

University of Nevada, Reno

**Population Genetics and Functional Connectivity of the Riparian Brush Rabbit
(*Sylvilagus bachmani riparius*):
Implications for the Conservation of an Endangered Lagomorph**

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science in Natural Resources and
Environmental Science

by

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May, 2017



THE GRADUATE SCHOOL

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prepared under our supervision by

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Entitled

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be accepted in partial fulfillment of the
requirements for the degree of

MASTER OF SCIENCE

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Abstract

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 to landscape often occur rapidly and over large spatial scales, challenging the adaptive potential of native organisms. Wide-spread 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). California's 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 organismal populations while augmenting genetic drift within populations. Using a combination of molecular data and graph theory approaches, I assessed the genetic diversity, population genetic structure, and structural and functional connectivity of the riparian brush rabbit (*Sylvilagus bachmani riparius*). Endemic to the riparian forests of the San Joaquin Valley, the riparian brush rabbit has lost over 95 percent of its habitat since European settlement. I 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. 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. While these findings highlight the extensive fragmentation of *S. b. riparius*' range, the augmented population at SJRNWR retains high levels of diversity and functional connectivity. As such, *S. b. riparius* would likely respond favorably to additional augmentation and restoration efforts.

Acknowledgements

First and foremost, I would like to thank my adviser, Dr. Marjorie Matocq, who has been a wonderful mentor throughout my undergraduate and graduate careers. With every challenge graduate school and life presented me, Marjorie was there with words of support, encouragement and guidance. I am incredibly grateful for her unwavering belief in my ability to “go forth and conquer”—even when I did not fully believe it myself. She has been an instrumental part of my growth as both a researcher and an individual.

I would also like to thank my committee members, Dr. Chris Feldman and Dr. Peter Weisberg for their excellent feedback and enthusiasm throughout this project. I thank the Evol-Doers—the extended Matocq-Feldman-Voyles-Ouyang lab group—both past and present, for your encouragement and inspiration. I thank Dr. Jason Malaney for his instruction in all things population genetics, and Dr. Angela Hornsby for her guidance in phylogenetic approaches, as well as her wisdom and support through all the ups and downs of grad school. I also thank Tom Dilts, for his guidance and support as I navigated the world of landscape genetics; Chapter 2 would have been impossible without him.

I am quite fortunate to have the support of my family, both the one I was born into and the one that I found along the way. To my mom, Sue Reeves: thank you for being there with words of encouragement, a shoulder to lean on and undying love and support. To my grandparents, Don and Carol Reeves: thank you for support and enthusiasm as I talked endlessly of school and bunnies. To my sister, Katelyn Smith: thank you for your amazing sense humor on the rough days, and being my sidekick to celebrate the good. To my Dad, Ed Rippert: thank you for your wisdom, perspective and inspiration. To Zach

Gierahn: thank you for helping me to rediscover my drive and my writing mojo, and for your endless support in good times and bad. To my best friends and fellow researchers, Jade Keehn and Danielle Walsh: I can hardly put to words how much your support has meant to me. Coffees, beers, pep talks and field adventures—I would not be where I am today without you two.

This project was largely funded by the Central Valley Project Conservation Program. I thank the Endangered Species Recovery Program for providing the biological samples and habitat maps used for this project. I would like to specifically thank Dr. Patrick Kelly and Bernadette Paul of the ESRP for their continued support and feedback throughout this project. I thank the UNR Graduate Student Association, the American Society of Mammalogists, and the Western Section of The Wildlife Society for the funding that enabled me to share this research with other wildlife and conservation professionals.

Last but not least, I owe a huge thank you to my four-legged companions, Stella, Icky and Skid. Their support—both in the field and at the desk—was immeasurable. I promise I will soon make up for all the hikes and dog park visits we missed while I was analyzing my data and writing my thesis...

Table of Contents

Abstract.....	i
Acknowledgements.....	iii
Chapter 1: Population Genetic Diversity and Structure of the Riparian Brush Rabbit (<i>Sylvilagus bachmani riparius</i>).....	1
Abstract.....	1
Introduction.....	2
Methods.....	5
Results.....	9
Discussion.....	12
Literature Cited.....	17
Tables and Figures.....	23
Chapter 2: Evaluating the Range-Wide Functional and Structural Connectivity of an Endangered Habitat Specialist, the Riparian Brush Rabbit (<i>Sylvilagus bachmani riparius</i>)	30
Abstract.....	30
Introduction.....	31
Materials and Methods.....	34
Results.....	38
Discussion.....	40
Literature Cited.....	43
Tables and Figures.....	48

Chapter 1: Population Genetic Diversity and Structure of the Riparian Brush

Rabbit (*Sylvilagus bachmani riparius*)

KEY WORDS: *Sylvilagus bachmani riparius*, endangered species, population genetics, San Joaquin Valley, California Floristic Province

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 primarily to habitat loss and fragmentation, but also periodic flooding, drought and wildfire, warranting its listing as an endangered subspecies at both the state and federal level. To better guide recovery efforts, I identified current genetic diversity and population genetic structure of five remnant populations of *S. b. riparius*, 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. However, 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 augmented population at the San Joaquin River National Wildlife Refuge 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. As such, *S. b. riparius* would likely respond favorably to additional augmentation and restoration efforts.

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 changes 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 (Williams et al. 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 1). 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 and Federally endangered subspecies in 2002 (Williams et al. 2004).

In 2001, the Endangered Species Recovery Program began a controlled propagation and release program to repatriate the San Joaquin River National Wildlife Refuge (SJRNR; Williams et al. 2004). From 2001 to 2012, six South Delta individuals were captured per year and bred in large pens. Captive-born individuals were released at SJRNR 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 SJRNR suffered dramatic declines in 2006 due to flooding. Additional translocations were used in conjunction with habitat restoration to recover those losses. Since founding SJRNR, brush rabbits have been observed at Buffington, Faith Ranch, and Durham Ferry (Figure 1B) but whether these animals are native or dispersers from SJRNR is unknown.

Here, I seek 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, I used a combination of mitochondrial sequences and microsatellite genotypes to address the following questions:

- 1) Do mitochondrial lineages exhibit differentiation between subspecies, suggesting historic divergence?
- 2) Do remnant native populations of *S. b. riparius* exhibit lower genotypic diversity than populations of neighboring subspecies that occupy areas of greater habitat availability and connectivity?
- 3) Are remnant populations of *S. b. riparius* genetically differentiated from one another, suggesting genetic drift within populations and restricted contemporary gene flow?
- 4) Does the translocated population at SJRNWR capture the genetic diversity and composition of natural populations of *S. b. riparius*?

METHODS

Genetic samples

I 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*. I 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; Fig. 1A). To represent diversity within *S. b. macrorhinus*, I 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 1A). *S. b. mariposae* is native to the foothills and western slopes of the Sierra Nevada, east of the San Joaquin Valley (Orr 1940; Fig. 1A). I 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 1A). 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). I 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*, I 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, I 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. I 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 μ l of H₂O, 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. I 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). I ran products on an ABI 3730 DNA Analyzer in the Nevada Genomics Center. I assembled and aligned fragments in Geneious v.7.0.6 (Kearse et al. 2012), and verified each sequence by eye. I aligned and trimmed the resulting sequences in MEGA 7.0 (Kumar et al. 2016). I then inferred phylogenetic relationships using a Neighbor-Joining approach implemented in MEGA 7.0 (Saitou and Nei, 1987, Tamura et al. 2004). I tested the robustness of nodes using 1000 bootstrap replicates (Felsenstein 1985), and computed evolutionary distances using the Maximum Composite Likelihood method (Tamura et al. 2004).

