FINAL REPORT

POPULATION GENETIC STRUCTURE OF THE RIPARIAN BRUSH RABBIT (*SYLVILAGUS BACHMANI RIPARIUS*): USING MULTIPLE MARKER SYSTEMS TO GAIN INSIGHT INTO HISTORIC AND ONGOING GENETIC CONNECTIVITY

SUBMITTED TO THE CVPIA HABITAT RESTORATION PROGRAM
GRANT AGREEMENT AWARD: F13AP00564
PERIOD OF PERFORMANCE: AUGUST 2013 - SEPTEMBER 2017

Marjorie Matocq[1], Patrick Kelly[2], Jennifer Rippert[1], and Scott Phillips[2]

[1]Department of Natural Resources and Environmental Science
Program in Ecology, Evolution, and Conservation Biology
University of Nevada, Reno

[2]Department of Biological Sciences
Endangered Species Recovery Program
California State University, Stanislaus

15 May 2017

POPULATION GENETIC STRUCTURE OF THE RIPARIAN BRUSH RABBIT (*SYLVILAGUS BACHMANI RIPARIUS*): USING MULTIPLE MARKER SYSTEMS TO GAIN INSIGHT INTO HISTORIC AND ONGOING GENETIC CONNECTIVITY

Marjorie Matocq[1], Patrick Kelly[2], Jennifer Rippert[1], and Scott Phillips[2]

[1]Department of Natural Resources and Environmental Science
Program in Ecology, Evolution, and Conservation Biology
University of Nevada, Reno

[2]Department of Biological Sciences
Endangered Species Recovery Program
California State University, Stanislaus

TABLE OF CONTENTS

TABLE OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENTS

This project would not have been possible without support from many quarters. We are grateful to the CVPIA Habitat Restoration Program for funding and other support in conducting the genetic research studies. We are also grateful to other federal and state agencies and programs for funding the collection of tissue samples during the course of the controlled propagation and reintroduction program (2001-2013), including the Central Valley Project Conservation Program, the U.S. Bureau of Reclamation, the U.S. Fish and Wildlife Service, the CalFed program, and the California Department of Fish and Wildlife. We are also grateful to Julie Constable who conducted the genetic analyses that this research tiered off. We are also grateful to Susan Dell'Osso for access to River Islands (Paradise Cut and other nearby areas), to the California Department of Parks and Recreation for access to Caswell Memorial State Park, to the Center for Natural Lands Management for access to Mossdale Preserve, and to David Meade and the San Joaquin County Office of Education for access to Durham Ferry. We are eternally grateful to the many ESRP biologists involved with brush rabbit fieldwork, including tissue sampling, since ESRP was established 25 years ago. Finally, we greatly appreciate the administrative support for this project we received at CSU Stanislaus and UN Reno.

ABSTRACT

California's San Joaquin Valley has faced dramatic changes in land composition over the last century, providing an example of a highly altered system with isolated remnants of native habitat. Over time, changes in habitat size and connectivity can impede gene flow between organismal populations while augmenting genetic drift within populations, resulting in long-term evolutionary consequences. Such is the case for the riparian brush rabbit (*Sylvilagus bachmani riparius*), a subspecies of brush rabbit endemic to riparian forests of the San Joaquin Valley. The riparian brush rabbit experienced substantial declines due to habitat loss (95% over the last century) and fragmentation, but also periodic flooding, drought and wildfire. As a result, the subspecies was listed as endangered by the state of California in 1994 and by the U.S. government in 2000.

To better guide recovery efforts, we identified current genetic diversity and population genetic structure of four natural remnant populations of *S. b. riparius*, three in the South Delta and Caswell Memorial State Park (Caswell MSP), as well as populations of the geographically adjacent subspecies *S. b. macrorhinus* and *S. b. mariposae*. Shared haplotypes across the current range of *S. b. riparius* suggest historic gene flow among populations. Nevertheless, nuclear genetic variation supports three distinct genetic clusters within the subspecies, suggesting recent divergence within this taxon, consistent with the history of fragmentation across the region. Despite this genetic structure, remnant populations of *S. b. riparius* maintain moderate levels of genotypic diversity, similar to populations of their neighboring subspecies. The restored population on the San Joaquin River National Wildlife Refuge (SJRNWR) holds high levels of diversity and a unique genetic composition, likely the result of its complex history of population declines, repeated translocations and natural gene flow from nearby populations, perhaps including Caswell MSP, leading us to believe that augmenting the Caswell MSP population with rabbits from the other natural populations (South Delta) or the restored population (SJRNWR) is not a concern.

By using a combination of genetic, Euclidean and effective (cost-weighted) distances in a graph theory framework, we evaluated structural and functional connectivity across the range of *S. b. riparius*. We found that genetic differentiation across this system is more strongly correlated with the effective habitat distances among populations, than it is with straight-line distance alone. Despite the overall level of fragmentation across this system, we found that habitat patches with the greatest importance for connectivity occur within and around the San Joaquin River National Wildlife Refuge (SJRNWR), where efforts to restore habitat and repopulate *S. b. riparius*' historic range have been most intense. As such, it appears that management and recovery efforts are making substantial strides in increasing both structural and functional connectivity for *S. b. riparius*. Furthermore, continued progress towards the goal of re-establishing connectivity across this system can be measured and even planned through the approaches described herein.

PART 1. PROJECT SUMMARY AND DISCUSSION

PROJECT OBJECTIVES

The overarching goal of the project was to develop the requisite genetic information to help guide management options for the riparian brush rabbit population at Caswell Memorial State Park. Specific project objectives to meet that goal are to:

1. Identify the evolutionary relationships between the riparian brush rabbit subspecies and the brush rabbit subspecies flanking the northern San Joaquin Valley.
2. Quantify genetic divergence among riparian brush rabbit populations currently inhabiting isolated or semi-isolated localities within the northern San Joaquin Valley.
3. Quantify average genetic relatedness within and recent gene flow between nearby occupied localities within the northern San Joaquin Valley.

PRIMARY CONTACT

Patrick A. Kelly

Professor of Zoology & Coordinator,
Endangered Species Recovery Program
Department of Biological Sciences
California State University, Stanislaus
One University Circle
Turlock, CA 95382

Telephone: (209) 664-6686
Email: pkelly@csustan.edu

PROJECT BACKGROUND AND RATIONALE

The riparian brush rabbit (*Sylvilagus bachmani riparius*) is a listed State and Federal endangered species (U.S. Fish and Wildlife Service 2000). It occupies riparian communities dominated by thickets of willows, wild roses, and other successional shrubs and trees in the northern San Joaquin Valley. The only known natural populations are confined to Caswell Memorial State Park (Caswell MSP) on the Stanislaus River and private lands (South Delta) along the San Joaquin River about 16 km north of Caswell MSP. In fact, when the subspecies was listed first by the State of California in 1994, the only known population was at Caswell MSP. Following a tip from a retired beekeeper, Mr. Clarence Edwards, ESRP biologists trapping in the South Delta confirmed a second population in 1998. A third population, consisting of animals translocated from the controlled propagation program (South Delta stock) and their progeny, is located on the San Joaquin River National Wildlife Refuge (SJRNWR) and some adjacent lands. Both natural populations are in San Joaquin County (Williams and Basey 1986; Williams et al. 2000), while the re-established population is in Stanislaus County (Figure 1).

The South Delta population is distributed in patches along Paradise Cut, Tom Paine Slough, the main channel of the San Joaquin River where it enters the Delta, two railroad right-of-ways near crossing points at the channel (Williams and Hamilton 2001), and at one site on the east side of the main river channel. All the land except for the interstate highway right-of-way is privately owned and is managed for cultivated agriculture, transportation, or flood control. Most of the historical habitat along the San Joaquin River and its tributaries has been lost or degraded beyond use (for brush rabbits) to agriculture, impoundment, and channelization.

Populations of riparian brush rabbits are under significant, proximate threats of extinction. Principal causes of endangerment can be linked directly to construction of dams on the Stanislaus and San Joaquin rivers, and to channelization of the valley floor portions of these rivers (U.S. Fish and Wildlife Service 1998). The population in Caswell MSP faces threats from inbreeding and loss of genetic diversity, random demographic events associated with small populations, wildfire, flooding, disease, predation exacerbated by high numbers of feral cats, and possibly from competition with desert cottontails (*S. audubonii*) (Williams and Basey 1986; Williams 1988, 1993; U.S. Fish and Wildlife Service 1998). The San Joaquin River NWR and South Delta populations face threats from stochastic demographic and genetic events, flooding, disease, predation, competition, and habitat conversion on private and state lands.

The size of the Caswell MSP population was last estimated in 1993, when we captured 41 individuals in a 3-week census in 3 sections of the Park, with an estimated population size of 213. Numbers have declined significantly since 1993. Between 1997 and 2001, no more than 6 individuals were captured in an annual census (Williams 1993, Williams et al. 2000, unpubl. data). In 2001, only 2 rabbits were captured. An average of 16 rabbits per year were captured from 2002 to 2004. In 2005 and 2006, 6 and 9 rabbits were captured, respectively, both as a result of the annual census and during trapping for a woodrat project. In 2007-2008, lower numbers were captured (1 in 2007, 2 in 2008) and the most recent survey in 2012 resulted in only 2 captures.

There has not been a population census of the South Delta population because of private property restrictions and logistic considerations. Spot trapping over a 6-month period on the parcels of private property in 1998-1999 captured 18 riparian brush rabbits. In 2001 we were given permission to trap more extensively along Paradise Cut on River Islands LLC property. During trapping over 4 nights and 5 days in August 2001, we captured 21 riparian brush rabbits at 3 sites. Brush rabbits were captured at three of four sites sampled at a combined rate 3.3 times higher than the highest capture rate in Caswell MSP (Williams and Hamilton 2001). The riparian community differed markedly from Caswell MSP. Caswell MSP consists mostly of old-growth valley oak

forest, with an abundance of grape vines cloaking trees and shrubs, and a large quantity of downed woody material littering the ground. The South Delta habitat represents various riparian successional stages from thickets of sand bar willows to large patches of an invasive weed, the white-topped peppergrass, *Lepidium latifolium* (Williams and Hamilton 2001).

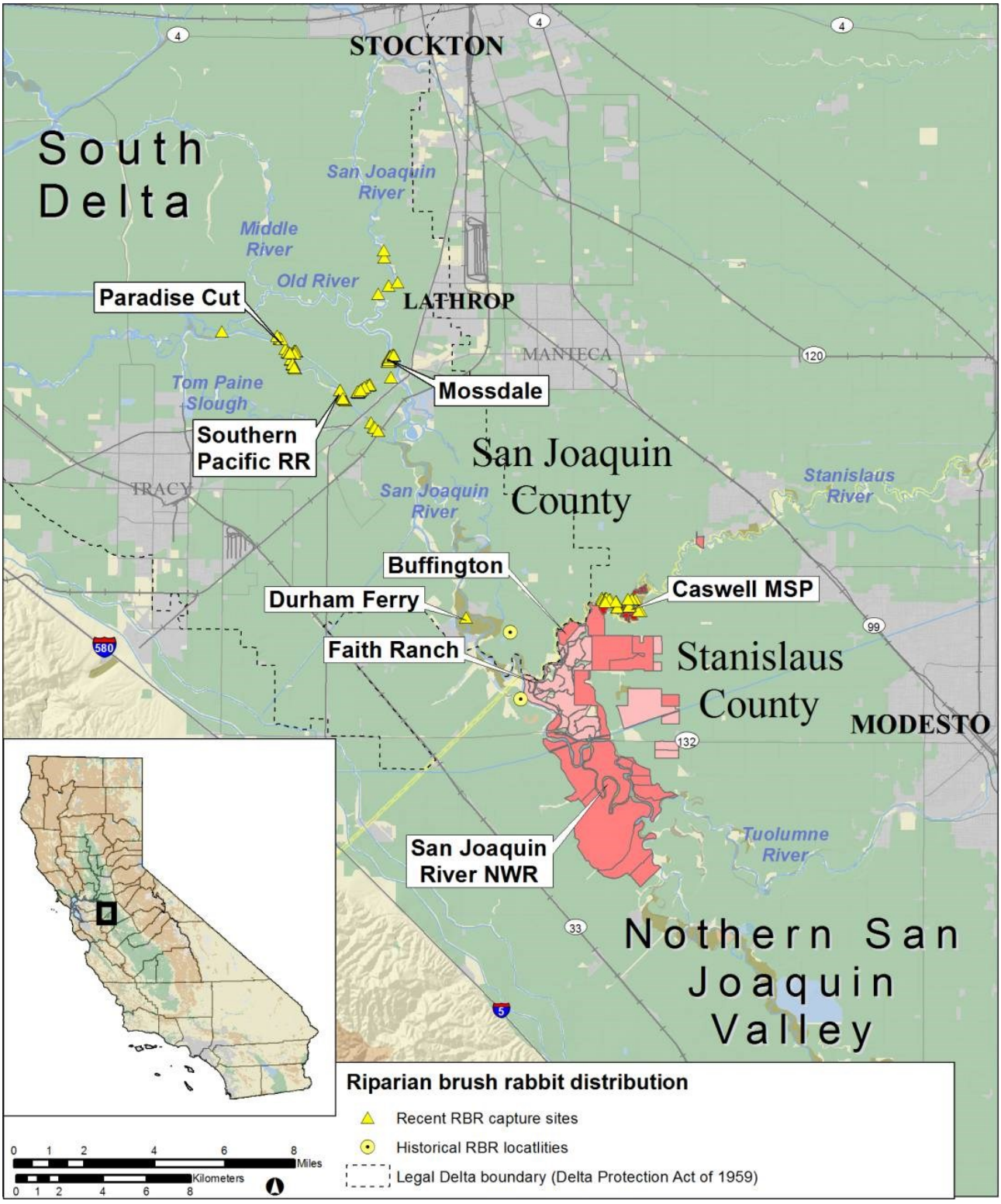

Figure 1. Locations of eight populations of *S. b. riparius*.

In February 2003, we discovered a new population of riparian brush rabbits in the South Delta on the east side of the San Joaquin River within the city limits of Lathrop. The site occupies 27 acres with natural vegetation located south of the Mossdale Landing development. We set 89 traps in the area and captured 13 rabbits during a 12-hour period. This is the only population that has been located in the South Delta on the east side of the main channel of the San Joaquin River. In 2004, Union Pacific Homes created the San Joaquin River Oxbow Preserve, also known as the Mossdale or Mossdale Oxbow Preserve, which is now owned and managed by the Center for Natural Lands Management, as mitigation for their development in the city of Lathrop. Because of its small size and proximity to the San Joaquin River, the resident brush rabbit population is highly vulnerable.

One potential strategy to augment the declining number of endangered riparian brush rabbits within their currently occupied range and to reduce the negative genetic impacts of small population size, is to translocate rabbits from areas with relatively high densities (i.e. South Delta), or their progeny (from the controlled propagation program). However, before attempting to mix animals from various native locales, it is imperative to understand the genetic history of the populations in question. Specifically, recent analyses of the partly co-distributed riparian woodrat (*Neotoma fuscipes riparia*) revealed a unique and complex evolutionary history of this subspecies in the northern San Joaquin Valley, that includes hybridization between the woodrats that currently occupy the western and eastern flanks of the Valley (Matocq et al. 2012). As such, it is entirely possible that riparian brush rabbits have an equally complex history in this region, but being able to confidently reveal this history requires the deployment of multiple genetic marker sets that each have the capacity to resolve different evolutionary depths of history.

The most recent genetic study of riparian brush rabbits revealed significant population and subspecies differentiation using a relatively small number of microsatellite markers (Constable et al. 2010). However, it is difficult to identify population and subspecies relationships with only rapidly evolving microsatellite markers because of their limited capacity to reveal deep history, and yet the information they do provide is absolutely central to identifying recent patterns of gene flow and patterns of individual relatedness. To more completely characterize the genetic history, both deep and recent, of extant Valley populations, our investigation coupled rapidly evolving microsatellite markers with more slowly evolving mtDNA sequence data, along with the deployment of new techniques allowing the estimation of population divergence and evolutionary relationships based on a broad view of genomic variation through single nucleotide polymorphisms (SNPs). This multifaceted approach provided us with deeper insights into the history of this endangered subspecies and its few remnant populations.

