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Non-Invasive Genetic Sampling of Southern Mule Deer (*Odocoileus hemionus fuliginatus*) Reveals Limited Movement Across California State Route 67 in San Diego County

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***Abstract.*—The Southern Mule Deer is a mobile but non-migratory large mammal found throughout southern California and is a covered species in the San Diego Multi-Species Conservation Plan. We assessed deer movement and population connectivity across California State Route 67 and two smaller roads in eastern San Diego County using non-invasive genetic sampling. We collected deer scat pellets between April and November 2015, and genotyped pellets at 15 microsatellites and a sex determination marker. We successfully genotyped 71 unique individuals from throughout the study area and detected nine recapture events. Recaptures were generally found close to original capture locations (within 1.5 km). We did not detect recaptures across roads; however, pedigree analysis detected 21 first order relative pairs, of which approximately 20% were found across State Route 67. Exact tests comparing allele frequencies between groups of individuals in pre-defined geographic clusters detected significant genetic differentiation across State Route 67. In contrast, the assignment-based algorithm of STRUCTURE supported a single genetic cluster across the study area. Our data suggest that State Route 67 may reduce, but does not preclude, movement and gene flow of Southern Mule Deer.**

Key Words.—dispersal, fragmentation, gene flow, movement, scat, southern California

Introduction

Understanding the effects of roads and habitat fragmentation on functional connectivity is a central issue in wildlife ecology (Forman and Alexander 1998; Fahrig and Rytwinski 2009). Direct observations of individual movement can be obtained with telemetry, camera traps, or other mark re-sighting techniques (Riley et al. 2006; Poessel et al. 2014; Alonso et al. 2015). However, these techniques can be time and resource intensive and capture and collaring can negatively impact individual animals (Dechen Quinn et al. 2014; Rachlow et al. 2014; Munerato et al. 2015). This may be especially problematic for rare, elusive, or large animals that are difficult to capture. Consequently, non-invasive genetic sampling and individual identification through genotyping has become a commonly used tool to assess individual movement, gene flow, and population parameters such as abundance and sex-ratios in wildlife species (Waits and Paetkau 2005; Luikart et al. 2010).

The Southern Mule Deer (*Odocoileus hemionus fuliginatus*) is one of six subspecies of Mule Deer and is distributed in southern California, USA, through Baja California, Mexico. It is a monitored species in the San Diego Multi-Species Conservation Plan (MSCP; Greer 2004), and connectivity among individual preserves within the highly urbanized Multi-Species Plan Area (MSPA) is of primary concern for this species (San Diego Management and Monitoring Program. 2014. Management Strategic Plan. Available from: http://sdmmp.com/reports_and_products/Management_Strategic_Plan.aspx [Accessed 10 March 2016]). Based on the mobile, long-lived, and generalist nature of this species, the Southern Mule Deer is thought to be doing relatively well in fragmented habitat of southern California. This assumption has been bolstered by regional wildlife corridor studies and inferences from other deer species in fragmented urban landscapes (Leberg and Ellsworth 1999; Peles et al. 1999; Ng et al. 2004; Doerner et al. 2005; Markovchick-Nicholls et al. 2008). However, prior DNA fingerprinting of Southern Mule Deer scat from the San Diego MSPA revealed significant population genetic structure and low levels of movement and gene flow (Valero 2004; Mitelberg 2010; Andrew Bohonak and Anna Mitelberg, unpubl. report. Available from: http://portal.sdmmp.com/view_article.php?cid=CiteID_1603251358358930 [Accessed 13 July 2016]). In particular, two regional populations were defined with genetic clustering techniques: a western and eastern population with evidence of mixed population assignment in eastern San Diego around the vicinity of State Route 67 (hereafter Route 67; Andrew Bohonak and Anna Mitelberg, unpubl. report). This region is also characterized by a gradient in urbanization from suburban to rural development, with higher density suburban housing to the west, and more open space and larger preserve space to the east (Fig. 1). However, the wide range of previous studies (designed to assess population-wide movement throughout the county), precluded

a thorough sampling along Route 67 and limited the ability of the authors to assess whether the road itself acts as a barrier to gene flow.

The goal of this study was to primarily assess east-west connectivity across Route 67 and secondarily, north-south connectivity across Scripps Poway Parkway and Poway Road, two highly trafficked roads to the west of Route 67 (Fig. 1). We collected Mule Deer scat piles from both sides of these road segments timed to roughly coincide with the spring rutting and fall mating seasons, when Mule Deer tend to move greater distances (Anderson and Wallmo 1984). Using previously developed microsatellite loci, we investigated movement distances among individuals genetically identified and resampled throughout the study period. We also assessed the distances between siblings and parent offspring pairs identified through pedigree reconstruction to capture past movement or dispersal patterns, and examined the impacts of roads on population genetic structure throughout the study area.

