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Efficacy of Three Methods for Collecting Behavioral Data on Urban San Joaquin Kit Foxes, *Vulpes macrotis mutica*

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Abstract.—Gathering behavioral data on mammalian carnivores is difficult due to their secretive and often nocturnal nature. Many methods are available for collecting behavioral data, but few direct comparisons of their accuracy and efficacy have been conducted. We used proximity logging collars with base stations at dens, direct observations, and remote cameras simultaneously to monitor parental behavior, particularly time spent at the den, in San Joaquin Kit Foxes (*Vulpes macrotis mutica*). Our objective was to compare the accuracy and efficacy of the three methods in describing kit fox behavior. Base stations worked erratically and did not record the presence of collared foxes at the den during any of the direct observation periods, so results could not be compared with other methods. Remote cameras significantly underestimated the time that foxes spent at the den because of the camera's limited field of view and the complex nature of kit fox den sites. Cameras also completely missed some parental behaviors, such as regular patrols around the den site. However, cameras were better than direct observations at capturing rapid events, such as a fox dropping off a small prey item at the den. Direct observation was the most accurate method for collecting most types of behavioral data. This method works well where visibility is good and animals are not unduly disturbed by human presence. Cameras may be a suitable replacement for many studies, especially where only relative measures, such as the relative amount of time spent at the den at different times of the day, are required.

Key Words.—behavior; camera stations; direct observations; kit fox; proximity collars

Gathering behavioral information about mammalian carnivores is essential to understanding their role in an ecological system and facilitating conservation (Caro 1999). Often, even small carnivores play significant roles in ecosystem function despite their relatively low abundance (Gompper et al. 2006). Direct observations can provide information on parental care (Strand et al. 2000; Elmhagen et al. 2014; Poessel and Gese 2013), offspring counts (McGee et al. 2005; Strand et al. 1999), territory defense (Fox 1969; Preston 1975; Iossa et al. 2008), circadian rhythms (Lemons et al. 2003; Poessel and Gese 2013), social interactions (Kitchen et al. 2006; Murdoch et al 2008), competition (Kamler et al. 2004), and resource needs (Tannerfeldt and Angerbjörn 1996; Strand et al. 1999; Elbroch and Allen 2013). However, direct observations are difficult or impossible in some species because small carnivores are often nocturnal and secretive and occur in low densities with relatively large home ranges (Crooks et al. 2008; Balme et al. 2009; Prange et al. 2011; Brawata et al. 2013). Furthermore, some species occupy habitats that are not conducive to observation (e.g., fossorial or arboreal species; Prange et al. 2006; Hauver et al. 2010). To overcome these limitations, many researchers have relied on the use of various modern technologies to enhance visibility of the study subjects (e.g., Brawata et al. 2013) or gather detailed information in other ways such as determining social relationships from proximity logging collar data (e.g., Ralls et al. 2013).

Available technologies include night-vision equipment (Murdoch et al. 2008; Brawata et al. 2013), remote cameras (Cutler and Swann 1999; Swann et al. 2004; Crooks et al. 2008), remote video surveillance (McGee et al. 2005; Brawata et al. 2013), and thermal imaging (Brawata et al. 2013). Recently, proximity logging collars have been used to document social and reproductive behavior in Island Foxes (*Urocyon littoralis*; Ralls et al. 2013) and den attendance patterns and tolerance of den visitations by conspecifics in Raccoons (*Procyon lotor*; Hauver et al. 2010). Each method for recording behavior has advantages and limitations, but few studies have made direct comparisons among the results obtained when several methods are used simultaneously.

San Joaquin Kit Foxes (*Vulpes macrotis mutica*) are small and nocturnal and unusual among canids in that they use earthen or subterranean dens during the daytime (Koopman et al. 1998; Moehrenschlager et al. 2004; Cypher 2010). Kit foxes live in family groups consisting of a mated pair and their current offspring as well as any offspring of the previous year that delayed dispersal and remained in their natal range (Ralls and White 2003; Cypher 2010). During the breeding season, philopatric young often assist the breeding pair by guarding pups and provisioning the mother and pups with food at a den (Moehlman 1989). Kit foxes typically inhabit arid and semiarid habitats in the Central Valley of California (Macdonald and Sillero-Zubiri 2004; Moehrenschlager et al. 2004; Cypher 2010), but have also adapted to living

in an urban environment (Cypher 2010). Bakersfield has a substantial population of San Joaquin Kit Foxes living throughout the city (Smith et al. 2006; Cypher 2010).

The close proximity of kit foxes to humans provides a unique and convenient situation to gather behavioral data. We compared the accuracy and efficacy of three methods used to collect data on parental behavior, particularly time spent at the den, in urban San Joaquin Kit Foxes during pup rearing. The three methods were proximity logging collars with base stations, remote cameras, and direct observations. These methods were used simultaneously to monitor family groups at dens during the breeding season so that results could be directly compared among data collection strategies.

METHODS

Our study sites were California State University, Bakersfield (CSUB) and Bakersfield College (BC) in Bakersfield, California. All family groups were monitored at college campuses because ambient light was sufficient to detect foxes without the aid of night vision equipment. Foxes living on school sites are relatively accustomed to human presence and binoculars were not even necessary for detection and identification of foxes. College campuses are also relatively safe and quiet at night so observations could be conducted without interference. All dens were located in flower beds and open manicured lawns.

We trapped foxes during late December 2010 to mid-January 2011 and in early January 2012 with wire-mesh box traps (38 × 38 × 107 cm; Tomahawk Live Trap, Hazelhurst, Wisconsin) baited with cat food, hot dogs, and sardines. We placed traps in secure locations away from well-trafficked areas and covered them with oiled cloth tarps to guard against the elements. We evaluated each fox to determine age, sex, and reproductive condition and applied a uniquely numbered ear-tag to every individual. Females were ear-tagged on the right and males were ear-tagged on the left to help distinguish fox sex at a glance. We marked each fox with a unique pattern using a perma-

nent non-toxic dye (Nyanzol-D; Albinal Dyestuff, Inc., Jersey City, New Jersey) to allow for the identification of individuals over the course of the project.

We applied proximity logging collars (Model E2C 162A; Sirtrack, Havelock North, New Zealand) to five foxes belonging to two family groups. Due to budget constraints, we had a limited number of collars, so we only collared adult foxes (> 2 y old) that were exhibiting signs of breeding (e.g., swollen vulva, enlarged testes). The collars consisted of an ultra-high frequency (UHF) transceiver bundled with a very-high frequency (VHF) transmitter (Prange et al. 2006). The VHF signal could be tracked with a receiver (Communications Specialists, Inc., Model R1000, Orange, California) and 3-element antenna (AF Antronics, Inc., Model F150-3FB, Urbana, Illinois) or omni-antenna (Teleonics, Model RA-5A, Mesa, Arizona). Each collar had a mortality sensor that would double the signal pulse rate if the animal remained motionless for more than 8 h. At each den where we had collared foxes and pups, we placed a proximity base station (Models E2C 162A and E2S 181A; Sirtrack, Havelock North, New Zealand) near the center of the den complex. To secure the base station and discourage foxes from moving it, we attached it to a 0.6-m (2-ft) wooden stake and hammered the stake into the ground until the base station was buried just below the surface (approximately 10 cm). The base stations were designed to receive the UHF signal from the collars and log the date, time, and collar ID of any collared animal that came within 10 m. We programmed the collars and base stations to collect data at the farthest range possible and preliminary controlled tests showed that collars and base stations were detecting one another at about 10 m apart.

Once we were reasonably sure that most, if not all, individuals from a natal den were captured, we began collecting data. Our objectives during direct observation periods were to record times when adult foxes were present and absent from the den, as well as to observe behaviors performed at the den. We observed the foxes at each den for a period of 2 h one to two times a week between January and May in 2011 and 2012 for some observa-

FIGURE 1. Camera station images of adult San Joaquin Kit Foxes (*Vulpes macrotis mutica*) provisioning at dens during the 2012 breeding season in Bakersfield, California. (Photographed by Bushnell Trophey XLT camera).

Figure 2. Camera station image of a San Joaquin Kit Fox (*Vulpes macrotis mutica*) pup at a den entrance and its mother patrolling the area around the den during the 2012 breeding season in Bakersfield, California. (Photographed by Bushnell Trophey XLT camera).

tions. For a given observation session, we tracked target foxes to a specific den at least 0.5 h prior to sunset. After tracking foxes, we found a nearby location from which to conduct observations. Generally, these locations were 10–50 m from the den and we began our 2-h observation session as soon as any foxes emerged from the den. Our study sites had sufficient ambient light to observe fox activity; at all locations foxes were identifiable to the individual without the aid of binoculars or night vision equipment. Only one den had vegetation that might obscure the presence of foxes at the den, but it was located in a stadium and we were able to sit high enough to see the den area clearly. We recorded the amount of time that each adult fox was present and absent from the den as well as other parental behaviors, such as delivering prey items to the den (Fig. 1). A fox was scored as being present anytime it was at the den, including when it was patrolling around the den site. During such patrolling, foxes could be as far as 100 m from the den and occasionally disappeared from sight for a few seconds (Fig. 2). As foxes moved away from the den behavior became an indicator of presence; if the fox was still vigilant and the pups remained outside the den, then the fox was still considered present and guarding.

We set up remote motion sensing cameras (Trophey XLT, Model 119456C; Bushnell Corporation, Hartford, Connecticut) at dens where pups were present and only used cameras with infrared flash to avoid disruption to the foxes. Cameras were attached to a 0.9-m (3-ft) u-post placed approximately 8–10 m from the den and pointed at the den entrance. In cases where there was more than one entrance, multiple cameras were installed to capture

all fox activity. Cameras were powered by eight AA batteries and images were recorded on an 8GB SD card. We programmed cameras to take three 8-MP pictures for each trigger with a 1-s interval between triggers.

