BUENA VISTA LAKE SHREW CONSERVATION: LOCATING EXTANT POPULATIONS USING NON-INVASIVE SURVEY TECHNIQUES, AND DEVELOPING GENETIC TOOLS TO FACILITATE POPULATION STUDIES

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March 2023

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ACKNOWLEDGMENTS

This project was funded by the U.S. Bureau of Reclamation through a grant from the Central Valley Project Conservation Program to the California State University-Stanislaus, Endangered Species Recovery Program. We thank Dan Strait, the CVPCP Manager, for project support and administrative assistance. For field and logistical assistance, we thank Bernadette Paul and Ellen Cypher at the CSUS Endangered Species Recovery Program; Geoff Grisdale, Katheryn (Heffernan) Jimenez, Miguel Jimenez, and Nick Stanley at the Kern and Pixley National Wildlife Refuges; Timothy Keldson and C.J. Chase from the U.S. Bureau of Land Management, Atwell Island Project; Francesca Cannizzo, Tina Rosa, and other staff at Westervelt Ecological Services and Austin Hope for access to the Lone Tree Mitigation Site; Jim Jones of South Valley Biology and Aaron Aguilera of AERA; Joe and Carolyn Belli at the Panorama Vista Preserve; Christopher Hon of California State Parks and Recreation; and Ben Ruiz of SWCA Environmental Consulting and the owners of the Goose Lake wetland area for access to that site.

EXECUTIVE SUMMARY

The Buena Vista Lake shrew (*Sorex ornatus relictus*; BVLS) formerly inhabited the interconnected seasonal and permanent lakes, wetlands, sloughs, and marshes in the Tulare Basin of the San Joaquin Valley. Approximately 95% of these habitats has been lost, leaving only isolated remnants of suitable habitat where BVLS still persist. Consequently, BVLS were federally listed as endangered in 2002. Additional information on BVLS distribution, detection methods, habitat preferences, compatible habitat management strategies, and genetic structure of populations would facilitate conservation and recovery efforts. Specific objectives of this project were to: (1) conduct additional surveys for BVLS in historic as well as previously unsurveyed locations, (2) evaluate the efficacy of cameras in detecting shrews, (3) develop an improved methodology for collecting fecal samples, (4) further assess habitat conditions preferred by BVLS, (5) further develop techniques for obtaining and analyzing genetic information on BLVS populations and individuals, and (6) develop conservation recommendations based on our findings.

Surveys for BVLS were conducted at nine locations using camera stations employing Reconyx close-focus cameras. Surveys had been conducted previously in all or portions of seven of the locations while no prior surveys had been conducted in two of the locations. BVLS were detected in one of the new locations. BVLS were confirmed to still be present at three other locations. BVLS were not detected at four other location including two where BVLS had been detected previously.

Efforts to assess the efficacy of camera stations in detecting BVLS included aiming two cameras at a single bait station and conducting repeat surveys (spaced one week apart) at specific camera station locations. The results did not conclusively demonstrate that camera stations might not occasionally miss detecting a shrew. However, cameras are still considered to be the most effective and safest technique for detecting shrews, particularly when multiple cameras are deployed throughout a given site for multiple nights.

Efforts to develop a better scat tube or other strategy for collecting BVLS scat were not successful. Issues include enticing shrews to enter devices, trampling of scats, and locating shrew scats among debris and scats from other species.

Identifying preferred habitat conditions for BVLS also proved difficult because attributes at sites with and without shrew detections were similar. The most common characteristics among sites with shrew detections were dense ground cover of vegetation or litter and moist soil conditions under the cover. BVLS appeared to adapt well to wetland management strategies designed to encourage use by winter waterfowl or nesting tricolored blackbirds. BVLS also appear to readily inhabit man-made wetlands. Thus, suitable habitat can be created for BVLS and managed for multiple uses.

The Smithsonian's Center for Conservation Genomics determined that adequate DNA for analysis could be obtained from BVLS fecal samples. Furthermore, the Center developed a new in-solution hybridization capture assay technique that can be an efficient and powerful tool when used on shrew DNA to simultaneously identify species, sex, population genetic variability, structure, and connectivity.

Recommendations include additional surveys for BVLS at new sites as access becomes available, continued use of cameras to survey for shrews, further investigation into non-invasive techniques for collecting BVLS scats, investigations of BVLS demography and ecology including habitat preferences using telemetry techniques, and additional collections of genetic samples to assess inbreeding levels, gene flow, and viability among BVLS populations.

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 2016, BVLS were known from only nine locations in the southern San Joaquin Valley (Cypher et al. 2017). 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 were considered to be the listed subspecies, *S. o. relictus* (J. Maldonado, unpubl. data; USFWS 2011; Cypher et al. 2017). Survey efforts conducted in 2016 and 2017 did locate BVLS populations at three new sites but shrews were not detected at a number of other sites (Cypher et al. 2017).

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). Cypher et al. (2017) attempted to characterize preferred habitat conditions for shrew but only was able to draw very general conclusions due to the difficulty in locating shrews and also because of the similarity between locations where shrews were and were not detected. Thus, the abundance and distribution of BVLS as well as preferred habitat attributes are still poorly understood.

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 undesirable under any circumstances but are even more concerning when working with a rare species such as BVLS. Shrews have been detected previously using non-invasive methods that may be less risky, including track tubes (e.g., Brehme et al. 2010). However, the relative efficacy of these techniques was unknown. Cypher et al. (2017) assessed the relative efficacy of several detection techniques including live-traps, track tubes, scat tubes, and close-focus field cameras. They found that the cameras had markedly higher detection rates than any of the other methods (Cypher et al. 2017, Tennant et al. 2020). As part of this effort, Maldonado (2017) determined that BVLS could be detected based on DNA from fecal samples.

Our goal with this project was to collect additional information that will contribute to conservation and recovery efforts for BVLS. Specific objectives were to: (1) conduct additional surveys for BVLS in locations within the historic range, (2) verify the efficacy of camera stations to reliably detect BVLS, (3) refine and verify the efficacy of scat tubes for non-invasive genetic sampling, (4) define optimal habitat attributes for BVLS, and (5) develop genotyping techniques to identify individuals characterize populations genetically.

STUDY AREA

The study area for this project was the southern San Joaquin Valley, California (Figure 1). The historic range for BVLS may have roughly conformed to the Tulare Lake Basin Hydrological Unit. 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).

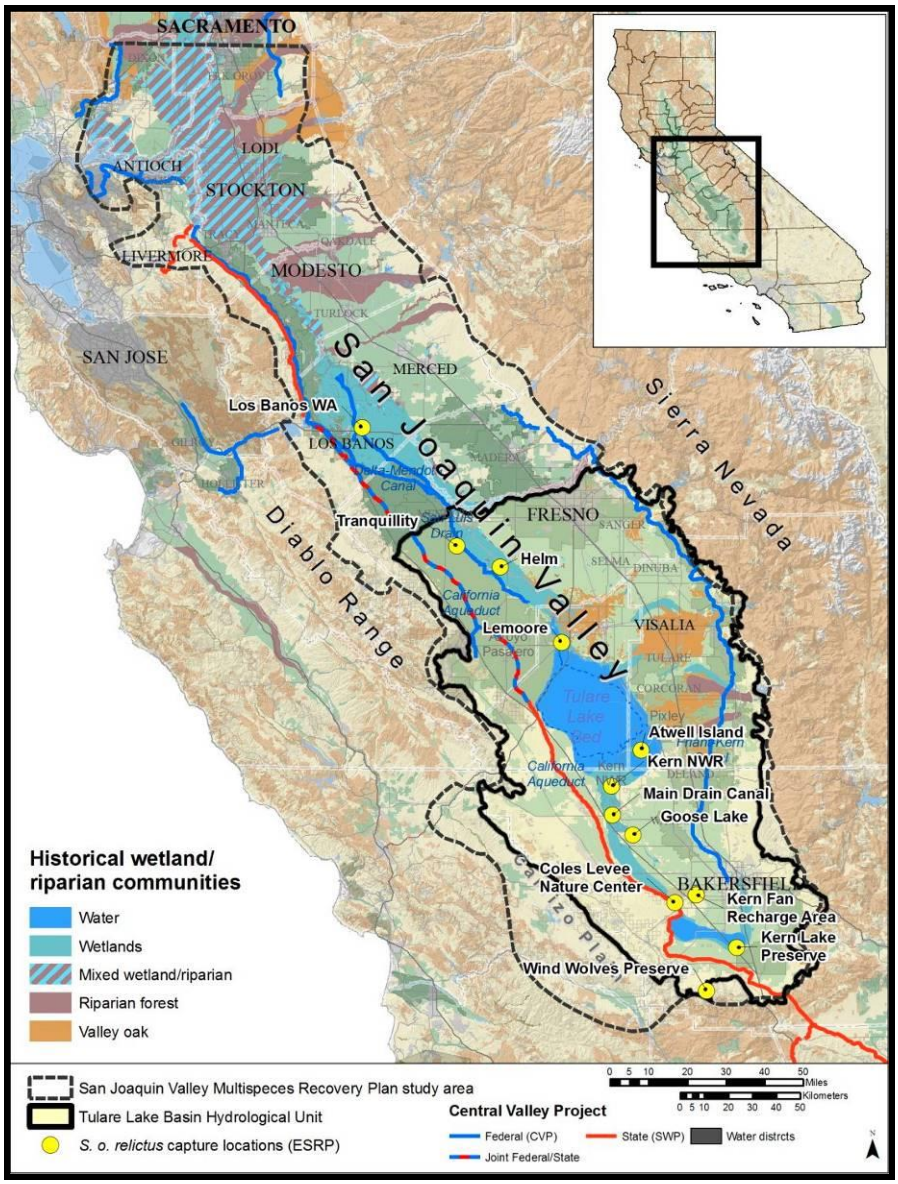

Figure 1. Locations where shrews have been detected prior to 2016 in the San Joaquin Valley, California.

