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DNA Fingerprinting of Southern Mule Deer (*Odocoileus hemionus fuliginatus*) in North San Diego County, California (2018–19)

Open-File Report 2019–1138

U.S. Department of the Interior
U.S. Geological Survey

Cover: **Top Left**: Biological technician Ryan Buck searching for Southern mule deer scat under the Lake Hodges Bridge, in San Diego County, with mule deer in the background; photograph taken by Julia G. Smith, December 11, 2018.
Bottom Right: Fresh Southern mule deer scat pellets; photograph taken by Anna Mitelberg, May 11, 2018.

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Conversion Factors

International System of Units to U.S. customary units

Multiply	By	To obtain
	Length	
kilometer (km)	0.6214	mile (mi)
meter (m)	1.094	yard (yd)
	Volume	
milliliter (L)	0.03381402	ounce, fluid (fl. oz)

Abbreviations

A	number of alleles
CNLM	Center for Natural Lands Management
DA	discriminant axis
DAPC	discriminate analysis of principal components
DNA	deoxyribonucleic acid
F_{ST}	fixation index
FS	full siblings
GPS	Global Positioning System
H_e	expected heterozygosity
HCA	habitat conservation area
HS	half siblings
I	interstate
k	number of assumed genetic groups
MCBCP	Marine Corps Base Camp Pendleton
MCMC	Markov Chain Monte Carlo
N_e	effective population size
p	probability
P_{ID}	probability of identity
P_{SIB}	probability of sibship
PC	principal component
PCR	polymerase chain reaction
PO	parent-offspring
SE	standard error
SR	state route
USGS	U.S. Geological Survey
v	version

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Abstract

Throughout the western United States, efforts are underway to better understand and preserve migration and movement corridors for mule deer and other big game and to minimize the impacts of development and other land-use change on populations. San Diego County is home to a unique non-migratory subspecies of mule deer, the Southern mule deer (*Odocoileus hemionus fuliginatus*; herein refered to as "mule deer"). Because it is the only large herbivorous mammal in San Diego, connectivity among mule deer groups is an important indicator of functional connectivity throughout San Diego County urban preserves and has therefore been monitored within central and eastern San Diego County using DNA fingerprinting since 2005. To continue this effort and to assess genetic connectivity in north San Diego County (herein "North County"), we genotyped scat samples from preserves in the area and tissue samples from Marine Corps Base Camp Pendleton. We used non-invasive capture/recapture analyses and pedigree analyses for assessing short-term movement and population clustering analyses to assess gene flow in North County. Additionally, we performed similar analyses on the combined San Diego County dataset, which was composed of the North County dataset collected for this study and a previously collected dataset from central and eastern San Diego County. Using recapture data, we found multiple instances of mule deer crossing roads in urban North County preserves, with several of these events occurring in areas where there are underpasses and culverts known to be used by mule deer. Corroborating previous studies in the region and statewide, pedigree and population structure analyses support the presence of two genetic clusters for mule deer in San Diego County—the "Coastal" and "Inland/Mountain" clusters. Low estimates of effective population size, especially in the Coastal cluster, suggest that to further understand potential vulnerabilities of mule deer in this region, it is important to continue to monitor connectivity, in particular, at the boundary between these two clusters.

Introduction

The Southern mule deer, *Odocoileus hemionus fuliginatus*, is one of six subspecies of mule deer in North America and is distributed in southern California, U.S.A. (fig. 1), through Baja California, Mexico. As the only large herbivorous mammal in western San Diego County (fig. 1), the Southern mule deer, herein "mule deer", occupies a unique ecological niche as a grazer and food source for mountain lions and coyotes. It is also a game species managed for hunting in portions of the county. Given these roles and its larger habitat requirements, the mule deer is considered an indicator of functional connectivity. Thus, monitoring connectivity among individual preserves within protected lands in San Diego County is of primary concern for this species (San Diego Management and Monitoring Program, 2011); indeed, the mule deer is a monitored species in several conservation plans throughout the region (City of San Diego, 2002; San Diego Association of Governments, 2003).

Several methods have been used to monitor mule deer populations in California, and San Diego County (fig. 1). These include (1) collecting radio-telemetry data (Colby, 2008), (2) conducting track and sign surveys (Markovchick-Nicholls and others, 2008; City of Carlsbad, 2015), (3) monitoring pinch points using wildlife cameras (Hayden, 2002; City of Carlsbad, 2015), (4) collecting genetic material directly from animals being handled or harvested (Pease and others, 2009), and (5) collecting genetic material from mule deer scat (that is, non-invasive methods; Valero, 2004; Mitelberg, 2010; Bohonak and Mitelberg, 2014; Mitelberg and Vandergast, 2016; Fraser and others, 2019). Genetic methods are particularly well suited for monitoring connectivity in urban landscapes for large and timid animals like mule deer. Whereas the first three approaches monitor only direct movement, population genetics analyses provide a measure of whether individuals are contributing their genes to their new territories through reproduction following movement (Schwartz and others, 2007).

As an additional benefit, genetic sampling using scat collected from an animal's approximate range over time offers a non-invasive method for monitoring direct movement of animals (in other words, a non-invasive form of capture-mark-recapture methods). Indeed, previous studies conducted in areas inhabited by mule deer in south and central San Diego County (Valero, 2004; Mitelberg, 2010; Bohonak and Mitelberg, 2014; Mitelberg and Vandergast, 2016) have shown that deoxyribonucleic acid (DNA) fingerprinting of mule deer scat can be an efficient and informative method for monitoring urban populations of mule deer in the region. These studies found population genetic structure and low levels of movement and gene flow through several areas in the region, revealing limited genetic exchange among resident groups of mule deer. Genetic connectivity appears to decline from east to west across the county and may be associated with increasing urbanization and barriers to movement along this east-west axis.

As with the rest of the county, many natural areas in north San Diego County (herein, "North County") are bordered or surrounded by urbanization. Potential barriers to mule deer movement include urban development, interstate (I) highways and state routes (SR; for example, I-15, I-5, SR-76, SR-78; appendix fig. 1.1), and heavily trafficked surface roads (for example, Rancho Santa Fe Road and Palomar Airport Road; appendix fig. 1.1). Monitoring connectivity across these potential barriers will help to elucidate their impacts on mule deer populations.

In this study, our primary objective was to assess mule deer movement and gene flow across North County. Our secondary objective was to assess mule deer population structure across the entire San Diego County region, incorporating previously collected datasets (Bohonak and Mitelberg, 2014; Mitelberg and Vandergast, 2016).

Methods

Study Site and Collections

To complement previous studies in the region (fig. 1; table 1), we focused on obtaining mule deer genetic samples from North County. We obtained both mule deer scat and tissue samples for population genetic analyses and used only the scat dataset for capture-recapture.

Scat Samples

We obtained mule deer scat samples from North County, and specifically, within or near preserves in the cities of Carlsbad, Escondido, Fallbrook, Pauma Valley, Valley Center, and Ramona (appendix fig. 1.1; table 2). Within the Carlsbad Habitat Management Plan preserve system, scat samples were collected by staff from Center for Natural Lands Management (CNLM) between April 11 and May 31, 2018, and again on February 28, 2019, by walking trails where mule deer presence has been observed (City of Carlsbad, 2015). We also obtained scat samples from the remaining sites by visiting sites within North County (between May 11, 2018, and February 28, 2019) where mule deer presence has previously been documented or by sending out collection kits to collaborators. We visually assessed scat samples for age and collected only samples that looked relatively fresh (see Bohonak and Mitelberg [2014] for details on scat age assessment). We collected and stored dry scat samples at room temperature in brown paper bags. We collected and stored moist scat samples (such as those collected very recently following defecation or during misty or rainy weather) in empty non-airtight pipette tip boxes to prevent contamination from seepage through the paper bags and to facilitate rapid drying before molding occurred. Along with sample collection date, exact Global Positioning System (GPS) data was collected for all scat samples at time of collection.

Tissue Samples

Tissue samples from Marine Corps Base Camp Pendleton (MCBCP; appendix fig. 1.1) came from animals harvested between September 3 and November 25, 2018. Samples were collected by the MCBCP game warden in screw cap tubes filled with 200-proof ethanol and stored at room temperature until we could extract DNA. Hunters self-reported the location to the MCBCP game warden by noting the kill on a map. The game warden recorded harvest date, gender, GPS coordinates (to within 100 meters of the kill). Based on location data, each sample was assigned to one of three collection sites: (1) Coastal—a coastal site dominated by coastal sage scrub, west of the Santa Margarita River and south of Basilone Road; (2) North—a northern site at higher elevation, dominated by chaparral, oak/savannah, west of the Santa Margarita River and north of Basilone Road; and (3) East—an eastern site characterized by more development, a mosaic of non-native vegetation, coastal sage scrub, and chaparral, east of the Santa Margarita River (appendix fig. 1.1; not all geographic locations labeled on map).

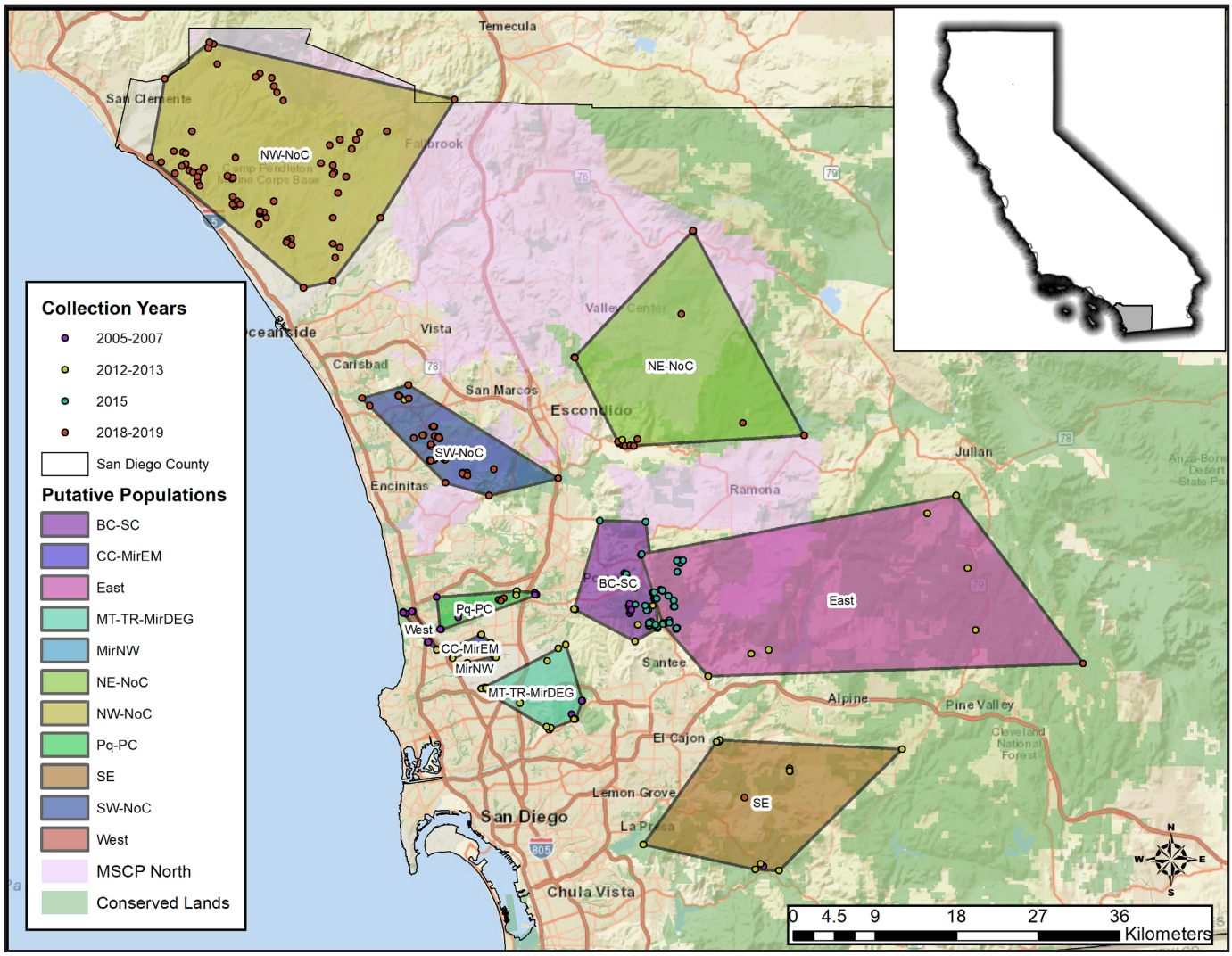

Figure 1. Successfully genotyped Southern mule deer in San Diego County, California (shaded in inset) between 2005 and 2019, with individuals grouped by putative geographic populations, following criteria described in Bohonak and Mitelberg (2014; see table 1).

