Pilot Study: Do California Highways Act as Barriers to Gene Flow for Ground-Dwelling Mammals?

December 2015

A Research Report from the National Center for Sustainable Transportation

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National Center for Sustainable Transportation

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Disclaimer

Acknowledgments

This study was funded by a grant from the National Center for Sustainable Transportation (NCST), supported by USDOT and Caltrans through the University Transportation Centers program. The authors would like to thank the NCST, USDOT, and Caltrans for their support of university-based research in transportation, and especially for the funding provided in support of this project. Funding for this project was provided by Caltrans (Agreement No 65A027, TO 009) through the National Center for Sustainable Transportation at the University of California, Davis. We are grateful to Ben Sacks for the expertise provided in the course of this study. We thank the following for their technical assistance: Zac Lounsberry, Alisha Goodbla, and Cate Quinn. Additionally, we thank the dedicated interns that helped with many hours of field collection and lab work in order to produce this study.

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EXECUTIVE SUMMARY

Roads have the potential to fragment wildlife populations, leading to genetic diversity loss, inbreeding, and increased extinction risk for small, isolated populations. In this study, we used coyote as a model to investigate how four Northern California highways affect gene flow of ground-dwelling mammals. We collected coyote scat samples from opposite sides of a stretch of I-580 and I-680 in the Bay Area and I-80 and US 50 in the Sierra Nevada foothills. We extracted DNA and genotyped each coyote at 13 microsatellite loci. We estimated genetic diversity and determined how that diversity was partitioned across the landscape in each region.

Genetic diversity levels in coyotes were high and comparable to other studies. We found significant genetic structure in both the Bay Area and Sierra Nevada foothills, although it didn't always correspond to highway presence. In the Bay Area, two populations were identified and although some evidence suggested I-580 was a significant barrier to gene flow, we identified migrants across the highway. One of the two populations in the Bay Area contained many second order relatives, suggesting limited gene flow into that population. There was evidence of dispersal out of that population, however. In the Sierra Nevada foothills, we identified three populations. Individuals from one population were sampled across highway I-80 suggesting it was not a significant barrier to movement. The most genetically divergent population in the Sierra Nevada foothills was also the most geographically distant and therefore it was difficult to determine whether gene flow into that population was limited by highway presence or simply geographic distance from other populations.

The conclusions drawn in our pilot study are limited by the small number of samples we were able to genotype completely in the timeframe of this project. We are going to continue analyzing samples that currently have only partial genotypes and add those to our regional datasets. Genetic analysis with these larger samples will allow us to better understand the role of highways in structuring coyote populations in the Bay Area and Sierra Nevada foothills.

Introduction

Roads can negatively affect wildlife by destroying important habitats, causing mortality through wildlife-vehicle collisions, and fragmenting populations (Coffin 2007). Population fragmentation occurs when roads act as physical or functional barriers to wildlife dispersal. Roads acting as barriers to dispersal will decrease gene flow between the populations they fragment (Gerlach and Musolf 2000, Clark et al. 2010, Delaney et al. 2010). Small, fragmented populations receiving little outside gene flow are more susceptible to genetic diversity loss and inbreeding. Populations with low genetic diversity are less able to adapt to environmental changes, particularly those occurring on a rapid timescale (e.g. Reusch et al. 2005). Inbreeding, or mating between close relatives, can lead to inbreeding depression which increases a population's extinction risk by decreasing the fitness of individuals (Frankham 1996). Therefore, by disrupting gene flow, roads can increase the likelihood that wildlife populations will be locally extirpated, particularly in urban areas (Riley et al. 2014a).

