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LOW SITE-SPECIFIC GENOMIC VARIABILITY IS CONSISTENT WITH THE HISTORY(S) OF FRAGMENTATION OF THE RIPARIAN BIOTA OF THE ARID SOUTHWEST

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ABSTRACT

Persistently low population sizes, when coupled with reduced interpopulation connectivity, can impede the long-term viability of species in fragmented landscapes. Riparian-associated species in the arid American Southwest now face a series of threats due to fragmented populations and changing environmental conditions. During the last century, riparian habitats have deteriorated due to the synergistic effects of livestock grazing, increasing incidence of fire, and other anthropogenic impacts potentially have made local populations smaller, less demographically stable, and susceptible to the negative impacts of genetic drift and stochastic events. We evaluated genomic variation within and across geographic areas (*i.e.*, mountain ranges and river systems) in the federally endangered New Mexico meadow jumping mouse (*Zapus luteus luteus*) using neutral and outlier loci to test whether observed genomic variation was influenced by 1) historical allopatric divergence, 2) recent

anthropogenic fragmentation, or 3) both of these factors. We sampled 145 specimens from across the range of *Z. l. luteus* and 44 samples of co-distributed, closely related taxa and obtained over 8,800 single nucleotide polymorphisms. Combining insights from population genomics and phylogenomics, we found that eight geographic areas that are significantly differentiated from one another and have exceptionally low variability and low effective population sizes (fewer than 50 effective individuals in most cases). These lineages, however, reflect a biogeographic history that is mismatched with hypothesized riparian connectivity, but instead point to possible mitonuclear discordance. Additionally, each lineage has genomic variation consistent with expectations of adaptation to local conditions. Combined, these results suggest that there may be insufficient genomic variation in these distinctive jumping mice populations necessary to sustain viable populations without active management efforts. This improved understanding of how drift and selection have likely shaped the genomic structure of this endangered mammal provides a foundation to develop thoughtful management decisions.

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1. Introduction

Imperiled species often occur in isolated, small populations due to the synergistic effects habitat fragmentation and degradation, climate change, and other anthropogenic impacts. Fragmentation often leads to population declines and loss of metapopulation connectivity (*i.e.*, gene flow) that can erode genomic variation due to drift (Frankham 2005; Frankham et al. 2010; Allendorf and Luikart 2013; Allendorf 2017). Reduced genomic variation lowers the capacity for adaptative responses to changing environmental conditions by decreasing evolutionary potential (Barret and Schluter 2008; Savolainen et al. 2013) and ultimately increasing the possibility of extinction. Therefore, an improved understanding of genomic variation across populations of imperiled species is key for robust management action.

Advances in genomic technologies provide new opportunities for evaluating how drift and selection shape genomic diversity of non-model species (Flanagan et al. 2018; Funk et al. 2018) and can provide precise estimates of fundamental evolutionary parameters, including demographic history, spatial structure, effective population size, and inbreeding, among others (Funk et al. 2012; Hohenlohe et al. 2020). These assessments are essential for recovery strategies for threatened or endangered species aimed at maintaining interactions between populations for their long-term persistence (Schwartz et al. 2007; USFWS 2016a; Flanagan et al. 2018; Funk et al. 2018; Smith et al. 2018). Population genomics studies also are key to identifying genomic loci and variants responsible for inbreeding and outbreeding depression or adaptation to changing environments. Furthermore, conservation efforts should aim to manage genomic variation in ways that maintain the capacity of populations and species to evolve and adapt in response to environmental change (Allendorf et al. 2010;

Whiteley et al. 2015). Here, we applied a genome-wide approach to gain insights from both neutral and putatively adaptive loci for a federally endangered subspecies. We aim to develop a robust foundation for possible recovery activities that may eventually include designation of optimal source populations for captive breeding, genomic augmentation, and/or repatriation of endangered and extirpated populations.

Study system

The New Mexico Meadow Jumping Mouse, *Zapus luteus luteus* (Miller 1911), ranges across the arid American Southwest, from southern Colorado to central New Mexico and eastern Arizona (Miller 1911; Hafner et al. 1981; Frey and Malaney 2009) and consists of relictual populations hypothesized to have been isolated to a series of disjunct riparian systems during the warming and drying of the early Holocene (Malaney et al. 2012, 2017, 2022). This taxon is ecologically, morphologically, and genetically distinct, consistent with its recognition as a subspecies (Malaney et al. 2017), but it has been reclassified taxonomically at least four times over the past 70 years as our understanding of taxonomic limits and evolutionary relationships was refined. First described as a distinct species, *Z. luteus* (Miller 1911; Bailey 1913), these southwestern populations were later thought to be a subspecies of the western jumping mouse (*Z. princeps*; Krutzsch 1954), but then reclassified as *Z. hudsonius luteus* based on allozyme variation (Hafner et al. 1981). Most recent studies show that these southwestern populations collectively form a distinct evolutionary lineage of jumping mice (Malaney et al. 2017) that diverged as it shifted westward during the Holocene (Malaney et al. 2012, 2022). A lack of genomic

perspective, however, has obscured important evolutionary parameters for this taxon, especially geographic structure and effective population sizes, which challenges our ability to design and implement effective recovery programs.

Jumping mice are typically found in temperate, snowy climates (Kottek et al. 2006; Peel et al. 2007), effectively restricting *Z. l. luteus* in the arid Southwest to riparian zones in the San Juan, Jemez, Sangre de Cristo, Sacramento, and White mountains. However, unlike many other montane-associated species in the region, some populations also occur in lower elevation riparian areas along major river systems (Figure 1), including the Middle Rio Grande Basin and tributaries of the San Juan River (Findley et al. 1975; Hoffmeister 1986; Morrison 1990; Frey and Malaney 2009).

Jumping mice are sensitive to riparian habitat change and especially the loss of tall, dense herbaceous vegetation (USFWS 2020). Surveys for *Z. l. luteus* failed to detect populations at 73% and 94% of historical localities in the Jemez Mountains and Sacramento Mountains, respectively (Frey and Malaney 2009) and 66% of historical locations in the White Mountains of Arizona (Frey 2017). Localized extirpations are likely a result of habitat degradation in riparian systems due to the synergistic impacts of livestock grazing, catastrophic wildfires, and climate change (Morrison 1992; Frey 2017) and are likely indicative of analogous declines for other, co-occurring species. Taken together, *Z. l. luteus* now represents a distinct taxonomic unit with an elevated conservation priority (Malaney and Cook 2013; Malaney et al. 2017, 2022).

Despite established management recommendations to conserve and enhance habitat for *Z. l. luteus*, some populations continue to decline or have been extirpated (Frey and Malaney 2009; Wright and Frey 2015; Frey 2017). Proposed recovery plans do not yet

include a robust understanding of geographic variation or demographic processes (USFWS 2014a; b, 2020; Leroy et al. 2018). Herein, using a genome-wide approach we evaluate three hypotheses that may have contributed to observed geographic genomic variation and demographics (Table 1). First, if disjunct populations of *Z. l. luteus* are the product of a deep divergence history (long-term allopatric hypothesis) due to isolation in cooler mountain tops and river systems since temperatures started rising after the Last Glacial Maximum (LGM), then we expect local populations to show independent signatures of divergence and adaptation due to site-specific selective pressures. Furthermore, we may expect to detect elevated allelic variation, heterozygosity, and no evidence of demographic declines in these populations that are no longer exchanging individuals. If, however, populations were functioning as a widespread, geographic metapopulation (*i.e.*, mountain ranges interconnected) that only recently fragmented and became isolated due to anthropogenic activities over the past 200 years (recent disturbance hypothesis), we would not expect to detect deep divergences or elevated fixation indices, but rather recent demographic declines and low genetic variation (allelic richness and heterozygosity). Third, through a combination of both scenarios, where populations were isolated since the LGM, but isolated populations subsequently were further, locally reduced due to habitat fragmentation by anthropogenic impacts in the last couple centuries, we expect to find genomic signatures of both evolutionarily divergence and recent deterioration of variability. Consequently, we expect to detect independent lineages with elevated fixation indices, coupled with eroded genomic variation that may include reduced allelic variation, heterozygosity, and effective population sizes at local sites. Thus, we

evaluate the impact of limited or lack of gene flow, genomic drift, and selection (signals of adaptation) on the generation and maintenance of genomic variation in *Z. l. luteus* and estimate whether the observed genomic signatures are consistent with expectations derived from the long-term allopatric, recent disturbance, or combined hypotheses. Additionally, because Hafner et al. (1981) reported evidence of potential hybridization between *Z. h. luteus* and *Z. princeps* at a single, high-elevation site in the Sangre de Cristo Mountains, we also evaluated for presence of potentially introgressed genomes in this system.

We first characterized the patterns of geographic genomic structure and gene flow across eight documented geographical areas (GAs) where they are known to occur. Second, we estimated effective population sizes (N_E) and evaluated the impact of genomic drift within each GA to assess the possibility of genomic erosion. Third, we screened thousands of loci for signals indicative of local adaptation. Fourth, we evaluated how local environments may influence genomic variation by identifying genotype-environment associations (GEAs). Finally, we tested for introgression with other closely related or sympatric zapodids.

2. Materials and Methods

2.1 Generating the SNP dataset

A total of 145 (135 specimens; 10 embryos) *Z. luteus luteus* were sampled between 1978 -2019 from 44 sites distributed across eight GAs (Figure 1; Supplemental Table 1), including the major mitochondrial lineages and phylogeographic groups identified previously (Malaney et al. 2012, 2022). An additional 44 samples represented by *Z. l. pallidus* (2 samples), *Z. hudsonius* (15 samples), and *Z. princeps* (27 samples) were used to test

hypotheses of divergence history and secondary contact with potential hybridization (Hafner et al. 1981), and to provide context for interpreting key evolutionary signals (see additional details below). Either liver or heart tissues were used from the Museum of Southwestern Biology (MSB) at the University of New Mexico, the Denver Museum of Nature and Science (DMNS), and other available samples (Frey Tissue - FT).

We applied double digest RAD sequencing (ddRADseq) to generate single nucleotide polymorphisms (SNPs) using modified published protocols (Peterson et al. 2012). This reduced-representation approach enables genotyping multiple individuals for thousands of markers (Nielsen et al. 2011; Peterson et al. 2012; Andrews et al. 2016). Samples were quantified using a Qubit 2.0 Fluorometer (Invitrogen) and we digested > 500 ng of genomic DNA using 20 units each of a common restriction enzyme *MspI* (restriction site 5′-CCGG-3′) and a rare site restriction enzyme *Sbf1 (*restriction site 5′-CCTGCAGG-3′) in a single reaction with the manufacturer recommended buffer (New England Biolabs) for 4 h at 37°C. Fragments were purified with Serapure SpeedBeads before ligation of barcoded Illumina adaptors. Libraries were size selected for 300–400-bp fragments using a Blue Pippin Prep size fractionator (Sage Sciences). The final library amplification used proofreading *Taq* and Illumina's indexed primers. Size distributions of fragments and pool concentrations were determined on an Agilent 2100 Bioanalyzer. Quantitative polymerase chain reaction (qPCR) was used to determine library concentrations before multiplexing equimolar amounts of each pool for sequencing on a single lane

of an Illumina HiSeq SE100 (100-bp, single-end sequenced) at UC Davis DNA Technologies Core.

The raw Illumina reads were filtered and demultiplexed using STACKS version 2.5.4 (Catchen et al. 2013; Rochette et al. 2019) and STACKS_PIPE-LINE version 2.4 (Portik et al. 2017) following the workflow outlined by (Rochette and Catchen 2017). No barcode mismatches were allowed during demultiplexing. We aligned sequences to the meadow jumping mouse genome (*Zapus hudsonius*; GCA_004024765.1) using the BWA short-read aligner with default parameters and the MEM alignment algorithm (Li and Durbin 2010). Reads potentially arising from PCR duplicates during sequencing were not explicitly accounted for because their low frequency presumably fails to significantly impact most population genomic parameter estimates (Schweyen et al. 2014). During filtering, sites with < 90% base call accuracy (Phred score =10) were converted to missing data and reads with ≥ 10% missing sites were discarded. Reads were aligned into stacks with a minimum coverage depth of 10x and a maximum of two nucleotide differences between stacks. The minor allele count was set to two to eliminate singletons, which reduces errors in model-based clustering methods (Linck and Battey 2019). Loci that were invariant, not biallelic, or absent from > 20% of samples were removed, as were samples with > 80% missing data (n = 17; Supplementary Table 1, equally distributed across GAs); calculated using VCFTOOLS v.0.1.16; (Danecek et al. 2011). A single randomly selected variable site per locus was sampled to minimize the chance of retaining physically linked SNPs. Paralogous loci can skew common downstream analyses for population genomics by artificially inflating levels of heterozygosity (Willis et al. 2017). Consequently, we removed potential paralogs by

excluding loci with an observed heterozygosity exceeding 0.75 using the populations module of STACKS (O'Leary et al. 2008; Willis et al. 2017).

