Cooperative ecosystem study of the endangered salt marsh harvest mouse (*Reithrodontomys raviventris)* population genetics

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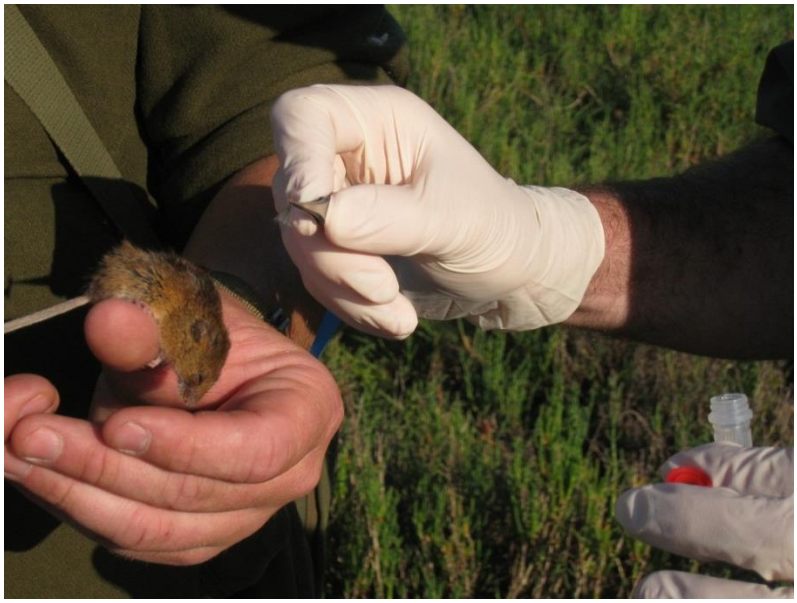

Salt marsh harvest mouse from Suisun in the process of having a hair sample taken for genetic analyses. Photo credit Mark Statham, June 10th 2011.

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Abstract

The salt marsh harvest mouse (SMHM, *Reithrodontomys raviventris*) is a California state and federally listed endangered species that is found only in the greater San Francisco Bay area. The population decline of the SMHM is mainly attributed to loss and fragmentation of habitat which is anticipated to increase with the advent of rising sea levels due to climate change. Management of the species is hampered by a lack of knowledge concerning current population substructure and genetic diversity, while monitoring is hindered by difficulty in morphological differentiation from the sympatric western harvest mouse (WHM, *R. megalotis*).

Our genetic analyses of 142 harvest mice using a 426 bp section of the mtDNA cytochrome *b* gene and 11 microsatellite loci identified deep subdivision consistent with the species *R. raviventris* and *R. megalotis*. Genetic methods for species identification indicated that the morphological assignment correctly identified 92% of SMHM and 44% of WHM. Over a quarter of WHM were misidentified as SMHM, indicating that in areas of relatively high abundance of WHM this would lead to an over estimation of SMHM during regular surveying and monitoring. The lowest species identification rate was in the San Francisco Bay population (50.9%), suggesting that the development of a new morphological key tailored to the southern subspecies of SMHM is warranted.

Genetic cluster analysis in the program structure identified major population differentiation within the salt marsh harvest mice consistent with the recognized subspecies *Reithrodontomys raviventris raviventris* from the San Francisco Bay, and *R. r. halicoetes* from San Pablo and Suisun bays. This subdivision was also evident in the mtDNA with most individuals (29 of 30) within the San Francisco Bay bearing one of two endemic haplotypes. Both of the northern bay populations had higher nuclear genetic diversity than that of the San Francisco Bay.

In the future, higher resolution mitochondrial markers and genomic sequences obtained through next generation sequencing (NGS) will be necessary to adequately quantify the magnitude of the divergence separating these subspecies. Additional sampling is needed from intervening areas between north and south, particularly along the Marin and San Mateo coastlines on the western side of the bay. Sampling from such areas will allow us to assess the possibility of gene flow between subspecies and to accurately characterize subspecies boundaries. Next generation sequencing approaches should be applied additionally to characterize functional adaptive

differences between subspecies, for example, related to salinity. Such information is necessary to inform management actions that maximize evolutionary potential and promote long-term persistence.

Introduction

The salt marsh harvest mouse (SMHM, *Reithrodontomys raviventris*) is a California state and federally listed endangered species that is found only in the San Francisco, San Pablo, and Suisun Bays of California (Shellhammer 1982; Whitaker 2008). The SMHM is restricted to salt and brackish marshes, and is critically dependent on dense cover for predator avoidance. The population decline of SMHM is mainly attributed to fragmentation and loss of habitat through reclamation of tidal areas, urban development, erosion, adverse water management, and vegetation change (Shellhammer 1982). This fragmentation is anticipated to increase with an estimated rise in sea level due to climate change between 56 and 200 cm over the 21st century (National Research Council 2010). Small and/or fragmented populations such as those of the SMHM are susceptible to inbreeding and localized extinction through random events. Deciding on management actions that promote persistence of these populations in the face of these trends depend on accurate information on current genetic structure and diversity as well as phylogenetic and adaptive divergence between subspecies.