Methods

Sampling and laboratory methods.—We collected scat piles in the spring (March-June) of 2015 and in fall (October) 2015, within a 100 km^2 region along Route 67 between Lakeside and Poway (Fig. 1), San Diego County, California, at sites where Mule Deer presence was previously confirmed by land managers, field researchers, members of the local community-based tracking team (San Diego Tracking Team; www.sdtt.org), or past successful collection efforts (Andrew Bohonak and Anna Mitelberg, unpubl. report). Upon arriving at the site, we searched for fresh deer sign (tracks and browse), which we tracked until we encountered fresh scat piles. Fresh scat appear shiny and smooth, versus older scat piles that appear dry and cracked (Mitelberg 2010). Previous studies suggest that fresher scat piles have higher amplification and genotyping success rates (Piggott 2005; Panasci et al. 2011). We air dried pellets of Mule Deer for two to four days at room temperature, and we collected the

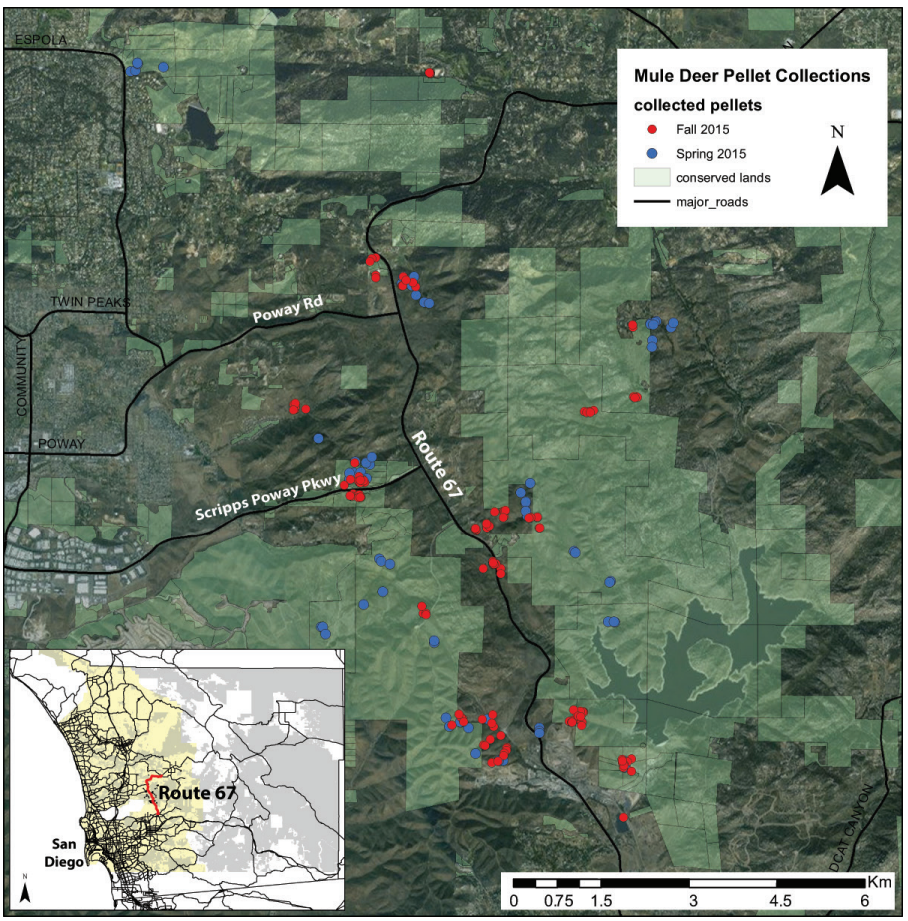

Figure 1. Location of study area showing major roadways and 238 scat piles collected of Mule Deer (*Odocoileus hemionus fuliginatus*). We assessed connectivity across California State Route 67, Scripps Poway Parkway and Poway Road, southern California. The insert shows the section of Route 67 examined in this study within San Diego County. The MSPA (Multi-Species Plan Area) is highlighted in yellow, and conserved lands across San Diego County are shown in gray. Road density and urban development increase to the west of the county, while open space and conserved lands increase to the east. (World Imagery Basemap source: ESRI, Digital Globe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo and the GIS User Community).

epithelial cells within two weeks of collecting pellets in the field (we stored dried pellets at 8° C for up to two weeks before performing this step). To collect epithelial cells from the surface of scat pellets, we dropped 3–5 scat pellets at a time into a small plastic bag and covered the pellets with approximately 2 mL of phosphate buffered saline solution (1X PBS). We proceeded to gently rub the surface of the pellets through the plastic bag to dislodge cells off the pellet, being careful not to break up the pellet. As some PBS was absorbed during this process, we added additional PBS as needed to keep the liquid volume in the bag at about 1 mL. We removed and disposed of the used pellets using tweezers and added more unprocessed pellets from the same scat pile to the bag, repeating the process until we washed about 12–20 pellets from a single scat pile in the same bag. We centrifuged this volume of PBS (about 1 mL), containing intestinal cells from 12–20 deer scat pellets for 2 min at 13,000 RPM to further concentrate the intestinal cells at the bottom of the tube. We transferred all but the bottom 250 µL of this solution to a 1.5 mL tube and stored at -20° C for future extraction attempts if necessary. We stored the remaining 250 µL at -20° C and extracted it within the following month using the DNA IQ kit (#DC6700; Promega, Madison, Wisconsin) according to the standard protocol (with the modifications that cell lysis was performed at 95° C and the lysed cell solution was filtered through a spin basket filled about half way with cheesecloth to filter out any sediment). To help detect contamination problems should they arise, each batch of washes included a negative control with only PBS and each batch of extractions included a negative control with water.