Population genetic diversity

To assess population-level diversity within the San Joaquin Valley, I 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*, I 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*, Mougél et al. 1997, *Sylvilagus palustris*, 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). I 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 H₂O, 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. I 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.

Analyses

I 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, I 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). I 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} . I 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, I 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, I 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. I 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, I 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).

RESULTS

Phylogenetic relationships of San Joaquin Valley brush rabbits

I find two modestly supported clades within *S. bachmani* (Figure 2). One clade predominates on the western flank of the Central 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 main 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, SP Railroad 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. Overall, *S. b. macrorhinus* retains greater haplotype diversity (19 haplotypes present in the 26 individuals sampled from the subspecies) than *S. b. riparius* (14 haplotypes present in the 78 individuals sampled from the subspecies), with one haplotype shared between the subspecies (Caswell, Pacheco and Mossdale).

Population genetic diversity

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 $n = 9$.

Population differentiation and genetic structure

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*, I 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 Mosssdale by an average $F_{ST} = 0.07$.

The first two coordinate axes of the PCoA explain 16.05 percent of the genetic variation among individuals and reveal overall genetic distinction among the subspecies, despite some overlap of certain individuals among groups (Fig. 3A). The Bayesian analysis of genetic structure gave strongest support for two genetic clusters within the three subspecies (Figure 3B), 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 $K = 4$ (Fig. 3C; blue and green, respectively), with the two additional genetic clusters corresponding to the *S. b. riparius* populations within the South Delta and Mosssdale (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 17.19 percent of the genetic variation among individuals and reveal distinction between the natural populations at CMSP (Fig. 4A; gold) and the South Delta and Mosssdale (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 4B). 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, I 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.

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

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 three haplotypes are present within the Pacheco population of *S. b. macrorhinus*, as well as the single haplotype from *S. b. mariposae*. 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 two additional haplotypes that fall within the clade predominant in *S. b. riparius* populations. 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 structure of natural S. b. riparius populations

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 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 (mostly green or light blue) 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 increasing 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 structure 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 augmented 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.

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TABLES AND FIGURES

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

Table 2. Diversity measures for 10 populations of brush rabbits. 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

	n	uH_E	A	A_4	A_9	A_{PS}	A_{PP}
<i>S.b. riparius</i>							
Paradise Cut	20	0.64	5.9	2.5	3.7		1
SPRR	9	0.67	4.9	2.6	3.9		1
Mossdale	21	0.68	5.1	2.6	3.6	20	3
Caswell MSP	17	0.62	5.0	2.4	3.5		2
SJRNWR	56	0.68	6.8	2.6	3.8		7
<i>S.b. macrorhinus</i>							
West Diablo	14	0.66	5.6	2.5	3.7	19	22
Pacheco	16	0.67	5.8	2.6	3.9		24
<i>S.b. mariposae</i>							
Longbarn	9	0.51	3.1	2.1	2.8		10
Coulterville	5	0.62	3.7	2.6	-	12	13
Piute	5	0.57	3.1	-	-		14

the average number of private alleles per population (A_{PP}).

Table 3. Two measures of genetic differentiation between brush rabbit populations. Pairwise F_{ST} 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 F_{ST} values are significantly different from zero with the exception of Paradise Cut and SPRR.

	<i>S. b. riparius</i>						<i>S. b. macrorhinus</i>			<i>S. b. mariposae</i>		
	Paradise Cut	SPRR	Mossdale	SJRNR	Caswell MSP	West Diablo	Pacheco	Longbarn	Coulterville	Piute		
Paradise Cut	-	0.03	0.18	0.17	0.36	0.53	0.36	0.63	0.60	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	0.60		
SJRNR	0.07	0.07	0.10	-	0.26	0.57	0.41	0.66	0.63	0.63		
Caswell MSP	0.15	0.12	0.16	0.11	-	0.47	0.40	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	0.20	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	0.20	0.23	0.23	0.23	0.25	0.18	0.23	0.11	-		

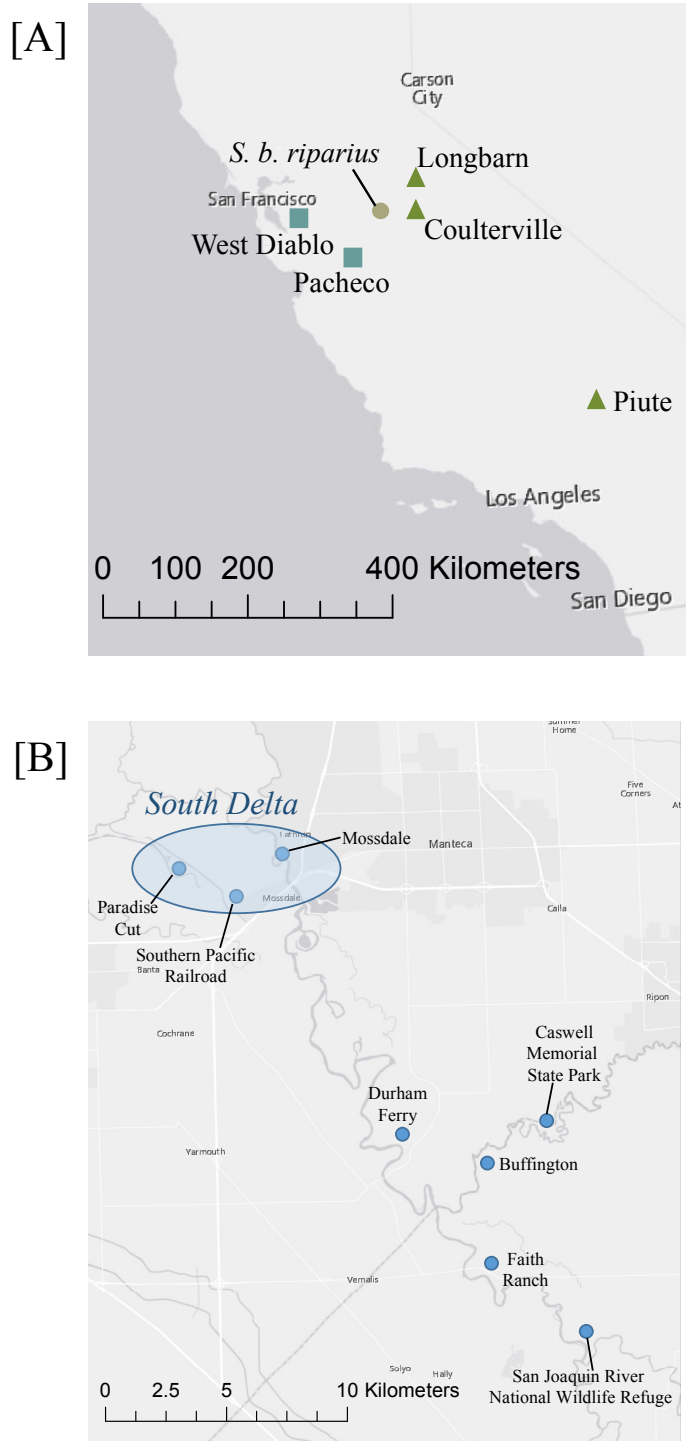


Figure 1. Locations of 11 brush rabbit populations across three subspecies [A], and eight populations of *S. b. riparius* [B].