An obstacle to basic monitoring has been difficulty identifying SMHM in the field due to extensive sympatry with the western harvest mouse (WHM, *R. megalotis*). Differentiating the two species in the field is difficult, requiring several morphological measurements to assign individuals to a species. This method often results in ambiguous cases, classified as unknown harvest mouse species, which has contributed to speculation that the two species could be undergoing hybridization (Laureen Barthman-Thompson personal communication). Discrepancies regarding how individuals take measurements can result in assignment of the same mouse to different species. In addition, juvenile and sub-adult mice are especially difficult to identify to species. Accurate species assignment of harvest mice is an obvious underlying requirement for surveying and monitoring of the SMHM.

Objectives

1. Quantify the genetic diversity and genetic effective population size in each of the three main bay areas (Suisun, San Pablo, and San Francisco Bay).
2. Determine how current populations are structured/fragmented both now and historically.
3. Assess the underlying genetic basis for the differentiation of the currently recognized northern and southern SMHM subspecies (*R. r. halicoetes* and *R. r. raviventris*).
4. Create tools for the accurate species identification and assess the possibility of hybridization between SMHM and WHM.

Materials and Methods

Samples

Sampling was carried out by personnel of the UC Davis *Mammalian Ecology and Conservation Unit* of the Veterinary Genetics Laboratory in coordination with personnel conducting ongoing SMHM monitoring (California Dept. of Fish and Wildlife, U.S. Fish and Wildlife Service, and the U.S.G.S.). We collected plucked hair samples from SMHM and sympatric WHM from three areas within the SMHM range (San Francisco, San Pablo, Suisun bays). These hair samples were then used as a source of DNA for population genetic analyses. We conducted analyses on 90 SMHM samples consisting of 30 individuals from each of the three bays (San Pablo: Napa plant restoration site [n=5], Fagan Slough North and South [n=16], Napa/Sonoma Marsh Wildlife Area ponds 4 and 5 [n=9]; Suisun; Hill Slough ponds 1, 2, and 4 [n=10], Point Edith [n=10], Denverton [n=10]; San Francisco; Mayhew's Landing [n=6], Warm Springs mouse pasture [n=4], and Eden Landing [n=20]), thus sampling 30 SMHM from the range of the southern subspecies and 60 from the northern. In addition we also sampled from 52 WHM trapped at the same locations (Appendix 1).

Lab Methods

We refined a method for extracting DNA from hair samples to better suit working with

rodent hair. Samples were digested overnight in solution consisting of 39 mM DTT (Dithiothreitiol), 100 mM NaCl, 3 mM $CaCl_2$, 8.13 mM TE (tris EDTA pH 8), 2% SDS (sodium dodecyl sulfate), and 0.2 mg proteinase K in a total volume of 400 µl. The digestion solution was purified using a modified phenol/chloroform method, and then cleaned using Amicon Ultra centrifuge filters (Millipore ltd). The resulting purified DNA was the used for polymerase chain reaction (PCR) amplification.

Mitochondrial DNA (mtDNA)

We carried out multiple trials to amplify via PCR mtDNA from both SMHM and WHM. We tested methods designed for other rodent species, methods designed in-house, and methods for different regions within the mtDNA (cytochrome *b* gene, D-loop). We achieved successful PCR amplification of a portion of the cytochrome *b* gene in both target harvest mouse species using the primers MVZ-05 and MVZ-04R (Smith et al. 1992; Brown 2003). PCR products were purified using ExoSap-IT (Affymetrix, Inc.) and sequenced in both directions using the BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Inc.). We then electrophoresed products on an ABI 3730 capillary sequencer (Applied Biosystems, Inc).

The resulting DNA sequences were aligned in the program Sequencher v5.1. We determined species by comparison to previously published sequences for the two species (Bell et al. 2001). We created a median-joining network (Bandelt et al. 1999) in Network 4.2.0.1 (www.fluxus-engineering.com) to assess the phylogenetic relationships among haplotypes. We used Arlequin 3.5 (Excoffier and Lischer 2010) to estimate haplotype and nucleotide diversity, and to estimate the degree of subdivision among bay-specific populations (Φ_{ST}).

Microsatellite Analyses

We obtained 67 DNA sequences containing microsatellite repeats from clones from a microsatellite-enriched library (GIS Ltd). Of these, 37 loci contained sufficient flanking sequence for primer design, which we screened for amplification in SMHM. We designed alternative primers where initial amplification success was poor. We identified ten loci that amplify in SMHM and/or WHM and developed to two multiplex assays for more efficient screening, which we published in Conservation Genetics Resources (Reponen et al. 2014; Appendix 2). We also

screened 10 microsatellite loci developed in *Reithrodontomys spectabilis*, a harvest mouse from Mexico (Vázquez-Domínguez and Espindola, 2013). One locus, R34, was polymorphic and was used for screening all harvest mice in this study.

