada foothills (Table 1). Gray fox showed a similar pattern, with an allelic richness of 7.29 for East SR 49 and 6.36 for West SR 49 sampling locations. Measures of Ho and He (estimates of gene diversity in a population) were high in all regions for both species. Coyote Ho ranged from 0.60-0.72 in the East Bay, 0.62-0.88 in the Sierra Nevada foothills, and 0.70-0.73 around SR 49 and 0.60 in gray fox on both sides of SR 49 (Table 1).

Mean pairwise relatedness values (r) within sampling locations for coyotes showed that most individuals were not closely related (mean r = 0.08-0.09 in the East Bay, 0.03-0.24 in Sierra Nevada foothills). However, some first order relationships (parent-offspring, full siblings, r ~0.50) were detected within the East Bay, all from West I-680. Second order relationships (grandparent-grandchild, half-siblings, r~0.25) were also detected, primarily from West I-680 (18 pairs) and one pair from East I-680. Within the Sierra Nevada foothills region, first order relationships were detected for two pairs, one on each side of SR 49. Second order relationships were also detected for four pairs, one each from South US-50 and South I-80, while the remaining two came from South I-80-North US-50. In all cases, the pair of individuals exhibiting these family relationships were sampled along the same side of the highways (Figure 4A).

For gray fox the mean pairwise relatedness value was 0.11 for both sides of the highway. Within East SR 49, second order relationships were detected for 25 pairs, while West SR 49 contained 7 pairs of second order relatedness. First order relatedness scores were recorded for 5 pairs within the East SR 49 and 2 in the West SR 49. Additionally, one first order pair (r = 0.54) was sampled on opposite sides of the highway, 9km apart. All other pairs of individuals were sampled on the same side of the highway (Figure 5A).

Genetic Connectivity

STRUCTURE revealed two genetic clusters in the East Bay (mean ln Pr(X|K) = -1226.13; Figure 4B). One cluster consisted of 11 individuals from the West I-680 sampling location while the second cluster contained individuals from all three sampling locations. Relatedness within the West I-680 sampling locations was high with a mean relatedness score of 0.10 for individuals sampled. Examining relatedness by cluster showed that the cluster comprised of individuals from all three sampling locations had a mean relatedness score of 0.05. The other cluster with individuals only from W I-680 had a mean relatedness score of 0.12. These higher values were driven by the presence of multiple first order relatives.

For coyotes within the Sierra Nevada foothills, two and four genetic clusters were best supported (mean ln Pr(X|K) = -3295.63 and -2971.70). In both the K = 2 and K = 4 scenarios revealed by STRUCTURE, neither cluster was associated with side of highway and there was no clear pattern associated with cluster assignment and sampling location, suggesting that K = 1 is more likely (Figure 6).

Within the gray fox samples, two genetic clusters were most likely (mean ln Pr(X|K) = -2059.78), with eight individuals split into a separate subpopulation (K1) (Figure 5B). Individuals within K1 were found throughout the study area, including on opposite sides of SR 49 (Figure 3). When we examined relatedness within K1, however, we found that the average relatedness value was 0.20 compared with a value of 0.09 for the cluster containing the other 49 individuals. All individuals within the K1 cluster have a second order relationship with at least one other group member. Three of the pairs within the group are first order relationships (r = 0.58-0.62).

Pairwise FST values, estimating genetic differentiation between sampling locations relative to sides of the highway, were not significant for either species, with two exceptions for coyotes. In coyotes, FST within each region was low, with significant differentiation only seen across highway I-80 for populations sampled adjacent to SR 49 (Table 2). The pairwise FST between the gray fox for side of highway was zero (FST = 0, p = 0.425). When examined for the K1 and K2 clusters pairwise FST was 0.34 (P = 0.001).

Mantel tests revealed no association between genetic and geographic distance for gray fox or coyotes in the Sierra Nevada foothills (r = 0.03, p = 0.24; r = -0.02, p = 0.58, respectively), but there was a weak association observed in the East Bay (r = 0.16, p = 0.05). The null low resistance landscape model performed as the top model in the East Bay region, where as in the Sierra Nevada foothills, the high resistance highway model performed better than the null model. None of the highway models met the criterion to be considered significantly better than the null (delta AIC >2). (Table 3).