2.2 Outlier detection

We screened the SNP dataset for outlier loci, which are either closer to, or farther from, fixation (F_{ST}) than expected from a neutral distribution and are often indicative of loci associated with traits under selection. Consequently, this effort enabled us to identify outlier loci potentially responsible for adaptive differentiation and to segregate potentially adaptive and neutral loci for population genomic analyses. Outlier loci were detected with a pair of complementary approaches—a Bayesian genome-scan method and unconstrained ordination.

Outlier loci were first detected via BayeScan 2.1 (Foll and Gaggiotti 2008), which is based on the multinomial-Dirichlet model and identifies differences in allele frequencies between subpopulations and the common gene pool of all subpopulations (measured as the subpopulation specific F_{ST} coefficient). We first defined populations as the geographic area (n = 8) and applied the default prior odds for neutrality of 10 (odds of a single locus being under selection for every 10 neutral loci). To control for the false discovery rate (FDR), we set the target FDR (q-value) to limit the proportion of false positives to < 5% (Foll and Gaggiotti 2008).

We executed the unconstrained ordination method in the R package *PCAdapt* (Priv et al. 2020). Unlike the BayeScan method, predefined populations are not required, and we specified the number of principal components (PCs) to retain (K parameter = 4) based on the inflection in the scree plot where the amount of variation explained by additional PCs sharply decreases. We defined outlier loci as those with q-values < 0.05, meaning that no more than

5% of loci identified as outliers are potentially false positives. In this study, the number of loci retained with *q*-values approach was identical to the Benjamini-Hochberg procedure.

We retained all loci identified by either procedure as a set of outlier loci to compare to putatively neutral loci in subsequent analyses, especially to determine the relative contribution of genomic drift and selection in shaping patterns of genomic structure.

2.3 Geographic genomic structure and characterizing differentiation

While GAs were derived from the spatial clustering of available samples and were generally associated with management units, the optimal number of genomic clusters may differ. Consequently, we used a pair of approaches to both identify and describe genomic clusters including a multivariate ordination approach and model-based analysis, with both analyses using the neutral dataset. We used a discriminant analysis of principal components (DAPC) in the R package *adegenet* (Jombart 2008; Jombart and Ahmed 2011) to generate a de novo clustering hypothesis using find.clusters. DAPC partitions both between-group and within-group components, maximizing variation between groups while minimizing variance within groups. We optimized the tradeoff between discrimination and overfitting and determined the optimal number of principal components (PCs) to retain using the *a*-score and randomization procedure (PC = 11; SF 2) that accounted for 62.5% of observed variation. We then used the K-means clustering method and defined the most likely number of genomic clusters using Bayesian Information Criterion (BIC). However, because the lowest BIC value may miss other valid clustering scenarios, we used a permutation approach to explore across *K*-values. We then assigned individual samples to a cluster using DAPC, plotted individuals

to display cluster membership, and generated a cluster membership probability plot for each individual.

Model-based approaches may outperform ordination-based approaches that fail to account for HWE when evaluating genomic structure. We compared the results of DAPC with those derived from population structure conducted in *Structure* 2.3.4 (Pritchard et al. 2000) using the neutral dataset but removing all missing loci (n = 1,391). We conducted 10 independent runs of Structure (Markov-Chain-Monte-Carlo [MCMC], burn-in 10,000 steps, 100,000 permutations) across 10 hypothesized genomic clusters (K = 1-10) using the admixture model with correlated allele-frequencies, but we did not apply a location prior. The optimal value of K was determined by comparing the ΔK value (Evanno et al. 2005) and the mean likelihood of K estimate (Ln Pr($X|K$); (Pritchard et al. 2010) using *Structure Harvester* (Earl and vonHoldt 2012). We then used the mean q-value (proportion of an individual's genome belonging to each cluster) calculated by *CLUMPP* (Jakobsson and Rosenberg 2007) and assigned individuals to a cluster based on q-values > 0.90 and considered individuals with lower mean q-values as intermediate (*i.e.*, hybrids). Finally, we conducted hierarchical Structure analyses with each inferred cluster run individually to help clarify patterns of hierarchical variation (Pritchard et al. 2000; Waples and Gaggiotti 2006; Janes et al. 2017).

After defining clusters, using both the outlier and neutral loci, we estimated genomic differentiation between GAs by calculating both pairwise allele fixation index (F_{ST}) and allele differentiation (Jost's D; Jost 2008; Jost et al. 2018) and then calculated 95% confidence intervals and p-values using the R package *hierfstat*

(Goudet 2005) by applying 999 bootstrap replicates. We additionally visualized genomic differentiation using principal components analysis (PCA) using GAs as putative clusters.

2.4 Genomic diversity and effective population size

Analyses of genomic diversity were performed on the full genomic dataset (neutral + outlier loci), neutral loci only, and outlier loci only, to ensure patterns of variation did not result from filtering bias (Table 2a-c). The R packages *adegenet* (Jombart 2008; Jombart and Ahmed 2011), *diveRsity* (Keenan et al. 2013), *poppr* (Kamvar et al. 2014), and *hierfstat* (Goudet 2005), plus GENALEX (Peakall and Smouse 2006, 2012) were used to calculate genomic diversity estimates among GAs and local sampling sites including the number of individuals genotyped (N_G), allelic richness (A_R), effective number of alleles (A_E), unbiased gene diversity (uH_E), observed heterozygosity (H_O), Shannon's information index (I), and Simpson's Index (l). Associated inbreeding coefficients (F_{is}) were assessed using 999 bootstrap replicates ($\alpha = 0.05$). Mean minor allele frequency (MAF), total alleles (A), and number of private alleles (PA) are provided for each sampling unit (Table 2a-c).

We estimated the effective population size (N_E) for each GA using a linkage disequilibrium-based estimator (LDN_E; Waples and Do 2008) for the neutral loci dataset with NEESTIMATOR v. 2.0 (Do et al. 2014). We calculated LDN_E to screen alleles at $P = 0.05$ and 95% confidence intervals were calculated using a jackknife method (Waples and Do 2008). SNP-based data may have significant linkage that can bias N_E estimates downward (Waples et al. 2016). Consequently, to account for the potential for linkage, LDN_E estimates were adjusted by the total number of haploid chromosomes (Chr = 72; Meylan 1968; Whitaker 1972) using the equation of (Waples et al. 2016).

2.5 Phylogenomics: Evolutionary History and Biogeography

Previous phylogeographic reconstructions of *Z. l. luteus* identified a strong signal of divergence between Western (White Mountains) and Eastern (all other samples) mitochondrial lineages (see below, Malaney et al. 2012, 2022). Phylogenetic reconstructions based on a single gene, however, can be prone to biases such as incomplete lineage sorting and introgression. Consequently, we estimated a time-calibrated Bayesian phylogeny for the RAD loci (no missing data, neutral only, n = 1,391 loci) using BEAST v.2.4.8. on the CIPRES v.3.3 computing cluster (Miller et al. 2010). To select the best-fit model of evolution, we used JMODELTEST v.2.1.7 applying the Bayesian information criterion (BIC) for the concatenated RAD loci (HKY). To calibrate the phylogeny, we applied published substitution rates and assigned the ucld.mean parameter, a lognormal distribution with a mean of 0.0024 substitutions/site/million years and a standard deviation of 0.43, resulting in a 95% highest probability density (HPD) ranging from 0.0011 to 0.0044 and spanning published mean substitution rates. We assigned the ucld.stdev parameter a gamma distribution with a mean of 0.45 after reviewing trace plots of posterior distributions of preliminary runs.

We used a lognormal relaxed clock model and constant-size coalescent tree prior and ran analyses for 50 million generations, retaining trees and parameters every 10k steps. Results were examined in TRACER v.1.7.1 (Rambaut et al. 2018) to evaluate convergence and effective sample sizes (ESS) for all estimated parameters. We discarded the first 20% of trees as burn-in and summarized the maximum clade credibility tree with median heights

using TREEANNOTATOR v. 2.4.8. The analysis was repeated three times with random starting seeds to confirm adequate mixing and consistent results.

Concatenation of phylogenomic data can contribute to overestimated credibility values in phylogenomic trees (Song et al. 2012; Xi et al. 2015). We therefore reconstructed a species tree using the multispecies coalescent model implemented by the SNAPP v.1.1.6 (Bryant et al. 2012) plugin in BEAST v.2.4.8 (Bouckaert et al. 2014). To reduce computational burden, we removed 90 samples and all missing loci for the neutral dataset (outlier loci already purged). The alignment consisted of 38 samples divided into eight GAs (putative Operational Taxonomic Units, OTUs; 4-5 samples each), 1,391 sites (loci), and 719 patterns. We applied a gamma prior for l (a = 10, b = 1,000), estimated mutation rates U and V (0.58 and 3.68, respectively), and set the coalescence rate to 10.0, with other parameters left default. We conducted three separate analyses by differing starting seeds with each run consisting of 1 million generations (10k burn-in) and sampling every 10,000 steps on the CIPRES Science Gateway (Miller et al. 2010). We confirmed MCMC convergence and acceptable ESS (all exceeded 200) values for parameters in TRACER v.1.7.1. We used TREEANNOTATOR v.2.4.8 to summarize the maximum clade credibility tree and visualized the posterior distribution of species trees after a 25% burn-in using DENSITREE v.2.1.11 (Bouckaert 2010) and FIGTREE v.1.4.3.

2.6 Outlier Association

We screened the outlier dataset to better understand the geographic patterns of allelic variation of outlier loci that may point to a relationship between the genome and environment consistent with localized natural selection and then associated those loci with a specific GA

or set of GAs. We expect this approach to help distinguish loci strongly associated with individual GAs or at a set of GAs consistent with populations located at higher elevation montane sites or lower elevation sites along large rivers (*i.e.,* Rio Grande or San Juan). Because rare or minor alleles have a disproportionate effect during adaptation (Gorlov et al. 2008; Hernandez et al. 2019), we first calculated the minor allele frequency (MAF) of each outlier locus for each GA. We then removed any loci that had missing data for at least one sample from each GA that would alter the MAF for that site leaving 205 loci shared across all individuals across all GAs. We then associated each MAF > 0.5 with individual GAs. For example, if the MAF was high (*e.g.,* 0.9) at a specific site but MAF low or absent at all other sites, then we associated that allele for that locus as potentially adapted to that site. We then tabulated the total number of loci associated with each GA or set of GAs (*i.e.,* montane or large rivers).

2.7 Evaluating differentiation and introgression with other zapodids

We assessed the potential for hybridization or asymmetric introgression of each of the GAs (n = 37) with three other taxa of jumping mice including co-occurring *Z. p. princeps* from northern New Mexico and southern Colorado (n = 27), *Z. l. pallidus* from Kansas (n = 4), and *Z. hudsonius campestris* from the Great Plains (n = 15) using ddRADseq data. This effort also enabled the evaluation of signatures of genomic differentiation among taxa. Sequences of all taxa were mapped, variants genotyped together, and a separate set of filters was applied to the multitaxon data, but generally followed the same bioinformatics procedures as above. After filtering,

the final multitaxon dataset included 83 samples (37 *Z. l. luteus*, 4 *Z. l. pallidus*, 15 *Z. h. campestris*, and 27 *Z. p. princeps*) and 7,568 biallelic SNP loci and 15,136 alleles.

Two methods were used to evaluate the possibility of introgression. First, levels of admixture were determined using the average *q*-values derived from STRUCTURE and CLUMPP as above. However, analyses were optimized by providing an assignment prior using putative taxonomic identifications. We also used DAPC clustering approach in adegenet (Jombart 2008; Jombart and Ahmed 2011) following similar methods as described above but providing putative taxonomic identifications rather than exploring the likely number of clusters. For both tests, if samples have a mixed genome (*i.e.*, hybrid), then we expect to find intermediate (< 0.90) *q*-values and posterior probabilities of assignment, but high *q*-values (> 0.90) if genomes are distinct between taxa.

Second, we compared genomic differentiation between designated taxa (species and subspecies) by estimating pairwise F_{ST}, Jost's *D*, PCA, and the number of private alleles (PA) as above for GAs. For these tests, if taxa have experienced hybridization, then we expect to detect low fixation indices (< 0.05), indistinct clusters and overlapping PC scores on primary axes, and relatively few private alleles (< 1%). If, however, genomes have not mixed, we expect to detect relatively high fixation indices (> 0.05), distinct clusters of variation without overlapping PC scores on primary PC axes, and an elevated fraction of private alleles (1-10%).