Table 1. Regional sites represented by Southern mule deer successfully genotyped between 2005 and 2019 in San Diego County.

[Sites added during 2018–19 include 1–6, 8–30, 59, and 61. Samples were also added to site 36 as part of 2018–19 collections]

Putative geographic population[1]	Site name	Site number
NW-NoC	Marine Corps Base Camp Pendleton - Coastal	1
NW-NoC	Marine Corps Base Camp Pendleton - North	2
NW-NoC	Marine Corps Base Camp Pendleton - East	3
NW-NoC	Santa Margarita Ecological Reserve	4
NE-NoC	Daley Ranch Preserve	5
NE-NoC	San Diego Zoo Safari Park - Beckman & Tram	6
NE-NoC	San Diego Zoo Safari Park	7
NE-NoC	San Diego Zoo Safari Park - Open Space	8
NE-NoC	Hellhole Canyon (Carter Residence)	9
NE-NoC	Plaisted Creek Preserve	10
NE-NoC	Pamo Valley	11
NE-NoC	Lake Sutherland	12
SW-NoC	City of Carlsbad Veterans Park	13
SW-NoC	City of Carlsbad Crossings Golf Course	14
SW-NoC	Carlsbad Oaks North HCA - North Faraday	15
SW-NoC	Carlsbad Oaks North HCA - South Faraday	16
SW-NoC	Dawson Los Monos Canyon Reserve	17
SW-NoC	Rancho La Costa HCA - Ridgeline	18
SW-NoC	Rancho La Costa HCA- Meadowlark	19
SW-NoC	Rancho La Costa HCA - San Marcos Creek	20
SW-NoC	Rancho La Costa HCA - San Elijo Road	21
SW-NoC	Rancho La Costa HCA - Denk	22
SW-NoC	Rancho La Costa HCA - Copper Creek	23
SW-NoC	Bumann Ranch Preserve	24
SW-NoC	Rancho La Costa HCA – Wildlife Corridor	25
SW-NoC	Rancho La Costa HCA – Choumas-Pappas	26
SW-NoC	Bridges and Sante Fe Creek Preserve	27
SW-NoC	Los Cielos Preserve - Cielos Estates	28
SW-NoC	Los Cielos Preserve - Meisha Canyon	29
SW-NoC	Lake Hodges - Bridge	30
MirNW	Miramar - Rose Canyon	31
MirNW	Miramar - Miramar Road - across Golf Course	32
PQ-PC	Del Mar Mesa	33
PQ-PC	Los Penasquitos Canyon Preserve - West	34
PQ-PC	Carmel Mountain	35
PQ-PC	Los Penasquitos Canyon Preserve - East	36
PQ-PC	Los Penasquitos Canyon Preserve - East of Black Mountain Rd	37
PQ-PC	Penasquitos Creek	38
CC-MirEM	Miramar - Eastgate Mall	39
CC-MirEM	Carrol Canyon - West	40
CC-MirEM	Carrol Canyon - East	41
West	Torrey Pines State Reserve	42
West	Los Penasquitos Marsh	43
West	Sorrento Valley	44
MT-TR-MirDEG	Miramar - Landfill	45
MT-TR-MirDEG	Miramar - Parcel G	46
MT-TR-MirDEG	Tierrasanta	47
MT-TR-MirDEG	Miramar - East	48
MT-TR-MirDEG	Mission Trails Regional Park	49
BC-SC	Beeler Canyon	50
BC-SC	Poway - North	51
BC-SC	Poway - South	52
BC-SC	Sycamore Canyon	53
East	East of SR-67	54
East	Lake Jennings	55
East	Julian	56
East	Cleveland National Forest - north	57
East	Cuyamaca Rancho State Park	58
East	Laguna Mountains	59
SE	South Crest Preserve	60
SE	Jamul (Fisher Residence)	61
SE	Sycuan Peak Ecological Reserve	62
SE	Japatul Valley	63
SE	San Miguel	64
SE	Rancho Jamul Ecological Reserve	65
SE	Hollenbeck Canyon	66

[1]Putative geographic populations, as defined in Bohonak and Mitelberg (2014). We grouped samples from the North County focus area into three additional populations: (1) “Northwest North County” (“NW-NoC”) — north of I-78, west of I-15; consisting of Marine Core Base Camp Pendleton and the Santa Margarita Ecological Reserve in Fallbrook; (2) “Northeast North County” (“NE-NoC”) — north of I-78, east of I-15; composed of samples from Escondido east of I-15, Pauma Valley, Valley Center, and Pamo Valley and Lake Sutherland in Ramona; and (3) “Southwest North County” (“SW-NoC”) — south of I-78, north of I-56, west of I-15; consisting of samples from Carlsbad and Escondido sites west of I-15. Due to denser sampling in these regions, samples from the “NW” population of Bohonak and Mitelberg (2014) have been reassigned to either the NW-NoC or the SW-NoC group of deer, and for clarity, the original “NE” population of Bohonak and Mitelberg (2014) was renamed as “East”.

Table 2. North San Diego County sites from which Southern mule deer scat or tissue samples were successfully obtained in 2018–19, putative geographic population, number of samples obtained, and number of samples yielding a reliable multilocus genotype (defined in Bohonak and Mitelberg, 2014).

[HCA, habitat conservation area]

Putative geographic population	Site name	Number of samples obtained	Number of samples yielding reliable multilocus genotype
SW-NoC	Bridges and Santa Fe Creek Preserve	3	1
SW-NoC	Bumann Ranch Preserve	1	1
SW-NoC	Carlsbad Oaks North HCA (North Faraday)	35	16
SW-NoC	Calrsbad Oaks North HCA (South Faraday)	35	14
SW-NoC	City of Carlsbad Veterans Park	1	1
SW-NoC	City of Carlsbad Crossings Golf Course	19	9
SW-NoC	Dawson Los Monos Canyon Reserve	9	7
SW-NoC	Lake Hodges Bridge	4	2
SW-NoC	Lake Hodges West	11	0
SW-NoC	Los Cielos Preserve (Cielos Estates)	17	14
SW-NoC	Los Cielos Preserve (Meisha Canyon)	20	7
SW-NoC	Rancho La Costa HCA (Choumas-Pappas)	59	22
SW-NoC	Rancho La Costa HCA (Copper Creek)	59	32
SW-NoC	Rancho La Costa HCA (Denk)	54	19
SW-NoC	Rancho La Costa HCA (East Ridgeline)	1	1
SW-NoC	Rancho La Costa HCA (Meadowlark)	10	9
SW-NoC	Rancho La Costa HCA (Ridgeline)	65	27
SW-NoC	Rancho La Costa HCA (San Elijo Road)	47	23
SW-NoC	Rancho La Costa HCA (San Marcos Creek)	23	8
SW-NoC	Rancho La Costa HCA (Wildlife Corridor)	17	13
SW-NoC	Rancho La Costa HCA (Winston)	3	3
NE-NoC	Boden Canyon Ecological Reserve	4	0
NE-NoC	Daley Ranch Preserve	12	7
NE-NoC	Grasslands	3	0
NE-NoC	Hellhole Canyon (Carter Residence)	16	6
NE-NoC	Hellhole Canyon Preserve	16	0
NE-NoC	Lake Sutherland	4	1
NE-NoC	Pamo Valley	5	2
NE-NoC	Plaisted Creek Preserve	13	7
NE-NoC	Rancho Guejito (adjacent land)	3	0
NE-NoC	San Diego Zoo Safari Park-Beckman & Tram	34	17
NE-NoC	San Diego Zoo Safari Park-Open Space	9	5
NE-NoC	Sycamore Creek and San Dieguito River Confluence	6	0
NW-NoC	Marine Corps Base Camp Pendeleton (Coastal)	38	38
NW-NoC	Marine Corps Base Camp Pendeleton (East)	8	8
NW-NoC	Marine Corps Base Camp Pendeleton (North)	23	23
NW-NoC	Monserate Mountain	1	0
NW-NoC	Santa Margarita Ecological Reserve	35	6
SE	Jamul (Fisher Residence)	6	1
East	Laguna Mountains	4	2
PQ-PC	Los Penasquitos Canyon Preserve east	2	2
		735	354

DNA Extractions and Genotyping

We extracted DNA from scat and tissue samples (extraction methods described below) and genotyped both sample types using a multiplex PCR (polymerase chain reaction) composed of 15 microsatellite loci and a sex marker (primers and conditions described in Bohonak and Mitelberg [2014]). We scored microsatellites using Genemarker version (v) 3.0.1 and binned using MsatAllele v 1.03 (Alberto, 2009).

Scat Samples

We extracted and genotyped DNA from scat piles following Bohonak and Mitelberg (2014) with a few minor modifications: (1) scat pellet surface washes were performed in a 5-milliliter (mL) Eppendorf tube set on a lab rotator for 30 minutes, (2) PCR volume was doubled to reduce potential inhibition from contaminants in scat extractions, and (3) a single PCR and fragment analysis run was used to assess scat DNA extraction quality by visually evaluating chromatographs. From this point on, we abandoned scat samples that failed to yield a clean, scorable chromatograph in this initial genotyping attempt. For samples with a scorable chromatograph in the first genotyping run, two additional PCRs were performed, resulting in three genotyping attempts for each qualifying scat DNA extraction.

Tissue Samples

We extracted DNA from tissues obtained from the MCBCP harvest using the DNeasy Blood and Tissue extraction kit (Qiagen), following manufacturer's recommended protocol. We genotyped each tissue sample with a single PCR, with the exception of 30 percent of tissue samples that we genotyped twice to assess the quality of tissue-derived genotypes.

Assessing Genotype Quality

Scat Samples

To reduce genotyping error (Bonin and others, 2004) in the scat-derived data, we analyzed the initial three-replicate multilocus genotypes for each scat sample with RELIOTYPE (Miller and others, 2002), a software that implements a maximum likelihood algorithm to assess the reliability of the multilocus genotype based on a reference set of allele frequencies. The software also recommends a replication strategy for samples that fail to pass the 99.49 percent reliability criterion. For this analysis, we used allele frequencies from the combined Bohonak and Mitelberg (2014) and Mitelberg and Vandergast (2016) datasets. We discarded all samples for which RELIOTYPE recommended more than six PCR replicates. We genotyped samples requiring six or fewer PCR replicates again (according to RELIOTYPE recommendations) and ran these through RELIOTYPE a second time. Following these additional PCRs, we discarded all data from scat samples failing to yield a reliable genotype. We used GIMLET 1.3.3 (Valière, 2002) to reconstruct consensus genotypes (from multiple genotypes per scat pile) for all scat piles with reliable DNA fingerprints.

