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RESEARCH ARTICLE

Landscape genetics of an endangered salt marsh endemic: Identifying population continuity and barriers to dispersal

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Abstract
Preserving the genetic diversity of endangered species is fundamental to their conservation and requires an understanding of genetic structure. In turn, identification of landscape features that impede gene flow can facilitate management to mitigate such obstacles and help with identifying isolated populations. We conducted a landscape genetic study of the endangered salt marsh harvest mouse (*Reithrodontomys raviventris*), a species endemic to the coastal marshes of the San Francisco Estuary of California. We collected and genotyped > 500 samples from across the marshes of Suisun Bay which contain the largest remaining tracts of habitat for the species. Cluster analyses and a population tree identified three geographically discrete populations. Next, we conducted landscape genetic analyses at two scales (the entire study area and across the Northern Marshes) where we tested 65 univariate models of landscape features and used the best supported to test multivariable analyses. Our analysis of the entire study area indicated that open water and elevation (> 2 m) constrained gene flow. Analysis of the Northern Marshes, where low elevation marsh habitat is more continuous, indicated that geographic distance was the only significant predictor of genetic distance at this scale. The identification of a large, connected population across Northern Marshes achieves a number of recovery targets for this stronghold of the species. The identification of landscape features that act as barriers to dispersal enables the identification of isolated and vulnerable populations more broadly across the species range, thus aiding conservation prioritization.

Keywords Circuitscape · Endangered species · Habitat fragmentation · Landscape genetics · Population substructure · Salt marsh harvest mouse

Introduction

Understanding the factors that shape genetic diversity is fundamental to multiple branches of biology including ecology, evolution, and conservation (Waits et al. 2015). In conservation, identification of discrete populations facilitates the preservation of genetic diversity within a species, which maximizes its evolutionary potential and resilience to environmental change (Allendorf and Luikart 2007). Population differentiation and genetic structure are a consequence of limited gene flow, genetic drift between subpopulations, and differential selection (Allendorf and Luikart 2007). On larger temporal and spatial scales, population structure can be caused by geological or climatic events, such as isolation into refugia with suitable climatic conditions during the Pleistocene (Avise 2009; Statham et al. 2014). On finer spatial and time scales, population substructure can arise from restriction into isolated patches of habitat separated by

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landscape features that are barriers to dispersal (Lorenzana et al. 2020). Human induced habitat loss has led to increased fragmentation, artificially creating small populations with an elevated chance of extirpation through stochastic events as well as reduced likelihood of recolonization (Allendorf and Luikart 2007). While it is important to be able to identify discrete populations, the underlying causes of population differentiation are also an important conservation consideration.

Knowledge of the functional connectivity (i.e., gene flow patterns) among populations is essential to endangered species management. Functional connectivity implies dispersal followed by successful reproduction. Direct methods for assessing dispersal, such as radio telemetry and capture-mark-recapture, can be difficult and costly, and require additional steps to determine if the dispersal resulted in successful reproduction and thus gene flow (Tucker et al. 2017). Gene flow also can take place over the course of multiple generations, linking locations that are further apart than can be connected by a single dispersal event (Bohonak 1999; Kanine et al. 2018). Genetic approaches provide powerful means of assessing both dispersal itself and the net magnitude of genetic exchange over a greater geographic area and time frame than traditional field methods (Lowe and Allendorf 2010; Tucker et al. 2017).

The field of landscape genetics combines the tools of landscape ecology and population genetics to quantify the effects of landscape heterogeneity on gene flow (Manel et al. 2003; Storfer et al. 2007). Gene flow can be affected by a variety of abiotic and biotic factors such as topography, microclimate, anthropogenic features, and vegetation, which can result in landscape- and species-specific patterns of genetic structure (Cushman et al. 2006; Tucker et al. 2017). Landscape genetic approaches are used to identify landscape features that are both positively and negatively correlated with observed genetic distance, thereby revealing barriers to gene flow and corridors of habitat associated with animal movement (e.g., Tucker et al. 2017; Aylward et al. 2020). In addition to illuminating general patterns, landscape genetic approaches can provide a basis for decisions about land purchases for habitat preservation and priorities for habitat restoration.

Here we examine the population structure and the role of landscape features in shaping gene flow in the salt marsh harvest mouse (*Reithrodontomys raviventris*), an endangered species endemic to the coastal marshes of the San Francisco Estuary of California (Shellhammer 1982). *R. raviventris* is the only mammal species fully restricted to coastal marshes (Greenberg 2006; Smith et al. 2018). This reliance on salt marshes has contributed to the species' vulnerability. Since the 1800s, over 90% of the tidal marshes of the San Francisco Bay Area have been lost to filling and diking (Shellhammer et al. 1982; Williams and Faber 2001). Continued destruction and fragmentation of the remaining habitat led to the species being listed as endangered by the state of California, the United States, and the International Union for the Conservation of Nature (Shellhammer et al. 1982; USFWS 1970; USFWS 2013; Whitaker and NatureServe 2018).

The recovery plan for *R. raviventris* sets out a number of requirements for the long-term persistence of the species (USFWS 2013). In addition to maintaining populations across the historical range, these requirements include preserving genetic diversity within populations sufficient to reduce the risk of inbreeding depression and a range of genetic variation among populations sufficient to allow for future evolution and resilience to environmental change. Both morphological and genetic data support the recognition of two distinct subspecies within *R. raviventris*: the southern *R. r. raviventris* of the South and Central San