We genotyped at 15 previously developed microsatellite loci (Valero 2004; Pease et al. 2009; Mitelberg 2010) and a sex specific marker (Gilson et al. 1998) in a single multiplex PCR using the Qiagen Multiplex PCR Plus kit (#206152; Qiagen, Hilden, Germany). Each 5 µL reaction contained 1.5 µL DNA, 2.5 µL Master Mix, 0.375 µL of primer mix containing all 16 primer sets in optimized proportions (see Appendix 1; #450056; ThermoFisher, Carlsbad, California), and 0.625 µL of water. Cycling conditions were as follows: 95° C for 5 min; followed by 37 cycles of 95° C for 30 s, 56° C for 3 min, 72° C for 30 s; and a final 68° C extension for 30 min. Each set of PCR reactions included two positive controls (one male extraction and one female extraction) and two negative controls. Eton Biosciences (San Diego, California) performed fragment analysis on an ABI 3730xl following submission of a 1.5 µL aliquot of the PCR product, combined with 10 µl formamide (#4311320,ThermoFisher, Carlsbad, California) and 0.5 µL of GeneScan 500 LIZ size standard (#4322682; ThermoFisher, Carlsbad, California). We compiled the raw data (chromatographs) into genotypes using GeneMapper v. 4.0 (Applied Biosystems, Foster City, California).

To identify and eliminate genotyping errors, we initially genotyped all scat piles twice at all loci; we analyzed these initial genotypes with RELIOTYPE (Miller et al. 2002), a software program that implements a maximum likelihood algorithm to assesses the reliability of the multilocus genotype based on allele frequencies and recommends a replication strategy for those samples failing to pass the 99.49% reliability criteria. At this point, we discarded all samples requiring more than six PCR replicates. We genotyped all remaining samples again (according to RELIOTYPE recommendations) and ran the data through RELIOTYPE a second time, following which we discarded all samples failing to yield a reliable genotype. We used GIMLET 1.3 (Valière 2002) to reconstruct consensus genotypes for all scat piles with reliable DNA fingerprints.

Identifying capture and recapture events.—We grouped the consensus genotypes for all scat piles with reliable DNA fingerprints by genotype, with missing alleles considered as any other alleles. Within this set of scat piles, we identified unique individual genotypes using GIMLET's group by genotype algorithm. We further sorted the remaining scat piles into two categories: either they were resampling events resulting from repeated sampling of the same scat pile of an individual at the same site and date, or they were true recaptures of the individual at a different site and/or date. We considered all scat piles collected within 1–2 d of each other and that matched the same unique genotype as resampling events; in these cases, we reduced all resampling events of an individual mule deer to one capture event with the first scat pile processed in the laboratory serving as a representative of that capture event. To verify whether resampling distances differed from recapture distance, we also examined the time span (in days) between all resampling and recapture events. These fell within three clustered time periods: either on the same day or the next day (within 1–2 d), within 16–26 d (within 1 mo), or within 130–200 d (across seasons). We examined box-plots of distances between samples for each of these three groups.

We implemented CERVUS v.3.0.7 (Kalinowski et al. 2007) on the subset of unique genotypes to calculate the average probability that two unrelated individuals (PID) and the more conservative average probability that two siblings (PSIB) in the present data set could have identical genotypes. We eliminated Locus B (which was found to have a high likelihood of null alleles) from all remaining analyses sensitive to methodological artifacts such as null alleles. We conducted all further analyses using the remaining 14 microsatellite markers. We used CERVUS to calculate all microsatellite summary statistics (Table 3).

Pedigree reconstruction.—We used the maximum likelihood pedigree reconstruction software COLONY v.2.0.5.9 (Jones and Wang 2010) to identify potential full siblings and parents. To assess stationarity in the pedigree solution, we performed two independent

COLONY runs with the following parameters: female and male polygamy, with inbreeding, long run, full-likelihood analysis method, medium likelihood precision, no sibship scaling or sibship prior; all other parameters were set to default. We used allelic frequencies and error rates calculated over the larger San Diego Southern Mule Deer population (Andrew Bohonak and Anna Mitelberg, unpubl. report). To avoid exclusion of parent-offspring pairs based on a single allele, we assigned to all loci the minimal recommended false alleles rate of 0.0001. We set the expected probability of detecting a father or mother to 0.05 and 0.15, respectively. We measured the Euclidean distance between resampling events, recaptures, first order relatives, and all unique captures in ArcMap 10.2.2 (Esri, Redlands, California). We assessed whether distances between recapture events and first order relatives differed from that among all pairs of non-related unique captures using t-tests of differences between means, calculated in DataDesk 6 (Ithaca, New York, New York; Velman 1997).