Tissue Samples

For tissue samples from MCBCP, we compared replicate genotypes to assess the reliability of tissue-derived genotypes. For each tissue sample, we also compared the gender assigned by PCR to the gender recorded at the time of harvest.

Identity and Capture-Recapture

Scat Samples

We identified individual mule deer from scat using GIMLET's "group by genotype" algorithm. We considered the first scat pile processed as an individual mule deer's "initial capture event" (the first time that individual was encountered). All matching scat piles collected after this capture were identified as "recapture events" (even if they occurred on the same day). We calculated the minimum, maximum, and average geographic (Euclidean) distances between all capture and recapture events in the R package gdistance (van Etten, 2017).

Tissue Samples

Tissue samples from MCBCP came from harvested individuals. Given the long distance between MCBCP and scat sampling sites (more than 8 kilometers [km]), it is unlikely that harvested mule deer were also sampled earlier in the year during scat collections. To confirm this, we combined the scat and tissue datasets and used CERVUS v 3.0.7 (Kalinowski and others, 2007) to identify all individuals.

Microsatellite Statistics

We performed all analyses from this point forward on the combined dataset of mule deer identified from the scat- and tissue-derived genotypes from North County (herein "North County dataset"). We used CERVUS to (1) calculate basic microsatellite statistics, (2) detect any loci with null alleles, and (3) calculate the average probability that two unrelated individuals (P_{ID}) or two siblings (P_{SIB}) in the dataset could have identical genotypes. Because pedigree and population genetic analyses can be sensitive to null alleles, we eliminated loci determined by CERVUS to have null alleles from all remaining analyses.

North County Pedigree Analyses

Family relationships can be used to complement capture/recapture data and infer long-term movement (over generations). We used the maximum likelihood pedigree reconstruction software COLONY v 2.0.6.5 (Wang, 2004; Wang and Santure, 2009; Jones and Wang, 2010) to identify potential first-order (full-siblings [FS] and parent offspring [PO] dyads) and second-order (half-sibling [HS]) relatives. We ran three repetitions of this analysis, each time with a different seed number and the following parameter options: female and male polygamy, with inbreeding, medium run length, full-likelihood analysis method, medium likelihood precision, no sibship scaling nor sibship prior; all other parameters were set to default. We used genotyping error rates from Bohonak and Mitelberg (2014), with the exception that the minimal recommended false alleles rate of 0.0001 was assigned to all loci to avoid exclusion of parent-offspring pairs based on a single allele. We set the expected probability of detecting a father or mother to 0.05 and 0.15, respectively. We accepted only dyads appearing in all three independent COLONY runs with ≥ 80 percent probability of relatedness ($P \geq 0.80$; Warner and others, 2016). We calculated the geographic distance between all related dyads using ArcMap v. 10.6.1.

Regional San Diego County Population Structure and Effective Population Size

We performed all analyses from this point forward on a "regional dataset" composed of all mule deer identified in this study and the "pre-2018" dataset of 223 mule deer identified in San Diego County by Bohonak and Mitelberg (2014) and Mitelberg and Vandergast (2016). To assess population structure in San Diego County, we first used CERVUS to confirm that no individual from the pre-2018 dataset was also sampled in the present study. We then ran a COLONY pedigree analysis to identify close relatives within the regional dataset. To reduce bias associated with sampling close relatives in population structure analyses (Rodríguez-Ramilo and Wang, 2012), we excluded one individual from each related dyad proposed by COLONY; we did this randomly, with the exception that per dyad, we preferentially retained an individual if it was the sole representative of a sampling site. We performed individual-based clustering analyses on all remaining unrelated individuals throughout the region in STRUCTURE v 2.3.4 (Pritchard and others, 2000; Falush and others, 2003; k = 1–10 clusters [where k is the number of assumed genetic groups]; 10 replicates per k; 500,000 burn in, 500,000 MCMC [Markov Chain Monte Carlo] replicates following burnin; admixture model; correlated alpha) and compiled the replicate runs in CLUMPAK (Kopelman and others, 2015). Individual-based clustering analyses, which search for the optimal number of genetic clusters (k), were based solely on individual genotype. We performed two analyses using STRUCTURE—one without and one with a prior assignment to a putative geographic population (as defined and shown in fig. 1). We used Evanno's ΔK or Pritchard's ln(Pr(X|K) to derive the optimal number of genetic clusters (k).

Additionally, we performed a discriminant analysis of principal components (DAPC; Jombart and others, 2010) using the adegenet package (v 2.1.1; Jombart, 2008) in R 3.5.1 (R Core Development Team, 2011). The package DAPC first uses a principal components analysis to identify population combinations and minimize variation within groups (Jombart and others, 2010). These principal component (PC) eigenvalues are then used in a discriminant analysis to find the discriminant functions that maximize differences among groups while minimizing variation within groups. We used the cross-validation procedure in adegenet to determine the optimal number of PCs to retain in the DAPC analysis, using 90 percent of the dataset as a training dataset and 10 percent as a validation dataset, and performing 30 replicates at each level of PC retention. We selected the number of PCs with the lowest root mean squared error for the final analysis. Because K-means clustering did not converge on an optimal K, we performed DAPC using putative geographic populations defined in Bohonak and Mitelberg (2014; see table 1 and fig. 1), as well as the two genetic clusters detected by STRUCTURE.

To estimate the extent of population differentiation for the entire San Diego County region, we ran a series of pairwise genetic differentiation (F_{ST}) tests using the R package STRATAG, with putative geographic populations as units (Archer and others, 2017). F_{ST} is a measure of population genetic differentiation as a result of population structure (Wright, 1965), ranging from no differentiation at $F_{ST} = 0$ to complete differentiation at $F_{ST} = 1$. We used 10,000 permutations to test the significance of each pairwise F_{ST} value and corrected for multiple tests using the Bonferroni correction ($p = 0.00091$ for 55 tests).

To estimate the effective population size (N_e) for the San Diego region, we used the linkage disequilibrium method implemented in N_e Estimator (Do and others, 2014), with the lowest frequency allele level of 0.05 (to limit inflation of N_e by rare alleles). We used the 95 percent confidence interval determined from permutation tests to obtain a range for the N_e estimates. N_e estimates were obtained for the 11 putative geographic populations; we also combined these populations into the larger regional level genetic clusters, as suggested by the STRUCTURE results, to assess N_e at this regional level.

Results

Collections

Scat Samples

We obtained 666 scat piles (table 2; appendix fig. 1.1). Of these, 437 samples (67 percent) were collected at CNLM managed properties in Carlsbad, 217 were collected from other regions in North County, and 12 samples came from San Diego County sites outside North County (Los Penasquitos Canyon Preserve, Jamul, and Laguna Mountains; these samples were only included in the regional analyses for all of San Diego County). Sampling at several sites in Fallbrook, Pauma Valley, Pala Indian Reservation, Temecula, and Rainbow were unsuccessful (appendix fig. 1.1; not all geographic locations labeled). As in previous studies in the region (Bohonak and Mitelberg, 2014, and Mitelberg and Vandergast, 2016), scat samples from the more rural eastern parts of San Diego County were more challenging to obtain, possibly because mule deer are more spread out in these areas.

Tissue Samples

We received 69 tissue samples (38 males and 31 females) from the 2018 harvest at MCBCP. Thirty-eight samples came from the Coastal, 23 from the North, and 8 from the East site (table 2; appendix fig. 1.1).

DNA Extractions and Genotyping

Scat Samples

We extracted and attempted to genotype all 666 scat piles at least once. Three hundred eighty (57 percent) of these scat piles yielded a scorable chromatograph in the initial genotyping attempt and qualified for further genotyping with two additional PCRs. We removed one of the 15 microsatellite loci (Locus F) from the scat dataset owing to scoring and binning inconsistencies.

Tissue Samples

We extracted DNA from 69 tissue samples and genotyped all 69 tissue samples for the 15 microsatellite loci and a gender identification marker. We scored and retained Locus F genotypes for the tissue samples because this locus amplified consistently in tissue extractions.

Assessing Genotype Quality

Scat Samples

Two hundred eighty-five scat piles (~43 percent of those collected and ~75 percent of those that qualified following a single genotype attempt) yielded a reliable genotype following the screening protocol implemented using RELIOTYPE; all 285 scat samples were unambiguously assigned a gender. Data from the remaining 381 scat piles were discarded from all further analyses.

Tissue Samples

We found no differences among replicate genotypes of tissue extractions. The gender assigned to each harvested mule deer based on the PCR-based gender marker matched the gender of all 69 mule deer as recorded at the time of sample collection.

Identity and Capture-Recapture

From the scat- and tissue-derived datasets collected, we identified a total of 170 individual genotypes (62 males and 108 females); 165 of these individuals came from North County (5 individuals came from outside our North County focus area—Jamul, Los Penasquitos Canyon Preserve, and Laguna Mountains and from this point on were included only in the regional analysis).

Scat Samples

We identified 101 individual mule deer in the scat-derived dataset (24 males and 77 females). Fifty-one mule deer had a single capture event; 50 mule deer, including 30 does and 20 bucks, were recaptured at least once (recapture rate = 50 percent). Recapture events per individual ranged from 1 to 21, and recapture distances ranged from 0 to 4,625 m, with an average of 353 m (table 3; scat piles at several sites did not have reliable GPS coordinates, and we excluded these from distance analyses). Approximately 22 percent of recaptures occurred within 100 m of each other, and 14 percent were recaptured more than 1 km apart.

Table 3. Fifty recaptured Southern mule deer, average Euclidean distance and range among recaptures per individual, potential roads crossed, and sampling sites (if different).