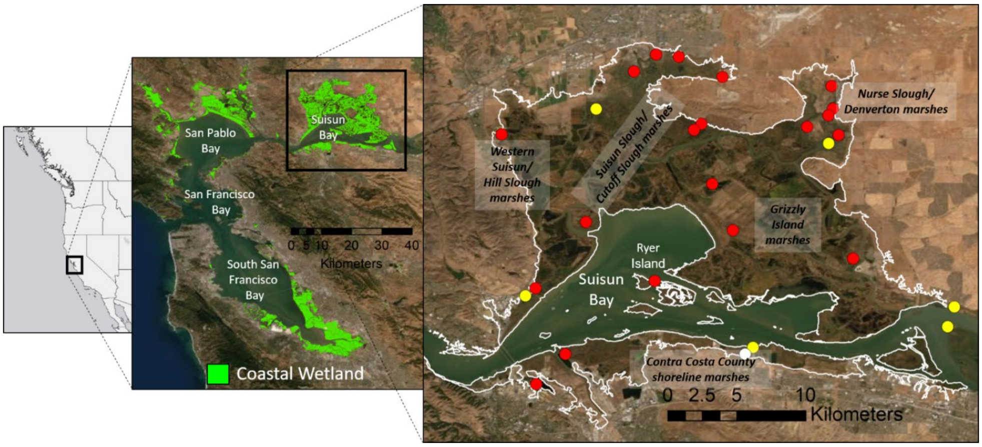

Fig. 1 *R. raviventris* trapping locations within the USFWS Suisun Bay Area Recovery Unit (white outline). Red circles indicate trapping locations where ≥ 10 mice were caught; yellow circles indicate where ≤ 3 *R. raviventris* were caught. We did not successfully trap any *R. raviventris* at Bay Point (indicated by a white circle). The five marsh management units (marsh complexes) are named

Francisco Bay, and northern *R. r. halicoetes* of San Pablo and Suisun Bays (Fig. 1; Fisler 1965; Statham et al. 2016; Statham et al. 2021). Beyond this large-scale subdivision, little is known about the presence of further distinct populations within the species. The tidal and diked marshes of Suisun Bay represent over half of the remaining habitat for the species (Fig. 1; Smith et al. 2020). Many of the marshes of Suisun contain high-density populations and collectively are likely to hold the largest remaining numbers of the species. Given the critical role of these marshes for the conservation of *R. raviventris*, it is important to better understand if multiple distinct populations are present.

Habitat specialists such as *R. raviventris* are often particularly vulnerable to habitat loss and fragmentation (Janecka et al. 2016; Walkup et al. 2017). For example, *R. raviventris* were found to avoid patchy or fragmented habitat relative to the sympatric and non-native generalist, *Mus musculus* (Bias and Morrison 2006). While considerable attention has been paid to understanding the species' habitat requirements (reviewed in Smith et al. 2018), little is known about how *R. raviventris* move through the landscape and which features limit animal movement and gene flow. Previous research indicated that the species' movements through open habitats were not restricted (USFWS 2013 and references cited therein). The species is also known to swim well and to cross canals (Shellhammer et al. 1982; Bias and Morrison 1999), although the degree to which they do so in sloughs or open water is unknown. The species is sometimes found in grasslands at the upper edge of marshlands, but the degree to which animals move through this habitat is unclear (USFWS 2013 and references cited therein). Therefore, it is important to understand the role that landscape features play in restricting connectivity and generating population substructure.

The primary objective of this study was to assess the population subdivision, genetic diversity, and levels of gene flow among *R. raviventris* within the marshes of Suisun Bay. The secondary objective was to assess which landscape features limit connectivity and resultant gene flow. The information gained from these objectives will facilitate the identification of populations of conservation concern and will allow an assessment of the species in relation to recovery targets.

Methods

Fieldwork and genetic sample collection

From 2010 to 2017, we trapped harvest mice (*Reithrodontomys* spp.) at 27 locations, including multiple sites within each of the five designated marsh complexes within the U.S. Fish and Wildlife Service (USFWS) Suisun Bay Area Recovery Unit (Fig. 1; Supplemental Fig. 1). At each site, we used ~ 100 Sherman live traps (H.B. Sherman Traps, Tallahassee, FL), spaced at ~ 10 m intervals, with the layout depending on the wetland size and shape. We baited traps with mixed bird seed and ground walnut, added cotton bedding for warmth, set the traps at dusk, and checked them at dawn for three to four consecutive days. We plucked hair as a source of DNA. Prior to sampling from an individual, we physically wiped down the forceps with a clean tissue, sterilized the forceps in a 2% bleach solution, rinsed with water to remove the bleach, and dried the forceps with a second tissue (Statham et al. 2016). We stored the hair in 95–100% ethanol until DNA extraction. Animal trapping, handing, and genetic sampling procedures were approved by UC Davis Institutional Animal Care and Use Committee (IACUC No. 19686) and authorized by the appropriate state and federal agencies (SC-011578, TE-35000), and under a cooperative agreement between the California Department of Fish and Wildlife and the U.S. Fish and Wildlife Service.

Laboratory methods

We extracted DNA from 777 harvest mouse samples using one of two methods. The first method involved digesting samples in a hair lysis buffer (Statham et al. 2016) and purifying with a modified phenol chloroform method. For the second method, we used a DNeasy Blood & Tissue kit (Qiagen Ltd), modifying the digestion buffer to include 20 µl 1 M Dithiothreitol (DTT), 300 µl buffer ATL, and 20 µl (mg/ml) proteinase K. Each extraction set included a negative control to monitor for contamination. Both *R. raviventris* and the morphologically similar *R. megalotis* (western harvest mouse) are present throughout the study area (Statham et al. 2016; Sustaita et al. 2018). We PCR-amplified and sequenced a portion of the cytochrome *b* gene, and used the associated > 10% sequence divergence to discriminate between species with certainty (Statham et al. 2016).

Microsatellite amplification

All subsequent analyses were restricted to the *R. raviventris* samples. We screened all samples with 20 microsatellite loci: Rrav 1, 6, 8, 10, 13, 18, 21, 29, 36 (Reponen et al. 2014), R34 (Vázquez-Domínguez and Espindola 2013), and new loci Rrav 40, 43, 44, 46, 47, 49, 51, 57, 61, 62 (Supplemental Table 1). We discovered and developed the ten new loci using next generation sequencing (see the Supplemental Information for full details). We PCR-amplified loci in 4 multiplexes using the Qiagen Multiplex PCR Kit (Valencia, CA, USA) according to manufacturer's guidelines (including Q-solution), and with the following thermal profile: 15 min at 95 °C, 33 cycles of 30 s at 94 °C, 1.5 min at 58 °C, and 1 min at 72 °C, and a 10-minute extension at 72 °C. We electrophoresed products on an ABI 3730 capillary sequencer and scored alleles relative to an internal size standard, Genescan 500 LIZ (Applied Biosystems), using STRand software (Locke et al. 2007). All hair samples were genotyped in duplicate.