3. Results

3.1 *Z. l. luteus* SNP dataset

Of the 145 *Z. l. luteus* samples, 16 samples with < 10x coverage, and one sample with > 20% missing loci were removed. From 515,716 loci, we removed 499,707 loci that did not pass sample or population constraints, leaving 16,009 loci, and 9,146 of those were filtered because they were not present in 75% of individuals in each population, so 8,849 variant sites remained. The resulting unlinked SNP data set was used to generate input files for downstream analyses included 128 individual samples, composed of 1,588,296 base pairs across 8,849 loci, and 17,411 alleles, with an effective per-sample coverage: mean=180.1x, stdev=153.1x, min=10.2x, max=754.2. The eight clusters or GAs defined (see below) were Johnson Mesa (JHM), Sangre de Cristo Mountains (SDC), the White Mountains (WHT), Sacramento Mountains (SAC), Jemez Mountains (JMZ), San Juan Mountains (SJN), Isleta (ISA) and Bosque del Apache (BDA). While the number of individuals genotyped was low for some GAs (ISA, SJN and SDC had ≤ 5 individuals), the number of alleles detected within each GA was > 10,000 and each had between 58% and 77% of total alleles (Table 2).

3.2 Outlier loci & geographic association

From the total set of loci, 8,138 loci (15,989 alleles) were identified as neutral, and the remaining 711 loci (1,422 alleles) were identified as putative outlier loci. Of the outliers, 205 were shared across all GAs and all samples (no missing data), making them suitable for comparing minor allele frequencies (MAF) across GAs (Figure 3). Most loci, in this reduced set of outlier loci, were associated with individual GAs (114/205; 55.6%) with BDA (30/205; 14.6%) and SAC (61/205;

29.8%) being disproportionately high, whereas JHM, JMZ, and SDC had just one outlier each (0.005%). Thirty-four (16.6%) of these outlier loci were associated only with a particular ecological setting, either high elevation sites (montane 10; 4.9%), or low elevation (Rio Grande Valley 24; 11.7%). However, because we lack access to an annotated genome, evaluating links between these primary ecological settings and specific gene function is not yet possible.

3.3 Geographic structure and differentiation

Geographic structure among samples is observed in the DAPC and Posterior Membership Probability analysis using both the neutral (n = 8,138) or outlier (n = 711) loci separately (Figure 2). Eleven PC eigenvalues were retained that accounted for ~60% of the variation and BIC determined nine optimal subclusters. But because two of the clusters identified (Rayado and Coyote sites) each had only two and three samples each and because they are spatially close and closely related to each other (see below), we combined these into a single cluster for subsequent analyses (SDC). Both neutral and outlier datasets failed to detect any level of admixture or proximity of samples to different clusters. PCA results were similar to DAPC, with all GAs clearly differentiated with the exception of the Rio Grande GAs (ISA and BDA), which clustered together in the first two axes but are differentiated in subsequent axes (SF1a). For outlier loci (SF1b), SJN clustered with JMZ and SDC clustered with JHM in the first two axes. The two first PCs captured 32.7% (neutral loci) and 49.1% (outlier loci) of the observed genomic variation. For neutral loci, PC1 explained 20.9% and PC2 explained 11.8% of the variation. For outlier loci PC1 explained 28.1% and PC2 explained 21.0% of the variation. In the STRUCTURE analysis, ΔK supported $K = 2$ as

optimal with the first cluster corresponding to JHM and SDC and the second represented by the remaining GAs (BDA, ISA, SAC, JMZ, SJN and WHT). However, STRUCTURE hierarchically separates each successive GA up to a K = 9. We present data on K = 5 and K = 8 to demonstrate successive perspectives of geographic structure that correspond with other analyses (SF3).

For neutral loci, pairwise F_{ST} and Jost *D* were elevated, indicative of fixed genomic differences between GAs. Pairwise F_{ST} is > 0.10 for all comparisons and ranged from 0.1013 - 0.5753 in neutral loci. Pairwise Jost *D* is generally > 0.05, with values for neutral loci ranging from 0.0157 - 0.0852. The GAs with the lowest differentiation observed in neutral loci were JHM and SDC (Table 3a). The highest degree of differentiation observed between GAs for F_{ST} were for SAC and BDA, while for Jost's *D* the highest value was between SDC and JHM. For outlier loci, pairwise comparisons were generally elevated indicating increased fixation compared to neutral loci, F_{ST} (0.4362 - 0.7686) and Jost's *D* values (0.0253 - 0.6745).

3.4 Genomic diversity

Using the full dataset (neutral + outlier loci), levels of genomic diversity were lowest in SAC and along the Rio Grande (BDA and ISA), whereas JHM, SDC, and JMZ had the highest genomic diversity measures overall. Allelic richness (A_R) across all GAs was low, often near 1. Unbiased gene diversity (uH_E) and observed heterozygosity (H_O) were generally low, often < 0.10. Across populations, A_R ranged from 1.085 - 1.301, H_O ranged from 0.049 - 0.1271, and uH_E ranged from 0.0592 - 0.1545. Observed heterozygosity was often lower than

expected ($Ho < uH_E$), which may be attributable to recent population declines or high levels of inbreeding leading to genomic erosion.

Inbreeding coefficients (F_{is}) ranged from -0.214 in ISA to 0.1845 in SDC. Values of F_{is} are elevated for some GAs, although careful interpretation is needed because not all GAs had large sample sizes and elevated estimates of F_{is} can be associated with low sample sizes or from single sampling bouts of closely related individuals (*e.g.,* siblings), which may have occurred in some cases (Supplemental Table).

Shannon-Wiener Index (I) is low (~ 1.0) for some GAs and may be indicative of depauperate genomic diversity. Similarly, Simpson's Index (lambda *l*) is low (<0.9) for some areas and may reflect low genomic diversity. Both low estimates for I and *l* are associated with few samples (<5), therefore careful interpretation is needed. Each GA has numerous private alleles (see outlier alleles below that differ) that range from 116 (SJN) – 1,136 (WHT), these likely reflect independent evolution and potentially suggest local adaptive differentiation.

Estimates of N_E ranged from 13.5 (BDA, n = 11) to 600.3 (JHM, n = 24 individuals). The combined GA (SDC) is represented by only 5 individuals yet had relatively high effective population estimates, whereas the two most densely sampled GAs (JMZ, WHT) had among the lowest estimates (Table 2a).

A summary of demographic statistics for GAs and for combined loci (Table 2a) and statistics for GAs for neutral and outlier loci (Table 2b and 2c, respectively) generally show similar values for neutral and outlier loci datasets, with outlier loci reflecting greater differences compared to the combined and neutral datasets.

3.5 Evolutionary (phylogenomic) relationships and biogeography

The time-calibrated BEAST tree inferred from concatenated RAD loci suggests monophyletic groups are concordant with the eight GAs detected using ordination and structure. The most ancestral node is consistent with a divergence estimate of approximately 20 kya. All ancestral nodes are supported with high confidence (> 0.95 posterior probability, Figure 4a) except for the ancestral node of the clade that includes BDA, ISA, SAC, JMZ and SJN, a node that appears to be associated with a period of rapid diversification. On the other hand, the SNAPP summary tree of posterior estimates of species trees reconstructed from unlinked SNPs, strongly supports this clade as well as the rest of the GAs (Figure 4b) and corresponds with the initiation of divergence about 20 kya.

3.6 Testing for introgression between jumping mice

F_{ST} values for subspecies of jumping mice, *Z. luteus luteus*, *Z. luteus pallidus*, *Z. hudsonius campestris*, and *Z. princeps princeps,* ranged from 0.18 to 0.82 (Table 4). The lowest level of fixation was between the subspecies *Z. l. pallidus* and *Z. l. luteus* (F_{ST} =0.18) and the highest was between the species *Z. p. princeps* and *Z. h. campestris* (F_{ST} =0.82). The *D* values ranged from 0.04 to 0.46, with *Z. l. pallidus* and *Z. l. luteus* showing the lowest differentiation and *Z. p. princeps* and *Z. l. luteus* the highest (Table 4). Private alleles (Table 4) show high differentiation across subspecies and overall, the private allele percentage for each subspecies was above 5%. The highest number of private alleles calculated was for *Z. l. luteus* (989, 9.0%, possibly due to the higher sampling; N=37) compared to the sister

subspecies, *Zapus l. pallidus*, which had the lowest number of private alleles PA=552 (5.8%), potentially due to small sample size (N=4).

Similarly, PCA (SF3), DAPC, and the Assignment Test (SF4) detected no evidence of significant hybridization or introgression between jumping mice species or subspecies despite close geographic proximity and in some cases close evolutionary relationships. Every individual was assigned to its corresponding taxon. For the PCA analysis, two PCs (PCA eigenvalues – inset) were sufficient to capture 67% of observed variation. For the DAPC analysis, three PCA eigenvalues and two DAs eigenvalues were retained and used to distinguish genomic variation among taxa.

4. Discussion

Using high-resolution, genome-wide SNP data, we gained new insights into how genomic diversity is partitioned within and among sampled GAs of *Z. l. luteus* using both putatively neutral and outlier SNP loci. We also failed to detect evidence of widespread introgression between *Z. l. luteus* and sympatric species or closely related subspecies (e.g., *Z. p. princeps* in the Sangre de Cristo Mountains or *Z. l. pallidus* and *Z. h. campestris* from the Great Plains). Taken together, these data allowed us to: i) characterize genomic geographic structure across sampled locations, ii) elucidate demographic processes, iii) compare the relative roles of drift and selection on divergence, and iv) identify geographic associations of outlier loci for this endangered taxon Finally, we contrast each of those signals with other imperiled vertebrates for which similar approaches have been applied. This first perspective into the genome-wide variation of *Z. l. luteus* yields valuable insight into the relative roles of

drift and adaptation to local conditions to help tailor management policy for this riparian-associated subspecies to mitigate the loss of genomic diversity.

4.1 No widespread introgression between jumping mice taxa

Introgression between wildlife species has evolutionary significance and often makes the development of conservation plans more complex (Allendorf and Luikart 2013). Therefore, an improved understanding of how gene flow, reinforcement, and introgression dynamics ultimately lead to divergence aids in managing populations and species. For example, investigations of introgression in stoats (*Mustela erminea* and *M. richardsonii*) provided insights into a complex history of diversification, wherein an extrinsic reproductive barrier (*i.e.,* insular oceanic isolation) that developed during glacial cycling led to the reinforcement of homoploid hybrid speciation (Colella et al. 2018b, 2021), with direct implications for conservation and management of endemicity on island archipelagos. Cryptic diversity and natural introgression in North American marten (*Martes americana* and *M. caurina*), coupled with translocation programs that have either failed or contributed to additional introgression and genomic swamping (Dawson et al. 2017; Colella et al. 2018a, 2019) reinforces the need for wildlife management to be mindful of introgression and take steps to ensure translocations are appropriate for management goals (Malaney et al. 2015). Introgression between red wolves (*Canis rufus*) and coyotes (*C. latrans*), for example, led to genetic swamping (VonHoldt et al. 2016, 2021; Heppenheimer et al. 2020). In that case, introgressive hybridization contributing to genomic extinction is the single greatest biological threat to the conservation efforts of red wolves

(Fredrickson and Hedrick 2006). Because of insights derived from these and other studies that show that introgression must be considered in proactive management efforts, we evaluated whether introgression played an important role in the evolutionary history of *Z. l. luteus* or might alter conservation priorities.

Phylogeographic data across all jumping mice has thus far failed to detect introgressive hybridization between closely related lineages or sympatric populations of divergent species (Malaney et al. 2012, 2017; Malaney and Cook 2013). However, previous allozyme analyses suggested the potential for genomic mixing between sympatric populations of *Z. p. princeps* and *Z. l. luteus* in the Sangre de Cristo Mountains (Hafner et al. 1981). Secondary contact is probable due to the dynamic biogeographic history, environmental heterogeneity, and high potential for ecotonal effects in the Southwest, as well as the suture zone documented for many species that exists where the Great Plains meet the montane habitats of the southern Rocky Mountains (Remington 1968; Swenson and Howard 2005). In particular, the Sangre de Cristo Range is an important zone of secondary contact and clinal variation resulting in genomic interactions between divergent lineages of pocket gophers (*Thomomys bottae*; Hafner et al. 1983) and their hematophagic chewing lice (*Geomydoecus actuosiy*; Nadler et al. 1990). Still, our analyses failed to detect signatures of mixed genomes between *Z. l. luteus* and *Z. princeps* within the Sangre de Cristo Range. In these analyses, we also failed to detect signals consistent with hybridization or introgression between *Z. l. luteus* and other sympatric or closely related zapodids. Fixation and differentiation measures (F_{st} and Jost *D*), as well as substantial number of private alleles and DAPC and assignment tests, all indicate that each of the four taxa included in the analyses

represent independent lineages despite some morphological and ecological similarities, spatial proximity, and evolutionary relatedness (Malaney et al. 2012, 2017, 2022).