[m, meter; f, female; NA, not applicable; m, male]

Mule deer	Gender	Average distance (m)	Range (m)	Road crossings (undercrossing or at grade? Pinch point?)	Across sampling sites? ( ) = site number assigned in table 1
MDn001	f	599	99–1,077	No	Rancho La Costa HCA-Denk (22)/Winston (002)
MDn003	f	207	10–352	No	No
MDn007	f	663	79–1,330	No	No
MDn008	f	645	4–1,859	No	Rancho La Costa HCA-Denk (22)/Copper Creek (23)
MDn043	f	366	2–1,227	No	No
MDn066	f	244	26–418	No	No
MDn098	f	1,059	NA	No	Rancho La Costa HCA-Copper Creek (23)/Wildlife Corridor (25)
MDn114	f	95	60–140	No	No
MDn116	f	316	1–697	Melrose Dr. (Melrose Road wildlife undercrossing bridge)	Rancho La Costa HCA-San Elijo Road (21)/San Marcos Creek (20)
MDn134	f	NA[1]	NA	NA	NA
MDn140	f	280	34–548	Melrose Dr. (Melrose Road wildlife undercrossing bridge)	Rancho La Costa HCA-San Elijo Road (21)/San Marcos Creek (20)
MDn150	f	95	28–184	No	No
MDn190	f	515	NA	Melrose Dr. (Melrose Road wildlife undercrossing bridge)	Rancho La Costa HCA-San Elijo Road (21)/San Marcos Creek (20)
MDn224	f	259	20–416	No	No
MDn227	f	48	36–72	No	No
MDn239	f	404	NA	No	No
MDn315	f	77	NA	No	No
MDn373	f	513	26–1,004	Faraday Ave. (tunnel; pinch point EW2-9)	Carlsbad Oaks North HCA-North Faraday (15)/South Faraday (16)
MDn383	f	1,067	NA	Melrose Dr. (Melrose Road wildlife undercrossing bridge)	Rancho La Costa HCA-San Marcos Creek (20)/Meadowlark (19)
MDn403	f	347	0–570	Faraday Ave. (tunnel; pinch point EW2-9)	Carlsbad Oaks North HCA-North Faraday (15)/South Faraday (16)
MDn425	f	134	0–223	No	No
MDn439	f	361	49–655	No	No
MDn463	f	49	0–87	No	No
MDn479	f	239	0–372	No	No
MDn508	f	38	NA	No	No
MDn549	f	29	NA	No	No
MDn559	f	170	NA	No	No
MDn573	f	NA	NA	NA	NA
MDn608	f	472	NA	No	No
MDn613	f	18	NA	No	No
MDn069	m	1,660	12–3,631	Rancho Santa Fe Rd. (probably at grade; pinch point EW3-6)	Rancho La Costa HCA-Copper Creek (23)/East Ridgeline (002)/Ridgeline (18)/Winston (00)
MDn104	m	43	NA	No	No
MDn127	m	NA	NA	NA	NA
MDn128	m	NA	NA	NA	NA

Table 3. Fifty recaptured Southern mule deer, average Euclidean distance and range among recaptures per individual, potential roads crossed, and sampling sites (if different).—Continued

[m, meter; f, female; NA, not applicable; m, male]

Mule deer	Gender	Average distance (m)	Range (m)	Road crossings (undercrossing or at grade? Pinch point?)	Across sampling sites? ( ) = site number assigned in table 1
MDn130	m	NA	NA	NA	NA
MDn165	m	NA	NA	NA	NA
MDn167	m	NA	NA	NA	NA
MDn195	m	2,340	21–4,625	Faraday Ave. (tunnel; pinch point EW2-9); El Camino Real Rd.; Cannon Rd.; Faraday Ave. (at grade; pinch point M5-2b)	Veterans Park (13)/Crossings Golf Course (14)/Carlsbad Oaks North-North Faraday (15)/South Faraday (16)/ Dawson Los Monos Canyon Reserve (17)
MDn201	m	219	7–376	No	No
MDn204	m	208	93–311	No	No
MDn208	m	7	NA	No	No
MDn209	m	76	NA	No	No
MDn223	m	233	42–375	No	No
MDn242	m	81	12–141	No	No
MDn351	m	576	4–1,213	Faraday Ave. (tunnel; pinch point EW2-9)	Carlsbad Oaks North HCA-North Faraday (15)/South Faraday (16)
MDn385	m	79	41–140	No	No
MDn386	m	57	NA	No	No
MDn563	m	280	NA	No	No
MDn615	m	11	NA	No	No
MDn716	m	9	7–11	No	No
Does		**333**	**0–1,859**		
Bucks		**392**	**7–4,625**		
All		**353**	**0–4,625**		

[1]NA Recaptured once or no distance calculated due to lack of exact GPS coordinates.

[2]00 Site does not have site number because all individuals collected at the site were recaptures.

We examined recapture events to obtain information on mule deer movement. All recapture events in which roads were crossed occurred in Carlsbad (table 3; figs. 2–5). Several of these recaptures occurred in areas where underpasses and culverts facilitate mule deer movement (City of Carlsbad, 2015). Four does had scat-based recaptures on both sides of Melrose Drive (table 3; fig. 2). These mule deer may have used the Melrose Drive Wildlife Undercrossing (fig. 2) to move between the San Marcos Creek and San Elijo Road sites of Rancho La Costa Habitat Conservation Area (HCA; to locate sites on map, refer to table 1 for site number, then to fig. 8 and appendix fig. 1.2). Scat from two does and one buck was found on both sides of Faraday Avenue; these animals may have used a tunnel under this road (table 3; fig. 3; pinch point EW2-9, City of Carlsbad, 2015). Previous monitoring efforts using cameras and mule deer sign surveys have shown mule deer moving through this tunnel frequently (City of Carlsbad, 2015).

We found two instances of bucks traversing relatively long distances in Carlsbad. Within a period of 13 days, scat from one buck (MDn069) was identified as far as 3.6 km apart in the Copper Creek, Ridgeline, and Winston sites of Rancho La Costa HCA (table 3; fig. 4). Within a period of 25 days, scat from one buck (MDn195) was captured at multiple sites as far as 4.6 km apart moving between Veterans Park, Crossings Golf Course, north and south of Faraday Avenue, and at the Dawson Los Monos Canyon Reserve (table 3; fig. 5).

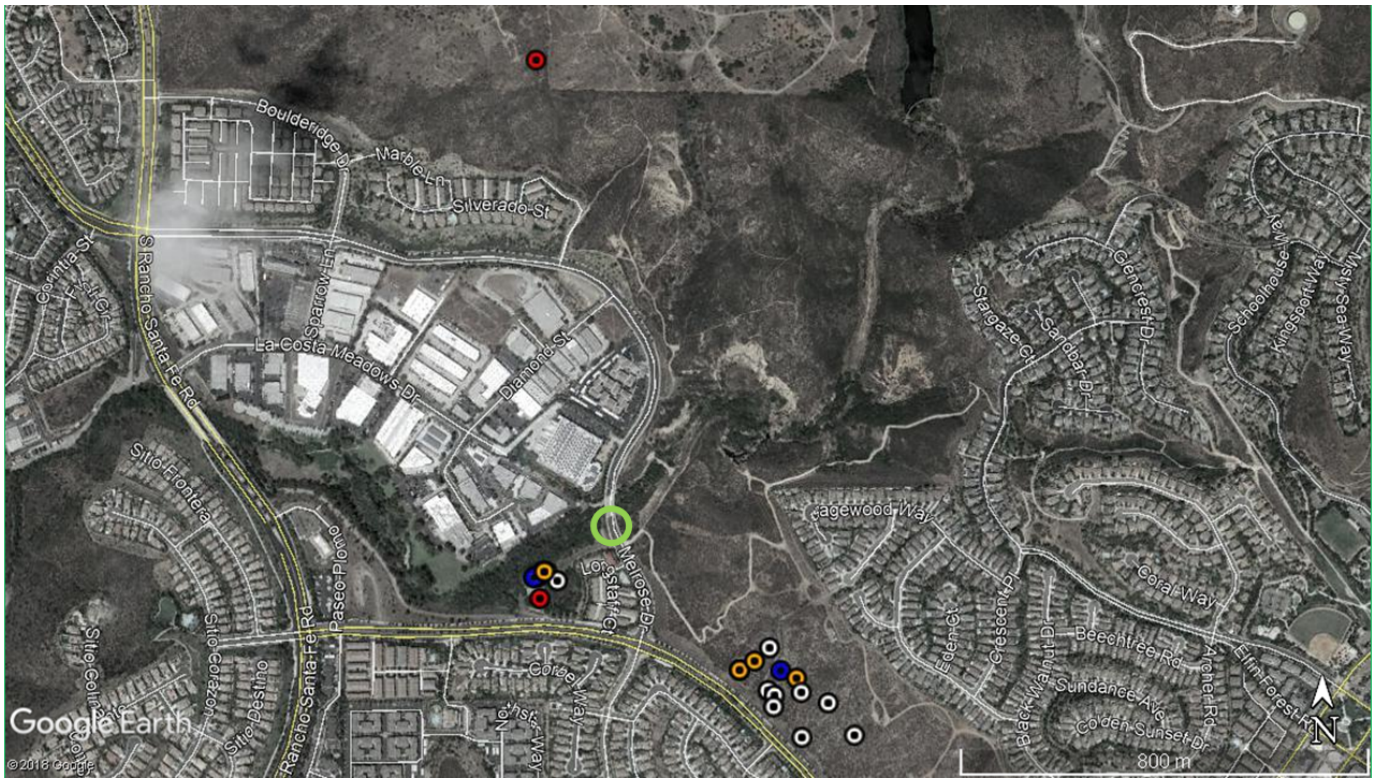

Figure 2. Four does (MDn190 [blue circles], MDn140 [orange circles], MDn383 [red circles], and MDn116 [white circles]) identified moving across Melrose Drive in the Rancho La Costa Habitat Conservation Area. Green circle = Melrose Road Wildlife Undercrossing Bridge.

Figure 3. One buck (MDn351 [red squares]) and two does (MDn373 [white circles], MDn403 [yellow circles]) identified moving across Faraday Avenue. Green circle = tunnel, pinch point EW2-9.

Figure 4. Buck MDn069 with all capture events (red squares). Green diamond = Rancho Santa Fe Road tunnel, pinch point EW3-6.

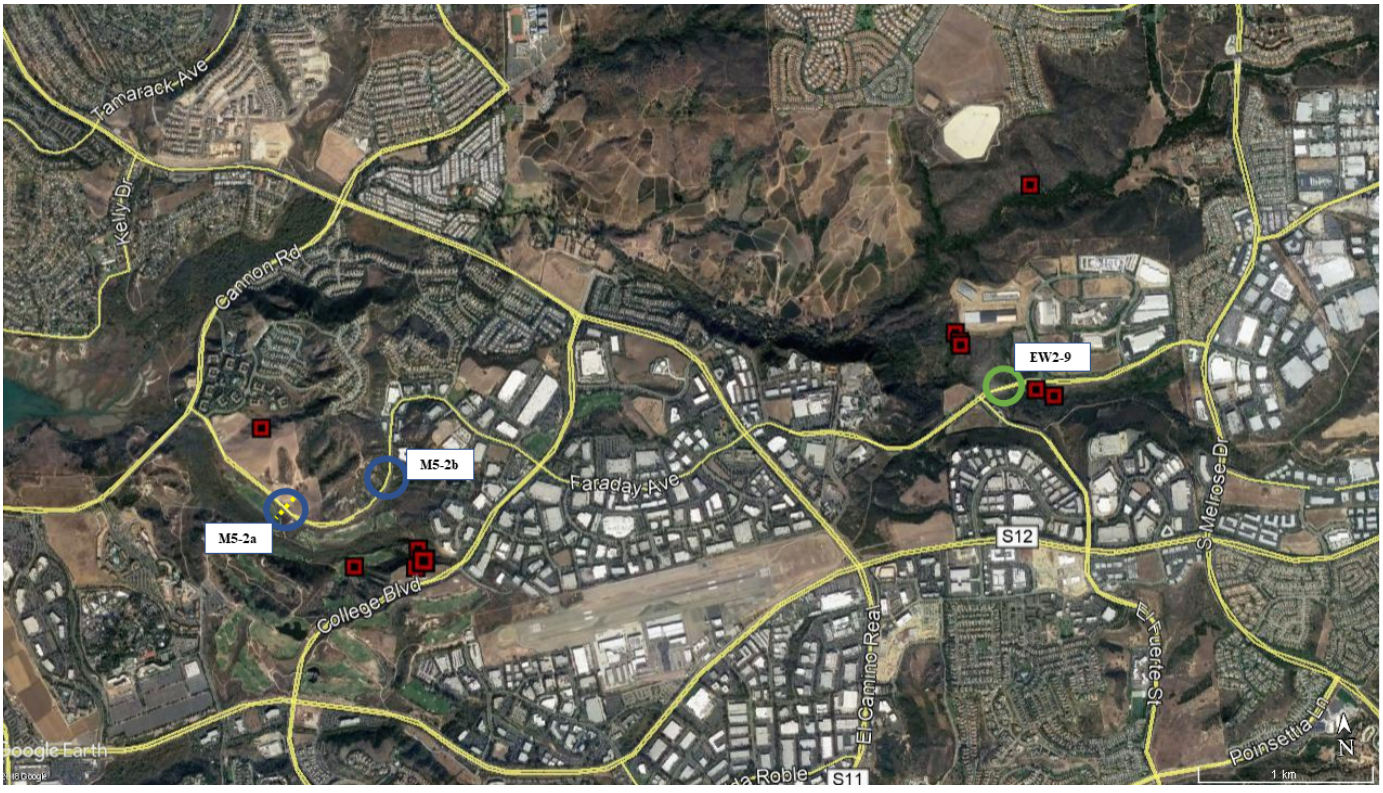

Figure 5. Buck MDn195, with all capture events (red squares). Blue circle = at grade crossing, pinch point M5-2b. Blue circle with yellow dotted line = tunnel, pinch point M5-2a. Green circle = tunnel (see fig. 3).