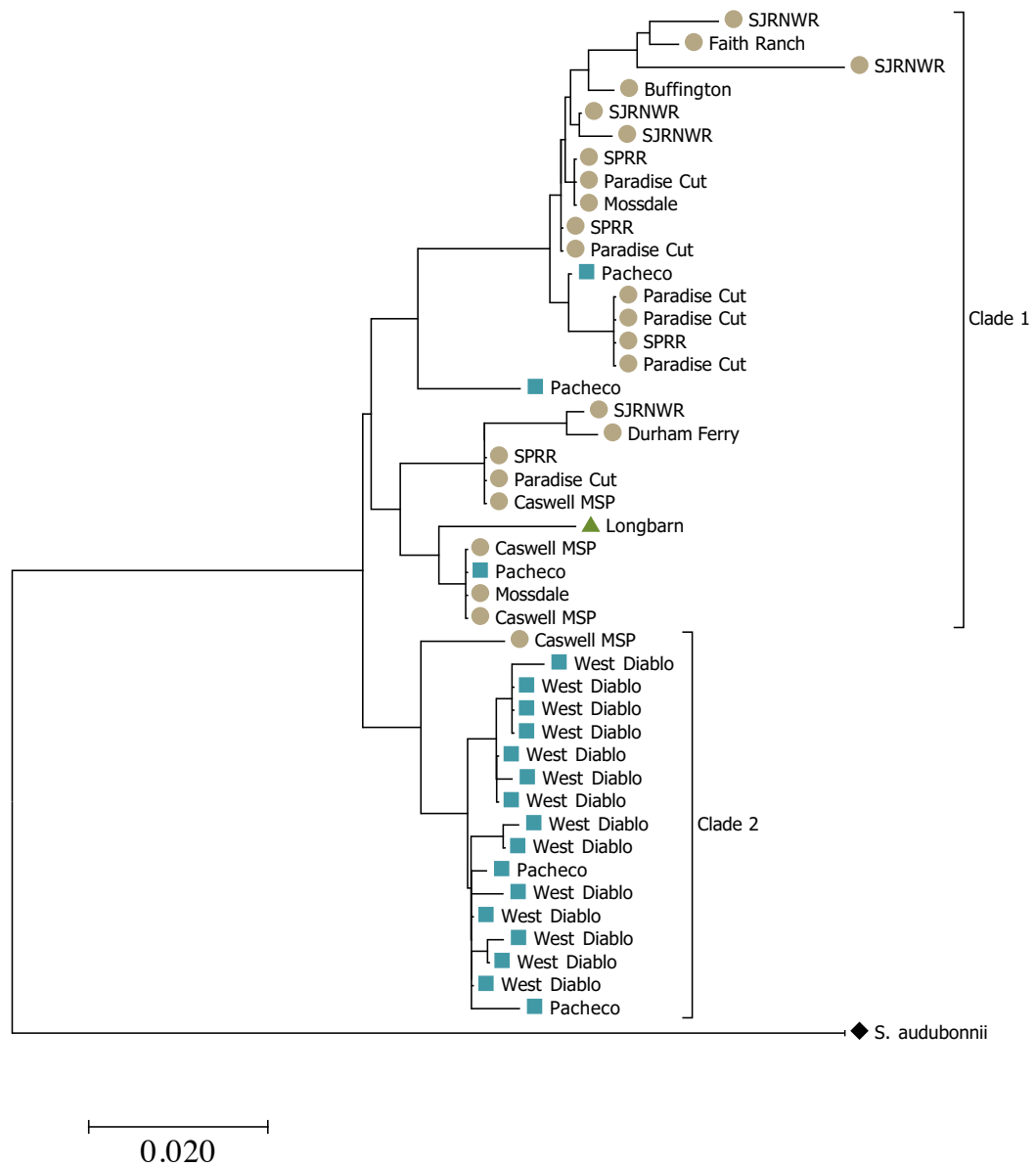


Figure 2. Phylogenetic relationships within *S. bachmani* based on mtDNA control region and partial threonine tRNA gene using a Neighbor-Joining approach. Each node represents a single haplotype per population of brush rabbit, and colors of the nodes correspond to subspecies (*S. b. riparius* in red, *S. b. mariposae* in green, and *S. b. macrorhinus* in blue). Branch lengths are based on the evolutionary distances between haplotypes, computed using a Maximum Composite Likelihood approach.