BVLS primarily have been found in wetland and riparian habitats that have moist soils of 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 1). Thus, survey efforts were focused on areas with remnant aquatic habitats, particularly areas where soils remained moist year-round.

METHODS, RESULTS, AND DISCUSSION BY OBJECTIVE

OBJECTIVE 1: CONDUCT SURVEYS FOR BVLS THROUGHOUT THE RANGE

On sites on which access permission had been granted, we conducted surveys for BVLS using camera traps. Camera taps consisted of a station to which shrews were attracted with a bait and then their images were captured. We used Reconyx PC600 and PC800 cameras (Reconyx, Holmen, WI). These cameras employ a close-focus lens that is set at the factory to a focal distance of approximately 40 cm. The cameras also employ an infrared flash to reduce scaring or disturbing animals.

A camera station was established at each selected survey location within a study site (Figure 2). At each station, a small Tupperware container (ca. 9-cm diameter, ca. 7 cm deep) was installed at ground level. The container was pinned to the ground with 15-cm nail to inhibit removal by larger animals. Five to 10 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. A 1-m t-post was hammered into the ground approximately 0.5 m from the bait cup and a camera was secured to the post approximately 20-30 cm above the ground with rubberized 0.5-m gear ties. The camera was angle downward such that the bait cup was in the center of the field of view. The cameras were programmed to capture five images in rapid-fire fashion at a fast shutter speed. The stations were operated for at least seven nights at a given location. Images captured by the cameras were carefully examined for visits by BVLS. Other species visiting the stations were noted as well.

Figure 2. Typical camera station for detecting Buena Vista Lake shrews.

Locating new sites to survey for BVLS proved difficult. We focused on locating sites south of Helm because the taxonomic classification of shrews north of Helm were considered questionable (J. Maldonado, unpublished data). However, few sites could be found that had appropriate habitat, that had not already been surveyed and where permission to access the sites could be secured. Thus, only two new sites were identified and surveyed. Additional surveys were conducted at sites that had been surveyed previously but where BVLS had not been detected in those previous surveys or had not been detected in many years. BVLS were detected at four of the nine sites surveyed during this project (Table 1, Figure 3).

Table 1. Areas, dates, methods, and results for Buena Vista Lake shrew surveys conducted in the southern San Joaquin Valley, California during 2020-22.

Area	Dates	Method	Trap nights or camera nights	BVLS detected
City of Bakersfield Recharge Area	20-27 Jun 2019 27 Jun-3 Jul 2019	10 cameras per session	130	No
City of Bakersfield Recharge Area	14-22 Aug 2019	10 cameras	80	No
Lone Tree Mitigation Site	17-24 Sep 2019	10 cameras	70	Yes
City of Bakersfield Recharge Area	13-22 Nov 2019	10 cameras	90	No
Coles Levee Pond	3-10 Feb 2020	10 cameras	70	No
Panorama Vista Preserve	12-18 Mar 2020	10 cameras	60	No
Coles Levee Pond	23 Feb-2 Mar 2021	20 cameras	140	No
Tule Elk Reserve	17-24 Mar 2021	10 cameras	70	No
Atwell Island Wetland and conveyances	25 May-2 Jun 2021	19 cameras	152	Yes
Pixley NWR wetland and Deer Creek	1-8 Jul 2021	16 cameras	112	No
Pixley NWR wetland	28 Oct-4 Nov 2021	12 cameras	84	Yes
CSU-Bakersfield	22-29 Jun 2022	6 cameras	42	No
Goose Lake duck club	14-23 Sep 2022	17 cameras	153	Yes

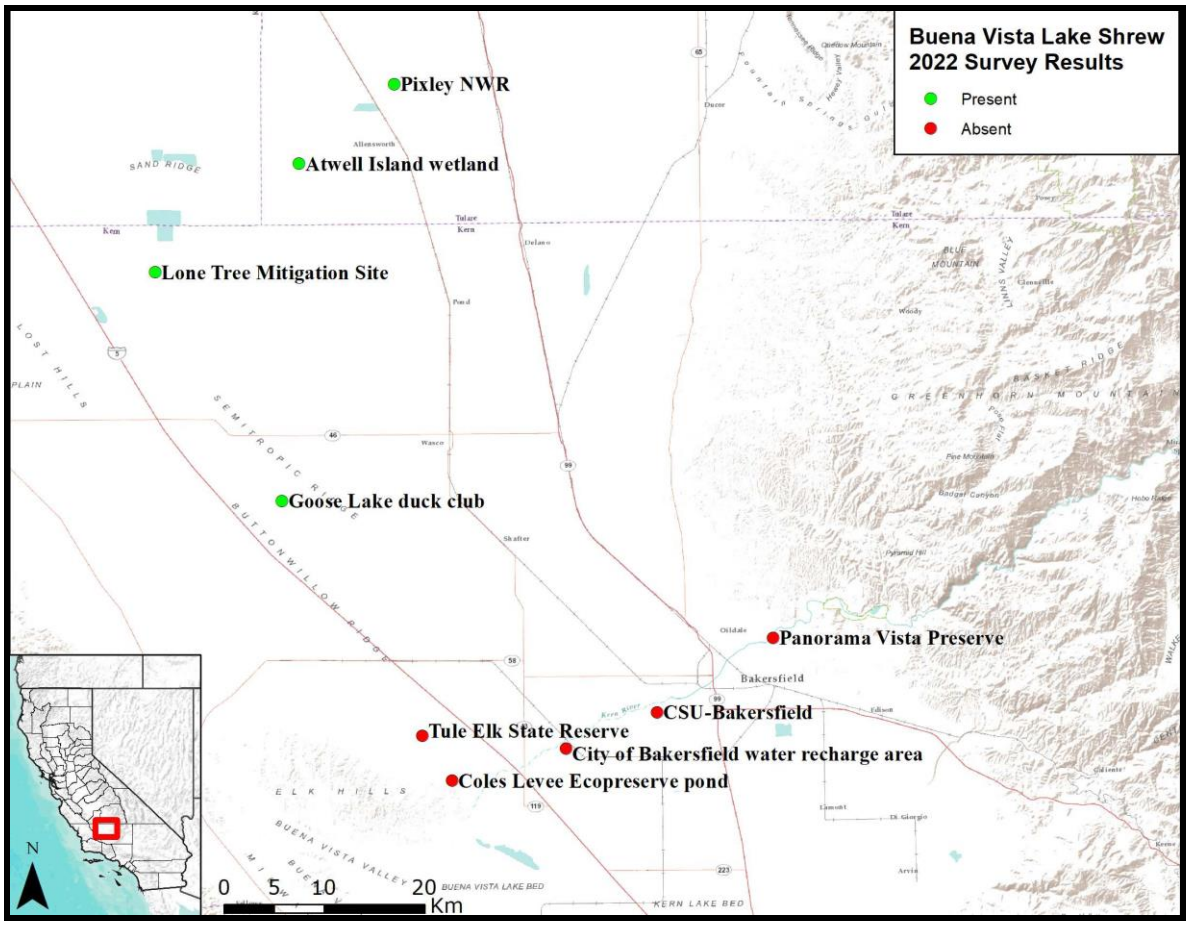

Figure 3. Locations in the southern San Joaquin Valley where surveys were conducted for Buena Vista Lake shrews during 2020-22.

One of the new sites surveyed was the California State University-Bakersfield campus. Two small (ca. 0.5 ac) man-made wetlands occur on the campus, but shrews were not detected at either location. The other new site was the Lone Tree Mitigation Site (LTMS) located adjacent to the west boundary of the Kern NWR. This site is a privately owned duck club that is being managed for BVLS by Westervelt Ecological Services under a conservation easement. The survey at this site was funded by Westervelt. This was the only new site where BVLS were detected. Detecting BVLS on this site was perhaps not surprising given the proximity of the site to the Kern NWR where BVLS appear to be abundant and wide-spread.

Surveys also were conducted at the Coles Levee Education Center pond (twice), Panorama Vista Preserve, and the Tule Elk State Reserve. All three of these sites had been surveyed during 2014-17 (Cypher et al. 2017). BVLS were not detected in those previous surveys and also were not detected in the current surveys (Table 1). The Coles Levee site was of particular interest because it has an abundance of what appears to be very suitable habitat, is undisturbed, and multiple shrews were captured there in surveys conducted in 1999 (Harpster and Williams 2001) and 2005 (ESRP, unpublished data). Water is provided to this site from a well that operates year-round. Therefore, conditions at the site are extremely stable. Thus, the inability to detect BVLS at this site in the three most recent surveys (2016, 2020, 2021) was concerning.