Genetic analyses

Unless otherwise stated, all statistical analyses were conducted on sampling locations with ≥ 10 individuals. We tested for deviations from Hardy-Weinberg and linkage equilibrium using Genepop http://genepop.curtin.edu.au/) with default Markov chain parameters. We corrected for multiple tests using the sequential Bonferroni method (Rice 1989). We calculated the observed heterozygosity (H_o), expected heterozygosity (H_e), and average number of alleles per locus (A) in the Excel Microsatellite toolkit (Park 2001). We calculated allelic richness (Ar; the mean number of alleles across loci) and private allelic richness (Pr; the mean number of private alleles across loci) rarified for 10 diploid individuals in HP-Rare v1.1 (Kalinowski 2005). We estimated pairwise F_{ST} among sampling sites using Arlequin 3.5 (Excoffier and Lischer 2010). We calculated Nei's D_A genetic distance (Takezaki and Nei 1996), in the program Populations 1.2.32 (Langella 1999; http://bioinformatics.org/~tryphon/populations/), and then used the program to generate a neighbor-joining tree with 200 bootstrap replicates.

We investigated population subdivision across Suisun Bay using all *R. raviventris* individuals (including those from populations with <10 individuals) in the Bayesian clustering program Structure 2.3.3 (Pritchard et al. 2000). We used the admixture model with correlated allele frequencies without *a priori* population assignment (Pritchard et al. 2000; Falush et al. 2003). Iterations were run assuming numbers of genetic clusters (K) ranging 1–10, with a burn-in of 50,000 steps followed by a run of 50,000 steps. Simulations were run five times at each value of K to assess consistency across runs. We followed the guidelines of Pritchard et al. (2000) and the Structure manual to infer K.

Genetic effective population size

We estimated the genetic effective population size (Ne) of *R. raviventris* populations using the bias-corrected linkage disequilibrium method (Waples and Do 2008) in the program NeEstimator 2.1 (Do et al. 2014). We used the random mating model, excluded low-frequency alleles at the 0.05 level, and calculated 95% confidence intervals. In this single sample method, we examined single year samples from each subpopulation identified in the program Structure.

Landscape genetic analyses

We examined how landscape variables restricted gene flow among *R. raviventris* populations using isolation by resistance modeling (McRae et al. 2008). We obtained spatial data for landscape variables from the U.S. Forest Service for cover types (Existing Vegetation: Region 5; https://data.fs.usda.gov/geodata/edw/datasets.php) and the Data Basin for elevation (90 m DEM of California; https://databasin.org/datasets/). We then clipped the layers to the study area in ArcMap 10.4 (Environmental Systems Research Institute (ESRI) 2015). We grouped cover types into the following broad categories: Barren, Cropland, Grassland, Urban (developed areas including roads), Water (including rivers, lakes, marine), Wetland, and Woodland (for further information see the Supplemental Information). *R. raviventris* are restricted to salt and brackish marshes that occur at sea level (Shellhammer 1982). The mean higher high water (MHHW) for Suisun is at 2 m and represents the highest elevation of salt marshes. Therefore, we separated our elevation layer into two categories ≤ 2 m and >2 m.

Rather than assigning *a priori* resistance values to landscape characteristics, we chose to follow a causal modeling approach (Cushman et al. 2006) to test a range of values, allowing the data to determine the optimal weighting scheme. For each landscape variable we generated resistance surfaces where the variable of interest was weighted 2, 5, 10, 25, 50, 100, 200, and 500, while other variables were weighted 1 (Roffler et al. 2016). In the case of elevation, we varied the resistance weight of elevations >2 m. We generated 65 models including a null model of Isolation by Distance (IBD). In the IBD model, all cell values in the resistance surface were weighted 1. This model is considered the appropriate surrogate for geographic distance for circuit theory analyses (Tucker et al. 2017). We estimated the resistance distance between all pairs of populations under each of the landscape resistance schemes using Circuitscape v 4.05 (McRae et al. 2008), and then evaluated each of these landscape distances against genetic distance (Nei's D_A). We tested the relationship at two different scales: the entire Suisun Bay dataset (hereby called Bay-Wide) and among the Northern Marshes. The Bay-Wide dataset consisted of 20 sites (each representing ≥ 10 individuals). The Northern Marshes consisted of 17 sites, excluding the two Contra Costa shore sites and Ryer Island. The Bay-Wide dataset allowed us to assess the causes of deeper subdivision, while the Northern Marshes dataset allowed an examination of more nuanced population subdivision among populations not separated by Suisun Bay.

We tested among resistance values for each landscape variable using maximum-likelihood population effects (MLPE) models (Clarke et al. 2002) estimated in the R package 'lme4' (Bates et al. 2015). We fitted sixty-five models for univariate analyses at both the Bay-Wide and Northern Marsh scales (Supplemental Table 2). Model support was assessed through the Information Theoretic approach using the Akaike Information Criterion of small sample sizes (AICc) (Burnham and Anderson 2002. The optimal resistance weight for each landscape variable was determined as the resistance value with the lowest AICc in univariate MLPE models (Shirk et al. 2018). Where AICc indicated similar support for two or more resistance values we used the resistance weight with the highest R^2 between the resistance distances and genetic distances among sites (calculated using linear regression in Microsoft Excel 2019). We excluded landscape variables from subsequent multivariable analyses when the best supported resistance weight was no improvement over IBD (within ΔAICc < 2.0 of IBD).

