CONSERVATION OF ENDANGERED BUENA VISTA LAKE SHREWS (*SOREX ORNATUS RELICTUS*) THROUGH INVESTIGATION OF TAXONOMIC STATUS, DISTRIBUTION, AND USE OF NON-INVASIVE SURVEY METHODS

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National Zoological Park

June 16, 2017

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ACKNOWLEDGMENTS

This project was funded by the U.S. Fish and Wildlife Service through an Endangered Species Conservation and Recovery Grant to the California Department of Fish and Wildlife (CDFW) and a subsequent sub-grant to the California State University-Stanislaus. We thank John Battistoni and Krista Tomlinson at CDFW for administrative assistance and project support. For field and logistical assistance, we thank Ellen Cypher, Bernadette Paul, Scott Phillips, and Kyle Tabor at the CSUS Endangered Species Recovery Program; Shana Carey, Christina Anderson, Dyana Mitchell, and Katie Hernandez and staff from or formally from the California Department of Fish and Wildlife; Tamra Nunes of the California Department of Transportation; Landon Peppel, Megan Lundin and staff at The Wildlands Conservancy's Wind Wolves Preserve; Geoff Grisdale, Nick Stanley, John Meriwether, Scott Frazier and other current or former staff at the Kern and Pixley National Wildlife Refuges; Mike White, Ellery Mayence and staff at the Tejon Ranch Conservancy; Art Chianello at the City of Bakersfield; John Laybourn, Karen McKenzie and staff at the Buena Vista Recreation Area; Jim Jones of South Valley Biology and Pam Willis of AERA; Carolyn Belli and Panorama Vista Preserve staff; Shauna McDonald and Jason Kirby of the U.S. Bureau of Reclamation; Bill Moffat and Carrie Bemis of California State Parks and Recreation; Rebecca Brooke, Carly Summers, Timothy Keldson, and Jihadda Govan of or formally from the U.S. Bureau of Land Management; Elizabeth Palmer and Jesse Bahm of the Natural Resource Conservation Service; Jonathan Pickett of the California Waterfowl Association; Tim Ashlock of the Buena Vista Water Storage District; Timothy Jessen at the University of Arizona; and Ken Hickman, Chris Wemmer, and Craig Fiehler for camera advice.

EXECUTIVE SUMMARY

The Buena Vista Lake shrew (*Sorex ornatus relictus*; BVLS) formerly inhabited the interconnected seasonal and permanent lakes, wetlands, sloughs, and marshes around historic Tulare, Kern and Buena Vista lakes in the Tulare Basin of the San Joaquin Valley. Approximately 95% of riparian and wetland habitat in the San Joaquin Valley has been lost, leaving only isolated remnants of suitable habitat where *S. o. relictus* still persists. Consequently, BVLS were federally listed as endangered in 2002. Information on taxonomic relationships to other ornate shrews, distribution, and habitat preferences are lacking. Our goal was to collect critical information that will contribute to conservation and recovery efforts for BVLS. Specific objectives were to: (1) complete an on-going taxonomic analysis of shrews in the San Joaquin Valley, (2) investigate the efficacy of non-invasive survey techniques for detecting shrews, (3) conduct surveys for BVLS in historic as well as previously unsurveyed locations, and (4) develop conservation recommendations based on our findings.

Taxonomic analyses were completed using BVLS genetic samples collected previously as well as during this project. The analyses were conducted by the Smithsonian Conservation Biology Institute. Results indicated that there is greater genetic connectivity and admixture between shrew populations in the northern and southern portions of the Valley than previously thought. The analysis also indicated that while some of the small populations retain moderate levels of genetic diversity, the southern San Joaquin Valley shrew populations retain unique alleles suggesting that conservation of these shrews is important to maintaining population-wide genetic diversity.

We compared the efficacy of track tubes, scat tubes, cameras, and live-traps for detecting shrews. Track tubes proved problematic in many regards, but in particular, positively identifying shrew tracks was difficult. This technique was abandoned. Among the other techniques, in direct comparison tests consisting of stations with all three techniques employed simultaneously in areas where BVLS were known to occur, detection rates were 3.3% for live-traps, 36.7% for scat tubes, and 88.9% for cameras. The cameras, which included a close-focus setting to facilitate small mammal identification, clearly performed the best in detecting shrews in a manner that minimizes risk for the animals and also is less labor-intensive than live-trapping. Scat tubes might be modified to enhance efficacy and could be useful to collect genetic samples non-invasively.

Using a combination of live-trapping and camera stations, we surveyed for BVLS in 16 population areas. BVLS were detected in seven of these areas. BVLS were detected previously in four of the areas, and they were detected for the first time in three areas. BVLS were not detected in nine other areas, including one where they had been detected previously. Of the seven areas where BVLS were detected, all or portions of five have some form of protected status. None of the areas with BVLS are being managed specifically for this species. BVLS were most often detected in locations with moist soils and dense cover consisting of rushes or cattails. Of note, one of the sites where BVLS were detected is an artificial wetland indicating that habitat restoration or creation may be possible for this species.

Based on our results, we offer 14 recommendations for conserving BVLS. In particular, protecting remaining suitable habitat and investigating habitat creation and shrew translocation may be critical to conserving and recovering this species.

INTRODUCTION

The Buena Vista Lake shrew (*Sorex ornatus relictus*; BVLS) formerly inhabited the interconnected seasonal and permanent lakes, wetlands, sloughs, and marshes around historic Tulare, Kern and Buena Vista lakes in the Tulare Basin of the San Joaquin Valley. By the early 1900s, when *S. o. relictus* was first described, diversion, draining, and dredging of the rivers and wetlands of the Tulare Basin for agricultural development had already begun to impact shrew populations (Grinnell 1932). Today, approximately 90-95% of riparian and wetland habitat in the San Joaquin Valley has been lost (Kelly et al. 2005, U.S. Fish and Wildlife Service [USFWS] 2011), leaving only isolated remnants of suitable habitat where BVLS still persists. Consequently, BVLS were federally listed as endangered in 2002 (USFWS 2011).

Prior to this study, BVLS were known from only nine locations in the southern San Joaquin Valley (Figure 1; Williams and Harpster 2001; USFWS 2011; California State University-Stanislaus, Endangered Species Recovery Program [ESRP] unpubl. data). Shrews also had been detected at several locations in the northern part of the valley (i.e., north of Kings County). At several locations where shrews have been detected, such as Wind Wolves Preserve and northern portions of the San Joaquin Valley, the taxonomic status was uncertain. Based on the current information from genetic analysis, only shrews south of Tranquility and Helm in Fresno County (Figure 1) were considered to be the listed subspecies, *S. o. relictus* (J. Maldonado, unpubl. data; USFWS 2011). Clarity on the taxonomic relationships of shrews in the San Joaquin Valley and the range of BVLS is needed to facilitate the development of effective conservation and recovery strategies.

The rarity of BVLS has contributed to a lack of information on basic aspects of their ecology. For example, while the majority of shrews have been captured in riparian and wetland habitat that is near water, shrews have also been captured in more xerophytic, upland areas and on retired farmland (USFWS 2011, ESRP unpubl. data). Furthermore, there have been very few targeted survey efforts to help define habitat preferences and no specific monitoring programs or population studies. Thus, the abundance and distribution of BVLS as well as preferred habitat attributes are still unknown.

Detecting the presence of shrews is challenging due to low capture rates and high trap mortality rates (e.g., Getz 1961, Yunger et al. 1992, Hays 1998, Do et al. 2013, Smith et al. 2017). Capture-related mortalities are even more problematic when working with a rare species such as BVLS. Shrews have been detected using other more non-invasive methods that may be less risky, including track tubes (e.g., Brehme et al. 2010). However, the efficacy of these techniques has not been evaluated.

Our goal was to collect critical information that will contribute to conservation and recovery efforts for BVLS. Specific objectives were to: (1) complete an on-going taxonomic analysis of shrews in the San Joaquin Valley, (2) investigate the efficacy of non-invasive survey techniques for detecting shrews, (3) conduct surveys for BVLS in historic as well as previously unsurveyed locations, and (4) develop conservation recommendations based on our findings.

METHODS

STUDY AREA

The study area for this project was the southern San Joaquin Valley, California (Figure 1). This area is within the region known as the San Joaquin Desert (Germano et al. 2011). The regional climate is Mediterranean in nature, and is characterized by hot, dry summers, and cool, wet winters with frequent fog. Mean maximum and minimum temperatures are 35°C and 18°C in summer, and 17°C and 5°C in winter. Annual precipitation averages ca. 15 cm and occurs primarily as rain falling between October and April (National Oceanic and Atmospheric Administration 2002).

BVLS primarily have been found in wetland and riparian habitats that have moist soils and dense cover of either herbaceous vegetation or leaf litter (USFWS 1998). Historically, extensive lakes, wetlands, and riparian areas occurred in the San Joaquin Valley and provided abundant habitat for BVLS. Indeed, most recent detections of shrews occur in areas where these aquatic features historically occurred (Figure 2). Thus, survey efforts were focused on areas with remnant aquatic habitats, particularly areas where soils remained moist year-round.

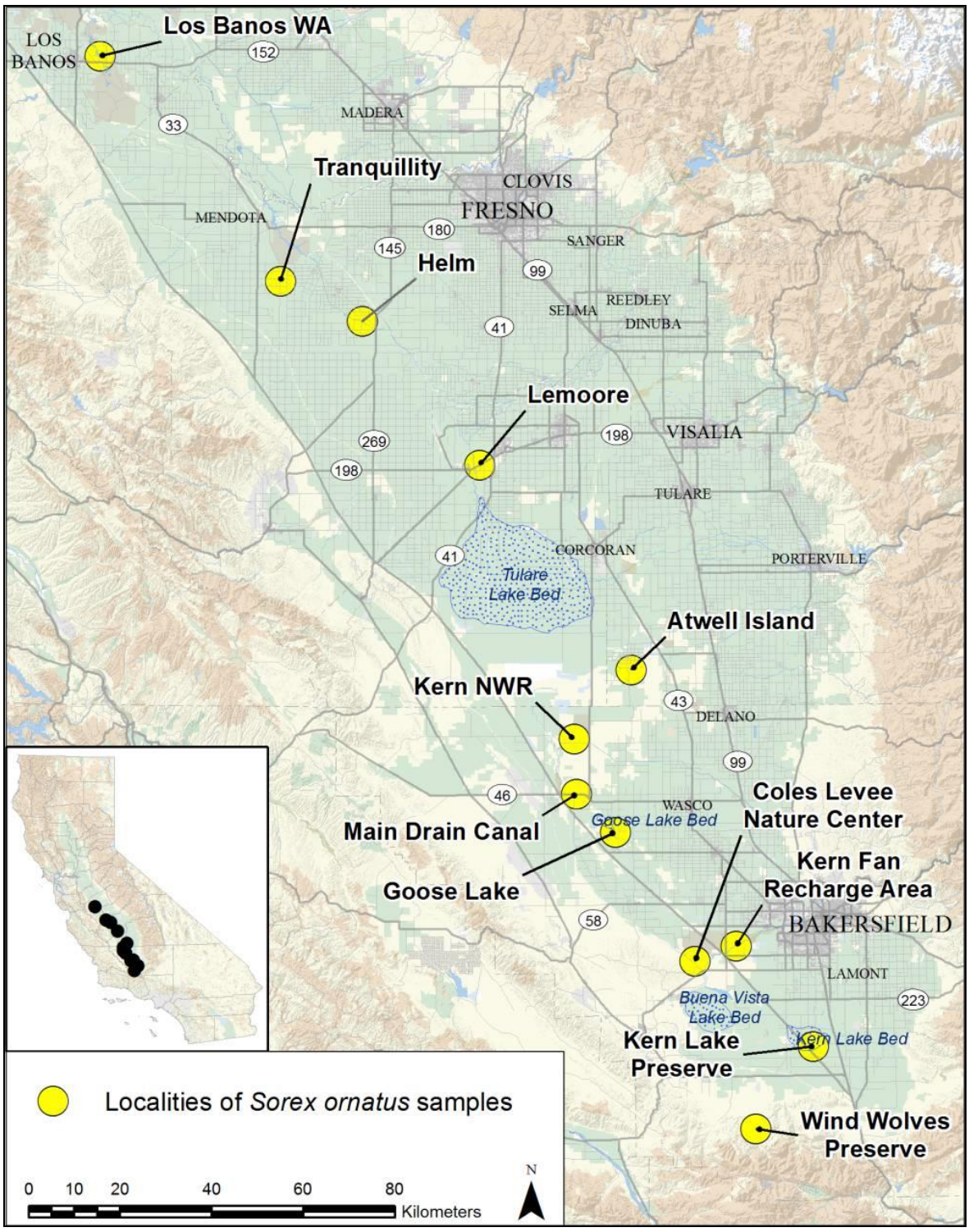

Figure 1. Locations where shrews have been detected and from where genetic samples have been collected previously in the San Joaquin Valley, California,

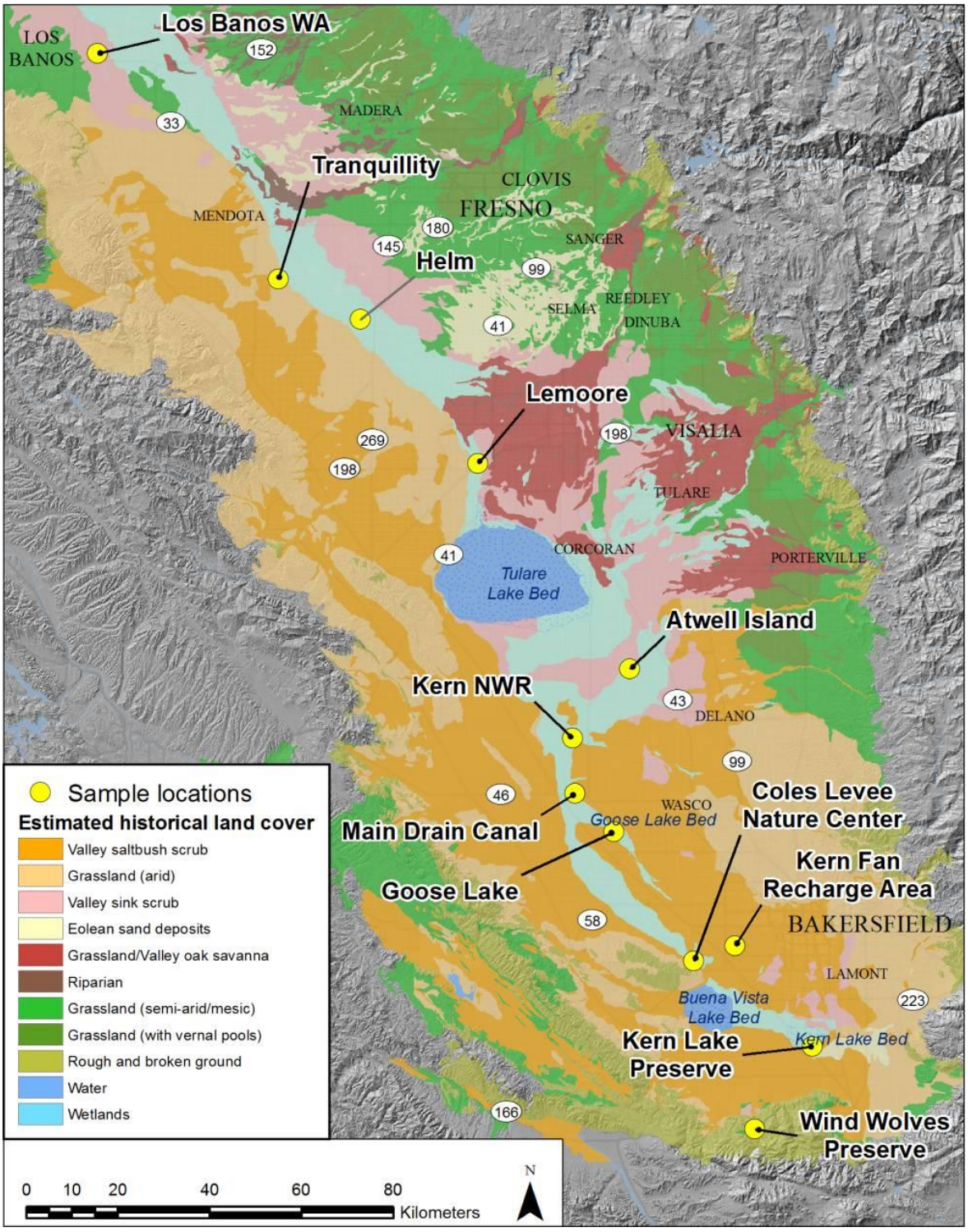

Figure 2. Locations where shrews have been detected and from where genetic samples have been collected previously in the San Joaquin Valley, California,

TAXONOMIC ANALYSIS

Dr. Jesus Maldonado at the Smithsonian Conservation Biology Institute has been assessing the taxonomic status of shrews in the San Joaquin Valley since the early 1990s. This assessment has been incremental as it has been dependent upon the availability of genetic

samples collected during periodic surveys for BVLS. Initial results have been presented in a previous report (Maldonado 2014). Additional genetic samples collected during this project (for genetic sampling techniques, see Surveys below) were added to this previous analysis. Details on methodology are provided in a companion report (Appendix B).

NON-INVASIVE DETECTION TECHNIQUES

The efficacy of three non-invasive detection techniques was evaluated: track tubes, scat tubes, and automated camera stations. Track tubes consisted of 2 15-cm long PVC pipes (6-cm diameter) connected by a 10-cm long 45-degree elbow (Figure 3). A flat wood block (5 cm x 5 cm; flat on the top and curved on the bottom to conform to the shape of the tube) was placed just inside both ends of the tube. The block was held in place by a bolt through both the wood and the PVC tube and secured with a wing nut. A piece of felt was wrapped around each block and secured with duct tape. Using a syringe, each piece of felt was saturated with a tracking medium consisting of 2 parts lamp black and 5 parts mineral oil. White notecards (7.5 x12.5 cm) were placed between the wood blocks and the elbow. When the track tube was set at a location in the field, mealworms (6-12) were then placed in the elbow. Shrews entering the tubes to get the worms would need to cross the felt pieces, thereby picking up tracking medium on their feet, and then cross over the notecards, thereby recording their tracks.

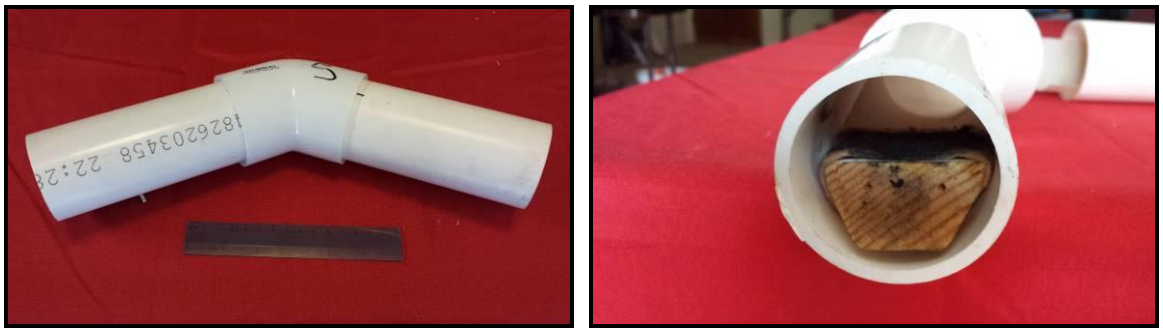

Figure 3. Track tube (left) with 15-cm ruler for scale, and wooden block with felt ink pad in entrance (right).

During initial testing of the track tubes in the field, we noticed that shrews occasionally left scats (i.e., feces) in the tubes. Thus, we modified the track tubes to create "scat tubes." The 15-cm pieces of PVC pipe were replaced with 30-cm long pieces without wood blocks. A piece of white paper 28.5 x 10.5 cm was taped to the inside bottom of each tube (Figure 4). As with the track tubes, mealworms were placed in the elbow when the scat tube was placed in the field. The longer tube gave shrews entering the device more time to deposit scats as they entered and exited the device.

Figure 4. Scat tube (left) with 15-cm ruler for scale, and entrance to scat tube (right) with paper to collect Buena Vista Lake shrew scats.