Tissue Samples

Each of 69 tissue-derived genotypes represented a single individual (38 males and 31 females). We identified no recaptures between the scat and tissue-based datasets.

Microsatellite Statistics

Across the 15 loci in the North County dataset, the P_{ID} of 4.9 x 10^{-11} and P_{SIB} of 4.6 x 10^{-5} were below the upper limits of 0.01 to 0.0001 recommended for genotypes in natural populations (Waits and others, 2001). Locus B was found to have a high probability of null alleles and was dropped from all pedigree and population structure analyses. The number of alleles (A) for the remaining 14 loci ranged from 2 to 12 per locus and was similar to the regional dataset, with a slightly higher average of 5 (table 4; versus 4.9 in the regional dataset). Average expected heterozygosity (H_e) was higher than observed (0.612 versus 0.577), which could indicate some inbreeding in the North County region (table 4).

North County Pedigree Analyses

We used first and second order relatives as another indicator of mule deer movement. Pedigree reconstruction of the North County dataset resulted in 11 full-sibling dyads (8 full-sibling family groups), no parent-offspring dyads, and 9 half-sibling dyads (7 half-sibling family groups; table 5; fig. 6). Six full-sibling dyads consisted of relatives located in different sampling sites and across roads of varying size. The five remaining dyads consisted of relatives within the same sampling site. Three individuals, one buck and two does, making up a single full-sibling group (FS4), spanned the north and south sides of Faraday Avenue within the Carlsbad Oaks North HCA. As in the case of the recaptures, these individuals could be using the tunnel to cross this road (fig. 3; pinchpoint EW2-9, City of Carlsbad, 2015). We identified one pair of female siblings (FS2) spanning the Carlsbad Oaks North and Rancho La Costa HCAs. The 8.5 km between these does is dissected by multiple roads, including Faraday Avenue, Palomar Airport Road, Ranch Santa Fe Road, Pointsettia Lane, Alga Road, and El Fuerto Street. We found two full-sibling does (FS7) within the San Diego Zoo Safari Park, approximately 2 km apart, with one found near the Beckman Center and the other in the eastern open space preserve portion of the park, with minor park roads between them. We identified one pair of full-sibling bucks (FS3) about 3 km apart within the Los Cielos Preserve, on opposite sides of the Del Dios Highway.

We found eight half-sibling pairs (table 5; fig. 6). Two half-sibling pairs (HS1 and HS2) were found within the San Diego Zoo Safari Park. One of these groups was a pair of does found 1.4 km apart. Four half-sibling family groups were found within the MCBCP, with three of these groups occurring between the Coastal and North collection sites at MCBCP and across two minor roads—Los Pulgas Canyon Road (approximately 13–14 km apart) and Basilone Road (approximately 3 km apart). Finally, we identified two half-sibling does (HS3) approximately 14 km apart between Hellhole Canyon (Carter residence) and Pamo Valley. There are four single lane roads between these sites.

Table 4. Microsatellite summary statistics for 14 Southern mule deer loci used for pedigree and population genetic analyses.

[A, number of alleles; N, number of individuals genotyped; H_o, observed heterozygosity; H_e, expected heterozygosity; PIC, polymorphic information content]

Locus	A	N	H_o	H_e	PIC
Locus C	4	165	0.412	0.428	0.389
Locus D	6	165	0.715	0.788	0.752
Locus F	3	69	0.406	0.437	0.380
Locus G	3	162	0.500	0.559	0.490
Locus H	2	165	0.376	0.414	0.327
Locus J	3	165	0.418	0.443	0.352
Locus K	5	165	0.685	0.695	0.639
Locus L	4	164	0.591	0.647	0.584
Locus M	5	165	0.642	0.703	0.651
Locus N	12	164	0.774	0.849	0.832
Locus P	5	165	0.661	0.679	0.616
Locus R	5	165	0.685	0.714	0.659
Locus S	9	160	0.756	0.764	0.728
Locus V	4	164	0.463	0.454	0.421
Average	5.0	157	0.577	0.612	0.559

Regional San Diego County Population Structure and Effective Population Size

We did not recapture any individuals from the previously analyzed regional San Diego dataset during this study. However, we found three full-sibling dyads and two half-sibling dyads in which North County dataset individuals were related to individuals collected as part of Bohonak and Mitelberg (2014) and Mitelberg and Vandergast (2016). In the first pair of full siblings, both mule deer were found at the San Diego Zoo Safari Park (6 years and 1.1 km apart). The second pair consisted of two siblings, both found in Carlsbad, one at Carlsbad Oaks North HCA and the other at Rancho La Costa HCA (5 years and 6.6 km apart). In the third pair, one sibling was detected in Hellhole Canyon (Carter residence), and its sibling was detected east of SR-67 and south of Foster Truck Trail (3 years and 31.4 km apart). We found one pair of half siblings between and Rancho La Costa (Choumas-Pappas) and Miramar Golf Course 6 years and 20.6 km apart. We found a second pair of half siblings between North MCBCP and Boulder Oaks Preserve east of SR-67, 3 years and 69 km apart.

Table 5. Family groups of Southern mule deer (full and half siblings with p ≥ 0.80) identified in the north San Diego County 2018–19 dataset, Euclidean distance between siblings, potential roads crossed, and whether crossing occurred across sampling sites.

[Samples in bold appear in more than one dyad. Number in parentheses corresponds to site number assigned in table 1. **Abbreviations**: m, meter; ff, female female; mm, male male; fm, female male]

Family group	Full-sibling (FS)/Half-sibling (HS) Dyad	Gender	Distance (m)	Road crossings?	Across sampling sites?
FS1	MDn479-MDn008	ff	30,489	Multiple, but exact route unknown – I-15; I-78	Hellhole Canyon - Carter Residence (9)/ Rancho La Costa HCA - Denk (22)
FS2	MDn098-MDn403	ff	8,458	Multiple possible routes and barriers — Faraday Ave.; Palomar Airport Rd.; Rancho Santa Fe Rd.; Pointesettia Ln.; Alga Rd.; El Fuerte St.	Carlsbad Oaks North HCA - North Faraday (15)/ Rancho La Costa HCA - Copper Creek (23)
FS3	SPn131-MDn165	mm	2,925	Del Dios Hwy.	Los Cielos Preserve-Cielos Estates (28)/ Los Cielos Preserve-Meisha Canyon (29)
FS4	**MDn439-MDn351**	fm	1,399	Faraday Ave. (tunnel; EW2-9)	Carlsbad Oaks North HCA - North Faraday (15)/South Faraday (16)
FS4	**SPn438-MDn351**	fm	1,386	Faraday Ave. (tunnel; EW2-9)	Carlsbad Oaks North HCA-North Faraday (15)/South Faraday (16)
FS4	SPn438-MDn439	ff	20	No	No
FS5	MDn608-MDn613	ff	47	No	No
FS6	SPn462-SPn468	ff	47	No	No
FS7	SPn580-**SPn588**	ff	2,142	Minor roads within San Diego Zoo Safari Park	San Diego Zoo Safari Park Beckman & Tram (6)/San Diego Zoo Safari Park Open Space (8)
FS7	**SPn588**-SPn568	fm	20	No	No
FS8	T652-T683	ff	1,899	No	No
HS1	MDn549-MDn563	fm	1,383	Minor roads within San Diego Zoo Safari Park	San Diego Zoo Safari Park Beckman & Tram (6)/San Diego Zoo Safari Park Open Space (8)
HS2	SPn557-MDn573	ff	639	Minor roads within San Diego Zoo Safari Park	No
HS3	SPn484-SPn707	ff	13,780	Santee Ln., Guejito Truck Trl., Pamo Rd., Lusardi Truck Trl.	Hellhole Canyon-Carter Residence (9)/ Pamo Valley (11)
HS4	T643-T625	fm	14,356	No	Coastal MCBCP (1) - North MCBCP (2)
HS5	T629-**T664**	ff	13,999	Las Pulgas Canyon Rd.	No
HS5	**T664**-T644	fm	13,453	Las Pulgas Canyon Rd.	Coastal MCBCP (1) - North MCBCP (2)
HS6	T654-T657	ff	2,973	Basilone Rd.	No
HS7	**T668**-T667	fm	12,897	No	No
HS7	**T668**-T689	fm	11,661	No	Coastal MCBCP (1) - North MCBCP (2)