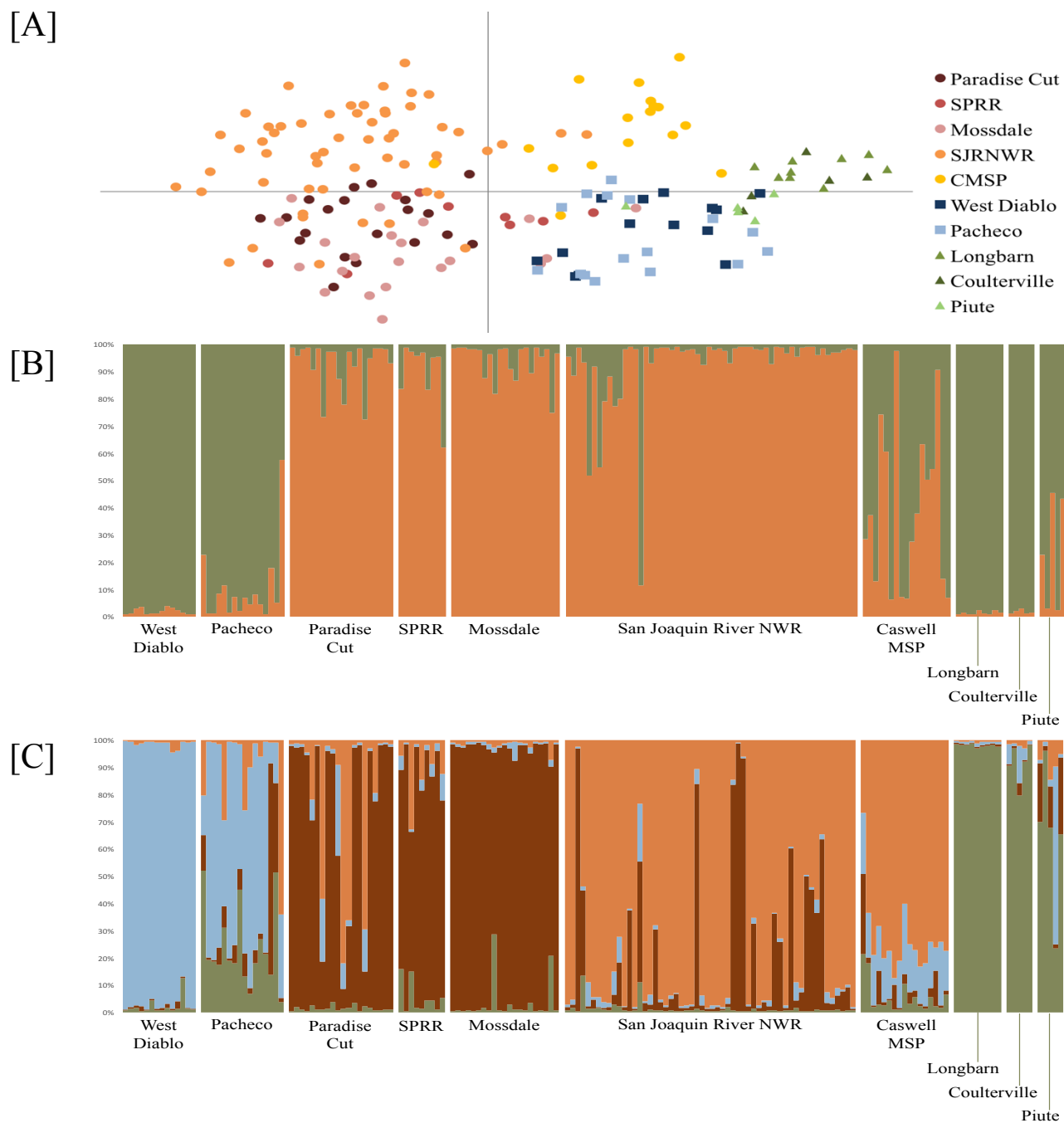


Figure 3. Genetic subdivision of brush rabbit subspecies in and flanking the San Joaquin Valley. [A] Principal Coordinates Analysis (PCoA) of 12 microsatellite loci for 172 individual brush rabbits. [B] Individual-based Bayesian clustering analysis at $K=2$, and [C] $K=4$ for 10 populations of brush rabbits across three subspecies. The genetic composition of each individual is represented by a single vertical bar.

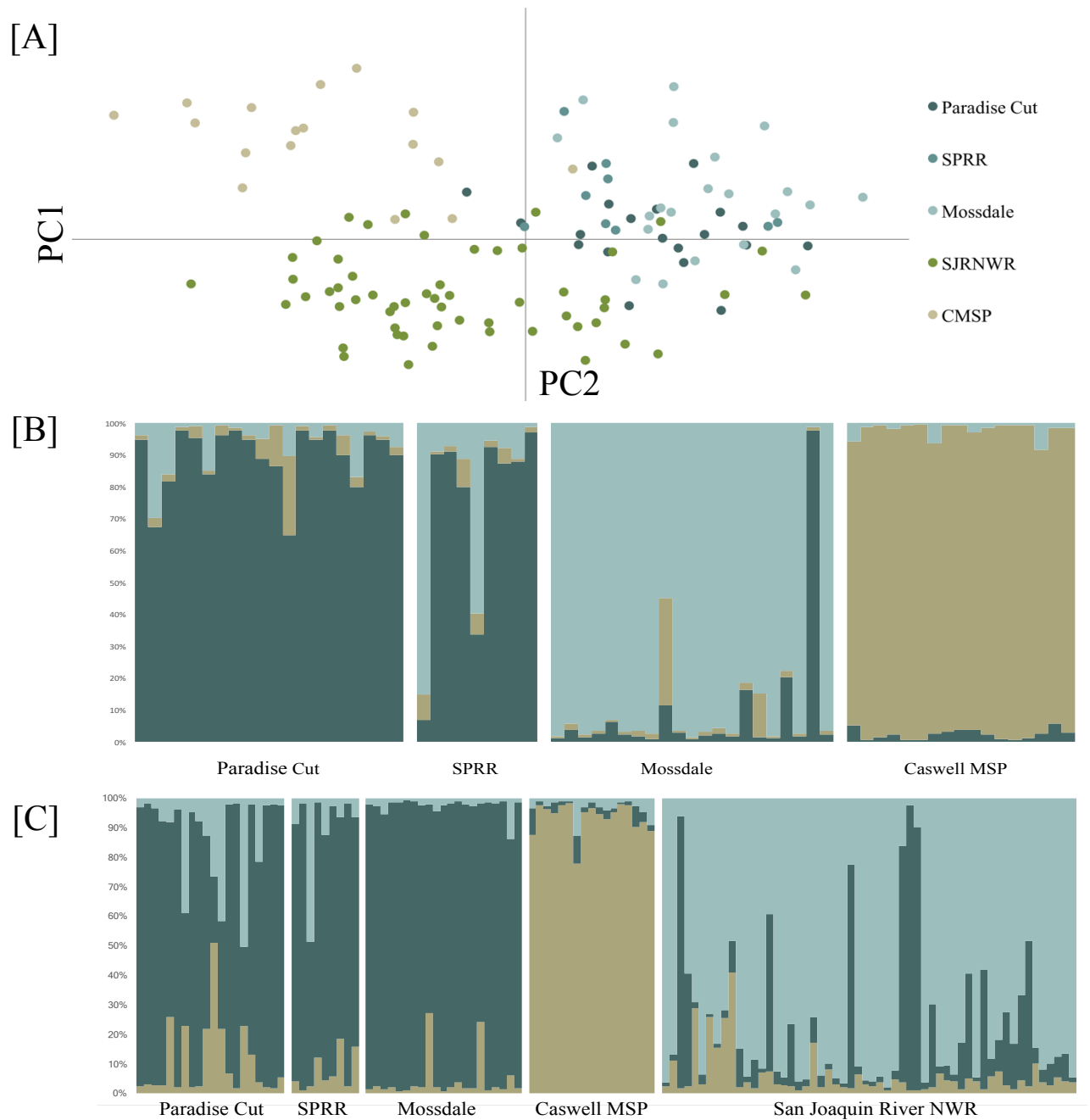


Figure 4. Genetic subdivision among populations of riparian brush rabbits. [A] Principal Coordinates Analysis of 12 microsatellite loci for 125 individual brush rabbits from 5 populations. [B] Individual-based Bayesian clustering analysis at $K=3$ for natural and [C] all populations of *S. b. riparius*. The genetic composition of each individual is represented by a single vertical bar.

Chapter 2: Evaluating the Range-Wide Functional and Structural Connectivity of an Endangered Habitat Specialist, the Riparian Brush Rabbit (*Sylvilagus bachmani riparius*)

KEY WORDS: graph theory, landscape genetics, conservation, habitat restoration

ABSTRACT

Anthropogenic changes to habitat structure and connectivity occur rapidly over large spatial scales, challenging the adaptive potential of native species. Such changes not only decrease the presence and configuration of habitat (structural connectivity), but also affect habitat quality and the behaviors of organisms moving within and among patches (functional connectivity). Graph theory is a powerful approach for quantifying and comparing structural and functional connectivity of fragmented landscapes. By using a combination of genetic, Euclidean and effective (cost-weighted) distances in a graph theory framework, I evaluate structural and functional connectivity across the range of the endangered riparian brush rabbit (*Sylvilagus bachmani riparius*). The riparian brush rabbit has lost over 95 percent of its habitat over the last century, and is limited to five known populations. I find 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. Functional connectivity is severely limited throughout *S. b. riparius*' range in consideration of effective habitat distances between habitat patches. Despite the overall level of fragmentation across this system, I find that habitat patches with the greatest importance for connectivity occur within and around the San Joaquin River National Wildlife Refuge, where efforts to restore habitat and repatriate *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.