Population structure.—The use of population genetic analyses aimed at detecting population structure provides an indirect method for inferring gene flow, i.e., movement and successful reproduction, or obstacles to it. Given the complexity of detecting population structure at the limited scale of this study for a large, mobile mammal such as the mule deer, we applied multiple analyses and used a consensus approach to determine population structure (Pearse and Crandall 2004). We employed two types of analyses, exact tests for population differentiation and individual clustering analyses, to indirectly assess whether Route 67 and/or either of the two highly trafficked roads in our sampling area function as barriers to mule deer gene flow. Because these kinds of analyses can result in biased conclusions in data sets containing related individuals, we randomly removed one of each pair of full siblings identified during pedigree reconstruction from the input files.

First, we used exact contingency tests to assess whether allele frequencies were significantly different among geographic groupings (Raymond and Rousset 1995). We performed Fisher's exact tests in GENEPOP (Rousset 2008) using 10,000 dememorisations, 100 batches, and 5,000 iterations per batch. In this type of analysis, groups are determined *a priori*, and the alternative hypothesis that these groups are genetically divergent is tested. We tested two scenarios: an East-West scenario, in which gene flow is limited by Route 67, resulting in two groups, East of Route 67 and West of Route 67; and a Roads scenario, in which gene flow is limited by Route 67, Poway Road, and Scripps Poway Parkway, resulting in four groups (North of Poway Road, South of Poway Road, South of Scripps Poway Parkway). Second, we performed individual-based clustering analyses in STRUCTURE v.2.2.4 (Pritchard et al. 2000; Falush et al. 2003), using an admixture model with correlated frequencies. We estimated the probability of K = 1–7 clusters using 1,000,000 Markov chain Monte Carlo (MCMC) iterations following a 500,000 iteration burn-in, with 10 replicate runs per K to verify consistency across chains. Individual-based clustering analyses search for the optimum number of gene pools based solely on individual genotype, without or with minimal value assigned to *a priori* population structure hypotheses. We combined replicate runs using CLUMPAK (Kopelman et al. 2015), which we also used to assist us in finding the preferred K using both, the method of Evanno et al. (2005) and Pritchard et al. (2000).

Results

Sampling and genotyping.—We collected 238 scat piles, 87 in the spring and 151 in the fall (Fig. 1). All 238 scat piles were extracted and genotyped. Although sample sizes were roughly equal on the east and west sides of Route 67, we were able to find and collect more pellets in the southern portion of the study area. About 53% (126) of the collected scat piles yielded reliable genotypes. Individual genotypic data and collection coordinates can be downloaded from the USGS (http://dx.doi.org/10.5066/F7KW5D32).

Capture and recapture events.—For the 15 loci in this study, the probability of PID of 2.5 × 10-10 and PSIB of 6.9 × 10-5 were both very low and well within reasonable limits of 0.01 to 0.0001 recommended for genotypes in natural populations (Waits et al. 2001). Excluding locus B, the number of alleles per locus ranged from 2 to 10, with an average of 4.21 alleles per locus (Table 1). Observed heterozygosity per locus ranged from 0.319 to 0.851, with an average of 0.563 (Table 1).

We identified 71 unique individuals (45 females and 26 males). We sampled 28 individuals more than once (some multiple times) for a total of 55 resampling or recapture events. Of these, we considered nine to be true recapture events (sampled at least 15 d apart). We considered the rest to be resampling events (sampled within one day of each other). With the exception of a few outliers, distances between samples acquired within one day tended to be closer together than those in the within month and among seasons groupings, and 95% confidence intervals around median distances did not overlap (Fig. 2). Of the 71 individuals, we found 38 on the east side of Route 67. Of the remaining 33 individuals that we found on the west side, 19 were south of Scripps Poway Parkway, 11 between Scripps-Poway Parkway and Poway Road, and three north of Poway Road (Fig. 3).

Six individuals (three males and three females) that we detected in the spring were recaptured during the fall collection season. The remaining three recapture events occurred within the same season. The average distance between recapture events was 816 m (ranging from 190 m to 1,564 m, Table 2). The average distance between recapture events for females and males was

Table 1. Summary statistics for 14 microsatellite loci of Southern Mule Deer (*Odocoileus hemionus fuliginatus*) encountered via scat genotyping at sites along Route 67 from Lakeside to Poway, San Diego County, California. Locus B was excluded from population genetic analyses due to presence of null alleles. Abbreviations are K = number of alleles; *n* = number of individuals genotyped; Hobs = observed heterozygosity; HExp = expected heterozygosity; PIC = polymorphic information content.

Locus	k	*n*	HObs	HExp	PIC
Locus C	3	69	0.319	0.363	0.326
Locus D	6	70	0.743	0.778	0.736
Locus F	3	45	0.378	0.417	0.375
Locus G	3	69	0.609	0.590	0.496
Locus H	2	64	0.406	0.378	0.305
Locus J	2	71	0.366	0.381	0.307
Locus K	4	71	0.634	0.606	0.548
Locus L	3	68	0.500	0.530	0.470
Locus M	3	71	0.648	0.597	0.525
Locus N	10	67	0.851	0.821	0.791
Locus P	5	71	0.648	0.635	0.581
Locus R	5	68	0.662	0.667	0.604
Locus S	7	71	0.761	0.795	0.760
Locus V	3	71	0.352	0.414	0.374
Average	4.214	67.571	0.563	0.569	0.514