Surveys also were conducted at the Atwell Island Land Retirement Demonstration Project and at Pixley NWR. BVLS had been detected at both sites previously (Cypher et al. 2017) and the purpose of the additional surveys was to gather information on the distribution of shrews on the sites. No shrews were detected during the first survey conducted at Pixley NWR but were detected at multiple sites in the wetland area during the second survey. BVLS were detected at a number of locations at the Atwell Island site including along the Alpaugh Canal and along several small irrigation canals in addition to the main wetland area. Another survey was conducted at the Goose Lake duck club. BVLS had been captured at this site during surveys in 2003 and 2005 (ESRP, unpublished data). The current survey was conducted to confirm that shrews were still present because the site is being considered for conservation. BVLS were detected at one station.

Finally, additional surveys were conducted at the City of Bakersfield Water Recharge Area. BVLS had been captured in two locations on the western edge of this site in 2000 (Harpster and Williams 2001) and 2014 (Cypher et al. 2017), although no shrews were detected during other surveys conducted in 2005 (ESRP, unpublished data) and 2014 (Cypher et al. 2017). The survey locations in 2019 were dispersed more extensively throughout the 2800-ac recharge area compared to previous surveys but also included the location where a shrew was captured in 2014. Despite three survey attempts, no BVLS were detected.

OBJECTIVE 2: VERIFY EFFICACY OF CAMERA STATIONS TO RELIABLY DETECT BVLS

The reliability of camera stations for detecting BVLS was assessed using two approaches. To determine whether cameras might "miss" capturing an image of a shrew when it visited a station, we set up 10 pairs of cameras with both cameras in each pair aimed at the same bait station. This test was conducted at the Kern NWR in an area where BVLS had been detected in previous surveys. The cameras were operated for six nights and then the results were compared. BVLS were detected at three of the 10 stations. At stations where shrews were detected, both cameras at the station captured images of shrews. We did not compare detection times between the two cameras to determine how the detections matched up temporally. We would not expect each camera to capture an image at the exact same time. Although both cameras at a station were programmed with the same settings, cameras still vary with regards to detection sensitivity and trigger speed, and we also have found in other testing with cameras that the flash of one can interfere with detection sensitivity of a nearby camera. Thus, we simply considered that detections by both cameras at a given station was a success. However, we also acknowledge that the sample size for this test was small (n = 3).

The second approach entailed operating camera stations at given locations, waiting one week, and then operating stations again at the same exact locations. An attempt to conduct this test at the Kern NWR in May 2020 was unsuccessful because BVLS were only detected on one of the 20 stations established. However, a test was successfully conducted in January 2022 at the Atwell Island wetland complex. We established 20 stations throughout the complex and stations were operated for seven nights from 12-19 January. BVLS were detected at 13 of the stations. The stations were operated again for seven nights from 26 January-2 February. BVLS were detected at 15 of the stations in the

second survey. However, five of these stations did not have detections in the first survey meaning that three of the stations with detections in the first survey did not have detections in the second survey.

Thus, the results of this test were somewhat ambiguous with a number of potential explanations for the results. One potential explanation is that the cameras are indeed inconsistent in their detections of BVLS. However, we feel that other explanations are more plausible. In particular, shrews may not have visited the stations that lacked detections in the second survey. Movements, spacing, and density of BVLS and the effects of temperature, moon phase, competitors, prey distribution, and habitat attributes on these parameters all are unknown. All of these factors could affect the presence of BVLS at a given station at a given time.

Whether a camera ever fails to detect a shrew is difficult to conclusively determine. Even if this does occur occasionally, we feel that the probability that the presence of shrews at a given study site would be missed completely is likely remote if certain protocols are followed. Those protocols include operating each camera station for multiple nights. We recommend at least three and preferably seven or more. Also, multiple stations should be distributed throughout the area of interest. Minimal or optimal spacing of stations is unknown in the absence of data on shrew activity, movement, spacing, and density patterns. The cost of the Reconyx cameras could potentially limit the number available for deployment. However, if a site was relatively large, then another strategy might be to employ all of the cameras available, operate them for a period of time, and then move the cameras to new locations. This process could be repeated until the area was adequately surveyed.

Cameras clearly are currently the best technique available for detecting BVLS. Camera station detection rates were compared via an experimental design to rates obtained using live-traps, scat tubes, and track tubes (Cypher et al. 2017, Tennant et al. 2020). Cameras significantly outperformed the other techniques. In addition to capturing images of visits by shrews, the cameras also captured images of shrews entering the live-traps without being captured or entering the scat tubes and track tubes without leaving any positively identified scats or tracks. The camera stations have the further advantage of not enclosing the shrews in any way thereby reducing risks such as being ambushed by a predator upon exiting a tube or the potential dangers from traps including exposure and ants. The techniques might be further improved as more information is gathered on BVLS behavior and ecology.

OBJECTIVE 3: REFINE AND VERIFY EFFICACY OF SCAT TUBES FOR NON-INVASIVE GENETIC SAMPLING

The scat tubes used in previous technique tests (Cypher et al. 2017) consisted of two 30-cm long PVC pipes (6-cm diameter) connected by a 10-cm long 45-degree elbow. A piece of white paper 28.5 x 10.5 cm was taped to the inside bottom of each tube and mealworms were placed in the elbow. In an effort to increase the time that shrews spent in the tubes, and therefore hopefully increase the opportunity for them to leave scats in the tubes, the size of the tubes was increased by adding two additional 30-cm straight pieces of PVC with a port to facilitate rebaiting (Figure 4). In field tests conducted at Kern NWR during 14-17 April 2020, 10 of these new tubes were deployed. Small mammal activity within the tubes was extensive but no obvious shrew scats were found. Most of the animals entering

the tubes likely were deer mice (*Peromyscus maniculatus*). Many of the fecal deposits had been trampled further complicating identification of shrew scats. We also were concerned that shrews, which are secretive and seem to stick to dense cover, might not want to enter the tubes and travel the long distance required to reach the bait.

Figure 4. Modified scat tube design with a 30-cm ruler for scale.

A new tube was designed consisting of two 15-cm PVC pieces connected by two 90-degree pieces (Figure 5). Furthermore, caps were screwed into the ends of the straight pieces and small holes 1-2 cm in diameter were drilled through them. The hope was that shrews might be more inclined to enter a smaller device and that the holes would impede entry by deer mice. Ten of the new tubes were deployed at Kern NWR during 25 June-2 July 2020. Cameras also were set pointed toward the ends of each tube to determine what species were entering. No shrews were detected approaching the tubes and deer mice were detected entering the tubes.

Figure 5. Modified scat tube (left) with a 30-cm ruler for scale, and the entrances to the tube modified to try to exclude deer mice (right).

The scat tubes were not difficult to construct and deploy and the materials were not expensive, particularly compared to the cost of cameras. However, several issues likely reduce the efficacy of this technique. Shrews did not appear to be inclined to enter the devices. However, deer mice readily and frequently entered. In addition to consuming the bait, the deer mice are considerably larger than the shrews and may have prevented shrews from entering the tubes either through aggression or simple intimidation based on their greater size. The deer mice also left considerable fecal material and some of this material was trampled by the frequent visits to the tubes by the mice. Finally, our confidence in identifying shrew scats macroscopically was diminished after obtaining from another site of a BVLS defecating (Figure 6). The resulting fecal deposit looked similar to those deposited by deer mice. Therefore, we determined that obtaining fecal samples using the tubes was not likely to be sufficiently reliable, and therefore we discontinued development and testing of scat tubes.

Figure 6. A Buena Vista Lake shrew on a bait cup at a camera station. The shrew has just defecated and the fecal deposit is visible just behind the shrew.

In further pursuit of obtaining fecal samples from BVLS, we tested another technique. We set out 8-gallon plastic buckets with bait cups set in the center of the bottoms of the buckets. Five small holes approximately 2.5 cm in diameter were drilled into opposite sides of the buckets near the bottom. A camera was mounted on the inside of the lid of the bucket to record any animals entering. We also set cameras facing the holes on both sides of the bucket to record animals approaching from the outside (Figure 7). Six

of these stations were set out at Kern NWR during 27-31 January 2020. BVLS were detected entering two of the buckets (Figures 8 and 9). A shrew came up to a third bucket but did not enter. Deer mice and house mice (*Mus musculus*) also entered the buckets. The buckets were redeployed at Kern NWR during 24-28 February 2020. Shrews entered all six buckets. A final test was conducted at Kern NWR during 14-17 April 2020 and 20 buckets were deployed. Conditions were drier at the refuge during this test and shrews were not detected at any of the stations.

Figure 7. A Buena Vista Lake shrew detection station with a bucket camera trap and two external cameras.

Figure 8. A Buena Vista Lake shrew entering a bucket camera trap.

Figure 9. A Buena Vista Lake shrew inside of a bucket camera trap.

Although shrews entered the buckets, we did not recover any shrew scats. One issue, similar to that of the scat tubes, was the other species also frequently entered the buckets, deposited abundant fecal material, and also trampled a lot of the scats. Sometimes the animals also dragged dirt and plant litter into the buckets with them. Also, dried worms were scattered around on the floor, partially due to the manner in which we dropped the worms on the top of the cup and partially due to dispersion by the animals that visited the bucket. All of the material on the floor of the bucket made it more difficult to locate scats. However, the bucket technique may warrant further testing. Significantly reducing the amount of dried worms or even eliminating them completely would reduce the material on the floor of the bucket. Also, it might help to check the bucket daily and clear out any debris from the previous night. Finally, if the camera detected a shrew visiting during the previous night, then there would be a greater chance that any scats found on the floor of the bucket might be from a shrew.