A better understanding of remnant populations is critically needed if we are to conserve brush rabbits and other species with fragmented distributions on the floor of the San Joaquin Valley. Kelly et al. (2005) estimate that approximately 70% of the San Joaquin Valley has been converted to irrigated agricultural, or to a lesser extent, urban use since the mid-19th century, and that 95% of historical riparian forest and oak woodland were converted to agricultural use by the year 2000. Loss of habitat associated with this land conversion is the primary cause of species endangerment for upland species of the San Joaquin Valley (U.S. Fish and Wildlife Service 1998). Loss of riparian habitat – both due to direct land conversion and diversion or alteration of stream channels – has been the primary threat to known RBR populations. Further, changes in climate, particularly precipitation, have the potential to directly impact the availability of suitable RBR habitat by changing the type and structure of available vegetation (Lenihan et al. 2003) and the severity of wet-season flood events (Brekke et al 2004). In addition to changes of habitat quality, vegetation changes may also alter the frequency of wildfires (Lenihan et al. 2003).

As previously mentioned, the known range of the riparian brush rabbit is extremely restricted today. This restricted distribution, the primary cause of their endangerment, also makes it particularly vulnerable to changes in vegetation and the frequency and severity of floods and wildfires. Because land outside of the Caswell MSP and SJRNWR boundaries are nearly all in private ownership, largely developed for agriculture or other purposes, and unlikely to harbor sustainable RBR populations (habitat is gone, degraded or mostly unprotected from flood and fire), there appears to be limited opportunity for RBR to migrate to new sites in response to vegetation or other changes unless existing and new habitat is restored to meet their needs. While habitat restoration and additional introductions (or re-introductions) of RBR to restored sites may be required in response to changing climatic conditions, we lacked a basic understanding of the genetic relationship between RBR the South Delta and Caswell MSP.

Because the population of riparian brush rabbits at Caswell MSP is small and a potential but so far unrealized candidate for augmentation by translocation, it was imperative to resolve its genetic history and relationship to nearby brush rabbit populations. As previously mentioned, the Constable et al. (2010) study showed that the Caswell MSP and South Delta populations are genetically distinct, but because their methods cannot distinguish the evolutionary depth of divergence, it was critical to couple their data with genetic markers that have the ability to resolve deeper relationships, like mitochondrial sequence data and single nucleotide polymorphisms (SNPs) from throughout the genome. Our methods are elaborated on in the following sections and Manuscripts. The coupled approach, Constable et al. (2010) with ours, would give us the insights we sought into the evolutionary depth of divergence between the populations and their relationships to nearby subspecies. Further, we sought to identify patterns of genetic connectivity between occupied areas, whether they were restored or natural populations.

Given the small size and particular vulnerability of the natural population at Caswell MSP, we felt that it was particularly urgent to resolve the question about its genetic relationship to the South Delta population. Despite its small size, the Caswell MSP population has not been augmented with progeny from the breeding program, in part because of genetic concerns. Nevertheless, one male rabbit (#40598M) initially captured in 2011 as a juvenile (220 g) on the U.S. Fish and Wildlife Service's Buffington Tract (a reintroduction site on the opposite [south] bank of the Stanislaus River) was captured as an adult (650 g) about 0.75 mi. to the east on Caswell MSP in Feb. 2012. This suggested that there might be recent and/or periodic gene flow between these locations. By combining various methods, we will be able to provide the most complete view of the genetic history of these populations, both deep and recent, to help develop and guide management options for the Caswell MSP population and contribute to its long-term persistence. Because of its small size, it is at higher risk of being lost to stochastic events such as flooding or wildfire. Management options developed in part from this research will hopefully prevent such an outcome.

METHODS

This genetics project focused primarily on known populations of the riparian brush rabbits (*S. b. riparius*) and secondarily on areas with populations of the brush rabbit subspecies occupying flanking areas, the California coastal range (Alamada and Santa Clara counties; *S. b. macrorhinus*) and the south-central Sierra Nevada foothills (*S. b. mariposae*).

Manuscript #1, below, details the laboratory methodology, analyses, and interpretation of data associated with this project. To briefly summarize, and in relation to major Tasks outlined for the project:

- Task 1: *Sample collection*. Not funded.
- Task 2: *Conduct DNA extraction/quantification, mtDNA sequencing, microsatellite and single nucleotide polymorphisms (SNP) genotyping*. The methods section of Manuscript 1, below, details our approach to DNA extraction, mtDNA sequencing, microsatellite genotyping, and the generation of a SNP dataset. Our initial proposal suggested that we would develop new microsatellite markers for brush rabbits, but we found that existing microsatellite markers that were designed in the years between the analyses of Constable et al. (2010) and the current project provided robust levels of diversity for our project. As such, we invested any cost savings into processing additional samples.
- Task 3: *Conduct analysis of mtDNA, microsatellite, and SNP results*. The analysis section of Manuscript 1 details our approach to data analysis of mtDNA sequences, microsatellite genotypes, and SNP genotypes. Our initial project proposal suggested that we would employ the software programs ARLEQUIN and FSTAT to conduct basic microsatellite analyses, but the program GenAlEx is a suitable alternative that allows the basic population genetic analyses of the initially proposed programs, but also Principal Coordinates Analysis, as presented in Manuscript #1. Likewise, for the analysis of SNPs, we initially suggested using the software STACKS, but ultimately chose to use custom PERL scripts designed at our institution for more efficient and customized analytical throughput.
- Task 4: *Conduct data interpretation and report preparation*. The discussion section of Manuscript 1 details our interpretation of the primary genetic results generated during this project. Periodic interim reports were generated during the project period, an initial draft final report was submitted at the end of September 2016, and the current report constitutes the second draft of the final report.

RESULTS AND DISCUSSION

Changes in landscape composition and connectivity can be powerful drivers of evolutionary change. While many natural changes occur at rates that allow organisms to adapt, anthropogenic changes often occur rapidly and over large spatial scales, challenging the adaptive potential of native organisms. Widespread anthropogenic changes not only decrease the presence and arrangement of habitat (structural connectivity), but also affect habitat quality and how organisms interact with the landscape (functional connectivity). The San Joaquin Valley provides an example of changes at both temporal scales. A product of millennia of hydrologic and geologic change, the San Joaquin Valley has experienced substantial changes in landscape composition over the last century, resulting in a highly altered system with isolated remnants of native habitat. The limited availability and connectivity of native habitat can impede gene flow between populations while augmenting genetic drift within populations, significant concerns for many endangered species in the San Joaquin Valley, including the riparian brush rabbit.

Using a combination of molecular data and graph theory approaches, we assessed the genetic diversity, population genetic structure, and structural and functional connectivity of the riparian brush rabbit. We find that remnant populations of *S. b. riparius* share mitochondrial haplotypes, suggestive of historic connectivity throughout their range. However, analyses of contemporary genetic differentiation and structure suggest the presence of three genetic clusters within the subspecies, corresponding to the geographic locations of natural populations, indicating that gene flow is likely limited by habitat fragmentation (Manuscript 2). Landscape analyses further support these data, indicating strong support for isolation by effective habitat distance and limited connectivity between habitat patches throughout the riparian brush rabbit's range (Manuscript 2).

While these findings highlight the extensive fragmentation of *S. b. riparius*' range, the restored population at the San Joaquin River National Wildlife Refuge shows high levels of genetic diversity and functional connectivity. As such, *S. b. riparius* would likely respond favorably to additional augmentation and restoration efforts. In particular, we are not concerned about augmenting the Caswell MSP population with rabbits from the other natural population (South Delta) or the restored population (SJRNWR). The restored population provides an encouraging perspective on the role of translocations and habitat restoration in increasing functional connectivity, a model that could be replicated at Caswell MSP or elsewhere in the subspecies' range (South Delta; Dos Rios, etc.). With regard to differentiation, the SJRNWR population retains a strong affinity to the South Delta, consistent with its augmentation history, yet exhibits less differentiation from the nearby Caswell MSP than do the South Delta populations.

Further, while genetic differentiation between the SJRNWR and natural populations no longer provides an unaltered measure of natural gene flow, our results suggest recent gene flow between the refuge and Caswell MSP—or other nearby, undocumented populations. In addition to augmentation through translocations, the SJRNWR, along with the geographically adjacent Faith Ranch and Buffington Tract, has been subject to substantial habitat restoration over the last 15 years. This has had the effect of increasing habitat connectivity among these restored populations and therefore supports the potential for migration and gene flow between the Caswell MSP and SJRNWR populations. Therefore, continued recovery and restoration efforts are not only essential for increasing the functional connectivity between RBR populations, but are likely the best options for management and recovery of this subspecies. Further, the functional connectivity of riparian habitat we have documented has the potential to benefit other sensitive or endangered species that use the habitats (e.g., riparian woodrat).

When this research project was proposed in 2012, specific outcomes were expected:

1. Additional occurrence records from the planned trapping effort.
2. Additional tissue samples for this and future genetic analyses.
3. The development of new microsatellite and SNP markers that will be useful in this and future studies of this species, as well as those of closely related species.
4. Deposition of all genetic data in publicly accessible databases such as Genbank for use by other researchers.
5. Reconstruction of phylogenetic affinities among populations of three brush rabbit subspecies in California.
6. Quantification of population divergence among Valley populations using three marker systems that, together, can resolve a wide range of evolutionary depths.
7. Timely publication of findings in widely read journals to attract further research interest and funding to this system.

Since Task 1 was not funded, outcomes 1 and 2 were largely unachieved, although we did acquire some additional tissue samples from ongoing work or other projects. Although new SNP markers were developed, it was not necessary to develop new microsatellite markers; the SNP markers will be useful in further studies of RBR and closely related species (outcome #3). To the extent possible and appropriate, genetic data resulting from this research will be made accessible to other researchers in publicly available databases (outcome #4); it is not necessary to make all the SNP data available. Outcome #5, achieved (Manuscript 1, Figure 3). Outcome #6, achieved (Manuscript 1). Outcome #7, in process: two manuscripts already in draft form (Manuscript 1, Manuscript 2).

SUMMARY OF EXPENDITURES

Labor

CSU Stanislaus

Regular Salaries and Wages	$18,405.99
Benefits	$10,140.28
General Operations	$2,275.38

Travel

Travel	$1,039.62

Contractual

University of Nevada, Reno

Graduate Salaries/Wages	$34,488.57
Benefits	$2,223.55
Tuition and Fees	$3,736.55
General Operations (lab costs)	$22,505.78

Indirect costs:

F&A (UNR)	$8,085.55
CSU Stanislaus indirect (CESU)	$9,950.73

Total	**$112,852.00**

PART 2. MANUSCRIPT 1: POPULATION GENETIC DIVERSITY AND STRUCTURE OF THE RIPARIAN BRUSH RABBIT (*SYLVILAGUS BACHMANI RIPARIUS*)

INTRODUCTION

Landscape change is a key driver of evolutionary change. This is evident in the California Floristic Province, where millennia of geologic and hydrologic change have molded one of the world's most biodiverse regions (Hewitt 1996, Mittermeier et al. 2000, Jacobs et al. 2004, Davis et al. 2008). As a result, this region harbors numerous pockets of endemic richness and a wide variety of intra- and interspecific diversity (Davis et al. 2008, Feldman & Spicer 2006, Rissler et al. 2006, Matocq et al. 2012).

California has undergone another wave of substantial change since European settlement. Since its first census in 1850, the human population of California has grown from just over 97 thousand individuals—less than 2 people per square mile—to over 37 million individuals in 2010—approximately 239 people per square mile (U.S. Department of Commerce 1996, U.S. Census Bureau 2012). As the human population of the state has increased, natural landscapes have increasingly been replaced by agricultural and urban landscapes (Cincotta et al. 2000, Kelly et al. 2005, U.S. Census Bureau 2012). When natural habitats are converted to anthropogenic landscapes, the effect is two-fold: fragmentation in the traditional sense—a change in the spatial arrangement of natural habitat from large contiguous patches to smaller patches surrounded by non- or lesser-habitat (Wilcove et al. 1986), and loss of habitat—in area, quality, or both (Lindenmayer & Fischer 2006). Gene flow between patches decreases with increasing patch isolation, while decreasing patch size or quality can limit the effective population size and augment genetic drift (Mendez et al. 2014).

California's San Joaquin Valley is a prime example of profound habitat changes at the human-wildlife interface. Since European settlement, the Valley has lost over 95 percent of its riparian gallery forests (U.S. Fish and Wildlife Service 1998; Kelly et al. 2005). Remaining patches of riparian habitat are small and highly fragmented, posing serious challenges to the endemic species relying on this habitat. The riparian brush rabbit (*Sylvilagus bachmani riparius*) is one such endemic. Unlike its neighboring subspecies, which occupy the chaparral habitats of the Diablo range to the west of the Valley (*S. b. macrorhinus*) and the Sierra foothills to the east (*S. b. mariposae*), *S. b. riparius* occupies the dense brush of the San Joaquin Valley's riparian forests. In addition to substantial habitat loss and fragmentation of the *S. b. riparius* historic range, remnant populations of *S. b. riparius* have also experienced declines due to flooding, fires, and drought. While the genetic impacts of the latter events are often mitigated by gene flow in subspecies with more robust habitat availability and connectivity, such as *S. b. macrorhinus* and *S. b. mariposae*, the combination of habitat loss and natural disasters are likely to have a profound impact on the genetic structure and differentiation of remnant populations of *S. b. riparius*.

By the late 20th century, the extent of *S. b. riparius* was limited to four natural populations referred to as: Paradise Cut, Southern Pacific Railroad (SPRR), and Mossdale, collectively termed the South Delta, and Caswell Memorial State Park (CMSP) to the south (Figure 2). The populations at the northern end of the subspecies' range occur over numerous small patches of riparian habitat along the San Joaquin River. These patches are punctuated by both agricultural and urban landscapes. In contrast, CMSP is comprised of one square kilometer of contiguous riparian habitat along the Stanislaus river approximately 15 kilometers south of the South Delta. Like the South Delta populations, CMSP is surrounded by agricultural lands. Due to its severely limited range and

small population sizes, the riparian brush rabbit was listed as a State endangered species in 1994 and Federally endangered species in 2000.

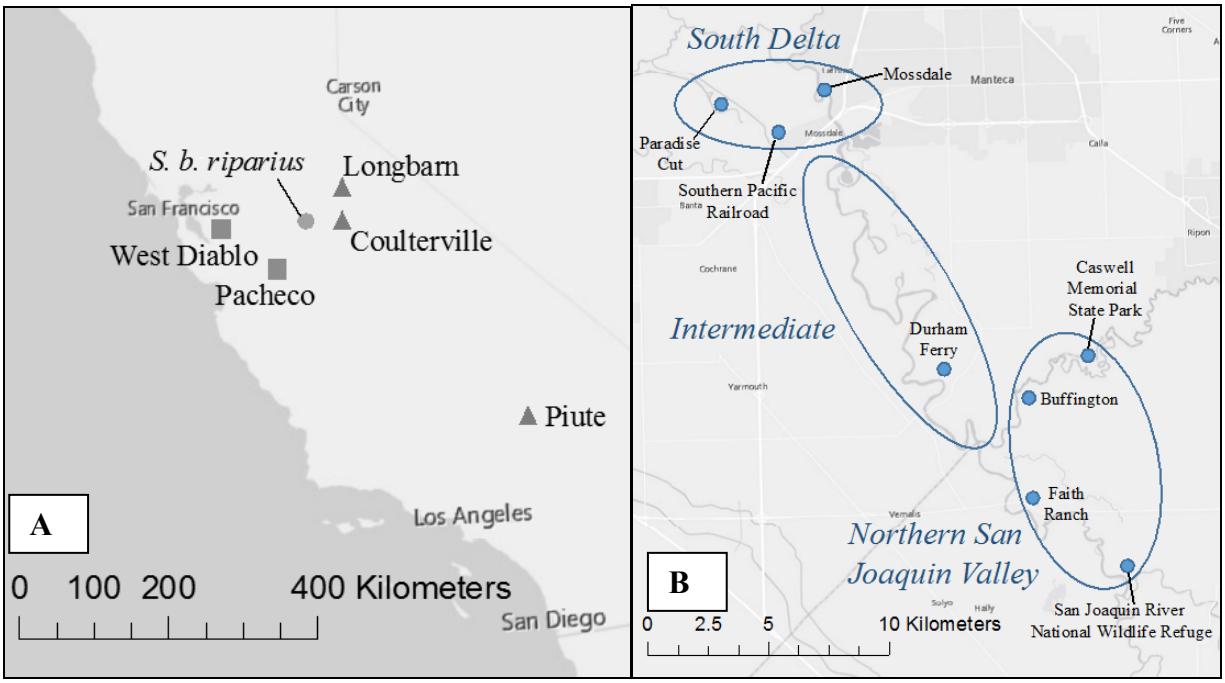

Figure 2. Locations of 11 brush rabbit populations across three subspecies [A], and eight populations of *S. b. riparius* [B].

