Coen et al Chapter 3 draft

ABSTRACT

INTRODUCTION

METHODS

Study Highways and Regions

We studied coyotes separated by Interstates 680 and 580 in the inland valleys of the East Bay (Bay Area), and Interstate 80 and State Route 50 in the lower Sierra Nevada Foothills (LSNF). These are 6-10 lane highways, with central median barriers, and are heavily trafficked. Bay Area highways were travelled by >180,000 vehicles daily within a heavily populated urban and suburban matrix. The LSNF 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, 2014 Traffic Volumes on California State Highways). The southern portion of the study area is comprised of urban matrix surrounding Sacramento with human population densities decreasing as the highways travel east and north from the city.

We studied both coyote and gray foxes separated by State Route 49 and 20 between the cities of Auburn and Grass Valley in the Sierra Nevada Foothills. These are 2 lane, undivided highways, traveled by 2000-40,000 vehicles daily (Caltrans, 2015 Traffic Volumes on California State Highways). The landscape flanking these state routes were 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), timber, and managed forest operated by land trusts and the US Forest Service.

Although both the East Bay and Sacramento region are highly developed, 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 Shackelton 1991, Grinder and Krausman 2001, Grubbs and Krausman 2009). Therefore, the highways are the only major landscape feature likely to disrupt gene flow in the absence of rivers or other geological features. In addition to the presence of the study highways in LSNF, 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 adjacent to the study highways or along road transects within 13 km of the SR 20 and 49. We collected mesopredator fecal samples 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 4⁰C 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 portion of the cytochrome b gene. Cytochrome b is a region of mitochondrial DNA commonly used to distinguish between mammal species (). 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 & Sacks 2014). Those samples identified as originating from gray fox were genotyped using 14/13 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 software (Locke et al. 2007). Negative 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; Peakall and Smouse 2012) using sequential Holms-Bonferroni corrections to account for multiple comparisons (Rice 1989). We used sides of SR 49 and 20 as sampling locations for samples collected along SR 49 and 20, and open space region as sampling location along the other study highways for these and later analyses. We then examined genetic diversity within and among coyote populations in our study areas by calculating the number of alleles, allelic richness, and 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 corrected for unequal sample sizes. Additionally, we measured pairwise relatedness (r) among coyotes within and among sampling locations in GenAlEx to identify close relatives (first and second order) in our dataset.

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. We also used the population admixture model with correlated allele frequencies. Each run consisted of 100,000 Markov chain Monte Carlo iterations following a burn-in period of 10,000 iterations. We tested the likelihood of K=1 through K=4 for the Bay Area and K=1 through K=6 for the Sierra Nevada foothills dataset, where K is the true number of populations. Ten replicates were conducted for each K. We determined 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 CLUMPP (Jakobsson and Rosenberg 2007) 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.

We also 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 999 permutations. We also calculated Nei’s genetic distance (Nei 1972; Nei 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 Ecodist (Goslee et al. 2015).

To determine whether the study highways or traffic rates have a significant effect on genetic distance between sampling locations, we measured the resistance distance between sampling locations using CIRCUITSCAPE v4.0.5 (McRae 2006). For highways alone as a barrier, all highway cells were coded as high resistance with all other landscape cells treated as low resistance. For traffic rates, sections of highway were binned into ten different traffic rates based on Caltrans Annual Average Daily Traffic counts from below 10,000 to over 202,000 (Caltrans gis source). 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 in order to generate an average resistance between points. Support for each resistance surface was 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 2017; Peterman 2018).

RESULTS

Sample collection and species identification

We collected a total of 251 and 327 mesopredator scats from our hiking transects 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 (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 BA region, samples were largely located north of the highway, while in the SNF region, few samples were located south of Hwy 50, in both cases due to access limitations. In gray fox, there were 37 samples on the East side of SR 49 and 20 samples on the West side.

Genetic Diversity

For populations that contained no close relatives (see below), no significant deviation from linkage equilibrium was observed at any loci after implementing the sequential Bonferroni correction (alpha = 0.0039). However, eight loci (AHT137, CXX374, CXX468, CPH11, CPH18, REN54P11, CXX279, and

REN162C04) deviated significantly from Hardy Weinberg equilibrium in at least on population for coyote. In gray fox, however, eight loci (CHP8, RFCHP2, FH2088, FH2004, AHTh171, FH2010, CXX402 and RF2001) were significantly out of equilibrium. For both species this was likely due to family structure in our gray fox 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 (SNF), 91 (East), and 75 (West), respectively. For gray fox, the number of alleles observed ranged from 96 (East), and 85 (West).