Transportation agencies are mandated to reduce the negative effects of roads on wildlife populations, including disruption of gene flow. 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 and which species are most affected. However, the degree to which roads impede wildlife movements and gene flow varies by road and species. Physical characteristics of roads (e.g. width, gradient, traffic volume) can affect their permeability to different species (Gerlach and Musolf 2000, Marsh et al. 2005, Charry and Jones 2009). In addition, a single road can affect different species to varying degrees due to species-specific behavior patterns. The Trans-Canada Highway was a significant dispersal barrier for grizzly bears (*Ursus arctos*) but not for black bears (Ursus *americanus*; Sawaya et al. 2014). Therefore the impacts of roads on wildlife gene flow cannot be generalized in space or among species.

Although others have shown that Southern California highways can significantly impede gene flow of numerous taxa (Riley et al. 2006, 2014, Delaney et al. 2010), few studies have investigated the effect of Northern California highways on wildlife gene flow. In this pilot study, we use the coyote, a wide-ranging mesopredator, as a model species to investigate how highways affect gene flow of ground-dwelling vertebrates in Northern California. The coyote is an ideal model for this type of investigation because it is abundant, occupies most habitats (pristine to urban), and leaves conspicuous scats that can be collected for genetic analysis. In this study we sample coyote scats in open space areas on either side of long stretches of I-580 and I-680 in the Bay Area and I-80 and US 50 in the Sierra Nevada foothills. We then use population genetic analysis to determine whether those highways acted as physical or functional barriers to coyote gene flow.

Materials and Methods

Study Highways

Interstates 580 and 680

We studied coyote separated by Interstates 680 and 580 in the inland valleys of the East Bay (hereafter referred to as Bay Area). Both highways have 10 lanes, center median barriers, and are heavily trafficked, travelled by >180,000 vehicles daily (Caltrans, 2014 Traffic Volumes on California State Highways). The East Bay region is a heavily populated urban and suburban matrix interspersed with regions designated as open space parkland (Figure 1A). Sampling was conducted in 115.8 square km of open space and parkland in regions adjacent to the study highways. All samples were collected ≤ 10 km from the highways. Although the East Bay region is 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.

Interstate 80 and US 50

Within the lower Sierra Nevada Foothills, we studied coyotes separated by Interstate 80 and State Road 50 (Figure 1B). Both highways are 6-10 lane highways with central median barriers and daily vehicle use that ranges from >140,000 in the southern section to 65,000 in the northern, more rural region of our study area. Sampling was conducted in 130 square km of open space and parklands in regions adjacent to the study highways. All samples were collected ≤ 10 km from the 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. In addition to the presence of the study highways, the American River 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

We collected mesopredator fecal samples along hiking transects in the study areas from November 2014 to August 2015. A fraction of each scat was preserved in 95% ethanol in the field for later DNA extraction. GPS points recorded the exact location where each sample was collected. Fecal samples were stored in the lab at 4^{o}C upon return to the lab. DNA was extracted using the QIAmp 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 for distinguishing between mammal species. All samples identified as non-target species (e.g. bobcat, gray fox) 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: AHTh171, AHT137, ANT142, CPH11, CPH18, CXX279, CXX374, CXX468, CXX602, INU055, REN54P11, REN162C04, and REN169O18 (Quinn & Sacks 2014). 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 0.5 μl of Genescan 500 LIZ size standard (Life Technologies; LT). Fragment analysis was performed on an ABI PRISM 3730 DNA Analyzer (LT) and alleles were scored with STRand software (Locke and Toonen 2007). Negative controls were included with each PCR run 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.

Data Analysis

Genetic Diversity

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 Bonferroni corrections to account for multiple comparisons (Rice 1989). 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.

Genetic Connectivity

We used STRUCTURE version 2.3.4 (Pritchard et al. 2000) to examine how coyote genetic diversity was partitioned across our sampling locations. STRUCTURE, a Bayesian clustering algorithm, inferred the most likely number of populations in the Bay Area and Sierra Nevada foothills study areas. Since our sampling was conducted on a relatively fine scale for a wide-ranging species, we expected population structuring to be weak, even if 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 number of true 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 ΔK (Evanno et al. 2005) in STRUCTURE HARVESTER (Earl and von Holdt 2012). The program CLUMPP (Jakobsson and Rosenberg 2007) was used to compile individual assignments across replicates and we used custom R code to create bar plots to visualize results.