The third technique was the use of automated cameras stations. Information from colleagues indicated that this technique could be effective if the proper baiting and camera set-up techniques could be determined. We experimented with several different camera models, including regular Reconyx HC600 HyperFire Covert Camera Traps, Busnell Trophy Cameras (of several models), Moultrie Wingscapes Birdcam Pros, and a "home-brewed" self-made camera trap put together by a naturalist colleague. We were able to detect shrews on several of these camera models, but the quality of the images on most of the cameras, particularly the commercial ones, was mediocre because the camera focal distance is set for larger wildlife.

We then were alerted to a "close-focus" model made by Reconyx. This camera (Reconyx HC600 HyperFire Covert, Reconyx, Holmen, WI) was a motion-activated, infrared field camera, and the focal distance was factory-set at 40 cm (ca. 16 in) to obtain clear images of small animals at close range. The cameras were programmed to capture 5 images in rapid-fire fashion at a fast shutter speed. Each camera was attached approximately 20 cm off the ground to a 0.5-m metal t-post. A bait station consisting of a small Tupperware container (ca. 9-cm diameter, ca. 7 cm deep) was installed at ground level approximately 50 cm in front of each camera (Figure 5). The container was pinned to the ground with 15-cm nail to inhibit removal by animals. Approximately 12 live mealworms (*Tenebrio molitor*) were placed in each container and approximately 40 dried mealworms were placed on top of each container as an additional attractant. At some stations, especially areas with standing water, a metal tea infuser ball with mealworms placed inside was used instead of or in addition to the Tupperware container.

To evaluate the efficacy of non-invasive techniques for detecting shrews, we conducted a series of direct comparison tests in fall 2016. These tests were conducted at 3 locations where BVLS had been consistently detected during surveys or initial non-invasive technique tests. The 3 locations were the Wind Wolves Preserve (WWP), Kern National Wildlife Refuge (Kern NWR), and Northern Semitropic Ridge Ecological Reserve (NSRER) and adjacent private lands. At each location, 10 sites were selected. At each site we placed a test station consisting of a live-trap, scat tube, and camera (Figure 6). The live-traps were set as described in the Survey section below. The scat tube was placed next to the trap, and both the trap and the scat tube were within the field of view of the camera. The stations were operated for three nights at Kern NWR and NSRER, and for two nights at WWP.

Using the combined data from all 3 locations, the proportion of stations with shrew detections was compared among the 3 techniques (live-traps, cameras, and scat tubes)

using contingency table analysis and a chi-square test statistic. For 2x2 analyses, a Yate's correction-for-continuity was employed (Zar 1984). Statistical tests were conducted using Social Science Statistics (http://www.socscistatistics.com/tests/Default.aspx). *P*-values ≤ 0.05 were considered significant.

Figure 5. Automated camera station for detecting Buena Vista Lake shrews.

Figure 6. Buena Vista Lake shrew detection test station consisting of an automated camera, a Sherman live-trap, and a scat tube.

SURVEYS

Surveys were conducted in 16 general population areas. In some areas, surveys were conducted at multiple sites that were within close proximity and appeared to have some connectivity. Initial surveys were conducted by live-trapping using small Sherman aluminum box traps (5.1 x 6.4 x 16.5 cm; H.B. Sherman Traps, Inc., Tallahassee, FL). Traps typically were set in sites with dense rushes (*Juncus* spp.) or cattails (*Typha latifolia*), or with deep leaf litter under willows (*Salix* spp.) and cottonwoods (*Populus fremontii*). Traps were opened in the evening, baited with mealworms, and provisioned with commercial small mammal pet bedding material or a small amount of polyester batting to provide thermal insulation. Trapping was conducted for 2 or 3 nights at each site. If nighttime temperatures were forecasted to drop below 50˚F, then traps were checked approximately 5 h after being opened and then closed for the remainder of the night. If temperatures were forecasted to remain above 50˚F, then traps were left open overnight and checked the next morning. All animals captured were weighed, and approximately 2-3 mm of the distal end of the tail was collected and placed in 95% ethyl alcohol for genetic analysis. After processing, shrews were released at the capture site. All live-trapping was conducted under a USFWS Recovery Permit issued to Brian Cypher (825573-5 and 6) and under a Memorandum of Understanding between the USFWS and CDFW.

Based on the results of the technique comparison tests, later surveys were conducted using automated camera stations. Methodology associated with setting up camera stations in the field was described previously. Camera stations were operated for 2-4 nights. Images collected by the cameras then were reviewed to determine whether shrews had been detected.

For each site where surveys were conducted, a rapid habitat characterization was conducted. Attributes characterized included tree species and canopy cover, litter depth, shrub species and density, ground cover species and density, and distance to open water (see Appendix A).

RESULTS

TAXONOMIC ANALYSIS

Results of the taxonomic analysis conducted by Dr. Jesus Maldonado at the Smithsonian Conservation Biology Institute are presented in a companion report that is included as Appendix B to this report.

NON-INVASIVE DETECTION TECHNIQUES

We compared the efficacy of track tubes, scat tubes, cameras, and live-traps for detecting shrews.

Track tubes

Track tubes proved to be a problematic detection technique for two reasons. First, positively identifying shrew tracks with any consistent confidence proved difficult. To

identify tracks, we consulted several track guides and also obtained known tracks by setting a captured shrew on one of the ink-soaked felt pieces or an ink pad, and then allowing it to run across recording paper (Figure 7). Also, during initial tests of the track tubes, we focused cameras on the tubes and thus could verify that shrews were entering the tubes. We thought that because shrews have five toes on both front and hind feet, whereas mice only have four toes on the front feet, we might be able to discern between shrews and sympatrically occurring rodents. However, even with the reference materials in hand and knowing that shrews had entered a given tube, when we examined the recording paper we did not feel confident that we could consistently identify shrew tracks, or conversely, to rule out that shrew tracks were not present. A second issue was that one or more shrews or other small mammal species, particularly deer mice (*Peromyscus maniculatus*), sometimes entered a given tube multiple times to retrieve mealworms. As a result, the recording papers commonly had a jumble of overlapping tracks that made it extremely difficult to single out and identify individual tracks (Figure 8). Thus, after some field trials, we abandoned pursuit of track tubes as an effective detection technique for BVLS.

Figure 7. Obtaining known tracks from a Buena Vista Lake shrew (left), and tracks from the front feet of a shrew (right).

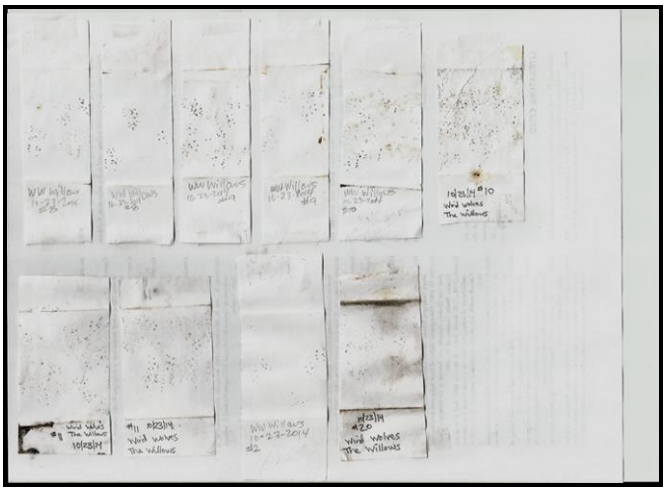

Figure 8. Track samples obtained from track tubes set to detect Buena Vista Lake shrews.

Scat tubes

Scat tubes proved to be an easier technique to employ compared to the track tubes, but still requires a good deal of expertise to correctly identify shrew scats. We obtained known shrew scats by allowing captured shrews to run around inside tubes. Also, we collected scats from clean traps in which shrews had been captured. The shrew scats, upon analysis under a dissecting scope, appeared to consist exclusively of invertebrate remains with no vegetation (Figure 9). Also, the scats seemed to be less well formed compared to rodent feces. Thus, we felt more confident about identifying shrew scats compared to identifying shrew tracks. That said, there also was a considerable number of scat samples deposited in tubes that we could not positively identify as shrew versus another species. However, our level of confidence in identifying shrew scat was sufficiently high that we included the scat tubes in our technique comparison tests.

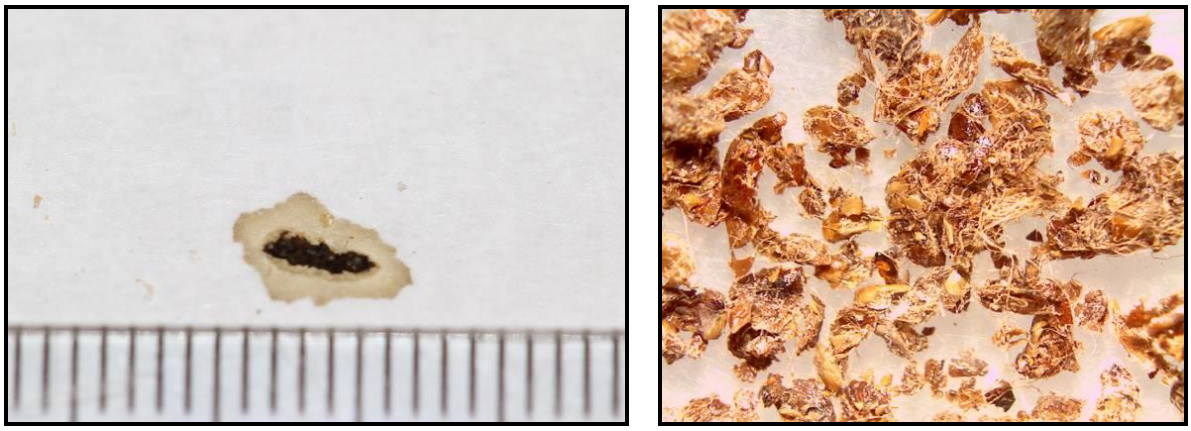

Figure 9. Known Buena Vista Lake shrew scat (left, scale increments are mm), and dissecting scope view of the contents of a shrew scat (right).

Automated camera stations

Cameras proved to be a very effective technique for detecting shrews, although detection efficacy varies among camera models. Although most cameras will capture images of small mammals, those with close-focus capability markedly facilitated the identification of shrews versus other small mammals (Figure 10). Cameras were also very effective because in the San Joaquin Valley area, only one species of shrews is found. Thus, we were highly confident that the close-focus cameras would be effective in detecting BVLS.

Figure 10. Images of Buena Vista Lake shrews taken with a regular Reconyx camera (left) and a close-focus Reconyx camera (right).

Detection technique comparison tests

During a detection technique comparison tests, the efficacy of three non-invasive detection techniques was evaluated: scat tubes, automated camera stations, and live-traps. During the tests, only one BVLS was captured at NSRER and none were captured at WWP and Kern NWR (Table 1). Shrew scats were detected at 1-6 scat tubes at the sites and shrews were detected on 7-9 cameras (Table 2). Two cameras at Kern NWR and one camera at NSRER malfunctioned due to improper factory settings. Excluding these cameras from analyses and summing results across all 3 sites, the proportion of stations with BVLS detections varied among techniques (χ^2 = 43.30, 2 df, $P < 0.0001$). The proportion of stations with detections (Table 2) was higher for cameras compared to traps (χ^2 = 42.24, 1 df, $P < 0.0001$) or scat tubes (χ^2 = 16.35, 1 df, $P < 0.0001$), the proportion for scat tubes was higher compared to traps (χ^2 = 10.42, 1 df, $P = 0.0012$). Also, the proportion of nights with detections (Table 2) was higher for cameras compared to traps (χ^2 = 63.68, 1 df, $P < 0.0001$). Of the 24 stations where BVLS were detected by cameras, shrews were first detected on the first night at 21 stations (87.5%) and on the second night at the remaining 3 stations (12.5%). Also, of the 24 stations where BVLS were detected by cameras, shrews entered scat tubes at 10 of these stations a total of 27 times, based on camera images, but scats were only found in 3 tubes. At these same 24 stations, shrews entered live-traps a total of 62 times, but only 1 shrew was captured.

SURVEYS

We conducted surveys in 16 general population areas (Table 2, Figure 11). Most of the live-trapping surveys were conducted during May-October 2014. Most of the camera station surveys were conducted during October 2016-March 2017. Live-trapping only was conducted in 5 areas, camera stations only in 3 areas, and both techniques were used in 8 areas. In some areas, more than one live-trapping session was conducted either to sample more sites within larger areas (e.g., Kern NWR), or because a considerable amount of seemingly suitable habitat was present but shrews were not captured during the first trapping session (e.g., City of Bakersfield Recharge area, Buena Vista Recreation Area).

Table 1. Results of comparisons of techniques for detecting Buena Vista Lake shrews at three sites in the San Joaquin Valley, California.

		Detections/station (%)			Detections/night (%)	
Site[1]	Dates	Traps	Scat tubes	Cameras	Traps	Cameras
WWP	10/25-27/16	0/10 (0%)	6/10 (60%)	9/10 (90%)	0/20 (0%)	17 (85%)
KNWR	10/17-20/16	1/10 (10%)	1/10 (10%)	8/8[2] (100%)	1/30 (3.3%)	20/24[2] (83.3%)
NSRER	10/31-11/3/16	0/10 (0%)	4/10 (40%)	7/9[3] (77.8%)	0/30 (0%)	9/27[3] (33.3%)
Totals	-	1/30 (3.3%)	11/30 (36.7%)	24/27 (88.9%)	1/70 (1.4%)	46/71 (64.8%)

[1] WWP = Wind Wolves Preserve; KNWR = Kern National Wildlife Refuge; NSRER = Northern Semitropic Ridge Ecological Reserve

[2] Two cameras malfunctioned resulting in 8 operable stations and 24 survey nights.

[3] One camera malfunctioned resulting in 9 operable stations and 27 survey nights.

BVLS were detected in 7 population areas (Table 2, Figure 11). Shrews were detected in 3 areas where only live-trapping was conducted, and one area where only camera stations were used. Shrews were detected in 3 areas where both techniques were used; in one of these areas shrews were only detected by live-traps, and in the other 2 areas they were only detected by cameras. Shrews were detected in multiple locations (>500 m apart) at the Kern NWR, Atwell Island area, Goose Lake Canal area, and WWP.

Although information on habitat attributes was recorded at most survey locations, these attributes were not rigorously quantified in this study, and therefore only some broad generalizations can be made regarding conditions at sites were shrews were detected. Tree canopy cover and shrubs were present at some sites but not all. Litter also was present at some sites but not others, and where present was not always deep. However, ground cover usually was very dense in sites where shrews were detected. Commonly, this cover consisted of rushes, sedges, or cattails, or some combination of these, occasionally with other wetland plant species present as well. Also, the soil in areas where shrews were detected tended to be moist (i.e., wet to the touch).

Table 2. Areas, dates, methods, and results for Buena Vista Lake shrew surveys conducted in the southern San Joaquin Valley, California during 2014-17. Multiple sites were surveyed in some population areas (see Figure 11 for population areas).

Area	Dates	Method	Trap nights or camera nights	BVLS detected
Wind Wolves Preserve	Oct 20-24, 2014	80 livetraps	320	Yes
Tejon Ranch	Jun 10-14, 2014	50 livetraps	200	No
	Mar 7-10, 2014	8 cameras	24	No
Buena Vista Recreation Area	May 13-16, 2014	36 livetraps	144	No
	Oct 6-10, 2014	40 livetraps	118	No
	Jan 24-27, 2014	10 cameras	30	No
Coles Levee Pond	Nov 7-10, 2016	10 livetraps	40	No
		10 cameras	40	No
Bakersfield City Recharge Area	May 19-22, 2014	79 livetraps	237	No
	Jun 17-20, 2014	57 livetraps	171	Yes
	Jan 24-27, 2014	5 cameras	10	No
Tule Elk Reserve	Nov 29-Dec 2, 2016	4 cameras	12	No
Panorama Vista Preserve	May 6-9, 2014	65 livetraps	190	No
	Jan 31-Feb 3, 2017	6 cameras	18	No
Hart Park	Oct 27-31, 2014	40 livetraps	160	No
	Jan 31-Feb 3, 2017	4 cameras	12	No
Kern River Overflow Canal at Semtropic Water Storage Canal Crossing	Mar 10-13, 2015	30 livetraps	120	No
	Mar 28-Apr 3, 2017	1 camera	6	Yes
Tumblin Lake	Mar 10-13, 2015	30-40 livetraps	150	No
Semitropic Ecological Reserve at the Semitropic Water District Overflow area	Mar 17-19, 2015	25-40 livetraps	99	No No
Kern River Overflow Canal at Interstate 5 and Hwy 46 (Goose Lake Canal population area)	Apr 8-10, 2014	30-40 livetraps	110	Yes
Semitropic Ecological Reserve at Goose Lake Canal	Oct 28-31, 2014	31-46 livetraps	169	Yes
Semitropic Ecological Reserve at Poso Creek Channel (Kern NWR population area)	Mar 19-21, 2014	30-41 livetraps	111	No
	Feb 27-Mar 3, 2017	6 cameras	21	No
Kern National Wildlife Refuge	Apr15-17, 2014	32-40 livetraps	108	Yes
Lake Woollomes	Dec 5-9, 2016	2 cameras	8	No
Atwell Island Wetland and surrounding ditches	Apr 23-25, 2016	35-45 livetraps	125	No
	Dec 5-9, 2016	2 cameras	8	Yes
	Mar 8-15, 2017	6 cameras	7	Yes
Pixley National Wildlife Refuge	Dec19-23, 2016	6 cameras	24	Yes

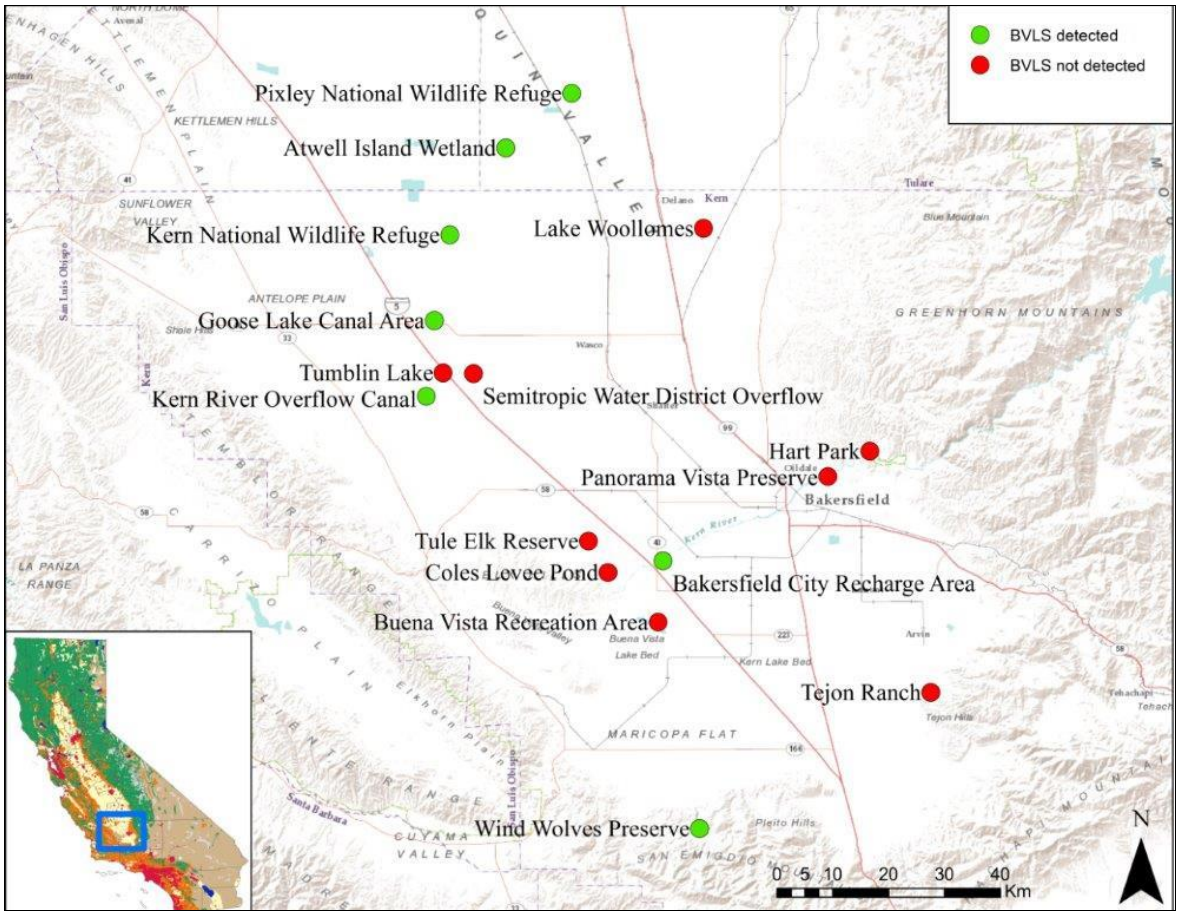

Figure 11. Population areas (n = 16) surveyed for Buena Vista Lake shrews in the southern San Joaquin Valley, California during 2014-17.