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 organism 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, I 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 1). 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 1) and between the refuge and the South Delta (Durham Ferry; Figure 1), 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 1). The South Delta is an aggregate of numerous, small patches of riparian habitat at the mouth of San Joaquin river, while NSJV hosts larger patches of habitat along the confluence of the Stanislaus and San Joaquin rivers. Patches in both

regions are punctuated by both agricultural and urban landscapes. My previous analyses 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 chapter. Briefly, I 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). I amplified DNA samples in four multiplex panels of three to five primers. Following amplification, I submitted samples to the Nevada Genomics Center and run on an ABI 3730 DNA analyzer (Applied Biosystems Inc.). I identified allele sizes using GeneMarker software v1.85 (SoftGenetics) and verified by eye. I 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 augmented 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, I 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). I assigned patches to putative regions based on genetic groups established in Chapter 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”. I 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). I 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). I 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. I 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. I 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.

I 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 has a strong correlation 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 the connectedness of a habitat throughout the network with the removal of each patch, giving in 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 patches 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 chapter, 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, Mosssdale; Table 1). Within the South Delta, the populations Paradise Cut and SPRR are not significantly differentiated ($F_{ST} = 0.02$), and differ from Mosssdale by an average $F_{ST} = 0.07$ (Table 1). 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 1), as well as CMSP ($F_{ST} = 0.11$, Table 1).

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 2). 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 3).

Structural connectivity and patch importance

Mean patch area varied from 22,033m² in the South Delta region to 121,488m² in the NSJV region (Table 2). 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).

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 4). 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 5) 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 ($dPC_{\text{connector}}$; Figure 6) 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 7), nor did the patch connector value (ANOVA, $p > 0.6$ for all pairwise comparisons; Figure 8). As with Euclidean distance, the most important patch for network connectivity was the SJRNWR ($dPC = 68.19$, $dPC_{\text{connector}} = 0.000002$). Patch migratory value varied only slightly between the NSJV and South Delta regions (ANOVA, $p = 0.05$; Figure 9) and not at all between these regions and Intermediate patches. Migratory value was highest at Faith Ranch ($dPC_{\text{flux}} = 35.5$)

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 alter movement 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 I 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 augmented 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 I have documented has the potential to restore natural patterns of occupancy and gene flow for many other species that comprise these native communities.

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TABLES AND FIGURES

Table 1. Genetic differentiation between brush rabbit populations, measured as F_{ST} .

Values were obtained from the analysis of genotypes resolved at 12 microsatellite loci.

All pairwise F_{ST} 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	0.10	0.11

Table 2. 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 (km²)		
	<i>minimum</i>	<i>maximum</i>	<i>mean</i>
South Delta	0.004	0.185	0.022
Intermediate	0.004	0.516	0.051
NSJV	0.004	5.737	0.121

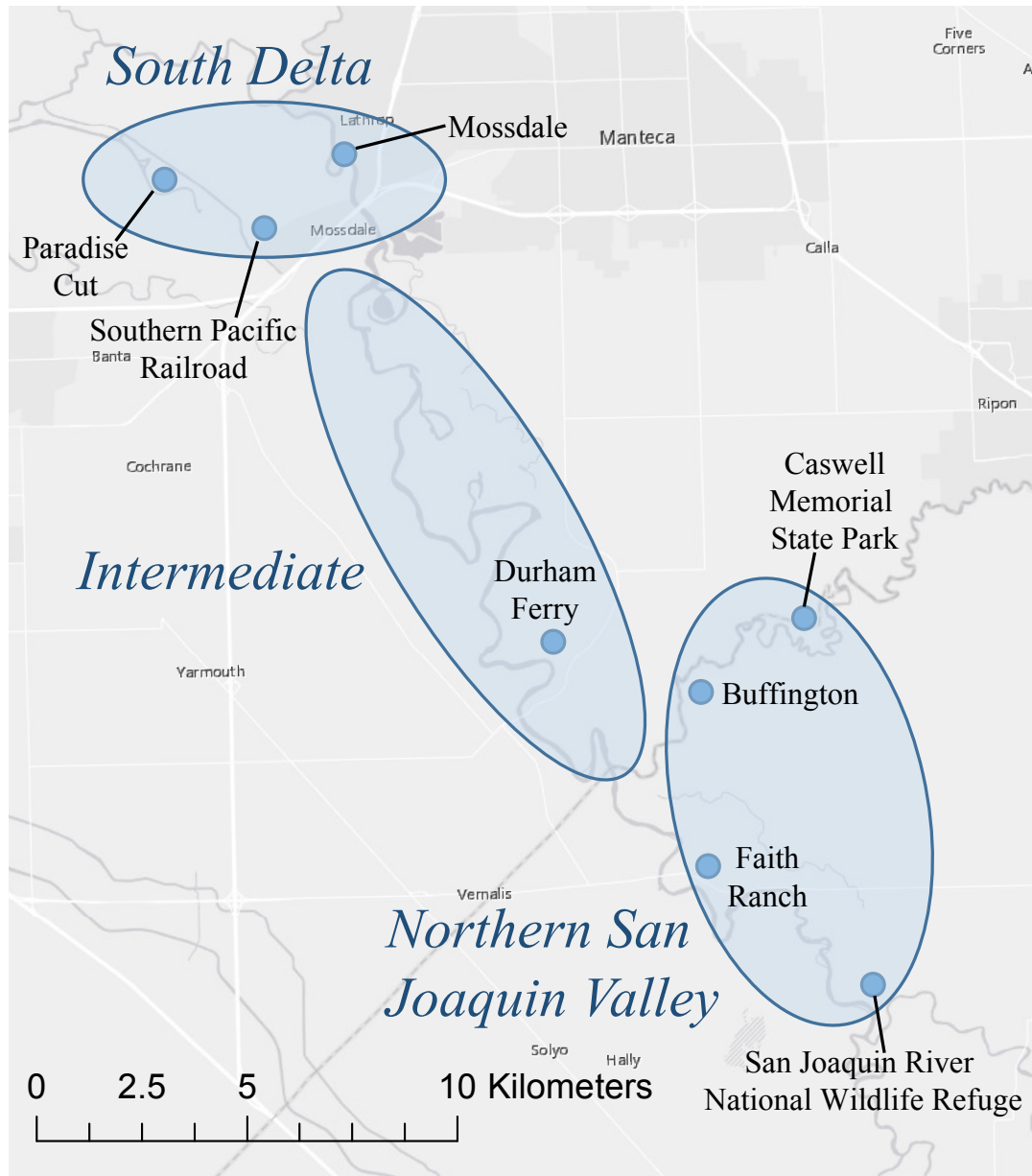


Figure 1. Locations of natural (Paradise Cut, Mossdale, Southern Pacific Railroad, and Caswell Memorial State Park) and augmented (San Joaquin River National Wildlife Refuge) *S. b. riparius* populations, and known occupied patches (Durham Ferry, Buffington and Faith Ranch). Putative habitat regions are indicated by elipses.