OBJECTIVE 4: DEFINE OPTIMAL HABITAT ATTRIBUTES FOR BVLS

For each site where surveys were conducted for BVLS, 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). This is the same information as has been collected in previous BVLS projects (e.g., Cypher et al. 2017).

This information may be of limited value in defining optimal habitat conditions for BVLS, as concluded by Cypher et al. (2017). The attributes at the sites where BVLS were not detected are not immediately discernible from the attributes at sites where BVLS were detected. Also, detection stations sometimes were established in the same exact locations during different surveys and in a number of cases shrews were detected at a given location during one survey but not the other. An excellent example is the surveys that were conducted at the Atwell Island wetland during 12-19 January and 26 January-2 February in 2022. The 20 camera stations were established in the same exact locations for each survey in order to assess the consistency of the cameras in detecting shrews. As was discussed under Objective 2, shrews were detected at 13 of the stations during the first survey and at 15 stations during the second survey. However, five of 15 stations with detections in the second survey did not have detections in the first survey and three of the stations with detections in the first survey did not have detections in the second survey. Habitat conditions had not changed between the two surveys.

These results suggest that the presence of shrews at a given location depend on other factors in addition to the habitat conditions immediately around a camera station. As discussed under Objective 2, a number of other factors potentially could influence the presence of shrews at a given site and time. These factors might include movements patterns, home range attributes, density, mortalities, intra-specific and intra-specific competition, temperature, moon phase, and prey distribution. Nothing is known about the effects of these factors on BVLS.

The attributes measured at camera stations essentially constitute microhabitat characteristics. Locations where shrews were detected generally had dense cover consisting of green herbaceous vegetation, green grass, litter (e.g., sticks and twigs), fallen leaves, or mats of stems (e.g., cattail, bulrush). A few sites lacked the materials above but deep mud cracks (usually >20 cm deep) were present and shrews may have been sheltering in these. The presence of moist soil also seemed to be a common characteristic of sites where shrews were detected. Moist soil most commonly occurs around wetlands, in riparian areas, along canals, under dense cover, and within deep mud cracks. It is unknown whether moist soil benefits shrews directly (e.g., providing moisture or higher humidity) or indirectly (e.g., supporting a greater abundance of invertebrate prey, supporting denser vegetative cover). In the winter and early spring during the "rainy season", dense green herbaceous vegetation is widespread and the soil is universally moist. This may be a time when suitable habitat conditions are more widespread and shrews are able to travel away from dry season refugia. Also, suitable conditions may be even more widespread or persist for longer in years with greater precipitation. Thus, habitat suitability for BVLS may include a pronounced temporal component consisting of both seasonal and annual variations.

Temporal variation in habitat suitability also is observed at sites in which water levels are anthropogenically manipulated as part of some management strategy. The

amount of water and the timing of water movement into and out of sites is based on the management objectives for each site. For example, at the Kern NWR, LTMS, Goose Lake duck club as well as other duck clubs, water levels are varied to encourage waterfowl, which are harvested at these sites. Impoundments on the sites are flooded to a depth of 0.5-2 m beginning in September or October, waterfowl hunting occurs from November to February, and then the water is allowed to percolate and evaporate, or in the case Kern NWR some of the water is drawn off and used elsewhere. At Pixley NWR, flooding of impoundments begins a bit later in the fall and the objective is to provide foraging and roosting habitat for sandhill cranes (*Grus canadensis*). At the Atwell Island wetland, water levels are varied to encourage nesting tricolored blackbirds (*Agelaius tricolor*). The wetland is flooded to a depth of 0.25-1 m beginning about February, the birds nest in dense stands of cattails or tules in the spring, and then the water is allowed to percolate or evaporate.

In an effort to gather at least some information on potential temporal patterns of shrew presence as well as to gather information on the response of BVLS to wetland management strategies, we conducted multiple surveys at several sites as conditions at the sites changed. At Kern NWR, surveys for BVLS were conducted during winter-spring 2020 in one impoundment. These surveys were conducted as a collaborative effort by Geoff Grisdale, the biologist for the Kern/Pixley NWR complex. At the LTMS, surveys were conducted in the created wetland during fall-spring 2019-20. At the Atwell Island wetland, surveys were conducted from spring 2021 to spring 2022. At all three sites, camera stations were established within 2 m of the edge of standing water or, as water levels receded, in vegetation that had previously been inundated. The objective of the surveys was to determine whether shrews were present along the margins of the wetlands when fully flooded, whether moved back into the previously flooded areas as water levels receded, and if so, how quickly.

At the Kern NWR (Table 2), BVLS were present along the margins of the wetland when it was fully flooded and then appeared to rapidly move into previously flooded areas as the water receded. A similar pattern also was observed at both the LTMS and Atwell Island wetlands (Table 2). Thus, there appears to be a general pattern of shrews being pushed to the margins during flooding and then recolonizing exposed areas (where soils likely are still moist) as the water recedes. Consequently, wetland management for waterfowl and tricolored blackbirds apparently is quite compatible with occupancy by BVLS. This result is very encouraging. The LTMS, which is being managed for BVLS under a conservation easement, and the Goose Lake site, which is being considered for conservation, both are privately owned "duck clubs" where access was granted and where BVLS are present. Numerous such duck clubs with similar water management patterns occur throughout the historic range of BVLS and shrews may be present on many of them. However, most duck clubs are privately owned and we were not able to gain access to survey for BVLS. Conservation groups also are seeking opportunities in the southern San Joaquin Valley to manage or create wetlands to encourage tricolored blackbird nesting. Managed wetlands for waterfowl and tricolored blackbirds could provide important habitat for BVLS and increase the number of extant populations.

Table 2. Results of surveys for Buena Vista Lake shrews (BVLS) conducted at three wetland sites to assess the response of shrews to variations in water levels.

Dates	Nights	Water conditions	BVLS detections
Kern NWR			
3/5/20-3/9/20	4	Wetland full	5/6 cameras
3/9/20-3/19/20	10		
3/19/20-4/29/20	41[a]	Water receding	2/4 cameras
5/14/20-6/2/20	19[a]	No standing water	6/8 cameras
6/11/20-6/24/20	13[a]		
Lone Tree Mitigation Site			
9/17/19-9/24/19	7	Completely dry	5/10 cameras
2/12/20-2/19/20	7	Water receding	10/16 cameras
4/20/20-4/28/20	8	No standing water	10/20 cameras
Atwell Island			
5/25/21-6/2/21	8	Wetland full	3/7 cameras
8/16/21-8/23/21	7	Completely dry	1/16 cameras
1/12/22-1/19/22	7	Dry, just prior to flooding	13/20 cameras
4/27/22-5/4/22	7	Wetland full	7/9 cameras

[a] Only the first 10 nights were counted in order to increase comparability between surveys and sites.

OBJECTIVE 5: DEVELOP GENOTYPING TECHNIQUES TO IDENTIFY INDIVIDUALS

This portion of the project was conducted by the staff at the Center for Conservation Genomics at the Smithsonian's National Zoo and Conservation Biology Institute. A detailed report on the results of this work is presented in Appendix B.

CONCLUSIONS AND RECOMMENDATIONS

Surveys for BVLS have now been conducted in most areas in the southern San Joaquin Valley where potentially suitable habitat is present and where access has been granted. To date, surveys have been conducted in at least 28 sites and BVLS have been detected at 20 of these sites (Fig. 10). A significant caveat is that some sites have not been resurveyed in many years and the current status of shrews on some sites is unknown. For example, the Kern Lake area has not been surveyed in over 30 years now. Unfortunately, it appears that BVLS can become extirpated on sites for reasons not completely understood. BVLS previously had been detected in abundance at the Coles Levee Ecosystem pond as recently as 2005. However, three additional surveys have since been conducted at this site and BVLS were not detected in these more recent surveys. No habitat disturbance or modifications appear to have been conducted at this site and conditions seem stable. The site is somewhat small in size (approximately 2 ha [5 ac]) and one possibility is that a habitat patch of this size is insufficient to support a viable population on a long-term basis. The site is adjacent to the Buena Vista Slough that might function as a dispersal corridor in wet years. Thus, the Coles Levee Pond possibly experiences periodic BVLS extirpation and recolonization.