We also examined population genetic structure by estimating pairwise F_{ST} values (a measure of genetic differentiation) among all sampling locations in the AMOVA framework in GenAlEx. Significance of pairwise F_{ST} values was determined through 999 permutations. We also 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 pairs of individual sample locations, recorded as GPS points (decimal latitude and longitude). For individuals that were detected twice in our sampling locations, we used two averaged locations to represent their detection center. The relationship between genetic and geographic distance in the Bay Area and Sierra Nevada foothills was assessed with Mantel tests in the R package Ecodist (Goslee et al. 2015). To determine whether the study highways have a significant effect on genetic distance between sampling locations, we performed partial Mantel tests, also in Ecodist, where we assigned a dummy variable to pairs of populations to designate whether they were on the same side (=0) or different side (=1) of the highway from each other.

Results

Sample Collection and Species Identification

We collected a total of 251 scats from our hiking transects. The species identification test revealed that 128 of these samples originated from coyote. We were able to obtain high quality genotypes (data at >85% of loci) for 59 individuals.

Genetic Diversity

For populations that contained no close relatives (see below), no significant deviation from Hardy-Weinberg equilibrium or linkage equilibrium was observed at any loci after implementing the sequential Bonferroni correction (alpha = 0.0039). However, one and four loci deviated significantly from Hardy Weinberg equilibrium in the Rancho Murieta (RNM) and Dublin (DUB) populations, respectively.

The total number of alleles observed within sampling locations ranged from 38-73 and 34-78 in the Bay Area and Sierra Nevada foothills, respectively. When rarefaction was conducted, allelic richness ranged from 2.6-2.9 in the Bay Area and 2.6 -3.9 in the Sierra Nevada foothills (Table 1). Measures of Ho and He were high in both regions with Ho ranging from 0.51-0.77 in the Bay Area and 0.46-0.79 in the Sierra Nevada foothills (Table 1). Both sampling regions showed high levels of genetic diversity with highly polymorphic loci (Bay Area = 0.94, Sierra Nevada foothills = 0.97; Table 1).

Mean pairwise relatedness values within sampling locations showed that most individuals were not highly related with mean r values ranging from 0.02 (Pleasanton; PLS) to 0.08 (DUB) in the Bay Area region and 0.03 (west of 80; W80) to 0.16 (Auburn; AUB) in the Sierra Nevada foothills (Figure 2A, B). However, second order relatives (grandparent-grandchild, half-sibling; r~0.25) were identified within DUB, AUB, and RNM. Within RNM (mean relatedness score 0.12) all 7 individuals had at least one second order relative in the group (Figure 2B). In the AUB location one pair of individuals had a relatedness score of 0.31 and within the DUB group, five

individuals had a second order relationship with another individual within the group (Figure 2B). In three cases close relatives were found in different sampling locations. One individual sampled west of 680 (W680) was a second order relative to an individual from DUB. Within the Sierra Nevada foothills one individual from American River Parkway (AMR) had a second order relative in Nimbus (NIM) while an individual from Folsom (FOL) showed a second order relationship with an individual from Rancho Murieta (RNM).

Genetic Connectivity

STRUCTURE runs revealed two distinct clusters in the Bay Area (mean ln Pr(X|K) = -865.75; Figure 3A). One cluster corresponded to the DUB sampling location while W680, east of 680 (E680), and PLS clustered together. One individual from the DUB group clustered with the W680/E680/PLS population and was also a second order relative to an individual from W680 (r = 0.22). Within the Sierra Nevada foothills, three clusters were detected (mean ln Pr(X|K) = -1560.44; Figure 3B). The AUB and FOL groups clustered as one population, while the NIM and the RNM were each distinct from the other sampling locations. A few individuals sampled on the northwest side of Folsom Lake seemed to originate from the RNM population and one individual sampled in the AMR assigned to the NIM cluster.