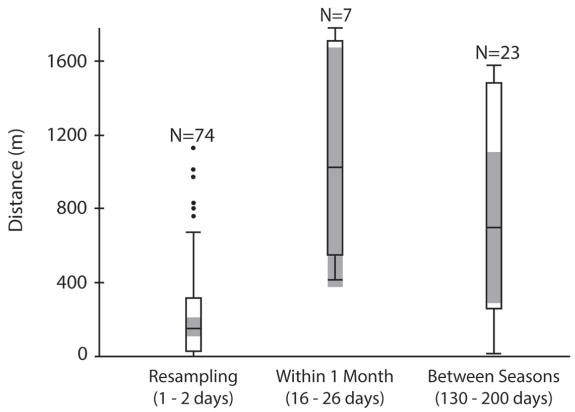

Figure 2. Euclidian distances between resampling/recapture events of Mule Deer (*Odocoileus hemionus fuliginatus*) across California State Route 67, Scripps Poway Parkway and Poway Road in southern California grouped into three time intervals. The box depicts the middle half of the data between the 25th and 75th percentiles, and the horizontal line marks the median. The shaded area indicates the 95% confidence intervals around the medians, and whiskers demarcate the main body of the data. The median distance between resampling events (occurring same or next day) was lower than the median distance between recapture events within the same month, and between seasons. *N* = number of recapture/resampling events within each time interval.

829 m and 1,342 m, respectively. The distance between recaptures was significantly less than the distances between all unique individuals in the study area (average distance 4,444 m; difference between means = -3,628.1; t = -21.58, df = 10, $P < 0.001$). We did not recapture any individuals across Route 67, Poway Road, or Scripps-Poway Parkway (Fig. 3).

Pedigree reconstruction.—The pedigree reconstruction analysis identified 11 full sibling and 10 mother offspring pairs (Table 3, Fig. 4). Of these, COLONY identified three full sibling pairs and one mother and her two offspring on opposite sides of CA67. The average distance between full siblings and mother offspring pairs was 1,738 m (ranging from same location to 5.7 km), and 1,988 m (ranging from 58 m to 4.3 km), respectively. The average distance between non-related individuals in the study area was 4,466 m. The average distance between first order relatives was significantly less than that between unrelated individuals in the study area (difference between means = -2,593.0; t = -6.15, df = 20, $P <$ 0.001).

Population structure.—Exact tests of genetic differentiation based on allele frequencies showed significant differentiation for the East-West scenario ($P < 0.001$). For the Roads scenario, we detected significant differentiation only between the region South of Scripps Poway Road and the region East of Route 67, as well as the region South of Poway Road and the region East of Route 67. These test results suggest some genetic differentiation exists across Route 67, but there was no evidence of this across Scripps Poway Road nor Poway Road. The best K analysis in CLUMPAK suggested K = 2 as the best solution using the method of Evanno et al. (2005) and K = 1 using the method of Pritchard et al. (2000). Because the Evanno et al. (2005) method cannot test the probability that K = 1, we conclude that a single gene pool (K = 1), as determined by STRUCTURE, is the most likely configuration in our study area (Fig. 5).

Discussion

The goals of this project were to assess east-west connectivity across California State Route 67 between Lakeside and Poway, and north-south connectivity across Scripps Poway Parkway and Poway Road for the Southern Mule Deer in San Diego County, California. We assessed connectivity using a combination of direct recapture and indirect population analyses. Recapture results suggest that deer remain resident in relatively small home ranges. We recaptured seven of the 71 Mule Deer and two deer were recaptured twice. None of the recaptures occurred across Route 67, Poway Road, or Scripps Poway Parkway, and all recaptures were found in close proximity to initial captures (within 1.5 km). This sug-

gests that over the sampling time frame, deer tended to remain in relatively small areas. Even when monitoring over a broader spatial extent (San Diego MSPA) and temporal period (8 y), Bohonak and Mitelberg (unpubl. report) reported similarly localized recaptures, with recapture distances ranging from 60 m to 1 km. Using telemetry and intense observations, Kie et al. (2002) also estimated small home ranges for Southern Mule Deer in San Diego County (average = 49 ha). These were between 2–20 times smaller than those estimated for other subspecies in other regions of California.

While individual mark recapture methods are limited to detecting movements over the time frame of the study, examining the spatial arrangement of parents and offspring and siblings can potentially provide information on longer term movement and dispersal patterns, over the time frame of a generation. Pedigree reconstruction identified some movement of first order relatives across Route 67. Pedigree analyses identified 21 first order relative dyads and of these, five pairs were found on opposite sides of Route 67 (representing 4–5 crossing events, 19–24%). This shows that movement across the road is possible, although movements on the same side of Route 67 were more frequently detected. First order relatives tended to be found farther apart than individual recaptures, up to 5.6 km. In their broader study, Bohonak and Mitelberg (unpubl. report) reported some first order relative pairs at distances up to 50 km apart, although the majority were within 2 km of each other. In combination, these results suggest that while most deer remain resident in small home ranges, long distance movement or dispersal events are possible throughout San Diego County.