In 2001, the Endangered Species Recovery Program began a controlled propagation and release program to translocate brush rabbits to the San Joaquin River National Wildlife Refuge (SJRNWR; Williams et al. 2004). From 2001 to 2012, six or more South Delta individuals were captured per year and bred in large pens. Captive-born individuals were released at SJRNWR from 2002 to 2011, while the founding breeders were returned to their capture locations within a year of capture. Despite the success of the translocation efforts, the population at SJRNWR suffered dramatic declines in 2006 due to flooding. Additional translocations were used in conjunction with habitat restoration to recover those losses. Since founding SJNWR, brush rabbits have been observed at Buffington, Faith Ranch, and Durham Ferry (Figure 2B) but whether these animals are native or dispersers from SJNWR is unknown.

Here, we sought to identify the genetic diversity and structure of *S. b. riparius*. Given the two distinct temporal scales at which landscape changes have occurred in the San Joaquin Valley, we used a combination of mitochondrial sequences and nuclear genotypes to achieve the following objectives:

8. Identify the evolutionary relationships between RBRs of the northern San Joaquin Valley and brush rabbit subspecies flanking the Valley.
9. Quantify genetic divergence among RBR populations currently inhabiting isolated or semi-isolated localities within the northern San Joaquin Valley.
10. Quantify average genetic relatedness within, and recent gene flow between, nearby occupied localities within the northern San Joaquin Valley.

METHODS

Genetic samples

We obtained ear biopsy and museum skin samples from 174 individuals across five localities of *S. b. riparius*, two localities of *S. b. macrorhinus*, and three localities of *S. b. mariposae*. We obtained samples from the remaining natural populations of *S. b. riparius* (Paradise Cut, N=20; SPRR, N=9; Mossdale, N=21, and CMSP, N=17), as well as the translocated population at SJRNWR (N=56). *S. b. macrorhinus* inhabits the chaparral and coastal scrub of the Coast and Diablo mountain ranges west of the San Joaquin Valley (Orr 1940; Figure 2A). To represent diversity within *S. b. macrorhinus*, we obtained samples from two localities within the Diablo range: West Diablo, a collection of individual samples from a mosaic of suitable habitat on the northern end of the Diablos surrounding Walnut Creek (N=14); and Pacheco State Park (Pacheco), a 27 square kilometer park northeast of Hollister (N=16, Figure 2A). *S. b. mariposae* is native to the foothills and western slopes of the Sierra Nevada, east of the San Joaquin Valley (Orr 1940; Figure 2A). We obtained samples from three localities in the Sierra foothills: Longbarn, east of Sonora (N=9); Coulterville, west of Yosemite (N=5); and Piute, east of Bakersfield (N=5; Figure 2A). Longbarn samples were collected in recent trapping efforts, while samples from Coulterville and Piute were obtained from historic museum specimens at the Museum of Vertebrate Zoology (MVZ 22929, 23619, 30026, 20027, 60318-60321, 208257). We extracted whole, genomic DNA from the tissue samples using DNeasy® Blood and Tissue Kit (Qiagen Valencia, CA, USA) following a modified protocol (Bell & Matocq 2011).

Phylogenetic approaches

To resolve phylogenetic relationships between *S. b. riparius*, *S. b. macrorhinus*, and *S. b. mariposae*, we amplified a 550 base pair section of the mitochondrial control region and threonine tRNA gene using lagomorph-specific primers (Waltari et al. 2004). In addition to the aforementioned *S. b. riparius* populations, we included two individuals from habitat between SJRNWR and CMSP (Buffington and Faith Ranch), and one individual from Durham Ferry, between SJRNWR and the South Delta. We carried out amplifications in 10 μL reactions consisting of 1 μM of each primer, 5 μl of Qiagen HotStarTaq PCR Master Mix (Qiagen Valencia, CA, USA), and 2 μl of H_2O, with thermocycler settings of: initial denaturation at 94°C for 15 minutes; 35 cycles of denaturation at 94°C for 30 seconds, annealing at 50°C for 30 seconds, extension at 72°C for 1 minute; and a final extension at 72°C for 5 minutes. We purified PCR products with EXOSAP-IT (Affymetrix, Santa Clara, CA, USA) and sequenced products in both directions using PCR primers using the BIGDYE TERMINATOR CYCLE SEQUENCING KIT 3.1 (Applied Biosystems Inc., Foster City, CA, USA). We ran products on an ABI 3730 DNA Analyzer in the Nevada Genomics Center. We assembled and aligned fragments in Geneious v.7.0.6 (Kearse et al. 2012), and verified each sequence by eye. jModeltest ver. 2.1.3 (Darriba et al. 2012) to find the best fit model of evolution. To reconstruct phylogenetic relationships, we ran MrBayes 3.1.2 (Ronquist et al. 2012) for 25 million generations and assessed nodal support using Bayesian posterior probabilities.

Population genetic diversity-microsatellites

To assess population-level diversity within the San Joaquin Valley, we resolved genotypes at 16 microsatellite loci. Microsatellites are highly repetitive, non-coding regions of nuclear DNA which accumulate mutations rapidly in the absence of selection pressure (Goldstein & Schlötterer 1999). Analysis of variation at these loci allows for distinction of recent patterns of divergence among

populations (Selkoe & Toonen 2006). In the absence of primers specifically designed for *Sylvilagus bachmani*, we used a combination of primers known to be polymorphic in other lagomorph taxa: A2, A10, A121, A124, A133, D103, D118, D126 (*Brachylagus idahoensis*, Estes-Zumpf et al. 2008), Sat5, Sat 7, Sat 8, Sat 12, Sat 16 (*Oryctolagus cuniculus,* Mougel et al. 1997, *Sylvilagus palustrus*, Tursi et al. 2013, *Brachylagus idahoensis,* Estes-Zumpf et al. 2010), Sol 44 (*Oryctolagus cuniculus*, *Sylvilagus sp., Lepus sp.,* Surridge et al. 1997, *Brachylagus idahoensis*, Estes-Zumpf et al. 2010), Sol 08, and Sol 30 (*Oryctolagus cuniculus*, Rico et al. 1994, *Syvilagus sp.,* Surridge et al. 1997, Tursi et al. 2013*, Lepus sp.,* Surridge et al. 1997, *Brachylagus idahoensis,* Estes-Zumpf et al. 2010). We carried out amplification in 10 µl multiplex reactions using 1 µM of each primer (forward primer labelled with one of four fluorescent tags: NED, VIC, PET, or 6FAM, Table 1), 4 µl of Qiagen HotStarTaq PCR Master Mix (Qiagen Valencia, CA, USA), and 3 µL of H_2O, with thermocycler settings of: initial denaturation at 94°C for 15 minutes; 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 1 minute 30 seconds, extension at 72°C for 1 minute; and a final extension at 72°C for 5 minutes. We combined amplified products with the LIZ size standard and HiDye, and resolved genotypes on an ABI 3730 DNA Analyzer (Applied Biosystems Inc., Foster City, CA, USA) at the Nevada Genomics Center. Allele sizes were identified using GeneMarker software v1.85 (SoftGenetics LLC, State College, PA, USA) and verified by eye.

Table 1. Four microsatellite multiplexes. Monomorphic loci (*) and those with a high frequency of null alleles (**) were omitted from subsequent analyses.

Multiplex	Primers	Dyes	Allele Size Range
A	A2	6FAM	**
	A124	6FAM	*
	D126	PET	175-199
B	A133	VIC	197-211
	Sat8	VIC	93-141
	D118	NED	239-275
	Sol44	NED	200-214
	D103	PET	102-122
C	A121	PET	194-244
	Sol08	6FAM	107-127
	Sol30	VIC	148-184
	Sat16	NED	113-139
D	A10	VIC	205-223
	Sat5	PET	*
	Sat7	NED	181-201
	Sat12	6FAM	104-124

We used GenAlEx (Peakall & Smouse 2006, 2012) to estimate observed, expected and unbiased heterozygosity and to test for deviations from Hardy-Weinberg and linkage disequilibrium. Due to disparate samples sizes among populations, we used a rarefaction method to estimate the average and effective number of alleles per population, corrected for sample size in ADZE 1.0 (Szpiech et al. 2008). We assessed genetic distance by calculating population pairwise F_{ST} (Weir & Cockerham 1984) and Nei's unbiased genetic distance (Nei 1978), followed by a Mantel test to test for isolation by distance among populations using linearized F_{ST}. We identified genetic subdivision using an individual-based Bayesian assignment approach implemented in the program STRUCTURE 2.3 (Pritchard et al. 2000). Given the recent augmentation efforts at the SJRNWR, we subdivided the data into three subsets: 1) natural populations of *S. b. riparius,* 2) all populations of *S. b. riparius,*

and 3) all populations across all three subspecies. Using an admixture model, we performed 10 independent Markov chain Monte Carlo (MCMC) runs with 1,000,000 burn-in steps and 1,000,000 search steps for each K from K=1 to 10. We used the ΔK approach to estimate the most probable number of clusters (Evanno et al. 2005). To further visualize genetic subdivision across the study area, we conducted a Principal Coordinates Analysis (PCoA) on the aforementioned subsets of data and plotted coordinate 1 versus coordinate 2 using GenAlEx (Peakall & Smouse 2006, 2012).

Population genetic differentiation- single nucleotide polymorphisms

Using the same DNA extractions as described above, we created a genomic library for each individual, by digesting whole genomic DNA with the restriction enzymes EcoR1 and MseI. We restricted our analysis to the 119 highest quality DNA samples. After digestion, we ligated adaptors to each individual's fragmented genomic DNA. Adaptors contain individual barcodes as unique identifiers (unique 8, 9, or 10 base sequences) as well as specific Illumina priming sites to allow high-throughput sequencing. Following ligation of adaptors, we performed two initial rounds of PCR amplification using Illumina primers. In order to reduce genome complexity we performed a size selection of the initial PCR products using a Pippin prep quantitative electrophoresis unit (Sage Science, Inc.) and extracting fragments in the 350-400 bp range. We combined uniquely-barcoded individuals into a single sample and distributed the sample across 3 Illumina sequencing lanes. We used custom Perl scripts to remove barcodes and perform initial quality filtering of reads. We assembled DNA sequences into homologous genetic regions using bwa (Burrows Wheeler Aligner; Li and Durbin 2009) and identified individuals single nucleotide polymorphisms (SNPs) within contigs using SAMtools and BCFtools (Li et al. 2009). We retained genotype data for loci with an average coverage depth of 10x per individual (meaning we had an average of ten DNA sequences per locus for each individual). We used a hierarchical Bayesian model to incorporate uncertainty arising from variable sequencing coverage depth to estimate allele frequencies and genotype parameters for each locus (Gompert and Buerkle 2011). We examined how differentiation is distributed across brush rabbit populations using Principal Components Analysis as implemented with the R function prcomp.

RESULTS

Phylogenetic relationships of San Joaquin Valley brush rabbits

S. bachmani of the San Joaquin Valley region maintain two modestly supported mitochondrial clades (Figure 3). One clade predominates on the western flank of the San Joaquin Valley in the Mt. Diablo region south to Pacheco State Park. One haplotype of this clade is also found in three individuals from CMSP. The second major clade is found throughout the sampled range of *S. b. riparius* although two of the haplotypes from this clade are found in six Pacheco individuals. Within the Central Valley locations, haplotype sharing is evident among South Delta populations in Paradise Cut, SPRR, and Mossdale, and between these localities and CMSP. The one *S. b. mariposae* sample from Longbarn falls within this second clade, but its placement is uncertain because of its level of differentiation from other haplotypes of this clade.

S. b. riparius and *S. b. macrorhinus* differ quite dramatically in overall level of mitochondrial diversity. Among the 52 *S. b. riparius* included in the mtDNA analysis, we found a total of 5 unique haplotypes for an overall haplotype diversity of 0.096 (5/52), while in the 24 individual *S. b. macrorhinus* examined, we found 13 unique haplotypes for a haplotype diversity of 0.542 (13/24).

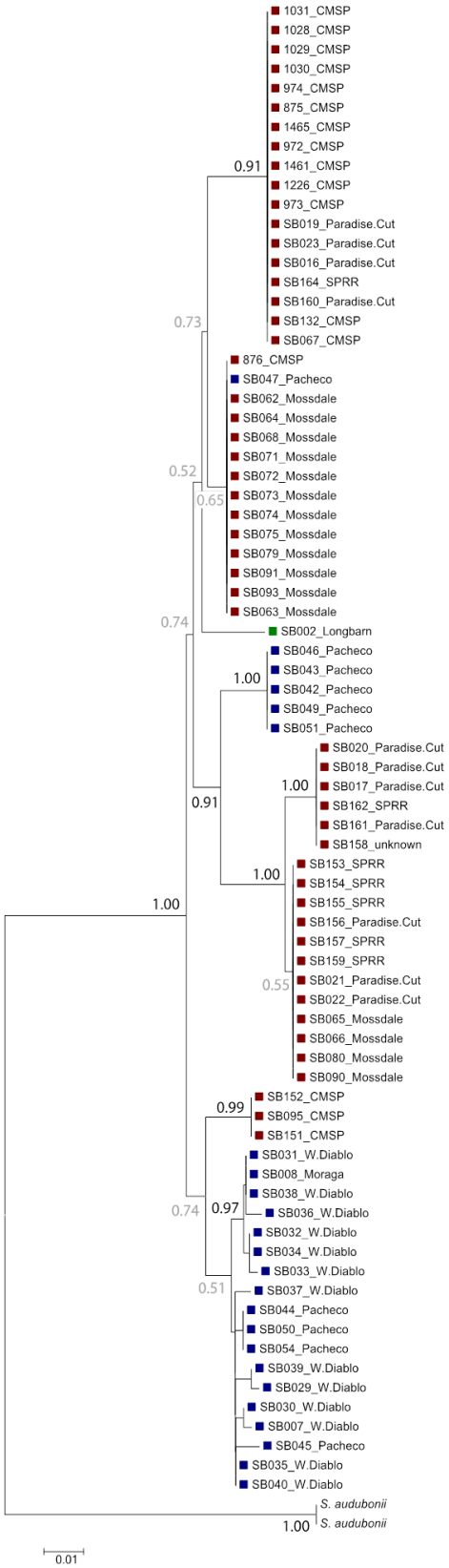

Figure 3. Phylogenetic relationships within *S. bachmani* based on mtDNA control region and partial threonine tRNA gene. Each node represents an individual and colors correspond to subspecies (*S. b. riparius* in red, *S. b. mariposae* in green, and *S. b. macrorhinus* in blue). Values on nodes are Bayesian posterior probabilities.

Population genetic diversity-microsatellites

Two loci (A124, Sat5) were monomorphic across all populations and were eliminated from further analyses. Two additional loci (A133 and A2) had a high frequency of null alleles and were also eliminated. As such, the following analyses are based on 12 microsatellite loci. Unbiased heterozygosity—the average observed heterozygosity of a population weighted by population size—ranged from 0.66 at Caswell to 0.73 at Buffington (Table 2). Observed heterozygosity did not vary significantly from expected heterozygosity, nor did it vary significantly among populations. The average number of alleles per population did not vary significantly between populations when corrected by the rarefaction method at n = 4, while the average number of alleles was significantly less ($p < 0.01$) within the Longbarn population of *S. b. mariposae* when corrected by the rarefaction method at n = 9.

Table 2. Standard genetic diversity metrics for 10 populations of brush rabbit. Sample size (n), unbiased expected heterozygosity (uH_E), average number of alleles per population (A), the resampled number of alleles per population based on the smallest sample size of n=4 individuals (A_4), the resampled number of alleles per population based on a sample size of n=9 individuals (A_9), the average number of private alleles per subspecies (A_{PS}), and the average number of private alleles per population (A_{PP}).

		n	uHE	A	A4	A9	APS	APP
S.b. riparius	Paradise Cut	20	0.64	5.9	2.5	3.7	20	1
	SPRR	9	0.67	4.9	2.6	3.9		1
	Mossdale	21	0.68	5.1	2.6	3.6		3
	Caswell MSP	17	0.62	5.0	2.4	3.5		2
	SJRNWR	56	0.68	6.8	2.6	3.8		7
S.b. macrorhinus	West Diablo	14	0.66	5.6	2.5	3.7	19	22
	Pacheco	16	0.67	5.8	2.6	3.9		24
S.b. mariposae	Longbarn	9	0.51	3.1	2.1	2.8	12	10
	Coulterville	5	0.62	3.7	2.6	-		13
	Piute	5	0.57	3.1	-	-		14

Population differentiation and genetic structure-microsatellites

The three subspecies are highly differentiated from one another at nuclear loci (Table 3), with *S. b. riparius* differing from *S. b. macrorhinus* by an average $F_{ST} = 0.15$ (min. = 0.13, max = 0.18) and from *S. b. mariposae* by an average $F_{ST} = 0.21$ (min. = 0.16, max. = 0.24). The two populations of *S. b. macrorhinus* differ by an $F_{ST} = 0.13$, while *S. b. mariposae* populations differ by an average $F_{ST} = 0.16$ (min.=0.11, max.=0.23). Within natural populations of *S. b. riparius*, we find substantial differentiation between CMSP and the populations in the South Delta with an average $F_{ST} = 0.14$ (min. = 0.12, max. = 0.16). Within the South Delta, the populations Paradise Cut and SPRR are not significantly differentiated ($F_{ST} = 0.02$), and differ from Mossdale by an average $F_{ST} = 0.07$.