DISCUSSION

TAXONOMIC ANALYSIS

The implications of the taxonomic analysis conducted as part of this project are described in detail in Appendix B. Two significant findings particularly relevant to the conservation and recovery of BVLS are that (1) certain microsatellite alleles and mtDNA haplotypes appear unique to shrews in the southern part of the San Joaquin Valley, particularly the Kern Lake area, and (2) genetic connectivity and admixture between shrews in the northern and southern part of the San Joaquin Valley appear to be higher than indicated by previous genetic results. These findings indicate the BVLS may have a larger range than originally estimated. If so, then this could expand the number of BVLS populations as well as the amount of remaining habitat. Additional populations and habitat could reduce the probability of extinction for this taxon. However, despite potentially being more abundant, widespread, and less imperiled, it appears that continued preservation of the southern populations may be important to maintaining range-wide genetic diversity.

NON-INVASIVE DETECTION TECHNIQUES

In our experience, track tubes were not an optimal technique for detecting shrews. Although shrews appeared to readily enter the tubes, obtaining clear tracks that could positively be identified proved difficult. A different track tube design potentially might be more effective or individuals with greater expertise might be able to more consistently and reliably identify shrew tracks. Others researchers have had difficulty identifying shrew tracks in track tubes (e.g., Connolly-Newman 2013), whereas yet others have reported greater success in identifying shrew tracks (e.g., Glennon et al. 2002, Wiewel et al. 2007). However, due to the combination of considerable labor and time necessary to construct the tubes, the difficulty in managing the felt ink pads (e.g., messy, dry out quickly), and the difficulty in identifying shrew tracks, we chose not to continue pursuing development of track tubes for detecting BVLS.

Scat tubes proved to be a bit more effective in detecting shrews. The scat tubes were easier to construct and deploy compared to track tubes, primarily because of not having to construct or manage the felt ink pads. Also, the tubes were inexpensive to construct with the cost of materials for each scat tube being less than $5. As with the track tubes, shrews appeared to readily enter the tubes and commonly left scats. However, although we felt confident in our identification of some scats as being those of shrews or other species, there still was a substantial proportion of scats that we could not confidently ascribe to shrews versus another species. Also, as indicated by camera images, shrews frequently entered the tubes without depositing scats. Thus, this technique also had a relatively high potential to not detect shrews that were present resulting in false-negative data.

The close-focus cameras proved to be extremely effective in detecting shrews. The camera stations were relatively easy to set up, although some care must be taken in ensuring that the cameras are correctly pointed at the bait container. The species present could not always be identified in some of the images. However, each time an animal visited a bait station, the cameras typically obtained multiple images, which markedly enhanced the opportunity to reliably distinguish shrews from other species. In our estimation, no visits by shrews were missed by the cameras due to an inability to identify the visitor, as opposed to the track and scat tubes. The rate at which cameras missed capturing an image of a shrew visiting a bait station is unknown. However, given that multiple images of shrews typically were captured by each camera, the potential for false-negatives at a given camera station probably is low, particularly if camera stations are operated for multiple nights. Also, given that shrews were detected on multiple cameras at each of the 3 test sites (78-100%), the probability that shrews would not be detected at a site where they are present is low.

Thus, cameras were extremely reliable in detecting shrews. Again, one of the primary reasons cameras work so well is because only one species of shrew is found in the San Joaquin Desert area. The principal drawback to this technique is the cost of the cameras. Currently, the only commercially available close-focus cameras we know of are those made by Reconyx, and each camera costs over $500. For most project budgets, this restricts the number of cameras that can be deployed. In an area where shrew distribution is patchy, a limited number of cameras could result in shrews not being detected in that area. An alternative is to construct “home brewed” units using less expensive non-field cameras and altering their electronics to essentially function similar to field cameras. Various websites provide directions for building such units. However, one drawback of

"home brewed" units is that they often require quite a bit of troubleshooting to operate effectively and reliably the majority of the time (K. Hickman, personal communication).

We felt that the attraction system we used was highly effective. Clearly, shrews were attracted to both the Tupperware containers and the tea infuser balls. Both provided scent and sound as an attractant for shrews, which was our intent as shrews may use both olfactory and auditory cues to find food (Pernetta 1977, Churchfield 1980). The dried mealworms provided a food reward. Given the high metabolic rates of shrews, we felt that such a reward was important because shrews were being distracted from their normal foraging patterns to come investigate the bait stations.

In our comparison tests of techniques, detection rates with cameras markedly exceeded those of the scat tubes or live traps. At most of the stations, cameras successfully detected shrews. Also, the cameras captured numerous images of shrews entering and exiting scat tubes and live-traps, but shrew scats were not detected in most of these tubes and only one shrew was captured. Thus, in our tests, cameras were significantly better at detecting shrews compared to other techniques.

Although we took care to set live-traps appropriately, clearly there were problems with traps malfunctioning and not capturing shrews. One issue we noticed was that mealworms or bedding would get under the treadle and prevent it from being depressed. This problem might be avoided with the use of different bait (e.g., dried mealworms) or bedding, or even different styles of traps. Smith et al. (2017) compared the efficacy of Longworth, medium Sherman, and small Sherman traps (like we used in this project) to capture shrews and found that capture rates were highest with the Longworth traps and lowest with the small Sherman traps. Shore et al. (1995) employed "treadle ramps" in Longworth traps and reported that this helped keep shrews and bait from fouling treadles. However, regardless of trap efficacy, there is still significant risk associated with live-trapping shrews. For example, trap mortality rates as high as 93% (Shonfield et al. 2013), 90% (Getz 1961), and 68% (Greenberg et al. 2007) have been reported for *Sorex* species, and Smith et al. (2017) reported a 30% mortality rate despite employing measures to prevent mortalities. We had one shrew die in a live-trap during this project, likely due to cold expose, and another shrew was killed by ants while in a trap during another project. Despite these low mortality rates, avoiding any deaths, particularly of a listed species, is desirable. Also, live-trapping is labor intensive and permits from the U.S. Fish and Wildlife Service and the California Department of Fish and Wildlife are necessary for live-trapping BVLS because of their protected status. Thus, if the goal is simply detection, then cameras are a better option than live-traps.

Although cameras clearly are the better technique for detecting shrews, use of live-traps might be necessary for projects in which genetic samples or capture of live individuals are needed (e.g., abundance estimation, ecological studies). In these situations, time and effort might be optimized by operating camera stations first, and then deploying multiple traps just at specific locations where shrews are detected.

If genetic samples are desired from an area but individual shrews otherwise do not need to be handled, then scat tubes might be used instead of live-traps. The tubes are less risky for shrews compared to live-traps because animals can enter and exit at will and are not confined in the tubes. Also, the tubes are less labor-intensive to operate. The Smithsonian Conservation Biology Institute was able to successfully extract BVLS DNA from known and putative shrew scats collected during this project. Scat tubes potentially could be

designed to increase efficacy as well. Different colored tubes might be more attractive to shrews. Also, longer tubes or even possibly some small obstacles in the tubes might increase the time that shrews remain in the devices, which would increase the potential that they might defecate while in the tubes. Another modification that might be helpful is a smaller diameter entrance to the tubes. This might discourage entry by deer mice and other small mammals, and this would both reduce the amount of non-shrew scat in tubes thereby increasing the probability that scats found in tubes actually were from shrews. In particular, use of scat tubes might be desirable for projects where the objective is shrew detection or genetic samples, but project budgets preclude the acquisition of close-focus cameras or the extensive labor required for live-trapping.

SURVEYS

We were only able to conduct surveys for BVLS in a limited number of areas (16) where access was granted. Additional areas may have suitable habitat but occur on private lands where we were unable to gain access. Thus, BVLS may occur in areas in addition to the 7 where we detected them during our surveys. Prior to our project, BVLS surveys were conducted previously in 8 of the 16 areas (Maldonado et al. 2001, Williams and Harpster 2001, ESRP unpublished data). BVLS were not detected at Lake Woollomes, the Tule Elk Reserve, or the Buena Vista Recreation Area in either past or current surveys. BVLS were not detected at the Coles Levee Pond in the current survey but were detected there in past surveys. BVLS were detected at Kern NWR, the Goose Lake Canal Area, Bakersfield City Recharge Area. and WWP in both previous and current surveys. Of the 8 areas not previously surveyed, BVLS were detected at 3: Pixley NWR, Atwell Island Wetland, and the Kern River Overflow Canal.

Of the 7 areas where BVLS were detected, all or portions of 5 areas have some sort of protected status. Pixley NWR and Kern NWR obviously are refuges managed by the U.S. Fish and Wildlife Service. BVLS occur in wetland and riparian habitats on these refuges. Management activities are conducted in these habitats primarily to benefit waterfowl. A designated critical habitat unit (USFWS 2013) for BVLS occurs on Kern NWR, although shrews also were detected in a number of other locations on the refuge outside of this unit. The Atwell Island Wetland is in an area managed by the U.S. Bureau of Land Management (BLM). Management at this site is for wetland communities in general. BVLS were detected in 2 large wetland and riparian areas at WWP. These 2 locations are the least impacted of all the areas where BVLS were detected and are kept in a mostly natural state (e.g., no water level manipulation). The preserve is owned and managed by The Wildlands Conservancy for conservation values, although much of the preserve is not under formal conservation easement. BVLS were detected in 2 sites in the Goose Lake Canal Area. One site is within a designated critical habitat unit that is largely within the NSRER managed by CDFW. Shrews were detected along canals in this area that are managed by the Semitropic Water Storage District. The other site is along a channelized slough that is owned and managed by the Semitropic Water Storage District, Buena Vista Water Storage District, or the Boswell Corporation (T. Ashlock, personal communication). This site has no specific protections for biological resources. BVLS were detected approximately 7 km further south along this same feature in an area designated as the Kern River Overflow Canal. This site is managed by the Buena Vista Water Storage District and also has no protections for biological resources.

The final area where BVLS were detected was the Bakersfield City Recharge area, but in a location much different than where shrews were detected previously in 2000 (Williams and Harpster 2001). The previous location is completely dry in most years and is unlikely to support shrews. In the current survey, we found shrews in a thin strip of riparian habitat that extends for approximately 650 m along a canal that is owned and operated by the City of Bakersfield. The canal is on the southwest edge of the City's water recharge area. Portions of the recharge area had been proposed as critical habitat for BVLS, but were not designated because the City developed a conservation plan for BVLS (USFWS 2013). However, the canal where we detected BVLS is not covered by this plan.

None of the areas where BVLS were detected are being managed specifically for BVLS. As mentioned previously, the locations with BVLS on WWP are in a mostly natural state and are subject to primarily natural processes. The San Emigdio Creek flows through the areas and flows are dictated by natural conditions as there are no impediments or diversions upstream from the wetland and riparian areas. Consequently, water is consistently available to this site although volumes vary with season. The Atwell Island site is managed for general wetland biological communities. Ground water is pumped into the main ponds of the wetland with some funding help from the Natural Resource Conservation Service. As long as pumps are functioning, water in the pond is present throughout the year and little or no habitat management occurs to the ponds themselves. In 2015, pumps malfunctioned and the ponds went dry for an extended period before the pumps could be fixed. Water in the surrounding ditches and canals adjacent or connecting to the wetland complex occasionally have water due to floodwaters in winter or farming needs. BLM is actively searching for opportunities to identify a more consistent source of water for the wetland complex that does not require pumping groundwater (R. Brooke, personal communication).

The habitats with BVLS at Kern NWR and Pixley NWR are primarily managed to benefit waterfowl. Water at Kern NWR is from surface allocations distributed each year to wildlife refuges in the Pacific flyway based on availability. These allocations are delivered from Lake Shasta through the California Aqueduct and canals and ditches managed by Semitropic Water Storage District (N. Stanley, U.S. Fish and Wildlife Service, personal communication). Pixley NWR, on the other hand, receives no surface water allocation. Groundwater pumping is used to fill the ponds at the Pixley wetland. The only exception to this is when Deer Creek, which borders the refuge, has flooding events, which happened during the winter of 2017.

For both Kern NWR and Pixley NWR, in typical years water is placed in the wetland pond complexes in the fall and winter and then dries up in the late spring and summer. However, areas with moist soils do persist in most years in some areas at both refuges. To prevent vegetation from choking the ponds, some areas are of the refuge are mowed or even disked in summer, typically after they dry out.

In the Goose Lake Canal Area, the canals through CDFW lands convey water on a seasonal basis (mostly in the fall and winter), primarily to deliver water to Kern NWR. Some small areas with moist soils persist during the periods when water is not being conveyed. Clearing of vegetation typically occurs when the canals are dry. In the channelized slough portion of the Kern River Overflow Canal, flowing water generally is not present except in rare years with extremely high precipitation levels. However, portions of this feature remain wet year-round due to seepage from adjacent canals or local ground water drainage patterns. Water is present much of the year in the City of

Bakersfield canal. However, occasionally the flow is stopped and the city conducts maintenance including vegetation clearing in the canal, although the vegetation up on the banks is usually not disturbed.

The effects of management activities on BVLS warrant investigation. Such activities could potentially impact local shrew populations, particularly if shrews are drawn into sites and then habitat conditions are rapidly altered by management actions.

Other than the WWP area, all of the other sites where BVLS were detected have been substantially anthropogenically altered. Wetlands or natural sloughs likely were present at one time in all of the areas. The Atwell Island site is of interest because it constitutes created habitat. This wetland was created in 2009 by the BLM and NRCS as a demonstration project and to provide habitat for wetland species. The origin and dispersal routes of shrews that colonized this site is unknown, although the site is connected to a network of canals and ditches that are used for farm water and flood control. During this project, BVLS were detected in a connecting ditch to the wetland complex approximately 2.5 kilometers away. BVLS also were found opportunistically at a residence approximately 1.5 km north of the wetland prior to this project. The previous owner of the property also maintained a duck club near the created wetland complex, although this club likely only had water during the fall and winter. Regardless of the means of colonization, the presence of BVLS at the Atwell Island site indicates that it may be possible to create additional habitat for this species.

HABITAT ATTRIBUTES

BVLS were reported to occur in marshes, wetlands, sloughs, lake edges, and riparian areas throughout the southern San Joaquin Valley (Grinnell 1933; Maldonado 1992; USFWS 1998, 2011). BVLS also occasionally have been captured in more upland habitats including fallow agricultural lands (USFWS 2011). However, optimal or preferred habitat attributes for BVLS have not been determined, both with regards to habitat types or microhabitat site conditions.

The sites where we detected shrews included riparian areas, marshes and wetlands, sloughs, and canal banks. Common plant species occurring in these areas included Fremont cottonwood, willows, mulefat (*Baccharis salicifolia*), rushes, alkali heath (*Frankenia salina*), wild rye (*Elymus* spp.), saltgrass (*Distichlis* spp.), stinging nettle (*Urtica dioica*), and cattails. Invasive tamarisk (*Tamarix* spp.) sometimes were present as well. Regardless of plant species composition, shrews appear to favor areas with abundant cover in the form of dense herbaceous vegetation or deep litter. We detected shrews most frequently in dense patches of rushes and sedges (Figure 12). BVLS also were detected in stands of cattails, particularly where there was a deep layer of old cattail stems on the ground (Figure 12). Less frequently, BVLS were detected in areas with deep leaf litter under cottonwood or willow trees (Figure 12). At one site, shrews were detected in dense vegetation along a canal (Figure 12). Moist soil was present at each of the sites where shrews were detected, and in many cases, the detection sites were within 1-2 m of standing water, but standing water does not appear to be a requirement.

A complicating factor in identifying preferred habitat attributes for BVLS is that the habitat conditions in which shrews are detected may vary temporally due to seasonal, annual, and management-related fluctuations in moisture. Shrews generally seem to occur in areas with dense ground cover vegetation and moist soils. During the wet season in the

San Joaquin Valley, which generally is from about December to March or April, areas with moist soil and dense vegetation can expand significantly, depending upon the timing and quantity of precipitation. Shrews may move into these new areas seeking resources, new home ranges, mates, dispersal opportunities, or for other unknown reasons. Consequently, they may be detected in these areas at certain times of the year. However, these areas are not permanently occupied because after the wet season they begin to dry out and eventually become unsuitable for shrews. If shrews are able to persist long enough to be detected during this drying phase, it could result in an inaccurate assessment of appropriate habitat conditions for shrews.

Similar to seasonal fluctuations, moisture levels and habitat suitability can vary with management activities. At Pixley NWR, Kern NWR, certain portions of the Goose Lake area, and the City of Bakersfield Canal site, water is pumped into these areas at particular times of year to meet specific management objectives that are unrelated to shrew conservation.

Finally, the presence of shrews in certain areas likely varies due to fluctuations in annual precipitation, which can be marked in the San Joaquin Valley. Shrews may be able to occupy certain areas in wetter years, but those same areas may be completely unsuitable in drier years. Our survey efforts primarily were conducted during a period of relatively dry conditions, and some locations where shrews had been detected in past projects were found to be currently unsuitable for shrews. Any retrospective analysis of the habitat in these locations during the drier years again would result in an inaccurate assessment of appropriate habitat conditions for shrews.

As described above, habitat conditions for shrews can be temporally and spatially dynamic due to seasonal, annual, or anthropogenic variation in moisture availability. Some areas appear to at least retain moist soils, if not standing water, on a year-round basis in most years. Such areas likely constitute "refugia" for BVLS. As suitable habitat conditions expand in seasons or years with more moisture or due to anthropogenic activities, BVLS appear to expand into these temporally suitable areas. As these areas dry, shrews either retreat back to refugia or eventually die out. If the latter, then these temporally suitable areas are in effect functioning as population sinks for BVLS.

Figure 12. Habitats where Buena Vista Lake shrews were detected during surveys in the southern San Joaquin Valley, California during 2014-17. Habitats included rushes (upper left), cattails (upper right), litter under willows (lower left), and along canals (lower right).

CONCLUSIONS

The results of the genetic analysis of shrews sampled in the San Joaquin Valley are discussed more thoroughly in the report included as Appendix B. In brief, the analysis indicated that there is greater genetic connectivity and admixture between shrew populations in the northern and southern portions of the Valley than previously thought. Further investigation should be conducted to clarify patterns of gene-flow and genetic structure within this region of the San Joaquin Valley because this will have significant implications for BVLS conservation and recovery. The analysis also indicated that while some of the small populations retain moderate levels of genetic diversity, the southern San Joaquin Valley shrew populations retain unique alleles and suggests that conservation of these shrews is important to maintaining population-wide genetic diversity.

Our investigation of non-invasive survey methods clearly demonstrated that the field cameras, particularly those with close-focus capability, were highly effective in detecting shrews in a manner that presents minimal risk to the animals. Compared to the traditional survey method of live-trapping, cameras are not only safer but also less labor-intensive. The primary drawback is the initial cost of the cameras. Track tubes and scat tubes were less expensive than cameras and less labor-intensive than traps, but also were less reliable due to difficulty in identifying shrew tracks and scats. The scat tubes might be used to obtain genetic samples from shrews in areas where cameras have confirmed presence.

We detected shrews in just 7 areas in the southern San Joaquin Valley. Some of the areas were quite small, connectivity is poor or non-existent between most areas, none of the areas are managed specifically for shrews, some of the areas have no permanent protections, most of the areas are altered to some degree, and habitat conditions in several areas vary markedly over short time periods due to management activities. The above substantially reduces the probability of shrew persistence in individual areas as well as range-wide persistence. The lack of connectivity between most of the areas significantly reduces the probability of recolonization if shrews become extirpated in a given area. Of the areas we were able to survey, the Wind Wolves Preserve and Kern NWR probably support the largest and most robust BVLS populations. These two areas are relatively large with a diversity of habitat conditions, and shrews were detected at multiple locations within these areas. A few of the areas where we did not detect shrews appeared to have appropriate habitat conditions, are not subject to routine disturbance, and are protected to at least some extent. These sites included the Panorama Vista Preserve, Tejon Ranch, Buena Vista Recreation Area, and the Coles Levee Pond. Shrews might have been present previously in these areas but then extirpated by some event. These areas might be candidates for introductions of shrews.