We tested for deviations from Hardy-Weinberg and linkage equilibrium using Genepop (http://genepop.curtin.edu.au/). We calculated the Observed (H_o) and Expected Heterozygosities (H_e), and average number of alleles per locus (A) in Microsatellite tool kit (Park 2001). We calculated the inbreeding coefficient (F_{IS}) in FSTAT v 2.9.3.2 (Goudet 1995). We calculated pairwise F_{ST} among sampling sites using Arlequin 3.5 (Excoffier and Lischer 2010).

We examined population substructure using the model based Bayesian clustering method implemented in the program Structure v 2.3.3 using the admixture model with correlated allele frequencies (Pritchard et al. 2000; Falush et al. 2003). This technique allowed us to evaluate population substructure without the need for *a priori* assignment of individuals to populations. Several loci amplified only in one of the two species (as ascertained through mtDNA sequencing). Where a null allele was evident in a locus all individuals of that species were coded with the same novel allele, allowing us to use informative data from all microsatellite loci. We conducted the structure analysis in a stepwise manner. We ran the first analysis on all specimens, regardless of species assignment. Subsequently, independent analyses were run on each of the highest supported clusters evident in the first analysis. Iterations were run at a range of K values, with a burn-in of 100,000 followed by a run of 500,000 generations. Simulations were repeated five times at each K value to assess consistency across runs. We determined the most meaningful K values by plotting the Ln P(D) values and determining where the greatest support was found (Pritchard and Wen 2002).

We also attempted to estimate effective population size (N_e) using multiple methods within the program NeEstimator v2.01 (Do et al. 2014). Results indicated that our microsatellite dataset did not have sufficient resolving power to generate useful estimates and that a higher resolution dataset (e.g. derived from a greater number of microsatellite or SNP [single nucleotide polymorphism] loci) would be required. Therefore, we based qualitative assessments on comparison of other genetic diversity measures.

Results

Mitochondrial DNA Analyses

We identified eight SMHM cytochrome *b* haplotypes (Table 1). SMHM haplotypes were all highly differentiated from WHM haplotypes, with 47 fixed nucleotide differences (~11% sequence divergence) between the two species. In contrast there were only 1-5 bases in difference between SMHM haplotypes, equivalent to 0.23 – 1.17% sequence divergence. The SMHM haplotypes divided into two clades; clade 1 was ubiquitous, while clade 2 was only found in San Pablo and Suisun bays (Figure 1). This pattern was indicative of greater ancestral diversity in the northern portion of the species range.

The highest haplotype and nucleotide diversity was found in San Pablo Bay (Table 2), while Suisun and San Francisco Bays had similar levels of diversity. The highest number of haplotypes was found Suisun Bay. Several haplotypes were shared between bays (e.g., haplotype A, Figure 1); however several haplotypes were geographically restricted (for example haplotypes C and L were found in multiple locations only in the San Francisco Bay).

Analyses of population subdivision using pairwise Φ_{ST} values indicated that all bays were significantly differentiated from one another (Table 3). There was a closer relationship between San Pablo and Suisun (with many shared and similar haplotypes), than between either of these bays and San Francisco Bay (with regionally restricted haplotypes, and no representatives of Clade 2 [Figure 2]).

Microsatellite Analyses

Nine of 11 microsatellite loci tested were polymorphic within the SMHM, with a range of 3-11 alleles per locus. One locus, Rrav15, was dropped from analyses due to difficulties with calling alleles, leaving 8 loci for analyses. Tests for linkage disequilibrium identified five population-specific locus-pairs that were statistically correlated, none of which was consistent across populations. We detected four loci with significant deviations from Hardy-Weinberg equilibrium, none of which was consistent across populations. Because the deviations from

linkage and Hardy-Weinberg equilibria were inconsistent among population, substructure in the total species was sufficient to explain them with no evidence of null alleles or physical linkage among loci. Therefore, we retained all 8 of these loci in subsequent analyses.

The San Francisco Bay population exhibited the lowest genetic diversity as indicated by average number of alleles per locus, observed heterozygosity, and expected heterozygosity (Table 4). All populations had positive F_{IS} values, reflecting a deficit of heterozygotes, likely caused by population substructure. Analyses of population pairwise F_{ST} revealed significant differentiation between all populations, with the San Francisco Bay population being more divergent from the two northern populations (average $F_{ST} = 0.144$), than they were from one another ($F_{ST} = 0.019$; Table 5). This pattern of genetic substructure is consistent with recognized subspecies restricted to the San Francisco Bay and the two northern bays respectively.

Bayesian cluster analysis in STRUCTURE of all harvest mice consistently indicated the greatest increase in highest posterior probabilities (Ln P[D]) support from K = 1 - 2. After K = 2 values plateaued, indicating only marginal increase in resolving power. The discrete groups identified at K = 2 were consistent with the mtDNA assignment indicating that they represented the SMHM and WHM species (Figure 3). All individuals were assigned ≥99% identity to one species cluster. Based on this basal division subsequent analyses were carried out on SMHM and WHM datasets separately. Analyses of SMHM indicated that K = 2 had the highest support across 5 iterations. Without any prior geographic information this separated mice from the southern San Francisco Bay from those is the North (consisting of San Pablo and Suisun bays). Separate analyses conducted on SMHM from the southern San Francisco Bay, and from San Pablo and Suisun did not support additional subdivision. Cluster analysis of the WHM dataset did not support subdivision of the population (Figure 3).