4.2 Geographic structure and demographics

Gaining a perspective about the geographic genomic structure within species and subspecies can provide insights into the factors responsible for promoting or eroding variation. For example, the collective impact of mutations and drift within finite populations, coupled with natural selection in adaptive response to local environmental conditions, play joint roles in population differentiation (Slatkin 1987; Barret and Schluter 2008; Frankham 2012). Conversely, gene flow between populations may constrain differentiation, in some cases by preventing adaptive alleles from increasing in frequency (Fedorka et al. 2012), or may promote differentiation through the spread of novel genes and gene combinations (Slatkin 1987; Barret and Schluter 2008; Laurent et al. 2016). Knowledge of geographic structure also yields insights into the biogeographic and demographic history of local and regional areas (Hewitt 2000; Lessa et al. 2003). In this study, consistent with expectations of the combined hypothesis, we find evidence that both allopatric divergence since the LGM and recent anthropogenic impacts are responsible for the observed genomic variation, and we highlight three primary themes. First, there is significant geographic structure, with each of the eight GAs showing genomic divergence. Second, phylogenomic differences reflect novel biogeographic histories that are somewhat distinctive from mtDNA-based phylogeographic signals. Third,

anthropogenically-linked declines (Frey 2005, 2013; Frey and Malaney 2009) may have contributed to low extant genomic variability. Each of these key themes warrant further discussion.

Spatial genomic structure

Evaluating spatial genomic structure is essential for gaining insights into the viability of populations. Accurately characterizing population subdivision and geographic patterns of diversity may help elucidate the divergent processes and events leading to speciation (Funk et al. 2012; Hoban et al. 2016). However, population structure also helps unravel demographic histories, including the imprint of migration or gene flow resulting from interactions between genetically distinct groups (Allendorf et al. 2010; Funk et al. 2012; Hohenlohe et al. 2020). Furthermore, the recognition of genetically (*i.e.*, evolutionarily) distinct population segments are foundational to conservation and management programs (USFWS 1973; Waples 1995). However, delineation of populations is a non-trivial exercise as poorly characterized hierarchical structure can skew evolutionary inference (Allendorf et al. 2012; Greenbaum et al. 2016).

For populations that are isolated due to habitat constraints, understanding how demographic and evolutionary histories ultimately shaped geographic structure is a fundamental initial step. Because jumping mice disperse a maximum of ~4 km annually (Schorr 2003) and distances between occupied habitats in montane ranges and major river systems are at a minimum 43 km and typically > 100 km, there appears to be low probability of migration or gene flow between GAs (USFWS 2020). Therefore, consistent with a deeper history of allopatric divergence (hypothesis 1 and 3), we predicted limited or no signal of

recent gene flow between GAs, and consequently high levels of geographic structure. Accordingly, we found that GAs were significantly structured, based on both neutral and outlier SNP loci regardless of ordination or model-based approaches, consistent with a lack of admixture. Furthermore, pairwise F_{ST} (> 0.10 for all comparisons) and Jost *D* (> 0.02) results indicated significant differentiation of allelic variation between GAs (Table 3). More specifically, JHM and SDC had the greatest degree of genomic differentiation between them, in ordination space for neutral loci and SAC was most distinct for outlier loci, while jumping mice from WHT (Western Lineage) have a significant, spatial genomic distinctness from other populations (Figure 2).

Taken together, spatial genomic structure indicates low connectivity with no measurable signal of current gene flow, consistent with the allopatric or combined hypotheses, suggesting long-term (beginning ~20 kya) demographic isolation (*i.e.*, since they last had contact in the lowlands). However, the mismatch of mtDNA phylogeographic structure (Malaney et al. 2022) between some GAs (exception is the White Mountains = Western Lineage) compared to the nuDNA perspective is suggestive of a more complex history of connectivity (since Last Glacial Maximum) that requires formal testing to distinguish past introgression from incomplete lineage sorting.

Phylogenomics reveal mitonuclear discordance and novel biogeographic patterns

In addition to contemporary estimates of genomic variation, an improved understanding of evolutionary history, especially spatiotemporal biogeographic factors, is important to characterizing conservation units and prioritizing conservation actions ((Crandall et al. 2000; Riddle et al. 2008; Richardson and Whittaker 2010;

Malaney and Cook, 2013). While our SNAPP summary tree and the time-calibrated BEAST tree (Figure 4) support eight monophyletic lineages consistent with geographically structured genomic variation (Figure 2, SF 1, SF 3), these results are inconsistent with mitochondrial variation (Malaney et al. 2012, 2022) that identified five phylogeographic haplogroups with White Mountains sharing an ancestral relationship with all other populations. Importantly, those mitochondrial data supported a close relationship among all populations in New Mexico and Colorado that were divergent from Arizona populations (White Mountains). In contrast, the nuclear data (Figure 4) indicate that SDC and JHM share a deep divergence from all other lineages. This preliminary signal of mitonuclear discordance will require more formalized tests and may be the product of stochastic, or incomplete lineage sorting (ILS). Alternatively, this pattern might be best explained by an ancient, asymmetric mitochondrial introgression between the ancestor of the SDC and JHM lineages with the ancestor of the rest of the New Mexico lineages. This hypothesis predicts that the ancestor of the SDC and JHM nuDNA lineages captured the mitochondria from the rest of the New Mexico clades perhaps around 10 kya based on divergence history captured in the SNAPP tree. Analogous mitonuclear discordance has been documented in other species in the American Southwest such as woodrats (genus *Neotoma*; Derieg in prep), and is now recognized to be relatively common across mammals (Alves et al. 2003; Hailer et al. 2012; Sullivan et al. 2014).

In addition to the potential for mitonuclear discordance, individual nuDNA lineages (Figure 4) are not coincidental with contemporary riparian connectivity. For example, the Jemez River is a tributary to the Rio Grande, so populations in the Jemez Mountains might be expected to be closely related to Rio Grande populations. Instead, SNP-based variation indicates that the JMZ lineage shares closer relationships with SJN (San Juan River is a

tributary of the Colorado River) and SAC (Pecos River drainage). These seemingly discordant relationships might be the result of either i) dynamic historical drainage systems (*e.g.,* Rio Puerco) that potentially interconnected the SJN, JMZ and SAC in the past or ii) that SJN, JMZ and SAC lineages were historically connected at lower elevations (perhaps in the middle Rio Grande Valley) but then shifted to higher elevations arriving at current distributions. Consistent with this later scenario, BDA and ISA may have been distributed further south along the Rio Grande and Edwards Plateau as previously predicted (Malaney et al. 2012) and subsequently replaced SJN, JMZ and SAC as they shifted northward to cooler climates. Both scenarios are consistent with fossil plant data that suggests more continuous woodlands were present in the southwestern USA and northern Mexico that were fragmented during the last 10,000 years, as grasslands and deserts displaced woodlands in lowland basins and warming caused cool-adapted species to retreat to higher elevations or shift northward (Betancourt, van Devender, & Martin, 1990). Additionally, widespread Pleistocene lakes likely provided abundant jumping mouse habitat across low elevations (Allen 2005). Similar hypotheses have been proposed for other co-distributed species in the region including the southwestern red squirrel (*Tamiasciurus fremonti*; Hope et al., 2016), Mogollon vole (*Microtus mogollonensis*; Crawford et al. 2011), Mexican jay (*Aphelocoma ultramarine*; McCormack et al. 2008), Mexican woodrat (*Neotoma mexicana* (Sullivan, 1994), and the Sacramento Mountain salamander (*Aneides hardii* Osborne et al. 2019).

Anthropogenic declines contribute to genomic drift

Understanding how anthropogenic impacts and associated population declines have affected genomic variation via drift is critical to assessing the long-term persistence of a population (Flanagan et al. 2018). For example, the loss of allelic richness and low heterozygosity can be detrimental to long-term viability of populations because allelic variation provides options for responding to changing environmental conditions. In addition, populations with small N_E often have elevated extinction risks due to the fixation of deleterious alleles (Jamieson and Allendorf 2012). Genomic variation across *Z. l. luteus* is generally consistent with a recent history of anthropogenically-induced population declines, with a few notable exceptions. In general, populations of *Z. l. luteus* have i) low allelic variation, ii) low heterozygosity, iii) elevated inbreeding coefficients, and iv) low N_E.

All areas of *Z. l. luteus* we sampled have depauperate genomic diversity when compared to recent studies of other vertebrates of conservation concern using similar data and analyses. For example, allelic richness (A_R) is generally lower for *Z. l. luteus* (1.085–1.301), than that reported for other imperiled species such as an A_R ranging from 1.22 – 1.49 in the eastern massasauga rattlesnake (*Sistrurus catenatus*; Sovic et al. 2018), 1.26 – 1.96 for Utah prairie dogs (*Cynomys parvidens;* Giglio et al. 2020), and 1.42 – 1.84 in Gila trout (*Oncorhynchus gilae*; Camak et al. 2021). Observed heterozygosity ranged between 0.049 – 0.1271 for jumping mice and is consistently lower than other imperiled species such as 0.132-0.190 in desert bighorn sheep (*Ovis canadensis nelson*; Jahner et al. 2019), 0.110-0.310 in Utah prairie dog (*Cynomys parvidens*; Giglio et al. 2020), and 0.106-0.335 in Gila Trout (*Oncorhynchus gilae*; Camak et al., 2021). Furthermore, H_o for *Z. l. luteus* was also lower than populations that have experienced recent bottlenecks including $H_o = 0.17$ in Eurasian beaver (*Castor fiber*; Senn et al. 2014), 0.16 in the brown bear (*Ursus arctos*; Miller

et al. 2012; Cronin et al. 2014) and 0.13 in the Arctic ringed seal (*Pusa hispida hispida*; (Olsen et al. 2011). Consequently, low genomic diversity suggests that *Z. l. luteus* has very small populations (Frey 2005, 2013; Frey and Malaney 2009) that have experienced genomic drift and/or elevated inbreeding (Hedrick 2000). This lack of variability portends a challenging future especially considering ongoing and expected climatic change.

Inbreeding depression leads to reduced survival and fertility of offspring of related individuals, which is suggested to be due primarily to recessive deleterious mutations in populations (Charlesworth and Willis 2009). Populations that show reduced levels of molecular genomic variation, which can be indicative of inbreeding depression, often have lower fitness and higher expression of abnormal phenotypes (Hedrick and Garcia-Dorado 2016), as observed in Florida panther (*Puma concolor coryi*; Roelke et al. 1993; Johnson et al. 2010), grey wolf (*Canis lupus*; Liberg et al., 2005), banner-tailed kangaroo rats (*Dipodomys spectabilis*; Willoughby et al. 2019), and Leadbeater's possum (*Gymnobelideus leadbeateri*; Zilko et al. 2020). In jumping mice, inbreeding depression should be evaluated, as some GAs had elevated F_{is} (*e.g.*, |>0.1|) values. In particular, F_{is} for the SAC is high and analogous to values detected in Mexican Grey Wolves (*Canis lupus baileyi*), a system characterized historically by very few breeding pairs that may have impacted sperm quality and decreased reproductive success with elevated F_{is} (Asa et al. 2007). However, because relatively few samples were available for most GAs, caution is warranted when interpreting these preliminary F_{is} values.

Deleterious mutations have an elevated probability of fixation in small populations and can have disproportionally negative consequences (Charlesworth and Charlesworth 1987; Charlesworth 2009; Dawson et al. 2011; Banks et al. 2013; Gasca-Pineda et al. 2013). From a conservation and management perspective, two types of concern are relevant. First, drift often leads to fixation of alleles, some of which can be deleterious, and some may contribute to rare diseases like facial tumor disease in Tasmanian devils (*Sarcophilus harrisii;* Morris et al. 2015) and cancer in pangolins (*Manis sp.* Hu et al. 2020). Second, functional or beneficial alleles can shift to lower frequencies and be lost (Whitlock 2000). When combined, inbreeding can lead to three types of genomic extinction including i) homozygosity of recessive deleterious mutations leading to depression of reproductive success, ii) mutational meltdown, where several slightly deleterious mutations become fixed due to strong genetic drift, and iii) maladaptation to changing environments (DeWoody et al. 2021; Kardos et al. 2021; Teixeira and Huber 2021). Thus, management programs should ensure that populations maintain higher N_E, and avoid dipping below 50 effective individuals (Franklin 1980; Slatkin 1987).

Based on both theoretical and empirical evidence, a minimum of 50 reproductive individuals (N_E) are needed to avoid the effects of drift and inbreeding in populations, first proposed by Franklin (1980) and Slatkin (1987), and discussed by others (Jamieson and Allendorf 2012; Frankham et al. 2014a), However, more recently some (Frankham et al. 2014b) have presented arguments that the 50 threshold is too small to avoid harmful effects of inbreeding and drift. N_E values for the NM meadow jumping mice are fewer than 50 for four of the GAs (JMZ, SAC, WHT, BDA), which suggests that these are at risk of losing genomic variability through drift-based processes. These estimates are similar to those found

in other threatened species including the eastern massasauga rattlesnake (*Sistrurus catenatus;* Sovic et al. 2018), the Florida bonneted bat (*Eumops floridanus*; Austin et al. 2022), and the Pribilof Island shrew (*Sorex pribilofensis;* Wiens et al. 2021). While such thresholds may not be justifiable in all cases, an improved perspective of both the historical and contemporary factors that have resulted in declines in both census and effective population sizes is needed to better offset risks of the deleterious effects of inbreeding depression and drift.