To compare methods, we conducted observations using multiple methods simultaneously. To standardize the observation time, we only used camera or proximity logger data collected during the 2-h direct observation session. For each individual fox, we tallied the total number of minutes spent at the den during a given 2-h observation session as determined by both direct observations and camera stations. Because cameras capture still frames, we considered foxes present if detections occurred no more than 5 min apart. This criterion was used to simulate patrolling behavior. Because data were non-normal and transformation did not normalize them, we used a Wilcoxon sign rank test ($\alpha = 0.05$) to determine if there were significant differences in the times foxes were present based on the observation method. We also counted the number of provisioning events (i.e., bringing food items back to the den) performed by each adult group member during the session using both direct observation and camera station data. These data allowed us to determine the efficacy of direct observations and camera stations in detecting instantaneous or rapid events that may be difficult to document.

Results

We used proximity collars and base stations on five foxes belonging to two family groups. One group was located at BC and consisted of a father and two helpers

TABLE 1. Identification number (Fox ID), family group (BC = Bakersfield College, CSUBC = California State University Bakersfield central, CSUBS = California State University Bakersfield south), sex, number of observation periods, and mean ± standard error of the number of minutes present at a den for each San Joaquin Kit Fox (*Vulpes macrotis mutica*) observed during the 2012 breeding season using direct observations and camera station observations in Bakersfield, California.

| | | | | Mean minutes | |
Fox ID	Family Group	Sex	Number of Observations	Direct	Camera Station
6524	BC	M	6	0.3 ± 0.2	0.7 ± 0.5
6525	BC	F	6	16.7 ± 7.8	13.0 ± 6.1
6566	BC	F	6	13.7 ± 7.4	3.2 ± 0.6
6584	BC	M	6	2.0 ± 1.3	2.5 ± 1.5
6578	CSUBC	M	3	21.0 ± 7.4	12.33 ± 9.4
6592	CSUBC	F	3	25.3 ± 13.0	17.0 ± 14.1
6065	CSUBS	M	5	2.6 ± 1.7	0.6 ± 0.4
6309	CSUBS	F	5	4.2 ± 1.6	1.6 ± 0.5
6585	CSUBS	F	5	1.2 ± 0.6	0.6 ± 0.4
6700	CSUBS	F	5	0.2 ± 0.2	0.4 ± 0.2

who we collared and a mother, two helpers, and three pups who we did not collar. One helper was an offspring from the previous year and the other was an adult offspring from at least two years prior. This group used a total of two dens during the breeding season, both of which were located in an unmaintained slope in the college stadium. The other group was located at CSUB and consisted of a father and a helper who we collared and two mothers and six pups not collared. The helper was an offspring from the previous year. This group used a total of four dens; one under a cement slab, one in a flowerbed, one in a manicured lawn, and one in an unmaintained field. A single base station was deployed at a central location at each den. Unfortunately, the base stations were unreliable and only occasionally recorded the presence of the collared foxes. They did not register any fox activity within the 2-h window used to collect simultaneous data using the other observation methods. Thus, we could not compare data from proximity logging collars with data collected using the other two methods.

We simultaneously collected data using direct observations and camera stations on 14 nights and observed 10 adult foxes from three family groups (Table 1). We usually collected data on more than one fox during each of these sessions, so our total sample size was 50 2-hr observation periods. The mean (± SE) number of minutes spent at the den by each fox was 7.52 ± 1.34 ($n = 50$) for direct observations and 4.40 ± 1.85 ($n = 50$) for camera station observations. Mean time present at the den was significantly higher for direct observations than for camera stations ($Z = 2.78$, df = 12, $P = 0.005$). Nine provisioning events were documented; seven were detected by cameras and four were detected by direct observations, but only two events were detected by both methods.

DISCUSSION

Proximity logging collars were not a reliable technique in our study of den attendance among urban San Joaquin kit foxes. The collars did not record kit fox presence at any time when kit foxes were known to be present at dens based on observations and cameras. Proximity logging collars have been successfully used to determine den attendance patterns in arboreal species (Hauver et al. 2010). However, controlled studies suggest that the closer the collars are to the ground the less reliable they are in recording contacts because the ground attenuates the signal (Prange et al. 2011). We buried base stations to prevent their removal by kit foxes and ground interference likely prevented the collar signal from reaching the base station. Future studies on den attendance using proximity loggers on fossorial species probably would be more effective if base stations are located above ground (e.g., attached to a post). However, in urban settings this could increase the risk of theft or vandalism.

Another factor that may have limited the effectiveness of proximity loggers to monitor den attendance is the structure of kit fox dens. Kit foxes tend to use large natal dens with multiple entrances (Egoscue 1956; Morrell 1972; McGrew 1979). Installing multiple base stations around the den complex might be necessary to ensure detection of foxes. Kit fox dens can also be deep and complex (Morrell 1972) and it may not be possible to determine kit fox presence if a collared individual is too far underground. With proximity loggers, den attendance information for fossorial species may be limited to time spent above ground at the den because time in the den and away from the den may be indistinguishable.

Finally, a detection distance setting of greater than 10 m should be used because foxes at times were observed to be present at dens but were more than 10 m from a base station (e.g., patrolling, den guarding).

Observing the den directly provides a wide field of view, which allows for documentation of behaviors occurring both at the den and in the surrounding area (Brawata et al. 2013). However, direct observations are limited by the amount of time a person can spend vigilantly watching a den (Weller and Derksen 1972) unless multiple observers are available (e.g., Poessel and Gese 2013). Direct observations are also limited by the distance from the point of interest. The ability of an observer to identify individuals and collect accurate behavioral information may decrease with distance, particularly if there are objects or vegetation obstructing the view (Sundell et al. 2006; Brawata et al. 2013). Conversely, if the observer is too close to a den, he or she could alter the natural behavior of the animal under observation (Brawata et al. 2013). Our observations were performed in an urban setting where animals were habituated to the presence of humans. A study conducted in natural lands would be more difficult due to increased wariness by foxes. In such situations, a blind might facilitate observations (Strand et al. 2000; Poessel and Gese 2013).

Camera stations are an effective method to collect behavioral information continuously over long periods of time (Cutler and Swann 1999). Because cameras can be left out for extended periods (e.g., several days), they can collect continuous data without the limitation of decreasing vigilance due to observer fatigue. Another advantage to using remote cameras is that they can be placed directly in front of the natal den without affecting the behavior of the animals under observation due to rapid habituation (Cutler and Swann 1999; Brawata et al. 2013). Camera stations detected more provisioning events than direct observations and may be better at detecting rapidly occurring events.

While there are several advantages to using cameras, they have some disadvantages. The biggest limitation when using cameras to monitor a den is the restricted field of view. Cameras will only detect activity in a field of view directly in front of the infrared sensor (Cutler and Swann 1999), but as previously stated, kit foxes use large dens with multiple entrances, some of which may be outside the field of view. Also, adult foxes often patrol around the area when guarding young and this behavior was not detected by the cameras (Westall 2015). Unless a camera can be installed at multiple den entrances and in the surrounding area, there is a risk of missing some behaviors. Another concern when using remote cameras is that human activity during deployment and collection of cameras or human scent on cameras or on the route into cameras may attract other species to den sites (Cutler and Swann 1999). This could be detrimental to the study animals if potential predators are attracted to a den area,

particularly when vulnerable young are present. Finally, while deployment and operation of cameras is less labor intensive than direct observations, analysis of the resulting photos can be a tedious and time consuming process (Weller and Derksen 1972).

Camera stations significantly underestimated the amount of time kit foxes spent at the den compared to direct observations. This was likely due to the limited field of view of cameras and possible failed triggering. During direct observations, foxes guarding pups would patrol the den area, moving in circles around the den and stopping at regular stations to keep watch (Westall 2015). Without multiple cameras in place, it could appear that a fox on patrol had left the den area when in fact it was still present but simply outside the camera field of view. While camera stations underestimate the amount of time present, they are better at detecting events that happen rapidly. Direct observation of provisioning may be less accurate because distance, obstruction, and the size of the provisioned item may limit visibility.

Direct observations provide the most accurate information on kit fox den attendance and behavior, but are limited to relatively short observation periods unless multiple observers are available (e.g., Poessel and Gese 2013). Camera stations can provide information over longer periods of time, but results are less accurate than direct observations. Either method could be used to gather valuable information, depending upon the study objectives, subject animals, and observation conditions. Direct observations may be used to focus on behaviors that are difficult to detect on camera, like patrolling, territory defense, or social interactions. Conducting direct observations is necessary when determining absolute values, such as the true amount of time devoted to various behaviors. Camera observations can be used to continuously document a wide variety of behaviors and are useful for determining relative behaviors, like the relative degree of activity at different times of day. Cameras could replace direct observations when documenting behaviors that are difficult to see from a distance which could include offspring counts, family group size, provisioning behavior, or types of items provisioned. Although proximity logging collars and base stations were unreliable in our study, proximity loggers could be used on kit foxes to obtain valuable information on social interactions, mating systems, and the potential for disease transmission as has been done with Island Foxes (Ralls et al. 2013; Sanchez and Hudgens 2015) and other species (Prange et al. 2006, 2011).

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Tory Westall has been working as a Research Ecologist with the Endangered Species Recovery Program of California State University, Stanislaus, since 2009. In that time she has studied various sensitive species in the San Joaquin Desert and surrounding areas. She is interested in the ecology and conservation of endangered species and primarily studies the San Joaquin kit fox. (Photographed by Christine Van Horn Job).