Pairwise F_{ST} values supported STRUCTURE results for both the Bay Area and Sierra Nevada Foothills. The DUB group was most differentiated from the other sampling locations in the Bay Area whereas the RNM and NIM groups were most distinct in the Sierra Nevada foothills (Table 2). There was no difference between the PLS and W680 samples but this was likely due to low sample sizes at both locations.

Mantel tests revealed a marginally significant positive association between genetic and geographic distance in the Sierra Nevada foothills (r= 0.19, p = 0.051), weakly supporting a pattern of IBD. No IBD was observed in the Bay Area (r = 0.30, p = 0.12). Partial Mantel tests in the Bay Area suggested that there was significant genetic divergence between populations on opposite sides of I-580 (r = 0.35, p = 0.05) while no significant difference was found on either side of I-680 (r = 0.10, p = 0.48). Within the Sierra Nevada foothills, US 50 had marginally significant levels of genetic divergence among sampling locations on opposite sides (r = 0.19, p = 0.06). Interesting, I-80 exhibited a negative relationship between side of highway and genetic divergence (r = -0.43, p = 0.01), suggesting that sampling locations on opposite sides of the highway were more genetically similar than those on the same side.

Discussion

Highways can act as a partial or total dispersal barrier for even wide ranging species, resulting in genetic differentiation between populations fragmented by roads over time due to a lack of gene flow (Riley et al. 2006, Ernest et al. 2014, Sawaya et al. 2014). 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. Our preliminary results are based on small samples from each location due to insufficient time to obtain complete genotypes for all coyote samples. However, we still discovered some evidence of genetic divergence among sampling locations related to highway presence.

We found that coyote populations within both study regions were genetically diverse, with high heterozygosity and allelic richness for all sampling locations. These results are in line with other findings of coyote genetic diversity throughout California (Sacks et al. 2005, Riley et al. 2006). Such high levels of genetic diversity suggest that both the Bay Area and Sierra Nevada foothills support large numbers of coyotes.

It is unclear from our current dataset whether highways form significant barriers to coyote movements in the Bay Area and Sierra Nevada foothills. In the Bay Area, only two genetic clusters were detected and they did not correspond perfectly to opposite sides of highways (Figure 1). The W680/E680/PLS cluster contained individuals distributed across both I-580 and I-680. Individuals assigning to the DUB cluster are concentrated on one side of I-580 and I-680 but this group was significantly differentiated from all other sample locations (Table 2), even those on the same side of the highways. On the other hand, pairwise relatedness analyses showed that all relatives found in the Bay Area dataset were located on the same side of I-580 and I-680. The large number of relatives in the DUB sample location and the partial Mantel test support little gene flow across I-580. However, STRUCTURE does not recognize PLS as a distinct genetic cluster and pairwise F_{ST} values show no difference between PLS and W680. Increasing the number of samples from this region will help clarify our results, as low sample size can bias measures of genetic divergence, particularly F_{ST} (see FUTURE WORK).

In the Sierra Nevada Foothills, the most genetically divergent sampling location, also found to be a unique genetic cluster (RNM; Figure 1; Table 2), was separated from all other groups by at least one highway. Every individual sampled in RNM was related to at least one other individual from that location, further suggesting its isolation. However, individuals from W80, FOL, and AUB assigned to a single genetic cluster despite the fact that they are found on opposite sides of I-80. Pairwise F_{ST} values suggest little genetic divergence in this region, as nearly all comparisons not involving RNM were not significant (Table 2), even for sampling locations on opposite sides of the highway. Migrants from the RNM and NIM cluster were found across US 50 and I-80, respectively, suggesting that neither highway is an impenetrable barrier to dispersal. The American River Parkway bike path provides a corridor for coyote dispersal under the freeway, which could explain the FOL migrant in AMR. The Mantel test detected a signal of IBD in the Sierra Nevada foothills, which provides an alternative explanation for the genetic distinctiveness of the RNM group relative to other samples in the region. Low gene flow into that RNM could be due to geographic isolation rather than the presence of highways