Multivariable models were constructed for all possible combinations of landscape variables supported by the univariate analysis. We used MLPE for multivariable modeling. Landscape resistance distance matrices from Circuitscape incorporate both the effects of landscape variables and geographic distance between sample locations. Therefore, we subtracted the geographic distance (the null resistance surface) from each landscape resistance distance to isolate the impact of the landscape variable on landscape resistance (Tucker et al. 2017). Additionally, we included geographic distance in each model as an independent covariate. Finally, we excluded all models with significant multicollinearity (one or more variables with Variance Inflation Factor, VIF > 10) and uninformative landscape variables (β coefficient 95% confidence intervals included 0). Final candidate models included a null model of IBD and model support was assessed using AICc. We used the most parsimonious model (i.e., the model with the lowest AICc) for downstream multivariable resistance surface analyses in ArcMap. This resistance surface was then used to generate a cumulative current map of *R. raviventris* connectivity in the program Circuitscape.

Results

Genetic species identification

Using mitochondrial DNA sequence analysis, we identified 566 *R. raviventris* and 211 *R. megalotis* samples. See Supplemental Table 3 for the number of *R. raviventris* sampled by year and site. We established the existence of *R. raviventris* for the first time at Lower Sherman Island, and potentially McAvoy Harbor and Gold Hills, and verified their continued occurrence at several rarely surveyed locations such as Ryer Island, McNabney Marsh, and Pt. Edith (Fig. 1; Supplemental Information). The only location

Table 1 Nuclear genetic variability of *R. raviventris* from Suisun Bay, California, USA, including locations with ≥ 10 individuals represented: mean number of alleles per locus (A), allelic richness (Ar), private allelic richness (Pr), expected heterozygosity (He), and observed heterozygosity (H_o). More information on sampling locations is provided in the Supplemental Information

Location	n	A	SD	Ar	SD	Pr	SD	H_e	SD	H_o	SD
Goodyear Sl.	58	5.9	3.4	4.5	1.9	0.08	0.18	0.65	0.04	0.61	0.02
Gold Hills	34	5.6	3.1	4.6	2.1	0.03	0.08	0.62	0.05	0.59	0.02
Peytonia Sl.	16	4.8	2.2	4.4	1.8	<0.01	0.00	0.63	0.04	0.55	0.03
Hill Sl. Ponds1 + 2	80	5.5	3.0	4.3	1.7	<0.01	0.00	0.64	0.04	0.60	0.01
Hill Sl. Pond 4	10	4.1	1.5	4.1	1.5	<0.01	0.00	0.63	0.04	0.62	0.04
Hill Sl. Pond 8	15	4.7	2.5	4.4	2.1	<0.01	0.01	0.63	0.04	0.62	0.03
Lower Joice Island	12	4.9	2.4	4.7	2.2	0.06	0.25	0.63	0.05	0.57	0.04
Joice Island Unit, Tidal	52	5.7	3.0	4.5	1.8	0.01	0.05	0.64	0.04	0.62	0.02
Joice Island Unit, Managed	20	5.1	2.1	4.4	1.6	0.04	0.11	0.63	0.05	0.60	0.03
Denverton Control	27	4.9	2.5	4.1	1.9	<0.01	0.00	0.64	0.04	0.66	0.02
Denverton Tidal	16	4.8	2.4	4.2	1.8	<0.01	0.00	0.61	0.05	0.55	0.03
Denverton 3	11	4.7	2.2	4.6	2.1	0.01	0.04	0.65	0.05	0.64	0.04
Bradmoor Island West	10	4.0	1.9	4.0	1.9	0.03	0.09	0.65	0.04	0.66	0.04
Bradmoor Island East	12	4.1	2.0	3.9	1.8	<0.01	0.00	0.58	0.06	0.51	0.04
Crescent Unit	48	5.6	3.0	4.4	1.8	0.07	0.12	0.64	0.04	0.61	0.02
Grizzly Is. Pond 15	25	5.1	2.4	4.3	1.7	0.03	0.10	0.63	0.04	0.58	0.02
Grizzly Is. Pond 1	15	4.5	1.9	4.1	1.6	0.02	0.08	0.60	0.04	0.58	0.03
Ryer Island	31	4.2	1.6	3.8	1.3	0.13	0.31	0.59	0.03	0.58	0.02
McNabney Marsh	12	3.9	1.8	3.8	1.7	0.01	0.04	0.60	0.04	0.51	0.04
Point Edith	17	4.6	2.2	4.2	1.8	0.01	0.02	0.63	0.05	0.60	0.03

Note: Sl = slough

where we did not catch any *R. raviventris* was at Bay Point Regional Shoreline on the Contra Costa shoreline.

Microsatellite analyses

We excluded the loci Rrav 13, 18, and 40 because of difficulties calling alleles. Locus Rrav 8 was previously found to be fixed for different alleles in *R. raviventris* and *R. megalotis* (Reponen et al. 2014; Statham et al. 2016). We used this locus to confirm the species identifications from the mitochondrial analysis, but because it continued to be monomorphic within *R. raviventris*, we excluded it from subsequent analyses. Among the remaining 16 loci, we noted a single occurrence each of significant deviation from linkage equilibrium in three different pairs of loci (Rrav 43 and 49; 6 and 62; 51 and 61), each in a different sampling location. We identified five occurrences where loci were significantly out of Hardy-Weinberg equilibrium; these consisted of three occurrences of Rrav 10 and two of Rrav 36. We also noted missing genotypes at Rrav 36, which taken together may indicate the presence of null alleles. The presence of null alleles has only a small effect on assignment tests and genetic distance estimates (Carlsson 2008). Therefore, we choose to retain these loci, and thus, all subsequent analyses were conducted using 16 loci.