Morphological versus Genetic Identification of Species

Multiple linear regression of morphological measurements for species assignment correctly identified 92% of SMHM (83 of 90), with one animal misidentified as WHM, and six as unidentified (Figure 4). Successful field identification of WHM was much lower at 44% (23 of 52). A large portion of WHM were misassigned as SMHM based on morphology (27%; 14 of 52) suggesting that the SMHM count is likely over estimated during annual monitoring in areas with a high prevalence of WHM. In addition the proportion of harvest mice correctly assigned to

species using morphology was not even across the three bays (Figure 5). Correct species assignment was substantially lower in the San Francisco Bay (50.9%), than either of the two northern bays (San Pablo, 100%; Suisun, 84.6%). The abundance of WHM relative to SMHM was similar in Suisun and San Francisco Bays (Table 6), yet the species identification rate was much lower in the San Francisco Bay, suggesting greater difficulty in identifying harvest mouse species in the southern portion of the SMHM range. This is consistent with the greater morphological variation within the southern subspecies (Laureen Barthman-Thompson, personal communication).

Discussion

Several lines of evidence identified major differentiation between salt marsh harvest mice from the San Francisco Bay and those from the two northern bays (San Pablo and Suisun). The majority (29 of 30) of SMHM from the San Francisco Bay had endemic mtDNA haplotypes (C or L), while majority of individuals from the two northern bays had shared haplotypes. Consequently we identified substantially higher Φ_{ST} values in pairwise comparisons between the San Francisco Bay population and either of the two northern populations, relative to that between the two northern bays. Analyses of nuclear microsatellites also identified the same major subdivision within SMHM using both pairwise F_{ST} and model based clustering methods. The observed differentiation within the SMHM is consistent with previously recognized subspecies, *Reithrodontomys raviventris raviventris* from the San Francisco Bay and *R. r. halicoetes* from San Pablo and Suisun Bays.

The San Francisco Bay population had lower diversity than populations in either of the northern bays. This trend was evident specifically in the multilocus nuclear microsatellite dataset, with consistent lower values across all diversity estimates (Table 4). Although the mtDNA haplotype and nucleotide diversity were similar between the San Francisco and Suisun bays, the San Francisco Bay population had the smallest number of haplotypes (Table 2). Given that the population in the San Francisco Bay corresponds to a genetically distinct, geographically restricted endemic subspecies, the finding that it also had the lowest genetic diversity is concerning. However, our samples may have reflected only a portion of the range of this subspecies,

necessitating additional sampling to characterize its extant genetic diversity.

The mtDNA and microsatellite datasets agreed in indicating clear differentiation between SMHM and WHM species. There were no shared mtDNA haplotypes between SMHM and WHM, and haplotypes from both species were highly divergent (~11%) from one another. This level of divergence is greater than typically found between interbreeding mammal species (Bradley and Baker 2001). These authors estimated the average sequence divergence between pairs of sister species within Rodentia at 9.55%, including estimates within the genus *Reithrodontomys* of ~8 and 13%. Nevertheless, because mtDNA is maternally inherited and therefore does not provide information regarding paternal inheritance, and consequently hybridization, we also assessed the relationship between species using the ten polymorphic bi-parentally inherited nuclear microsatellites. These markers similarly indicated unambiguous assignment of all individuals to a single species, strongly arguing against the possibility of any ongoing gene flow between species despite their similar morphology and overlapping ranges.

The clear genetic differentiation between species allowed us to assess the efficacy of species assignment based on morphology. Morphological identification of SMHM was much more accurate (92%) than identification of WHM (44%), implying that although 92% of SMHM are correctly identified, 27% of WHM are also included in the SMHM counts. Depending on the relative numbers of these species caught, the estimated number of SMHM overall could be significantly inflated. This problem is most significant when the WHM is relatively abundant and differences in space or time in the relative abundances of the two species can confound comparisons. This problem has potentially significant ramifications for ongoing surveying and monitor efforts. We also noted a substantially lower species identification rate in the southern portion of the species range, despite a similar ratio of WHM to SMHM being trapped in Suisun Bay. With a success rate just above 50%, it is clear that the morphological species identification keys used in the San Francisco Bay are insufficient to accurately discriminate between species.