Our results contrast with the findings of Riley et al. (2006), who studied coyote movements and gene flow across Highway 101 in Southern California. In that study, STRUCTURE detected two populations, corresponding to the north and south sides of Highway 101 (N = 68). In our study areas, there is no distinct break between populations that can be attributed to highways alone. Both Riley et al. (2006) and this study identified migrants across highways although the levels of population structure in Riley et al. (2006) suggested little gene flow occurred. The lack of population structure in our study areas suggests there is gene flow across highways, which may be facilitated by crossing points such as culverts and underpasses. For example the American River Bike Trail follows along the river from Sacramento towards Folsom Lake and passes under

Interstate 80, creating the potential for genetic exchange from west of 80, down through Sacramento, then northward through the American river and Folsom Lake system.

Future Work

The results in this report are preliminary due to the small number of complete genotypes we were able to obtain by the end of 2015. With some additional labwork, we will fill in missing data for ~60 additional samples, effectively doubling our sample size. We intend to continue genotyping these samples, at no cost to Caltrans, to achieve a more adequate sample size for genetic analysis. In addition, Dr. Ben Sacks, a coyote expert at UC Davis, has offered to mine his extensive coyote genotype database for samples collected by his lab within our study areas. If he finds samples that were collected within 10km of our study sections of I-580, I-680, I-80, and US 50, he will share that genotype data with us to further increase our sample sizes. Once we have a more adequate collection of samples, we will re-analyze the data and update this report to Caltrans.

Tables and Figures

Table 1. Genetic diversity summary statistics for Bay Area and Sierra Nevada foothill coyotes.

Sampling Location	N	A_T	A_L	AR	Ho	He	%P
Bay Area (BA)	22	103	3.9		0.66	0.60	94.2
West of 680 (W680)	3	45	2.6	2.7	0.51	0.56	84.6
East of 680 (E680)	4	47	3.6	2.7	0.77	0.61	100
Dublin (DUB)	13	73	5.6	2.59	0.63	0.65	100
Pleasanton (PLS)	2	38	2.9	2.92	0.73	0.59	92.3
Sierra Nevada Foothills (SNF)	37	115	4.2		0.68	0.64	97.4
West of 80 (W80)	8	78	6	3.88	0.67	0.75	100
Auburn (AUB)	3	34	2.6	2.62	0.79	0.52	92.3
Folsom (FOL)	8	69	5.3	3.7	0.77	0.74	100
American River (AMR)	3	34	2.6	2.62	0.46	0.51	92.3
Nimbus (NIM)	8	58	4.5	3.17	0.59	0.65	100
Rancho Murrieta (RNM)	7	52	4	3.2	0.78	0.65	100

N = sample size.
A_T = total number of alleles
A_L= 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 F_{ST} values for the Bay Area (BA) and Sierra Nevada Foothills (SNF) sampling locations. P values are above the diagonal. Bolded values are statistically significant with a sequential Bonferroni correction. BA alpha = 0.0125, SNF alpha = 0.0038)

BA	W680	E680	DUB	PLS
W680	0	0.014	0.002	0.482
E680	0.089	0	0.001	0.002
DUB	**0.112**	**0.110**	0	0.008
PLS	0.000	**0.158**	**0.121**	0

SNF	W80	AUB	FOL	AMR	NIM	RNM
W80	0	0.029	0.049	0.312	0.013	0.001
AUB	0.051	0	0.018	0.277	0.003	0.004
FOL	0.022	0.067	0	0.052	0.005	0.001
AMR	0.009	0.033	0.049	0	0.053	0.048
NIM	0.039	**0.126**	0.052	0.054	0	0.001
RNM	**0.073**	0.144	**0.084**	0.070	**0.127**	0