Table 3. Two measures of genetic differentiation between brush rabbit populations. Pairwise FST values are below the diagonal and Nei's unbiased genetic distances are above the diagonal. S. b. riparius populations are in white, S. b. macrorhinus populations are highlighted in light brown, and S. b. mariposae populations are highlighted in dark brown. All pairwise FST values are significantly different from zero with the exception of Paradise Cut and SPRR.

	Paradise Cut	SPRR	Mossdale	SJRNWR	Caswell MSP	West Diablo	Pacheco	Longbarn	Coulterville	Piute
Paradise Cut	-	0.03	0.18	0.17	0.36	0.53	0.36	0.63	0.6	0.47
SPRR	0.02	-	0.13	0.18	0.28	0.53	0.22	0.45	0.39	0.42
Mossdale	0.08	0.05	-	0.28	0.44	0.54	0.35	0.65	0.66	0.6
SJRNWR	0.07	0.07	0.1	-	0.26	0.57	0.41	0.66	0.63	0.63
Caswell MSP	0.15	0.12	0.16	0.11	-	0.47	0.4	0.36	0.28	0.55
West Diablo	0.18	0.17	0.17	0.17	0.17	-	0.39	0.62	0.59	0.68
Pacheco	0.13	0.08	0.12	0.13	0.14	0.13	-	0.43	0.34	0.52
Longbarn	0.24	0.2	0.24	0.22	0.17	0.24	0.17	-	0.19	0.47
Coulterville	0.23	0.18	0.23	0.21	0.16	0.23	0.13	0.16	-	0.33
Piute	0.23	0.2	0.23	0.23	0.23	0.25	0.18	0.23	0.11	-

The first two coordinate axes of the PCoA explain 16% of the genetic variation among individuals and reveal overall genetic distinction among the subspecies, despite some overlap of certain individuals among groups (Figure 4A). The Bayesian analysis of genetic structure gave strongest support for two genetic clusters within the three subspecies (Figure 4B), with *S. b. macrorhinus* and *S. b. mariposae* grouping together (green), and *S. b. riparius* comprising the second group (orange). Distinction between *S. b. macrorhinus* and *S. b. mariposae* does not resolve until K = 4 (Figure 4C; blue and green, respectively), with the two additional genetic clusters corresponding to the *S. b. riparius* populations within the South Delta and Mossdale (dark brown) and at CMSP (orange). The translocated population at SJRNWR displays considerable admixture of the two *S. b. riparius* genetic clusters.

Within *S. b. riparius*, the first two coordinate axes explain 17% of the genetic variation among individuals and reveal distinction between the natural populations at CMSP (Figure 5A; gold) and the South Delta and Mossdale (blue), with minimal overlap of individuals between the population groups. The translocated population at SJRNWR appears to be intermediate to these two groups (green). The Bayesian analysis of genetic structure supports this distinction, with strongest support for three genetic clusters within the natural populations (Figure 5B). Paradise Cut and SPRR are largely comprised of one genetic cluster (dark green), while Mossdale and CMSP are each dominated by unique genetic clusters (light blue and gold, respectively). To place SJRNWR within this natural structure, we forced K=3 for all *S. b. riparius* populations using the previously identified genetic groups to train the model. All three of the natural genetic clusters are present within SJRNWR, though the genetic cluster associated with the populations at Paradise Cut and SPRR is most prevalent within the translocated population.

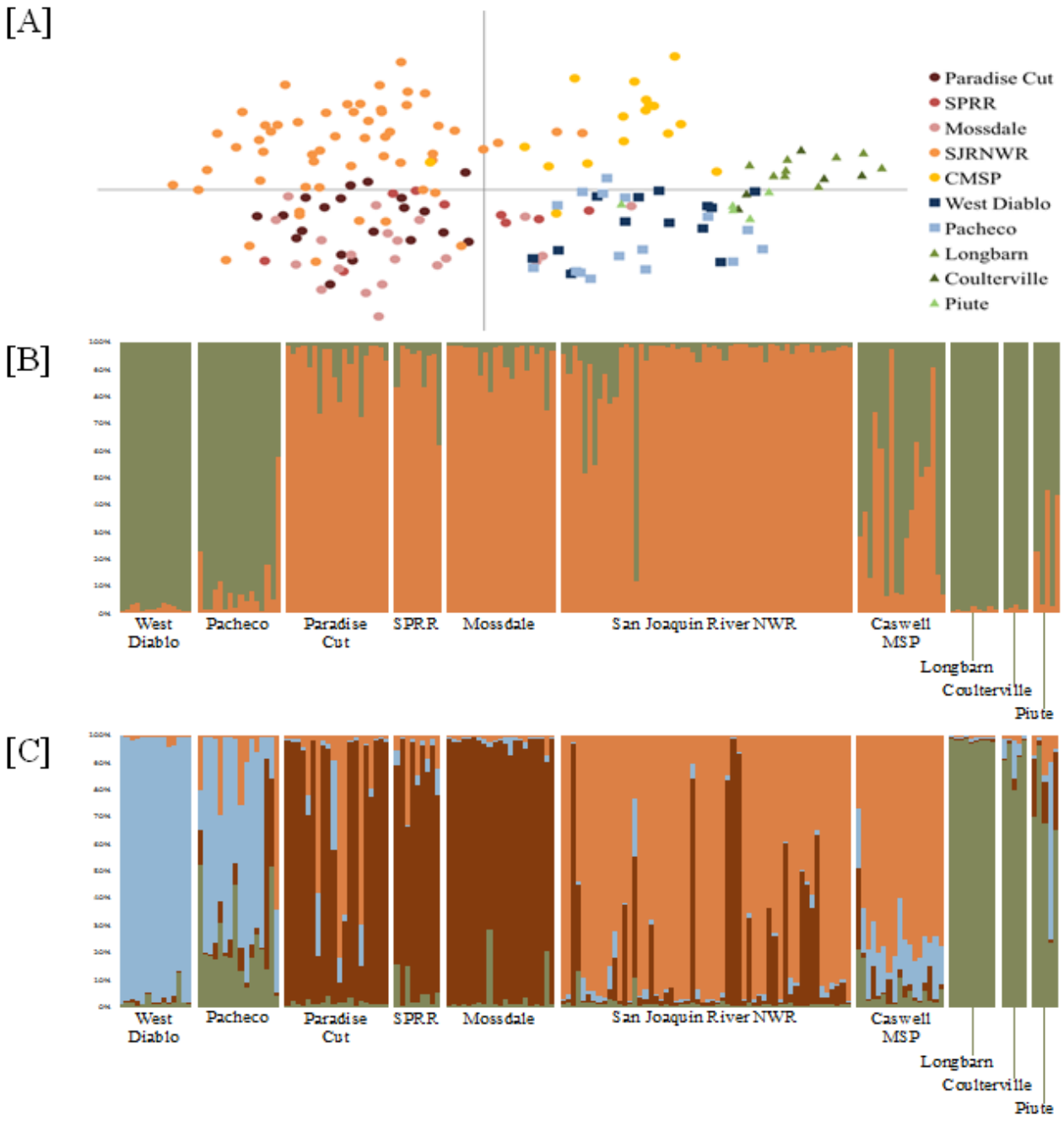

Figure 4. Genetic subdivision of brush rabbit subspecies in and flanking the San Joaquin Valley. [A] Principal Coordinates Analysis (PCoA) of 12 microsatellite loci for 172 individual brush rabbits. [B] Individual-based Bayesian clustering analysis at K=2, and [C] K=4 for 10 populations of brush rabbits across three subspecies. The genetic composition of each individual is represented by a single vertical bar

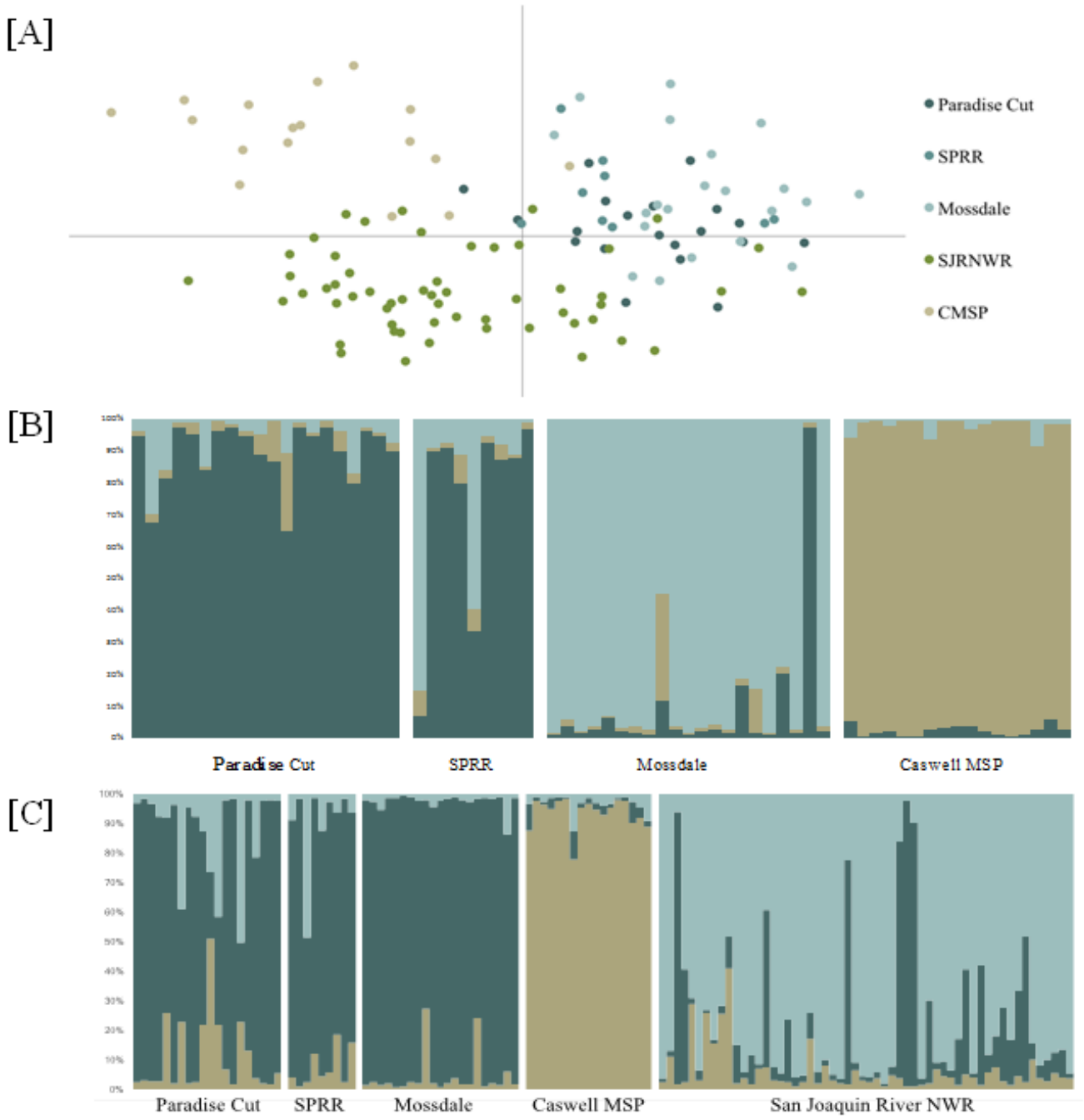

Figure 5. Genetic subdivision among populations of riparian brush rabbits. [A] Principal Coordinates Analysis of 12 microsatellite loci for 125 individual brush rabbits from 5 populations. [B] Individual-based Bayesian clustering analysis at K=3 for natural and [C] all populations of *S. b. riparius*. The genetic composition of each individual is represented by a single vertical bar.

Population differentiation- single nucleotide polymorphisms

After quality filtering of the data, we were able to retain 16,573 SNPs for 95 riparian brush rabbit individuals. Population differentiation and overall evolutionary affinities based on the SNP dataset were consistent with the control region and microsatellite analysis (Figure 6). South Delta populations cluster together, with Paradise Cut (dark blue, Figure 6) and Southern Pacific Railroad (medium blue) overlapping one another substantially, and being differentiated from most individuals at Mossdale (light blue). The Caswell Memorial State Park population (beige) is largely distinct from the South Delta *S. b. riparius* populations. The SJRNWR population falls intermediate to the South Delta and Caswell individuals, as in the microsatellite analysis, and consistent with its mixed ancestry. Because of the overall consistency between our nuclear microsatellite dataset and the SNP dataset, at the time of this final report we base our discussion on the control region and microsatellite dataset alone. Further analysis of the SNP dataset is ongoing and will lead to a separate publication beyond the preliminary analysis included here.

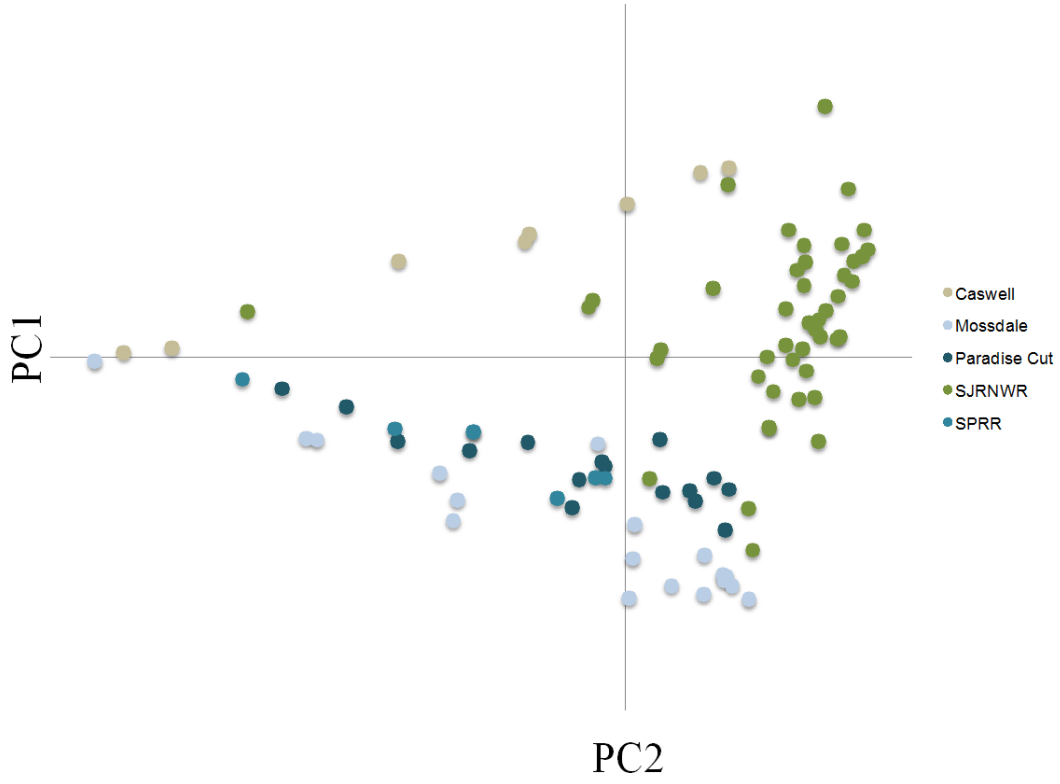

Figure 6. Genetic subdivisions among populations of riparian brush rabbits. Principle Coordinates Analysis of 16,573 single nucleotide polymorphisms.

DISCUSSION

As residents of the California Floristic Province, subspecies of brush rabbit have been subject to substantial changes in habitat connectivity and availability at a variety of spatial and temporal scales. While millennia of geological change promoted differentiation within species throughout the California Floristic Province (Feldman & Spicer 2006, Matocq et al. 2012), rapid anthropogenic change of local landscapes within the Province have further altered the genetic structure of populations and subspecies (Vandergast et al. 2007, Barr et al. 2015).