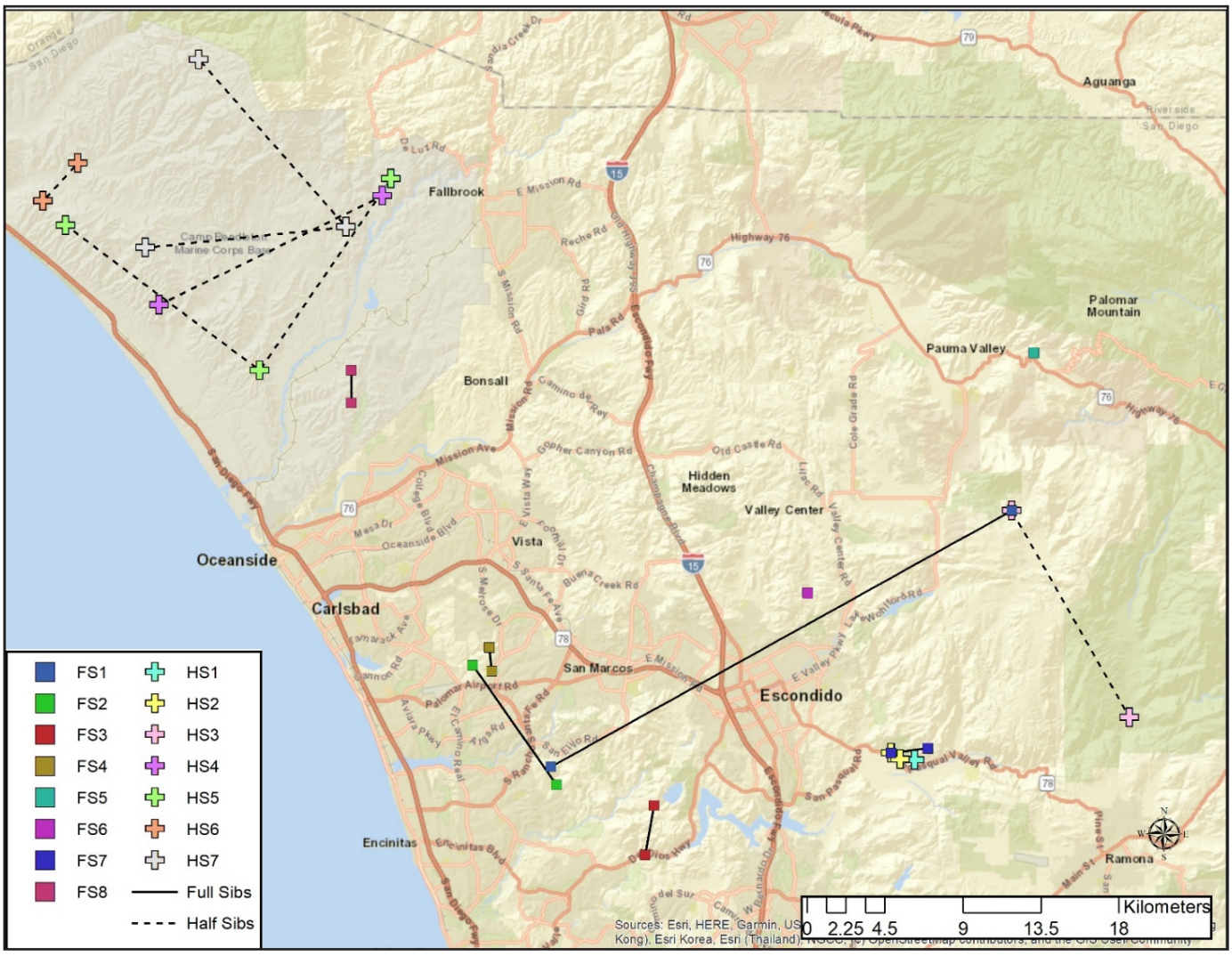

Figure 6. Full- and half-sibling Southern mule deer groups ($P \geq 0.80$) identified by COLONY in the north San Diego County focus area.

To reduce bias associated with sampling family groups in population clustering analyses, we removed 36 individuals from the regional dataset that appeared in one or more full-sibling dyads. STRUCTURE analyses with and without prior location information indicated k = 2 as the most likely number of genetic clusters in the regional dataset, regardless of which method we used to evaluate k (Evanno's ΔK or Pritchard's ln(Pr(X|K); figs. 7, 8). Across San Diego County, the first cluster (from here on referred to as "Coastal") consists of sites south of SR-78 and west of I-15, including sites in the following six putative geographic populations in San Diego, La Mesa, and Carlsbad (SW-NoC, West, MT-TR-MirDEG, MirNW, CC-MirEM, and Pq-PC, as identified in fig. 1). The second genetic cluster (from here on referred to as "Inland/Mountain") is composed of sites mostly east of I-15, as well as northern sites north of SR-76 and west of I-15 and includes sites in the following five putative geographic populations: BC-SC, East, NE-NoC, NW-NoC, and SE. In north San Diego County, the Coastal cluster is composed of individuals from Carlsbad and western portions of Escondido, and the Inland/Mountain cluster is composed of mule deer from Fallbrook, Valley Center, Pauma Valley, and Lake Sutherland. The Inland/Mountain cluster also contains all mule deer sampled on MCBCP. Mule deer from the eastern portions of Escondido (Daley Ranch Preserve and San Diego Zoo Safari Park) derive approximately 50 percent of their genetic background from each of these clusters.

After cross-validating the DAPC, 40 PCs achieved the lowest root mean square error with a value of 40 (0.4471375), resulting in an assignment rate of 0.56. We detected some separation along axis 1 between most Coastal putative geographic populations (that is, West, MT-TR-MirDEG, MirNW, CC-Mir, and Pq-PC) and the remaining sampling sites (fig. 9), although Coastal and Inland/Mountain clusters overlapped (fig. 10). These patterns were similar to the results from the STRUCTURE analyses, with individuals assigning to both clusters at intermediate sampling areas.

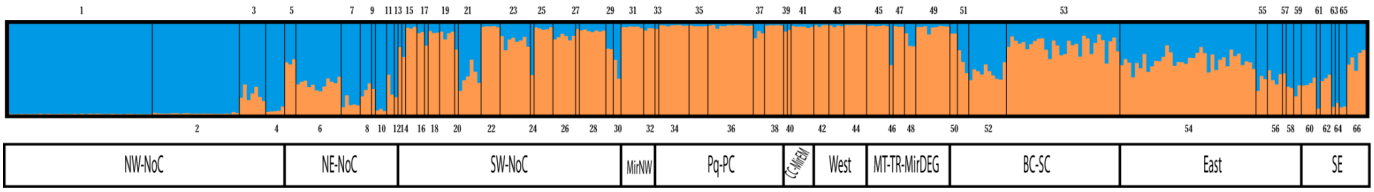

Figure 7. STRUCTURE plot for the regional San Diego County dataset (2005–19). Each bar in the plot represents one of 359 unique unrelated Southern mule deer included in the analysis with the colors representing the proportion of each individual's genetic background assigned to one of two clusters proposed by STRUCTURE. Numbers represent sites, and rectangles represent putative populations as detailed in table 1.

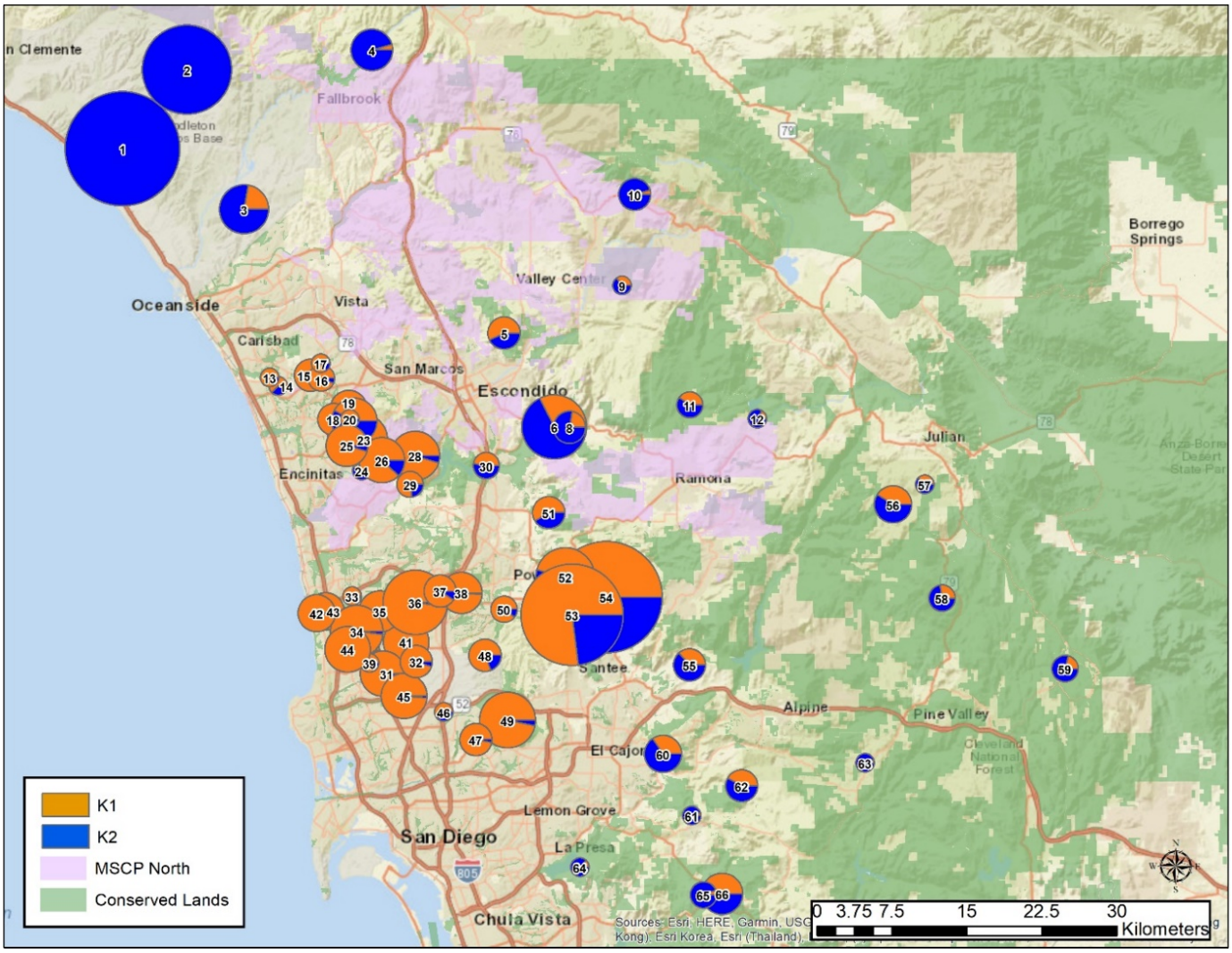

Figure 8. Sites sampled in San Diego County between 2005 and 2019, with each pie chart representing the proportion of the site's genetic background assigned to each of two genetic clusters, as identified by STRUCTURE (a priori site information included in analysis). Pie charts are proportional to the number of Southern mule deer fingerprinted at the site, with the smallest pie consisting of one individual. For accuracy, pies are placed at the center of each sampling site, resulting in some overlap; a map with finer detail of the central and southern areas is provided in appendix 1 (appendix fig. 1.2).

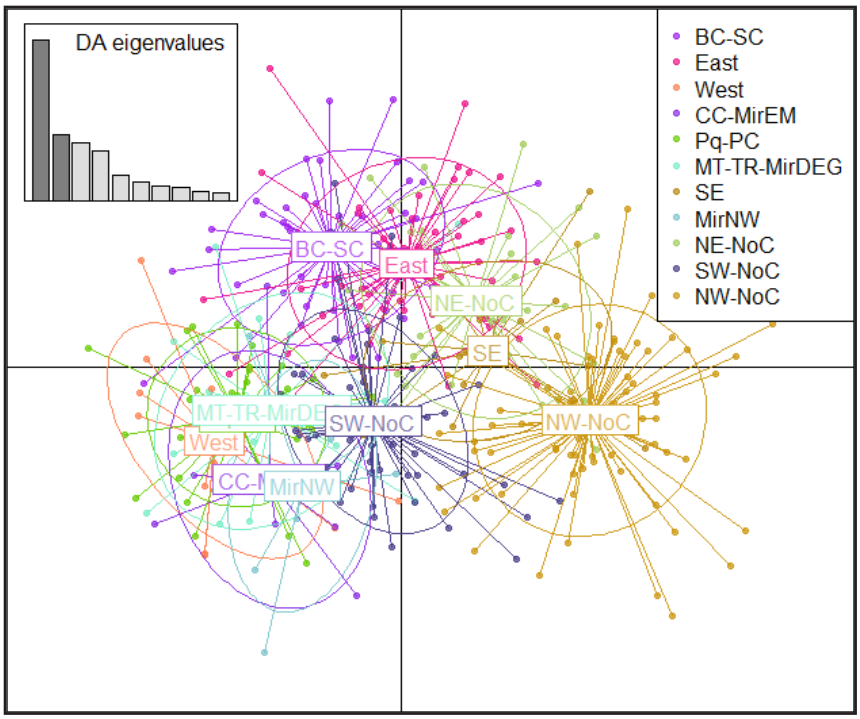

Figure 9. Sampled Southern mule deer by discriminant analysis (DA) eigenvalues axes 1 (x-axis) and 2 (y-axis). Individuals are colored by their population assignments. Color scheme corresponds to fig. 1.