Taken together, and when placed in the context of other published species of concern, these low evolutionary metrics for *Z. l. luteus* portend severe conservation challenges ahead. Ongoing anthropogenic habitat fragmentation is a major cause of decline for a variety of species, but low sample availability in some GAs, including the SDC, ISA, and SJN (fewer that 5 samples in all cases) can compromise evolutionary measures (allelic variation, heterozygosity, N_E, among other), therefore, in this system, careful interpretation is needed for all estimates from these poorly sampled geographic areas. Obtaining additional contemporary sampling should be a priority for these regions to allow more robust genomic testing of contemporary population status (Waples 2014).

4.3 Outlier loci and GEA – potential signatures of local adaptation

New Mexico meadow jumping mice are distributed across distinctive riparian environments spanning approximately 1,500 m elevation (~1,500 m at Bosque del Apache to ~3,000 m at Taos Ski Valley) and occurring in five USGS Level III ecoregions (Figure 1; (Malaney et al. 2022). Consequently, we predicted a high proportion of alleles

restricted to local areas and environmental conditions. The most prominent differences are associated with elevation including samples from higher elevation sites (montane GAs; JHM, SDC, JMZ, SAC, WHT and SJN) compared to the lowest elevation sites (Rio Grande GAs; BDA and ISA). Similarly, work on prairie dogs (*Cynomys parvidens*, Giglio et al. 2020), hummingbirds (*Coeligena violifer* and *Colibri coruscans*, Lim et al. 2021), and tree frogs (*Boana platanera,* Medina et al. 2021) have found elevation-associated differences in alleles attributable to local adaptation to environmental conditions where temperature and precipitation differences have appeared to contribute to adaptive potential. While we did not perform genome-wide association assessments to formally associate outlier loci to bioclimatic variation, we note that these preliminary data suggest that locally adapted variation may exist among lineages despite low allelic variation, depauperate heterozygosity, potentially elevated inbreeding, and exceptionally low N_e in individual GAs.

From the 205 outlier loci with no missing data detected, the association analysis is consistent with 16% of the loci potentially associated with ecological characteristics of local populations (low elevation Rio Grande vs montane elevations). These loci would be potential candidates for further evaluation and more formal tests of selection. In particular, jumping mice from SAC and BDA have a genomic signature consistent with natural selection when compared to other GAs. However, like evolutionary measures of variation, it is important to emphasize that low sampling availability across most GAs may constrain insights into the effects of selection in this system. Nevertheless, these preliminary associations provide hints that future exploration should attempt to decouple signals of spatial genomic variation due to geographic isolation from adaptive response to local environmental conditions.

4.4 Conservation and Management Implications

Habitat loss and degradation due to livestock grazing, fire intensity, and climate change have resulted in significant population declines for multiple riparian associated vertebrates in the Southwest, including Gila trout (*Oncorhynchus gilae*, Camak et al. 2021), the narrow-headed garter snake (*Thamnophis rufipunctatus*, Wood et al. 2018), southwestern willow fly catcher (*Empidonax traillii extimus*, Busch et al. 2000), and the New Mexico meadow jumping mouse (Frey and Malaney 2009), among others. New Mexico meadow jumping mouse conservation and management programs remain incompletely implemented across all occupied areas and the amount of habitat protected is not enough for population recovery. For example, in the Sacramento Mountains, detections of jumping mice in the last 20 years are restricted to four of the 23 historically occupied localities (17.4%, (Frey and Malaney 2009) and may now be restricted to a single locality (Chambers 2017, 2018). Furthermore, there is persistent livestock grazing violations in the region (Silver 2021), in addition to transformation of riparian habitat by higher densities of wild ungulates (i.e., elk grazing) that have further damaged stream habitats. Because some jumping mouse populations have likely persisted at chronically low numbers, an improved understanding of the genomic architecture of inbreeding depression is also needed (insufficient sample sizes preclude sufficient power in these tests). Consequently, increasing population numbers alone (USFWS 2014b, 2016, 2020, 2022) will likely be an untenable management goal (Allendorf and Luikart 2013; Whiteley et al. 2015).

Because of the depauperate genomic variation and low N_E within GAs, coupled with high genomic differentiation between GAs, a comprehensive management strategy

(including developing annotated genomes for each GA) is needed to ensure that remaining genomic variation is optimally preserved. Genomic management should strive to maintain site specific variation and heterozygosity levels and monitor the potentially deleterious effects of genetic drift. Moreover, if proposed translocation strategies are expected to be successful (USFWS 2022), a genomic management framework will be required. Without a genomic perspective on geographic variation, captive breeding and translocations of jumping mice may face multiple, complex problems, as documented in other threatened wildlife (Malaney et al. 2015; Colella et al. 2019; Jahner et al. 2019). Importantly, a genomic map of geographic variation is essential to translocation strategies, otherwise these actions may be risky (Moritz 1999; Weeks et al. 2011) and create unnecessary conservation challenges (Thomas et al. 2013; Whiteley et al. 2015).

More broadly, however, a comprehensive sampling strategy is required for long-term population monitoring. Herein, we emphasize four primary points. First, of the 115 specific localities where *Z. l. luteus* have been detected, only 44 (38.3%) have cryopreserved tissues that are essential for building genomic datasets (Malaney et al. 2022). Thus, > 60% of known localities have no tissues for basic genetic or genomic tests ultimately compromising conservation and management. Second, none of the type localities used to formally describe geographic variation (*i.e.,* subspecies) has available topotype sampling. Therefore, our understanding of the taxonomic limits and validity of subspecies withing this system is compromised. Third, of the 135 specimens (excluding 10 embryos) used in this study, most (78, 57.8%) were obtained prior to 2000. For some GAs of concern, most of the available samples (e.g. SAC 82% and BDA 93%) were collected prior to 1980, and recent samples are needed to critically evaluate the impacts of climate change and management programs on

genomic variability. Fourth, the potential geographic range of the species remains poorly sampled. Eight new localities have been detected since 2017, but new sampling efforts are compromised due to the legal status of the subspecies (Malaney et al. 2022). Climate change is projected to render half of the USFWS designated critical habitats for this mammal unsuitable by 2070 (Malaney et al. 2022). Consequently, insights into how this species is responding will be compromised during this period of habitat degradation and climate change. Taken together, incomplete sample availability continues to obscure windows into ecological and evolutionary processes. More broadly, however, as emerging technologies such as cloning become increasingly viable conservation options (Ryder 2002; Sandler et al. 2021a; Segelbacher et al. 2022), both temporal and geographic sample availability will be key to conservation success for not only this species, but any imperiled taxon. Genomic augmentation and effective conservation cloning (Sandler et al. 2021b) will require robust views of historic levels of variation. Regular spatiotemporal sampling is essential for capturing genomic variation relevant to conservation and management now and into the future and should not be underappreciated.

Data availability Statement

Raw sequence data generated for this study are available at the Sequencing Read Archive (SRA) on NCBI GenBank: *to be completed after manuscript is accepted for publication*. Genotypic data for neutral and outlier SNP loci are available on DRYAD DOI: *to be completed after manuscript is accepted for publication*. All code or software applications were submitted to GitHub

(https://github.com/xxxxxx). Data and code are also available from the corresponding author upon request.

5. Literature cited

ALLEN, B. D. 2005. Ice age lakes in New Mexico. New Mexico's ice ages. New Mexico Museum of Natural History and Science Bulletin, 28:107–113.

ALLENDORF, F. W. 2017. Genetics and the conservation of natural populations: allozymes to genomes. Molecular Ecology 26:420–430.

ALLENDORF, F. W., P. A. HOHENLOHE, AND G. LUIKART. 2010. Genomics and the future of conservation genetics. Nature Reviews Genetics 11:697–709.

ALLENDORF, F. W., AND G. LUIKART. 2013. Conservation and the genetics of populations. Second Edi. John Wiley & Sons, Ltd, Hoboken, NJ.

ALLENDORF, F. W., G. H. LUIKART, AND S. N. AITKEN. 2012. Conservation and the Genetics of Populations. 2nd Edition. Wiley-Blackwell.

ALVES, P. C., N. FERRAND, F. SUCHENTRUNK, AND D. J. HARRIS. 2003. Ancient introgression of *Lepus timidus* mtDNA into *L. granatensis* and *L. europaeus* in the Iberian Peninsula. Molecular Phylogenetics and Evolution 27:70–80.

ANDREWS, K. R., J. M. GOOD, M. R. MILLER, G. LUIKART, AND P. A. HOHENLOHE. 2016. Harnessing the power of RADseq for ecological and evolutionary genomics. Nature Reviews Genetics 17:81–92.

ASA, C. ET AL. 2007. Relationship of inbreeding with sperm quality and reproductive success in Mexican gray wolves. Animal Conservation 10:326–331.

AUSTIN, J. D. ET AL. 2022. Strong population genetic structure and cryptic diversity in the Florida bonneted bat (*Eumops floridanus*). Conservation Genetics 1:1–18.

BAILEY, V. 1913. Ten new mammals from New Mexico. Proceedings of the Biological Society of Washington 26:129–134.

BANKS, S. C. ET AL. 2013. How does ecological disturbance influence genetic diversity? Trends in Ecology & Evolution 28:670–679.

BARRET, R., AND D. SCHLUTER. 2008. Adaptation from standing genetic variation. Trends in Ecology & Evolution 23:38–44.

BETANCOURT, J. L., T. R. VAN DEVENDER, AND P. S. MARTIN. 1990. Packrat middens: the last 40,000 years of biotic change:467.

BOUCKAERT, R., J. HELED, D. KÜ HNERT, T. VAUGHAN, AND C. H. WU. 2014. BEAST 2: A Software Platform for Bayesian Evolutionary Analysis. PLoS Computational Biology 10:1003537.

BOUCKAERT, R. R. 2010. DensiTree: making sense of sets of phylogenetic trees. Bioinformatics 26:1372–1373.

BRYANT, D., R. BOUCKAERT, J. FELSENSTEIN, N. A. ROSENBERG, AND A. ROYCHOUDHURY. 2012. Inferring Species Trees Directly from Biallelic Genetic Markers: Bypassing Gene Trees in a Full Coalescent Analysis. Molecular Biology and Evolution 29:1917–1932.

BUSCH, J. D., M. P. MILLER, E. H. PAXTON, M. K. SOGGE, AND P. KEIM. 2000. Genetic Variation in The Endangered Southwestern Willow Flycatcher. The Auk 117:586–595.

CAMAK, D. T., M. J. OSBORNE, AND T. F. TURNER. 2021. Population genomics and conservation of Gila Trout (*Oncorhynchus gilae*). Conservation Genetics 22:729–743.

CATCHEN, J., P. A. HOHENLOHE, S. BASSHAM, A. AMORES, AND W. A. CRESKO. 2013. Stacks: an analysis tool set for population genomics. Molecular Ecology 22:3124–3140.

CHAMBERS, C. L. 2017. New Mexico meadow jumping mouse habitat and diet on the Lincoln National. Lincoln National Forest Jumping Mouse Annual Report for 2017. New Mexico

CHAMBERS, C. L. 2018. New Mexico meadow jumping mouse habitat and diet on the Lincoln National. Lincoln National Forest Jumping Mouse Annual Report for 2018. New Mexico

CHARLESWORTH, B. 2009. Effective population size and patterns of molecular evolution and variation. Nature Reviews Genetics 10:195–205.

CHARLESWORTH, D., AND B. CHARLESWORTH. 1987. Inbreeding depression and its evolutionary consequences. Annual Review of Ecology and Systematics. Vol. 18:237–268.

CHARLESWORTH, D., AND J. H. WILLIS. 2009. Fundamental Concepts in Genetics: The genetics of inbreeding depression. Nature Reviews Genetics 10:783–796

COLELLA, J. P., L. M. FREDERICK, S. L. TALBOT, AND J. A. COOK. 2021. Extrinsically reinforced hybrid speciation within Holarctic ermine (*Mustela* spp.) produces an insular endemic. Diversity and Distributions 27:747–762.

COLELLA, J. P., E. J. JOHNSON, AND J. A. COOK. 2018a. Reconciling molecules and morphology in North American *Martes*. Journal of Mammalogy 99:1323–1335.

COLELLA, J. P., T. LAN, S. C. SCHUSTER, S. L. TALBOT, J. A. COOK, AND C. LINDQVIST. 2018b. Whole-genome analysis of Mustela erminea finds that pulsed hybridization impacts evolution at high latitudes. Communications Biology 2018 1:1 1:1–10.

COLELLA, J. P., R. E. WILSON, S. L. TALBOT, AND J. A. COOK. 2019. Implications of introgression for wildlife translocations: the case of North American martens. Conservation Genetics 20:153–166.

COYNER, B. S., P. J. MURPHY, AND M. D. MATOCQ. 2015. Hybridization and asymmetric introgression across a narrow zone of contact between *Neotoma fuscipes* and *N. macrotis* (Rodentia: Cricetidae). Biological Journal of the Linnean Society 115:162–172.