Phylogenetic relationships of San Joaquin Valley brush rabbits (Objective 1)

Phylogenetic analyses reveal two clades within the three subspecies of *S. bachmani* sampled. The first clade is predominant in the *S. b. macrorhinus* subspecies to the west of the Valley, though a single haplotype of this clade is found in the CMSP population of *S. b. riparius.* The second clade is predominant within *S. b. riparius,* though two haplotypes are present within the Pacheco population of *S. b. macrorhinus.* Shared haplotypes within this clade, not only between populations of *S. b. riparius* but also between subspecies, suggest either retention of ancestral diversity, or recent gene flow within and across the Valley. While the hydrogeological history of the region would suggest greater connectivity between the San Joaquin Valley and the northern end of the Diablo range, the population at Pacheco not only shares a haplotype with CMSP and Mossdale, but also harbors another haplotype that falls within the predominant *S. b. riparius* clade. Unlike the West Diablo population, which is located along the western slopes of the Diablos, the Pacheco population is located on the eastern slopes near waterways that drain east into the Valley and the San Joaquin River. Presence of riparian habitat along these drainages may have enabled gene flow as recently as the early 20th century, resulting in the shared haplotypes between this population and populations of *S. b. riparius* in the Valley.

Genetic diversity, population differentiation and genetic connectivity of natural S. b. riparius populations (Objectives 2 and 3)

Despite the substantial difference in habitat connectivity and availability in their respective ranges, *S. b. riparius* maintains levels of heterozygosity and allelic diversity equal to sister taxa that, presumably, have not experienced such profound range contractions. While both *S. b. riparius* and *S. b. macrorhinus* exhibit greater genetic diversity than the Longbarn population of *S. b. mariposae*, the small sample size limits inferential power and warrants further investigation both within the Longbarn population and throughout *S. b. mariposae's* range. Within *S. b. riparius*, natural populations maintain average to high levels of heterozygosity and low numbers of private alleles. However, mitochondrial diversity is much higher within *S. b. macrorhinus*, suggesting loss of historical diversity within *S. b. riparius*. As such, it is likely that genetic drift has worked independently within these fragmented populations, shifting allele frequencies without profoundly altering heterozygosity.

Natural populations of *S. b. riparius* exhibit substantial differentiation from one another, with the exception of Paradise Cut and SPRR in the South Delta. These localities are the least isolated of the remnant natural populations, and it is very likely that small patches of suitable habitat between the localities allow gene flow. The other South Delta population at Mossdale is significantly differentiated from both Paradise Cut and SPRR, consistent with the greater geographic distance and diminished habitat connectivity between these populations. Bayesian analyses indicate that the South Delta is comprised of two genetic groups, one associated with the Paradise Cut/SPRR

complex, while the other predominates within the Mossdale population. Despite this distinction, recent or ongoing gene flow is evidenced by the mixture of some pure individuals within populations and admixture of both genetic groups within individuals.

The southernmost natural population at CMSP is highly differentiated from the other natural *S. b. riparius* populations. The genetic distance between CMSP and the South Delta populations of Paradise Cut and Mossdale is only slightly less than the genetic distances between the West Diablo population of *S. b. macrorhinus* and populations of *S. b. riparius* (Table 1). The PCoA analyses indicate distinction between CMSP and the South Delta, which is further supported by the Bayesian analyses, and indicates that CMSP is comprised of a third genetic group distinct from the two predominating in the South Delta. The substantial geographic distance between CMSP and the South Delta, coupled with limited habitat connectivity, has likely limited contemporary gene flow between the two population groups, promoting increased differentiation as genetic drift has acted independently on CMSP and the South Delta.

Translocated population at the San Joaquin River National Wildlife Refuge

Consistent with its translocation history, the population at SJRNWR retains a strong affinity to the populations at Paradise Cut and SPRR (Table 1). However, PCoA analysis places it intermediate to the populations of the South Delta and Caswell, while Bayesian analyses indicate that the population is comprised of a third genetic cluster independent of the South Delta and Caswell. Given the close proximity of SJRNWR to CMSP, and the restoration of habitat between the two localities, gene flow between the restored population at the refuge and the natural population at Caswell—or other undocumented, nearby populations—is possible. The combination of SJRNWR's complex history of population declines, repeated translocations and natural gene flow have likely contributed to the unique genetic composition and high levels of diversity within this population.

Conservation implications

While habitat loss and fragmentation have made an impact in the genetic structure of natural *S. b. riparius* populations, habitat restoration and animal translocation efforts have succeeded in establishing a diverse and persisting population at the SJRNWR and has enabled natural gene flow between the refuge and native populations. As such, it is likely that this resilient subspecies would respond favorably to continued restoration and augmentation efforts.

PART 3. MANUSCRIPT 2: EVALUATING THE RANGE-WIDE FUNCTIONAL AND STRUCTURAL CONNECTIVITY OF AN ENDANGERED HABITAT SPECIALIST, THE RIPARIAN BRUSH RABBIT (*SYLVILAGUS BACHMANI RIPARIUS*)

INTRODUCTION

One of the most significant landscape-level threats to genetic diversity is anthropogenically-induced habitat fragmentation (Aguilar et al. 2008). Unlike many natural landscape changes that may occur over centuries to millennia, anthropogenic change can have profound, large-scale impacts on habitat quality and availability over very short timeframes. For endemic species and habitat specialists, these changes can severely threaten both the evolutionary legacy and adaptive potential of remnant populations (Palumbi 2001). Small populations are often characterized by low genetic variation and inbreeding because of their small effective population sizes and the associated genetic drift. Further exacerbating rates of genetic drift and loss of variation is the fact that human-altered landscapes create a largely impermeable matrix, which reduces the potential for gene flow among populations (Mendez et al. 2014).

The effects of habitat loss and fragmentation are multifaceted. From a purely spatial perspective, fragmentation reduces the total area of habitat while increasing the distance between habitat patches (Wilcove et al. 1986; Lindenmayer and Fischer 2006). Thus, fragmentation reduces structural connectivity, or the spatial continuity of habitat (Auffret et al. 2015). As patches are subdivided, the amount of patch edge is increased relative to the area of the patch. While not necessarily as inhospitable as the anthropogenic matrix, patch edges often vary substantially from the patch interior in both biotic and abiotic properties, further altering the quality of the remnant habitat patch (Lidicker 1998).

Loss of habitat area and quality can alter the behaviors of resident organisms as they are exposed to novel pressures from both edge and matrix (Ewers and Didham 2006, Haynes et al. 2006). Functional connectivity addresses these behaviors as well as the structural arrangement of the landscape by incorporating patterns of organismal movement and use with habitat presence (Tischendorf and Fahrig 2000). One approach for evaluating functional connectivity is the comparison of genetic differentiation of organismal populations to the geographic distance between habitat patches. Genetic differentiation among populations increases as gene flow decreases and as genetic drift acts independently on isolated populations. In the simplest case, genetic differentiation among populations can be the result of simple geographic distance alone, referred to as a pattern of isolation by distance (Wright 1943). While this pattern is often evident in population genetic studies (Slatkin 1995, Whitlock and MacCauley 1999), it is based on the shortest, straight-line (Euclidean) distances between populations without regard to underlying habitat quality or composition (Waits and Storfer 2016). In order to explore the role of habitat structure and configuration in determining movement among populations, distances between patches or populations can be weighted by the cost distance, or impedance of the habitat on an organism's movement, to generate an "effective" distance between populations (Andriaensen et al. 2002). This is not to say that spatial distance alone is not an important driver of differentiation in dispersal limited systems, in fact, it is the baseline expectation. Nonetheless, if habitat quality and configuration also play a role in determining genetic connectivity, methods of analysis that incorporate such data, in addition to straight-line distance, should be explored.

A simple yet powerful way to compare structural and functional connectivity of a fragmented system is through the implementation of graph theory (Murphy et al. 2016). Graph theory refers to

the mathematical graphs used to model pairwise relationships between pairs of populations and habitat patches (Urban and Keitt 2011). Landscape and genetic data are inherently graph-like; each patch or population serves as a node—or point within the graph—while genetic and geographic distances between populations and patches serve as connections—or edges. The resulting networks can then be investigated for pairwise correlation between genetic and geographic or effective distances through the use of Mantel tests (Mantel 1967).

Here, we use a combination of genetic data and graph theory approaches to assess structural and functional connectivity of the endangered riparian brush rabbit (*Sylvilagus bachmani riparius*). *S. b. riparius* is a subspecies of brush rabbit endemic to the riparian gallery forests of California's San Joaquin Valley. A riparian specialist, *S. b. riparius* relies heavily upon runways through dense vegetation for movement, breeding, and refuge from predators (Orr 1940). Over the last century, the San Joaquin Valley has lost more than 95% of its riparian forests (Kelly et al. 2005), and only four natural populations of *S. b. riparius* are known to remain (Williams et al. 1998): Paradise Cut, Southern Pacific Railroad (SPRR), and Mossdale, collectively termed the South Delta, and Caswell Memorial State Park (CMSP; Figure 2B). Following its listing as an endangered subspecies in 2002, the Endangered Species Recovery Program began a controlled propagation and release program at the San Joaquin River National Wildlife Refuge (SJRNWR) using captive-bred progeny from South Delta breeding stock (Williams et al. 2004). Concurrently, wildlife managers worked to restore habitat both within the refuge and between the refuge and the nearby population at CMSP. Since these efforts, riparian brush rabbits have been observed in habitat patches between SJRNWR and Caswell (Buffington and Faith Ranch; Figure 2B) and between the refuge and the South Delta (Durham Ferry; Figure 2B), leading managers to question the extent to which natural gene flow is possible between populations. In order to aid future management and recovery efforts, this research seeks to address the following questions:

1. Is the genetic differentiation between riparian brush rabbit populations a product of distance alone, or do habitat features play a role in functional connectivity?
2. Does the habitat presently available allow for natural gene flow between populations?
3. Where is habitat connectivity highest and lowest within the subspecies' range?

MATERIALS AND METHODS

Study system

Remnant populations of *S. b. riparius* have been divided into two putative population groups: the South Delta, comprised of the populations of Paradise Cut, SPRR, and Mossdale, and the Northern San Joaquin Valley (NSJV), comprised of CMSP and the SJRNWR (Figure 2B). The South Delta is an aggregate of numerous, small patches of riparian habitat at the mouth of the San Joaquin river, while NSJV hosts larger patches of habitat along the confluence of the Stanislaus and San Joaquin rivers. Patches in both regions include both agricultural and urban landscapes. Our previous analyses (Manuscript 1) of genetic differentiation and structure within natural populations of *S. b. riparius* indicate the presence of two distinct genetic groups within the subspecies associated with the geographic location of populations—one within the South Delta, and one associated with CMSP. The augmented population at the SJRNWR forms a third genetic group intermediate to the South Delta and CMSP.

Genetic differentiation and structure

The molecular data used for these analyses were derived from the larger, multi-subspecies microsatellite genotypes presented in the previous Manuscript. Briefly, we extracted whole, genomic DNA from 123 *S. b. riparius* tissue samples using the Qiagen DNEasy extraction kit (Qiagen, Inc), following a modified protocol (Bell and Matocq 2011). We amplified DNA samples in four multiplex panels of three to five primers. Following amplification, we submitted samples to the Nevada Genomics Center to run on an ABI 3730 DNA analyzer (Applied Biosystems Inc.). We identified allele sizes using GeneMarker software v1.85 (SoftGenetics) and verified by eye. We estimated genetic distance by calculating population pairwise F_{ST} (Weir and Cockerham 1984) in GenAlEx (Peakall and Smouse 2006, 2012) for all natural populations, as well as the restored population at SJRNWR.

Defining patches and inter-patch distances

Phillips et al. (2013) identified regions of habitat suitable for *S. b. riparius* based on vegetative composition, cover and density throughout the San Joaquin Valley. Their model prioritized vegetation most important for persistence of *S. b. riparius* (Kelly et al. 2011): large patches of dense riparian brush, ecotonal edges of brush to grasses and herbaceous forbs, open tree overstory, and scaffolding plants which would allow climbing riparian plant species to grow tall enough to withstand flood events. Each cell of their resulting habitat suitability map was ranked on a scale of 0 (inhabitable) to 100 (optimal), with the highest values assigned to areas with shrub cover greater than 20 percent, shrub density greater than 35 percent, and canopy density less than 90 percent. Urban areas, rivers and regions devoid of shrub cover were designated as uninhabitable. Using these habitat values, we selected habitat patches of moderate quality or better (Phillips et al. 2013). Any patches less than 400 meters in area were omitted from subsequent analyses as these small patches are unlikely to be permanently occupied by these rabbits (Chapman 1974). We assigned patches to putative regions based on genetic groups established in Manuscript 1; patches at and surrounding the South Delta region were collectively grouped as "South Delta," patches at and between CMSP, SJRNWR, Buffington Tract and Faith Ranch were grouped as "NSJV", and patches between South Delta and NSJV were grouped as "Intermediate". We calculated total area for each patch in ArcGIS, and compared mean patch areas between putative regions using a one-way ANOVA and post-hoc Tukey test in R (R Core Team 2015). Euclidean distance between the patches was calculated in ArcGIS and exported to input files using the Conefor Inputs extension in ArcGIS (Jenness 2011). We used a cost surface developed by Phillips et al. (2013) to generate effective distances between habitat patches. The cost surface used the values from their habitat suitability analyses to assign each cell of the habitat map with a cost value. Costs ranged 1 (completely permeable habitat ranked with a suitability value of 90 or better on a scale of 1-100) to 10,000 (impermeable; urban, agricultural and barren landscapes, waterways). We generated cost distances between patches using the Landscape Genetics toolbox (Etherington 2010), and converted these to effective distances using the methods outlined in Andriaensen et al. (2002).

Evaluation of functional and structural connectivity

While suitable habitat exists in patches throughout *S. b. riparius'* range, many of these patches occur on private lands that have been inaccessible for research purposes. As such, no range-wide occupancy data exist for explicit analyses of functional connectivity between all existing habitat patches. We used the genetic differentiation between known populations to determine if functional connectivity was a product of pure distance alone, or by the availability and arrangement of habitat structure. We generated pairwise euclidean, cost, and genetic distance matrixes between occupied

patches using the R package ADE4 (R Core Team 2015) and conducted Mantel tests to test for isolation by Euclidean and effective distance.

We used Conefor 2.6 (Saura and Torne 2009) to generate a range-wide connectivity network and assess individual patch importance to the connectivity of the network as a whole. Patch connectivity was evaluated using the PC probabilistic index (Saura and Pascual-Hortal 2007), a measure of the likelihood of connectivity between habitat patches. In absence of dispersal data for the species, dispersal distances were calculated from home range size (Harestad and Bunel 1979). Home range size is strongly correlated to maximum dispersal distance in many small mammals, *S. bachmani* included (Bowman et al. 2002). Maximum dispersal, or the furthest distance an individual will travel to colonize a new habitat patch, was calculated as 2116 meters, associated with a five percent probability of connection, or likelihood that an individual will travel the maximum dispersal distance. Patch importance for connectivity was evaluated by three metrics: overall value (dPC), value for migration (dPC_{flux}) and stepping stone value ($dPC_{connector}$). The dPC metric evaluates the change in connectedness of a habitat throughout the network with the removal of each patch, giving an indication to the importance of individual habitat patches to network cohesion (Saura and Rubio 2010). The dPC_{flux} metric weights the amount of connections to and from a given habitat patch by the patch's area, indicating the importance of each patch as a migratory destination (Saura and Rubio 2010). The more accessible (more connections) and the larger a patch is, the higher the flux value will be. Similarly, $dPC_{connector}$ measures the importance of a habitat patch by the amount of connections, but does not weight connections by area (Saura and Rubio 2010). This provides a metric of movement alone without excluding the importance of smaller patches as potential migratory stopovers. Differences in patch importance and connectivity within and between putative regions were evaluated for significance using a one-way ANOVA with post-hoc pairwise tukey tests in R.

RESULTS

Functional connectivity

As discussed in the previous Manuscript, the natural population of *S. b. riparius* at CMSP is substantially differentiated from populations in the South Delta with an average $F_{ST} = 0.14$ (min. = 0.12, SPRR, max. = 0.16, Mossdale; Table 4). Within the South Delta, the populations Paradise Cut and SPRR are not significantly differentiated ($F_{ST} = 0.02$), and differ from Mossdale by an average $F_{ST} = 0.07$ (Table 4). Despite augmentation with animals of South Delta origin, SJRNWR is significantly differentiated from all South Delta populations with an average $F_{ST} = 0.08$ (min. = 0.07, SPRR and Paradise Cut, max = 0.10, Mossdale, Table 4), as well as CMSP ($F_{ST} = 0.11$, Table 4).

Table 4. Genetic differentiation between brush rabbit populations, measured as FST. Values were obtained from the analysis of genotypes resolved at 12 microsatellite loci. All pairwise FST values are significantly different from zero with the exception of Paradise Cut and SPRR. Asterisk (*) indicates augmented population.