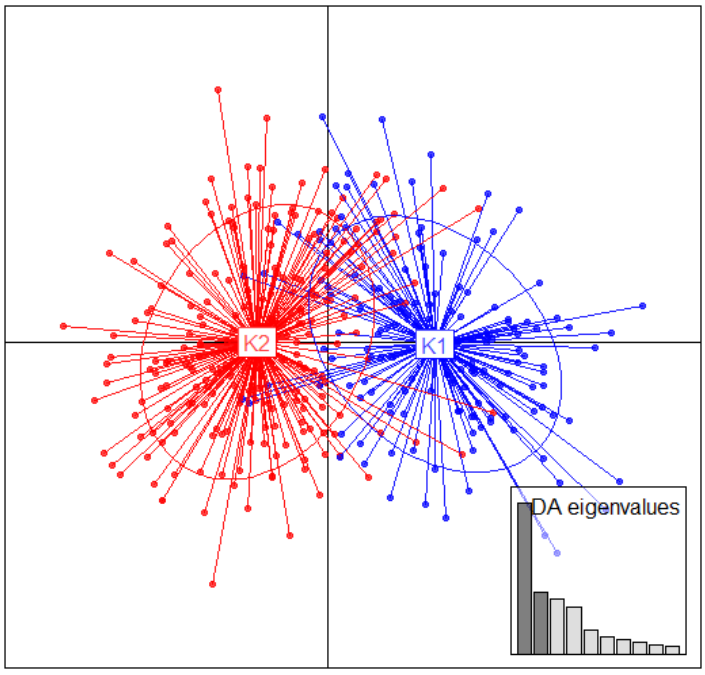

Figure 10. Sampled Southern mule deer by discriminant analysis (DA) 1 (x-axis) and DA 2 (y-axis). Individuals are colored by their STRUCTURE genetic cluster assignments, where individuals are assigned to one of two genetic clusters, K1 or K2, from which it derives more than 50 percent of its genetic background.

Fifty-one of fifty-five pairwise F_{ST} values were significant ($p \leq 0.00091$) after Bonferroni correction (table 6). Significant F_{ST} values varied from 0.024, between BC-SC and East (both sites east of I-15), to 0.146, between NW-NoC and CC-MirEM. Consistent with the STRUCTURE results, sites within the same cluster have lower F_{ST} values than sites between clusters.

The N_e estimate for the Coastal cluster (64.8; 51.1–83.9) is approximately two-thirds that of the Inland/Mountain cluster (100; 81.5–125.3; table 7). The average number of alleles is also higher in the Inland/Mountain cluster than in the Coastal cluster (4.3 versus 3.5; table 7), suggesting the latter is less diverse.

Table 6. Pairwise F_{ST} (genetic differentiation) tests performed in StrataG.

[Numbers in **bold** are significant at 0.05 after Bonferroni correction for 55 tests.]

	NW-NoC	NE-NoC	SW-NoC	MirNW	Pq-PC	CC-MirEM	West	MT-TR-MirDEG	BC-SC	East
NE-NoC	**0.037**									
SW-NoC	**0.064**	0.055								
MirNW	**0.108**	0.081	**0.078**							
Pq-PC	**0.116**	**0.100**	**0.080**	0.038						
CC-MirEM	**0.146**	**0.146**	**0.127**	**0.088**	**0.055**					
West	**0.145**	**0.139**	**0.093**	**0.104**	**0.060**	**0.083**				
MT-TR-MirDEG	**0.096**	**0.092**	**0.050**	**0.071**	**0.032**	**0.072**	**0.030**			
BC-SC	**0.063**	**0.059**	**0.048**	**0.098**	**0.057**	**0.106**	**0.093**	**0.040**		
East	**0.053**	**0.061**	**0.040**	**0.092**	**0.085**	**0.113**	**0.110**	**0.059**	**0.024**	
SE	**0.029**	0.027	**0.085**	**0.063**	**0.078**	**0.112**	**0.131**	**0.080**	**0.049**	**0.049**

Table 7. N_e (effective population size), as estimated in N_e Estimator, using LD, at 0.05 frequency for the lowest allele.

Genetic cluster	Putative geographic population	Sample size	Alleles	N_e (minimum, maximum)
Inland/Mountain (north of I-76, east of I-15)		213	4.3	100.2 (81.5, 125.3)
	NW-NoC	73	4.6	178.2 (93.2, 796)
	NE-NoC	30	4.4	17.8 (12.6, 26.4)
	BC-SC	45	3.9	77.3 (39.5, 333.4)
	East	48	4.4	53.9 (35, 98.2)
	SE	17	4.1	39.3 (16, infinite)
Coastal (coastal, south of I-78; west of I-15)		146	3.5	64.8 (51.1, 83.9)
	SW-NoC	59	4.0	45.1 (31.7, 69.4)
	West	14	3.1	17.5 (7.2, 127.5)
	MT-TR-MirDEG	22	3.9	21 (11.9, 47.9)
	MirNW	9	3.2	11.9 (3.1, 21917.2)
	Pq-PC	34	3.9	180.1 (54.6, infinite)
	CC-MirEM	8	2.8	19.3 (3, infinite)

Discussion

Mule Deer Movement and Connectivity in North County

Our primary objective was to assess mule deer movement and gene flow across North County. Scat recaptures and locations of first-order relatives indicate mule deer movement is limited, occurring for the most part within the putative geographic populations rather than between them. Additionally, we found that the mule deer fall into one of two regional genetic clusters identified in the regional San Diego County analyses (that is, Coastal and Inland/Mountain). Below, we discuss connectivity within and between each cluster.

Connectivity Within the Coastal Genetic Cluster in North County

Several lines of evidence suggest movement and gene flow within and between urban preserves in North County. First, regional population clustering analyses show that mule deer in this region (putative geographic population SW-NoC) derive most of their genetic background from the Coastal genetic cluster.

Second, we identified multiple recaptures between preserves. Two bucks were identified at multiple sites within and among preserves, traveling up to 4.6 km in less than 3 weeks, potentially using tunnels and crossing roads directly at grade. Within a period of two weeks, buck MDn069 was identified in the Copper Creek, Ridgeline, and Winston sites of Rancho La Costa HCA (table 3; fig. 4). There is a long and dark underpass in this area, but use by mule deer has not been observed (Markus Spiegelberg, written commun., 2019; City of Carlsbad, 2015); therefore, we suspect this individual crossed Rancho Santa Fe Road at grade (pinch point EW3-6; City of Carlsbad, 2015). Also, within a period of 25 days, buck MDn195 was identified at multiple sites as far as 4.6 km apart moving between Veterans Park, Crossings Golf Course, north and south of Faraday Avenue, and the Dawson Los Monos Canyon Reserve (table 3; fig. 5). Although we cannot trace this mule deer's exact route, this individual is likely moving along Agua Hedionda Creek, through agricultural fields and riparian areas of Agua Hedionda Lagoon and is likely using underpasses and(or) bridges at El Camino Real Road and Cannon Road; it may have crossed Faraday Avenue at grade (pinch point M5-2b; City of Carlsbad, 2015), as the undercrossing (pinch point M5-2a; City of Carlsbad, 2015) is locked at night. A recent mule deer hit on Faraday Avenue near Crossing Golf Course and Veterans Park indicates that mule deer are in fact attempting to cross Faraday Avenue here at grade (Markus Spiegelberg, written commun., 2019).

Third, pedigree analyses identified full- and half-sibling relatives at different preserves and(or) sites within the preserves in the short- (~ scat collected up to 1 month apart) and long-term (~ scat collected 5 or more years apart). Preserves in western Escondido share a pair of full siblings between the two sites in the Los Cielos Preserve located across Del Dios Highway, suggesting recent movement between these sites.

Connectivity Within the Inland/Mountain Genetic Cluster in North County

Evidence for relatively short-term gene flow within the Inland/Mountain genetic cluster includes (1) half siblings between the North and Coastal sites at MCBCP and (2) a half-sibling pair between Hellhole Canyon (Carter residence) and Pamo Valley. A long-term connection is supported by population clustering results, which show that individuals collected at sites in the NW-NoC and NE-NoC putative geographic populations derive a large proportion of their genetic background from the same genetic cluster. F_{ST} values between NW-NoC and NE-NoC are low (0.037) and are almost half that of NW-NoC and SW-NoC (0.064), suggesting that there has been more gene flow between the former putative geographic populations across I-15 than between the latter across SR-78.

Connectivity Between Genetic Clusters in North County

We found no recaptures or first order relatives and identified only one half-sibling pair between Rancho La Costa and Hellhole Canyon in Valley Center (although this pair had low support just above our cut-off value of $p \geq 0.80$). However, populations clustering results indicate that gene flow has occurred between the two genetic clusters in North County. Connectivity is likely being maintained under the I-15 overpass, via Lake Hodges as well as Daley Ranch Preserve and San Diego Zoo Safari Park, at sites where individuals derive ~ 50 percent of their genetic background from each genetic cluster (fig. 7). Finally, the eastern MCBCP site contains individuals with a shared genetic background between the two clusters (fig. 7), suggesting that mule deer may have been able to move more freely through the San Marcos Mountains in the recent past.

Regional San Diego County Mule Deer Connectivity, Diversity, and Effective Population Size

Our secondary goal was to assess regional mule deer connectivity across all of San Diego County. We found evidence for two regional clusters in San Diego County, corroborating results from previous regional (Bohonak and Mitelberg, 2014; Mitelberg and Vandergast, 2016) and statewide landscape genetic studies (Pease and others, 2009). Sites in the SW-NoC putative geographic population (Carlsbad and west Escondido) and coastal sites south of I-56 and west of I-15 (including sites in MirNW, Pq-PC, CC-MirEM, West, Mt-TR-MirDEG) belong to the same genetic cluster. Sites in the NW-NoC and NE-NoC belong to the same genetic cluster as sites east of I-15 (including sites in BC-SC and East), as well as sites in the southeast part of the county (SE). This cluster likely extends northward through the Santa Ana Mountains based on overlap with previously published studies that include more northern collection locations (Pease and others, 2009; Fraser and others, 2019).

Interestingly, sites that are far apart geographically, but within the Inland/Mountain genetic cluster, such as those in the NW-NoC putative geographic population and sites in SE and East, have small and significant F_{ST} values of 0.029 and 0.053 (fig. 1; table 6). In contrast, sites that are closer together geographically, but are in different genetic clusters, such as those in the West and NW-NoC, or West and SE, have larger, significant F_{ST} values of 0.145 and 0.131, respectively. This could indicate an effect of urban barriers inhibiting connectivity among some geographic populations or behavioral differences in movement patterns that are related to genetic differences.