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Katherine Ralls is a Senior Research Zoologist Emeritus with the Smithsonian Conservation Biology Institute. She is interested in the behavioral ecology, genetics, and conservation of mammals. Since 1980, she has focused on conservation biology, especially the genetic problems of small captive and wild populations, and studies of threatened and endangered mammals, including San Joaquin Kit Foxes and Sea Otters (*Enhydra lutris*). (Photographed by Vickie Pearse).

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. Manage-

ment 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
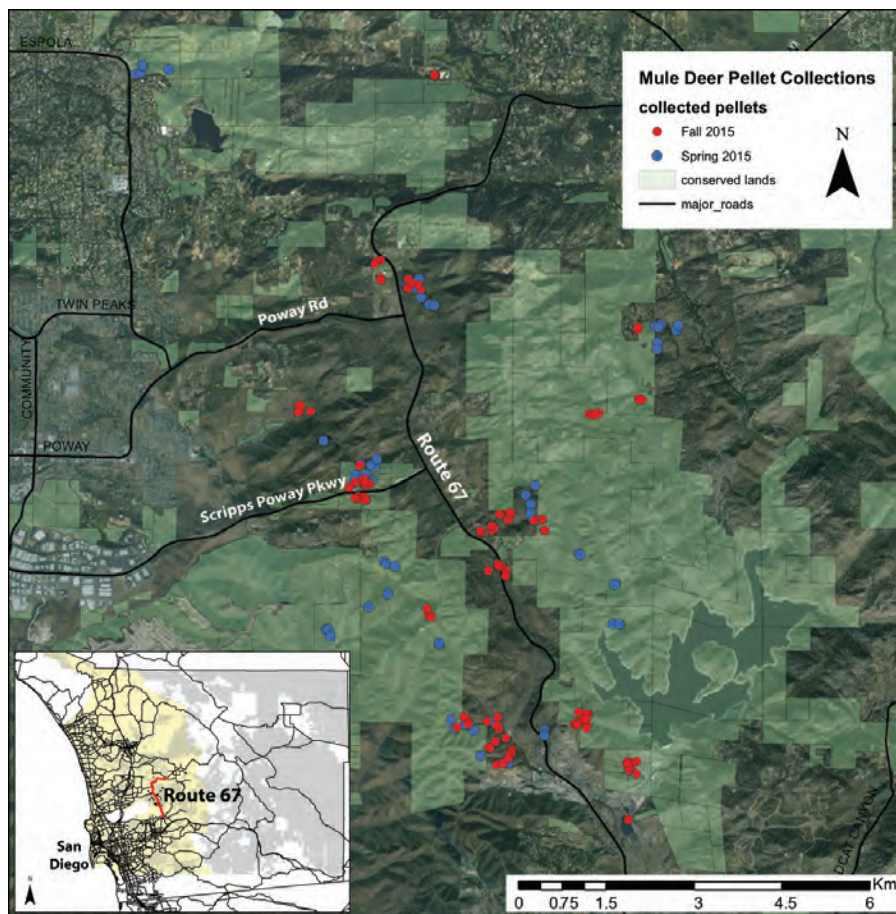

FIGURE 1. Location of study area showing major roadways and 238 scat piles collected of Mule Deer (*Odocoileus hemionus fuliginatus*). We assessed connectivity across California State Route 67, Scripps Poway Parkway and Poway Road, southern California. The insert shows the section of Route 67 examined 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 GENE-POP (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

| 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
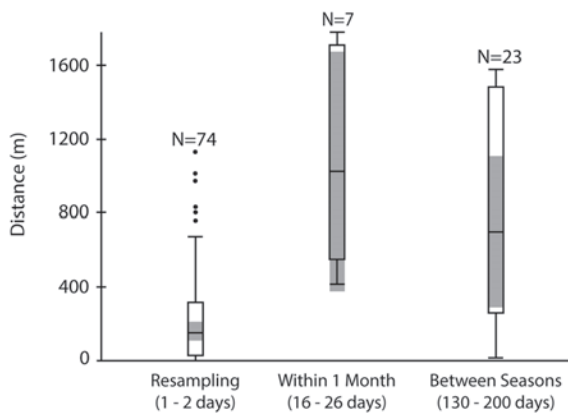
FIGURE 2. Euclidian distances between resampling/recapture events of Mule Deer (*Odocoileus hemionus fuliginatus*) across California State Route 67, Scripps Poway Parkway and Poway Road in southern California grouped into three time intervals. The box depicts the middle half of the data between the 25th 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
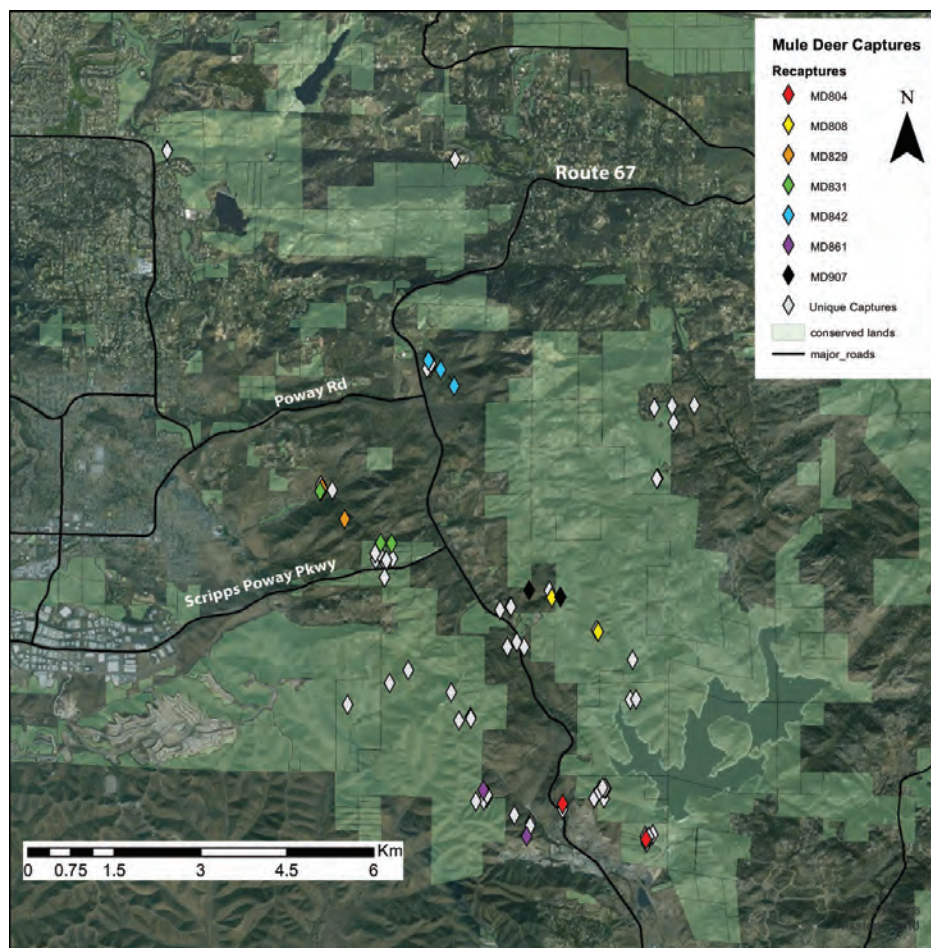

FIGURE 3. Locations of 80 capture events of Mule Deer (*Odocoileus hemionus fuliginatus*) across California State Route 67, Scripps Poway Parkway and Poway Road in southern California. Individuals captured once are in gray and individuals captured multiple times are color coded. Two individuals (MD831, 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		
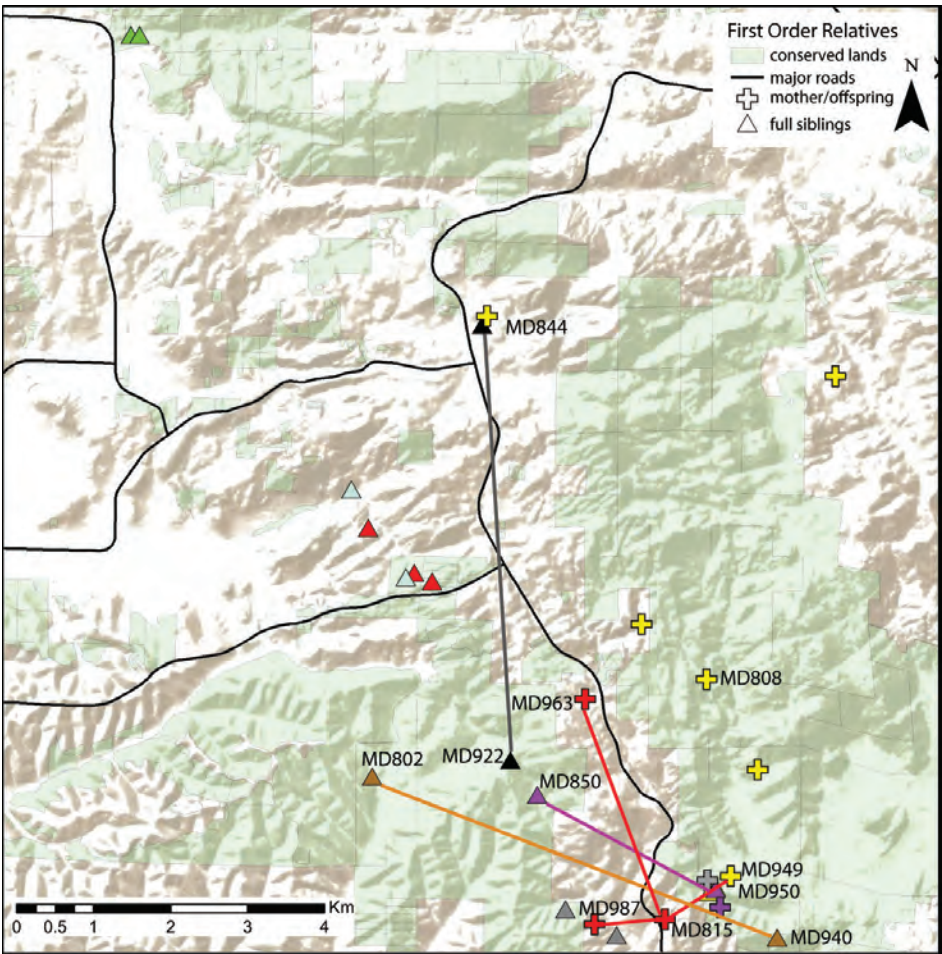
FIGURE 4. Locations of first-order relatives of Mule Deer (*Odocoileus hemionus fuliginatus*) across California State Route 67, Scripps Poway Parkway and Poway Road in southern California, as identified by the program COLONY (P > 0.05). Family groups are color coded with unique symbols. Lines are drawn between first-order relatives found on either side of Route 67. (World Terrain Basemap sources: Esri, USGS, NOAA).