CRANDALL, K. A., O. R. P. BININDA-EMONDS, G. M. MACE, AND R. K. WAYNE. 2000. Considering evolutionary processes in conservation biology. Trends in Ecology & Evolution 15:290–295.

CRAWFORD, D. L., J. W. DRAGOO, F. A. SMITH, AND A. N. CHAVEZ. 2011. Diversification within the Mexican Vole (*Microtus mexicanus*) and the Role of Post-Pleistocene Climate Change. https://doi.org/10.3398/064.071.0205 71:176–194.

CRONIN, M. A. ET AL. 2014. Molecular Phylogeny and SNP Variation of Polar Bears (*Ursus maritimus*), Brown Bears (*U. arctos*), and Black Bears (*U. americanus*) Derived from Genome Sequences. Journal of Heredity 105:312–323.

DANECEK, P. ET AL. 2011. The variant call format and VCFtools. Bioinformatics 27:2156–2158.

DAWSON, N. G., J. P. COLELLA, M. P. SMALL, K. D. STONE, S. L. TALBOT, AND J. A. COOK. 2017. Historical biogeography sets the foundation for contemporary conservation of martens (genus Martes) in northwestern North America. Journal of Mammalogy 98:715–730.

DAWSON, T. P., S. T. JACKSON, J. I. HOUSE, I. C. PRENTICE, AND G. M. MACE. 2011. Beyond Predictions: Biodiversity Conservation in a Changing Climate. Science 332:53–58.

DERIEG, K. M., K. GUITIÉRREZ, S. W. LIPHARDT, J. L. MALANEY, AND J. A. COOK. 2022. Mitonuclear discordance in woodrats (Genus Neotoma) points to historical mitochondrial introgression. In prep.

DEWOODY, J. A., A. M. HARDER, S. MATHUR, AND J. R. WILLOUGHBY. 2021. The long-standing significance of genetic diversity in conservation. Molecular Ecology 30:4147–4154.

DO, C., R. S. WAPLES, D. PEEL, G. M. MACBETH, AND B. J. TILLETT. 2014. NEESTIMATOR v2: re-implementation of software for the estimation of contemporary effective population size (*Ne*) from genetic data. Molecular Ecology Resources 14:209–214.

EARL, D. A., AND B. M. VONHOLDT. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources 4:359–361.

EVANNO, G., S. REGNAUT, AND J. GOUDET. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology 14:2611–2620.

FEDORKA, K. M., W. E. WINTERHALTER, K. L. SHAW, W. R. BROGAN, AND T. A. MOUSSEAU. 2012. The role of gene flow asymmetry along an environmental gradient in constraining local adaptation and range expansion. Journal of Evolutionary Biology 25:1676–1685.

FINDLEY, J. S., A. H. HARRIS, D. E. WILSON, AND C. JONES. 1975. Mammals of New Mexico. University of New Mexico Press.

FLANAGAN, S. P., B. R. FORESTER, E. K. LATCH, S. N. AITKEN, AND S. HOBAN. 2018. Guidelines for planning genomic assessment and monitoring of locally adaptive variation to inform species conservation. Evolutionary Applications 11:1035–1052.

FOLL, M., AND O. GAGGIOTTI. 2008. A Genome-Scan Method to Identify Selected Loci Appropriate for Both Dominant and Codominant Markers: A Bayesian Perspective. Genetics 180:977–993.

FRANKHAM, R. 2005. Genetics and extinction. Biological Conservation 126:131–140.

FRANKHAM, R. 2012. How closely does genetic diversity in finite populations conform to predictions of neutral theory large deficits in regions of low recombination. Heredity 108:167–178.

FRANKHAM, R., C. J. A. BRADSHAW, AND B. W. BROOK. 2014a. Genetics in conservation management: Revised recommendations for the 50/500 rules, Red List criteria and population viability analyses. Biological Conservation 170:56–63.

FRANKHAM, R., C. J. A. BRADSHAW, AND B. W. BROOK. 2014b. 50/500 rules need upward revision to 100/1000: – Response to Franklin et al. Biological Conservation, 176:286–286. https://doi.org/10.1016/J.BIOCON.2014.05.006

FRANKHAM, R., D. A. BRISCOE, AND J. D. BALLOU. 2010. Introduction to conservation genetics. Second Ed. Cambridge University Press.

FRANKLIN, I. R. 1980. Evolutionary change in small populations. Pp. 135–149 in Conservation Biology: An Evolutionary- Ecological Perspective (M. Soule & B. Wilcox, eds.). Sinauer Associates, Sunderland, MA.

FREDRICKSON, R. J., AND P. W. HEDRICK. 2006. Dynamics of Hybridization and Introgression in Red Wolves and Coyotes. Conservation Biology 20:1272–1283.

FREY, J. K. 2005. Status Assessment of Montane Populations of the New Mexico Meadow Jumping Mouse (*Zapus hudsonius luteus*) in New Mexico.

FREY, J. K. 2013. Survey for the meadow jumping mouse (*Zapus hudsonius luteus*) at Bosque del Apache National Wildlife Refuge, Spring 2013 Use and Methodological Advances for using Species Distribution Models in Wildlife Conservation and Management View project Habitat Suitability Mapping of Dusky Grouse (*Dendragapus obscurus*) in the American Southwest View project.

FREY, J. K. 2017. Landscape scale and microhabitat of the endangered New Mexico meadow jumping mouse in the White Mountains, Arizona. Journal of Fish and Wildlife Management 8:39–58.

FREY, J. K., AND J. L. MALANEY. 2009. Decline of the meadow jumping mouse *(Zapus hudsonius luteus*) in two mountain ranges in New Mexico. Southwestern Naturalist 54:31–44.

FUNK, W. C., B. R. FORESTER, S. J. CONVERSE, C. DARST, AND S. MOREY. 2018. Improving conservation policy with genomics: a guide to integrating adaptive potential into U.S. Endangered Species Act decisions for conservation practitioners and geneticists. Conservation Genetics 20:115–134.

FUNK, W. C., J. K. MCKAY, P. A. HOHENLOHE, AND F. W. ALLENDORF. 2012. Harnessing genomics for delineating conservation units. Trends in Ecology and Evolution 27:489–496.

GASCA-PINEDA, J., I. CASSAIGNE, R. A. ALONSO, AND L. E. EGUIARTE. 2013. Effective Population Size, Genetic Variation, and Their Relevance for Conservation: The Bighorn Sheep in Tiburon Island and Comparisons with Managed Artiodactyls. PLoS ONE 8:78120.

GIGLIO, R. M., T. E. ROCKE, J. E. OSORIO, AND E. K. LATCH. 2020. Characterizing patterns of genomic variation in the threatened Utah prairie dog: Implications for conservation and management. Evolutionary Applications:1–16.

GORLOV, I. P., O. Y. GORLOVA, S. R. SUNYAEV, M. R. SPITZ, AND C. I. AMOS. 2008. Shifting Paradigm of Association Studies: Value of Rare Single-Nucleotide Polymorphisms. The American Journal of Human Genetics 82:100–112.

GOUDET, J. 2005. HIERFSTAT, a package for R to compute and test hierarchical *F*-statistics. Molecular Ecology Notes 5:184–186.

GREENBAUM, G., A. R. TEMPLETON, AND S. BAR-DAVID. 2016. Inference and analysis of population structure using genetic data and network theory. Genetics 202:1299–1312.

GREENBAUM, G., A. R. TEMPLETON, Y. ZARMI, AND S. BAR-DAVID. 2014. Allelic Richness following Population Founding Events-A Stochastic Modeling Framework

Incorporating Gene Flow and Genetic Drift. https://doi.org/10.1371/journal.pone.0115203

HAFNER, D. J., K. E. PETERSEN, AND T. L. YATES. 1981. Evolutionary Relationships of Jumping Mice (Genus *Zapus*) of the Southwestern United States. Journal of Mammalogy 62:501–512.

HAFNER, J. C., D. J. HAFNER, J. L. PATTON, AND M. F. SMITH. 1983. Contact zones and the genetics of differentiation in the pocket gopher *Thomomys bottae* (Rodentia: Geomyidae). Systematic Biology 32:1–20.

HAILER, F. ET AL. 2012. Nuclear Genomic Sequences Reveal that Polar Bears Are an Old and Distinct Bear Lineage. Science 336:344–347.

HEDRICK, P. W., S. T. KALINOWSKI. 2000. Inbreeding Depression in Conservation Biology. I. Annual Review of Ecology and Systematics 31:139–162.

HEDRICK, P. W., AND A. GARCIA-DORADO. 2016. Understanding Inbreeding Depression, Purging, and Genetic Rescue. Trends in Ecology and Evolution 31:940–952.

HEPPENHEIMER, E. ET AL. 2020. A Genome-Wide Perspective on the Persistence of Red Wolf Ancestry in Southeastern Canids. Journal of Heredity 111:277–286.

HERNANDEZ, R. D., L. H. URICCHIO, K. HARTMAN, C. YE, A. DAHL, AND N. ZAITLEN. 2019. Ultrarare variants drive substantial cis heritability of human gene expression. Nature Genetics 51:1349–1355.

HEWITT, G. 2000. The genetic legacy of the Quaternary ice ages. Nature 405:907–913

HOBAN, S. ET AL. 2016. Finding the Genomic Basis of Local Adaptation: Pitfalls, Practical Solutions, and Future Directions. The American Naturalist 188:379–397.

HOFFMEISTER, D. 1986. Mammals of Arizona. University of Arizona Press, Tucson, Arizona.

HOHENLOHE, P. A., W. C. FUNK, AND O. P. RAJORA. 2020. Population genomics for wildlife conservation and management. Molecular Ecology 30:62–82.

HOPE, A. G. ET AL. 2016. Revision of widespread red squirrels (genus: Tamiasciurus) highlights the complexity of speciation within North American forests. Molecular Phylogenetics and Evolution 100:170–182.

HU, J. Y. ET AL. 2020. Genomic consequences of population decline in critically endangered pangolins and their demographic histories. National Science Review 7:798–814.

JAHNER, J. P. ET AL. 2019. The genetic legacy of 50 years of desert bighorn sheep translocations. Evolutionary Applications 12:198–213.

JAKOBSSON, M., AND N. A. ROSENBERG. 2007. Genetics and population analysis CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23:1801–1806.

JAMIESON, I. G., AND F. W. ALLENDORF. 2012. How does the 50/500 rule apply to MVPs? Trends in Ecology and Evolution 27:578–584.

JANES, J. K. ET AL. 2017. The K = 2 conundrum. Molecular Ecology 26:3594–3602.

JOHNSON, W. E. ET AL. 2010. Genetic restoration of the Florida panther. Science 329:1641–1645.

JOMBART, T. 2008. Adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24:1403–1405.

JOMBART, T., AND I. AHMED. 2011. Adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. Bioinformatics 27:3070–3071.

JOST, L. 2008. *Gst* and its relatives do not measure differentiation. Molecular Ecology 17:4015–4026.

JOST, L., F. ARCHER, S. FLANAGAN, O. GAGGIOTTI, S. HOBAN, AND E. LATCH. 2018. Differentiation measures for conservation genetics. Evolutionary Applications 11:1139–1148.

KAMVAR, Z. N., J. F. TABIMA, AND N. J. GRÜNWALD. 2014. Poppr: An R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. PeerJ 2014:1–14.

KARDOS, M. ET AL. 2021. The crucial role of genome-wide genetic variation in conservation. Proceedings of the National Academy of Sciences 118.

KEENAN, K., P. MCGINNITY, T. F. CROSS, W. W. CROZIER, AND P. A. PRODOHL. 2013. diveRsity: An R package for the estimation and exploration of population genetics parameters and their associated errors. Methods in Ecology and Evolution 4:782–788.

KOTTEK, M., J. GRIESER, C. BECK, B. RUDOLF, AND F. RUBEL. 2006. World Map of the Köppen-Geiger climate classification updated 15:259–263.

KRUTZSCH, P. 1954. North American jumping mice (genus *Zapus*). University of Kansas Publications. Museum of Natural History 7:349–472.

LAURENT, S. ET AL. 2016. The population genomics of rapid adaptation: Disentangling signatures of selection and demography in white sands lizards. Molecular Ecology 25:306–323.

LEROY, G. ET AL. 2018. Next-generation metrics for monitoring genetic erosion within populations of conservation concern. Evolutionary Applications. 11:1066-1083

LESSA, E. P., J. A. COOK, AND J. L. PATTON. 2003. Genetic footprints of demographic expansion in North America, but not Amazonia, during the Late Quaternary. Proceedings of the National Academy of Sciences 100:10331–10334.

LI, H., AND R. DURBIN. 2010. Fast and accurate long-read alignment with Burrows-Wheeler transform. Bioinformatics 26:589–595.

LIBERG, O. ET AL. 2005. Severe inbreeding depression in a wild wolf Canis lupus population. Biology Letters 1:17–20.

LIM, M. C. W., K. BI, C. C. WITT, C. H. GRAHAM, AND L. M. DÁVALOS. 2021. Pervasive Genomic Signatures of Local Adaptation to Altitude Across Highland Specialist Andean Hummingbird Populations. Journal of Heredity 112:229–240.