DISCUSSION

Highways have the potential to disrupt connectivity of wildlife populations, acting as a partial or total dispersal barrier for even wide-ranging species. Populations fragmented by roads over time can exhibit genetic differentiation due to a lack of gene flow via dispersal (Riley et al. 2006, 2014a, Sawaya et al. 2014). Barrier permeability is tied to an organism’s perception of risk and tolerance for disturbance (Clevenger and Waltho 2005, Jaeger et al. 2005, Ferris 2017). Highways present a landscape feature with many associated environmental disturbances, producing light, noise, and movement beyond the range typically encountered by organisms in a natural environment. Tolerance to high levels of disturbance can increase the connectivity of species across highway barriers. Those species that are disturbance averse or more sensitive to edge and open habitats are more at risk of experiencing disruptions in dispersal imposed by road networks. The aim of this study was to determine whether highways disrupt coyote gene flow in the East Bay and the Sierra Nevada foothills. Additionally, tolerance for disturbance was examined by comparing genetic connectivity within gray fox and coyote populations across SR 49.

We found that coyote and gray fox populations within the study regions were genetically diverse, with high heterozygosity and allelic richness for all sampling locations. These results are in line with other findings of canid genetic diversity throughout California (Sacks et al. 2008, Deyoung et al. 2009). High levels of genetic diversity suggest that both study regions support genetically healthy populations of both species.

If highways were disrupting gene flow, we would expect to see distinct populations corresponding to each side of the study highway. For example, if the East Bay highways were impermeable to gene flow, we would expect to see three distinct populations of coyote, corresponding to the west side of I-680, the east side of I-680, and south of I-580/west of I-680 (Figure 3). In contrast, Structure analysis found only two genetic clusters in the East Bay and they did not correspond to opposite sides of the highways (Figure 2). Similarly, gray fox showed no evidence of genetic structuring relative to side of SR 49, with members of each cluster spanning both sides of the highway. These genetic clusters identified by Structure corresponded to family groups rather than side of highway, based on estimates of pairwise relatedness. The presence of close relatives in a sample can create spurious patterns of population structure and create Hardy Weinberg disequilibrium (Rodríguez-Ramilo and Wang 2012). The fact that we found no significant genetic differentiation due to highways within the East Bay study region supports our conclusion that the finding of two genetic clusters is an artifact of having close relatives in W680 sample location. It is unlikely that any of the focal highways form a complete barrier to movement for either species. There was no genetic structuring for coyotes or gray foxes across SR 49. Direct dispersal was observed for gray fox across SR 49. One pair of second order relatives for gray fox were detected across SR 49 from each other, separated by approximately nine kilometers. Even for the more heavily trafficked highways in the East Bay and Sacramento regions (AADT average >180,000 and >84,000, respectively), coyotes exhibited no evidence of genetic structuring relative to side of highway.

In the Sierra Nevada foothills study area, both STRUCTURE and pairwise FST analysis indicated presence of only a single coyote population. This result was unexpected because an extensive network of camera traps in the study area has not observed coyotes using crossing structures in the Sierra Nevada foothills study area (F. Shilling, personal communication). However, camera traps have revealed that coyotes are using higher elevation crossing structures (northeast of our study area), (F. Shilling, personal communication). These coyotes could be migrating into and reproducing in the study area. Additionally, the American River bike trail, which follows a riparian corridor along the Sacramento River from Folsom Lake into the city of Sacramento, may provide passage across I-80.

These results contrast with a genetic study conducted in Southern California which found that the Ventura freeway was a significant barrier to gene flow in coyote (Riley et al. 2006). It is possible that the highly urbanized environment of Los Angeles imposes additional constraints on coyote movements that are not present in the regions of Northern California studied. In the Ventura freeway study, coyotes were able to cross the highway, but authors suggested that migrants could not breed successfully due to territorial conflicts (Riley et al. 2006). There is more habitat available in both study areas in comparison to the regions examined around Southern California. Sacramento hosts a chain of parks connected by a riparian corridor that further connects to several U.S. National Forests north of the highly urbanized city of Sacramento. The East Bay inland valleys are ringed by a network of land trusts and parks in the Diablo Range. In both regions, migrants may be able to acquire territory and reproduce which would reduce signals of population isolation.