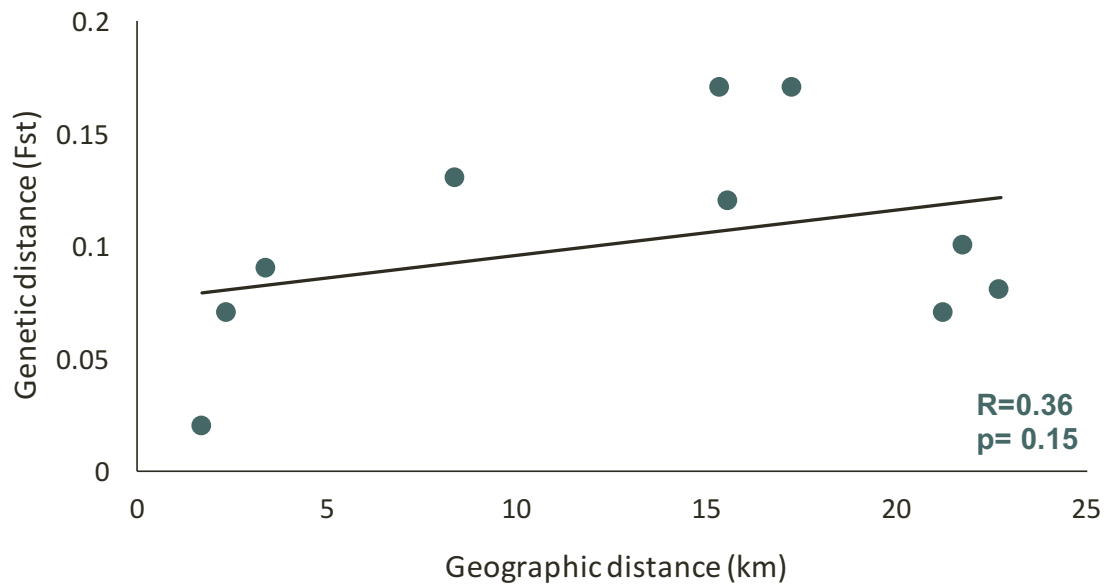


Figure 2. Isolation by Euclidean distance between natural populations of *S. b. riparius*, evaluated as the correlation between genetic distance and geographic distance.

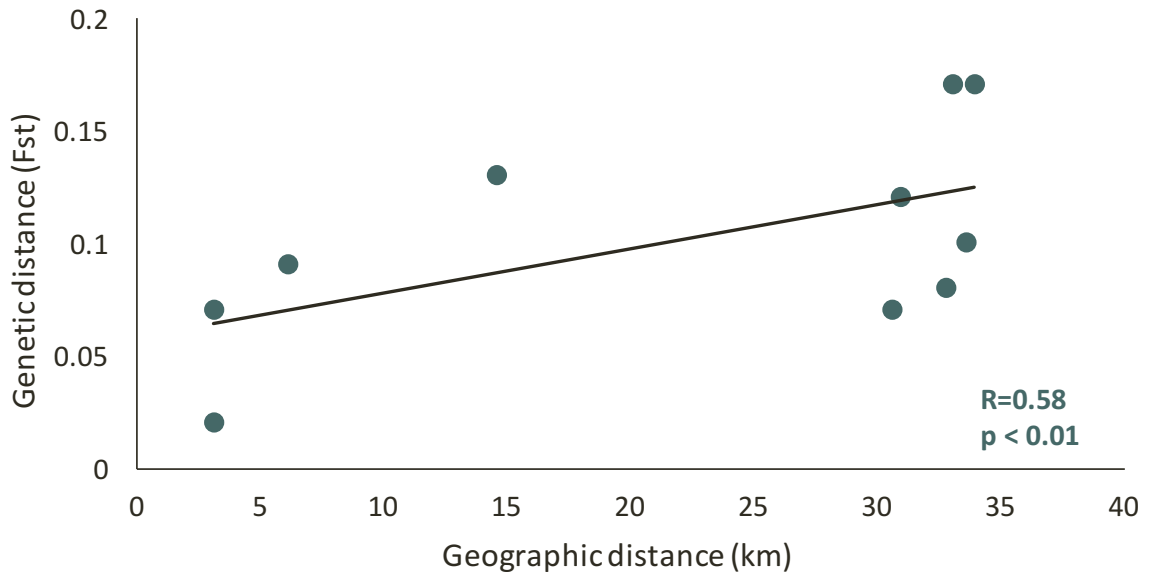


Figure 3. Isolation by effective distance between natural populations of *S. b. riparius*, evaluated as the correlation between genetic distance and geographic distance weighted by habitat structure.

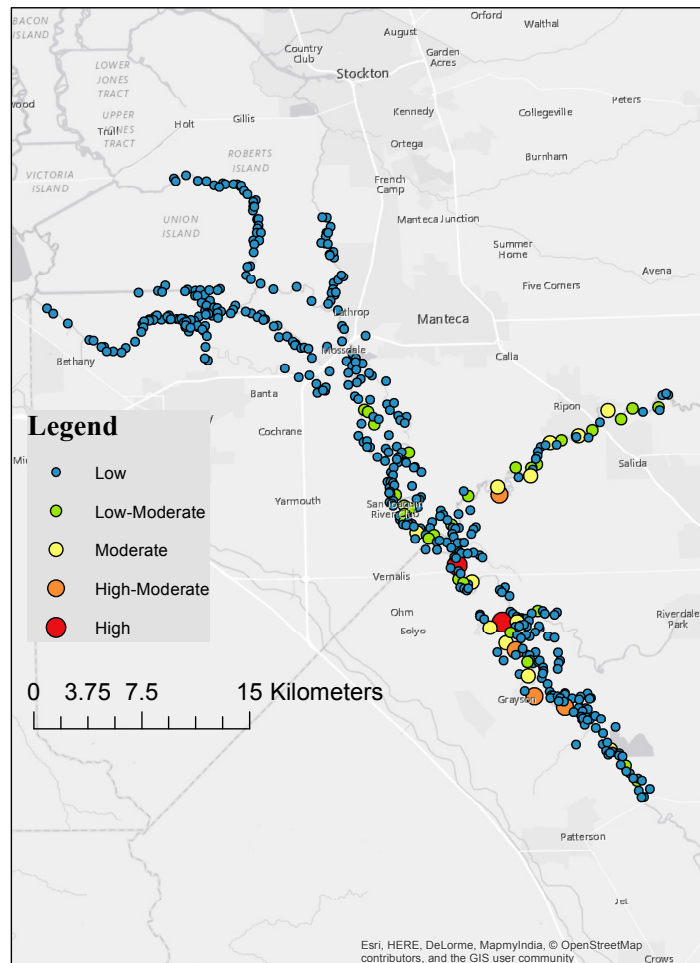


Figure 4. Overall patch value (dPC) calculated using Euclidean distance between habitat patches. Importance is indicated by the size and color of the point; the larger and warmer in color the patch marker, the greater the patch value for overall connectivity. Patch value was significantly higher in the 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 ($p = 0.85$).