LINCK, E., AND C. J. BATTEY. 2019. Minor allele frequency thresholds strongly affect population structure inference with genomic data sets. Molecular Ecology Resources 19:639–647.

MALANEY, J. L. ET AL. 2022. Wagering with an incomplete deck - new records and phylogeographic insights provide a predictive basis for future conservation plans for endangered jumping mice (*Zapus luteus*). *In Review*.

MALANEY, J. L., AND J. A. COOK. 2013. Using biogeographical history to inform conservation: The case of Preble's meadow jumping mouse. Molecular Ecology 22:6000–6017.

MALANEY, J. L., J. R. DEMBOSKI, AND J. A. COOK. 2017. Integrative species delimitation of the widespread North American jumping mice (Zapodinae). Molecular Phylogenetics and Evolution:137–152.

MALANEY, J. L., C. R. FELDMAN, M. COX, P. WOLFF, J. D. WEHAUSEN, AND M. D. MATOCQ. 2015. Translocated to the fringe: genetic and niche variation in bighorn sheep of the Great Basin and northern Mojave deserts. Diversity and Distributions 21:1063–1074.

MALANEY, J. L., J. K. FREY, AND J. A. COOK. 2012. The biogeographic legacy of an imperilled taxon provides a foundation for assessing lineage diversification, demography and conservation genetics. Diversity and Distributions 18:689–703.

MATOCQ, M. D., AND P. J. MURPHY. 2007. Fine-scale phenotypic change across a species transition zone in the genus Neotoma: disentangling independent evolution from phylogenetic history. Evolution 61:2544–2557.

MCCORMACK, J. E., B. S. BOWEN, AND T. B. SMITH. 2008. Integrating paleoecology and genetics of bird populations in two sky island archipelagos. BMC Biology 6:28.

MEDINA, R. ET AL. 2021. Phenotypic and genomic diversification with isolation by environment along elevational gradients in a neotropical treefrog. Molecular Ecology 30:4062–4076.

MEYLAN, A. 1968. Formules chromosomiques de quelques petits mammiferes nord-americains. Revue Suisse de Zoologie 75:691–696.

MILLER, G. S. 1911. A new jumping-mouse from New Mexico. Proceedings of the Biological Society of Washington 24:253–254.

MILLER, M. A., W. PFEIFFER, AND T. SCHWARTZ. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Pp. 1–8 in 2010 Gateway Computing Environments Workshop (GCE). IEEE.

MILLER, W. ET AL. 2012. Polar and brown bear genomes reveal ancient admixture and demographic footprints of past climate change. Proceedings of the National Academy of Sciences 109 (36) E2382-E2390.

MORITZ, C. 1999. Conservation units and translocations: Strategies for conserving evolutionary processes. Hereditas 130:217–228.

MORRIS, K. M., B. WRIGHT, C. E. GRUEBER, C. HOGG, AND K. BELOV. 2015. Lack of genetic diversity across diverse immune genes in an endangered mammal, the Tasmanian devil (*Sarcophilus harrisii*). Molecular Ecology 24:3860–3872.

MORRISON, J. L. 1990. The Meadow Jumping Mouse in New Mexico: Habiat Preferences and Management Recommendations. Proceedings of the symposium on managing wildlife in the Southwest:136–143.

MORRISON, J. L. 1992. Persistence of the Meadow Jumping Mouse, *Zapus hudsonius luteus* in New Mexico. Proceedings of the Biological Society of Washington 24:253–254.

NADLER, S. A., M. S. HAFNER, J. C. HAFNER, AND D. J. HAFNER. 1990. Genetic differentiation among chewing louse populations (Mallophaga: Trichodectidae) in a pocket gopher contact zone (Rodentia: Geomyidae). Evolution 44:942–951.

NIELSEN, R., J. S. PAUL, A. ALBRECHTSEN, AND Y. S. SONG. 2011. Genotype and SNP calling from next-generation sequencing data. Nature Reviews Genetics 12:443–451.

O'LEARY, S. J., J. B. PURITZ, S. C. WILLIS, C. M. HOLLENBECK, AND D. S. PORTNOY. 2008. These aren't the loci you're looking for: Principles of effective SNP filtering for molecular ecologists. Molecular Ecology 27:3193–3206.

OLSEN, M. T. ET AL. 2011. A simple route to single-nucleotide polymorphisms in a nonmodel species: identification and characterization of SNPs in the Artic ringed seal (*Pusa hispida hispida*). Molecular Ecology Resources 11:9–19.

OSBORNE, M. J., S. J. CORDOVA, A. C. CAMERON, AND T. F. TURNER. 2019. Isolation by elevation: mitochondrial divergence among sky island populations of Sacramento Mountain salamander (*Aneides hardii*) 20:545–556.

PEAKALL, R., AND P. E. SMOUSE. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6:288–295.

PEAKALL, R., AND P. E. SMOUSE. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 28:2537–2539.

PEEL, M. C., B. L. FINLAYSON, AND T. A. MCMAHON. 2007. Updated world map of the Köppen-Geiger climate classification. Hydrology and Earth System Sciences 4:439–473.

PETERSON, B. K., J. N. WEBER, E. H. KAY, H. S. FISHER, AND H. E. HOEKSTRA. 2012. Double Digest RADseq: An Inexpensive Method for De Novo SNP Discovery and Genotyping in Model and Non-Model Species. PLoS ONE 7:e37135.

PORTIK, D. M. ET AL. 2017. Evaluating mechanisms of diversification in a Guineo-Congolian tropical forest frog using demographic model selection. Molecular Ecology 26:5245–5263.

PRITCHARD, J. K., M. STEPHENS, AND P. DONNELLY. 2000. Inference of Population Structure Using Multilocus Genotype Data. Genetics 155:945–959.

PRITCHARD, J. K., W. WEN, AND D. FALUSCH. 2010. Documentation for structure software: Version 2.3. Documentation for Structure softwar: version 2. Springer, Boston.

PRIV, F., K. LUU, B. J. VILHJ ALMSSON, AND M. G. B. BLUM. 2020. Performing Highly Efficient Genome Scans for Local Adaptation with R Package pcadapt Version 4. Molecular Biology and Evolution 37:2153–2154.

RAMBAUT, A., A. J. DRUMMOND, D. XIE, G. BAELE, AND M. A. SUCHARD. 2018. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. Systematic Biology 67:901–904.

REMINGTON, C. L. 1968. Suture-zones of hybrid interaction between recently joined biotas. Evolutionary Biology:321–428.

RICHARDSON, D. M., AND R. J. WHITTAKER. 2010. Conservation biogeography - foundations, concepts and challenges. Diversity and Distributions 16:313–320.

RIDDLE, B. ET AL. 2008. The role of molecular genetics in sculpting the future of integrative biogeography: http://dx.doi.org/10.1177/0309133308093822 32:173–202.

ROCHETTE, N. C., AND J. M. CATCHEN. 2017. Deriving genotypes from RAD-seq short-read data using Stacks. Nature Protocols 12:2640–2659.

ROCHETTE, N. C., A. G. RIVERA-COLÓN, AND J. M. CATCHEN. 2019. Stacks 2: Analytical methods for paired-end sequencing improve RADseq-based population genomics. Molecular Ecology 28:4737–4754.

ROELKE, M. E., J. S. MARTENSON, AND S. J. O'BRIEN. 1993. The consequences of demographic reduction and genetic depletion in the endangered Florida panther. Current Biology 3:340–350.

RUNCK, A. M., M. D. MATOCQ, AND J. A. COOK. 2009. Historic hybridization and persistence of a novel mito-nuclear combination in red-backed voles (genus Myodes). BMC Evolutionary Biology 9:1–15.

RYDER, O. A. 2002. Cloning advances and challenges for conservation. Trends in Biotechnology 20:231–232.

SANDLER, R. L., L. MOSES, AND S. M. WISELY. 2021. An ethical analysis of cloning for genetic rescue: Case study of the black-footed ferret. Biological conservation 257:109118.

SAVOLAINEN, O., M. LASCOUX, AND J. MERILÄ. 2013. Ecological genomics of local adaptation. Nature Reviews Genetics 14:807–820.

SCHORR, R. A. 2003. Meadow Jumping Mice (*Zapus hudsonius preblei*) on the U.S. Air Force Academy El Paso County, Colorado: Populations, Movement and Habitat from 2000-2002. Colorado State University, Fort Collins, Colorado.

SCHWARTZ, M. K., G. LUIKART, AND R. S. WAPLES. 2007. Genetic monitoring as a promising tool for conservation and management. Trends in Ecology and Evolution 22:25–33.

SCHWEYEN, H., A. ROZENBERG, AND F. LEESE. 2014. Detection and removal of PCR duplicates in population genomic ddRAD studies by addition of a degenerate base region (DBR) in sequencing adapters. Biological Bulletin 227:146–160.

SEGELBACHER, G. ET AL. 2022. New developments in the field of genomic technologies and their relevance to conservation management. Conservation Genetics 23:217–242.

SENN, H. ET AL. 2014. Nuclear and mitochondrial genetic structure in the Eurasian beaver (*Castor fiber*) – implications for future reintroductions. Evolutionary Applications 7:645–662.

SHURTLIFF, Q. R., P. J. MURPHY, J. D. YEITER, AND M. D. MATOCQ. 2013. Experimental evidence for asymmetric mate preference and aggression: Behavioral interactions in a woodrat (*Neotoma*) hybrid zone. BMC Evolutionary Biology 13:1–13.

SILVER, R. 2021. Documented grazing violations 2016-2021 and counting by two Lincoln NF permittees without a single notice of non-compliance letter. Field report from the Lincoln national forest. November 4, 2021. Center for Biological Diversity, Flagstaff, AZ.

SLATKIN, M. 1987. Gene flow and the geographic structure of natural populations. Science 236:787–792.

SMITH, D. R. ET AL. 2018. Development of a Species Status Assessment Process for Decisions under the U.S. Endangered Species Act. Journal of Fish and Wildlife Management 9:302–320.

SONG, S., L. LIU, S. V. EDWARDS, AND S. WU. 2012. Resolving conflict in eutherian mammal phylogeny using phylogenomics and the multispecies coalescent model. Proceedings of the National Academy of Sciences of the United States of America 109:14942–14947.

SOVIC, M., A. FRIES, S. A. MARTIN, AND H. LISLE GIBBS. 2018. Genetic signatures of small effective population sizes and demographic declines in an endangered rattlesnake, *Sistrurus catenatus*. Evolutionary Applications 12: 664-678.

SULLIVAN, J. ET AL. 2014. Divergence with gene flow within the recent chipmunk radiation (*Tamias*). Heredity 113:185–194.

SULLIVAN, R. M. 1994. Micro-Evolutionary Differentiation and Biogeographic Structure Among Coniferous Forest Populations of the Mexican Woodrat (*Neotoma mexicana*) in the American Southwest: A Test of the Vicariance Hypothesis. Journal of Biogeography 21:369.

SWENSON, N. G., AND D. J. HOWARD. 2005. Clustering of contact zones, hybrid zones, and phylogeographic breaks in North America. The American Naturalist 166:581–591.

TEIXEIRA, J. C., AND C. D. HUBER. 2021. The inflated significance of neutral genetic diversity in conservation genetics. Proceedings of the National Academy of Sciences 118.

THOMAS, M. A., G. W. ROEMER, C. J. DONLAN, B. G. DICKSON, M. D. MATOCQ, AND J. L. MALANEY. 2013. Gene tweaking for conservation. Nature 501:485–486.

USFWS. 1973. Endangered Species Act of 1973, As Amended through the 108th Congress.

USFWS. 2014a. Recovery Outline New Mexico Meadow Jumping Mouse (*Zapus hudsonius luteus*).

USFWS. 2014b. Species Status Assessment Report New Mexico meadow jumping mouse (*Zapus hudsonius luteus*). Albuquerque, NM.

USFWS. 2016. Endangered and Threatened Wildlife and Plants; Designation of Critical Habitat for the New Mexico Meadow Jumping Mouse. Federal Register 81:14264–14325.

USFWS. 2020. Species Status Assessment Report for the New Mexico meadow jumping mouse (*Zapus hudsonius luteus*).

USFWS. 2022. Endangered and Threatened Wildlife and Plants; Draft Recovery Plan for the New Mexico Meadow Jumping Mouse. Albuquerque.

VONHOLDT, B. M. ET AL. 2016. Whole-genome sequence analysis shows that two endemic species of North American wolf are admixtures of the coyote and gray wolf. Science Advances 2:1–14.

VONHOLDT, B. M. ET AL. 2021. Persistence and expansion of cryptic endangered red wolf genomic ancestry along the American Gulf coast. Molecular Ecology. DOI: 10.1111/mec.16200.

WAPLES, R. K., W. A. LARSON, AND R. S. WAPLES. 2016. Estimating contemporary effective population size in non-model species using linkage disequilibrium across thousands of loci. Heredity 117:233–240.