Finally, we caught shrews in a variety of habitats. Although preferred habitat types and microhabitat conditions still are poorly defined, based on our surveys BVLS were most commonly detected in areas with moist soils and dense cover primarily consisting of rushes, sedges, or cattails. Further investigation of optimal habitat conditions for BVLS is warranted. Of some encouragement is the detection of shrews at the Atwell Island Wetland. The presence of BVLS at this site indicates that habitat areas might be created for this species. The primary challenge to habitat creation is securing a reliable source of water to keep the area wet year-round.

RECOMMENDATIONS

Based on the results of this project, the following recommendations are offered for BVLS conservation.

1. ADDITIONAL SURVEYS

Additional surveys for BVLS should be conducted as opportunities become available. In particular, surveys should be conducted on any lands with potential habitat that were not surveyed during our project. In particular, we were not able to access sites in the Kern Lake and Goose Lake areas where BVLS have been detected in previous years. Suitable habitat still appears to be present in these areas. Also, there are many private duck clubs in the Northern Semitropic Ridge Ecological Reserve area that may have potential BVLS habitat. Upon initial assessment, we thought that many of these sites likely did not have enough suitable habitat to warrant BVLS surveys. However, the finding of shrews at Pixley NWR, which is managed very similar to a duck club, indicates that BVLS might occur on some duck clubs as well. Although on private property, many duck clubs in the Tulare Basin have had conservation easements placed on them by USFWS and may be willing to allowing surveys on their property. However, it would be advantageous to develop some strategies for protecting landowners who may worry about potential Endangered Species Act regulatory impacts, such as exploring potential Safe Harbor

Agreements options. It also may be worthwhile repeating surveys in areas that we surveyed but did not detect shrews. In a recent survey that was not part of this project, shrews were not detected during an initial 7-day camera session, but were detected during a subsequent 7-day survey conducted approximately 6 weeks later at the same site (CSUS ESRP unpublished data).

2. USE OF CAMERAS

Use of cameras is strongly encouraged in any future surveys for BVLS because detection rates are so much higher with this technique versus other techniques. We highly recommend the use of close-focus cameras to more accurately identify any shrews coming to camera stations.

3. HABITAT PROTECTION

Sites where BVLS currently are known to be present should be protected. Some sites already have some protections, and others, particularly on private lands, might be protected through acquisition or conservation easements.

4. HABITAT MANAGEMENT

On sites where BVLS are present, consideration should be given to managing habitat in manner that is beneficial for shrews. Such practices might include slowly lowering or raising water levels, attempting to keep soils moist even if standing water is not present, and not clearing or disturbing beneficial vegetation, particularly dense patches of rushes or cattails.

5. RESPONSE TO TEMPORAL VARIATION

The response by shrews to temporal variation in habitat conditions should be investigated. Such investigation should include variation due to both natural (e.g., seasonal and annual precipitation) and anthropogenic (e.g., management actions, disturbance) sources.

6. HABITAT PREFERENCES

Investigations should be conducted to define optimal habitat conditions for BVLS. This should include both habitat type (e.g., riparian areas, marshes, sloughs, canals) and microhabitat conditions. Variables that should be included in any such investigation include vegetation composition, vegetation structure and density, litter type and depth, soil moisture, distance to standing water, and invertebrate abundance.

7. HABITAT ENHANCEMENT AND CREATION

Additional wetland enhancement, restoration or creation should be conducted and colonization of such areas by BVLS should be encouraged and monitored. The Goose Lake Canal area, where BVLS were detected, would be an excellent place to enhance habitat connections to the Kern NWR population. The Goose Lake Canal, which delivers water to the Kern NWR in the fall and winter, is currently being managed only for surface water delivery and is devoid of any substantial vegetation. Creating habitat along the canal would likely provide amble opportunity for shrews to colonize and expand their population in this area. Several parcels along or adjacent to the canal are managed by CDFW as part

of the Northern Semitropic Ridge Ecological Reserve and may provide opportunities for habitat enhancement beyond the canal banks. The Kern River Overflow Canal, which is part of the Goose Lake Canal area, also provides similar opportunities. The obstacle to habitat creation would be determining how the water districts manage the canal beds and banks for water flow and developing optimal strategies for water and vegetation management. Another area that has great potential for enhancement is the habitat near Pixley NWR and the Atwell Island wetland complex. There are several creek beds, ditches and canals in these areas that, if restored, could provide opportunities to connect shrew populations.

8. TRANSLOCATIONS TO SUITABLE, UNOCCUPIED HABITAT

Translocation of BVLS to suitable but apparently unoccupied sites should be investigated. Such sites could include protected areas with existing habitat (e.g., Panorama Vista Preserve, Tejon Ranch, Buena Vista Recreation Area) or areas with restored/created habitat. Some regulatory protection, such as a Safe Harbor Agreement, could enhance the willingness of landowners to host introduced BVLS populations.

9. DEMOGRAPHICS AND ECOLOGY

Investigations should be conducted to define BLVS demographics and ecology. Topics of particular importance due to a lack of data include survival rates, sources of mortality, reproductive attributes, food preferences, space use, and dispersal distances. Data on these characteristics would enhance the preparation of conservation strategies for BVLS.

10. NORTHERN VALLEY SHREWS

Additional genetic analysis should be conducted on shrews in the northern portion of the San Joaquin Valley to determine whether they align more closely with BVLS or with more common subspecies of ornate shrews. If they align more closely with BVLS, then additional surveys and habitat protection in the northern valley may be warranted.

11. INDIVIDUAL IDENTIFICATION VIA GENETICS

Additional investigation should be conducted on genetically identifying individual shrews. This would facilitate population studies such as estimating abundance as well as genetic exchange between areas.

12. GENETIC SAMPLES

Preliminary genetic analysis suggest that DNA can be extracted and amplified from shrew fecal samples (see genetics report in Appendix B). This suggests that the use of scat tubes to collect genetic samples from shrews is of great potential for increasing sample sizes for refining genetic analysis and warrants further investigation. In particular, the tube design might be modified to make them more inviting to shrews such that they are more likely to enter and stay longer period of time and increasing the potential for scat deposition.

13. OUTREACH

Unlike larger, more visible, and more charismatic species, BVLS are virtually unknown to the public. Outreach could increase awareness of BVLS and the threats to their continued

existence, and potentially increase public support for their conservation. A major challenge is that many water districts have demonized BVLS because it is a species that might impact water deliveries to agriculture. Thus, outreach and possible solutions, like Safe Harbor Agreements, are needed.

14. CAPTIVE BREEDING

The efficacy of captive breeding should be investigated for BVLS. Captive bred shrews could be used for introduction trials to unoccupied or created habitat. Use of captive bred animals would negate the need to capture and remove individuals from natural populations. Stock for captive breeding colonies could be obtained from areas where habitat is being converted, areas that appear to function as population sinks in wet years, or robust natural populations such as those at WWP or Kern NWR. Captive propagation has been successfully conducted with other species of *Sorex* type shrews such as the common shrew (*S. araneus*; Searle 1984) and desert shrew (*Notiosorex crawfordi*; Punzo 2003).

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APPENDIX A. FORM USED TO ASSESS HABITAT ATTRIBUTES ON SITES SURVEYED FOR BUENA VISTA LAKE SHREWS.

Buena Vista Lake Shrew Surveys
Site Assessment

General location: _____

Specific camera site (GPS coordinates): _____

Dates camera set: _____ **Pictures of site:** Y N

Tree canopy

Present: Yes No

Extent: (if present)
_____ Tree cover over most of site
_____ Intermittent tree cover
_____ Only occasional tree

Species:
_____ Willows
_____ Cottonwood
_____ Tamarisk
_____ Other

Litter cover

Density:
_____ Shallow (up to 4")
_____ Medium (4-8")
_____ Deep (>8")

Shrubs (woody plants >1 meter tall)

Present: Yes No

Density: (if present)
_____ Sparse (just occasional shrub)
_____ Medium (patches of shrubs)
_____ Dense (fairly continuous)

Species (check if more than just 1 or 2 are present on site; put a "D" by the dominants):

_____ Mule fat
_____ Elderberry
_____ Tamarisk
_____
_____
_____
_____ Other _____(***or collect a sample or take pictures***)

Ground cover

Density: _____ Sparse (>30% bare ground)
_____ Medium (10-30% bare ground)
_____ Dense (<10% bare ground)

Species (check all that appear abundant at the site):

_____ salt grass _____ _____
_____ other grass _____ _____
_____ rushes/sedges _____ _____
_____ cattails _____
_____ bulrush (tules) _____
_____ mugwort _____

(For abundant "unknowns", collect samples and/or take pictures)

Proximity attributes:

Distance to open water (m): _____

Moist soil present: Y N ("yes" if feels wet to the touch)

Disturbances within 10 m of camera station (check all that apply):

_____ road
_____ disking
_____ clearing or scraping
_____ crops

SHREWS DETECTED? Y N

APPENDIX B. LEVELS OF GENETIC STRUCTURE OF ORNATE SHREWS IN THE SAN JOAQUIN VALLEY AND SURROUNDING LOCALITIES BASED ON ADDITIONAL SAMPLING FROM INTENSIVE SURVEY EFFORTS

LEVELS OF GENETIC STRUCTURE OF ORNATE SHREWS IN THE SAN JOAQUIN VALLEY AND SURROUNDING LOCALITIES BASED ON ADDITIONAL SAMPLING FROM INTENSIVE SURVEY EFFORTS

Buena Vista Lake Shrew captured in the Kern Lake Preserve by JE Maldonado
Photo of by Moose Peterson

Prepared by:
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Center for Conservation Genomics
Smithsonian Conservation Biology Institute
National Zoological Park

June 15, 2017

OBJECTIVES

The overall objective of this research project is to determine the levels of genetic variability in subpopulations throughout the southern San Joaquin Valley (SJV) and use additional samples from outside the SJV to determine levels of genetic structure within SJV and also between populations that occur in the surrounding areas in the Sierra Nevada Mts., the Salinas Valley and the Tehachapi Mts. It is thought that ornate shrew populations in the SJV have been separated from each other due to loss of habitat (primarily through agricultural land conversion). The results of this study with additional samples from localities that were previously poorly sampled will help us complement the results of previous genetic surveys that were conducted in an attempt to determine the patterns of genetic variability and structure within and among the geographic subpopulations of Buena Vista lake shrews (*Sorex ornatus relictus*; Maldonado 2006, Maldonado and Dutta 2014*)*. While previous results showed signatures of genetic structure in the SJV, the relative degree of genetic differentiation was questioned because representatives from other subspecies were not included and also because of the small sample sizes in some of the surveyed localities. Therefore, in this report, we continue to address those concerns by screening additional *Sorex ornatus* samples that were collected during intensive live trapping surveys from May-October 2014. This study incorporates 12 new samples from previously sampled localities that yielded small sample sizes and reevaluates the results of previous analyses conducted by Maldonado and Dutta 2014. Sampling was previously obtained from surrounding areas of the SJV and also by including populations of ornate shrews from the Southern and Northern Clades from a previous phylogeograpic study of ornate shrews (Maldonado et al 2001) and using the same microsatellite loci designed specifically for *Sorex ornatus* (Maldonado et al 2006). This was done in an attempt to better calibrate the levels of genetic differentiation in the SJV, by understanding the levels of differentiation between populations outside the SJV and in the different mtDNA clades; Northern, Central and Southern clades as in Maldonado et al 2001 and improving sample sizes. In this study, we also report on preliminary results of genetic analyses that demonstrate that we can extract DNA from non-invasively collected fecal pellets in PVC tracking tubes and identify the ornate shrew mtDNA haplotype from that scat sample. Our results will be useful for conservation planning and implementing recovery actions for the remaining populations of the endangered Buena Vista Lake shrew.

Keywords

Sorex ornatus relictus, ornate shrew, mtDNA, microsatellites, Soricidae, non-invasive.

INTRODUCTION

The ornate shrew (*Sorex ornatus*) is restricted to coastal marshes and riparian communities of California, from 39°N latitude southward discontinuously to the tip of Baja California (Mexico). Currently, 9 subspecies are recognized, and a number of populations presumably have existed in small, isolated areas for long periods of time, such as those in montane meadows in southern California, in small coastal salt marshes in northern Baja California, and on Santa Catalina Island (Owen & Hoffmann 1983). Other populations have existed in widespread habitats, such as the large coastal marshes of the Los Angeles Basin and San Joaquin Valley (Williams 1986). Recently, however, some of these habitats have been altered by development, resulting in extensive habitat fragmentation. Three subspecies are included in the list of mammalian species of special concern in California, and the Buena Vista Lake shrew (*S. o. relictus)* has been listed as endangered (USFWS, 2002; Federal Register Vol. 67, 44) due to loss of habitat through agriculture and urban development.

Past subspecies of the ornate shrew often were described using body size and pelage coloration, which may be the result of environmental induction rather than genetically based differences, and sometimes based on only one or two specimens (Owen and Hoffmann 1983). However, the validity of the nine named subspecies of ornate shrews has recently been confirmed using univariate and multivariate statistical analyses of cranial measurements (Maldonado *et al.* 2004). Because of their short life span (Rudd 1953, Newman 1976), semi-fossorial habit, habitat specialization, high metabolism (McNab 1991), and small size, dispersal between patches of mesic habitat is limited and the high degree of local geographic morphological and genetic variation in shrews is expected. Furthermore, faced with a high abundance of invertebrate food, shrew populations can achieve high local densities. Therefore, the evolution of multiple subspecies in coastal and inland marshes in this species is also not surprising.

Although the existence of nine morphologically distinct subspecies of ornate shrew is well founded, a molecular genetic analysis of this species using mtDNA and allozymes conducted by Maldonado *et al.* (2001) found that the ornate shrew was phylogeographically separated into 3 clades representing southern, central, and northern localities. Clades have a high genetic divergence (4.2 -- 4.9% cytochrome *b* sequence divergence) that suggests a relatively long evolutionary independence from one another. Based on molecular data, populations in the northern clade diverged from the central and southern populations > 1 million years ago and genetically are more similar to neighboring populations of wandering shrews. Results of that genetic study also suggested that the central clade, where the presumed *S.o relictus* haplotype fall, had a relatively shallow phylogeny and several localities shared mtDNA haplotypes. This study only had representative samples from two distantly geographically separated localities; Los Banos from the Central SJV and Kern Lake Preserve, from the southern part of the Valley. Since then, several surveys conducted by personnel from ESRP and the California Department of Fish and Wildlife have discovered and sampled shrews from 10 additional localities distributed in between these populations (Figure 2; Appendix A). In addition, a survey carried out in September of 2010 by ESRP personnel at the Wind Wolves Preserve yielded an additional 11 shrew samples and live trapping surveys conducted in May-October 2014 yielded 12 additional samples from Southern San Joaquin Valley populations that had very

small sample sizes. All of these samples have now been analyzed and integrated into the results presented in this report.

Table 2. Subspecies, sampling locality and sample size used in the mtDNA and microsatellite analyses for *S. ornatus* from the Central-Southern San Joaquin Valley and representative populations from other subspecies located outside the San Joaquin Valley. Locality names correspond to localities in Figure 2. Highlighted in yellow are sample sizes indicating localities where the 2014 surveys yielded additional shrews for genetic analysis.

Local code	Subspecies	Locality	County	State	Previous Sample size	Actual sample size
	Ornate shrews					
1	*S.o. californicus*	Los Banos Wildlife Area*	Merced	California	14	14
2	*S.o. californicus*	Tranquillity	Fresno	California	76	76
3	*S.ornatus ssp.*	Helm	Fresno	California	9	9
4	*S.ornatus ssp.*	Lemoore	Kings	California	12	12
5	*S.ornatus ssp.*	Atwell Island	Kern	California	5	5
6	*S.ornatus ssp.*	Kern National Wildlife Refuge	Kern	California	7	11
7	*S.ornatus ssp.*	Main Drain Canal	Kern	California	2	4
8	*S.ornatus ssp.*	Goose Lake	Kern	California	11	14
9	*S.o. relictus*	Coles Levee Nature Center	Kern	California	8	8
10	*S.ornatus ssp.*	Kern Fan Recharge	Kern	California	2	3
11	*S.ornatus ssp.*	Kern Lake Preserve*	Kern	California	17	17
12	*S.ornatus ssp*	Wind Wolves Preserve	Kern	California	11	13
13	*S.o. californicus*	El Portal, Sierra Nevada*	Mariposa	California	9	9
14	*S.o. ornatus*	Kern River, Sierra Nevada*	Kern	California	14	14
15	*S. o. salarius*	Mouth of Salinas River*	Monterey	California	16	16
16	*S.o. sinuosus*	San Pablo Bay*	Solano	California	14	14
17	*S.o. ornatus*	Torrey Pines State Reserve*	San Diego	California	36	36
18	*S.o. willetti*	Santa Catalina Island	Los Angeles	California	22	22
				Total	285	295

* mtDNA haplotype data for these localities were obtained from Maldonado *et al* 2001.

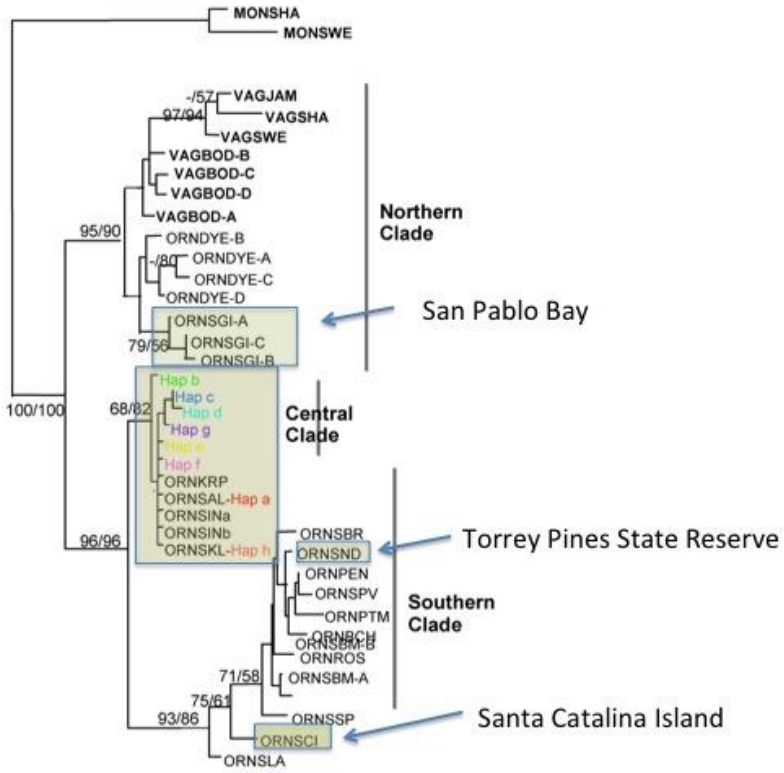


Figure 1. **Phylogenetic trees showing relationships between haplotypes found in ornate shrews in this survey and those form northern, central, southern California and Baja California from Maldonado et al 2001. Neighbor joining (NJ) tree using Kimura two-parameter sequence divergence values based on 392 bp of cytochrome *b* sequence. Codes for haplotypes correspond to those in Table 2 in Maldonado *et al* 2001. In color, are the eight haplotypes detected in this survey. Note that our most common haplotype in this survey (haplotype a) was the same haplotype as previously designated as "ORNSAL" in Maldonado *et al* 2001 and the "ORNSKL" haplotype found previously in Kern lake was designated as "Haplotype h" in this study. In bold, are haplotypes corresponding to *S. vagrans* (VAG) and *S. monticolus* (MON). Similar topologies were obtained using parsimony (MP) and maximum likelihood analysis in both cases. Percentage of support in 1,000 bootstrap NJ (numerator) and MP (denominator) replicates is indicated by the node when it is over 50%.**

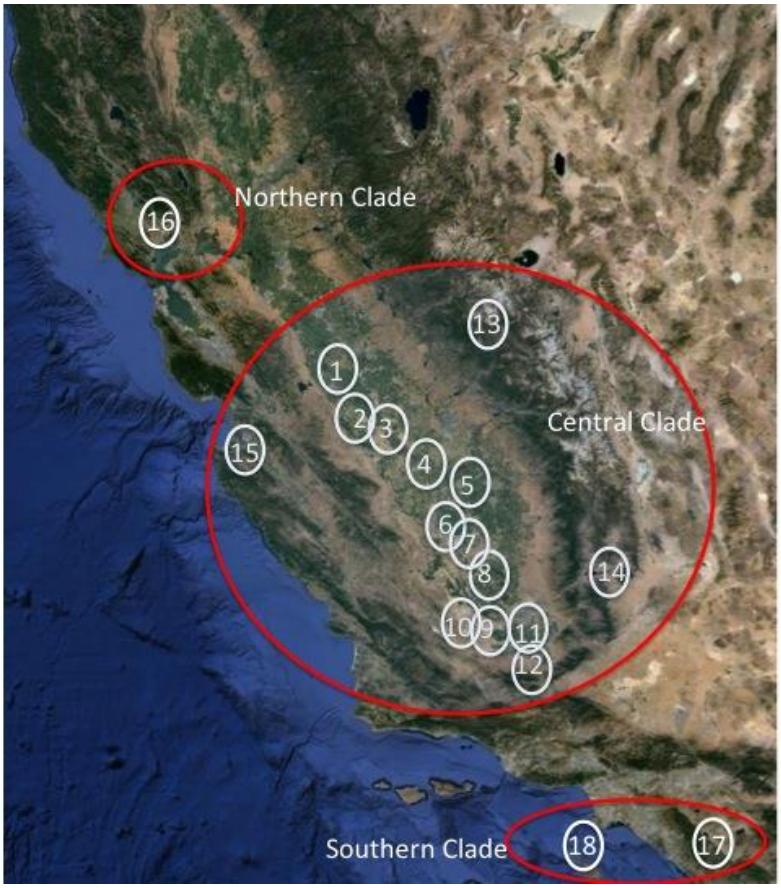


Figure 2. Location of the 18 ornate shrew (*S. ornatus*) representative populations for the microsatellite analysis in this study. Locality code numbers as indicated in Table 2. Note that the population selected from the Northern mtDNA clade (San Pablo Bay) and the two populations from the Southern clade (Santa Catalina and Torrey Pines) were included as outgroups for assessing hierarchical levels of differentiation using microsatellite data.