In a study of mule deer throughout California, Pease and others (2009) described two major genetic clusters for mule deer in southern California. The San Diego cluster consisted of samples from San Diego County, west of the Peninsular Range in the coastal plain. The geographically larger southern cluster included individuals collected east of San Diego in Imperial County, MCBCP, and northward into the southern Sierra Nevada Mountains and Coastal Range (Pease and others 2009, fig. 1). The Pease and others (2009) genetic clusters also roughly corresponded to subspecies' range limits. The San Diego cluster corresponds to the Southern mule deer (*Odocoileus hemionus fuliginatus*), whereas the southern cluster overlaps most of the California mule deer (*Odocoileus hemionus californicus*) subspecies range. These clusters correspond spatially to the two San Diego County clusters recovered in our study, but we identify several zones of admixture, possibly as a result of finer-scale sampling efforts. If the Coastal cluster is equivalent to the Southern mule deer subspecies, then the subspecies appears to be restricted to a smaller portion of San Diego County than previously described, mainly residing in coastal San Diego, west of I-15. Further sampling in eastern San Diego County might resolve the eastern contact zones. The genetic diversity and effective population size of the Coastal cluster are lower than in the Inland/Mountain cluster, and N_e for the Coastal cluster is below the recommended threshold of 100 to avoid inbreeding depression (Frankham and others, 2014). This may be because the Inland/Mountain genetic cluster encompasses a much larger area and number of individuals than the Coastal cluster with fewer restrictions to movement. Pease and others (2009) also reported that genetic diversity for the San Diego County Southern mule deer was lower than other subspecies of mule deer in California. Finally, the Southern mule deer subspecies range also extends into Baja California, Mexico, although we have no samples from south of the United States/Mexico border to which to compare diversity and gene flow estimates.

Comparing among North County and other sampling areas in San Diego, levels of genetic diversity within North County ($A = 5.0$ and $H_e = 0.61$) are slightly lower than all of San Diego County samples combined ($A = 6.0$ and $H_e = 0.63$). Likewise, genetic diversity appears slightly higher across San Diego geographic populations than reported in other regions of southern California, using the same microsatellite markers. Fraser and others (2019) examined geographic populations of mule deer in Orange and Los Angeles Counties and reported H_e ranging from 0.52 in Chino Hills to 0.59 in the Verdugo Mountains and Hollywood Hills (all in Los Angeles County) and N_e ranging from 16.6 in the San Joaquin Hills (Orange County) to over 236 in the Santa Ana Mountains.

Conclusions and Future Work

We have shown that short-term genetic monitoring in relatively small regions of interest, and where mule deer presence has been documented, can provide useful information on mule deer movement. Until now, no region has been sampled as intensively as the city of Carlsbad preserves, which may, in part, explain why previous DNA fingerprinting efforts resulted in low recapture rates and distances (no more than 1.56 km apart; Valero, 2004; Bohonak and Mitelberg, 2014; Mitelberg and Vandergast, 2016). In the study presented here, we were able to capture mule deer movements of up to 4.6 km within 3 weeks. Additionally, our short-term intensive genetic monitoring indicates that mule deer successfully use infrastructure, such as tunnels and culverts as well as cross roads at grade. At-grade crossings can put drivers and mule deer at risk. Providing additional deer-appropriate undercrossings in areas where at-grade crossings are apparent could help avoid accidents and maintain gene flow. This type of intensive, short-term genetic monitoring effort could be implemented in other urban areas in the county where mule deer movement throughout the landscape is a concern.

Obtaining fresh scat samples in less urban areas, and where mule deer are presumably less concentrated, has proven more challenging. Therefore, evidence for connectivity from recaptures and first-order relatives may be limited owing to small sample sizes and dispersion of sampling sites. Suggestions for improving sampling success in relatively large areas of interest include (1) continuing scat collections over time and storing scat for later analysis (also suggested in Bohonak and Mitelberg, 2014), (2) notifying and encouraging the public to track mule deer sightings through iNaturalist or other apps, and (3) enlisting the help of preserve managers for opportunistic scat collections.

Finally, there are other research efforts employing the same genetic markers to study mule deer populations in San Diego County and other parts of southern California (Fraser and others, 2019). Efforts to combine these datasets could provide a more comprehensive picture of subspecies ranges and connectivity among local geographic populations of mule deer. Such efforts could assist in monitoring mule deer for long-term persistence in the region.

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Appendix 1

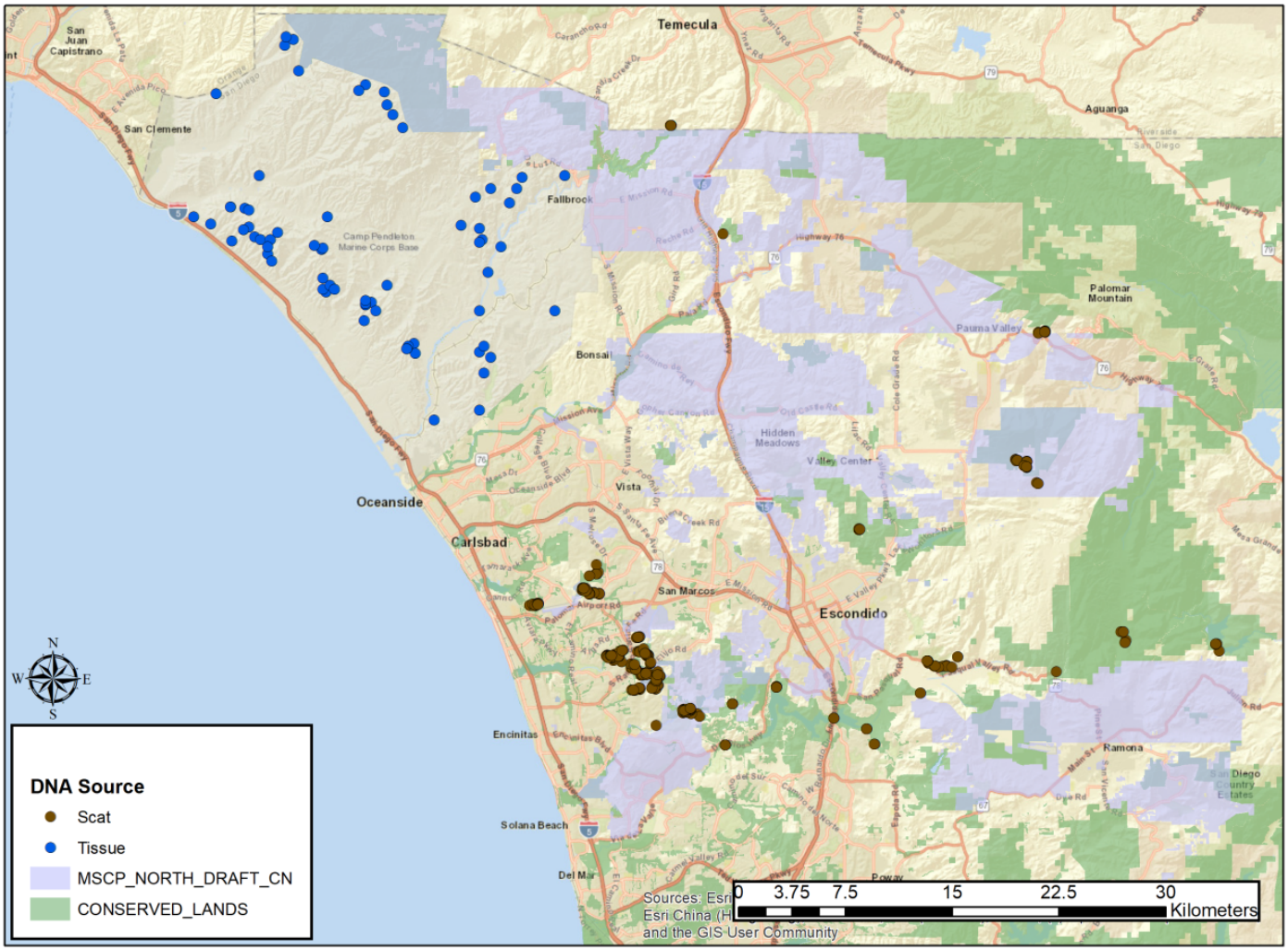

Figure 1.1. Southern mule deer tissue and scat samples obtained in 2018–19.

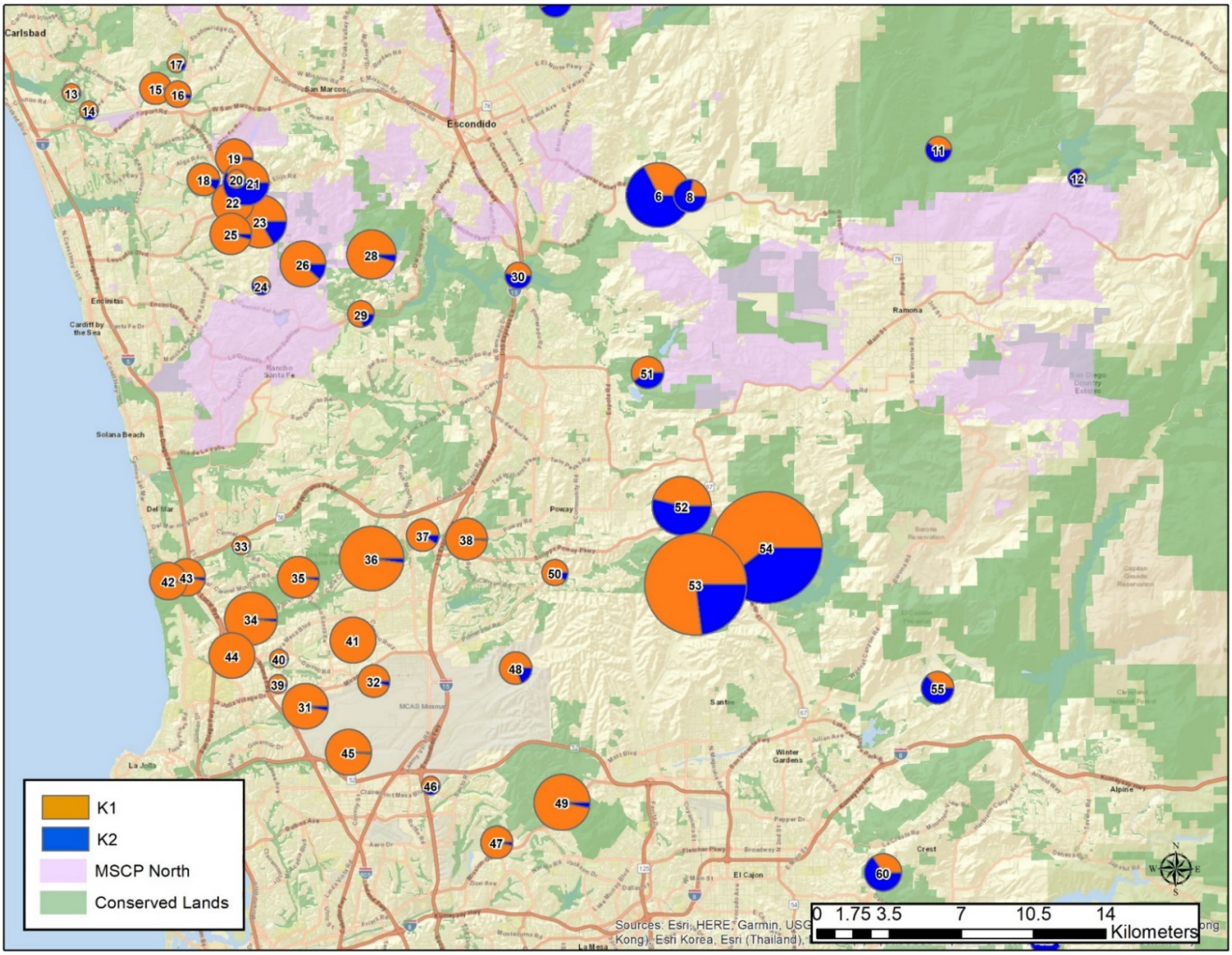

Figure 1.2. Finer detail of central and southern areas of fig. 8, showing the regional San Diego County STRUCTURE analysis.

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