As coyotes showed no evidence of genetic structuring across large, highly trafficked highways, it was expected that this adaptable species would exhibit genetic connectivity across a smaller highway with a lower average traffic rate (AADT <20,300). In comparison, gray fox populations were expected to show evidence of disrupted genetic connectivity due to the presence of SR 49. Whereas coyotes have been shown to inhabit a wide range of human-modified habitats, including heavily urbanized cities, gray fox, tend to be more elusive, and less frequently observed in heavily modified human settings (Lombardi et al. 2017).

Interestingly, pairwise relatedness analyses showed that almost all detections of related individuals for gray fox were clustered on the same side of the highway. The same pattern was observed for coyote in the East Bay. Coyotes sometimes exhibit delayed dispersal of individuals from their natal habitat, where individuals stay with the parental pair and help with rearing siblings (Harrison 1992). Delayed dispersal and helper offspring occur in other fox species, has been proposed but not directly observed in gray fox (Geffen et al. 1996, Weston Glenn et al. 2009). This behavior may increase the chance of sampling family groups. Additionally, Riley (2006) showed that younger dispersers hold the territories adjacent to highways, with these being smaller and denser than territories away from highways. This pile up of dispersers along highway edges may also contribute to increased relatedness observed in a single season.

While divergence between populations for either species was not observed, traffic rates may be negatively impacting dispersal. Landscape resistance models indicated that traffic rates may be starting to reach levels that could reduce gene flow between populations of coyotes sampled on opposite sides of highways in the East Bay. Traffic rates in the East Bay were markedly higher than those observed in the Sierra Nevada foothills, with all highway segments of I-680 and I-580 having traffic rates of greater than 106,000 average vehicles daily.

Unexpectedly, gray fox samples were encountered more frequently than coyote in the SR 49 road transects. Contrary to our expectations, we found that gray fox were distributed throughout the entire study area, even in urban areas such as Auburn. Grays foxes tend to be most abundant in places where potential predators (coyotes, bobcats) are less abundant (Fedriani et al. 2000, Farias et al. 2005, Temple et al. 2010). Competitive exclusion by the presence of coyote has been linked to increased use of urban habitats in gray fox (Crooks and Soulé 1999, Riley 2006). Prevalence of coyote within the urban centers along SR 49 may explain the lower detection rates of gray fox in these cities. Contrastingly, the high detection rates of gray fox in the suburban and suburban-rural matrix might be the due to the proportion of private lands (lower detection probability) and human wildlife conflict (Poessel et al. 2017) resulting in lower abundances of coyotes.

Anthropogenic change to the environment occurs often over short time scales. As an example, construction of Interstate 5 through California, a 2,222 km stretch of highway running the length of the state, was completed in 25 years. How these changes impact the health and stability of populations is of increasing importance, particularly in the face of increased anthropogenic landscape alteration. Unfortunately, genetic stability within a population after a disturbance is not immediate, requiring time for the population to reach a new equilibrium (Landguth and Cushman 2010). This creates a disconnect between the event that can change the genetic composition of a population and the time until this change is able to be detected in what is referred to as a time lag (Epps and Keyghobadi 2015).

Several factors, both inherent to the focal species and the system, influence the length of a time lag. For example, the permeability of a barrier between bisected populations determines the rate of gene flow. Within a species, factors such as generation time, dispersal distances, population size and genetic variability of the population at the break of connectivity all play a role in time lags (Epps and Keyghobadi 2015). For small populations, those with short generation time, or those that have low initial genetic variation, time lags will be short and signals of disrupted gene flow will manifest quickly. For species like gray fox and coyote, which have large populations, high genetic variability, are capable of long-range dispersal, and have relatively long generation times, detection of a disruption in genetic connectivity may take many generations to manifest.