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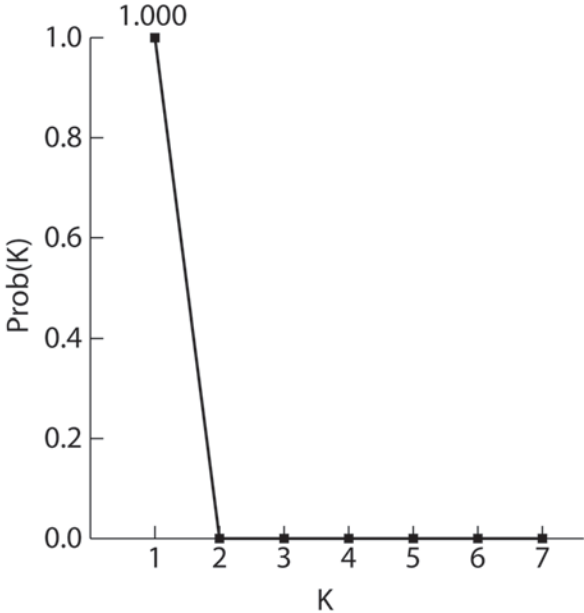

FIGURE 5. Probability plot of STRUCTURE results of Mule Deer (*Odocoileus hemionus fuliginatus*) across California State Route 67, Scripps Poway Parkway and Poway Road in southern California.

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

Locus	Size range	Forward primer Sequence	Conc. (μM)	Reverse Primer 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

Evaluation and Management of California Monarch Winter Sites

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Abstract.—The proposed management system for the California winter sites of Monarch Butterflies (*Danaus plexippus*) is based on environmental and biological variables that govern three central factors: (1) the diurnal field activities of monarchs, (2) their cluster behavior, and (3) their occupancy of winter groves. Key to understanding these variables is the development of a Grove Seasonal Wind Profile (GSWP) that will depict the following: (1) disruptive winds, ≥2 m/s entry and path through the grove; (2) the protectiveness of grove trees against disruptive winds, ≥ 2 m/s; (3) the best area to plant seedlings to buffer strong winds; and (4) the area within the wind protective zone where the butterflies are forming winter aggregations. This information can be used to evaluate and manage the suitability of the grove for winter aggregations and to make informed decisions regarding selective pruning or removal of trees, planting of replacement tree species, planting of grasses and winter flowering plants, and the need to evaluate trees for public safety.

Key Words.—butterflies; conservation; *Danaus plexippus*; Monarch Butterflies; winter habitat management

Introduction

Each fall North American Monarch Butterflies (*Danaus plexippus*) make their pilgrimage to winter sites in Mexico and California. In California, their winter aggregations are found in selected forested areas generally located along a narrow 1.6 km coastal strip from Mendocino County to Baja California. Their distribution (winter sites and population numbers) displays a bell-shaped curve with the greatest numbers found within the central coast (Santa Cruz, Monterey, San Luis Obispo and Santa Barbara Counties; Leong et al. 2004).

Leong et al. (1991) also showed that overwintering Monarch Butterflies are found only in forest groves that offer a specific set of microclimate conditions. Conditions for winter aggregations are similar for all California winter sites even though such sites may vary in forest structure, density, tree species composition, grove configuration, and topography (Leong 1990; Leong et al. 1991; 2004). These conditions have been created by topography and an ever-changing, fragile, dynamic plant community (forested area as well as the surrounding vegetation) and should be managed by winter habitat mangers or conservationist to maintain their suitability for overwintering butterflies.

Because the winter occupancy of monarchs depends upon the stability of a grove conditions, Leong et al. (2004) proposed two terms to describe winter sites: Transition and Climax. These terms reflect the dynamic nature of overwintering sites and should be the focus of all habitat management. Transitional winter sites can provide suitable conditions for winter aggregations for only a few weeks. They reflect an evolving, interim winter habitat, progressing into (1) those that will provide stable grove conditions to support winter aggregations for the entire season, (2) those that have transformed from stable conditions due to grove-tree senescence or tree losses to diseases and/or storm winds, and (3) those that are non-progressive and will eventually become a non-winter site. Climax sites are groves that provide suitable conditions for winter aggregation the entire winter season and may support overwintering Monarch Butterflies for a few years or for several decades. Eventually, if not managed, climax grove conditions will transform into to a transitional state due to normal grove maturation or tree losses due to winter storms, diseases such as pine pitch canker, or indiscriminate tree thinning by property managers.

The frequent labeling of winter sites as autumnal and permanent (Nagano and Sakai 1990) should not be used in the context of habitat management, because these terms do not reflect the changing nature of plant communities that support overwintering butterflies. Designating a winter site as permanent, for example, implies that plant communities do not change and that habitat management is unnecessary. Similarly, the term autumnal sites suggest that the butterflies occupy such sites as way stations in the fall as they progress toward permanent sites. Further, labeling winter sites as autumnal or permanent also suggests that they are non-successional sites, which in the majority of instances is not the case. These two terms, in fact, reflect an outmoded philosophy regarding the unchanging nature of winter sites. For example, in 1988, the California Legislature allocated $2 million from a bond issue of $776 million for the acquisition of land where monarchs overwintered along the Pacific coastline (Malcolm 1993), but no monies were allotted to manage the winter sites precisely because they were considered to be either autumnal or permanent sites. A portion of the Escalona Gulch winter site in Santa Cruz County was also purchased under this bond issue, and as time passed,

the misleading assumption that the site was permanent resulted in a forested area without overwintering butterflies.

Under the California Tree Culture Act of 1868 (Assembly Bill No. 583), the introduction and planting of eucalyptus trees allowed the State's Monarch Butterflies to expand their winter distribution. However, because there have been no new large tree plantings in recent years in California, the mass winter aggregations of Monarch Butterflies are limited to current discrete and forested areas that are undergoing ecological succession. Unless these sites are managed, grove conditions that presently favor winter aggregations will eventually transform to the point that they will longer support overwintering butterflies. The purpose of the present paper is to propose a management system for California winter sites that is based on the environmental and biological factors governing the monarchs' diurnal field activities, their cluster behavior, and the occupancy levels of their winter groves. This approach facilitates the evaluation of winter groves and ensures informed decisions regarding (1) selecting and replacing trees to buffer disruptive winds or to serve as cluster trees, (2) estimating the need for plantings of winter flowering plants, (3) determining the value of growing milkweeds at the winter groves, and (4) assessing the health of grove trees for public safety.

MATERIALS AND METHODS

Aerial photographs.—I obtained aerial photographs of the study groves were either from Google Earth or by the agency that requested this study. These photographs give us an overview of the study sites as well as to the pattern of vegetation, topography and/or building associated with the winter sites. I converted some aerial photographs to line drawings to better illustrate the location where the butterflies were aggregating, imbibing on water or nectar, sunning, and mating.

Monarch population estimates.—I observed butterflies from 0730–0830 PST when ambient temperatures were below flight threshold (13° C [55°F]; Masters et al. 1988) and when the butterflies were inactive in their clusters. I visually estimated the population of each cluster using binoculars, counting the number of butterflies in a given area, and multiplying this number by the number of times that area would cover the cluster expanse. I determined the overall population by summing the totals of each cluster.

Field activities.—I surveyed the diurnal activities of Monarch Butterflies (clustering, sunning, foraging for water or nectar, and mating) at Lighthouse Field State Beach winter site, Santa Cruz, California, during the winter season 2001–2002 and at San Simeon winter site, San Simeon, California, during the winter season 2005–2006. I made surveys during sunny winter days at 2-h intervals,

starting at 0800 at the cluster arena and terminating at 1400. Each survey was performed at 30 m, 60 m, and 90 m radii distances, starting from the cluster trees and then recorded the type of activity, number of butterflies, and their location.

I took solar radiation measurements using a Kahlsicon radiation balance meter (cal cm^{-2} m^{-1}; Kahl Scientific Instrument Corporation, El Cajon, California) and wind measurements using a Kestrel 4500 Weather Meter (Nielsen-Kellerman Company, Boothwyn, Pennsylvania) in the field where the butterflies were actively foraging for water or nectar or sunning. Additional observations and findings referred to in this paper result from field research conducted by the author at the following locations during the indicated dates: Pismo North Beach, San Luis Obispo County, California, 1990; Los Osos, San Luis Obispo County, California, 1997; Lighthouse Field State Beach, Santa Cruz, California, 2002; Purple Gate, San Rafael, California, 2004; San Simeon, San Luis Obispo, California, 2004; Fort Baker, San Francisco, California, 2006; Woodland Estates, Nipomo, California, 2008; and Sweet Spring Preserve, Los Osos, California, 2013.