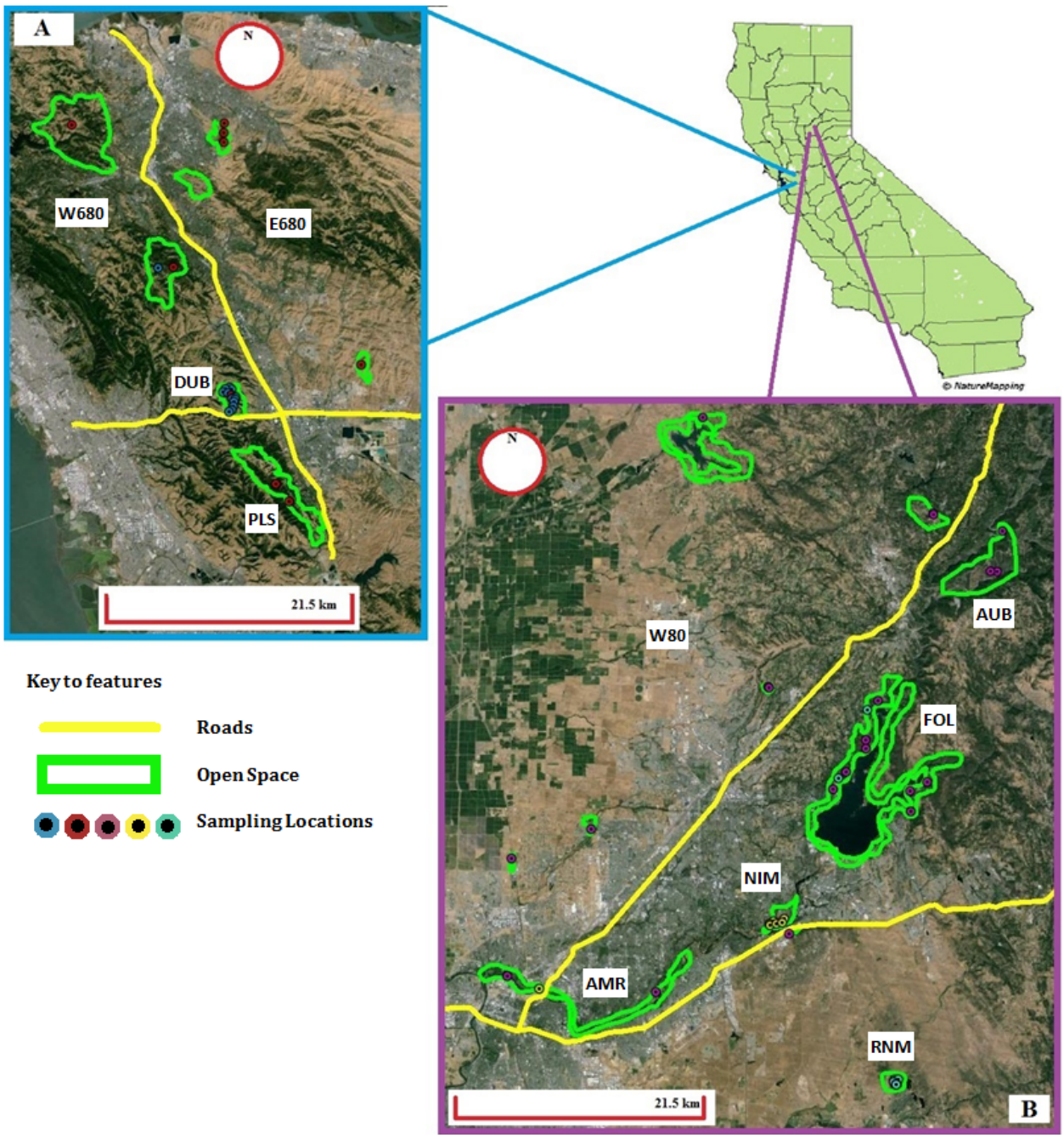


Figure 1. Map of study area and coyote sampling locations. A) Bay Area sampling locations along I-580 and I-680. I-580 runs West-East, I-680 runs North-South. B) Sierra Nevada Foothill sampling locations along US 50 and I-80. US 50 runs West-East and I-80 runs Southwest-Northeast. Colors of symbols represents membership in a genetic cluster identified by STRUCTURE.