We performed two types of analyses to assess genetic population structure. While contingency tests showed statistically significant population differentiation between deer to the west and east of Route 67, the results of the individual based clustering analyses suggested that a single gene pool was optimal for the region. Previous studies have found that exact tests for population structure are more sensitive to detecting fine-scale genetic structure that may indicate recent barriers to movement than individual-based clustering methods (Waples

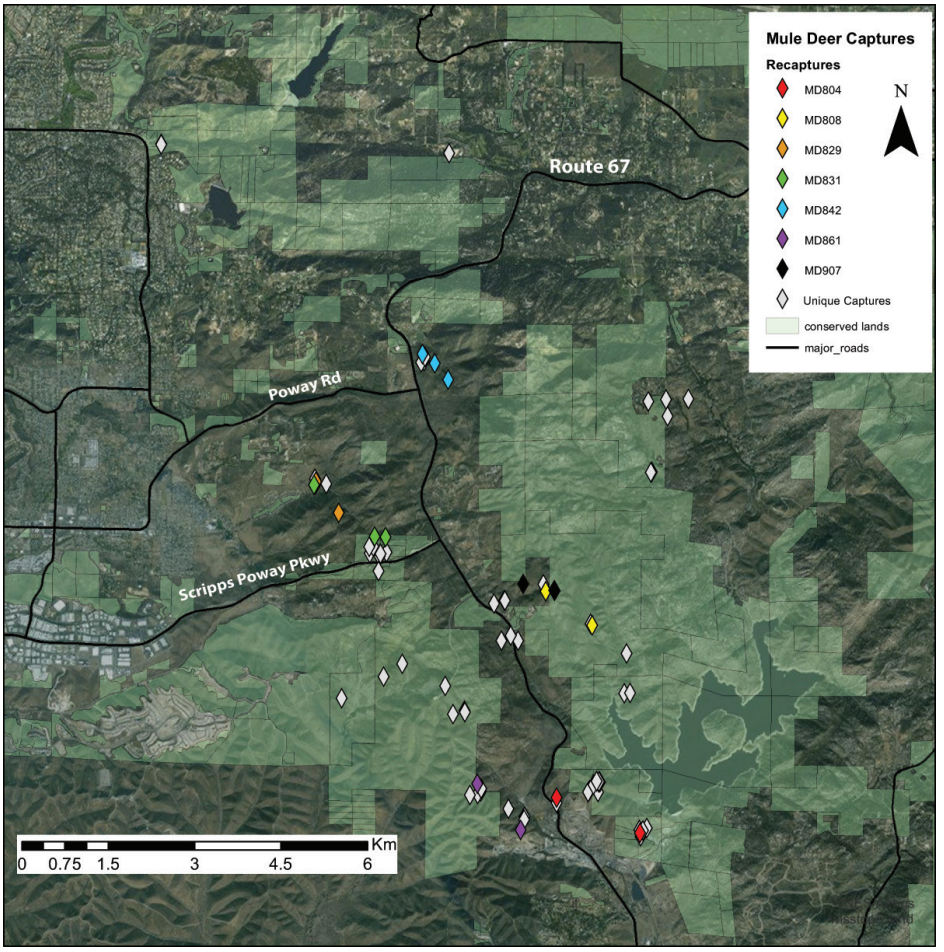

Figure 3. Locations of 80 capture events of Mule Deer (*Odocoileus hemionus fuliginatus*) across California State Route 67, Scripps Poway Parkway and Poway Road in southern California. Individuals captured once are in gray and individuals captured multiple times are color coded. Two individuals (MD831, in green, and MD842, in blue) were recaptured on two separate occasions. (World Imagery Basemap sources: ESRI, Digital Globe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo and the GIS User Community).

and Gaggiotti 2006; Barr et al. 2015). Weak structure across Route 67 may reflect the social structure of Mule Deer. Female Mule Deer offspring are known to set up territories near their mothers, resulting in what has been described as rose-petal population structure (Porter et al. 1991). A partial barrier to movement may cause a build-up of family structure along the road, resulting in the detected allele frequency differences.

A small number of successful crossings, however, may be adequate to maintain a single genetic cluster across Route 67 over the long term. Theoretically, migration rates of 1–10 individuals per generation are sufficient to counteract drift in an ideal population at Hardy-Weinberg equilibrium (Mills and Allendorf 1996; Wang 2004). Additionally, Mule Deer social structure is characterized by a polygamous mating system, with male biased dispersal and female philopatry, a social and breeding group structure that has been shown to preserve individual heterozygosity, while reducing the number of migrants necessary to maintain gene flow (Chesser 1991a, b; Sugg et al. 1996; Stortz 1999; Parreira and Chikhi 2015). One male deer crossing every few years may therefore be enough to introduce his genetic diversity to a region through multiple matings in a single year, or consecutive years. Male competition may force older or weaker males to move on as new males enter a region. Because males do not establish permanent territories, males tend to move greater distances and have larger home ranges than females (Anderson and Wallmo 1984). Although our sample size is quite small and the study area extent fairly limited, male recapture distances averaged 1.6 times farther than female recaptures, and first order relative pairs that included a male were on average 1.9 times farther apart than female relatives. Long male dispersal events may be difficult to detect directly through mark-recapture if they are infrequent, but appear to be reflected in the overall population genetic structure.