Protectiveness of winter grove against disruptive winds.—The protection from disruptive winds (2 m/s) is an important factor governing the winter occupancy of Monarch Butterflies at California winter sites. To determine the ability of a grove to buffer disruptive winds, I divided the grove into grids 30 m apart (except for Sweet Springs winter site, where the grids were 20 m apart). At each grid intersection (i.e., sample point), I measured wind velocity (m/s) and wind direction (azimuth/heading) using a Kestrel 4500 Weather Meter. At the conclusion of the study, I graphically depicted the winter wind profile of each grove, showing the path and general direction of disruptive winds. I did this by indicating sample stations that recorded at least one wind measurement of ≥ 2 m/s with blue circles and measurements below this threshold with white circles.

RESULTS AND DISCUSSION

Field activities.—Because few, if any, flowering plants are available for the millions of overwintering butterflies that descend upon the high mountains of Mexico, the migrating insects primarily survive the winter months by living off their body fat reserves (Brower 1995). In California, Monarch Butterflies have an added survival benefit from the nectar of winter flowering plants. To survive, Monarch Butterflies must, during sunny winter days, warm their body temperatures enough to leave their clusters and drink water or nectar from winter flowering plants. This allows them to metabolize fat reserves for maintenance of their physiological well-being, and by the second week of January for the development of reproductive organs (Leong et al. 2012). After a the activity of a day, butterflies must return to the cooler tem-

peratures of the grove to re-integrate into their winter aggregations and thereby lower their body temperatures and metabolism to conserve their body fat reserve (Chaplin and Wells 1982; Masters et al. 1988; Wells et al. 1992; Frey et al. 1992; Leong et al. 2004).

I observed butterflies flying, sunning, or foraging for water or nectar beginning at 0800, reaching peak activity at 1000, and declining by 1200 (Fig. 1). The number of active butterflies and activity appears to be closely related to the amount of radiant solar energy available (Fig. 1). By 1400, I observed only a few butterflies in the field, while the majority of the butterflies I saw were reforming their winter aggregations in the grove (Fig. 2).

The field activities of overwintering butterflies were limited to a kilometer range of the winter grove and in mainly sunlit areas (Fig. 3). This behavior was documented by my field studies conducted at San Simeon, California and at the Lighthouse Field State Beach, Santa Cruz, California (Fig. 4). Notably, the limits of the foraging distance of butterflies suggest an innate conservation of body energy reserves that is critical for their winter survival. The end of an overwintering season is signaled by intense mating activity. The males capture the females by chasing (Hill et al. 1976) or by capturing them while they bask on the canopy foliage (Leong 1995) and both types of mating activities occur in sunlit open field areas and foliage.

Grove management considerations.—Planting of winter flowing plants and open sunlit areas for sunning, imbibing water and nectar, and mating should be established within a kilometer of the grove and mainly along the southern area of the overwintering colony. Unlike in Mexico, Monarch Butterflies overwintering in California are found in forested areas close to the coastline, where temperatures rarely reach freezing and where relative humidity is high (Leong et al. 2004). The winter occupancy and survival of Monarch Butterflies in California are primarily dependent upon two key environmental variables: wind and the access of butterflies to solar radiant energy, mainly filtered (morning and afternoon) sunlight as it is streamed through the foliage of grove trees.

Monarch Butterflies are very sensitive to winds, forming winter aggregations on trees exposed to minimal winds and on foliage that buffers prevailing winds (Leong 1990). Field studies of butterfly sites I conducted at Los Osos, Purple Gate, Nipomo, and Sweet Springs, in California, have consistently showed that strong winds have a direct negative effect on the winter occupancy of a grove by butterflies (Leong 1990, 1997; Leong et al. 1991, 2004). Winds ≥ 2 m/s are disruptive to the aggregating butterflies by blowing them from their roosting branches or dislodging them by shaking the branches. When the butterflies observed in this study were subjected to winds above flight threshold (about 16° C), they either flew to a more sheltered area of the grove or, if no refuge area was available, abandoned the grove temporarily or for the re-
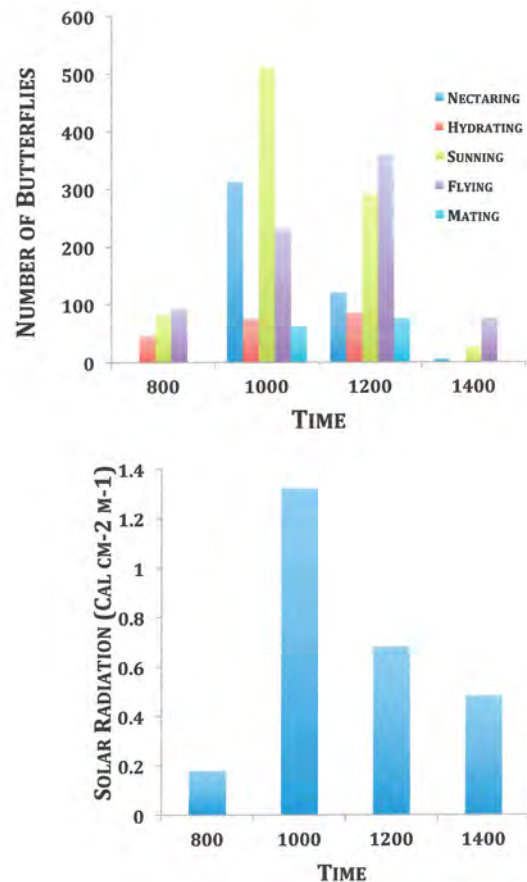

FIGURE 1. The pattern of the number of Monarch Butterflies (*Danaus plexippus*) active during the day is bell-shaped (A), which corresponds closely with solar radiant energy (B).
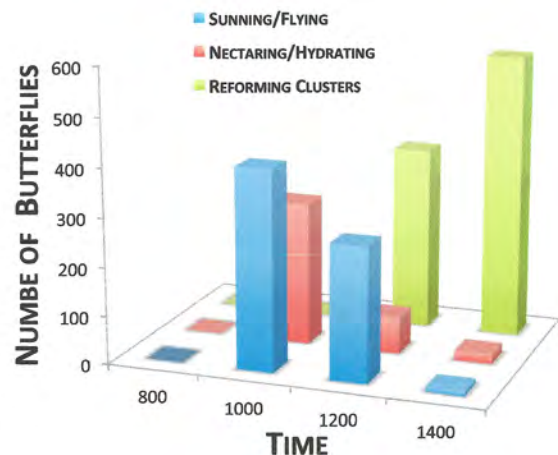

FIGURE 2. After a day of activities, Monarch Butterflies (*Danaus plexippus*) at San Simeon returned to the cool temperatures of the grove to reform their winter aggregations and to conserve their body energy reserve by lowering their body temperatures and metabolism.

mainder of the season. If subjected to these winds below flight threshold, they were dislodged from their roost and blown to the ground where they lay like scattered leaves until warmed by ambient temperatures and able to fly to neighboring foliage or to ultimately abandon the grove. I have found that the mortality of the downed butterflies of
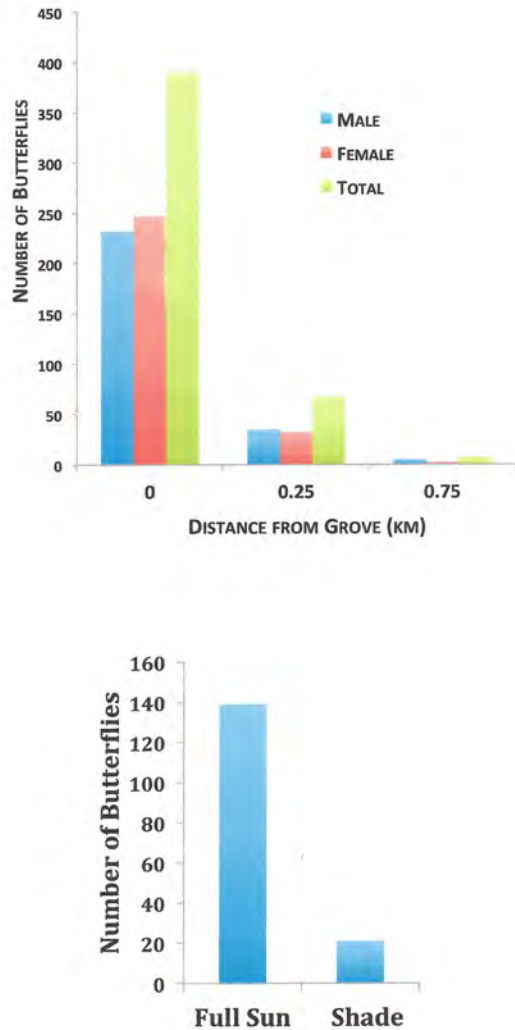
FIGURE 4. The seasonal summary of two winter sites of Monarch Butterflies (*Danaus plexippus*) at San Simeon, California (A) and at the Lighthouse Field State Beach, Santa Cruz, California (B). I found that the field activities of the butterflies occurred within 1 km of the grove and in sunlit field and foliage.

FIGURE 3. Monarch Butterflies (*Danaus plexippus*) will forage for water or nectar within a kilometer of the grove (A) and mostly in sunlit areas (B).

Pismo North Beach winter site is < 1% (Kingston Leong, unpubl. data).