When rarefaction was conducted, coyote allelic richness ranged from 3.5-3.9 in the Bay Area, 2.8-4.8 (Table 1). Gray fox showed a similar pattern, with an allelic richness of 7.29 for the East and 6.36 for the West sampling location. Measures of Ho and He (estimates of gene diversity in a population) were high all regions for both species with coyote Ho ranging from 0.60-0.72 in the Bay Area, 0.62-0.88 in the Sierra Nevada foothills, and 0.70-0.73 around SR 49 and 0.60 in gray fox (Table 1).

Mean pairwise relatedness values (r) within sampling locations for coyotes showed that most individuals were not closely related (0.08-0.09 in the Bay Area, 0.03-0.24 in SNF). First order relationships (parent-offspring, full siblings, r ~0.50) were detect within the Bay Area, all from W680. Second order relationships (grandparent-grandchild, half-siblings, r~0.25) were also detected, primarily from W680 (18 pairs) and one pair from E680. Within the SNF region, first order relationships (parent-offspring, full siblings, r ~0.50) were detect for two pairs. One each in the N80-E49 and N80-W49 in the East. Second order relationships (grandparent-grandchild, half-siblings, r~0.25) were also detected for four pairs, one each from S50 and S80-E49, while the remaining two came from S80-N50. In all cases, the individuals in the relationship were sampled along the same side of the highway (Figure 2).

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

Genetic Connectivity

STRUCTURE revealed two genetic clusters in the Bay Area (mean ln Pr(X|K) = -1226.13; Figure 2). One cluster consisted of 14 individuals from the W680 sampling location and one from the E680 location while the second cluster contained individuals from all three locations. Relatedness within the W680 sampling locations was high …

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 (Figures 3, 5).

On the other hand, two genetic clusters were most likely in the gray fox data, with eight individuals split into a separate subpopulation (K1) (Figure 4, 6). Individuals within K1 were found throughout the study area, including on opposite sides of SR 49 (Figure 6). 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. In Coyotes, FST within each region was low, with no significant differentiation across highways (Table 2). The pairwise FST between the gray fox for side of highway was low. When examined for the K1 and K2 clusters pairwise FST was 0.34 (P = 0.001) but this was largely driven by the number of close relatives in the K1 group.

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 Bay Area (r = 0.16, p = 0.05). Neither highways or traffic rates seemed to influence genetic distance with the equally permeable landscape as the best supported resistance surface for both gray fox and both populations of coyotes (table XXX).

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 (Riley et al. 2006, Ernest et al. 2014, Sawaya et al. 2014). Barrier permeability is tied to an organism’s perception of risk and tolerance for disturbance. Highways, as a system, are environments filled with 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 barriers to dispersal imposed by road networks. The aim of this study was to determine whether highways disrupt wildlife gene flow in the Bay Area and the Sierra Nevada foothills, using coyote as a model species. 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 region were genetically diverse, with high heterozygosity and allelic richness on both side of SR 49. These results are in line with other findings of canid genetic diversity throughout California (Sacks et al. 2008; Deyoung et al. 2009; Coen et al. 2015). High levels of genetic diversity suggest that the focal regions supports a thriving population of both species.

It is unlikely that any of the focal highways forms a complete barrier to movement for either species. There was no genetic structuring for coyotes or gray foxes across SR 49. Even for the more heavily trafficked highways in the Bay Area and Sacramento regions (AADT average >180,000 and >64,000, respectively) there was no evidence of genetic structuring relative to side of highway. Genetic clusters identified by Structure corresponded to family groups rather than side of highway. Indeed, members in each genetic cluster for both species were found on east and west sides of SR 49. These results suggest that at least some coyotes or gray foxes have crossed SR 49 either under bridges or through culverts (Figure 1) or across the road surface when traffic rates are low. We did find evidence of IBD and a positive association between side of highway and coyote genetic distance, but these results may be partially explained by small sample size and the species’ relatively diffuse distribution across the study area.

Unlike Riley et al. (2006), who found significant genetic structure between the north and south sides of Highway 101 (N=68), we did not detect any signal of population structure related to highway presence. If highways were disrupting coyote gene flow, we would expect to see distinct populations corresponding to each side of the study highway. For example, if the Bay Area highways were impermeable to coyote gene flow, we would expect to see three distinct populations, corresponding to the west side of I-680, the east side of I-580, and south of I-580/west of I-680 (Figure 3). In contrast, Structure analysis found only two genetic clusters in the Bay Area and they did not correspond to opposite sides of the highways (Figure 2). The presence of close relatives in a sample can create spurious patterns of population structure and create Hardy Weinberg disequilibrium. The fact that we found no significant genetic differentiation due to highways within the Bay Area study region supports our conclusion that the finding of two genetic clusters is an artifact of having close relatives in W680 sample location. Increased sample sizes for E680 and S580 would likely improve the resolution of population structure in this region.