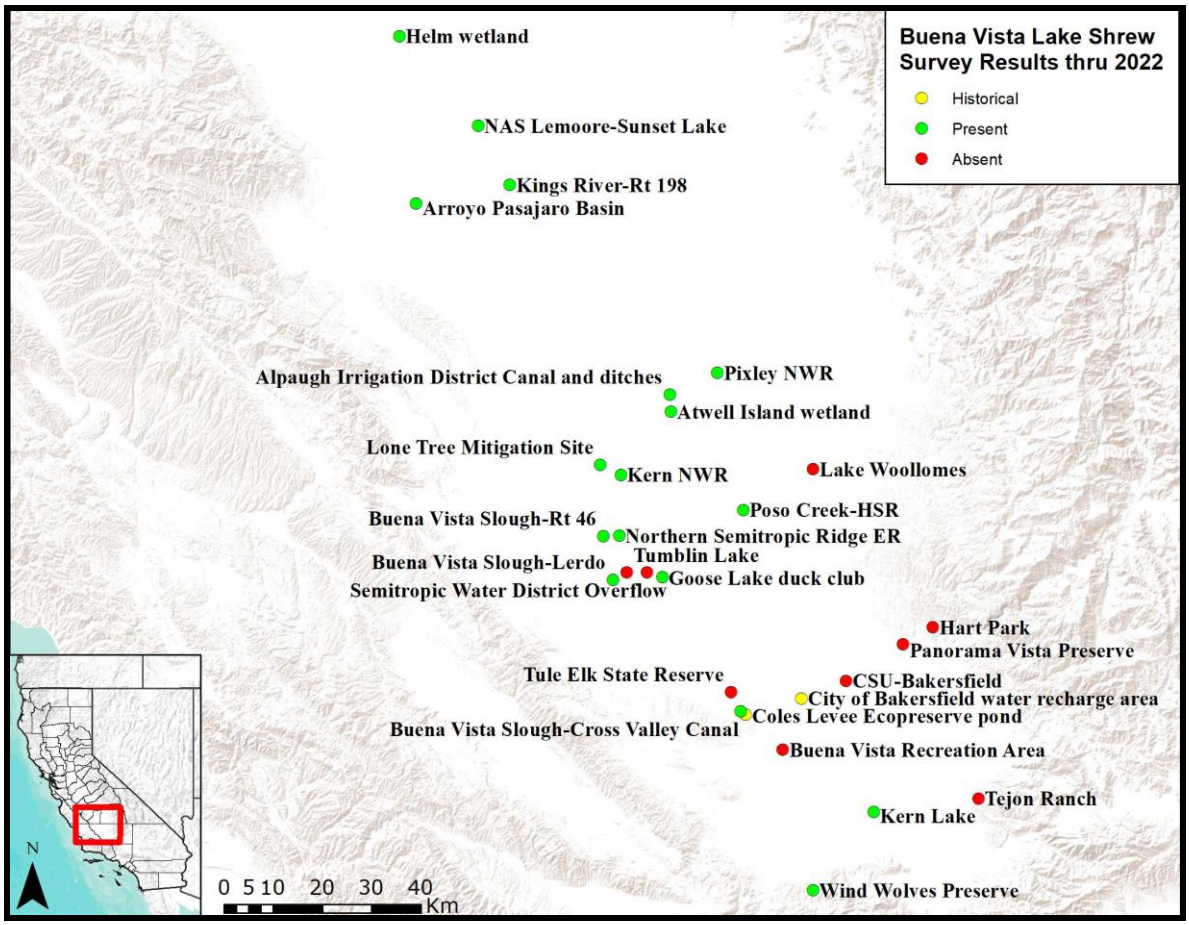

Table 10. Locations in the southern San Joaquin Valley where surveys have been conducted for Buena Vista Lake shrews (BVLS) and where BVLS have been detected through 2022.

Another site where BVLS may have become extirpated is the City of Bakersfield recharge area. In wet years, water flows down the Kern River bed through this area and some or all of the recharge basins fill with water. During these years, apparently suitable habitat for BVLS appears to be abundant over about a 1,000-ha (2,500-ac) area. However, these suitable conditions usually only last for a few months. There frequently are multiple dry years in the intervals between wet years and suitable conditions for BVLS do not appear to be present during these dry years. Furthermore, as part of the management routine in this area, vegetation that might have supported BVLS commonly is cleared from the basins and banks after they have dried. A short stretch of canal (ca. 700 m) with riparian vegetation on its banks occurs on the southwestern edge of the recharge area. Water commonly is present in this canal for one or more periods each year. BVLS have been detected previously in this canal segment but were not detected during a survey conducted in 2019. This site also is relatively small (approximately 2.5 ha [6.5 ac]) and also may be insufficient to support a viable population of BVLS on a long-term basis.

The presence and persistence of BVLS at sites may depend upon several factors. Suitable habitat conditions certainly need to be present, particularly dense ground cover of some sort (e.g., live vegetation, matted stems, leaf litter, etc.). To create and maintain suitable conditions, water needs to be present on the site for some portion of the year. Also, the presence of water may need to occur every year. Otherwise, the site may become too dry to provide sufficient cover and prey. Finally, the site may need to be of a certain size in order for BVLS populations to persist. During this project, we documented apparent extirpation of BVLS at two sites (Coles Levee Ecopreserve Pond, City of Bakersfield recharge area) that had less than 4 ha (10 ac) of suitable habitat. We also did not detect BVLS at three ponds (two on the CSU-Bakersfield campus and one at the Tule Elk Reserve) that were less than 0.5 ha (1 ac) in size. To date, BVLS have been consistently detected at sites such as the Kern NWR, Pixley NWR, Atwell Island Wetland, and Goose Lake duck Club where water is present annually and that are at least 16 ha (40 ac) in size. The probability of persistence of BVLS on a given site likely increases with the size of the site, frequency of water presence, frequency of disturbance, and quality of the habitat.

Cameras clearly are currently the best technique available for detecting BVLS. They clearly are more reliable in detecting shrews and also safer as they do not enclose or confine the shrews in any way. Usually, multiple images of an individual are obtained enhancing the opportunity for a positive species identification and providing a permanent record of the detection. Another reason cameras work so well is that only one species of shrew is found in the San Joaquin Desert area. Thus, cameras are extremely reliable in detecting BVLS. A drawback of this technique is the initial cost of acquiring cameras. Hopefully, less expensive close-focus cameras will eventually become available. Until a more effective technique is developed and proven, camera stations are the recommended method for conducting surveys for BVLS.

Methods, particularly non-invasive ones, for collecting genetic samples from BVLS warrant further investigation. For investigations for which it is necessary to live-capture shrews (e.g., telemetry studies, studies requiring marking, morphological studies, etc.), samples can easily be collected from captured individuals. However, when live-capture is not necessary but samples are needed for genetic studies, then non-invasive sampling methods would be desirable. Fecal samples likely would be the easiest samples to obtain non-invasively. We were not successful in developing an effective and reliable technique

for non-invasively collecting genetic samples. However, further investigation and development efforts are warranted.

Preferred habitat types and microhabitat conditions for BVLS still are poorly defined. These attributes have been difficult to quantify for reasons discussed previously. In general, BVLS have most commonly been detected in areas with moist soils and dense cover primarily consisting of rushes, sedges, or cattails. However, plant species composition may be less important than structure and conditions on the ground beneath the vegetation. Dense ground cover overlaying moist soils that support an abundance of invertebrates may be the primary habitat components for BVLS. The presence of these conditions may be dynamic, varying with season, annual precipitation, and management actions.

An encouraging finding from this and previous projects is that BVLS can colonize and apparently thrive in created wetlands. Kern NWR, Pixley NWR, Atwell Island wetland, and the LTMS all are anthropogenic wetlands with populations of BVLS. Further encouraging is that BVLS appear able to adapt to the fluctuating water levels associated with the management of these wetlands. Consequently, suitable habitat can be created for BVLS and such sites can be managed for multiple benefits. This significantly expands the options and potential for conserving and recovering BVLS. The primary challenge is securing a reliable source of water to wet such areas for some period of time annually thereby maintaining suitable habitat conditions for BVLS.

The genetic analyses conducted at the Smithsonian demonstrated that a newly developed in-solution hybridization capture assay can be an efficient and powerful tool that can be implemented in non-invasive studies of ornate shrews to simultaneously identify species, sex, population genetic variability, structure, and connectivity. This is significant as it means that considerable information about the genetic status of the entire BLVS subspecies, individual populations, and even individual shrews can be obtained from DNA collected non-invasively such as fecal samples.

RECOMMENDATIONS

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

1. CONDUCT 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 area where BVLS have been detected previously. Suitable habitat still appears to be present in this area. Also, there are many private duck clubs in the Tulare Basin region 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, BVLS have been detected at Pixley NWR, Goose Lake duck club, and Lone Tree Mitigation Site, all of which are managed n a similar manner to duck clubs in the southern San Joaquin Valley.

Other locations that appear to have a large quantity of habitat that might be suitable for BVLS and warrant surveys include:

- Wetland areas on lands owned by the Boswell Corporation in Tulare County (occasionally referred to as Creighton Ranch).
- Wetland areas in Kings County where water runoff from agricultural lands is collected and treated and where a portion of the area is managed for shorebirds.
- Wetland and riparian areas along the Kings River and tributaries in the Lemoore area of Kings County

2. USE CAMERAS FOR SURVEYS

Use of cameras is strongly encouraged in any surveys for BVLS. Close-focus cameras should be used to minimize misidentifications and false-negative results. To increase the probability of detecting BVLS at a given site, multiple stations should be operated for multiple nights at the site. The number of stations will depend on the size of the site and the importance of knowing more precisely where shrews are present across the site. We recommend operating stations for at least three nights and preferably seven nights.

3. FURTHER INVESTIGATE NON-INVASIVE SAMPLING TECHNIQUES

A reliable technique for collecting genetic samples, particularly scats, would be useful. A form of scat tube may warrant additional investigation, as would any other technique where shrews could be attracted to visit a location in the hope that they would defecate during the visit.