The seasonal wind profile of a grove (GSWP) is a valuable management analytical tool to determine protectiveness or the ability of winter groves to buffer disruptive winds. If disruptive winds permeate the cluster arena, the overwintering butterflies will either abandon the grove temporarily or for the rest of the season. The GSWP is also a means to determine the area of the grove that requires wind protection and where planting of seedlings are needed to buffer future disruptive winds. The following scenarios (1, 2, and 3) of winter grove analyses using GSWP are based on my field observations of monarch winter groves in five California locations: Monarch Lane (Leong 1997), Los Osos, Purple Gate, San Simeon, Fort Baker, and Sweet Springs (Kingston Leong, unpubl. data).

Monarch Lane, Los Osos, California.—The GSWP for winter season 1994–1995 showed that strong NW winds (≥ 2 m/s) entered the grove along its western border and permeated to trees supporting winter aggrega-

tions of Monarch Butterflies (Fig. 5). When exposed to these winds, 2,000–3,000 butterflies abandoned the grove for the season. Analysis of the perimeter trees of the grove showed that the break in wind protection was attributed to a dead perimeter tree (Leong 1997).

In March 1995, eight 2 m (6 ft) eucalyptus seedlings (*Eucalyptus globulus* var. *compacta* Labill.) were planted in the sunlit area west of the dead perimeter tree to close the gap in wind protection of the remaining perimeter trees. Within two winter seasons, the GSWP of the 1996–1997 winter season revealed that the disruptive winds (blue sample station circles) entered the grove from the NW, NE and SW and were effectively buffered before reaching the trees supporting 3,000 overwintering butterflies (orange circles; Fig. 5). Notably, the butterflies did not form winter aggregations randomly within the protective zone of disruptive wind, but only on trees that provided the best access to filtered morning and afternoon sunlight (Fig. 5). On these trees, the butterflies remained at this locale of the grove the entire winter. Use of GSWP provided graphic analyses of the protectiveness of the grove against disruptive winds for the butterflies and where plantings of new grove trees are needed to buffer such winds.
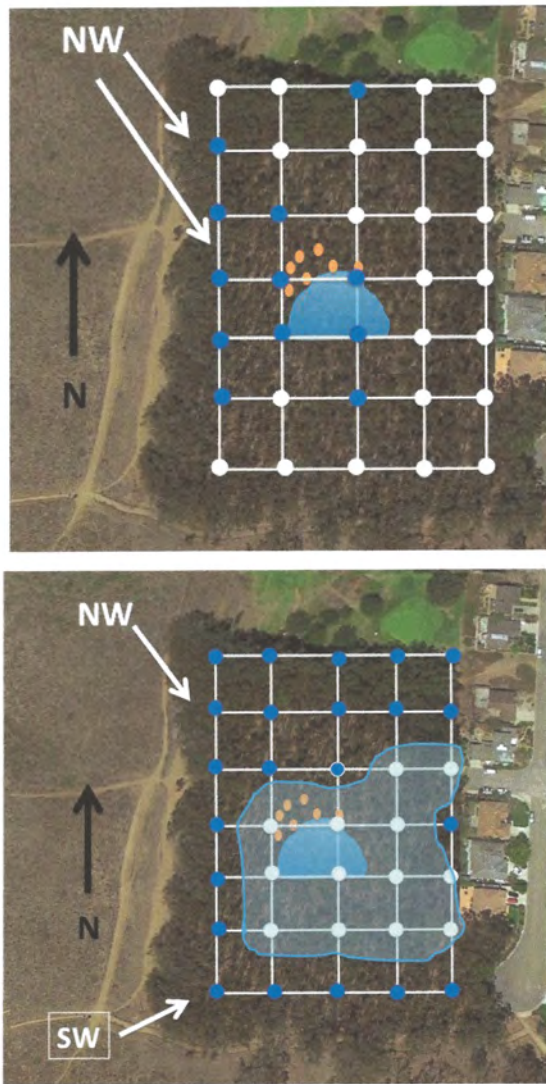
FIGURE 5. (Top) The seasonal wind profile of the Monarch Lane grove in 1994–95 shows disruptive winds entering the northwest and permeating the cluster arena. (Bottom) The seasonal wind profile of the Monarch Lane grove in 1996–97 shows that the disruptive winds entering from the northwest and southwest. The grove trees effectively buffered the velocities of these winds before they reached the cluster arena, creating a wind buffered zone (shaded light blue).

Purple Gate, San Simeon, California.—The GWSP of the winter site at San Simeon indicated no disruptive wind measurements (Fig. 6), while those at Purple Gate had many readings of disruptive winds ≥ 2 m/s, especially those entering the grove from the northwest (Fig. 7). Notably, the residential home located NW of the trees supporting the winter aggregation of monarchs shielded the butterflies from strong winds and likely accounted for the approximately 2,000 overwintering butterflies at Purple Gate. Although GWSP of San Simeon showed no disruptive wind recordings, there was a winter storm from the southwest a few days prior to the sample on 8 January. The butterflies abandoned the main cluster area, and a small number of butterflies (325 of 6,850) were

found clustering on perimeter trees in area A, a refuge location in response to southeastern storm winds. The few butterflies that remained never returned to the main aggregation area, but instead clustered toward the end of the season on perimeter trees in area B. When exposed to storm winds, the butterflies will seek refuge areas within the grove and if none are available will abandon the grove. Refuge areas can be other grove trees or man-made structures as a residential home as was depicted for Purple Gate.

Fort Baker (Duncan Hill) and Sweet Springs, Los Osos, California.—Both winter sites are transitional sites and show similar GSWP where much of the habitat is susceptible to disruptive winds, particularly during winter storms (≥ 2 m/s). These winds permeated the winter site, especially the areas where the butterflies were once or formed (Fort Baker and Sweet Springs, respectively) winter aggregations (Figs. 8 and 9). Both sites share similar characteristics in that they are made up of mature grove trees (at Fort Baker, eucalyptus; at Sweet Springs, Monterey Cypress, *Cupressus macrocarpa*, and Blue Gum eucalyptus, *Eucalyptus globulus globulus*). The groves have heavy canopy foliage with little to no lower foliage to buffer strong horizontal winds, especially during winter storms. The restoration of the two sites is possible with strategic plantings of trees to create a buffer zone against strong winds entering below the canopy foliage. As with most winter sites, however, there is no commitment to manage the site beyond what has been shown in traditional analyses of the habitat. Without proper management, these sites are consequently destined to degrade until they cease serving as overwintering site for the butterflies. Mature grove trees offer minimal protection against storm winds because they lack sufficient lower branches (foliage) to buffer storm winds that enter and permeate horizontally through the grove. When storm winds permeate through the aggregation area, the butterflies will often use these winter sites, at best, as transitional.

The winter aggregations of Monarch Butterflies are not randomly distributed within the wind-protective boundaries of the grove (i.e., light blue shaded area of Fig. 5; Leong et al. 1991; Frey et al. 1992), and I often observed them at Los Osos, Purple Gate, Lighthouse Field State Beach and San Simeon winter sites on trees providing best access to filtered morning and afternoon sunlight. Exposure to morning sunlight, mainly radiant energy, is important for the diurnal activities of butterflies, such as flying, sunning, foraging for water or nectar, and seeking mates.

Afternoon filtered sunlight directs the location within the wind protective zone where the butterflies will form their winter aggregations. Although measurements of actual solar radiant energy were not possible during the present study due to cluster heights, the cluster reforming activities of butterflies could be readily observed beginning at 1200 (Fig. 2). I observed butterflies that were

Figure 6. The seasonal wind profile of the San Simeon grove in 2003–2004 shows no recordings of disruptive winds. The butterflies clustered in the main cluster area until they were exposed to a January winter storm from the southeast. The few butterflies that remained move to a refuge area (A) between sample areas 10 and 11.

Figure 7. The seasonal wind profile of the Purple Gate grove in 2003–2004 showed disruptive winds entering from the northwest. The butterflies were found behind the wind protective area (shadow) of a two-story residential home.

reforming their winter aggregations fluttering above and settling on branches exposed to filtered sunlight. As the sunlight traverses to other wind-sheltered sunlit branches, the former branches, now shaded, will have roosting butterflies, while the latter will have active butterflies, forming their winter aggregations. Significantly, the formation of winter aggregations of Monarch Butterflies seems to occur only on foliage or branches exposed to af-

ternoon sunlight and seldom on those that are in constant shade or are exposed to direct sunlight.

The influence of filtered sunlight on the winter aggregations of monarchs was demonstrated when I (Leong 1998) selectively topped southern trees prior to the 1996–1997 winter season to allow better morning and afternoon sunlight onto a given area of the Monarch Lane winter site, Los Osos, California. The butterflies clustered on trees within the targeted area and remained on these trees throughout the winter season. I observed a similar response during 2011–2012 winter season at Butterfly Grove, Trilogy, at Nipomo, California. A historical cluster tree was shaded during critical afternoon hours by the new growth of neighboring trees and did not support winter aggregation throughout winter season 2010–2011. Prior to the beginning of the 2011–2012 winter season, I removed trees that were blocking the exposure of historical cluster trees to filtered afternoon sunlight. The butterflies returned to form winter aggregations on the historical tree from the beginning of the season until they abandoned the grove in December. If the access of butterflies to filtered sunlight is the limiting factor for winter aggregations, the butterflies will return to cluster on a targeted tree once southern trees and limbs are removed to permit increased afternoon sunlight exposure.