Our findings suggest that all focal highways in this study are not barriers to coyote gene flow and that SR 49 is a permeable barrier to dispersal for gray fox, although a time lag effect may be present. The intensity of use for these highways is more recent than their appearance on the landscape. Interstate 80, for example, was designated for construction in 1956. This highway was widened to its current six to eight lane width in Sacramento in 1973 and again in 2011 to accommodate increased traffic densities generated by growing populations in Sacramento (Faigin 2020). The more rural Sierra Nevada foothills have also experienced tremendous growth over the previous two decades, between 7-11% for various cities within Placer County’s foothill region (Center for Strategic Econimic Research 2014). Following the trend of increased human populations, the AADT for this section of SR 49 has seen steady increases in traffic volumes. Within the study region, there is an increase in the proportions of segments that are under moderate to high traffic volumes. Looking forward, the cities within the Sierra Nevada foothills are projected to see an additional growth of 17.3%, which will result in a corresponding increase in traffic volumes. Currently dispersal may not be limited for either of these species across highways, but these landscape features are likely a much more serious obstacle to genetic connectivity in small and less vagile species. While current road use patterns appear to still allow the passage of both gray fox and coyote, this pattern may not persist with projected increased vehicle use.

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Table 1 – Genetic diversity summary statistics for coyotes and gray fox

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Species | Sampling Location | N | AT | AL | AR | Ho | He | %P |
| Coyote | East Bay | 30 | 113 | 8.9 | 8.8 | 0.63 | 0.76 | 100 |
|  | East I-680 | 6 | 55 | 4.2 | 3.5 | 0.72 | 0.62 | 100 |
|  | West I-680 | 20 | 99 | 7.6 | 3.9 | 0.60 | 0.73 | 100 |
|  | South I-580 | 4 | 54 | 4.2 | 3.9 | 0.69 | 0.66 | 100 |
|  | Sierra Nevada foothills | 67 | 152 | 11.9 | 10.2 | 0.72 | 0.82 | 100 |
|  | North I-80 | 9 | 83 | 6.4 | 4.3 | 0.62 | 0.75 | 100 |
|  | East SR 49 | 11 | 105 | 8.1 | 4.8 | 0.74 | 0.82 | 100 |
|  | West SR 49 | 8 | 80 | 6.2 | 4.3 | 0.74 | 0.76 | 100 |
|  | South US-50 | 3 | 37 | 2.9 | 2.8 | 0.89 | 0.59 | 100 |
|  | South I-80 | 3 | 36 | 2.8 | 2.8 | 0.81 | 0.56 | 100 |
|  | S I-80-N US-50 | 33 | 106 | 8.2 | 4.1 | 0.71 | 0.78 | 100 |
| Gray fox | East SR 49 | 37 | 96 | 7.4 | 7.29 | 0.60 | 0.71 | 100 |
|  | West SR 49 | 20 | 85 | 6.5 | 6.36 | 0.60 | 0.65 | 100 |
| N = sample size.  AT = total number of alleles  AL= mean number of alleles per locus.  AR = allelic richness, standardized to sample size.  Ho = observed heterozygosity.  He = expected heterozygosity.  %P = percent polymorphic loci. | | | | | | | | |

Table 2 – Pairwise FST values for the Bay Area (BA) and Sierra Nevada Foothills (SNF) sampling locations. P values are above the diagonal. Sequential Bonferroni corrected alpha = 0.0167.

|  |  |  |  |
| --- | --- | --- | --- |
| East Bay | East I-680 | West I-680 | South I-580 |
| East I-680 | 0 | 0.174 | 0.174 |
| West I-680 | 0.058 | 0 | 0.174 |
| South I-580 | 0.131 | 0.064 | 0 |

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Sierra Nevada foothills | North I-80 | East SR 49 | West SR 49 | South US-50 | South I-80 | South I-80-North US-50 |
| North I-80 | 0 | 0.399 | 0.052 | 0.399 | 0.399 | 0.385 |
| East SR 49 | 0.007 | 0 | 0.385 | 0.099 | 0.245 | **0.003** |
| West SR 49 | 0.039 | 0.018 | 0 | 0.3 | 0.216 | **0.014** |
| South US-50 | 0.027 | 0.071 | 0.053 | 0 | 0.099 | 0.052 |
| South I-80 | 0.020 | 0.051 | 0.068 | 0.171 | 0 | 0.144 |
| South I-80- North US-50 | 0.010 | 0.028 | 0.034 | 0.063 | 0.047 | 0 |