Our results suggest that State Route 67 may reduce, but not preclude movement and gene flow in Mule Deer between Lakeside and Poway. These results are concordant with those of other ongoing connectivity investigations. For example recent camera trap and road kill observations along Route 67 include instances of Mule Deer using or approaching two culverts and two reports of deer road kill, suggesting that Mule Deer may be able to use these underpasses, and attempt at-grade crossings (Megan Jennings and Rebecca Lewison, unpubl. report). We did not detect recaptures or first order relatives on either side of Scripps-Poway Parkway or Poway Road; however, we detected no significant differentiation using contingency tests. This may indicate that these roads do not pose a barrier to deer gene flow, although these results may also be impacted by small sample sizes north of Poway Road (three individuals) and between Poway Road and Scripps-Poway Parkway (11 individuals). Larger sample sizes will be needed to better assess the genetic impacts of these roads.

Table 2. Euclidean distances in meters between Southern Mule Deer (*Odocoileus hemionus fuliginatus*) recaptured at sites along Route 67 from Lakeside to Poway, San Diego County, California, and average distance across all recaptures and by sex.

Mule Deer	Sex	Distance (m)	Season
MD804	M	1,564	Spring-Fall
MD808	F	998	Spring-Fall
MD829	F	669	Spring-Fall
MD831	M	190	Spring-Fall
MD831	M	1,382	Fall
MD842	M	628	Spring
MD842	M	262	Spring-Fall
MD861	F	1,095	Spring-Fall
MD907	F	553	Fall
Average		816	
Average M		1,342	
Average F		829	

Table 3. Euclidean distances in meters between Mother-Offspring and Full Sib Pairs of Southern Mule Deer (*Odocoileus hemionus fuliginatus*) at sites along Route 67 from Lakeside to Poway, San Diego County, California, as identified by the program COLONY.

Relationship		Distance (m)	Sex	Across Road
Mother	Offspring			
MD808	MD826	4,292	M	no
MD808	MD868	1,105	M	no
MD808	MD877	1,349	F	no
MD808	MD903	5,505	F	no
MD808	MD949	2,730	F	no
MD815	MD963	2,988	F	Route 67
MD815	MD987	841	F	Route 67
MD949	MD815	832	F	no
MD949	MD943	185	F	no
MD949	MD957	58	F	no
Average		1,988		
Sibling 1	Sibling 2			
MD802	MD940	5,735	FM	Route 67
MD815	MD943	744	FF	no
MD829	MD831	837	FM	no
MD829	MD840	1,076	FM	no
MD831	MD840	250	MM	no
MD835	MD1004	1,329	MM	no
MD844	MD922	5,691	MF	Route 67
MD848	MD980	738	FF	no
MD850	MD950	2,613	MM	Route 67
MD883	MD883	4	FF	no
MD952	MD956	105	FF	no
Average		1,738		