Other winter habitat management considerations.—To increase adult nutritional resources, plantings of winter flowering plants should be within a kilometer of the grove and in sunlit areas. Overwintering Monarch Butterflies seem to limit their field activities (foraging for water, consuming nectar, sunning, and mating) within a kilometer of the grove, generally in adjacent open, southern areas of the grove. Replacement trees should be planted as soon as possible because they may take 10–20 y to grow to a height and foliage density sufficient enough to effectively buffer disruptive winds or serve as cluster trees. Monterey Cypress, Monterey Pine (*Pinus radiata*), and Blue Gum eucalyptus are fast growing tree species and have supported winter aggregations in mixed or in pure (except Monterey Cypress) stands. The choice of tree replacements should be based on whether a given tree species will serve as a cluster or buffering tree.

Monterey Cypress trees are resistant to pine pitch canker disease and will produce dense foliage ideal for buffering strong winds (State Board of Forester 1908). Planting of Monterey Cypress in the southern region of the grove, however, should be carefully evaluated, because its dense foliage may prevent filtered sunlight from reaching trees supporting winter aggregations. In mixed stands of grove trees, Monterey Cypress trees provide excellent foundations for winter aggregations.

Blue Gum eucalyptus or Monterey Pine may be used as cluster trees or general grove trees, particularly in the southern section because their foliage can effectively buffer gusty and storm-force winds and still allow fil-
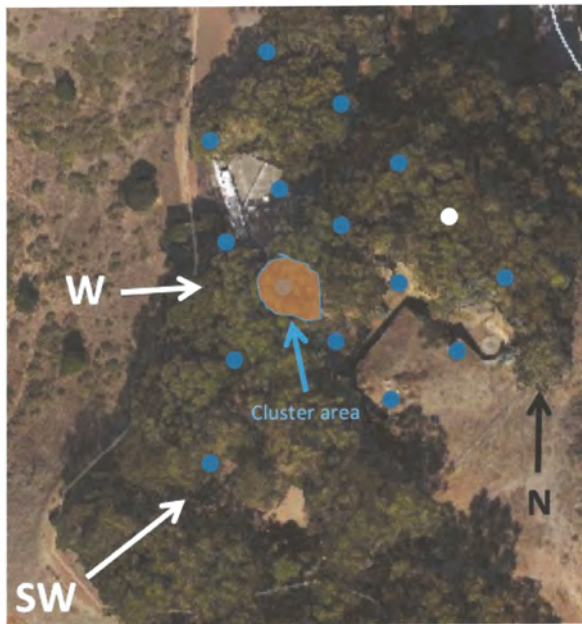
FIGURE 8. The seasonal wind profile of the Fort Baker grove in 2005–2006 revealed a degraded winter site where disruptive winds permeated through the former aggregation area (shaded orange) and 15 of 16 sample stations.
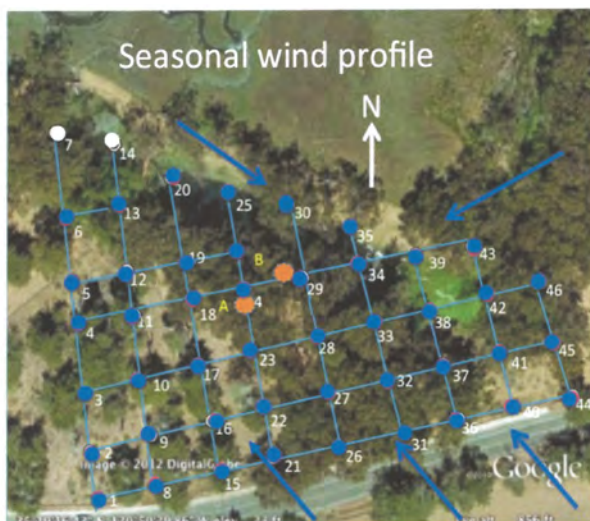

FIGURE 9. The seasonal wind profile of the Sweet Springs grove in 2013–2014 recorded disruptive winds from 44 of 46 sample stations, indicating a degraded winter site.

tered sunlight into the cluster arena. Although eucalyptus trees, particularly Blue Gum, are considered to be an invasive species, they can be used in a managed grove because volunteer seedlings can be easily eliminated and thereby controlled. Monterey Pine trees should be considered only if a resistant variety to pine pitch canker is developed. Once these trees are planted, their loss due to pine pitch canker will set back the grove recovery several years and will increase the cost of management.

Trees within a managed grove should always be evaluated for public safety as well as for their role in maintaining habitat integrity. The removal of dead or dying trees should be of paramount importance for public safety where they pose a risk to people or property in a monarch grove. No unauthorized visitors should be allowed to enter an overwintering site during winter storms.

The native Narrow-leaf Milkweed, *Asclepias fascicularis*, or Indian or Woollypod Milkweed, *A. eriocarpa*, are the primary larval food sources for Monarch Butterflies and are not available because they die back to their rhizomes during the winter months. Docents at some California winter sites have planted Tropical or Bloodflower Milkweed, *A. curassavica*, during the winter season in an attempt to increase the dwelling numbers of Monarch Butterflies and/or to provide visitors live examples of their life stages. This practice is not advisable and should be discouraged because the majority of the butterflies are in reproductive diapause (Leong et al. 1995). Planting at this time will encourage the few non-diapausing adults to lay eggs, resulting in starving caterpillars or adult butterflies that are not in synchrony with the natural milkweed seasonal cycle.

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Kingston Leong received his Bachelor's and Master's degree from Unversity of Hawaii and his Ph.D. degree from Oregon State University in Entomology. He has taught at Cal Poly University, San Luis Obispo, in the Biological Science Department since 1970 and retired in 2006. He has worked on various aspects of the biology of Monarch Butterfly biology and for the past 25 y and his research was centered on environmental factors affecting the winter occupancy of overwintering Monarch Butterflies at California winter groves and how these factors can be used to manage winter sites. (Photographed by Carol Leong).

NOTES

UNUSUAL MATING POSITION BY A PAIR OF BLAINVILLE'S HORNED LIZARDS (*PHRYNOSOMA BLAINVILLII*) IN THE SAN JOAQUIN DESERT, CALIFORNIA

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Abstract.—Here we report on and present a photograph of the unusual belly-to-belly mating of Blaineville's Horned Lizard (*Phrynosoma blainvillii*). This species is the only horned lizard that mates in this manner.

Key Words.—California; lizards; mating; San Joaquin Desert

During the course of a two year study of Blainville's Horned Lizards (*Phrynosoma blainvillii*) at the Atwell Island Restoration Project of the US Bureau of Land Management in Tulare County, California (Hult and Germano 2015a, b), we came upon a pair of *P. blainvillii* on 22 April 2009 at 0917 that were mating with one lizard on its back on the ground and the other on top, in a belly-to-belly posture (Fig. 1). We do not know how long the lizards had been in this position, but the lizard on top was still fairly dark in color and lightened considerably by the time they pulled apart at 0942. The air temperature (ca. 1.2 m above the ground) was 28.4° C, the surface temperature (2 cm aboveground) was 30.5° C, and the soil temperature (2 cm under the surface) was 31.5° C when recorded about 5 min after we sighted the pair. The lizard on its back sporadically heaved its chest; two heaves were given in quick succession. Pairs of heaves would be spaced 5–10 s apart over an interval encompassing 10–15 pairs of heaves, and then there would be long pauses of 1–3 min without heaving. We think the chest heaving may have been respiration. This sporadic heaving lasted over the 25 min we watched this pair until they disengaged. Unfortunately, they pulled apart so quickly while we were not directly watching them that we are uncertain which sex was on top. Each ran about 1 m from where they were mating, which was in the open, with the female (73 mm SVL, 22.5 g) running into a Heermann's Kangaroo Rat (*Dipodomys heermanni*) burrow and the male (69 mm SVL, 18.5 g) running under a Goldenbush (*Isocoma acradenia*) shrub about 0.25 m from the burrow.

Most horned lizards mate with the female upright and the male on top or to her side, with tails positioned to allow cloacal contact (Sherbrooke 2003). Mating usually lasts 15–30 min (Sherbrooke 2003). The belly-to-belly mating position of *P. blainvillii* that we observed

typically occurs with the male on top (Montanucci 1968; Tollestrup 1981) and is unique to this species among horned lizards (Sherbrooke 2003), though mating in *P. blainvillii* also occurs with the female upright (Tollestrup 1981). Although we did not determine which sex was on top during mating, males are known to bite the female on the throat and then flip her onto her back (Tollestrup 1981). The significance of mating belly-to-belly is not understood (Tollestrup 1981).

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FIGURE 1. Apparent copulatory position of a male and female Blaineville's Horned Lizard (*Phrynosoma blainvillii*) found 22 April 2009 at the Atwell Island Restoration site in Tulare County, California. Note that the individual on top (presumably the male) has grasped the throat skin of the individual on the bottom. (Photographed by Susan Hult).

NOTES

HABITAT USE AND MANAGEMENT CONSIDERATIONS FOR THE THREATENED ALAMEDA WHIPSNAKE (*MASTICOPHIS LATERALIS EURYXANTHUS*) IN CENTRAL CALIFORNIA

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Abstract.—Uncommon or declining species are often relatively enigmatic, with large and numerous gaps in our understanding of the natural history of the species. The basking activity of the Alameda Whipsnake (*Masticophis lateralis euryxanthus*) is a gap in knowledge that may contribute to mismanagement of the species. We looked at 365 reported observations for this threatened subspecies and found 25% of the observations were reported in open habitat, such as trails, roads, parking areas, or other sites. This resulted in mortality to nearly 4% of all reported animals that were basking in open areas that allowed bicycle and motor vehicle traffic. Understanding the risk to anthropogenic modifications to habitat used by this subspecies may aid in recovery of the Alameda Whipsnake.