Next steps

Although we have identified genetic subdivisions within the SMHM consistent with

recognized subspecies the geographic position and strength of the subspecies boundary is unclear. We were only able to sample SMHM from the southern extreme of the San Francisco Bay and thus did not include mice from any of the numerous fragmented marshes in the intervening areas between north and south, particularly those along the Marin and San Mateo coastlines on the western side of the bay. This is important as the dividing line between SMHM subspecies is considered to be between San Rafael and Richmond Marsh (Shellhammer, 1989). Sampling these intervening marshes would allow assessment of whether the observed deep differentiation corresponds to the putative subspecies dividing line, indicating a degree of reproductive isolation, or whether intervening populations bridge gene flow between northern and southern populations more consistent with an isolation-by-distance pattern or porous boundary (i.e., hybrid zone). Second, in addition to geographically confirming/locating the subdivision and assessing its porosity (if any), characterizing the depth of phylogenetic divergence and adaptive differentiation are essential to management actions to ensure the maintenance of adaptive difference, evolutionary potential, and persistence of both subspecies in the face of future sea-level rise

SMHM exist in fragmented patches in the greater San Francisco Bay where they are exposed to different selective pressures, including variation in salinity and associated with different habitats, vegetation cover, competitors, and predators. Genetic screening at the genome-wide scale using next generation sequencing (NGS) can provide the resolving power necessary for the quantification of phylogenetic divergence between subspecies/genetic units, and also the identification of genomic regions associated with adaptive differentiation between subspecies and among populations in different ecological conditions.

We identified major deficiency in the morphological keys used to identify harvest mice in the San Francisco Bay. This inaccuracy is particularly worrying as SMHM populations in this area are the sole representatives of the subspecies *R. r. raviventris*. Accurate species identification is vital for monitoring this cryptic endangered species, thus the development of a new morphological key to discriminate between harvest mice species in San Francisco Bay is warranted. This key should be developed in concert with genetic analyses to confirm species identification.

Future research should therefore prioritize the following objectives:

(1) Use existing markers to characterize genetic populations from previously unsampled locations

(2) Use NGS to quantify phylogenetic divergence between subspecies/genetic units.

(3) Use NGS to preliminarily screen for genomic regions associated with adaptive differentiation between subspecies and among populations in different ecological conditions.

(4) Determine if centrally located populations are in habitats that facilitate gene flow or if subspecies are reproductively isolated from one another.

(5) Develop an accurate morphological key for harvest mice in the southern San Francisco Bay.

Personnel and Cooperators

SMHM survey cooperators include Laureen Barthman-Thompson, Sarah Estrella (DFW, Suisun Marsh), Karen Taylor (DFW Napa/Sonoma Wildlife Area), Stacy Martinelli (DFW, Fagen Marsh), John Krause (DFW, Eden Landing Ecological Reserve), USFWS staff Rachel Tertes (Don Edwards SFB NWR), Meg Marriott (USFWS, San Pablo Bay NWR), and Isa Woo (USGS, San Pablo Bay). UC Davis Personnel include Sini Reponen, Luis Hernandez, Susan Fresquez, Natalie Goddard, Michelle Holtz, Siobhan Aamoth. Thank you for to Bill Burkhard (DWR), Peter Moyle (UC Davis) for provision of student funding.

References

Bell DM, Hamilton MJ, Edwards CW, Wiggins LE, Martinez RM, Strauss RE, Bradley RD, Baker RJ (2001) Patterns of karyotypic megaevolution in Reithrodontomys: evidence from a cytochrome-b phylogenetic hypothesis. Journal of Mammalogy. 82 (1), 81-91

Bandelt HJ, Forester P, Rohl A (1999) Median-joining networks for inferring intraspecific phylogenies. Molecular Biology and Evolution 16:37-48.

Bradley RD, Baker RJ (2001) A test of the genetic species concept: cytochrome-b sequences and mammals. Journal of Mammalogy, 82(4):960–973

Brown SK. (2003) Conservation Genetics of Salt Marsh Harvest Mice (*Reinthrodontomys raviventris*) in Suisun Marsh, CA. Master Thesis, San Luis Obispo, Calif. California Polytechnic State University.

Do C, Waples RS, Peel D, Macbeth GM, Tillett BJ, Ovenden JR (2014) NeEstimator V2: re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. Molecular Ecology Resources, 14(1), 209-214.

Excoffier L, Lischer HEL (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10:564-567.

Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* **164**,1567-1587.

Goudet J (1995) FSTAT (Version 1.2): A Computer Program to Calculate F-Statistics. J. Hered (1995) 86(6): 485-486

Park SDE (2001) "The Excel microsatellite toolkit." *Trypanotolerance in West African Cattle and the Population Genetic Effects of Selection*. PhD Thesis, University College Dublin.

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

Pritchard JK, Wen W (2002) Documentation for Structure Software Version 2. http://pritch.bsd.uchicago.edu

Reponen SEM, Statham MJ, Barthman-Thompson L, Sacks BN (2014) Microsatellite primer development for the salt marsh harvest mouse (Reithrodontomys raviventris) and cross-amplification in the western harvest mouse (R. megalotis). Conservation Genetics Resources. 1-3.

Shellhammer HS (1982) Reithrodontomys raviventris. Mammalian species (American Society of Mammalogists) No. 169 :1-3.

Shellhammer HS (1989) Salt marsh harvest mice, urban development, and rising sea levels. Conservation Biology, Vol. 3, No. 1, pp. 59-65

Smith MF, Thomas WK, Patton JL (1992). Mitochondrial DNA-like sequence in the nuclear genome of an akodontine rodent. Mol Biol Evol. 1992 Mar;9(2):204-15.