In this study, we report on the levels of genetic variation within and between populations of ornate shrews incorporating a total of eighteen localities, of which eleven are from the South-Central SJV, and three other localities are from surrounding areas within the same mitochondrial Central clade, but geographically located outside the SJV. We quantified variation in cytochrome *b*, known to have moderate rates of evolution in ornate shrews (Maldonado *et al.* 2001) and over a wide range of mammalian taxa with relatively recent divergence times (Irwin *et al.* 1991; Smith & Patton 1993; Baker *et al.* 1994; Mouchaty *et al.* 1995). We complement the mitochondrial DNA data with a survey of 9 nuclear microsatellite loci designed specifically for Buena Vista Lake shrews and known to have adequate levels of variability in several subspecies of ornate shrews (Maldonado *et al.* 2006).

MATERIALS AND METHODS

DNA EXTRACTION, PCR AMPLIFICATION AND SEQUENCING

Tissue samples from 144 individuals were obtained and analyzed from 14 localities in the SJV and Tulare Basin by personnel in a previous study for ESRP (Maldonado 2006). In a subsequent survey of genetic variability of shrews from the San Joaquin Valley and surrounding areas (Maldonado and Dutta 2014), we also included 11 additional samples from the Wind Wolves Preserve population and 22 samples from Catalina Island shrews available from the frozen DNA collection at the Center for Conservation Genomics. For this report, we have added 12 additional samples from areas that were poorly sampled in the previous survey, Kern National Wildlife refuge, Main Drain Canal, Goose Lake, Kern Fan Recharge and Wind Wolves Preserve (**Table 2** and Appendix A).

Minimally invasive tissue samples were obtained from the tip of the tail of ornate shrews and stored in ethanol (95%) or, in a few cases, from a liver sample if the entire specimen was obtained (accidental trap deaths) and preserved at -20 °C. Whole genomic DNA was extracted using the DNA extractions following standard DNeasy® kit (QIAGEN®) protocols established for tissue samples. Because the amount of tissue obtained from tail clips is very small, we used the entire sample in our DNA extraction protocol. All extractions were performed in a separate laboratory room devoted only to DNA extractions to minimize problems with contamination. Our extraction method has proven to be reliable for DNA extraction of small amounts of tissue in our laboratory and we were able to obtain amplifiable mtDNA from all samples.

We utilized two universal primers (H15149 Kocher et al. 1989; L14724 Meyer and Wilson 1990) that were used to amplify 425 bp of the mitochondrial cytochrome b gene which has been previously sequenced for ornate shrew samples from throughout the area and is ideal for comparison with our 12 recently obtained samples. Products were amplified via the polymerase chain reaction (PCR), and each mixture contained 20 ng of genomic DNA, 0.2 μM each primer, 1x PCR Gold Buffer (150 mM Tris-HCl, pH 8.0, 500 mM KCl), 2.0 mM of MgCl2, 0.2 mM of each dNTP´s, and 1U AmpliTaq Gold® DNA Polymerase. Cycling conditions consisted of an initial denaturation step at 95°C for 5 minutes, followed by 40 cycles of denaturation at 95°C for 1 min, annealing at 50°C for 1 min , and extension at 72 °C for 1 min . To ensure that all reactions had gone to completion, a final extension of 72 °C for 10 min was used. Negative controls, which did not include template DNA, were set up with all PCR reactions as checks for contamination of PCR reagents. Thermal cycling was performed in a PTC-100 Programmable Thermal Cycler (MJ Research Inc. Watertown, MA, USA) following the optimization cycling program described above. The PCR products were then cleaned with a 1:10 dilution of ExoSAP-It ® (Affymetrix) using 1μL of the 1:10 dilution for each 10 μL of PCR product, with incubation at 37°C for 30 minutes, followed by inactivation at 80°C for 15 minutes. We performed the cycle sequencing reactions using BigDye® Terminator v3.1 (Thermo Fisher Scientific), and the cleaned PCR products were detected in an automated capillary DNA sequencer (3100 Model; Applied Biosystems). Forward and reverse sequences were then aligned using the program SEQUENCHER® 5.2.4 (Gene Codes Corp Ann Arbor, MI) and verified visually.

SEQUENCE ANALYSIS

The cytochrome b sequence data without primer sequences totaled 392 bp and were aligned with previously published haplotypes of the same cytochrome b region of ornate, vagrant and montane shrews from Maldonado et al. 2001 and analyzed using three phylogenetic methods: maximum parsimony and neighbor joining. We conducted an analysis to determine the most parsimonious tree, each molecular data partition was tested by maximum parsimony (MP), distance-based neighbor-joining, and maximum likelihood (ML) analyses using PAUP* 4.0b10 (Swofford 2002). Confidence in estimated relationships was determined using 1,000 bootstrap pseudoreplicates (Felsenstein 1985). Lastly, the genetic distance between haplotypes was estimated by the Kimura 2-parameter model (Kimura 1981) and used to calculate a neighbor joining tree (Saitou & Nei 1987).

For this study, we incorporated the haplotype frequency data from Los Banos, Kern Lake, El Portal, Kern River Salinas River, San Pablo Bay and Torrey Pines from Maldonado et al. (2001). We used the analysis of molecular variance (AMOVA; Excoffier et al. 1992) to investigate the proportion of total genetic variation within and among described populations. We calculated f_{ST} statistics based on corrected sequence distances among haplotypes. We also generated pairwise genetic distances to calculated fixation indices (f_{ST}) between all populations pairs. Statistical significance was ascertained by conducting 10,000 permutations in the software ARLEQUIN 2.0 (Schneider et al. 2000). We also used this program to perform Fisher's exact test of population differentiation as described in Raymond & Rousset (1995). Genetic variability within populations was estimated in terms of haplotypic (H) and nucleotide (π) diversity, also as implemented in the program ARLEQUIN 2.0 (Schneider et al. 2000). MEGA 2 (Kumara et al., 2001) was also used to estimate mtDNA nucleotide diversity within population and a neighbor joining tree based on average sequence divergence (392 bp of cytochrome b sequence) between populations.

We used TCS version 1.13 (Clement et al. 2000) to generate and unrooted haplotype network and to assess the intra-specific phylogeny of the mitochondrial cytochrome b haplotypes we used a statistical parsimony network according to Templeton et al (1992). The statistical parsimony networks were constructed using the latest version of TCS (v.1.18) software package (Clement et. al, 2000). This method uses parsimony (as defined by Templeton et al. 1992) to construct pair-wise distances (number of mutational steps) between all haplotypes until the probability exceeds 95%. The matrix just above this cutoff point represents the maximum number of mutational steps justified by the 95% parsimony criterion. This method is particularly appropriate for population level analysis, as it does not involve many of the assumptions of phylogenetic reconstruction methods. For instance, it does not assume that the ancestral sequence is missing and does not require bifurcating relationships (Gentile et. al, 2002). The TCS program then connects the haplotypes based on these criteria into a network with the number of mutational steps indicated on the lines connecting haplotypes. On the basis of coalescent theory, this program also identifies the most probable ancestral haplotype among the collection of samples (Donnelly and Tavare, 1986; Castelloe and Templeton, 1994).

MICROSATELLITE SCREENING

Samples used for mtDNA sequence analysis were also used to screen for microsatellite variation (Table 1). We were able to amplify all of our nine microsatellite loci for all 12 samples increasing our total sample size for microsatellite analysis to 284 samples. Here, we present results that included data from an additional 12 samples from four localities in the southern San Joaquin Valley and also improved our previous analysis by amplifying samples and loci that had not yielded data earlier from additional populations in the SJV and outgroups as in Maldonado and Dutta (2014). The nine polymorphic microsatellite loci that we amplified were designed specifically for the Buena Vista Lake shrew but were also known to be polymorphic in other subspecies of ornate shrews (Maldonado et al. 2006). PCR conditions consisted of an initial denaturation at 95 °C for 5 min, followed by 30 cycles at 95 °C for 1 min, Ta for 1 min (see Maldonado et al. 2006 for each primer condition), and 72 °C for 1 min, and a final extension of 72 °C for 10 min. Amplifications of microsatellites was carried out in a 10 μL volume containing 20 ng of DNA, 0.2 μM of each primer, 0.2 mM of each dNTP, 1x PCR Gold Buffer (150 mM Tris-HCl, pH 8.0, 500 mM KCl), and 0.5 U of AmpliTaq Gold® DNA Polymerase. PCR products were separated using capillary electrophoresis on an ABI 3730xl sequencer, using GeneScan™ –500 ROX® size standard and scored alleles on GeneMapper-4.1 (Applied Biosystems).

MICROSATELLITE ANALYSIS

We measured genetic diversity using estimates of the number of alleles per locus (A), allelic richness (AR), private allelic richness (PR), observed (Ho) and expected (He) heterozygosity. We used the Excel Microsatellite tool kit 3.1 (Park 2001) to estimate descriptive statistics for each population, such as the proportion of polymorphic loci, and observed and expected heterozygosity and mean number of alleles per locus. Since we had unequal sample sizes from the 14 populations, we used HP-Rare (Kalinowski, 2004, 2005), which uses a rarefaction method to compensate for unequal sample sizes, to compute AR and PR for each population. We conducted tests for global and population-level deviations from Hardy–Weinberg equilibrium (HWE) and linkage disequilibrium (LD) using GENEPOP 4.0.10 (Rousset, 2008) with a Bonferroni correction (Rice, 1989) applied for multiple comparisons. We used ARLEQUIN with 10,000 permutations to test the statistical significance of pairwise F_{ST} values (Weir & Cockerham, 1984) as a measure of genetic differentiation among the different populations.

We used the model-based clustering method of the program *STRUCTURE* (Pritchard et al. 2000) to infer population structure among localities and to probabilistically assign all individuals to the detected clusters (*k*). Subsequent analysis of each subset tested $K = 1$ to $K = c+3$ (the number of collections (c) included in the subset plus three), with a burn-in of 500,000 followed by 500,000 iterations, and 12 analyses for each *K*. Individual assignment success to the cluster of origin was recorded both as the highest likelihood of assignment (*q*) and the percentage of individuals in a cluster with $q \geq 0.70$ (Pritchard *et al.* 2000; Pritchard and Wen 2004). The mean and standard deviation of Pr (X|K) from the 10 replicate runs were used to find the most likely value of K. We also used the ΔK method of Evanno et al. (2005) based on the second-order rate of change in log Pr (X|K) as implemented by the program STRUCTURE HARVESTER (Earl & von Holdt, 2011).

NON-INVASIVE SAMPLES

DNA EXTRACTION, PCR AMPLIFICATION AND SEQUENCING

DNA was also extracted from small fecal pellets taken from PVC track tubes that were known to have detected shrews. Scats were stored in sealed Eppendorf Nunc Tubes ™ dry and at room temperature and sent to the lab for analysis (Figure 3). All of the scat samples tested were from the Wind Wolves Preserve where shrews had been successfully detected previously and had defecated in the PVC pipe track tubes. DNA was extracted from a single pellet using the QIAamp DNA stool mini kit (QIAGEN®) with modifications from the manufacturer's protocol as in Eggert et al. (2005) and an extended overnight incubation in lysis buffer and proteinase K at 56°C on a shaker. Extractions were carried out in a separate room dedicated to DNA extractions of samples from a diversity of sources including scat, hair, blood and tissue samples. This room has a positive pressure air handling system to separate the extraction laboratory air supply from sample preparation and downstream PCR applications in the main lab. Negative controls (no scat) accompanied each set of extractions and were used to check for contamination. In addition, in order to check for repeatability, DNA was extracted twice from a small subset of samples; once the methods were validated, the rest of the samples were extracted only once.

Species Identification We designed a pair of primers that amplify a shorter 255 bp fragment from the Cytochrome b sequences that we had produced in the past using all of the ornate shrew haplotypes recovered in previous surveys. We then selected a region that contains most of the variable sites that identify the different ornate shrew haplotypes in the San Joaquin Valley. The primer sequences are as follows: SO287L: 5' - TACGAAAAACCCACCCCTTA – 3' and SO287H: 5' – TCCGACGTGAAGGAATAAGC-3'. Products were amplified via the polymerase chain reaction (PCR), and each mixture contained 0.2 μM each primer, 1x PCR Gold Buffer (150 mM Tris-HCl, pH 8.0, 500 mM KCl), 2.0 mM of MgCl2, 0.2 mM of each dNTP´s, and 1U AmpliTaq Gold® DNA Polymerase. Cycling conditions consisted of an initial denaturation step at 95°C for 5 minutes, followed by 40 cycles of denaturation at 95°C for 1 min, annealing at 50°C for 1 min, and extension at 72 °C for 1 min and a final extension of 72 °C for 10 min was used. Negative controls, which did not include template DNA, were set up with all PCR reactions as checks for contamination of PCR reagents. Thermal cycling was performed in a PTC-100 Programmable Thermal Cycler (MJ Research Inc. Watertown, MA, USA) following the optimization cycling program described above. The PCR products were then cleaned with a 1:10 dilution of ExoSAP-It ® (Affymetrix) using 1μL of the 1:10 dilution for each 10 μL of PCR product, with incubation at 37°C for 30 minutes, followed by inactivation at 80°C for 15 minutes. We performed the cycle sequencing reactions using BigDye® Terminator v3.1 (Thermo Fisher Scientific), and the cleaned PCR products were detected in an automated capillary DNA sequencer (3100 Model; Applied Biosystems). Forward and reverse sequences were then aligned using the program SEQUENCHER® 5.2.4 (Gene Codes Corp Ann Arbor, MI) and verified by eye.

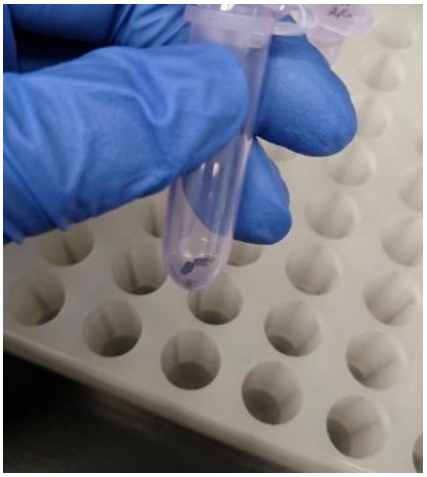


Figure 3. Small mammal fecal pellets obtained from PVC track tubes for non-invasive genetic analysis.

RESULTS

SEQUENCE VARIATION AND PHYLOGEOGRAPHY OF HAPLOTYPES

We found 11 different haplotypes in the 213 ornate shrews from samples in the 15 surveyed areas within the central region (Table 2). The phylogenetic analysis comparing the 11 haplotypes to the published haplotypes from Maldonado *et al.* 2001, recovered the same three distinct geographic clades (Northern, Central and Southern) in phylogenetic trees based on the cytochrome b gene from all samples (Figure 1). These clades generally were supported in more than 80%, and 90% of bootstrapped trees. Within each of the three major clades, the topology varies among tree building methods, few nodes were supported in more than 50% of the bootstrap iterations and no support was evident for the subspecies that are distributed in several of the sampled populations. The 11 central clade haplotypes where derived from 15 populations in the coastal Salinas Valley, San Joaquin Valley, Sierra Nevada populations north of the Tehachapi Mountains in central California and south of the San Francisco Bay area. Based solely on distribution range, the central clade includes shrews attributable to four different subspecies: *S. o. californicus, S. o. salarius, S. o. relictus* and *S. o. ornatus*.

Table 2. *Sorex ornatus* haplotype distribution at 15 sampling localities in Central-Southern San Joaquin Valley and surrounding areas in Central California. Note that the haplotype distribution for populations located outside the Central Clade (San Pablo Bay, Torrey Pines and Santa Catalina Island) were not included because they have unique and divergent haplotypes that are not shared with Central clade populations. Highlighted in yellow are haplotypes frequencies for localities that changed after samples collected in the 2014 surveys were added.

Haplotype	El Portal Sierra N	Salinas River	Los Banos	Tranquillity	Helm	Lemoore	Atwell Island	Kern NWR	Main Drain Canal	Goose Lake	Coles Levee	Kern Fan Recharge Area	Kern Lake	Wind Wolves	Kern River reserve	Total
Haplotype A		16	14	73	6	11	4	9	2	6	1	3		9	12	166
Haplotype B											7					7
Haplotype C						1	1	2	2	8						14
Haplotype D				1	2									4		7
Haplotype E					1											1
Haplotype F				1												1
Haplotype G				1												1
Haplotype H													17			17
Haplotype ORNKRP*															2	2
Haplotype SIN-A*	8															8
Haplotype SIN-B*	1															1
TOTAL	9	16	14	76	9	12	5	11	4	14	8	3	17	13	14	225

*Haplotypes obtained from Maldonado et al. 2001

A total of 9 variable sites were found in the 392 bp fragment and 8 of these were transitions and 1 was a transversion. Haplotype A was the most common haplotype in the area surveyed and it was detected most of the Valley localities except in Kern Lake Preserve. In addition, haplotype A was previously recovered in Salinas, Los Banos and the Kern River Preserve and was present in 42 individuals sampled from these three localities in Maldonado *et al.* 2001. Kern River also had a unique haplotype (ORNKRP). Haplotype B was only recovered in Coles Levee. Haplotype C was recovered with increasing frequency from North to South in Lemoore, Atwell Island, and Main Drain Canal and was the most common haplotype in Goose Lake. Haplotype D was only found once in Tranquillity and in two individuals in Helm but it was also recovered in our southernmost locality 4 of the 11 samples from Wind Wolves preserve. Haplotype E was only recovered in one individual in Helm and Haplotypes F and G were only found once in Tranquillity (Table). The two "Southern" outgroup populations from Torrey Pines (San Diego) and Catalina Island each had a single unique but highly divergent haplotypes. The "Northern" outgroup population had 3 closely related haplotypes. In this study, we found that most of

the localities that we surveyed and that had sample sizes greater than n= 2 had more than one haplotype. The only exception was the Kern National Wildlife refuge and the populations screened in Maldonado *et al.* 2001 from Salinas, Los Banos and Kern Lake. Tranquillity, our best sampled locality, had the highest number of haplotypes (4) and the nucleotide diversity (π, Nei 1987) within populations was similar in the northern (presumably larger) populations than in the southern populations where it ranged from 0.005 to 0.007, the rest had values of zero.

The statistical parsimony network revealed that most of the cytochrome *b* haplotypes were closely linked in a star shaped phylogenetic pattern including haplotypes from central clade populations outside the SJV. The most frequent haplotype A was internal, while the majority occurred at low frequencies in single populations and presented distal positions in the network. Haplotype E differs from A by one transversion and haplotypes F, G and H, each differ by one transition from A. Haplotype B differs by 2 transitions from A and haplotype C by 3 transitions from A. Haplotype D is the most divergent haplotype and differs by two transitions from C (Figure 3). Two of the substitutions occurred at first position codons and eight of the substitutions occurred at third position codons.