Table 3 - Model selection results for linear mixed-effects models. AIC is the Akaike information criterion, AIC is the difference in AIC between the best model and each competing model. The best supported model for each region and species is bolded. For all but the East Bay, the highways have no affect. In the East Bay, traffic rates influence patterns of genetic distance.

|  |  |  |  |
| --- | --- | --- | --- |
|  | Surface | AIC | DAIC |
| East Bay |  |  |  |
| Coyote | **Permeable landscape** | 711.63 | 0.010 |
|  | Permeable landscape + all highway impermeable | 711.63 | 0.010 |
|  | Permeable landscape + highway permeability binned to traffic | 711.64 | 0.000 |
| Sierra Nevada Foothills | |  |  |
| Coyote | Permeable landscape | 2371.77 | 0.000 |
|  | **Permeable landscape + all highway impermeable** | 2370.78 | 0.987 |
|  | Permeable landscape + highway permeability binned to traffic | 2371.02 | 0.743 |
|  |  |  |  |
| Gray Fox | Permeable landscape | 2780.15 | 0.000 |
|  | **Permeable landscape + all highway impermeable** | 2779.74 | 0.414 |
|  | Permeable landscape + highway permeability binned to traffic | 2779.92 | 0.238 |

Map

Description automatically generatedFigure 1 - Map of the East Bay study area and coyote sampling locations along I-580 and I-680. I-580 runs West-East, I-680 runs North-South. Annual Average Daily Traffic (AADT) volumes are indicated along highways. Coyote sampling locations are indicated by triangles, color denotes genetic cluster assignment.

Map

Description automatically generated

Figure 2 - Map of the Sierra Nevada Foothill study area and coyote sampling locations along US-50, I-80, and SR 49. SR 50 runs West-East, I-80 runs Southwest-Northeast, SR 49 runs North and then East. Annual Average Daily Traffic (AADT) volumes are indicated along highways. Coyote sampling locations are indicated by triangles, color denotes genetic cluster assignment.

Map

Description automatically generated

Figure 3 - Map of the Sierra Nevada Foothill study area and gray fox sampling locations SR 49. SR 49 runs North and then East before connecting back to I-80. Annual Average Daily Traffic (AADT) volumes are indicated along highways. Gray fox sampling locations are indicated by triangles, color denotes genetic cluster assignment.

Chart, histogram

Description automatically generated

Figure 4. Genetic relationships between coyotes sampled within the East Bay region. A) Pairwise relatedness matrix of individuals within the each of the sampling locations. Individuals are arranged along the axis according to their population assignment. Order on the y axis is the reverse order. Warmer colors indicate higher relatedness between individuals, with red boxes identifying pairs that have relationships near or at 1st order levels (r ~ 0.5). B) Bar plot depicting individual assignments for coyotes sampled in the East Bay. Each color corresponds to a genetic cluster identified by STRUCTURE, each bar corresponds to an individual sample, and the proportion of color in each bar depicts an individual’s proportional ancestry in each genetic cluster. Cluster assignment is largely driven by a group of closely related individuals in West I-680.

A picture containing shape

Description automatically generatedFigure 5. Genetic relationships between gray foxes sampled along SR 49. A) Pairwise relatedness matrix of individuals within the each of the sampling locations. Individuals are arranged along the axis according to their population assignment. Order on the y axis is the reverse order. Warmer colors indicate higher relatedness between individuals, with red boxes identifying pairs that have relationships near or at 1st order levels (r ~ 0.5). B) Bar plot depicting individual assignments for gray foxes sampled along SR 49. Each color corresponds to a genetic cluster identified by STRUCTURE, each bar corresponds to an individual sample, and the proportion of color in each bar depicts an individual’s proportional ancestry in each genetic cluster.

Chart, bar chart, treemap chart

Description automatically generatedFigure 6. Bar plots depicting individual assignments for coyotes sampled in the Sierra Nevada foothill study region. Each color corresponds to a genetic cluster identified by STRUCTURE, each bar corresponds to an individual sample, and the proportion of color in each bar depicts an individual’s proportional ancestry in each genetic cluster. Relationships between related pairs are indicate above the bar plots (red = first order, orange = second order)