Genetic diversity

We obtained genotype data for 558 of 566 *R. raviventris* samples. Per locus genotype replicates were nearly all identical, at 99.1% agreement. Where there was a difference in genotype between replicates, alleles were only included when they amplified in both replicates. We identified and removed the genotypes of 20 replicated individuals, resulting in a dataset of 538 *R. raviventris* individuals. Across this Bay-Wide dataset, the number of alleles per locus ranged from 3 to 18. Within populations, genetic diversity indexes varied only slightly (Table 1). For example, allelic richness ranged from 3.8 (SD = 1.3) to 4.7 (SD = 2.2), while H_e ranged from 0.58 (SD = 0.06) to 0.65 (SD = 0.04). Mean private allelic richness was generally low across sites (0.03; SD = 0.03), but with the greatest number at Ryer Island (0.13; SD = 0.31).

Population subdivision

The majority of F_{ST} comparisons between locations indicated statistically significant, but low to moderate, differentiation (Table 2). The highest F_{ST} (0.138) was identified between Ryer Island and McNabney Marsh. Most pairwise comparisons between each of these populations and others also were relatively high, indicating that mice at these locations were generally isolated from those elsewhere. Correspondingly, the neighbor-joining tree of *R. raviventris* populations identified the population on Ryer Island as the most distinct, followed by McNabney Marsh (Fig. 2). Both populations were on long branches indicative of increased genetic distance from other populations. The two populations from the Contra Costa shoreline (McNabney Marsh and Point Edith) were more closely related to one another than to those elsewhere.

Analyses with the program Structure consistently identified Ryer Island as distinct at all *K* values from 2 to 10. We identified increased support for $K = 2$–4, beyond which support dropped (Supplemental Fig. 2). At $K = 3$, individuals from McNabney Marsh separated, along with a portion of individuals from the neighboring Point Edith. At $K = 4$ (the value with the highest support), we resolved discrete populations in Ryer Island, McNabney (along with a portion of neighboring Pt. Edith), and to a lesser extent in Benicia/Goodyear which were the sites in the southwestern extreme of the Northern Marshes (Fig. 3).

Genetic effective population size

Among the genetically distinct populations identified in this study, the highest *N*e was located within the Northern Marshes (Table 3). The Ryer Island population was significantly lower than the Northern Marsh population. The population at McNabney Marsh had the lowest *N*e among all populations examined within Suisun Bay.

Landscape genetics

For the Bay-Wide dataset, we tested 8 multivariable models containing Elevation (resistance weight = 500), Urban (500), and Water (200), as well as IBD. The highest performing model included Elevation and Water, while the second just included Water (Table 4). These two models accounted for > 99% of the cumulative AICc weight. For the Northern Marshes dataset, the best supported resistance weight for each landscape variable either was IBD or no better supported than IBD. Therefore, we did not conduct a multivariable analysis for the Northern Marshes. The cumulative current map (Fig. 4) based on the best supported model for the Bay-Wide dataset (i.e., Elevation and Water) identified the lowest current density in Suisun Bay and in the Potrero Hills, while higher densities occurred in marshlands and low elevation grasslands of the Northern Marshes and on the Contra Costa Shoreline.

Table 2 Pairwise F_{ST} values among 20 *R. raviventris* sampling locations with ≥10 individuals. Locations are in geographic order, first across the Northern Marshes, then Ryer Island, then the Contra Costa Shoreline. Significant differentiation (α = 0.05) after Bonferroni correction for multiple tests is indicated by an underline

Location	GD	GH	PY	HS1 + 2	HS4	HS8	LJ	JIM	JIT	DC	DT	D3	BIW	BIE	CU	GI15	GI1	RI	MN
GD																			
GH	0.024																		
PY	0.033	0.043																	
HS1 + 2	0.010	0.018	0.022																
HS4	0.025	0.035	0.026	0.003															
HS8	0.016	0.009	0.031	0.005	0.011														
LJ	0.016	0.030	0.024	0.021	0.031	0.028													
JIM	0.012	0.020	0.023	0.010	0.034	0.026	0.013												
JIT	0.014	0.011	0.033	0.005	0.015	0.008	0.027	0.008											
DC	0.031	0.023	0.037	0.018	0.019	0.024	0.028	0.026	0.006										
DT	0.014	0.006	0.028	0.004	0.014	0.001	0.021	0.012	0.001	0.003									
D3	0.015	0.023	0.022	0.003	0.016	0.010	0.014	0.014	< 0.001	0.007	< 0.001								
BIW	0.020	0.025	0.050	0.020	0.010	0.014	0.020	0.038	0.008	0.014	0.014	0.021							
BIE	0.046	0.034	0.065	0.030	0.047	0.044	0.029	0.047	0.021	0.015	0.016	0.026	0.033						
CU	0.017	0.018	0.026	0.021	0.032	0.017	0.024	0.022	0.014	0.017	0.010	0.025	0.031	0.042					
GI15	0.017	0.029	0.034	0.016	0.023	0.027	0.029	0.009	0.011	0.014	0.005	0.003	0.029	0.037	0.015				
GI1	0.031	0.043	0.038	0.028	0.063	0.042	0.024	0.034	0.018	0.030	0.033	0.014	0.060	0.038	0.037	0.031			
RI	0.097	0.102	0.120	0.094	0.120	0.101	0.115	0.095	0.084	0.107	0.102	0.085	0.098	0.119	0.095	0.088	0.091		
MN	0.078	0.096	0.098	0.072	0.090	0.083	0.075	0.067	0.062	0.084	0.079	0.056	0.090	0.120	0.079	0.077	0.082	0.138	
PE	0.030	0.023	0.052	0.026	0.040	0.021	0.031	0.026	0.016	0.016	0.008	0.025	0.027	0.023	0.026	0.024	0.039	0.096	0.064

BIE = Bradmoor Island East, BIW = Bradmoor Island West, CU = Crescent Unit, D3 = Denverton 3, DC = Denverton Control, DT = Denverton Tidal, G = Goodyear, GD = Gold Hills, GI1 = Grizzly Island Pond 1, GI15 = Grizzly Island Pond 15, HS1 + 2 = Hill Slough Ponds 1 + 2, HS4 = Hill Slough Pond 4, HS8 = Hill Slough Pond 8, JIM = Joice Island Managed, JIT = Joice Island Tidal, LJ = Lower Joice Island, MN = McNabney Marsh, PE = Point Edith WA, PY = Peytonia Slough ER, RI = Ryer Island