4. HABITAT PREFERENCES

Further investigation into BVLS habitat preferences, particularly microhabitat, are warranted. Such investigations likely will require more intensive research approaches including live-capture, detailed sampling around capture locations (e.g., soil moisture, invertebrate abundance, vegetation structure, etc.), or even telemetry studies to better assess habitat selection by BVLS.

5. HABITAT ENHANCEMENT AND CREATION

Clearly, it is possible to enhance or even create habitat suitable for BVLS. Investigations into strategies for enhancing habitat (e.g., retaining vegetation on canal and impoundment banks, limiting the clearing of vegetation in wetlands, etc.) are warranted. Also, additional efforts to create wetlands to benefit BVLS as well as other wildlife are warranted.

6. 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.

Collecting some of these data would be significantly facilitated by the development of telemetry techniques for BVLS.

7. GENETIC SAMPLING

Additional genetic samples should be collected whenever the opportunity presents itself. Samples can be collected from individuals found dead, tissue samples from live-captured individuals, or fecal samples deposited by shrews. Genetic analyses indicate that DNA can be extracted and amplified from shrew fecal samples. Additional samples from multiple locations can be used to provide a more detailed assessment of the genetic status of populations and the entire subspecies including taxonomic relationship to other shrews, levels of inbreeding, gene flow between populations, and population viability.

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APPENDIX A. DATA COLLECTED 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

Methods

Genomic sequence variant discovery and bait design to enable the use of non-invasive samples for genomics

Dr. Jesús Maldonado of the Center for Conservation Genomics (CCG), Smithsonian National Zoo and Conservation Biology Institute, previously demonstrated that it was possible to extract quantifiable DNA from putative BVLS scat samples collected in our scat collection tubes deployed at Wind Wolves Preserve (Cypher et al., 2017). To determine the species of these samples using traditional methodologies, the cytochrome *b* (*cytb*) mitochondrial gene was amplified by PCR and sequenced in both directions using standard Sanger sequencing methods. The sequences were edited by eye and BLASTn was used to determine the species that deposited the feces and to identify if the scat sample was from an ornate shrew.

Recent studies conducted at the CCG have demonstrated that in-solution DNA hybridization capture methods can be used to generate multi-locus genotypes from canids using even low-quality, low-quantity DNA extracted from scat samples (Parker et al., 2022). These methods can be very powerful, enabling genomics research without needing to capture or even see an animal in the wild. For the BVLS, scat-derived genomic data could be used for multiple applications in addition to species identification, including analyses of population genetic variability, structure, demography, and taxonomic classification.

To test if these capture methods can be used to genotype BVLS scat samples, it was first necessary to design a large panel of in-solution capture hybridization probes targeting Single Nucleotide Polymorphic (SNP) sites in *Sorex ornatus*. Because there are currently no published North American *Sorex* species genomes publicly available to develop these probes, the first step in this study was to sequence eight *Sorex ornatus* from the San Joaquin Valley including five genomes from putative BVLS (Table 1). We used DNA extracted from tissue samples that were stored at the CCG and used in previous studies focusing on the genetic structure of ornate shrews in the San Joaquin Valley and surrounding areas (See Cypher et al., 2017). DNA samples from ornate shrews from Goose Lake, Buena Vista Slough, Kern NWR, Wind Wolves, Tranquility, Atwell Island and Catalina Island were selected (Fig. 1, Table 1) and sequenced on an Illumina NovaSeq at the Oklahoma Medical Research Foundation (OMRF). The sequences were mapped to the reference genome of a Eurasian shrew (*Sorex araneus*, GenBank, SorAra2.0; RefSeq, GCF_000181275.1). After mapping sequence reads to the reference genome, SNPs were identified using the program BCFtools v1.9 (Li et al., 2009). Variants were filtered to include only those sites with sufficiently high quality and sequence coverage.

From the set of filtered SNP variants, bait sequences were designed targeting each SNP using BaitsTools v1.7.0 (Campana, 2018) with the same program options as in Parker et al. (2022). To enable species identification, a single, 80 base pair sequence matching the mitochondrial cytochrome *b* gene was included in the bait set. Additionally, for sex

identification, probes were designed to enrich for portions of the sex-linked zinc finger protein (*ZFX*/ZFY) and sex-determining region-Y protein (*SRY*) genes. The final bait set was further filtered by Daicel Arbor Biosciences (Ann Arbor, MI) to remove sequences with multiple BLAST hits to the reference genome and synthesized.

Enrichment and sequencing

Ten additional DNA samples that were previously collected from five localities were used to test the probe set, including five scat samples collected in Wind Wolves Preserve using scat tubes (Tennant et al., 2020) and five tissue-derived DNA samples from four localities: Kern Water Bank, Tranquility, Atwell Island, and Catalina Island (Fig. 1). A recently published, single-stranded DNA library preparation method was used to prepare genomic DNA libraries (Kapp et al., 2021). This method was originally designed for ancient DNA and was selected because of its ability to successfully prepare genomic DNA libraries from even very low-quantity, low-quality DNA. After library preparation, we followed the manufacturer's protocol (Arbor Biosciences myBaits protocol v.5) to enrich the libraries for the set of selected SNPs. The libraries were combined and sequenced on an Illumina NovaSeq at the OMRF.

Analysis of whole genome sequence data

After variants were identified using the whole genome sequence data as described above, filters were applied to remove low quality genotypes. A principal components analysis (PCA) was then performed with the SNPRelate package (v1.30.1, Zheng et al., 2012) in R (version 4.2.1) to visualize relationships between individuals. To estimate demographic histories, the Pairwise Sequentially Markovian Coalescence model (PSMC) v0.6.5 (Li & Durbin, 2011) was run with 100 bootstrap replicates. Finally, observed and expected heterozygosity and inbreeding coefficients (F_{IS}) were calculated using VCFtools v0.1.16 (Danecek et al., 2011).

Analysis of SNP enrichment data

Post-enrichment sequence reads were mapped to the Eurasian shrew genomic reference and variants were called as described above. Variants were filtered for quality. A PCA was performed as described above and observed and expected heterozygosity and inbreeding coefficients (F_{IS}) were calculated using VCFtools v0.1.16 (Danecek et al., 2011).

Species Identification

To confirm the species that deposited the scat samples, unique sequence reads that did not map to the nuclear genome were imported into Geneious Prime® v2021.2.2 (Biomatters, Ltd., Auckland, New Zealand) and mapped to the tundra shrew (*Sorex tundrensis*) *cytb* reference sequence (Genbank KM067275) using the Geneious algorithm. Consensus sequences were generated in Geneious, aligned using the MAFFT plugin, and checked for stop codons (a measure of correctness). The consensus sequences were then compared by eye to published BVLS *cytb* sequences to confirm identity.

Sex identification

To determine the sex of individuals, sequence reads were mapped to the female and male *ZFX*/*ZFY* and *SRY* consensus sequences that were used to design the baits. For *ZFX/ZFY* genes, consensus sequences were generated for each assembly in Geneious and aligned with the reference sequences using the MAFFT plugin. A sample was considered to be from a male if reads mapped to the *SRY* reference and SNPs were identified in both the *ZFX* and *ZFY* consensus sequences. A sample was considered female if no reads mapped to the *SRY* reference and no SNPs were identified in the *ZFX* and *ZFY* consensus sequences.

Results

Whole genome sequencing and probe design

After applying filters, 11,471 SNP variants remained in the whole genome sequencing data. The PCA of these data shows that the two individuals from Tranquility (putatively of the *Sorex ornatus ornatus* subspecies), cluster together and separate from the BVLS (*S. ornatus relictus*) individuals (Fig. 4). The individual from Wind Wolves Preserve falls between Tranquility and the remaining *S. o. relictus* individuals. It is interesting to note that the Catalina Island shrew (*Sorex ornatus willetti),* which was used here as an outgroup for comparison, shows a wide separation from the remaining San Joaquin Valley individuals. We also found higher average levels of heterozygosity in shrews from the San Joaquin Valley (average=33%, stdev=6.9%), compared to the Catalina Island shrew (17%) and lower average inbreeding coefficient (average=0.032, stdev=0.204) compared to the Catalina Island shrews (0.50). The average observed heterozygosity of the Tranquility shrews (*n*=2) was 37% while the average observed heterozygosity of the BVLS (*n*=5) was 34%.

Our probe design targeted 4,001 SNPs. With an average of four probes covering each locus, the total number of SNP probes was 16,475. Including probes for *SRY, ZFX/ZFY* and *cytb*, the total number of probes in the set was 16,720.

Sex identification

The results of sex determination for each of the eight shrews matched the morphologically determined sex except one that was identified as a female morphologically but as a male by sequencing. This likely indicates the difficulty of determining shrew sex morphologically – immature males without visible, descended testes can be misidentified as females.

Demographic history

The demographic history inferred by PSMC showed a decreasing, small effective population size for the Catalina Island shrew (Fig 2a.) and a much larger effective population size for the Kern NWR (BVLS) shrew (Fig. 4b.). Note that the vertical line after 10^5 years ago indicates that there are not enough segregating sites to infer demographic history in the recent past and not an increasing population size. The Kern

individual shows oscillating population size which is consistent with known population cycles previously documented in *Sorex ornatus* (Maldonado et al., 2001).