	Paradise Cut	SPRR	Mossdale	Caswell MSP
SPRR	0.02			
Mossdale	0.08	0.05		
Caswell MSP	0.15	0.12	0.16	
SJRNWR*	0.07	0.07	0.1	0.11

Isolation by euclidean distance alone explained over 35 percent of the genetic differentiation between populations; however, the correlation was not significant (Mantel test, $r^2 = 0.357$, $p = 0.144$, Figure 7). By contrast, isolation by effective distance explained greater than 57 percent of the genetic differentiation between populations and was highly significant (Mantel test, $r^2 = 0.575$, $p < 0.01$ Figure 8).

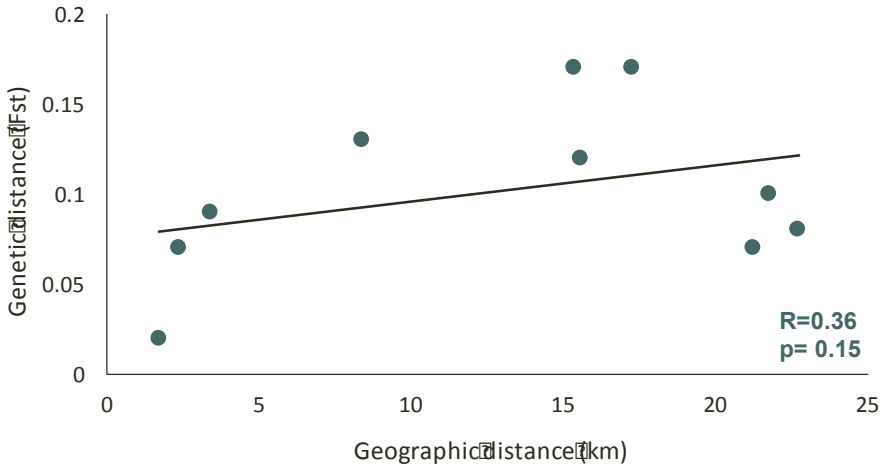

Figure 7. . Isolation by Euclidean distance between natural populations of *S. b. riparius*, evaluated as the correlation between genetic distance and geographic distance.

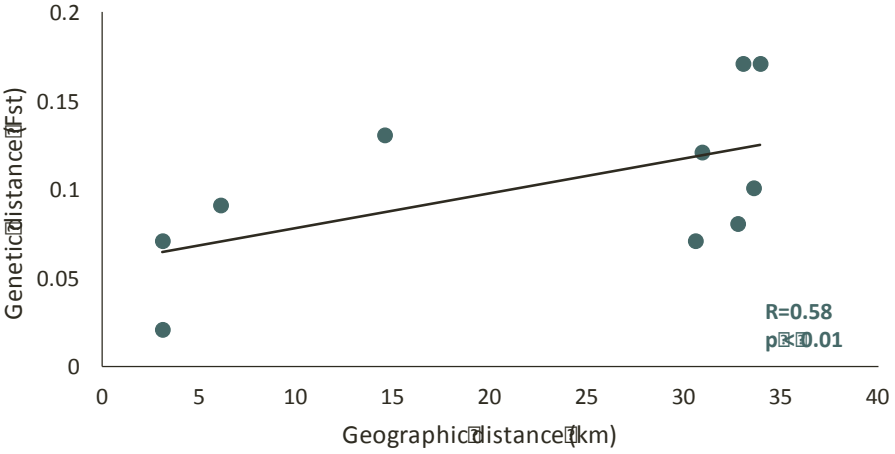

Figure 8. . Isolation by effective distance between natural populations of *S. b. riparius*, evaluated as the correlation between genetic distance and geographic distance weighted by habitat structure.

Structural connectivity and patch importance

Mean patch area varied from 22,033 m^2 in the South Delta region to 121,488 m^2 in the NSJV region (Table 5). Overall, the South Delta patches were significantly smaller than those in the NSJV (ANOVA, $p < 0.01$). Patches in the Intermediate region between the South Delta and the NSJV did not vary significantly from either of these regions (ANOVA, $p = 0.75$ and $p = 0.17$, respectively).

Table 5. Minimum, maximum and mean patch areas by region. The largest maximum and mean patch areas were within the Northern San Joaquin Valley region. The smallest minimum patch area was within the Intermediate region, while the South Delta held the smallest mean area.

Patch	Patch area (km^2)		
	minimum	*maximum*	*mean*
South Delta	0.004	0.185	0.022
Intermediate	0.004	0.516	0.051
NSJV	0.004	5.737	0.121

When evaluated by euclidean distance alone, importance to overall connectivity (dPC) was higher in the NSJV than Intermediate and South Delta patches (ANOVA, $p = 0.05$ and $p < 0.01$, respectively; Figure 9). Overall connectivity values ranged from 0 in the South Delta to 62.5 in the NSJV, with the most important patches coinciding with the populations at the SJRNWR (dPC = 62.5) and the geographically adjacent Faith Ranch. Intermediate patches between the NSJV and the South Delta population groups did not vary significantly in overall importance from the South Delta (ANOVA, $p = 0.85$). Patch migratory value ($dPC_{flux;}$ Figure 10) was higher in the NSJV populations than both the South Delta and Intermediate patches (ANOVA, $p < 0.01$ and $p < 0.05$, respectively), with values ranging from 0 in the South Delta to 33.9 in the NSJV, with the SJRNWR and Faith Ranch holding the highest migratory values (33.9 and 13.2, respectively). The difference in mean patch value between the South Delta and Intermediate patches was not significant (ANOVA, $p=0.87$). The connector value ($dPC_{connector;}$ Figure 11) of patches ranged from 0 in the South Delta to 31.1 at Faith Ranch in the NSJV. Connector values varied significantly between the NSJV and South Delta patches (ANOVA, $p < 0.001$), but did not vary significantly between Intermediate patches and either the South Delta or the NSJV (ANOVA, $p = 0.83$ and $p = 0.15$, respectively).

These same analyses yielded dramatically different results when effective distance was used in place of Euclidean distance. Overall connectivity (dPC) did not vary between regions (ANOVA, $p > 0$ .1 for all pairwise comparisons; Figure 12), nor did the patch connector value (ANOVA, $p > 0.6$ for all pairwise comparisons; Figure 13). As with Euclidean distance, the most important patch for network connectivity was the SJRNWR (dPC = 68.19, $dPC_{connector} = 0.000002$). Patch migratory value varied only slightly between the NSJV and South Delta regions (ANOVA, $p = 0.05$; Figure 14) and not at all between these regions and Intermediate patches. Migratory value was highest at Faith Ranch ($dPC_{flux} = 35.5$).

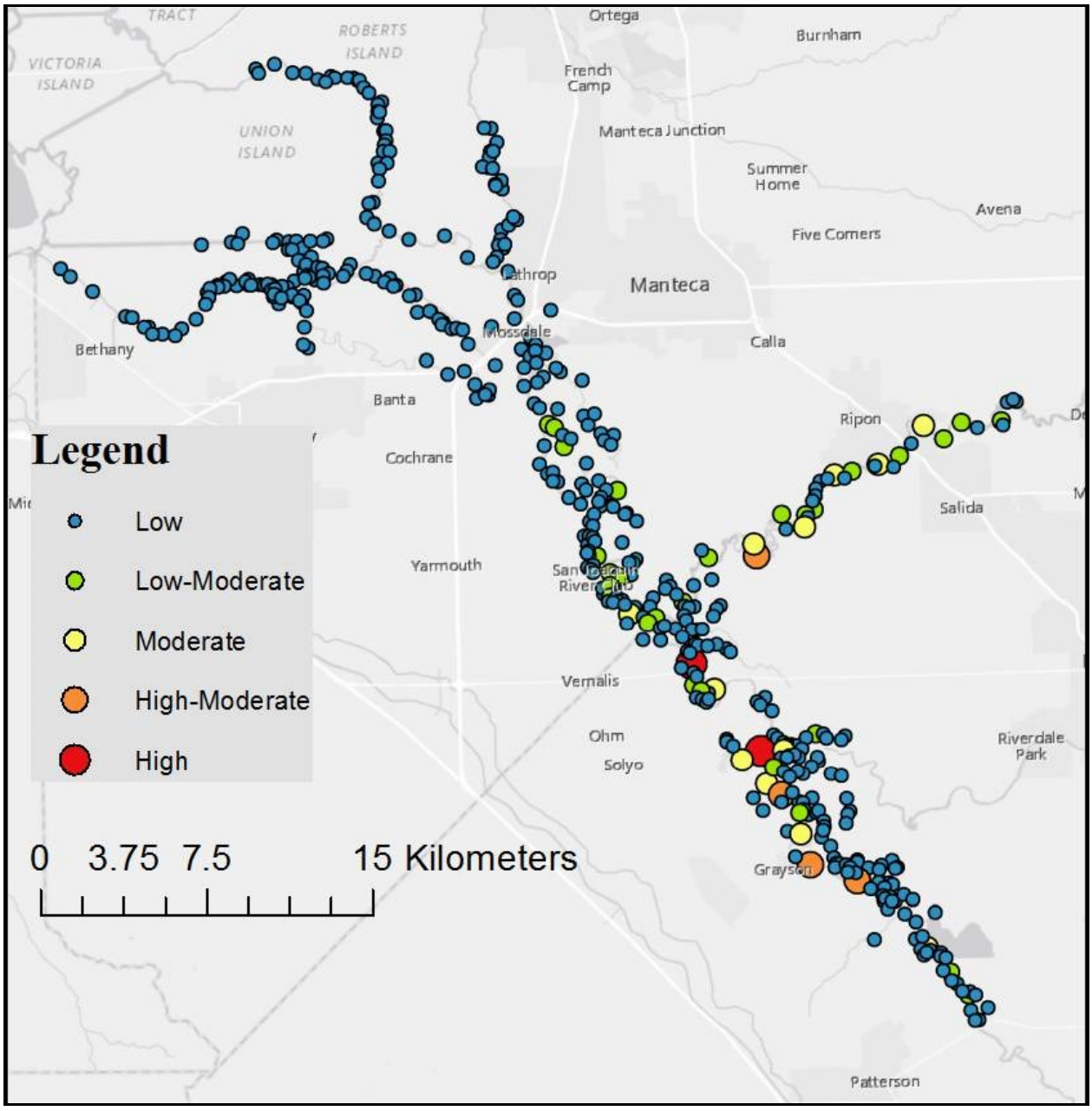

Figure 9. Overall patch value (dPC) calculated using Euclidean distance between habitat patches. Importance is indicated by the size and color of the point; the larger and warmer in color the patch marker, the greater the patch value for overall connectivity. Patch value was significantly higher in the NSJV region than Intermediate and South Delta patches ($p = 0.05$ and $p < 0.01$, respectively). Intermediate patches between the NSJV and the South Delta population groups did not vary significantly in overall importance from the South Delta ($p = 0.85$).

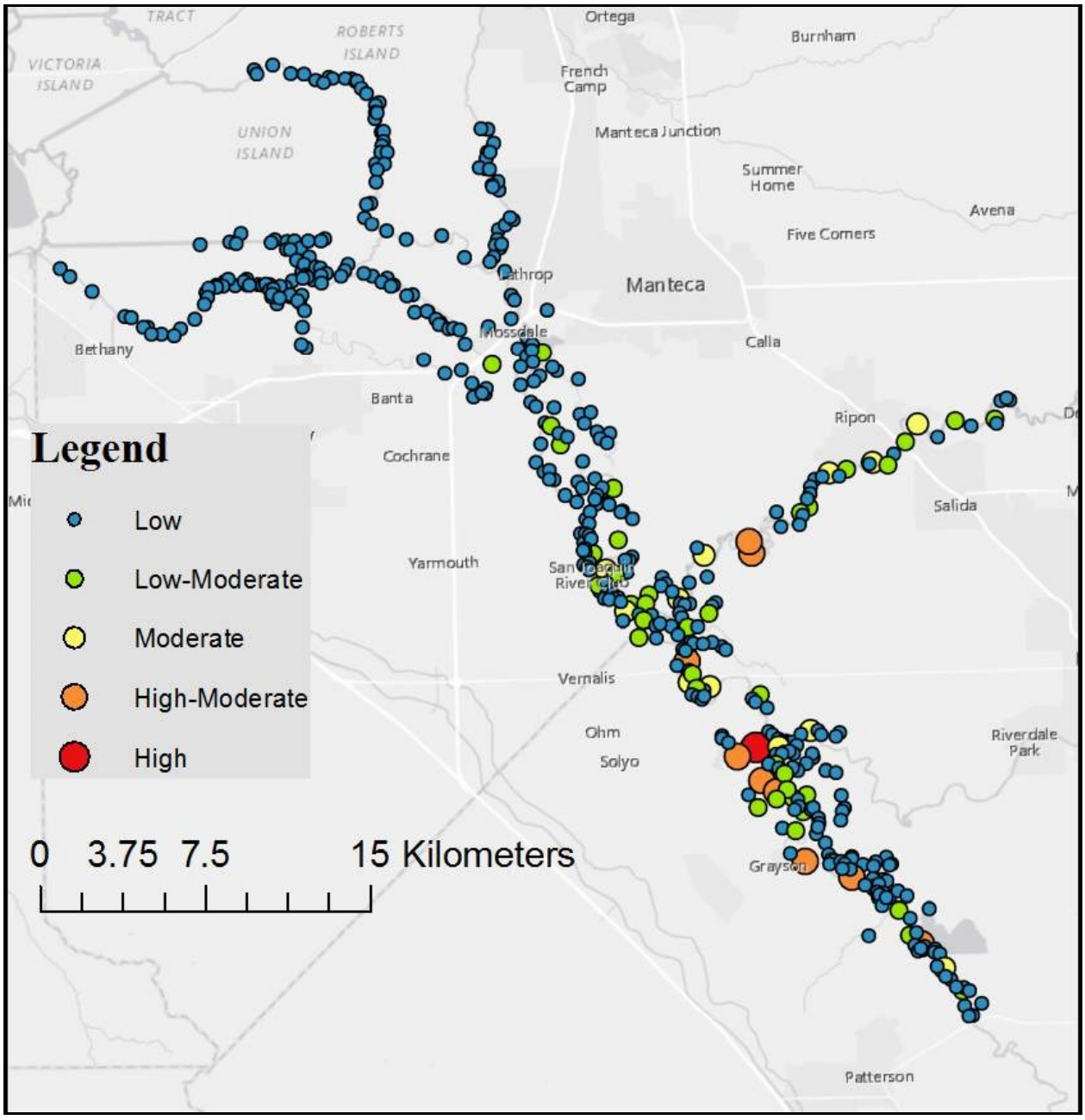

Figure 10. Patch migratory value (dPC_{flux}) calculated using Euclidean distance between habitat patches. Importance is indicated by the size and color of the point; the larger and warmer in color the patch marker, the greater the patch value for overall connectivity. Patch migratory value was higher in the NSJV populations than both the South Delta and Intermediate patches ($p < 0.01$ and $p < 0.05$, respectively), but did not vary significantly between the South Delta and Intermediate regions ($p = 0.87$).