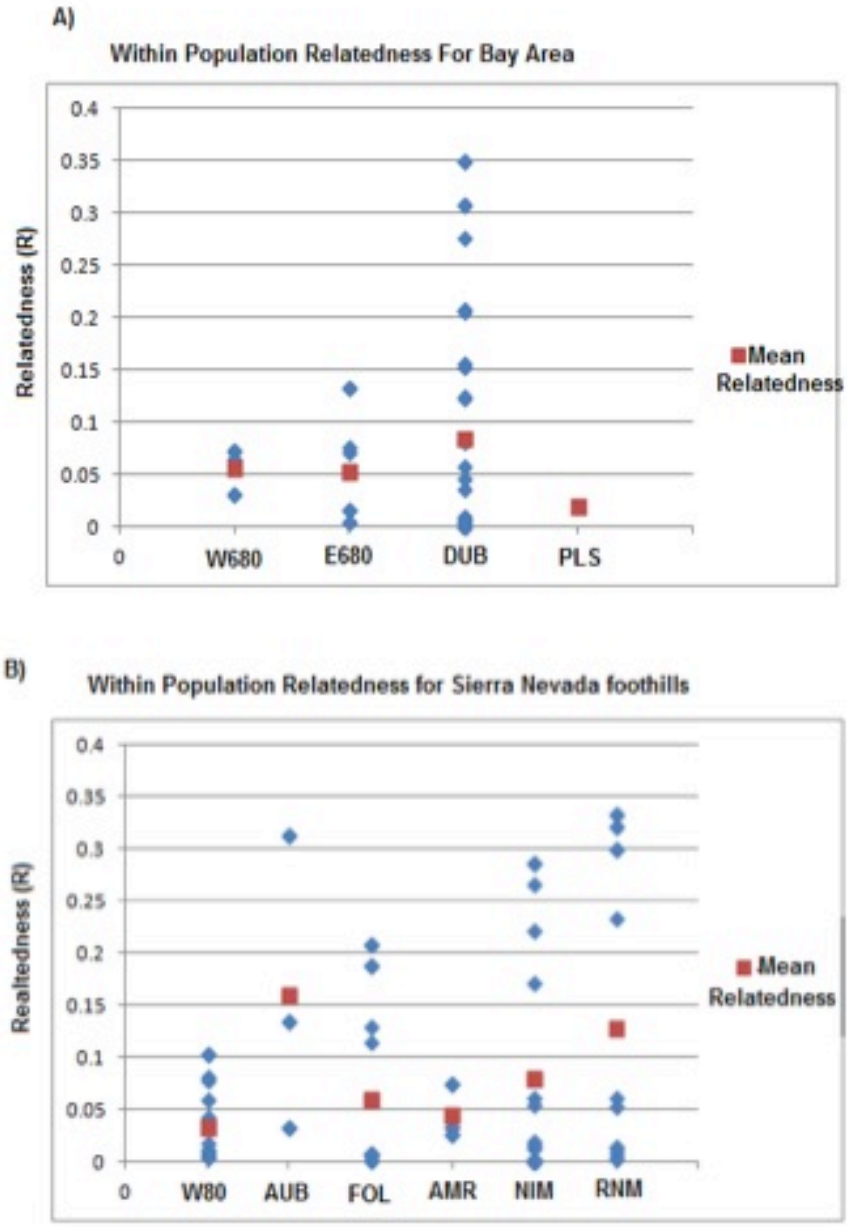
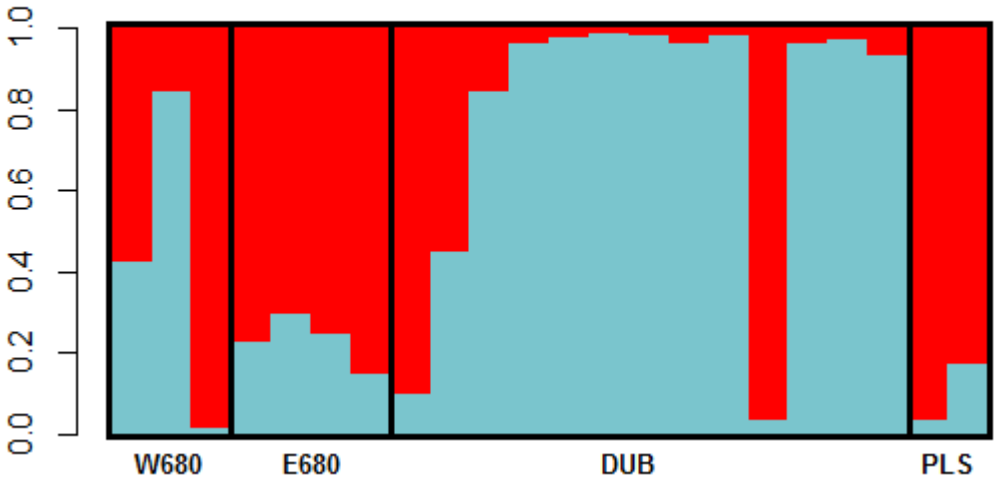