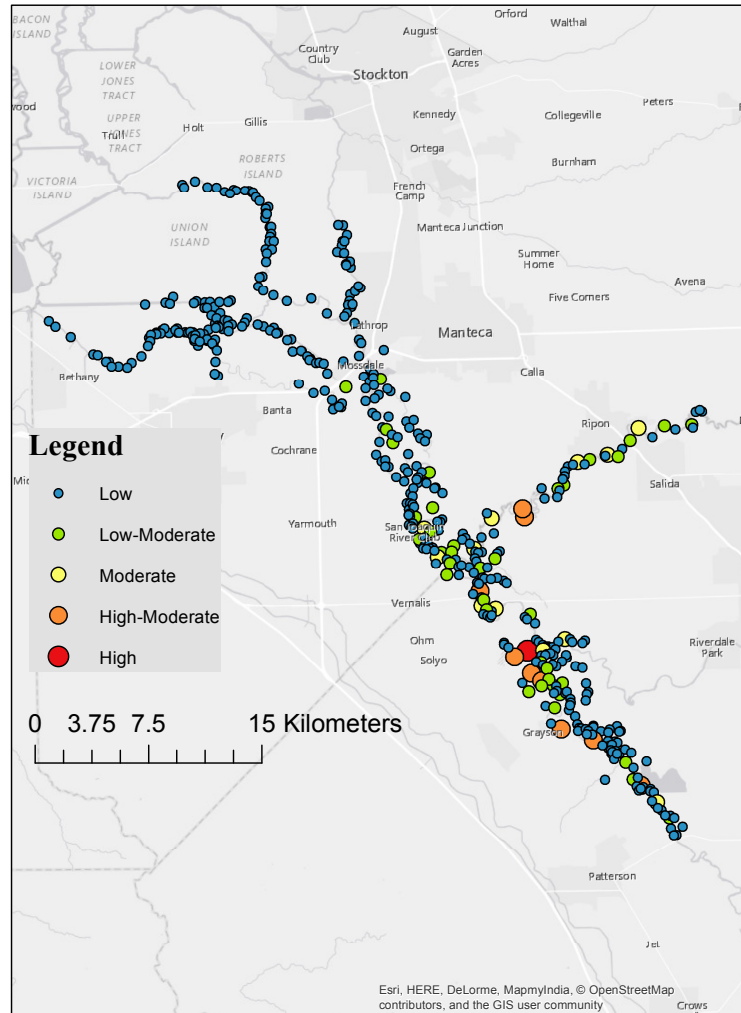


Figure 5. Patch migratory value (dPC_{flux}) calculated using Euclidean distance between habitat patches. Importance is indicated by the size and color of the point; the larger and warmer in color the patch marker, the greater the patch value for overall connectivity. Patch migratory value was higher 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 ($p = 0.87$).

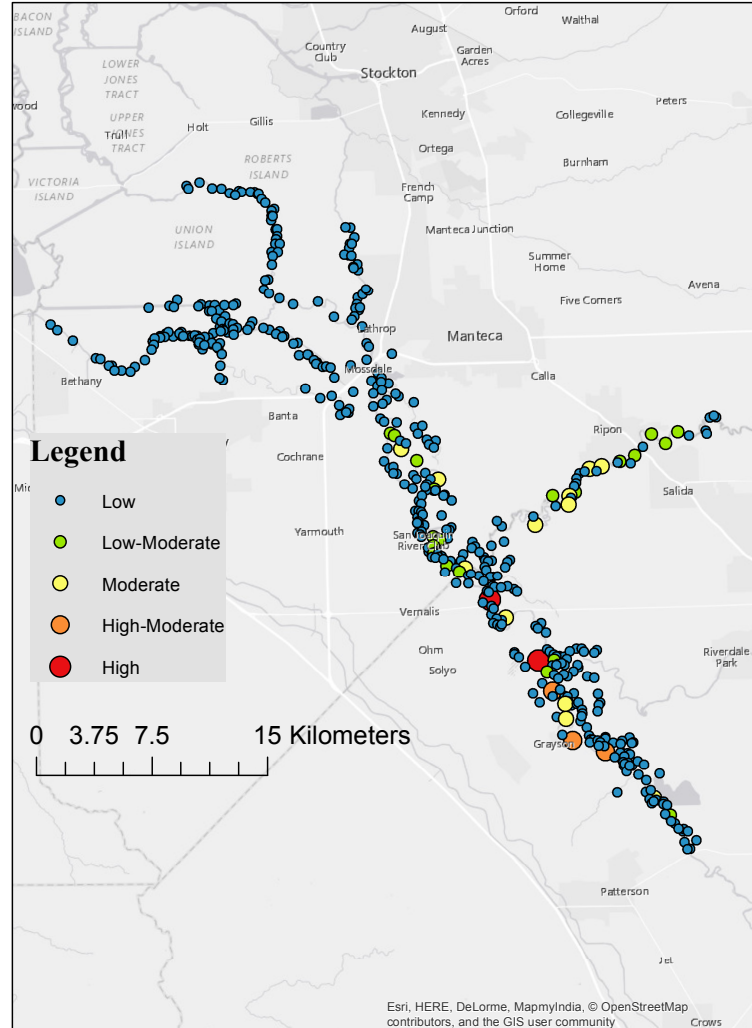


Figure 6. Patch value as a stepping stone ($dPC_{connector}$) calculated using Euclidean distance between habitat patches. Importance is indicated by the size and color of the point; the larger and warmer in color the patch marker, the greater the patch value for overall connectivity. Connector values 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).

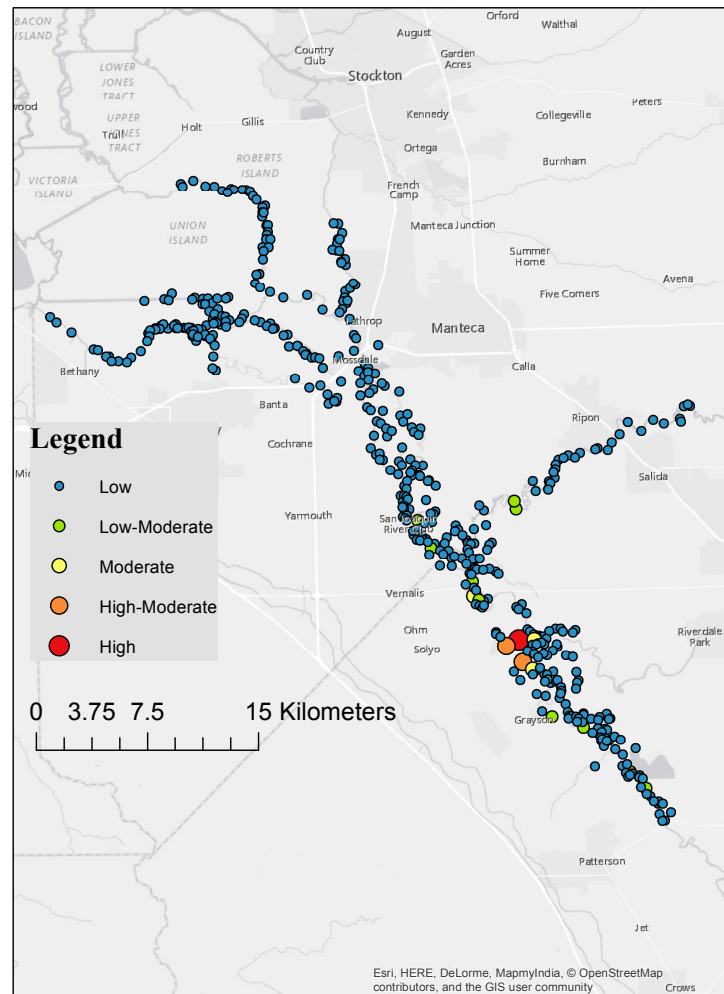


Figure 7. Overall patch value (dPC) calculated using effective distance between habitat patches. Importance is indicated by the size and color of the point; the larger and warmer in color the patch marker, the greater the patch value for overall connectivity. Overall connectivity did not vary between regions ($p > 0.1$ for all pairwise comparisons).