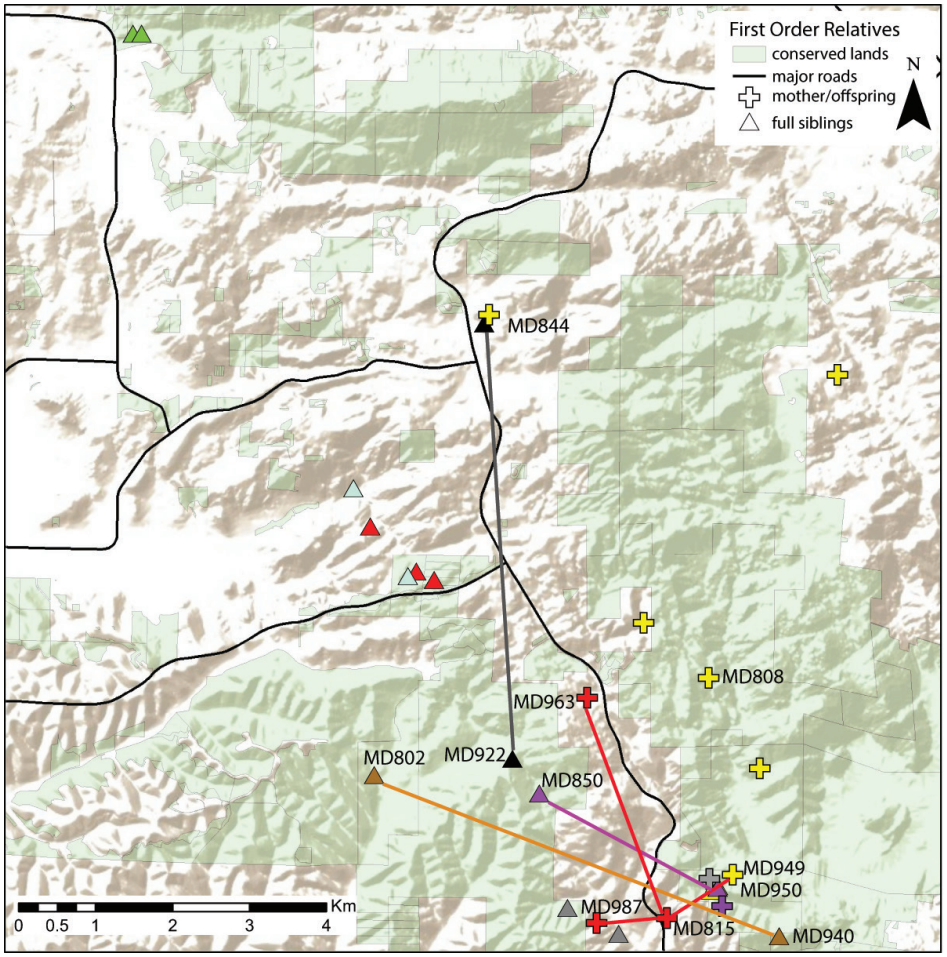

Figure 4. Locations of first-order relatives of Mule Deer (*Odocoileus hemionus fuliginatus*) across California State Route 67, Scripps Poway Parkway and Poway Road in southern California, as identified by the program COLONY (P > 0.05). Family groups are color coded with unique symbols. Lines are drawn between first-order relatives found on either side of Route 67. (World Terrain Basemap sources: Esri, USGS, NOAA).

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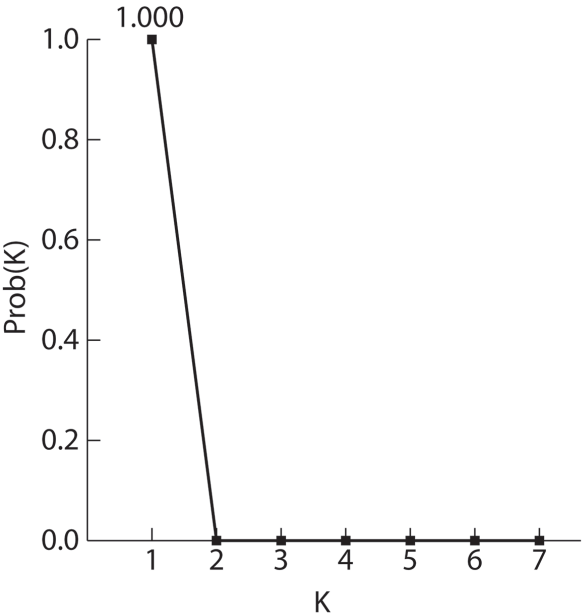

Figure 5. Probability plot of STRUCTURE results of Mule Deer (*Odocoileus hemionus fuliginatus*) across California State Route 67, Scripps Poway Parkway and Poway Road in southern California.

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Appendix 1. Mule Deer (*Odocoileus hemionus*) primers, primers sequences (5' to 3'), and concentrations in the multiplex PCR reactions.

		Forward primer		Reverse Primer	
Locus	Size range	Sequence	Conc. (μM)	Sequence	Conc. (μM)
B	149–153	6FAM - GCTGCTCTCCTACTGCTCTG	0.038	CTATTCGTCTTCTTCCTCTCTG	0.038
C	311–327	6FAM - CAACCATTCATCCATCTTG	0.203	AAAGGTAGAAAGGGTGAGC	0.203
D	162–186	PET - AGAGCCTCGTCTTTTCATTC	0.127	TTGCTGCTTGCTTGTCTAAT	0.127
F	157–165	VIC - AAGGAGTCTTTCAGTTTTGAGA	0.025	GGTTCTGTCTTTGCTTGTTG	0.025
G	318–330	VIC - TATGGTCACAGCAACATTGT	0.038	GTTCCCTTCCTTTTTCAGG	0.038
H	349–353	PET - GCTGCCATTGCCAGATA	0.241	CCCCTCCTGTGCTCTCA	0.241
J	238–246	6FAM - CACGCAACCACTCATTTACC	0.101	TGGGTGAAAGGATTATGTGC	0.101
K	193–209	6FAM - GCAGGAAGGAGGAGACAGTA	0.051	GCTGGTTCGTTATCATTTAGC	0.051
L	260–296	PET - CCCTGTGGTCTAGCAAA	0.177	ATAGGCACATGCTCATAAG	0.177
M	142–170	NED - AGGGAAACCTCTGTTCAGGA	0.025	ACCAAGCAAAATGCCTTACA	0.025
N	289–330	NED - TCCAGAGAAGCAACCAATAG	0.127	GTGTGCCTTAAACAACCTGT	0.127
P	215–235	6FAM - TTTCACTGTTTTCTCCTTCAGA	0.152	TGCCCAATCAGATGTTGTAG	0.152
R	264–296	VIC - GGGGTCTTCTCAATCCA	0.127	TCAGTTTCTGGAACTCTAAAGT	0.127
S	191–219	VIC - GCAAAGAGACAGAAGACAATAG	0.101	GACCAGGAAACCCAGAAT	0.101
V	84–96	6FAM - GCAAACAGAAATAGCCACAG	0.025	TCAGGATGGGTTGAATAAATC	0.025
SRY	223	NED - CCCATGAACGCATTCATTGTGTGG	0.101	ATTTTAGCCTTCCGACGAGGTCGATA	0.101