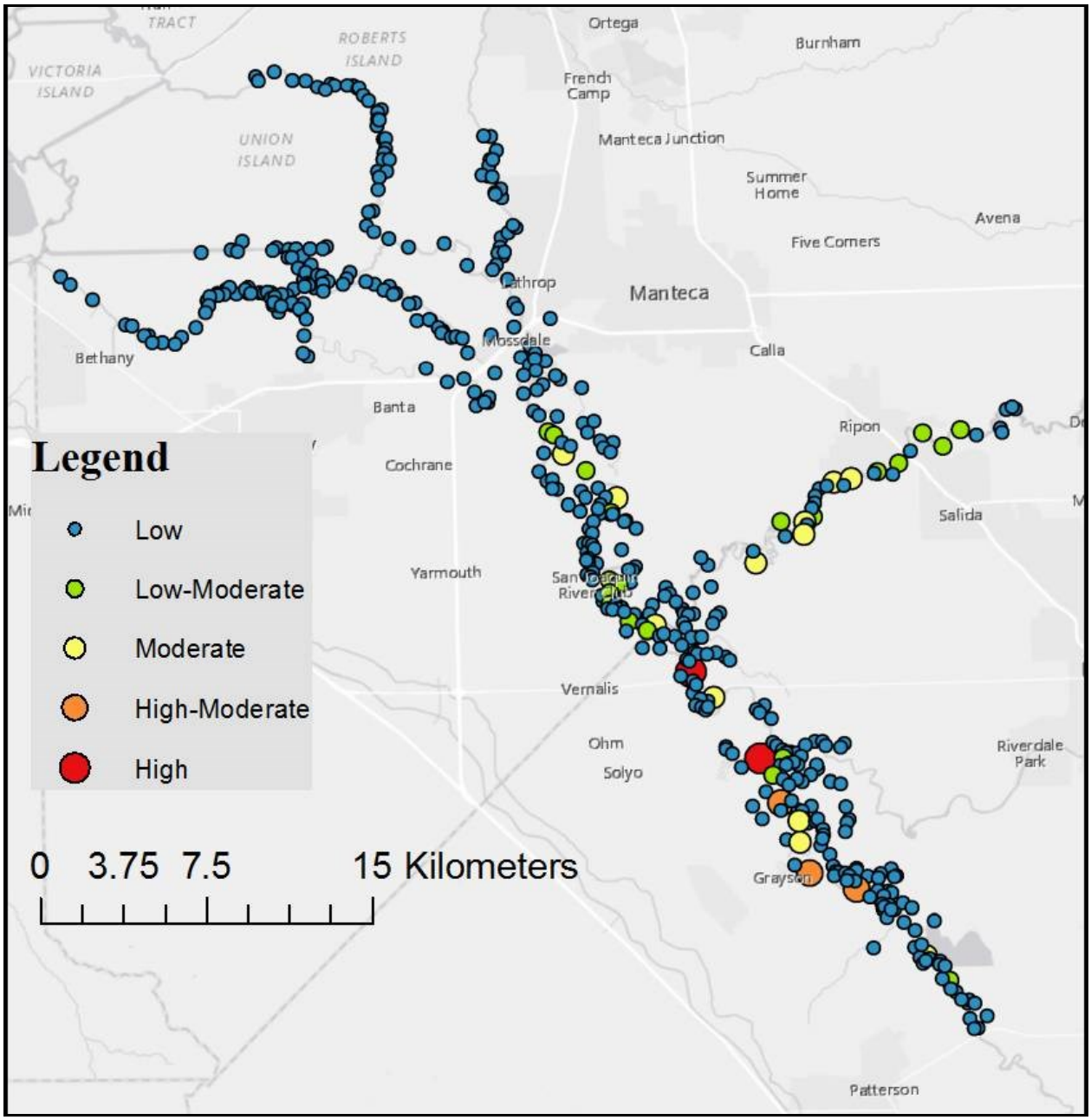

Figure 11. Patch value as a stepping stone ($dPC_{connector}$) calculated using Euclidean distancebetween habitat patches. Importance is indicated by the size and color of the point; the larger and warmer in color the patch marker, the greater the patch value for overall connectivity. Connector values varied significantly between the NSJV and South Delta patches ($p < 0.001$), but did not vary significantly between Intermediate patches and either the South Delta or the NSJV ($p = 0.83$ and $p = 0.15$, respectively).

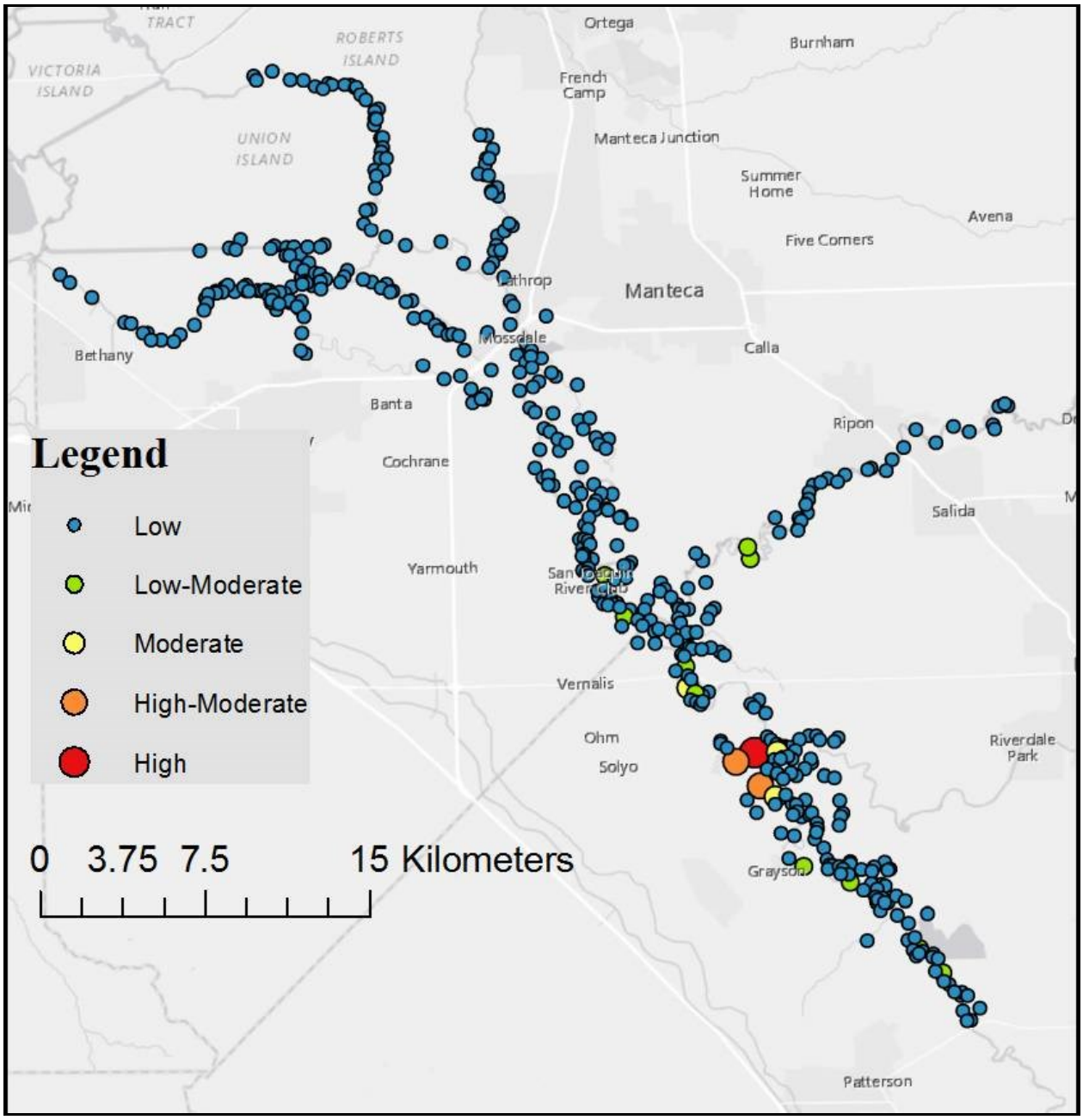

Figure 12. Overall patch value (dPC) calculated using effective distance between habitat patches. Importance is indicated by the size and color of the point; the larger and warmer in color the patch marker, the greater the patch value for overall connectivity. Overall connectivity did not vary between regions ($p > 0.1$ for all pairwise comparisons).

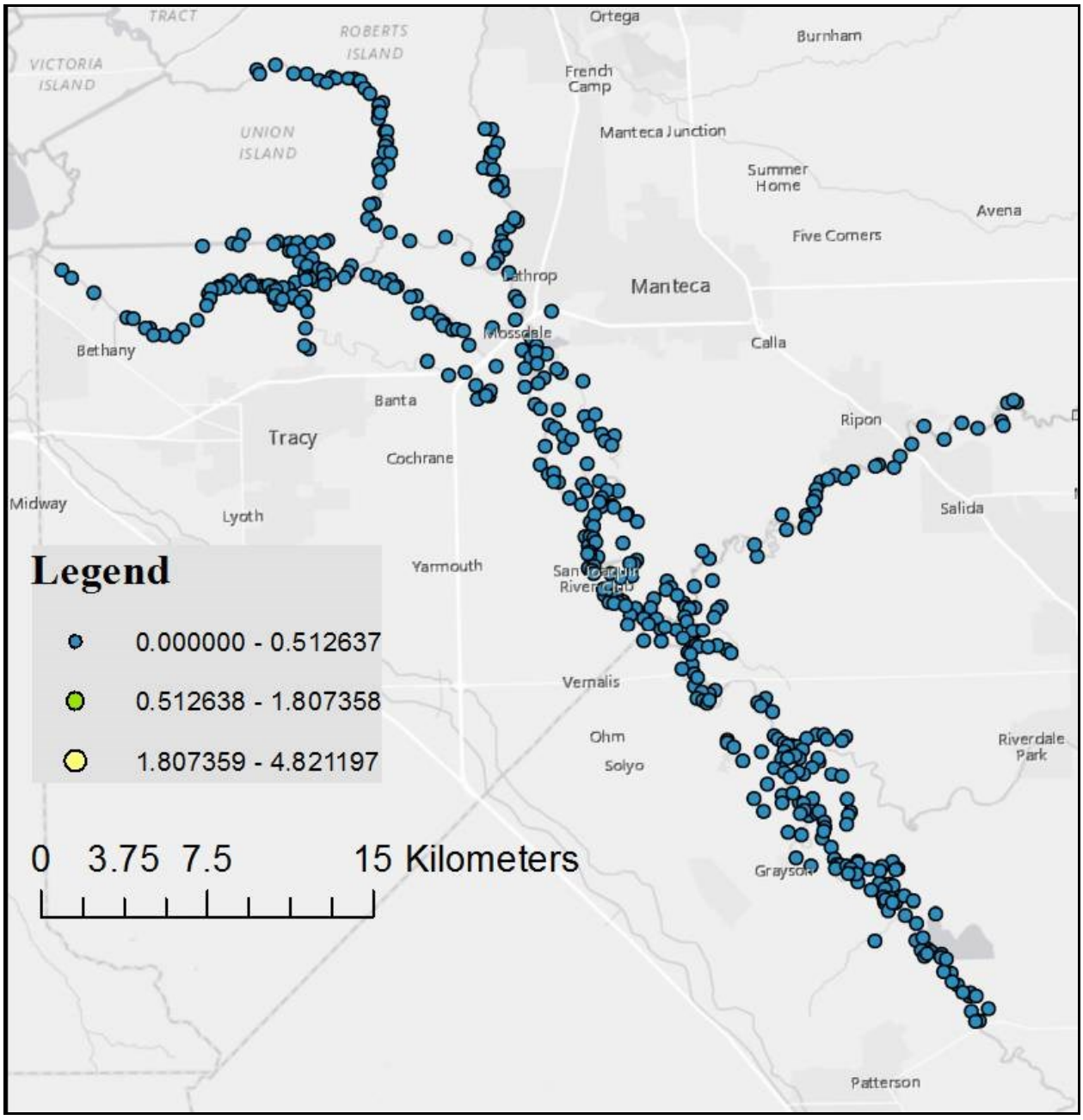

Figure 13. Patch value as a stepping stone ($dPC_{connector}$) calculated using effective distance between habitat patches. Importance is indicated by the size and color of the point; the larger and warmer in color the patch marker, the greater the patch value for overall connectivity. Connector value did not vary between regions ($p > 0.6$ for all pairwise comparisons).

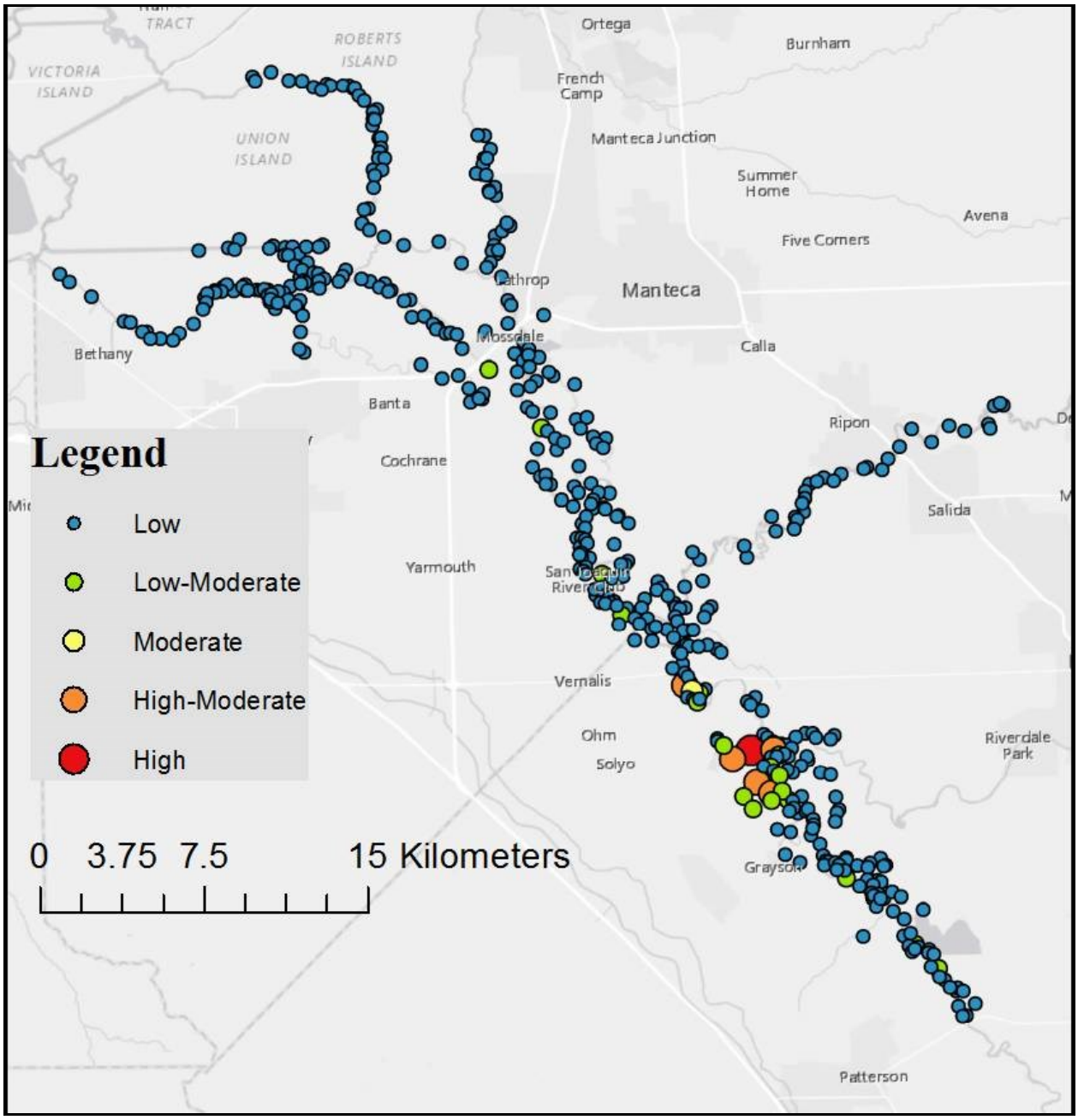

Figure 14. Patch migratory value (dPC_{flux}) calculated using effective distance between habitat patches. Importance is indicated by the size and color of the point; the larger and warmer in color the patch marker, the greater the patch value for overall connectivity. Patch migratory value varied only slightly between the NSJV and South Delta regions (p = 0.05) and not at all between these regions and Intermediate patches.

DISCUSSION

The rapid pace and large scale at which anthropogenic habitat fragmentation occurs challenges the adaptive potential of endemic species. For habitat specialists, like *S. b. riparius*, these challenges are compounded by the additional impermeability of a new, anthropogenic matrix, which can limit dispersal and gene flow between populations.

The greatest genetic differentiation between natural populations of *S. b. riparius* occurs between CMSP and the South Delta populations of Mossdale and Paradise Cut. These populations exhibit the greatest geographic distance from one another; however, Euclidean distance alone was not significantly correlated with genetic distance, suggesting additional factors influence genetic differentiation between these populations. Effective distance explained an additional 20 percent of the genetic differentiation between populations, and was statistically significant, indicating that habitat availability and connectivity are critical to brush rabbit gene flow. Given the relatively restricted nature of suitable riparian habitat along rivers we would have expected that Euclidean distances, often spanning non-riparian regions, would poorly reflect the functional connectivity represented by genetic distances. Similar patterns have been observed in other species associated with riparian habitat (Vignieri 2005, Watts et al. 2004).

While the NSJV holds significantly higher patch values than the Intermediate and South Delta regions when evaluated by Euclidean distance, only the migratory patch value varied significantly between regions when evaluated by effective distance. Though riparian corridors mirror straight-line distances, it is likely that the arrangement of edge and matrix substantially increases the effective distances between habitat patches, both within the NSJV region and throughout *S. b. riparius'* range. Because migratory value is weighted by patch area, the significant difference in migratory value between the NSJV and South Delta is largely a result of the larger habitat patches within the NSJV region.