SNP enrichments

After filtering for quality but not for missingness, 3,897 SNP sites remained out of the 4,001 targeted by our probes, indicating that our enrichments were successful. After filtering for missingness (maximum missing 60%), 1,863 SNP sites remained. We removed three scats due to missing data (<10 sites) and performed a PCA with all five tissue samples and two scat samples. One scat sample was an outlier in the plot (plot not shown). This individual had extremely low heterozygosity (9.4%), and this likely influenced its outlying position in the PCA. We hypothesize that the sample's low heterozygosity values were due to allelic dropout. We therefore removed this sample and re-ran the PCA with the five tissue samples and one scat individual (Fig. 4). The PCA shows a very similar pattern to the WGS data. As expected, there was a wide separation between the Catalina Island shrew (*S. ornatus willetti*) and the remaining shrews from the San Joaquin Valley. The two individuals from Tranquility clustered together and form a separate group from the *S. ornatus relictus* individuals. The individual from Wind Wolves Preserve (scat sample) is plotted between Tranquility and the remaining *S. o. relictus* individuals. Similar to the WGS results, the Catalina Island shrew has very low heterozygosity (4.3%) compared to the average (average=23.5%, stdev=9.6%), and a higher inbreeding coefficient (0.87) compared to the average (average=0.29, stdev=0.29), while the Tranquility and San Joaquin valley shrews had similar observed heterozygosity (average=32%).

Species Identification

For species confirmation, all of the tissue and scat samples had enrichment sequence reads that mapped to the *S. tundrensis cytb* reference (range 9-837 reads); the average number of mapped reads was higher for tissues (average=359) than for scats (average=159). The translation of the consensus sequences showed no stop codons, and the sequences were identical to published BVLS *cytb* fragments. Two sequences derived from Sanger sequencing matched *S. ornatus*; two matched *H. sapiens*, and one matched *Reithrodontomys megalotis* (the western harvest mouse). This indicates that our enrichment method was more sensitive in detecting BVLS sequences in the scat-derived DNA.

Sex identification

The results of sex determination for each of the tissues were congruent for each marker (*ZFX/ZFY* and *SRY*). Two samples were identified as male and three as females. For the scat samples, three samples failed; i.e., they resulted in too few sequence reads to determine sex. The sample that generated a successful SNP genotype was identified as female. The sample that appeared as an outlier in the PCA analysis had *ZFX* sequences that were not found in other *S. ornatus* individuals. A search of the NCBI BLASTn database showed that these *ZFX* sequences matched *Peromyscus spp.* (mouse) and not *Sorex spp.* This indicates that the Zinc Finger probes hybridized with DNA sequences present in the scat sample from another species that could have been a prey or a predator species. This is

likely due to the higher level of sequence conservation in the Zinc Finger protein coding gene relative to the noncoding SNP markers that would allow the probes to hybridize with other non-target species DNA present in the scat.

Discussion

The whole genome sequence (WGS) data that we produced represent the first and only nuclear genomes from any North American *Sorex* species – the closest available published genome is the Eurasian shrew. Using these WGS data, we were able to compare heterozygosity levels between different populations of *Sorex ornatus* with those of the BVLS. We found that the Catalina Island shrew has extremely low heterozygosity, a high inbreeding coefficient, and a small effective population size (Fig. 3), which means that they may be at a higher risk for extinction compared to shrews from the San Joaquin Valley.

Our next steps will be to analyze protein coding regions of the genome to determine if there is evidence of deleterious mutation accumulation or allelic purging. This will give us more information about the genetic health of the different ornate shrew populations. We will also use phylogenetic methods to determine the timing of the diversification of the different ornate shrew populations that will allow us to better estimate the level of differentiation of the BVLS from the other subspecies. The BVLS subspecies showed low inbreeding coefficients and their demographic history indicates a pattern of fluctuating effective population sizes concordant with episodes of population cycling (Fig. 3). The observed heterozygosity of the BVLS shrews was similar to that of the Tranquility shrews (34% and 37%, respectively). Next, we will generate phylogenetic trees with our mitochondrial and nuclear WGS data in order to determine the evolutionary relationships between the BVLS and other *Sorex* species as well as the relationship of California *Sorex* to the Eurasian clade. We will use this information to determine taxonomic boundaries.

Validation of our newly developed in-solution hybridization capture assay

We found that the newly designed probe set and in-solution hybridization capture methodology developed for this study were highly sensitive and able to detect BVLS mitochondrial DNA sequences in all of the scat and tissue-derived DNA samples. We were also able to sequence sex-linked markers that allowed us to determine or confirm the sex of all tissue-derived samples and one scat-derived sample.

Interestingly, one of the scat samples produced *Peromyscus spp.* (mouse) sex-linked *ZFX* sequences. This could be the result of the scat-derived DNA containing sequences that came from an animal that consumed and/or a one that was consumed by the shrew – in the future, we could use this to identify other species that are in the area and linked through predation.

One of the five scat samples produced several hundred SNPs. This was enough information to place this sample in a PCA with the other tissue-derived samples (Fig. 4). This indicates that our method is capable of producing genotype data with sufficient loci to investigate population structure using DNA extracted from scat, even at extremely low

quantities: the sample that produced the successful SNP genotype contained only 1.8 nanograms of DNA.

We showed here that our newly developed in-solution hybridization capture assay can be an efficient and powerful tool that can be implemented in non-invasive studies of ornate shrews to simultaneously identify species, sex, population genetic variability, structure, and connectivity. This novel genomic method enables finer-scale resolution than the traditional methods that used PCR and microsatellite technologies. This will be particularly important in areas where the BVLS populations have dramatic population declines but can still be detected by implementing large-scale non-invasive surveys using scat tubes described in Cypher et al. (2017). That study demonstrated that the scat tubes are an efficient way to collect scat samples that is less risky for shrews compared to live traps because animals can enter and exit at will and are not confined in the tubes. The scat tubes are also less labor-intensive to operate than traditional survey methods using pitfall or live traps.

Anticipated products

We aim to produce two publications based on the results of this study. The first will be a molecular phylogenetic analysis of the mitochondrial and nuclear WGS data, and the second will be a description of the WGS data as well as the design, implementation, and analysis of the enrichment data.

Data Availability

The raw whole genome sequence data and SNP genotype data will be made publicly accessible in the GenBank database and NCBI Sequence Read Archive at the time of publication of the above mentioned manuscripts.

Table 1. Individuals sequenced. WGS=whole genome sequence.

Locality	Subspecies	Observed heterozygosity	Expected heterozygosity	Number of SNPs	Inbreeding coefficient	Data type
Wind Wolves Preserve - Willows	*S. o. relictus*	0.26	0.32	492	0.18	Enrichment
Wind Wolves Preserve - Willows	*S. o. relictus*	NA	NA	0	NA	Enrichment
Wind Wolves Preserve - Willows	*S. o. relictus*	1.00	0.20	1	-3.29	Enrichment
Wind Wolves Preserve - Willows	*S. o. relictus*	0.09	0.29	320	0.68	Enrichment
Wind Wolves Preserve - Willows	*S. o. relictus*	0.29	0.29	7	-0.02	Enrichment
Kern Water Bank	*S. o. relictus*	0.25	0.34	2114	0.26	Enrichment
Tranquility	*S. o. ornatus*	0.27	0.34	2114	0.19	Enrichment
Tranquility	*S. o. ornatus*	0.29	0.34	2116	0.15	Enrichment
Atwell Island	*S. o. relictus*	0.30	0.34	2113	0.11	Enrichment
Catalina Is.	*S. o. willetti*	0.04	0.34	2116	0.87	Enrichment
Goose Lake Canal x Hwy 46	*S. o. relictus*	0.36	0.34	11471	-0.07	WGS
Buena Vista Slough at Hwy 46 x I-5	*S. o. relictus*	0.33	0.34	11471	0.02	WGS
Kern NWR Unit 7	*S. o. relictus*	0.32	0.34	11471	0.05	WGS
Wind Wolves Preserve - Willows	*S. o. relictus*	0.32	0.34	11471	0.06	WGS
Atwell Island	*S. o. relictus*	0.37	0.34	11471	-0.10	WGS
Catalina Island	*S. o. willetti*	0.17	0.34	11471	0.49	WGS
Tranquility	*S. o. ornatus*	0.35	0.34	11471	-0.03	WGS
Tranquility	*S. o. ornatus*	0.40	0.34	11471	-0.18	WGS

Figure 1. Locations previously surveyed for BVLS showing collection localities of the shrew samples used in this study – map reproduced from Buena Vista Lake Ornate Shrew Species Status Assessment, USFWS, 2020.