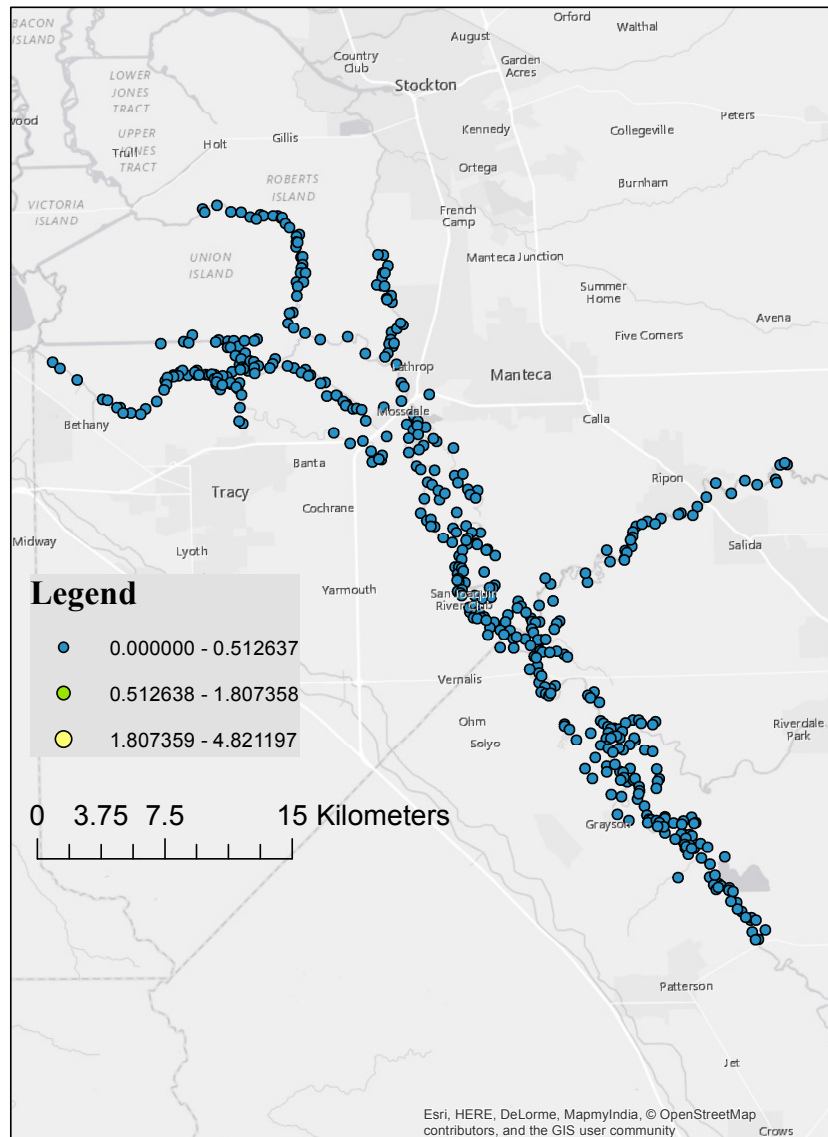


Figure 8. Patch value as a stepping stone ($dPC_{connector}$) calculated using effective distance between habitat patches. Importance is indicated by the size and color of the point; the larger and warmer in color the patch marker, the greater the patch value for overall connectivity. Connector value did not vary between regions ($p > 0.6$ for all pairwise comparisons).

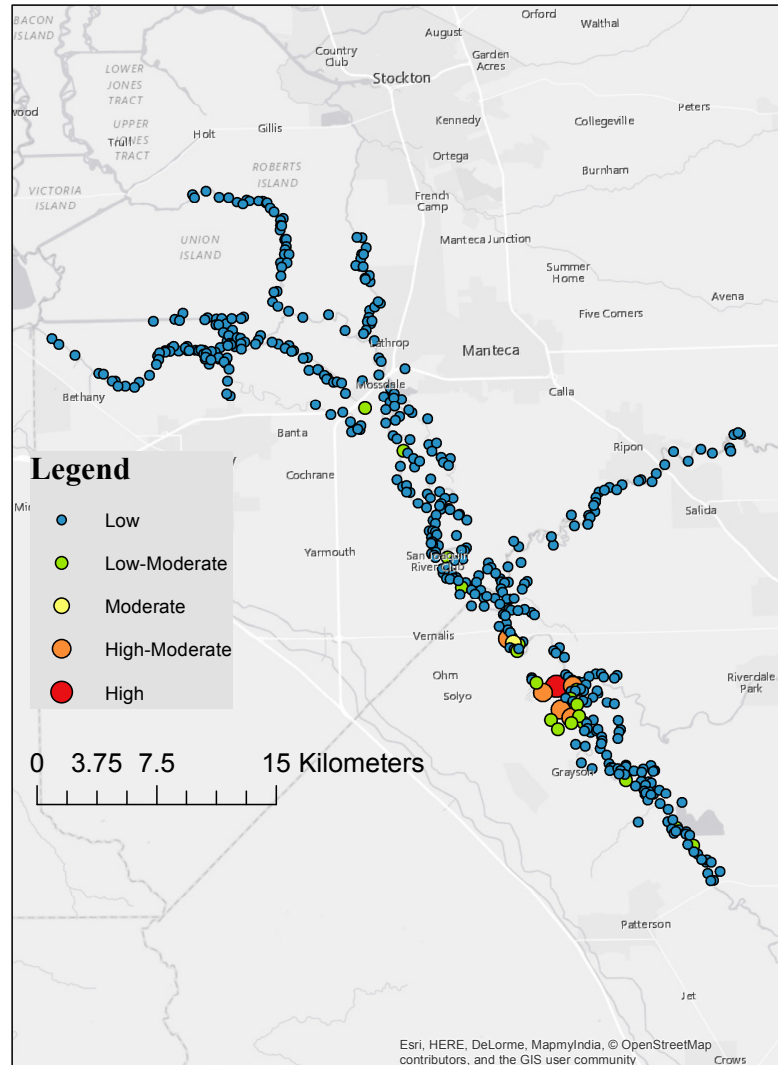


Figure 9. Patch migratory value (dPC_{flux}) calculated using effective distance between habitat patches. Importance is indicated by the size and color of the point; the larger and warmer in color the patch marker, the greater the patch value for overall connectivity. Patch migratory value varied only slightly between the NSJV and South Delta regions ($p = 0.05$) and not at all between these regions and Intermediate patches.