National Research Council (2010) Sea Level Rise and the Coastal Environment. Advancing the Science of Climate Change. Washington, D.C.: The National Academies Press. p. 245.

Vázquez-Domínguez E, Espindola S (2013) Characterization of ten new microsatellite loci from the endangered endemic rodent, *Reithrodontomys spectabilis*. Conservation genetics resources. 5: 251–253

Whitaker Jr. JO, NatureServe (2008) Reithrodontomys raviventris. In: Hammerson, G. & Williams, D.F. IUCN 2013. IUCN Red List of Threatened Species. Version 2013.2. www.iucnredlist. org. Downloaded on 10 Dec 2013

Table 1. mtDNA haplotype occurrence in SMHM in all sampling locations across the three bays.

Location		Haplotype							
	n	A	B	C	D	E	I	L	M
San Pablo Bay									
Napa/Sonoma Marshes P4/5	*15*	10	-	-	4	-	1	-	-
Fagan Slough N/S	*10*	-	10	-	-	-	-	-	-
Napa Plant Restoration Area	*5*	3	-	-	2	-	-	-	-
Suisun Bay									
Hill Slough P1,2,4	*10*	6	-	-	2	2	-	-	-
Point Edith	*10*	10	-	-	-	-	-	-	-
Denverton	*10*	6	2	-	-	1	-	-	1
San Francisco Bay									
Warm Springs	*4*	-	-	2	-	-	-	2	-
Eden Landing	*20*	-	-	18	-	-	-	2	-
Mayhew's Landing	*6*	1	-	-	-	-	-	5	-
Total	*90*	36	12	20	8	3	1	9	1

Table 2. Mitochondrial DNA diversity statistics within SMHM.

Location	# Haplotypes	Haplotype Diversity	SD	Nucleotide Diversity	SD
San Pablo	4	0.68	0.04	0.0043	0.0030
Suisun	5	0.46	0.11	0.0021	0.0017
San Francisco	3	0.48	0.07	0.0021	0.0017

Table 3. Population subdivision within SMHM as evident with mtDNA. Values indicate pairwise Φ_{ST} between the three bays.

Population	San Pablo	Suisun	San Francisco
San Pablo	0	-	-
Suisun	0.09	0	-
San Francisco	0.36	0.38	0

Note: SF v SP, and SF v SU P = 0.0000. SU v SP P = 0.045

Table 4. Microsatellite diversity statistics within SMHM. A = average number of alleles per locus; H_E = expected heterozygosity, H_O = Observed heterozygosity, F_{IS} = inbreeding coefficient.

Population	n	A	H_E	SD	H_O	SD	F_{IS}
San Pablo	30	4.9	0.63	0.06	0.56	0.03	0.11
Suisun	30	4.8	0.59	0.09	0.57	0.03	0.04
San Francisco	30	3.9	0.52	0.09	0.49	0.03	0.06

Table 5. Population subdivision within SMHM as evident with nuclear microsatellites. Values indicate pairwise F_{ST} between the three bays.

Population	SP	SU	SF
San Pablo	-		
Suisun	0.019	-	
San Francisco	0.145	0.142	-

All pairwise comparisons were significant at 0.05.

Table 6. Number of genetically identified SMHM and WHM from each bay and ratio of abundance of both species.

Bay	WHM	SMHM	Abundance of WHM relative to SMHM
San Pablo	3	30	0.10 : 1
Suisun	22	30	0.73 : 1
San Francisco	27	30	0.90 : 1

Figure 1: Network of Salt Marsh Harvest Mouse mtDNA haplotypes, based on 426 bp of cytochrome *b* from 90 animals.