Figure 2. Pairwise relatedness values between individuals within sampling locations. Means for each population are denoted by a red square. Within the Bay Area (A), the DUB location contains many individuals with relatedness scores consistent with second order relatives (r ~ 0.25). In the Sierra Nevada foothills region (B), the AUB, FOL, NIM, and RNM groups contained second order relatives. All individuals in the RNM group were related to at least one other individual in the group.

A)



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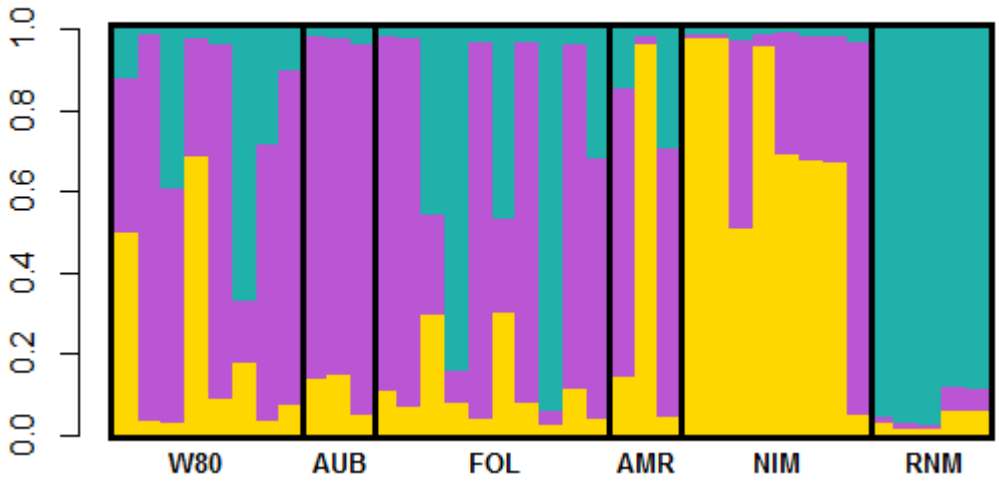

Figure 3. Bar plots depicting individual assignments for coyotes sampled in the Bay Area (A) and Sierra Nevada foothills (B). Each color corresponds to a genetic cluster identified by STRUCTURE, each bar corresponds to an individual sample, and the proportion of color in each bar depicts an individual's proportional ancestry in each genetic cluster.

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