The California Whipsnake (*Masticophis lateralis*; formerly Striped Racer) is a near-endemic species that ranges through the mountains of California and south into Baja California, Mexico (Stebbins and McGinnis 2012). It is divided into two subspecies: the Alameda Whipsnake (*M. l. euryxanthus*), which occurs primarily in Alameda, Contra Costa, San Benito, San Joaquin, and Santa Clara counties in California, and the Chaparral Whipsnake (*M. l. lateralis*), which occurs in the remainder of the north and south Coast Range, the southern Cascade and Sierra Nevada ranges, and also Baja California (Ortenburger 1928; Richmond et al. 2011). Currently there is a paucity of published information on the natural history of the species, and little information related to the behavior of either subspecies. Efforts to understand and report information that can support management of this species, in particular Alameda Whipsnake, which is state and federally listed as threatened, are critical to maintaining, monitoring, and managing populations.

Published reports do provide insight into the characteristics and dimensions of the morphology, types of prey, associated habitats, thermal ecology, and reproductive ecology of the entire species. However, there is little information on habitat use, particularly how fragmentation of habitat could affect management of this subspecies. In particular, understanding how trails, roads, and developed areas impact the habitat of this snake could benefit its recovery.

To better understand habitat use, it is important to consider the thermal ecology of the snake. This aspect of the Alameda Whipsnake was carefully studied by Hammerson (1979), who found the subspecies to be highly thermophilic. This work suggested that basking, particularly morning basking, is critical to supporting the physiology of the snake in pursuing and capturing prey (typically lizards). Both subspecies of the California Whipsnake are known to bask in the tops of trees and have been reported there by several researchers (Grinnell and Grinnell 1907; Ortenburger 1923; Shafer and Hein 2005). More frequently, however, the Alameda Whipsnake, has been reported basking in open areas, presumably seeking exposure to direct solar radiation (Hammerson 1979; Sullivan 1981a). Sullivan (1981b) reported six Alameda Whipsnakes basking on a single road in Contra Costa and San Joaquin counties, California.

For our analysis, we reviewed 365 reported observations of the threatened subspecies, including our own observations, and attempted to understand aspects of its behavior that could potentially influence management considerations; particularly how often Alameda Whipsnakes were observed on roads or trails, or in developed areas. These 365 observations included all known California Natural Diversity Database (CNDDB) occurrences (California Natural Diversity Database. 2015. *Masticophis lateralis euryxanthus*. California Natural Diversity Database Version 5.1.1. Biogeographic Data Branch. Department of Fish and Wildlife. Available at: https://www.dfg.ca.gov/biogeodata/cnddb/mapsanddata. asp. [Accessed 9 October 2015]), specimens from the California Academy of Sciences (CAS; California Academy of Sciences. 2015. CAS Herpetology Collection Database. Available at: http://researcharchive.calacademy. org/research/herpetology/catalog/index.asp. [Accessed 4 October 2015]) and the Museum of Vertebrate Zoology

Figure 1. Basking Alameda Whipsnake (*Masticophis lateralis euryxanthus*) on an open trail, Contra Costa County, California. (Photographed by Kendall Oei).

(MVZ; Museum of Vertebrate Zoology. 2015. University of California, Berkeley. Available at: http://mvz.berkeley.edu. [Accessed 4 October 2015]), and individual Alameda Whipsnake sightings reported at parks, preserves, and open spaces within its range. We acknowledge the biases associated with using reported observations (which may include misidentification, missing data, examples that may not represent typical behavior, etc.), but feel that the information used herein can support certain conclusions. We excluded from analysis all reported observations associated with snake trapping events and attempted to determine what type of habitat was associated with the individual snakes, whenever possible. Notwithstanding the limited information provided by the remaining data, we were able to detect an important pattern of behavior. We were able to determine for 40% of these 365 observations whether or not the snake was using a road, trail, or developed area at the time of encounter.

Numerous reports (109, or 30%) of the 365 included information specifying whether the observation occurred within either natural (grassland, rock outcrops, oak woodland, chaparral) or developed areas (i.e., anthropogenic structures, such as driveways, parking areas, graded areas around campgrounds, etc.). Ninety observations (25%) indicated that the specimen was using an open area described as one of the following: hiking trail, fire access road, paved roadway, or developed area (i.e., public bathroom area or picnic area). Based on the thermal ecology of the snake (Hammerson 1979), it is quite possible that such open areas are favored by these snakes because they provide suitable basking habitat and exposure to direct solar radiation, or support surface basking when the substrate radiates heat. Three of the behaviors reported from roads and trails were perceived as sunning or basking (Fig. 1).

Ground-level basking, which is frequently how the species is observed, can facilitate predation and lead to direct or indirect mortality from this and other causes (Sullivan 1981b; Andrews et al. 2015). In the case of the Alameda Whipsnake, at least 13 of the 365 observations we analyzed included specimens that had apparently succumbed to anthropogenic-related mortality; four of these were described as roadkill, presumably killed by cars when the snake was on a roadway. At least one snake on a hiking trail was presumed killed by a mountain bike (MVZ 223176; Fig. 2). The direct causes of mortality for the remaining eight reported dead snakes were ambiguous (i.e., found on road dead). Although roadkill was the likely cause in all eight cases, it could not be determined with certainty.

Andrews and Gibbons (2005) reported that smaller species of snakes may avoid open spaces (including roadways) to reduce the risk of predation. This is less likely to be true of the larger of the two subspecies of California Whipsnakes. When Sullivan (1981) incorporated the tendency of snakes to bask on roads in formulating a methodology for detecting them, he included the whipsnake as an exemplar.

When formulating management decisions, it is important to acknowledge these findings. Specifically, management of the Alameda Whipsnake and its habitat requires careful consideration of habitat alterations and fragmentation. More specifically, habitats that are opened or bisected for placement of trails and roadways may increase the likelihood of ground basking by Alameda Whipsnakes. This may add to interactions between snakes and cars or bikes, and result in increased injury, mortality, and other types of disturbance (i.e., collection, predation, etc.). It is clear that this threatened subspecies can persist in areas bisected by roads and trails, but such infrastructure should be limited or its use restricted, whenever possible, to reduce likelihood of increased mortality of the species.

Figure 2. Alameda Whipsnake (*Masticophis lateralis euryxanthus*) presumably killed on a trail when struck by a mountain bike, Alameda County, California. (Photograph courtesy of University of California, Berkeley, Museum of Vertebrate Zoology).

Zoology, Berkeley who assisted us in obtaining the photo of specimen #223176. We are also grateful to all those who report observation of sensitive species with such detail and clarity that data-mining becomes a reasonable methodology for data collection.

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Ariel Miller is a Wildlife Biologist with over 5-y experience working with California fauna; much of her experience has included work with reptile and amphibian species, such as California Tiger Salamander (*Ambystoma californiense*), California Red-legged Frog (*Rana draytonii*), Giant Garter Snake (*Thamnophis gigas*), and Western Pond Turtle (*Actinemys marmorata*), among others. She is especially interested in conservation and improving upon existing knowledge of natural histories of species. (Photographed by Sarah M. Foster).

Jeff A. Alvarez is a Herpetologist who has specialized in California reptiles and amphibians for 30 y. He has worked with California Red-legged Frogs (*Rana draytonii*), California Tiger Salamanders (*Ambystoma californiense*), Western Pond Turtles (*Actinemys marmorata*), and Alameda Whipsnakes for much of his career. His focus is on the conservation and natural history of herpetofauna and survey techniques development. (Photographed by David Wyatt).

2016 Annual Meeting Review

Program Chair: Rachel Sprague

Attendance: 507 Individual Registrants, including 120 students

Restoration Activity: Chino Hills State Park

Plenary Theme: Transforming the process of conservation to create sustainable solutions. **Speakers**: Francine Madden, HWCC; Jill Lewandowski, BOEM; Marc Kenyon, California Department Fish & Wildlife; MJ Mazurek, Island Conservation; Danny Martorello, Washington Department of Fish & Wildlife

Keynote Address: Conflict in Conservation, A 360 View. **Speakers**: Vic Leipzig, Amigos de Bolsa Chica (1995); Wayne Spencer, Conservation Biology Institute (2008); Ed LaRue, Desert Tortoise Council (2004); Frazier Haney, Mojave Desert Land Trust (2011)

Awards Bestowed

Chapter of the Year – San Francisco Bay Area
Conservationist of the Year – The Santa Monica Mountains Conservancy
Dasmann Professional of the Year – Rocky Gútierrez
TWS Distinguished Service – Jon Hooper
James D. Yoakum for Outstanding Service – Ken Mayer

Student Presentation Awards

Best Posters:

1st Place – **Jennifer Gansberg**, University of Nevada, Reno, "Genetic diversity and population structure of the riparian brush rabbit (*Sylvilagus bachmani riparius*)"

2nd Place – **Rachel Chock**, UCLA, "Exploitation, interference, and niche partitioning: Competition and coexistence in a rodent community"

3rd Place – **Courtney Silver**, California State University, Chico, "Lexicon of Love: Vocalizations in Multiple Populations of *Rana boylii*"

Best Presentations:

1st Place – **Shannon Murphy**, Humboldt State University, "Parental care behaviors in Brandt's Cormorant (*Phalacrocorax penicillatus*): Effects on reproductive success and use as indicators of the marine environment"

2nd Place – **Jade Keehn**, University of Nevada, Reno, "Predation ecology of vertebrates at a California wind farm"

3rd Place – **Carly Creley**, California State University, Los Angeles, "Changes in the distribution of the eastern gray squirrel, *Sciurus carolinensis*, in California from 1900-2015 with projections for its potential future range"

2016 Western Section Membership

Regular	563
Student	99
New Professional	80
Retired	34
Life	22
Life 2/3	1
Honorary	1
Total:	800

TWS Western Section Board Members