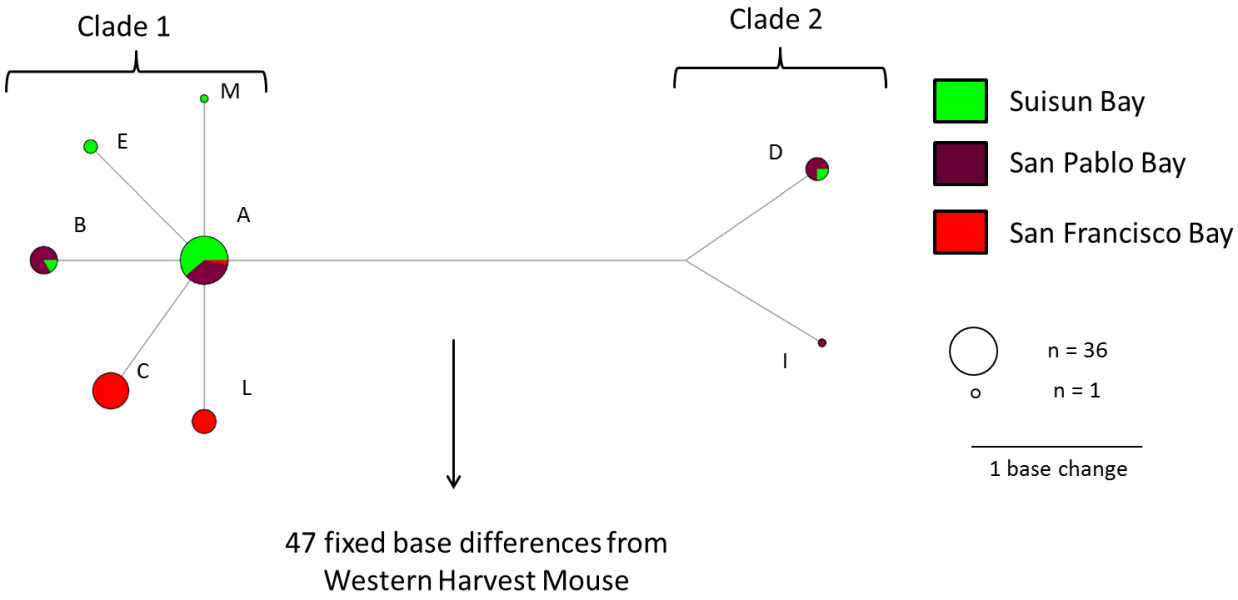


Figure 2: Map of the San Francisco Bay area. Sampling locations are indicated with a small yellow circle. The pie charts indicate the proportional representation of the mitochondrial clades in each of the three main bays. Pairwise Φ_{ST} values are given between each of the bays.

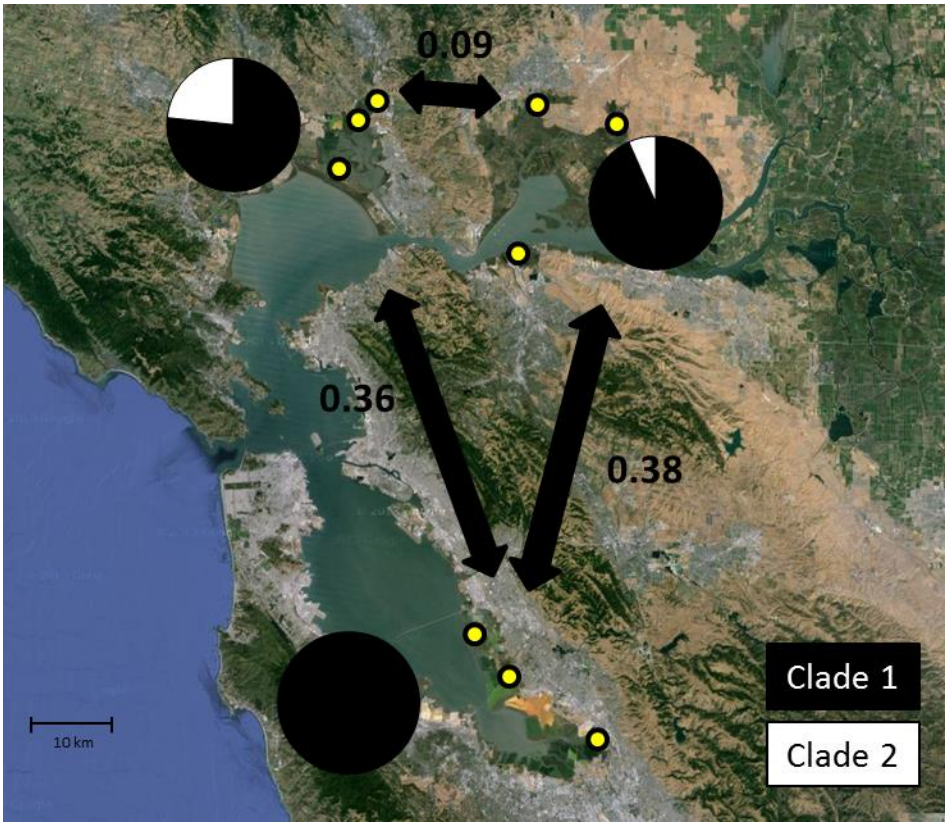


Figure 3. Harvest mouse genetic subdivision as evident from 10 microsatellite loci and cluster analysis using the program structure. All analyses were performed without prior information regarding the population of origin or putative species. a) Genetic cluster analysis of all harvest mice separated animals into two groups, Salt Marsh Harvest Mouse and Western Harvest Mouse, consistent with the species assignment from mtDNA analyses. b) Cluster analysis separated animals from the south bay from those in two northern bays, consistent with previously described subspecies. c) There was no evidence of population subdivision within the western harvest mouse dataset.