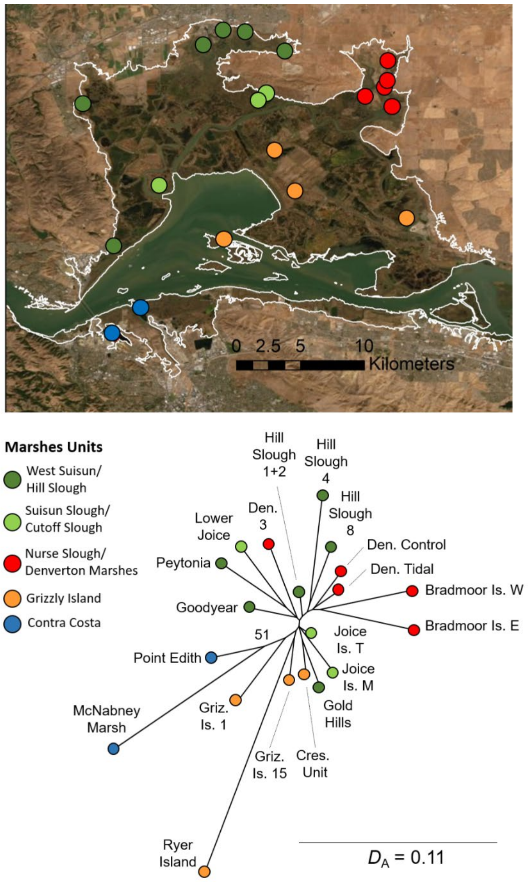

Fig. 2 Population tree of *R. raviventris* sampling locations across the marshes of Suisun Bay, CA. Based on locations with > 10 individuals. Distance is Nei's D_A. The value at the node of Point Edith and McNabney is bootstrap support based on 200 replicates. Support at all other nodes was < 50%, consistent with a little population substructure across the Northern Marshes

Discussion

Here we identified distinct populations of the endangered *R. raviventris* within the marshes of Suisun Bay. These marshes are the largest remaining stronghold of the species, making the preservation of this land and these populations important for the species as a whole. We characterized the diversity, genetic effective population size, and the genetic connectivity among and between these populations, facilitating evaluation of the species against recovery targets and aiding conservation prioritization. Landscape genetic analyses identified large, open tracts of water and areas of higher elevation as features that impede gene flow. This information can be used to assess connectivity more broadly across the species range, which subsequently can identify populations that may be evolutionarily distinct or functionally isolated and susceptible to extirpation due to habitat fragmentation. Accurate detection of such populations can enable conservation practitioners to take appropriate action to ensure persistence while potentially also benefiting other less vagile vulnerable species inhabiting the reduced and fragmented marshes of the San Francisco Estuary.

Population substructure and diversity

Multiple lines of evidence resolved population subdivision among *R. raviventris* across Suisun Bay. Distinct populations were identified at Ryer Island, along the Contra Costa County shoreline (Point Edith and McNabney Marsh), and a large contiguous population across the Northern Marshes. Cluster analysis, population tree branch lengths, and pairwise F_{ST} values indicated that Ryer Island formed a discrete genetic unit, highly differentiated from all others. Small,

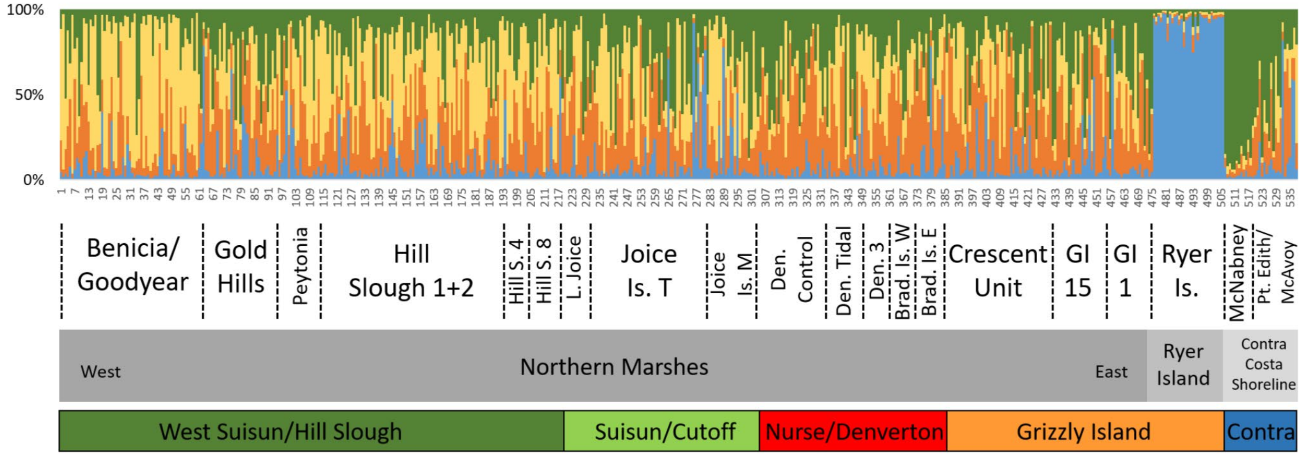

Fig. 3 Population structure of *R. raviventris* across the Suisun Bay, California, based on $K = 4$ as estimated in the program Structure. Representing all 538 salt marsh harvest mice genotyped at 16 microsatellite loci. Sampling sites are arranged in order from west to east, first considering the Northern Marshes, then Ryer Island, and finally the southern marshes along the Contra Costa Shoreline. Marsh complexes are indicated at the bottom

Table 3 Genetic effective population size (*N*e) of *R. raviventris* populations identified in the Suisun Bay Area Recovery Unit, CA