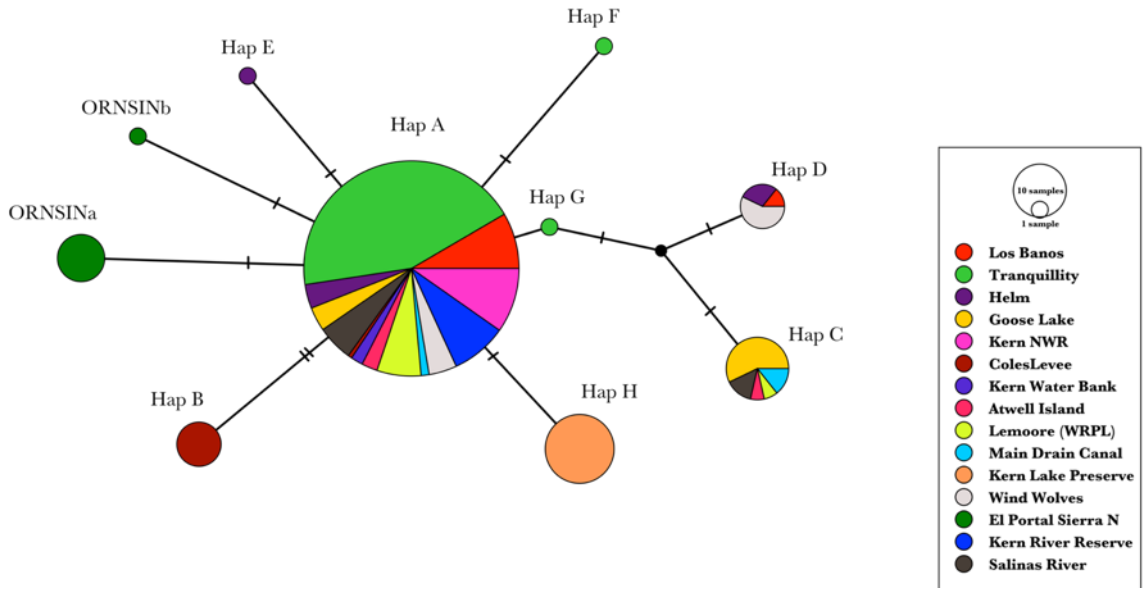


Figure 3. Statistical parsimony network of cytochrome *b* haplotypes. Localities where each haplotype was found are color coded. Each line connecting a circle indicates one base pair substitution. Small black circles denote hypothetical internodes. Note that haplotype A was designated as the ancestral haplotype and is a common haplotype found in most of the SJV populations except Kern Lake and El Portal populations.

MICROSATELLITE VARIATION

Genotypes at 9 microsatellite DNA loci were determined for 284 samples that included the outgroup populations outside the Central clade (Appendix C). The outgoup populations showed significant levels of genetic differentiation with the central clade and we therefore conducted finer scale analysis of the 197 samples of *Sorex ornatus* from the 14 localities

along the Central-Southern SJV (Table 2, Figure 2). All populations were polymorphic for most loci, except for a few localities where microsatellite loci were not very polymorphic. Locus SH-5 and locus A3-26 had the lowest levels of polymorphism, whereas SH-22, A3-35 and SH-25 had the highest number of alleles. Allelic richness (Ar) ranged from 2.11 (Kern Fan recharge) to 2.75 (Tranquility). We used allelic richness and private allele richness as a measure of diversity since measures such as number of alleles are strongly biased by sample size (n ranged from 3 to 76) (Table 3). Private allele richness (Pr) was highest in the Wind Wolves population, and this is true even after accounting for sample size. Observed heterozygosity ranged from 39% in Lemoore to 65% in Kern NWR (Table 4). Most of the polymorphic loci were in Hardy-Weinberg equilibrium with the exception of locus A3-26 that yielded a deficiency of heterozygous genotypes. This level of deviation is likely due to one or a combination of factors including sub-structuring of the sample (i.e., Wahlund effect), inbreeding, or the presence of null alleles. Overall, no linkage disequilibrium was detected (maximum-likelihood ratio test with Bonferroni correction for multiple comparisons, $p > 0.05$). Fst values were high and significant between several pairs of populations, indicating genetic subdivision of ornate shrews in this region (Table 5)

Table 3. The number of alleles for each population across the 9 microsatellite loci. Number of alleles that are private to only that population shown in square brackets. Ar and Pr are the Allelic richness and private allele richness, which are measures of allelic and private allele diversity to account for unequal sampling.

	A3-35A	A4-5A	SH-22A	A3-26A	A4-1A	A3-5A	SH-5A	A4-20A	SH-25A	Ar	Pr
Atwell Isl	6	4	5	2	2	4	2	3	5	2.5	0.04
Coles Levee	4	6	5	2	2	4	2	5	3	2.26	0.08
Helm	5	6	7	3	3	7	3	5	10	2.74	0.21
Goose Lake	9	8	7	3	2	5	3	5	9	2.69	0.09
Main Drain	3	4	3	1	1	2	2	3	4	2.56	0.36
Wind Wolves	9	7	9	2	2	8	3	8	9	2.72	0.39
Kern Lake	8	5	4	2	2	6	3	5	6	2.35	0.21
Kern NWR	8	10	7	2	3	7	3	6	8	2.65	0.18
Kern River	7	6	8	2	2	7	2	6	7	2.5	0.13
Kern Fan Recharge	2	3	2	2	1	2	2	3	2	2.11	0.02
Lemoore	7	7	8	2	2	6	3	8	10	2.6	0.16
Salinas	5	6	10	1	3	11	2	9	9	2.41	0.31
Sierra North	7	6	5	2	2	7	2	6	5	2.49	0.24
Tranquility	18	9	7	3	3	11	3	10	19	2.75	0.18

Table 4. Unbiased observed and expected heterozygosity, along with the mean number of alleles for the 14 sampled populations with 9 microsatellite loci. Sample sizes are presented in brackets.

	Ho	He	Na
Atwell Isl (5)	0.6333	0.5906	3.67
Coles Levee (8)	0.4603	0.5276	3.67
Helm (9)	0.4938	0.6742	5.44
Goose Lake (16)	0.5417	0.6838	5.67
Main Drain (2)	0.5000	0.4583	2.56
Wind Wolves (14)	0.5486	0.6758	6.33
Kern Lake (17)	0.4591	0.5592	4.56
Kern NWR (11)	0.6515	0.6476	6.00
Kern River (14)	0.5390	0.6136	5.22
Kern Fan Recharge (2)	0.5000	0.3889	2.11
Lemoore (12)	0.3948	0.6183	5.89
Salinas (16)	0.4444	0.5495	6.22
Sierra North (9)	0.5910	0.5981	4.67
Tranquility (74)	0.5387	0.7113	9.22

Results from the S*TRUCTURE* analyses identified 6 genetic clusters (*K*= 6) among the 14 localities of *S. ornatus* genotyped at 9 microsatellite DNA loci (Figure 4). The 6 clusters were subdivided as follows: [1]Atwell Island, Coles Levee, Main Drain, Kern NWR, Kern Fan Recharge, [2] Goose Lake, Helm, Lemoore, Wind wolves, Sierra north, [3] Tranquility, [4] Salinas, [5] Kern River, and [6] Kern lake. It is evident from this analysis, that populations within the central clade have finer scale levels of genetic differentiation at non-coding nuclear microsatellite markers. Salinas and Kern River are the most geographically distant from the main valley, and they form very distinct genetic clusters with low levels of admixture. Within the valley, Tranquility forms a distinct genetic cluster, although it has a higher signature of admixture than Salinas and Kern River. Kern Lake is located in the southern part of the SJV, but it forms a highly differentiated genetic cluster with both nuclear and mtDNA markers. Kern NWR, Coles Levee, Kern Fan Recharge, Atwell Island and Main drain are all neighboring populations in the valley, and form a well admixed but distinct genetic cluster. Figure 4 and Table 6 show the admixture levels of these populations with respect to the 6-identified population genetic clusters. Kern Lake, Kern River and Salinas, which form independent genetic clusters, have the least amount of admixture, while all other populations have moderate to high levels of admixture. The degree of differentiation for Kern River and Salinas is not unexpected, given that these populations are geographically distant from the main SJV populations. However, even within the main San Joaquin Valley we find significant genetic differences and very interesting clustering patterns, where Kern Lake, Tranquillity form independent genetic clusters.

Table 5. Matrix of genetic differentiation (Fst) values between each pair of 14 sampled populations. Significant Fst values are shown in bold.

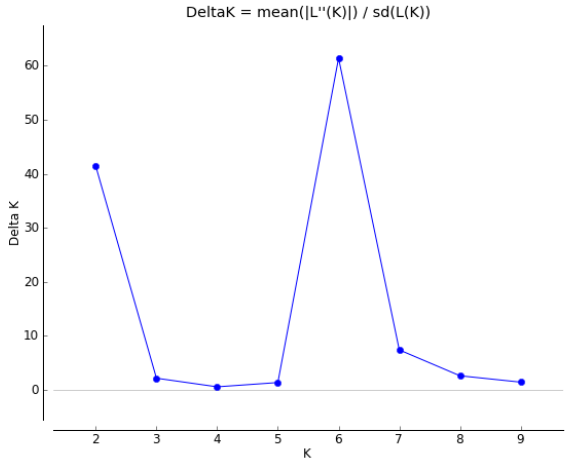
	Atwell Isl	Coles Levee	Helm	Goose Lake	Main Drain	Wind Wolves	Kern Lake	Kern NWR	Kern River	Kern Fan Recharge	Lemoore	Salinas	Sierra North	Tranquility
Atwell Isl	*													
Coles Levee	**0.070**	*												
Helm	0.035	**0.090**	*											
Goose Lake	0.039	**0.111**	0.020	*										
Main Drain	**0.130**	**0.232**	0.088	0.073	*									
Wind Wolves	**0.065**	**0.117**	**0.051**	**0.057**	**0.110**	*								
Kern Lake	**0.075**	**0.135**	**0.070**	**0.092**	**0.227**	**0.098**	*							
Kern NWR	0.030	**0.096**	**0.053**	**0.038**	**0.101**	**0.073**	**0.108**	*						
Kern River	**0.071**	**0.123**	**0.051**	**0.069**	**0.155**	**0.061**	**0.095**	**0.086**	*					
Kern Fan Recharge	0.106	**0.199**	0.126	**0.118**	0.280	**0.126**	**0.138**	**0.083**	**0.193**	*				
Lemoore	**0.101**	**0.198**	**0.098**	**0.074**	0.115	**0.094**	**0.162**	**0.121**	**0.102**	**0.222**	*			
Salinas	**0.147**	**0.157**	**0.125**	**0.129**	**0.233**	**0.122**	**0.129**	**0.128**	**0.141**	**0.191**	**0.216**	*		
Sierra North	**0.098**	**0.137**	**0.067**	**0.074**	**0.188**	**0.072**	**0.088**	**0.111**	**0.067**	**0.151**	**0.138**	**0.136**	*	
Tranquility	**0.056**	**0.113**	**0.027**	**0.047**	**0.104**	**0.047**	**0.081**	**0.064**	**0.060**	**0.122**	**0.069**	**0.121**	**0.089**	*

Table 6. The admixture levels between different sampled populations that make up the 6 distinct genetic population clusters. Highlighted in light red are the localities with the highest levels of admixture within each of the 6 clusters.

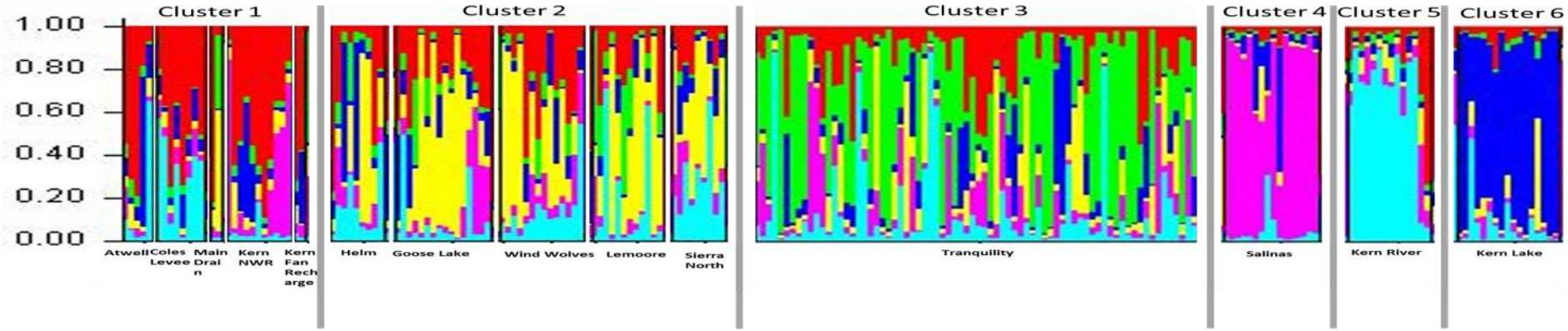
Sampled Population	n	Genetic Pop 1	Genetic Pop 2	Genetic Pop 3	Genetic Pop 4	Genetic Pop 5	Genetic Pop 6
Atwell Isl	5	0.4448	0.0719	0.0544	0.0487	0.1711	0.2090
Coles Levee	8	0.4538	0.0431	0.0246	0.0640	0.3165	0.0980
Helm	9	0.2344	0.2708	0.0755	0.0788	0.1635	0.1770
Goose Lake	16	0.2356	0.4031	0.0664	0.1092	0.0858	0.1000
Main Drain	2	0.4257	0.3325	0.1749	0.0399	0.0156	0.0114
Wind Wolves	14	0.2437	0.3866	0.0540	0.0882	0.1440	0.0835
Kern Lake	17	0.0416	0.0628	0.0210	0.0278	0.0771	0.7697
Kern NWR	11	0.4665	0.0761	0.0366	0.2603	0.0408	0.1198
Kern River	14	0.1197	0.0583	0.0285	0.0787	0.6798	0.0351
Kern Fan Recharge	2	0.6353	0.0648	0.0298	0.0481	0.0285	0.1934
Lemoore	12	0.1566	0.4086	0.1649	0.0493	0.1675	0.0532
Salinas	16	0.0284	0.0313	0.0154	0.7792	0.0459	0.0998
Sierra North	9	0.1179	0.3936	0.0220	0.0893	0.2704	0.1069
Tranquility	74	0.1395	0.1201	0.3968	0.1118	0.1336	0.0981

Figure 4. Estimated population structure inferred from STRUCTURE analysis A). Plot from Structure Harvester showing the highest value of ΔK (60) on K=6 genetic clusters and a smaller peak (ΔK 40) at K= 2 clusters. B) Bar graphs representing the average ancestry coefficient (q) of each individual for 10 replicates of K clusters. Each individual is represented as a thin horizontal line with sampling locations designated on the bottom. Dotted lines separate the individuals from different sampling locations.

A)


DeltaK = mean(|L''(K)|) / sd(L(K))
Delta K
K
0
10
20
30
40
50
60
2
3
4
5
6
7
8
9

B)


Cluster 1
Cluster 2
Cluster 3
Cluster 4
Cluster 5
Cluster 6
1.00
0.80
0.60
0.40
0.20
0.00
Atwell
Coles Levee
Main Drain
Kern NWR
Kern Fan Recharge
Helm
Goose Lake
Wind Wolves
Lemoore
Sierra North
Tranquility
Salinas
Kern River
Kern Lake

RESULTS OF GENETIC ANALYSIS OF NON-INVASIVE SAMPLES

We were able to successfully extract DNA from the fecal samples using our scat extraction protocol. We then attempted to amplify the scats with the universal CytB primers (H15149 Kocher et al. 1989; L14724 Meyer and Wilson 1990) that were used to amplify 425 bp of the mitochondrial cytochrome b gene using the same conditions used for the tail and tissue samples. However, our amplification success was very low and of the samples that amplified and that were sequenced, most yielded dirty poor-quality sequences or sequences that revealed contamination from human DNA. One scat sample (Scat 8B) gave a clean amplification using the universal primers yielding a 425bp fragment of sequence. This sample, however, blasted 100% identical to a harvest mouse (*Reithrodontomys megalotis)* haplotype published in Genbank (Acc# KR11944.1) from Mendocino Co. CA. This is not surprising because harvest mice are common in the Wind Wolves area.

We then attempted to amplify a fragment of the Cytb gene with the newly developed pair of short primers (SO287L and SO287H) designed specifically to amplify degraded shrew DNA. These primers successfully amplified 4 additional scat samples and sequence analysis revealed that they were all from ornate shrew Haplotype A (Table 7). This haplotype is the common haplotype found in Wind Wolves Reserve and therefore confirms the presence of other shrews with this same haplotype. However, because we did not attempt to develop microsatellite protocols for non-invasive sampling, we were able to determine if these samples came from one or several individuals. Therefore, future efforts should be devoted to develop markers that can reliably identify different individuals and sex of each individual from DNA extracted from fecal samples.

Table 7. Scat samples collected in Wind Wolves Preserve with trap # and their respective species identification and haplotype.

SAMPLE	*HAPLOTYPE*	*LOCATION*
SCAT-9P	*SOREX ORNATUS –HAP A*	*WIND WOLVES*
SCAT-5L	*SOREX ORNATUS –HAP A*	*WIND WOLVES*
SCAT-1D	*SOREX ORNATUS –HAP A*	*WIND WOLVES*
SCAT-2C	*SOREX ORNATUS –HAP A*	*WIND WOLVES*
SCAT 8B	*REITHRODONTOMYS MEGALOTIS*	*WIND WOLVES*

DISCUSSION

The ornate shrew is one of the most threatened small mammals in central, southern (Williams 1986), and Baja California (Elliot 1903; Huey 1964; Woloszyn *et al.* 1985; Maldonado 1999). It is thought to be threatened primarily due to destruction of wetlands and riparian habitats and several subspecies are listed as California Mammal Species of Special Concern and the Buena Vista Lake shrew is federally endangered (USFWS 2002). It is thought that ornate shrews may once have had a continuous distribution along the marshlands of Tulare Basin in the SJV (Grinnell 1932) but this habitat is now greatly fragmented due to cultivation and the recent disappearance of lakes and sloughs (Williams

1986). Here we provide information regarding patterns of genetic variation in these areas that is required for implementing major recovery actions for the Buena Vista Lake shrew.

PHYLOGEOGRAPHY OF HAPLOTYPES

Three well-defined geographic population groupings were discovered in phylogenetic analyses based on mitochondrial DNA sequences of ornate shrews in Maldonado *et al*. 2001. The results of our phylogenetic analysis comparing the 8 haplotypes recovered from the sequencing of the samples from the central- southern SJV with those of Maldonado *et al*. 2001 clusters them in a well-supported central clade that includes haplotypes from the coastal Salinas Valley, the central-southern SJV and Sierra Nevada populations north of the Tehachapi Mountains in central California and south of the San Francisco Bay area. This confirms previous findings that the southern, central and northern populations of the ornate shrew form distinct and well supported clades that have long and separate ancestry and are reciprocally monophyletic and divergent in haplotype frequencies (Maldonado *et al*. 2001).

Moreover, within the SJV region, mtDNA suggest that gene flow is low among some of the populations and although one mtDNA haplotype (Haplotype A) is common and/or present in most of the localities, six other haplotypes had never been detected in the central ornate shrew clade and five of those haplotypes were unique to single populations (Haplotypes B, E, F, G, and H). Interestingly, there appears to be a pattern of increasing frequency for haplotype C and decreasing frequency of haplotype A in southern localities (Figure 5). These haplotype frequency differences result in four localities that are significantly differentiated relative to a random collection of genotypes and show low levels of gene flow with high fst values and high mean population genetic distances (Goose Lake, Main Drain, Coles Levee and Kern Lake). This level of inter-haplotypic divergence is greater than previously reported from this area by Maldonado *et al*. (2001).

In addition, Maldonado *et al*. (2001) found 24 different haplotypes in 20 populations and the occurrence of unique haplotypes in most localities suggests that genetic subdivision was a common characteristic of ornate shrews throughout most of their range. In general, therefore, these samples do exhibit a degree of geographic structuring perhaps larger than might be expected, given the limited geographic scale over which the samples were collected. Clearly, populations of this species have the capacity to exchange genes, or at least be genealogically connected, over respectable distances. The relatively high connectedness among some samples from the central valley contrasts with data for samples from shrews from the Southern California clade.