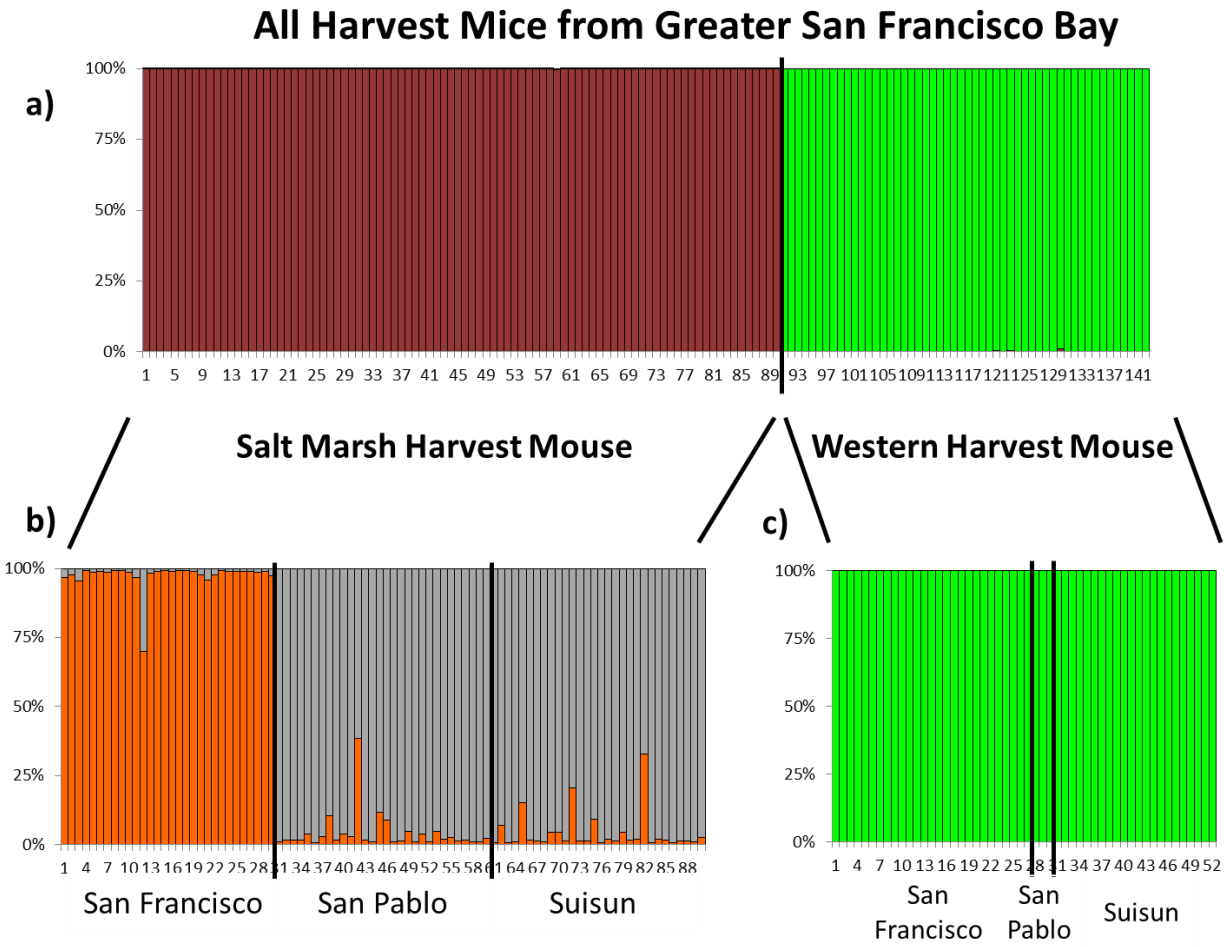
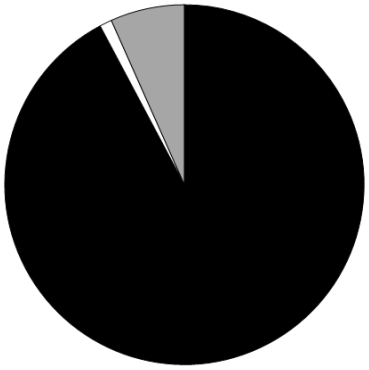


Figure 4. Comparison of genetic and morphological species assignment of harvest mouse species. 92% of SMHM were correctly identified in the field. 44% of WHM were correctly identified in the field.

Genetic Identification as SMHM (n = 90)



Morphological Species Assessment	SMHM	%	WHM	%	Color Code
SMHM	83	92	14	27	
WHM	1	1	23	44	
Unknown	6	7	15	29	
Total	90		52		

Genetic Identification as WHM (n = 52)

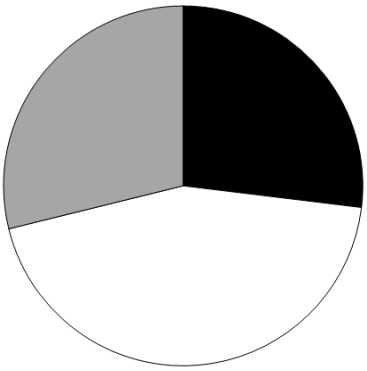


Figure 5. Species identification rates across the three bays. Unassigned in Field indicates the portion of harvest mice that were unassignable to species using the morphological key.

Morphological Species Assessment	Key	San Pablo		Suisun		San Francisco	
		n	%	n	%	n	%
Correct		33	100	44	84.6	29	50.9
Incorrect		0	0	5	9.6	10	17.5
Unassigned in Field		0	0	3	5.8	18	31.6