Location	No. Samples	*Ne*	95% Confidence Intervals
Northern Marshes (2014)	131	329	204–735
Northern Marshes (2016)	162	249	175–402
Ryer (2013)	31	47	30–91
McNabney Marsh (2016)	12	15	8–42

Table 4 Landscape resistance models of *R. raviventris* from across Suisun Bay, CA (Bay-Wide dataset) ranked by AICc and ΔAICc. Top models were those where AICc weight (W) contributes to 99% of the cumulative AICc weight (Wcum)

Model	AICc	ΔAICc	W	Wcum
Water + Elevation	-1142.87	0.000	0.926	0.926
Water	-1137.80	5.073	0.073	1.000
IBD	-1125.51	17.362	0.000	1.000
Elevation	-1125.14	17.737	0.000	1.000
Urban	-1124.80	18.069	0.000	1.000

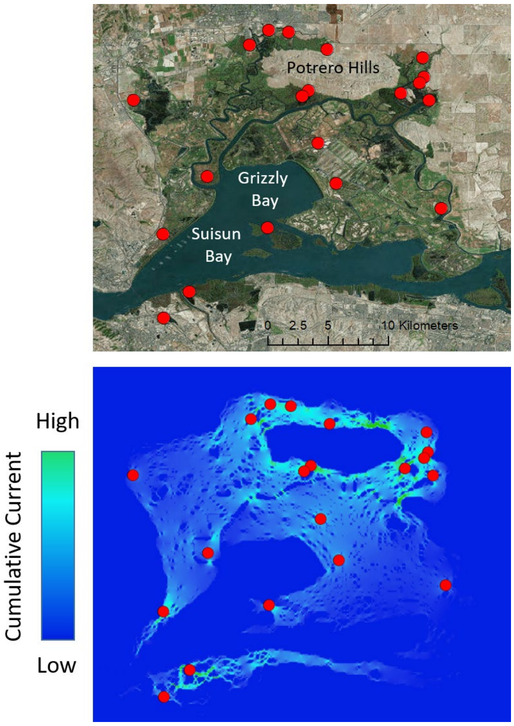

Fig. 4 Cumulative current map modelling connectivity among 20 *R. raviventris* trapping locations within USFWS Suisun Bay Area Recovery Unit, CA. The current map was generated in the program Circuitscape based on the best supported model that contained Elevation (resistance weight = 500) and Water (200). Darker blue indicates areas with higher resistance that may restrict gene flow. Areas shaded in light blue to green have higher current density and may facilitate gene flow (higher connectivity). Narrower areas of conductance tend to have higher density current, while broader areas tend to have more diffuse current

isolated populations such as Ryer Island are more susceptible to loss of diversity and genetic drift, resulting in accelerated divergence (Allendorf and Luikart 2007). Therefore, it is worth exploring whether the observed substructure could be attributed to either of these factors. On Ryer Island, heterozygosity levels were low but comparable to values from other locations and while the genetic effective population size was smaller than that of the Northern Marshes, it was significantly larger than that of McNabney Marsh. Moreover, the greatest occurrence of private alleles was observed on Ryer Island. Taken together, these results indicated that the distinct population that we identified on Ryer Island was best explained by long-term isolation (rather than small population size and accelerated genetic drift). Ryer Island is just above sea level, thus leaving the endemic mouse population vulnerable to sea level rise. Currently there are no specific conservation measures to conserve this population.

Although Ryer Island was differentiated from all other populations, the Circuitscape current map modeled connectivity across the narrowest stretches of water connecting it and Grizzly Island. This represents the path of least resistance; however, it does not necessarily mean that there has been recent gene flow between locations. While there has not been any study assessing how far the species can swim, Geissel et al. (1988) reported mice crossing channels of 1.5 and 2 m, with a maximum potential crossing of < 10 m. This is also consistent with our own observations. The closest distance between Ryer Island and Grizzly island is just over 500 m. Therefore, our results are more consistent with historical colonization from or connectivity with populations on Grizzly Island, which may have been connected to Ryer Island when sea levels were much lower.

As expected, given their separation from other populations by Suisun Bay, the mice located on the Contra Costa County shoreline at Point Edith and McNabney Marsh were also genetically distinct. Additionally, the McNabney Marsh population was differentiated from its neighbor at Point Edith and all other locations. The McNabney Marsh population consistently had the lowest genetic diversity estimates (i.e., allelic richness and heterozygosity) and genetic effective population size in the study. Furthermore, McNabney Marsh had a very low number of private alleles, which is consistent with recent isolation. Taken together, these results indicate that isolation and small population size have resulted in genetic drift and a reduction of genetic diversity, which have contributed to this population becoming genetically distinct. This finding is consistent with the history of the site. This area is a restored marsh that is almost completely surrounded by elevated urban areas. Additionally, it was heavily impacted by an oil spill in 1988. Thus, the population was likely founded by a small number of individuals with limited subsequent immigration and gene flow from other locations. Effort should be made to improve the genetic diversity of this population through enhanced habitat and population connectivity with neighboring locations.

R. raviventris from the Northern Marshes of Suisun Bay form one large population that is genetically distinct from those along the Contra Costa County shoreline and on Ryer Island. Based on the inhabited acreage and density of mice (USFWS 2013), these marshes likely hold the greatest remaining numbers of *R. raviventris* across the species' range, and are therefore of particular concern for the persistence of the species as a whole. The population tree, pairwise F_{ST} values, and cluster analyses indicated little population substructure across the area. These results were consistent with the Circuitscape current map, which modeled high connectivity among sites within the Northern Marshes, with the possible exception of areas of higher elevation, and wider expanses of water. The Circuitscape current map and the population tree indicated connectivity between locations to the north of the Potrero Hills. While this area is currently covered in grassland, it is at very low elevation and is likely important to conservation, especially in light of rising sea level which may transform this area into marsh habitat in future decades. Estimates of genetic diversity were generally high across the Northern Marshes, with no indication of a systemic loss of diversity. Similarly, most locations had little to no private alleles, thus indicating that genetic diversity was largely shared across the area. The genetic effective population size estimated from two independent sample sets was consistently the highest in the study.