While these findings highlight the extensive fragmentation of *S. b. riparius'* range, the restored population at SJRNWR provides an encouraging perspective on the role of translocations and habitat restoration in increasing functional connectivity. With regard to differentiation, the SJRNWR retains a strong affinity toward the South Delta, consistent with its augmentation history, yet exhibits less differentiation from the nearby CMSP than do the South Delta populations. While genetic differentiation between the SJRNWR and natural populations no longer provides an unaltered measure of natural gene flow, these results suggest recent gene flow between the refuge and CMSP—or other nearby, undocumented populations.

In addition to augmentation through translocations, SJRNWR and the geographically adjacent Faith Ranch have been subject to substantial habitat restoration over the last 15 years. As a result, these patches hold the highest overall and migratory patch values when evaluated by both Euclidean and effective distance. The high levels of habitat connectivity among these restored populations supports the potential for migration and gene flow between CMSP and the SJRNWR. As such, continued recovery and restoration efforts are not only essential for increasing the functional connectivity between *S. b. riparius* populations, but are likely the best options for management and recovery of this subspecies. Further, the augmented functional connectivity of riparian habitats we have documented has the potential to restore natural patterns of occupancy and gene flow for many other species that comprise these native communities.

LITERATURE CITED

Aguilar R, Quesada M, Ashworth L, Herrerias-Diego Y, Lobo J (2008) Genetic consequences of habitat fragmentation in plant populations: susceptible signals in plant traits and methodological approaches. Molecular Ecology, **17**, 5177-5188.

Andriaensen, FA, Chardon JP, De Blust G, Swinnen E, Villalba S, Gulinck H, Matthysen E (2002) The application of 'least-cost' modelling as a functional landscape model. Landscape and Urban Planning, **64**, 233-247.

Auffret GA, Plue J, Cousins SAO (2015) The spatial and temporal components of functional connectivity in fragmented landscapes. AMBIO, **44**, 51-59.

Barr KR, Kus BE, Preston KL, Howell S, Perkins E, Vandergast AG (2015). Habitat fragmentation in coastal southern California disrupts genetic connectivity in cactus wren (Campylorhynchus brunneicapillus). Molecular Ecology, **24**, 2349-2363.

Bell KC, Matocq MD (2011) Regional genetic subdivision in the Mohave ground squirrel: Evidence of historic isolation and ongoing connectivity in a Mojave Desert endemic. Animal Conservation, **14**, 371–381.

Bowman J, Fahrig L (2002) Dispersal distance of mammals is proportional to home range size. Ecology, **83**, 2049-2055.

Brekke, L.D., N. L. Miller, K.E. Bashford, N.W.T.Quinn, and J.A. Dracup (2004). Climate change impacts uncertainty for water resources in the San Joaquin River Basin, California, J. Amer. Water Resoures Assoc., 40, 149-164.

Chapman JA (1974) Sylvilagus bachmani. Mammalian Species, **34**, 1–4.

Cincotta RP, Wisnewski J, Engelman R (2000) Human population in the biodiversity hotspots. Nature, **404**, 990–992.

Constable, J., S. Phillips, D. Williams, J. Youngblom, and P. Kelly. 2010. Characterization of Genetic Structure and Phylogenetic Relationships of Riparian Brush Rabbit Populations. Unpublished report to the Central Valley Project Conservation Program and Habitat Restoration Program. CSU Stanislaus – Endangered Species Recovery Program, Turlock CA.

Davis EB, Koo MS, Conroy C, Patton JL, Moritz C (2008) The California Hotspots Project: Identifying regions of rapid diversification of mammals. Molecular Ecology, **17**, 120–138.

Estes-Zumpf WA, Rachlow JL, Waits LP (2008) Ten polymorphic microsatellite markers for the pygmy rabbit (*Brachylagus idahoensis*). Molecular Ecology Resources, **8**, 360-362.

Estes-Zumpf WA, Rachlow JL, Waits LP, Warheit KI (2010) Dispersal, gene flow, and population genetic structure in the pygmy rabbit (*Brachylagus idahoensis*). Journal of Mammalogy, **91**, 208-219.

Etherington TR (2011) Python based GIS tools for landscape genetics: visualizing genetic relatedness and measuring landscape connectivity. Methods in Ecology and Evolution, **2**, 52-55

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology, **14**, 2611-2620.

Ewers RM, Didham RK (2006) Confounding factors in the detection of species responses to habitat fragmentation. Biological reviews, **81**, 117-142.

Feldman CR, Spicer GS (2006) Comparative phylogeography of woodland reptiles in California: Repeated patterns of cladogenesis and population expansion. Molecular Ecology, **15**, 2201–2222.

Felsenstein J (1985) Confidence limits on phylogenies: An approach using the bootstrap. Evolution, **39**, 783-791.

Goldstein DB, Schlötterer C (1999) Microsatellites—Evolution and Applications. Oxford University Press, Oxford.

Gompert, Z. and Buerkle, C.A. (2011) A hierarchical Bayesian model for next-generation population genomics. Genetics, **187**, 903-917.

Harestad AS, Bunnel FL (1979) Home range and body weight—a reevaluation. Ecology, **60**, 389-402.

Haynes KJ, Cronin JT, de Roos A (2006) Interpatch Movement and edge effects: The role of behavioral responses to the landscape matrix. Oikos, **113**, 43-54.

Hewitt GM (1996) Some genetic consequences of ice ages, and their role, in divergence and speciation. Biological Journal of the Linnean Society, **58**, 247–276.

Jacobs DK, Haney TA, Louie KD (2004) Genes, diversity and the geologic process on the Pacific coast. Annual Review of Earth and Planetary Sciences, **32**, 601-652.

Jenness J (2016) Conefor Inputs Tool for ArcGIS 10.x. Jenness Enterprises, Flagstaff, Arizona. http://www.jennessent.com/arcgis/conefor_inputs.htm

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Mentjies P, Drummond A (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics, **28**, 1647-1649.

Kelly PA, Edgarian TK, Lloyd MR, Phillips SE (2011) Conservation principles for the Riparian Brush Rabbit and Riparian Woodrat. Unpublished report prepared for the U.S. Fish and Wildlife Service, Bay-Delta Fish and Wildlife Office, the California Department of Fish and Game and the California Department of Water Resources. California State University, Stanislaus—Endangered Species Recovery Program, Turlock, CA.

Kelly PA, Phillips SE, Williams DF (2005) Documenting ecological change in time and space: The San Joaquin Valley of California. In: Mammalian Diversification: From Chromosomes to Phylogeography (A Celebration of the Career of James L. Patton), pp. 57–78.

Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution, **33**, 1870-1874.

Lenihan J.M., R. Drapek, D. Bachelet, and R.P. Neilson (2003). Climate Change Effects on Vegetation Distribution, Carbon, and Fire in California. *Ecological Applications*, 13(6).Storfer, A. 1999. Gene flow and endangered species translocations: a topic revisited. Biological Conservation 87:173-180.

Li, H et al. 1000 genome project data processing subgroup (2009). The sequence alignment/map format and SAMtools. Bioinformatics, **25**, 2078-2079.

Li, H. and Durbin R. (2009) Fast and accurate short read alignment with Burrows-Wheeler Transform. Bioinformatics, **25**, 1754-60.

Lidicker, WZ (1998) Responses of mammals to habitat edges: an overview. Landscape Ecology, **14**, 333-343.

Lindenmayer DB, Fischer J (2006) Habitat loss. In Habitat Fragmentation and Landscape Change: an ecological and conservation synthesis. Island Press, Washington, D.C.

Mantel N (1967) The detection of disease clustering and a generalized regression approach. Cancer Research, **27**, 209-220.

Matocq MD, Kelly PA, Phillips SE, Maldonado JE (2012) Reconstructing the evolutionary history of an endangered subspecies across the changing landscape of the Great Central Valley of California. Molecular Ecology, **21**, 5918–5933.

Mendez M, Vogel M, Tella JL, Godoy JA (2014) Joint effects of population size and isolation on genetic erosion in fragmented populations: finding fragmentation thresholds for management. Evolutionary Applications, **7**, 506-518.

Mittermeier RA, Mittermeier CG, Myers N, da Fonseca NA, Kent J (2000) Biodiversity hotspots for conservation priorities. Nature, **403**, 853–858.

Mougel F, Mounolou JC, Monnerot M (1997) Nine polymorphic microsatellite loci in the rabbit, *Oryctolagus cuniculus*. Animal Genetics, **28**, 58-71.

Murphy M, Dyer R, Cushman SA (2016) Graph theory and network models in landscape genetics. In *Landscape Genetics: Concepts, Methods and Applications.* John Wiley and Sons, Ltd., West Sussex, UK.

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics, **89**, 583-590.

Orr RT (1940) The rabbits of California. California Academy of Sciences, San Francisco, CA.

Palumbi SR (2001) Humans as the World's Greatest Evolutionary Force. Science, **293**, 1786-1790.

Peakall R, Smouse PE (2006) GenAlEx 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes, **6**, 288-295.

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research. Bioinformatics, **28**, 2537-2539.

Phillips SE, Edgarian TK, Lloyd MR, Kelly PA (2013) Habitat Suitability and Connectivity for Riparian Brush Rabbits in the Northern San Joaquin Valley and Sacramento/San Joaquin River Delta. Unpublished report to the U.S. Fish and Wildlife Service, San Luis National Wildlife Refuge Complex. California State University, Stanislaus, Turlock, CA.

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics, **155**, 945–959.

R Core Team (2015) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Rico C, Rico I, Webb N, Smith S, Bell D, Hewitt G (1994) Four polymorphic microsatellite loci for the European wild rabbit, *Oryctolagus cuniculus*. Animal Genetics, **25**, 367.

Rissler LG, Hijmans RJ, Graham CH, Moritz C, Wake DB (2006) Phylogeographic lineages and species comparisons in conservation analyses: a case study of california herpetofauna. The American Naturalist, **167**, 655–666.

Saitou N, Nei M (1987) The neighbor-joining method: A new method for reconstruction phylogenetic trees. Molecular Biology and Evolution, **4**, 406-425.

Saura S, Pascual-Hortal L (2007) A new habitat availability index to integrate connectivity in landscape conservation planning: comparison with existing indices and application to a case study. Landscape and Urban Planning, **83**, 91-103.

Saura S, Rubio L (2010) A common currency for the different ways in which patches and links can contribute to the habitat availability and connectivity in a landscape. Ecography, **33**, 523-537.

Saura S, Torné J (2009) Conefor Sensinode 2.2: a software package for quantifying the importance of habitat patches for landscape connectivity. Environmental Modelling & Software, **24**, 135-139.

Selkoe KA, Toonen RJ (2006) Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. Ecology Letters, **9**, 615-629.

Slatkin M (1995) A measure of population subdivision based on microsatellite allele frequencies. Genetics, **139**, 457-462.

Surridge AK, Bell DJ, Rico C, Hewitt GM (1997) Polymorphic microsatellite loci in the European rabbit (*Oryctolagus cuniculus*) are also amplified in other lagomorph species. Animal Genetics, **28**, 302-305.

Szpiech ZA, Jakobsson M, Rosenberg NA (2008) ADZE: a rarefaction approach for counting alleles private to combinations of populations. Bioinformatics, **24**, 2498-2504.

Tamura K, Nei M, Kumar S (2004) Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences (USA), **100**, 11030-11035.

Tischendorf L, Fahrig L (2000) On the usage and measurement of landscape connectivity. Oikos, **90**,7-19.

Tursi RM, Hughes PT, Hoffman EA (2013) Taxonomy versus phylogeny: evolutionary history of marsh rabbits without hopping to conclusions. Diversity and Distributions, **19**, 120-133.

U.S. Census Bureau (2012) California: 2010: Population and housing unit counts. U.S. Government Printing Office, Washington, D.C.

U.S. Department of Commerce, Bureau of the Census (1996) Population of states and counties of the United States: 1790 to 1990, from the twenty-one decennial censuses. U.S. Government Printing Office, Washington, D.C.

U.S. Fish and Wildlife Service (1998). Recovery plan for upland species of the San Joaquin Valley, California. Region 1, Portland, OR 319 pp.

U.S. Fish and Wildlife Service (2000). Endangered and threatened wildlife and plants; final rule to list the riparian brush rabbit and the riparian, or San Joaquin Valley, woodrat as endangered. Federal Register 65 (36):8881-8890.

Urban D, Keitt T (2011) Landscape connectivity: a graph-theoretic perspective. Ecology, **82**, 1205-1218.

Vandergast, AE, Bohonak AJ, Weissman DB, Fisher RN (2007) Understanding the genetic effects of recent habitat fragmentation in the context of evolutionary history: phylogeography and landscape genetics of a southern California endemic Jerusalem cricket (Orthoptera: Stenopelmatidae: Stenopelmatus). Molecular Ecology, **16**, 977-992.

Vignieri SN (2005) Streams over mountains: influence of riparian connectivity on gene flow in the Pacific jumping mouse (Zapus trinotatus). Molecular Ecology, **14**, 1925-1937

Waits LF, Storfer A (2016) Basics of population genetics: quantifying neutral and adaptive genetic variation for landscape genetic studies. In *Landscape Genetics: Concepts, Methods and Applications.* John Wiley and Sons, Ltd., West Sussex, UK.

Waltari E, Demboski JR, Klein DR, Cook JA (2004) A molecular perspective on the historical biogeography of the northern high latitudes. Journal of Mammalogy, **85**, 591-600.

Watts PC, Rouquette JR, Saccheri IJ, Kemp SJ, Thompson DJ (2004) Molecular and ecological evidence for small-scale isolation by distance in an endangered damselfly, *Coenagrion mercuriale.* Molecular Ecology, **13**, 2931-2945.

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. Evolution, **38**, 1358–1370.

Whitlock MC, McCauley DE (1999) Indirect measures of gene flow and migration: FST doesn't equal 1/(4*Nm* + 1). Heredity, **82**, 117-125.

Wilcove DS, MacLellan CH, Dobson AP (1986) Habitat fragmentation in the temperate zone. In: Conservation Biology (ed. Soule ME), pp. 237-256. Sinauer, Sunderland, MA.

Williams DF, Lloyd MR, Hamilton LP, Williams EA, Kelly PA (2004) Controlled propagation and reintroduction of riparian brush rabbits (*Sylvilagus bachmani riparius*): annual report for 2003-2004. California State University, Stanislaus, Endangered Species Recovery Program.

Williams, D. F. (1993). Population censuses of riparian brush rabbits and riparian woodrats at Caswell Memorial State Park during January 1993. Final Report, California Dept. Parks and Recreation, Lodi, CA 15 pp.

Williams, D.F. (1988). Ecology and management of the riparian brush rabbit in Caswell Memorial State Park. California Dept. Parks and Recreation, Final Report, Interagency Agreement, 4-305-6108, Lodi, CA 38 pp.

Williams, D.F., and G.E. Basey (1986). Population status of the riparian brush rabbit, *Sylvilagus bachmani riparius*. California Dep. Fish and Game, Sacramento, Wildl. Manage. Div., Nongame Bird and Mammal Section, Contract Final Report, 21 pp.

Williams, D.F., and L.P. Hamilton (2001). Riparian Brush Rabbit Survey: Paradise Cut along Stewart Tract, San Joaquin County, California, August 2001. Report to Califia LLC, Lathrop, CA, and California Department of Fish and Game, Sacramento, 10 pp.

Williams, D.F., L.P. Hamilton, J.J. Youngblom, C. Lee, and P.A. Kelly (2000). Riparian brush rabbit studies, 1997-2000. Report prepared for the U.S. Bureau of Reclamation and Fish and Wildlife Service, Endangered Species Recovery Program, Fresno, CA 13 pp.

Wright S (1943) Isolation-by-distance. Genetics, **28**, 114-138.

APPENDIX A. GLOSSARY OF TERMS

allele	One of two or more alternative forms of a gene that arise by mutation.
haplotype	A group of genes within an organism that was inherited together from a single parent.
heterozygosity	A diploid organism is heterozygous at a gene locus when it has two different alleles for that gene. The heterozygosity of a population is the proportion of individuals heterozygous for a particular locus or gene.
genotype	The genetic constitution of an organism, or typically, the portion of the full genotype sampled for a particular study.
microsatellite	A tract of repetitive DNA in which certain DNA motifs (ranging in length from 2–5 base pairs) are repeated, typically 5–50 times.
gene flow	The transfer of alleles or genes from one population to another.
effective population size	The number of individuals in a population who contribute offspring to the next generation.
genetic drift	Random changes in allele frequency from one generation to the next due to chance alone. Smaller populations experience higher rates of genetic drift.
F_{ST}	A measure of population differentiation.
mitochondrial DNA	Extranuclear DNA found in mitochondria that in most eukaryotes is a circular molecule that is only maternally inherited—called also mtDNA.