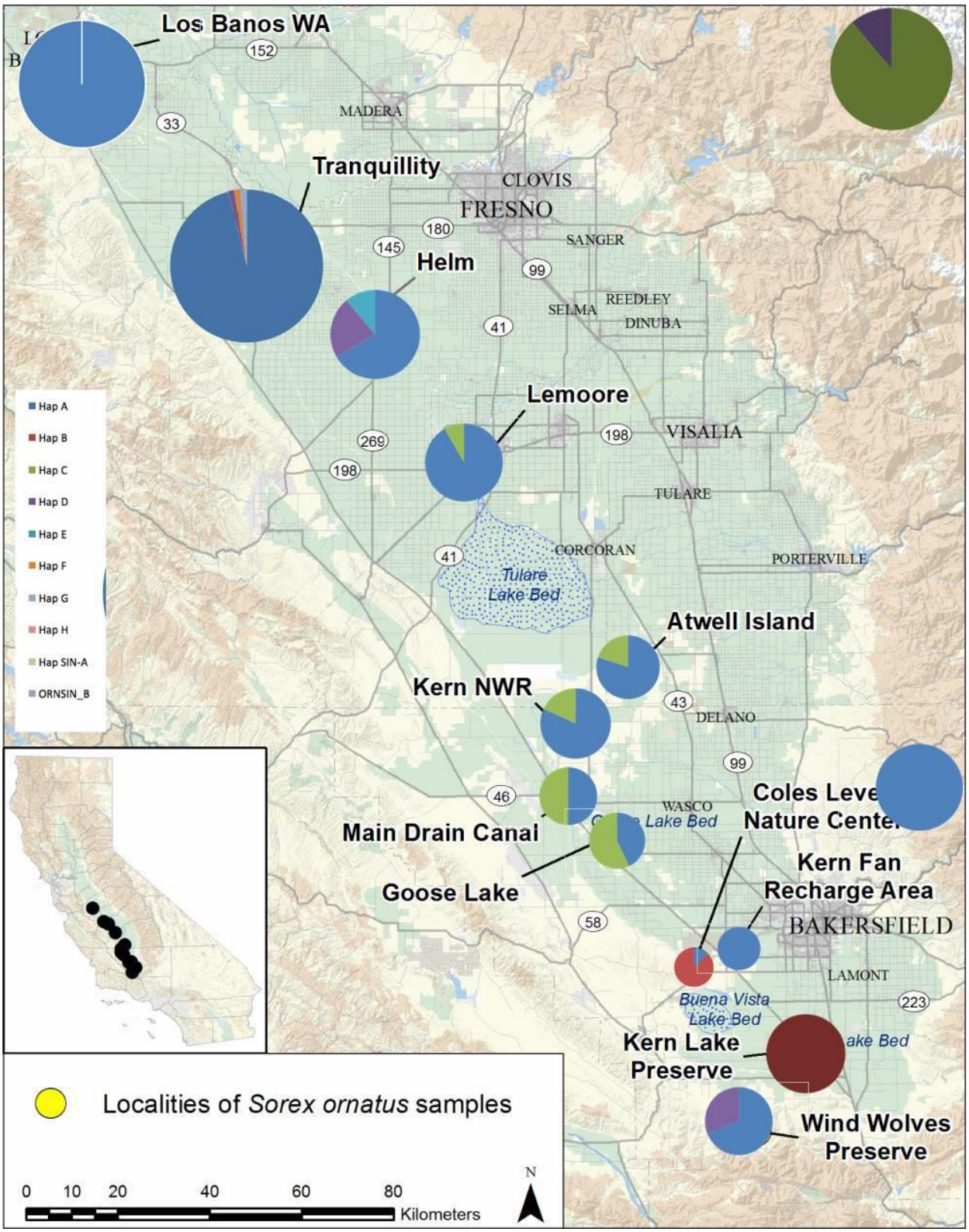


Figure 5. Map showing the haplotype distribution at 12 sampling localities in Central-Southern San Joaquin Valley and mtDNA haplotype frequencies for each site.

DIVERGENCE WITHIN AND AMONG POPULATIONS

The net sequence divergence between southern and central clades is 4.2%, and assuming a mutation rate of 2% per million years (Wilson *et al*. 1985), the two clades diverged about 1.1 million years ago (Maldonado *et al*. 2001). Finally, the mean divergence between sequences within the central- southern San Joaquin Valley clade is 0.7%. These values correspond to divergence times of approximately 0.150 Mya. The central clade has a lower mean divergence than the other two clades and suggests a more recent radiation of haplotypes (Maldonado *et al*. 2001). The central clade exhibited a more "shallow" gene genealogies, an intraspecific pattern not uncommonly seen within small mammal species (Tiemann-Boege *et al*. 2000; Edwards and Bradley 2001; Jaarola and Searle 2002). Also, the star shaped parsimony network of haplotypes suggests a pattern of past demographic change in these populations (Figure 3)

Previous evolutionary hypotheses concerning the radiation of shrews have drawn on the conventional wisdom that Pleistocene climatic cycles precipitated a large portion of speciation events between extant sister taxa (Findley 1955). The tripartite division of ornate shrew clades dates to the early Pleistocene and does not reflect isolation in recent ice age refugia (Maldonado *et al*. 2001). In contrast, past patterns of genetic divergence within clades appear to be erased by population contraction during inter-glacials and re-established during glacial period expansions and suggests that ice age effects may have more pronounced impact on regional within clade diversity than on speciation (Maldonado *et al*. 2001). During these periods, wetland habitat available to shrews was more limited and fragmented. As suggested by low rates of gene flow, shrews are poor dispersers and the imprint of past events may be long retained in present day populations.

Low estimates of inter-locality gene flow suggest that several of the southern San Joaquin localities have been genetically relatively independent for a reasonable period of time. Furthermore, local populations must have remained sufficiently large to prevent the loss of genetic variation, despite opportunities for drift, but it is unlikely that these populations have remained of similar size with the degree of habitat modification that they have experienced recently. Shrews have been reported to undergo occasional population demographic expansions leading to periodic increases in interpopulation "connectedness" and interspersed temporally with more typically small-sized but persistent local populations. However, this is a process that is currently being prevented by fragmentation and loss of wetland habitat connectivity.

GENETIC VARIATION AND POPULATION GENETIC STRUCTURE

While mitochondrial is DNA is more typically used for phylogeographic inference, it nonetheless represents a single locus that accumulates mutations relatively slowly (0.2-11.3% sequence divergence per million years for vertebrates (Martin and Palumbi 1993). Therefore, clear and pronounced differences between groups at the mitochondrial level typically reflect historical separation of groups rather than more recent population level differences. However, microsatellite loci are highly variable markers widely accepted as appropriate for detecting finer scale population level subdivision and for measuring gene flow (Quellar *et al*. 1993; Jarne and Lagoda 1996; Goldstein and Pollock 1997).

In this study, we found higher levels of mtDNA genetic diversity (6 additional haplotypes) than were previously reported within shrew populations that fall in the central clade in Maldonado *et al.* (2001). We also found lower levels of divergence in mitochondrial

sequence than with microsatellites between populations. However, four populations (Main Drain, Coles Levee, Goose Lake and Kern Lake) show moderate but significant levels of mtDNA differentiation. These populations contain at least one haplotype that is not found in Tranquillity or Los Banos. The haplotype network suggests a pattern more typical of a past population expansion event.

The microsatellite DNA dataset contained sufficient allelic diversity to elucidate 6 distinct clusters among the 14 localities in the SJV central clade localities and delineates significant subdivision between the populations (Figure 4) with at least 3 subpopulations in the extreme south-central SJV region (Cluster 2, 3, 4). Our analysis does not support the null hypothesis of a homogeneous gene pool among the southern SJV localities. The magnitude of the observed differentiation was considerable and was supported by significant values for several statistical comparisons, regardless of the genome under consideration. Given the disjunct distribution of selected localities and the structuring of nuclear multi-locus genotypes, combined with the presence of some locality specific mtDNA haplotypes, supports genetic discontinuities observed throughout the study area. In addition, the patterns of genetic subdivision revealed by the *STRUCTURE* analyses for this region may not necessarily stem from very recent repeated population bottlenecks or founder effects and isolation, which reduced the microsatellite alleles to a subset of those present in neighboring populations, rather it appears to be a signature of a more historic level of divergence as suggested by the presence of several private alleles not present in Tranquillity and present in the southern localities. This interpretation is consistent with mtDNA analyses, which does not does not show a dramatic loss of haplotypes and lower nucleotide diversity relative to sample sizes in southern localities. Population based differentiation estimates (Fst values) generally agree with the *STRUCTURE* analysis, showing higher and significant Fst values between populations assigned to different genetic clusters (Table 6).

Results from a previous study (Maldonado 2006) conducted using a smaller number of representative localities (10 localities) within the Central and Southern SJV showed 5 genetic clusters with samples collected from the localities in 1) Main Drain canal, 2) Coles Levee and 3) Kern Lake each appearing as distinctly defined groups and Lemoore, Atwell, Kern WRF, and Kern Fan were clustered together to form a fourth group. Finally, samples from Helm clustered with Tranquillity to form a fifth group, although Helm had one individual with a mtDNA haplotype (**e**) that had a single nucleotide polymorphism from the common haplotype (**a)** found throughout the localities surveyed in the SJV. Our present study included analysis of an expanded data set of additional samples from outside the SJV to better calibrate levels of genetic differentiation and structure within the San Joaquin Valley and also between populations that occur in the surrounding areas in the Sierra Nevada Mts., the Salinas Valley and the Wind Wolves Preserve in the Northern slope of the Tehachapi Mts. Our results show that ornate shrew populations located outside the SJV and that were previously considered to be separated into two mtDNA Northern clade (represented by San Pablo Bay population) and Southern clade (represented by the San Diego and Santa Catalina Island populations) also revealed strong signatures of nuclear DNA differentiation based on analysis of allelic frequencies at our 9 polymorphic microsatellite loci but including them in the structure analysis completely erased any signature of population structure within the Central clade localities. Therefore, a hierarchical structure analysis which included additional populations surrounding the SJV that were previously determined to be within the mtDNA "Central" clade, including

samples from the Salinas Valley, the Northern Sierra Nevada populations in El Portal and the Southern Sierra Nevada populations in Kern River Reserve revealed signatures of finer scale population subdivision in the San Joaquin Valley.

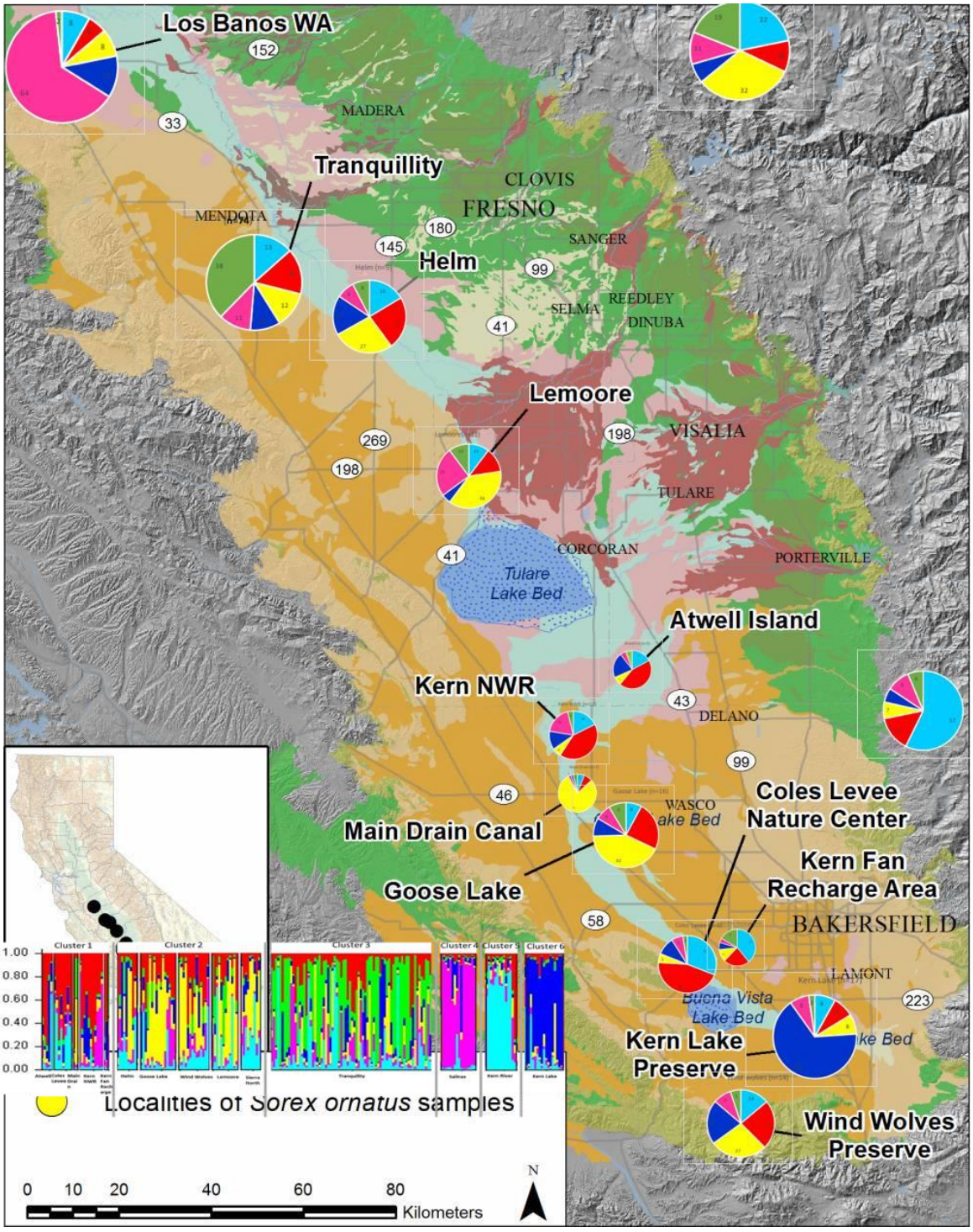


Figure 6. Map showing pie charts with the admixed proportions of genetic clusters for each locality and correlate to the STRUCTURE bar plot (lower).

EFFECT OF SAMPLE SIZES IN THE INTERPRETATION OF RESULTS

Intense small mammal surveys by ESRP and CDFW personnel resulted in the discovery of several small populations of shrews along the southern part of the SJV. Despite the repeated surveys and intense small mammal trapping, the number of samples recovered from each locality is still small. Through additional surveys conducted by ESRP personnel, we were able to obtain 12 additional samples from 4 localities that had previously yielded small sample sizes. This is probably a reflection of the rarity and difficulty of capturing shrews in these areas and non-invasive techniques developed by Cypher and Tennant will result in better detection probabilities in future surveys.

Despite the problems with sampling in the southern San Joaquin Valley localities, it is worth noting that this is by far the largest sample size of shrews ever obtained from this geographic region. Our results suggest that some of the populations are differentiated as indicated by difference in mtDNA haplotype frequencies and nuclear DNA microsatellite allele frequencies; however, because sample sizes in some of the localities that were important for this study were small, we note that it is difficult to draw firm conclusions from them. For the most part, it was not possible to estimate reliable measures of haplotype and nucleotide diversity for some locality samples, as the number of individuals examined in each was less than 5 (for e.g. Kern Fan and Main Drain). However, we feel more confident on estimates for the other eight valley populations that had sample sizes between 5 and 17.

We also note that the sample size from Tranquillity is 3 times larger in collection size and it also appears to be currently a demographically stable and large population compared to any of the southern SJV populations and this should be taken into consideration when drawing conclusions regarding comparisons of mtDNA haplotypic and nuclear allelic diversity. Furthermore, it is also important to note that Tranquillity has the largest sample of ornate shrews ever sequenced (n=76) and/or genotyped with microsatellite loci (n=74) from a single locality and this provides more power in detecting the presence of private haplotypes or alleles in the smaller sampled localities. Four haplotypes were detected in Tranquillity, more than in any single locality throughout the entire *S. ornatus* species range. This is also why we recommend using the richness values (allelic and private allele richness) when comparing between locations with unequal sample sizes.

As emphasized by Moritz (1994a,b), even with statistically adequate sample sizes it is difficult to obtain estimates of population size or gene flow that are accurate in the short term through the use of any set of genetic markers. This is particularly true for mtDNA genes, which are more prone to the stochastic effects of drift because of their smaller effective size relative to nuclear markers. Moreover, the maternal inheritance of mtDNA means that any gene flow estimates are those of females, and are thus likely to be biased towards low levels in organisms, like mammals, where female philopatry and male dispersal are typical.

MANAGEMENT RECOMMENDATIONS AND CONSIDERATIONS FOR CONSERVATION PLANNING

The ornate shrew (*Sorex ornatus*) is restricted to the vanishing wetlands of California, USA and Baja California, Mexico. Several subspecies of ornate shrews are considered "mammal species of special concern" in California by the Department of Fish and Game and one (*S. o. relictus*) has been listed as endangered by the USFWS. Populations of

shrews around Buena Vista Lake have been diminished or extirpated due to habitat deterioration and human development.

The mtDNA haplotype network revealed that most of the cytochrome *b* haplotypes were closely linked in a star shaped phylogenetic pattern including haplotypes from central clade populations outside the SJV. This pattern suggest that populations underwent a rapid demographic change. Our results suggest that some of the populations have different mtDNA haplotype frequencies although one mtDNA haplotype (Haplotype A) is common and/or present in most of the localities, six other haplotypes had not been previously detected in the central ornate shrew clade and five of those haplotypes were unique to single populations (Haplotypes B, E, F, G, and H). It is important to note that if populations south of Helm go extinct, three mtDNA haplotypes that to date have not been detected elsewhere will be lost. Wind Wolves, which was a population with almost no previous information, showed the presence of 2 haplotypes that were also found in other SJV populations. We also detected 8 private alleles and the highest private allele richness in the landscape using microsatellite markers in this locality.

We found genetic signatures of 6 population genetic clusters with moderate levels of admixture between them within the sampled areas of the SJV as follows (Figure 7). The distribution of the clusters does not follow a clean geographic pattern and are partitioned as follows: 1) Kern NWR+ Coles Levee+ Kern Fan Recharge + Atwell Island + Main Drain; 2) Goose Lake + Helm + Lemoore+ Wind Wolves; 4) Salinas; 5) Kern River Preserve; 6) Kern Lake. These populations have also retained moderate levels of genetic diversity and show levels of genetic differentiation at microsatellite loci and suggests that some of these populations have been recently isolated but the levels of admixture suggest that in the past these populations may have had higher levels of connectivity than what we see today. Note that the map in figure 7 shows the historic habitat characteristics and most of the localities in the Central Valley where shrews have been detected, are currently in areas that in the past had continuous riparian habitats and supports our results that Southern San Joaquin Valley populations have had higher levels of habitat connectivity than we see today.

Our analysis shows the power of sampling unknown locations, and how these previously undetected/ unsampled populations at the fringes of their range (such as Wind Wolves) have retained significant levels of genetic diversity. Furthermore, an overall goal of defining units for conservation management should become one that conserves both the products and the processes of evolution (Moritz 2002), a shift in focus from defining isolated products of evolution to investigating and protecting historical levels of gene flow between them (DeWeerdt 2002). Therefore, the results of the genetic structure of these localities should be considered for the overall management of the populations as they may respond independently to environmental changes (Moritz 1995). Also, an important point to consider is that if the small remnant populations currently occupying the southern SJV disappear due to habitat alteration, they are unlikely to be recolonized from elsewhere.