Landscape genetics

The Bay-Wide dataset identified both Water and Elevation as the most predictive landscape features restricting gene flow and animal movement within the current Suisun Marsh landscape. Although Urban was not included in our top resistance surfaces, urban land was highly correlated with areas > 2 m in elevation; thus, urbanization may be a contributing factor to the negative association between elevation and gene flow. Our results suggest that *R. raviventris* in other locations that are separated by elevation and/or open water likely represent isolated and genetically distinct populations. Thus, several additional unsampled islands within Suisun Bay may harbor distinct populations of the species. The same is also likely to be true in the few remaining marshes in the central San Francisco Bay. These marshes are highly fragmented and isolated by both water and areas of higher elevation as well as associated urban features (USFWS 2013). Any relict populations at these sites are susceptible to inbreeding and extirpation due to stochastic events, with limited likelihood for natural recolonization, which will be exacerbated by rising sea level due to climate change.

Within the Northern Marshes, geographic distance was the most predictive landscape variable of genetic distance. This finding was in keeping with other analyses (e.g., cluster analysis and the population tree). Neither Urban (primarily small roads at this scale), Elevation, Water, Grassland nor Barren areas appear to explain the observed genetic distance within the Northern Marshes. This finding agrees with the results of radio telemetry where barren areas were not found to be barriers to movement, and that the species crossed levees, roads, and canals (Bias and Morrison 1999). While live-trapping efforts in Suisun found that *R. raviventris* inhabited adjacent upland grass-dominated microhabitats, they did so at substantially lower density than in wetland habitats (Sustaita et al. 2011). Potentially none of these landscape variables substantially restrict the species movement and gene flow at the scale of the Northern Marshes. While *R. raviventris* may respond negatively to habitat fragmentation and smaller patch sizes (Bias and Morrison 2006; Marcot et al. 2020), the Northern Marshes are composed of a contiguous mosaic of relatively large diked marshes separated by relatively narrow levees, roads, and canals, which are semi-permeable to *R. raviventris* (Bias and Morrison 1999). In addition, several of the landscape cover types assessed (e.g. urban, cropland or woodland) were limited within the area, and it may be necessary to analyze locations with a greater representation or variation of these features to detect their impact on *R. raviventris* gene flow (Short Bull et al. 2011; Aylward et al. 2020).

Conservation and management implications

The populations that we have identified at Ryer Island, on Contra Costa Shoreline, and across the Northern Marshes of Suisun are genetically distinct and geographically discrete. One or more of these populations may be consistent with a "distinct population segment" under the U.S. Endangered Species Act (61 Fed. Reg. 4722 1996). Such a designation may allow a population to qualify for down-listing to "Threatened" prior to other segments if it meets the specified criteria. Therefore, it is worth considering our findings for each of these populations in regard to the recovery plan for the species (USFWS 2013). The plan states that it is necessary to maintain well-distributed populations throughout the geographic range and that the range of genetic variation must be maintained. We found that *R. raviventris* are well-distributed across the majority of the Suisun Bay Area Recovery Unit (with the possible exception of the eastern portion of Contra Costa Shoreline) and that genetic diversity was similar across much of Suisun Bay (with an exception of a small number of more isolated or peripheral locations, such as

Ryer Island and McNabney Marsh). One of the criteria for down listing or delisting *R. raviventris* is that viable habitat areas within marsh complexes must be connected to allow them to function as one large population over time (USFWS 2013). Our research indicated that *R. raviventris* were effectively one large population across the Northern Marshes of Suisun Bay. This large area constitutes the majority of four out the five marsh complexes within the Suisun Bay Area Recovery Unit. Taken together, our research indicated that the large, genetically diverse, and connected *R. raviventris* population in the Northern Marshes of Suisun Bay meets many of the genetic conditions necessary for down listing to threatened. However, before such changes can be made, additional considerations would need to be met, including reduced destruction and modification of the species habitat, as well as reaching specified requirements for marsh acreage and population densities.

Conclusions and future research

Protecting the distinct populations that we have identified and the connectivity within them will assist the survival of *R. raviventris* for the future. Continued and enhanced management and recovery effort should focus on portions of the range that have been highly impacted by habitat loss and fragmentation, such as the Contra Costa Shoreline, which is represented by a narrow band of marshland hemmed in and fragmented by urban features. More broadly across the species range, particular attention should be paid to the southern subspecies which has a smaller and more fragmented geographic range (Smith et al. 2018) and lower genetic diversity (Statham et al. 2016), and where the inventory of genetic (i.e., variation, subdivision, Ne, gene flow) and demographic (i.e. range and population trends) characteristics are limited. The identification of a genetically distinct population on Ryer Island raises important questions about the presence of other unique island and isolated populations throughout the species' range. Additionally, a genomic analysis focusing on the timing of separation of Ryer Island as well as other distinct populations would allow us to determine if these populations are differentiated on an evolutionary time scale to better appreciate how valuable they are from a conservation standpoint. Beyond *R. raviventris*, multiple other species of conservation concern (4 U.S. federally listed and 11 California species of concern) occur within the coastal marshes of the San Francisco Estuary (USFWS 2013). These species are broadly impacted by the same major threats that caused the decline of *R. raviventris*. Thus, the identification and preservation of vulnerable populations of *R. raviventris* may also aid the preservation of other less vagile species of conservation concern across the marshes of the San Francisco Estuary.

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Author contributions MJS designed the research; LBT coordinated the fieldwork; MJS performed the research and analyzed the data; CMA and EMK contributed to analytical methods; MJS wrote the initial manuscript and all authors assisted with revising the manuscript.

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Availability of data and material Novel primer sequences are in the supplemental information. Data from this paper is available on Dryad https://doi.org/10.25338/B81062

Code availability Not applicable.

Declarations

Conflicts of interest/Competing interests No.

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