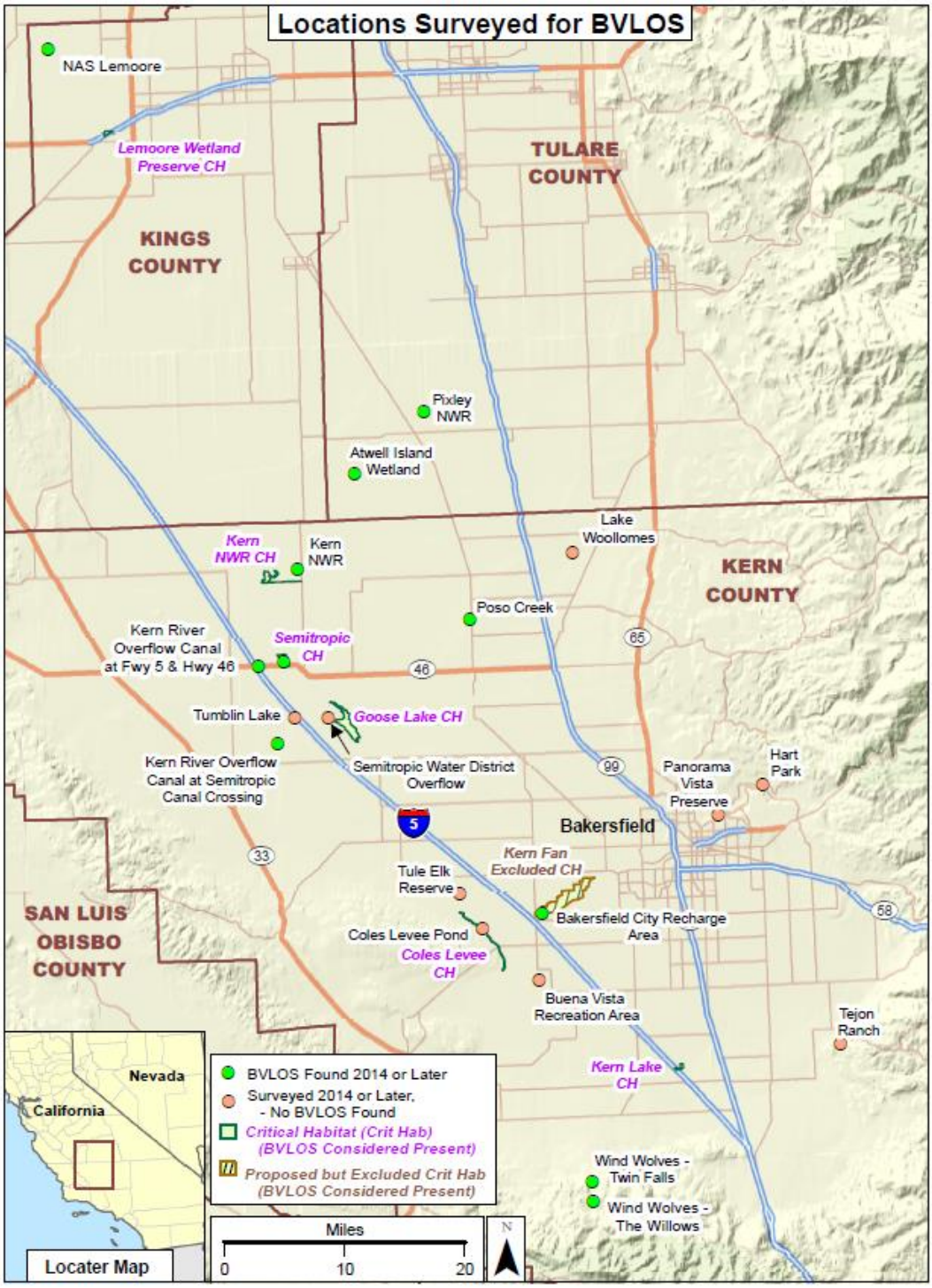


Figure 2. Whole genome variants principal components analysis (PCA). PC1 accounts for 19.5% of variation in the data; PC2 accounts for 17.4%.

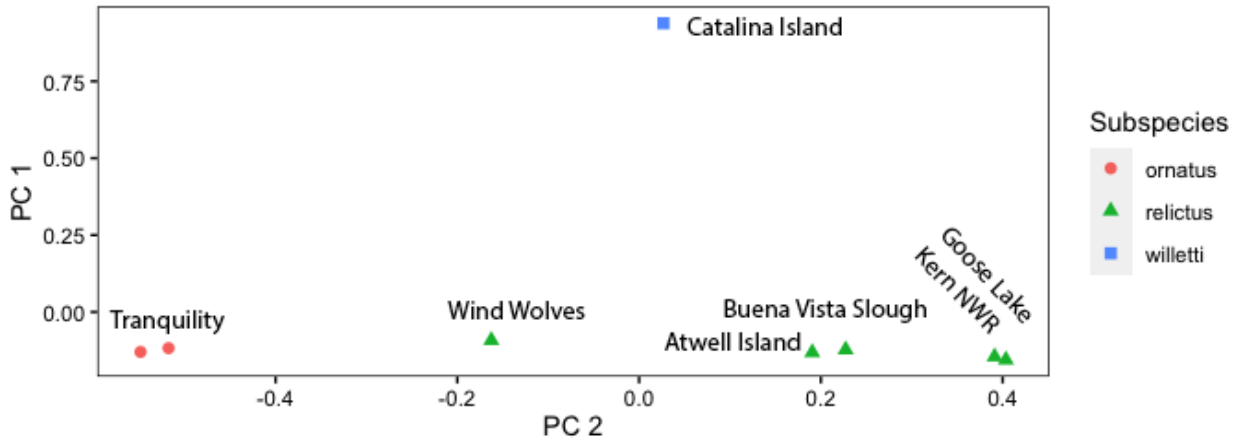


Figure 3. Demographic trends inferred by PSMC for Catalina Island (a, left) and Kern NWR (b, right).

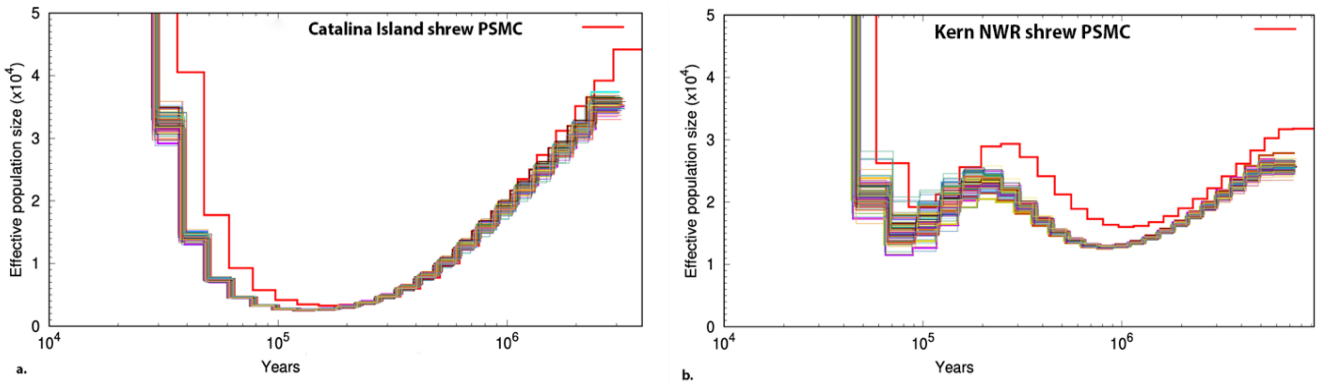
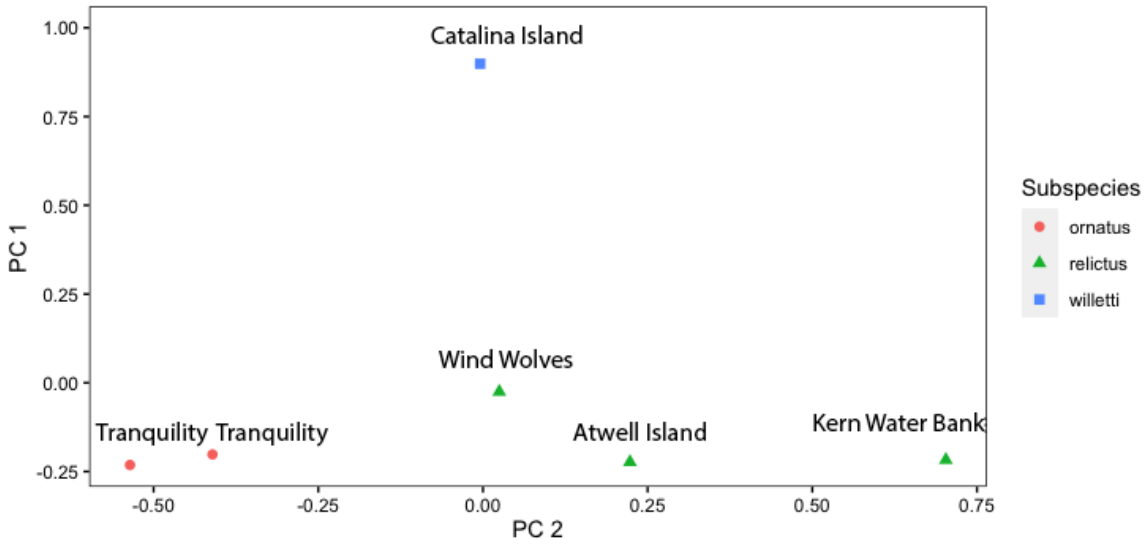


Figure 4. SNP enrichment data PCA. PC1 accounts for 41.2% of variation in the data; PC2 accounts for 20.7%. The Wind Wolves data point was derived from a scat sample; the remaining data points were derived from tissue samples.



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