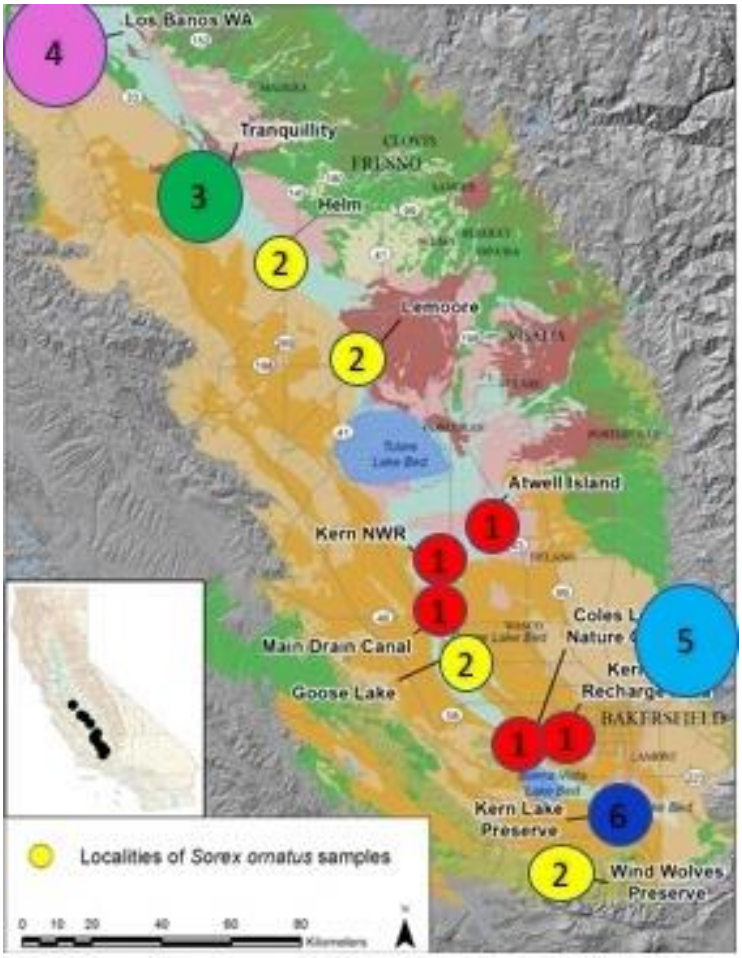


Figure 7. Simplified version of the geographic distribution of the 6 main genetic clusters detected with STRUCTURE using microsatellite loci overlaid over a San Joaquin Valley map showing the historic habitat characteristics. Note that the light blue color denotes riparian habitat connecting all of the Southern San Joaquin Valley populations.

ACKNOWLEDGEMENTS

This project was funded by the U.S. Fish and Wildlife Service through an Endangered Species Conservation and Recovery Grant to the California Department of Fish and Wildlife (CDFW) and a subsequent sub-grant to the California State University-Stanislaus. We thank ESRP personnel, B. Cypher. E. Tennant, P. Kelly, F. Vang, D. Newman, K. Garcia for assistance in conducting the field surveys and collection of samples for DNA analysis. S. Phillips for all his help with map figures and the formatting of a previous draft of a report prepared in 2006. J. Ortega, S. Young, L. Terwilliger, A. Rivara and A. Martinez, A. Carter for help with laboratory analyses. USFWS and California Department of Fish and Game for granting permits and allowing access to State and Federal wildlife reserves in the area. The current work was largely funded by an IAA between USFWS and Smithsonian Institution (IAA-F12PG00198). Previous work was also funded by the Central Valley Project Conservation Program (USBR and USFWS) and the Bureau of Reclamation SCCAO (Fresno USBR office) also helped with some funding of BVLS field work. Some of the Tranquillity tissues were collected as part of a US Dept. of Interior Land Retirement. Demonstration. Project work (also headed up by USBR, but with

USFWS and BLM as partners). The Center for Conservation Genomics at the Smithsonian Conservation Biology Institute provided equipment and support for laboratory analyses.

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APPENDIX A. LIST OF SAMPLES

Table 1. List of samples with locality, collection date, type of sample (T=tail clip, W= whole specimen, B= blood) geographic coordinates, plot number, and haplotype obtained by ESRP and CDFW for genetic analysis. Note that the last 12 samples highlighted in yellow are the newly added samples to the genetic analysis.

Sample No.	Location	Date	Sample	T,R,S or Lat/Long	Plotid	Haplotype
SO 15	Tranquillity	9-Jun-00	W	T15S R15E Sec 16	13BP2	A
SO 16	Tranquillity	9-Jun-00	W	T15S R15E Sec 16	8EP1	A
SO 17	Tranquillity	9-Jun-00	W	T15S R15E Sec 16	5AP1	A
SO 18	Tranquillity	9-Jun-00	T	T15S R15E Sec 16	6AC	A
SO 19	Tranquillity	9-Jun-00	T	T15S R15E Sec 16	11DC	A
SO 20	Tranquillity	9-Jun-00	T	T15S R15E Sec 16	7CP2	A
SO 21	Tranquillity	9-Jun-00	T	T15S R15E Sec 16	12AP1	A
SO 22	Tranquillity	9-Jun-00	T	T15S R15E Sec 16	13AC	A
SO 23	Tranquillity	9-Jun-00	T	T15S R15E Sec 16	11DP3	A
SO 24	Tranquillity	9-Jun-00	T	T15S R15E Sec 16	12AP1	A
SO 25	Tranquillity	9-Jun-00	T	T15S R15E Sec 16	10AP1	A
SO 27	Tranquillity	9-Jun-00	T	T15S R15E Sec 16	11CC	A
SO 28	Tranquillity	9-Jun-00	T	T15S R15E Sec 16	6AC	A
SO 29	Tranquillity	9-Jun-00	T	T15S R15E Sec 16	13BP1	F
SO 30	Tranquillity	9-Jun-00	T	T15S R15E Sec 16	12CP1	A
SO 31	Tranquillity	20-Jun-01	T	T15S R15E Sec 16	13EP3	A
SO 32	Tranquillity	20-Jun-01	T	T15S R15E Sec 16	20DP1	A
SO 33	Tranquillity	20-Jun-01	T	T15S R15E Sec 16	20CC	A
SO 34	Tranquillity	20-Jun-01	T	T15S R15E Sec 16	20DP3	A
SO 35	Tranquillity	20-Jun-01	T	T15S R15E Sec 16	20AP1	A
SO 36	Tranquillity	20-Jun-01	T	T15S R15E Sec 16	7DP2	A
SO 37	Tranquillity	20-Jun-01	T	T15S R15E Sec 16	20AP1	A
SO 38	Tranquillity	21-Jun-01	T	T15S R15E Sec 16	15DC	A
SO 39	Tranquillity	21-Jun-01	T	T15S R15E Sec 16	20AP3	A
SO 40	Tranquillity	21-Jun-01	T	T15S R15E Sec 16	20CP2	A
SO 41	Tranquillity	21-Jun-01	T	T15S R15E Sec 16	8AP1	A
SO 42	Tranquillity	21-Jun-01	T	T15S R15E Sec 16	20B	A
SO 43	Tranquillity	21-Jun-01	T	T15S R15E Sec 16	17BC	A
SO 44	Tranquillity	21-Jun-01	T	T15S R15E Sec 16	17EP3	A
SO 45	Tranquillity	21-Jun-01	T	T15S R15E Sec 16	7CC	A
SO 46	Tranquillity	21-Jun-01	T	T15S R15E Sec 16	17BC	A
SO 47	Tranquillity	21-Jun-01	T	T15S R15E Sec 16	17DC	A
SO 48	Tranquillity	21-Jun-01	T	T15S R15E Sec 16	17DP3	A
SO 49	Tranquillity	21-Jun-01	T	T15S R15E Sec 16	11BP1	A
SO 50	Tranquillity	21-Jun-01	T	T15S R15E Sec 16	20C	A
SO 51	Tranquillity	22-Jun-01	T	T15S R15E Sec 16	20AP1	A
SO 52	Tranquillity	22-Jun-01	T	T15S R15E Sec 16	13BP3	A
SO 53	Tranquillity	22-Jun-01	T	T15S R15E Sec 16	18AP2	A
SO 54	Tranquillity	22-Jun-01	T	T15S R15E Sec 16	13DC	A
SO 55	Tranquillity	22-Jun-01	T	T15S R15E Sec 16	20DC	A
SO 56	Tranquillity	17-Apr-02	T	T15S R15E Sec 16	18CC	A

Sample No.	Location	Date	Sample	T,R,S or Lat/Long	Plotid	Haplotype
SO 57	Tranquillity	17-Apr-02	T	T15S R15E Sec 16	19AC	A
SO 58	Tranquillity	17-Apr-02	T	T15S R15E Sec 16	7DC	A
SO 59	Tranquillity	17-Apr-02	T	T15S R15E Sec 16	17DP2	A
SO 60	Tranquillity	17-Apr-02	T	T15S R15E Sec 16	5BC	A
SO 61	Tranquillity	17-Apr-02	W	T15S R15E Sec 16	13C-P1ORP3	A
SO 62	Tranquillity	17-Apr-02	W	T15S R15E Sec 16	13C-P1ORP3	A
SO 63	Tranquillity	17-Apr-02	W	T15S R15E Sec 16	2BC	A
SO 64	Tranquillity	17-Apr-02	W	T15S R15E Sec 16	6DP3	A
SO 65	Tranquillity	17-Apr-02	W	T15S R15E Sec 16	18	D
SO 66	Tranquillity	19-Apr-02	T	T15S R15E Sec 16	1CP1	A
SO 67	Tranquillity	19-Apr-02	T	T15S R15E Sec 16	18DP1	A
SO 68	Tranquillity	19-Apr-02	W	T15S R15E Sec 16	1CP1	A
SO 69	Tranquillity	19-Apr-02	W	T15S R15E Sec 16	1CP1	A
SO 70	Tranquillity	19-Apr-02	W	T15S R15E Sec 16	4BP2	A
SO 71	Tranquillity	19-Apr-02	W	T15S R15E Sec 16	1DC	A
SO 72	Tranquillity	8-May-03	W	T15S R15E Sec 16	12EC	A
SO 73	Tranquillity	9-May-03	W	T15S R15E Sec 16	12EC	A
SO 74	Tranquillity	20-May-03	W	T15S R15E Sec 16	5CP2	A
SO 75	Tranquillity	20-May-03	W	T15S R15E Sec 16	6EC	A
SO 76	Tranquillity	20-May-03	W	T15S R15E Sec 16	1BP2	A
SO 77	Tranquillity	20-May-03	W	T15S R15E Sec 16	19BP1	A
SO 78	Tranquillity	20-May-03	W	T15S R15E Sec 16	11AP3	A
SO 79	Tranquillity	20-May-03	W	T15S R15E Sec 16	12BC	A
SO 80	Tranquillity	21-May-03	W	T15S R15E Sec 16	10AP3	G
SO 81	Tranquillity	22-May-03	W	T15S R15E Sec 16	15EP1	A
SO 82	Tranquillity	22-May-03	W	T15S R15E Sec 16	15DP3	A
SO 83	Tranquillity	23-May-03	W	T15S R15E Sec 16	6EC	A
SO 94	Tranquility	9-May-03	W	T15S R15E Sec 17	12EC	A
SO 95	Tranquility	9-May-03	W	T15S R15E Sec 18	8DP3	A
SO 96	Tranquility	9-May-03	W	T15S R15E Sec 19	8DP3	A
SO 97	Tranquility	9-May-03	W	T15S R15E Sec 20	8DP3	A
SO 98	Tranquility	9-May-03	W	T15S R15E Sec 21	8DP3	A
SO 99	Tranquility	9-May-03	W	T15S R15E Sec 22	8DP3	A
SO 100	Tranquility	9-May-03	W	T15S R15E Sec 23	8DP3	A
SO 101	Tranquility	9-May-03	W	T15S R15E Sec 24	8DP3	A
SO 102	Helm	4-Mar-04	T	T16S R17E Sec 9	H7	A
SO 103	Helm	4-Mar-04	T	T16S R17E Sec 9	I9	A
SO 104	Helm	4-Mar-04	T	T16S R17E Sec 9	H8	D
SO 105	Helm	5-Mar-04	T	T16S R17E Sec 8	D7	A
SO 106	Helm	5-Mar-04	T	T16S R17E Sec 9	H14	D
SO 107	Helm	5-Mar-04	T	T16S R17E Sec 9	I9	A
SO 108	Helm	5-Mar-04	T	T16S R17E Sec 9	G2	A
SO 114	Helm	4-Mar-04	W	T16S R17E Sec 9	G14	E
SO 115	Helm	5-Mar-04	W	T16S R17E Sec 9	G3	A
SO 89	Goose Lake	31-Jan-03	T	T27S R23E Sec 32	B2-S8	C

Sample No.	Location	Date	Sample	T,R,S or Lat/Long	Plotid	Haplotype
SO 90	Goose Lake	31-Jan-03	T	T27S R23E Sec 32	B2-S9	A
SO 91	Goose Lake	31-Jan-03	T	T27S R23E Sec 32	B2-S10	C
SO 93	Goose Lake	31-Jan-03	T	T27S R23E Sec 29	B4-S8	C
SO 137	Goose lake	11-Feb-05	T	N35.54604 W119.52214	Trap F,S14	C
SO 138	Goose lake	11-Feb-05	T	N35.53042 W119.51383	TrapES10	A
SO 139	Goose lake	15-Mar-05	T	N35.54761 W119.52476	Trap 2CS16	A
SO 140	Goose lake	16-Mar-05	T	N35.52975 W119.51454	Trap 2ES13	C
SO 141	Goose lake	16-Mar-05	T	N35.54572 W119.52203	Trap 2DS23	A
SO 142	Goose lake	17-Mar-05	T	N35.55565 W119.52410	Trap 2AS2	C
SO 143	Goose lake	17-Mar-05	T	N35.54645 W119.52359	Trap 2DS1	C
SO 1	Kern NWR	2-Mar-99	T,B	T25S R22E Sec 29		A
SO 2	Kern NWR	2-Mar-99	T,B	T25S R22E Sec 29		A
SO 3	Kern NWR	3-Mar-99	T,B	T25S R22E Sec 29		A
SO 4	Kern NWR	3-Mar-99	T,B	T25S R22E Sec 29		A
SO 5	Kern NWR	3-Mar-99	T,B,F	T25S R22E Sec 29		A
S.BAIRD	KERN NWR	?	T	Found dead by warden-Sue Baird		A
MC1998	KERN NWR	?	T	Collected by Mario Castellanos (UCLA)		A
SO 6	Coles Levee	17-Mar-99	T,B	T30S R25E Sec 30		B
SO 7	Coles Levee	18-Mar-99	T,B	T30S R25E Sec 30		B
SO 8	Coles Levee	18-Mar-99	T,B	T30S R25E Sec 30		B
SO 9	Coles Levee	18-Mar-99	T,B	T30S R25E Sec 30		B
SO 10	Coles Levee	18-Mar-99	T,B	T30S R25E Sec 30		B
SO 11	Coles Levee	18-Mar-99	T,B	T30S R25E Sec 30		A
SO 12	Coles Levee	18-Mar-99	T,B	T30S R25E Sec 30		B
SO 154	Coles Levee	6-May-05	T	N35.29007 W119.33089	Trap CL12	B
SO 13	Kern Water Bank	28-Mar-00	T	T30S R25E Sec 13		A
SO 14	Kern Water Bank	30-Mar-00	T	T30S R26E Sec 18		A
SO 84	Atwell Island	26-Apr-02	T	T24S R23E Sec 10	27C	A
SO 85	Atwell Island	26-Apr-02	T	T24S R23E Sec 10	18C	C
SO 86	Atwell Island	26-Apr-02	W	T24S R23E Sec 10	29P2	A
SO 87	Atwell Island	26-Apr-02	W	T24S R23E Sec 10	25P3	A
SO 88	Atwell Island	26-Apr-02	W	T24S R23E Sec 10	25C	A
SO 109	Lemoore	18-Mar-04	T	T19S R20E Sec19	D22	A
SO 110	Lemoore	19-Mar-04	T	T19S R20E Sec19	G7	A
SO 111	Lemoore	20-Mar-04	T	T19S R20E Sec19	G11	A
SO 112	Lemoore	20-Mar-04	T	T19S R20E Sec19	H5	C
SO 113	Lemoore	20-Mar-04	T	T19S R20E Sec19	H10	A
SO 145	Lemoore	20-Apr-05	T		LBS7C	A

Sample No.	Location	Date	Sample	T,R,S or Lat/Long	Plotid	Haplotype
SO 146	Lemoore	21-Apr-05	T		LAS5A	A
SO 147	Lemoore	21-Apr-05	T		LP8	A
SO 148	Lemoore	22-Apr-05	T		LA57C	A
SO 149	Lemoore	22-Apr-05	T		LA56C	A
SO 150	Lemoore	23-Apr-05	T		LBS7C	A
SO 151	Lemoore	24-Apr-05	T		LDS6D	A
SO 152	Main Drain	29-Apr-05	T	N35.61856 W119.61306	Trap MD10	A
SO 153	Main Drain	29-Apr-05	T	N35.61839 W119.61315	Trap MD11	C
SO 144	Wind Wolves Preserve	15-Apr-05	T		ESRP	D
SR001	Wind Wolves Preserve	24-Sept 10	T	N34.95638 W119.18601	Willows	A
SR002	Wind Wolves Preserve	24-Sept 10	T	N34.95674W119.18385	Willows	D
SR003	Wind Wolves Preserve	28-Sept 10	T	N34.97944 W119.18446	Twin Farm	A
SR004	Wind Wolves Preserve	28-Sept 10	T	N34.97943 W119.18449	Twin Farm	D
SR005	Wind Wolves Preserve	28-Sept 10	T	N34.95597 W119.18340	Willows	A
SR006	Wind Wolves Preserve	28-Sept 10	T	N34.95562 W119.18303	Willows	A
SR007	Wind Wolves Preserve	28-Sept 10	T	N34.95496 W119.18302	Willows	A
SR008	Wind Wolves Preserve	29-Sept 10	T	34.97817 W119.18513	Twin Farms	D
SR009	Wind Wolves Preserve	29-Sept 10	T	N34.97950 W119.18452	Twin Farms	A
SR0010	Wind Wolves Preserve	29-Sept 10	T	N34.95573 W119.18307	Willows	A
SR0011	Wind Wolves Preserve	29-Sept 10	T	N34.95568 W119.18313	Willows	DNW
BLS001	Goose Lake Canal x Hwy 46	30-Oct-14	T	N35.617788 W119.613891	Trap#5	A
BLS002	Goose Lake Canal x Hwy 46	30-Oct-14	T	N35.617788 W119.6138919	Trap#40	C
BLS003	Buena Vista Slough at Hwy 46 x I-5	10-Apr-14	T	N35.616693 W119.6484561	Trap#20	A
BLS004	Kern NWR U- 1	17-Apr-14	T	N35.731906 W119.579999	Trap#3A	A
BLS005	Kern NWR U-1	16-Apr-14	T	N35.731906 W119.579999	Trap#7	A
BLS006	Buena Vista Slough Hwy 46 x I-5	10-Apr-14	T	N 35.617211 W119.648669	Trap#11	C
BLS007	Bak City Recharge Canal	20-Jun-14	T	N 35.617 W 119.648	GPS1-#156	A

Sample No.	Location	Date	Sample	T,R,S or Lat/Long	Plotid	Haplotype
BLS008	Kern NWR U-7	5-Jun-14	T	N35.731906 W119.579999	GPS1-#139 WT #4	C
BLS009	Kern NWR U- 7	6-Jun-14	T	N35.731906 W119.579999	GPS 1-#139 WT #3	C
BLS010	Wind Wolves Preserve	21-Oct-14	T	N34.95568 W119.18313	Trap#7	A
BLS011	Wind Wolves Preserve	24-Oct-14	T	N34.95568 W119.18313	Trap#17	A
BLS012	Goose Lake Canal x Hwy 46	29-Oct-14	W	N35.617788 W119.613891	Dead in trap	A

APPENDIX B. TOTAL NUMBER OF HAPLOTYPES PER LOCALITY

Cytochrome be haplotype counts for each of the 15 sampling localities in Central-Southern San Joaquin Valley and surrounding areas in Central California.

Tranquillity
SampleSize=76
73 hap a
1hap d
1 hap g
1 hap f

Helm (Fresno Slough WRPG)
SampleSize=9
6 hap a
2 hap d
1 hap e

Goose Lake
SampleSize=14
6 hap a
8 hap c

Kern NWR
Sample Size=11
9 hap a
2 hap c

Coles Levee Nature Center
Sample Size=8
7 hap b
1 hap a

Kern Water Bank
Sample Size=3
3 hap a

Atwell Island
Sample Size=5
4 hap a
1 hap c

Lemoore (WRPL)
Sample Size=12
11 hap a
1 hap c

Main Drain Canal
Sample Size=4
2 hap a
2 hap c

Los Banos
Sample Size =14
14 hap a

Kern Lake Preserve
Sample size =17

17 hap h

Wind Wolves Reserve
Sample size = 13
9 hap a
4 hap d

El Portal Sierra N
Sample size= 9
8 SIN-a
1 SIN-b

Kern River Reserve
Sample size= 14
12 Hap a
2 ORNKRP

Salinas River
Sample size= 16
16 hap a