WAPLES, R. S. 1995. Evolutionarily Significant Units and the Conservation of Biological Diversity under the Endangered Species Act. American Fisheries Society Symposium 17:8–27.

WAPLES, R. S. 2014. Testing for Hardy–Weinberg Proportions: Have We Lost the Plot? Journal of Heredity 106:1–19.

WAPLES, R. S., AND C. DO. 2008. LDNE: a program for estimating effective population size from data on linkage disequilibrium. Molecular Ecology Resources 8:753–756.

WAPLES, R. S., AND O. GAGGIOTTI. 2006. What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. Molecular Ecology 15:1419–1439.

WEEKS, A. R. ET AL. 2011. Assessing the benefits and risks of translocations in changing environments: a genetic perspective. Evolutionary Applications 4:709–725.

WHITAKER, J. O. 1972. *Zapus hudsonius*. Mammalian Species 11:1.

WHITELEY, A. R., S. W. FITZPATRICK, W. C. FUNK, AND D. A. TALLMON. 2015. Genetic rescue to the rescue. Trends in Ecology & Evolution 30:42–49.

WHITLOCK, M. C. 2000. Fixation of new alleles and the extinction of small populations: drift load, beneficial alleles and sexual selection. Evolution 54:1855–1861.

WIENS, B. J., F. J. COMBE, AND A. G. HOPE. 2021. Genetic drift drives differentiation of an endangered insular shrew (*Sorex pribilofensis*). *In prep*.

WILLIS, S. C., C. M. HOLLENBECK, J. B. PURITZ, J. R. GOLD, AND D. S. PORTNOY. 2017. Haplotyping RAD loci: an efficient method to filter paralogs and account for physical linkage. Molecular Ecology Resources 17:955–965.

WILLOUGHBY, J. R., P. M. WASER, A. BRÜNICHE-OLSEN, AND M. R. CHRISTIE. 2019. Inbreeding load and inbreeding depression estimated from lifetime reproductive success in a small, dispersal-limited population. Heredity 123:192–201.

WOOD, D. A., I. D. EMMONS, E. M. NOWAK, B. L. CHRISTMAN, A. T. HOLYCROSS, AND A. G. VANDERGAST. 2018. Conservation genomics of the Mogollon Narrow-headed Gartersnake (*Thamnophis rufipunctatus*) and Northern Mexican Gartersnake (*Thamnophis eques megalops*): U.S. Geological Survey Open-File Report 2018 –1141.

WRIGHT, G. D., AND J. K. FREY. 2015. Habitat selection by the endangered New Mexico meadow jumping mouse on an irrigated floodplain. Journal of Fish and Wildlife Management 6:112–129.

XI, Z., L. LIU, AND C. C. DAVIS. 2015. Genes with minimal phylogenetic information are problematic for coalescent analyses when gene tree estimation is biased. Molecular Phylogenetics and Evolution 92:63–71.

ZILKO, J. P., D. HARLEY, B. HANSEN, A. PAVLOVA, AND P. SUNNUCKS. 2020. Accounting for cryptic population substructure enhances detection of inbreeding depression with genomic inbreeding coefficients: an example from a critically endangered marsupial. Molecular ecology 29:2978–2993.

6. Figures legends

Figure 1. Museum-based specimens (green) for the New Mexico Meadow Jumping Mouse (Zapus luteus luteus). Samples used in this project included museum-based frozen tissues (orange) for genomic assessments. Grey background areas represent montane regions with elevational contours (black = 1,500 m, white = 3,000 m). Labels with eight Geographic Areas (GAs - colored) used in analyses for this project. GAs include five major mountain ranges (SDC - Sangre de Cristo [green], JMZ – Jemez [orange], SAC – Sacramento [yellow], and WHT - White mountains [blue], plus JHM - Johnson Mesa [red]) and three major river

areas (ISA – Isleta [purple] and BDA - Bosque del Apache [magenta] of the Rio Grande and tributaries of the SJN - San Juan River [brown]).

Figure 2. Scatter plot representing Discriminant Analysis of Principal Components (above) and Posterior Membership Probability (Assignment Test; below) for Geographic Areas (GA) of *Zapus luteus luteus* across the American Southwest using (a) putatively neutral loci (outlier loci removed) and (b) outlier loci. Axes in a, b represents the first two linear discriminants (LD1 and LD2). Points represent individuals, circles represent 95% confidence intervals around clusters, and colors represent each lineage. For both tests, 11 PC eigenvalues and 2 DAs eigenvalues (insets – top) were used (e). For assignment tests, individual samples were randomly removed to determine posterior membership probability (below). These analyses indicate the proximity of samples to different clusters and measures of potential admixture between groups, which was zero for both datasets (neutral and outlier).

Figure 3. Outlier Loci Associations (potentially adaptive). 205 candidate outlier loci, screened for frequencies <0.50 and then associated with 1) eight geographic areas (GA; 55.6%), 2) two ecological associations (16.6%) including montane (EC-Mont) with high MAF scores for at least three high-elevation areas and the lower elevation Rio Grande (EC-Rio) with high MAF scores for both BDA and ISA, 3) geographic regions (4.9%) including the NE (RN-NE; JHM and SDC) and NW (RN-NW; JMZ and SJN), 4) at least four GAs (Multi; 4.9%), or 5) unknown (Unk; 18.0%).

Figure 4. Evolutionary (phylogenomic) relationships and biogeography across Zapus luteus luteus. (A) Time-calibrated BEAST tree inferred from concatenated RAD loci. (B) Summary tree of posterior estimates of species trees reconstructed from unlinked single nucleotide polymorphisms using SNAPP. In both trees, nodes with large dots received ≥ 0.95 posterior probability support, and major geographical groups (colors) match those in other figures.

7. Figures

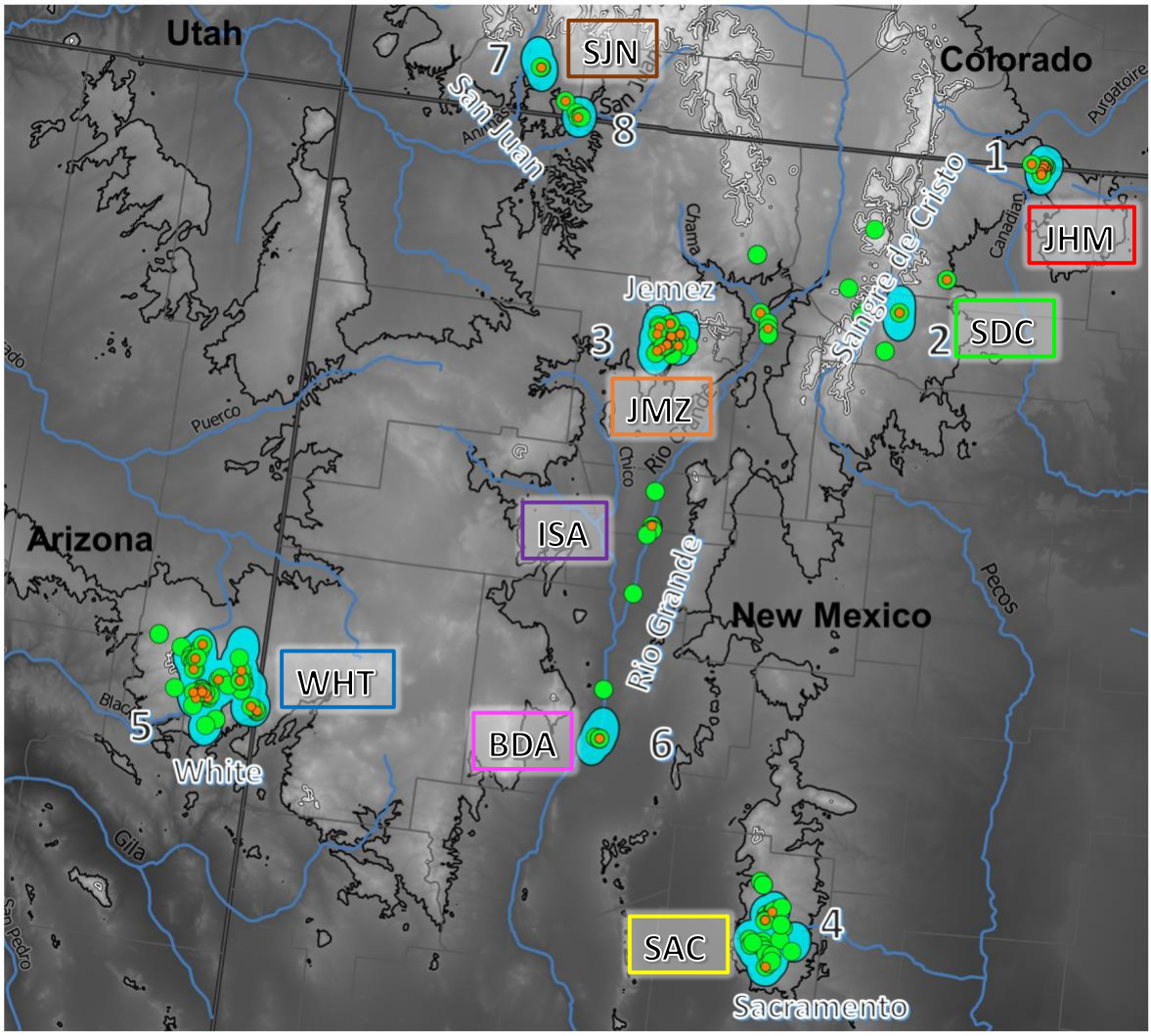


Figure 1. Distribution map of samples used in this project

Neutral Loci (n = 8,138) **Outlier Loci (n = 711)**

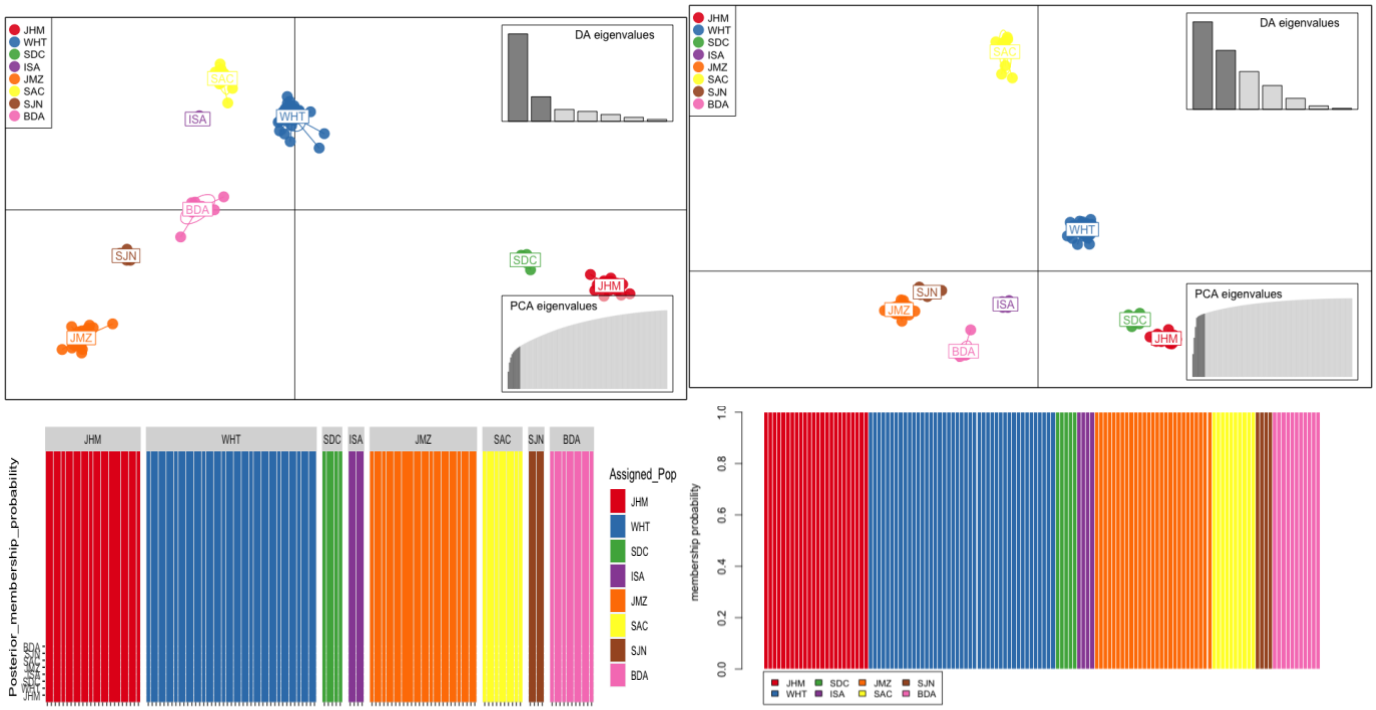


Figure 2. Discriminant Analysis of Principal Components plot (above) and Posterior Membership Probability (Assignment Test; below).

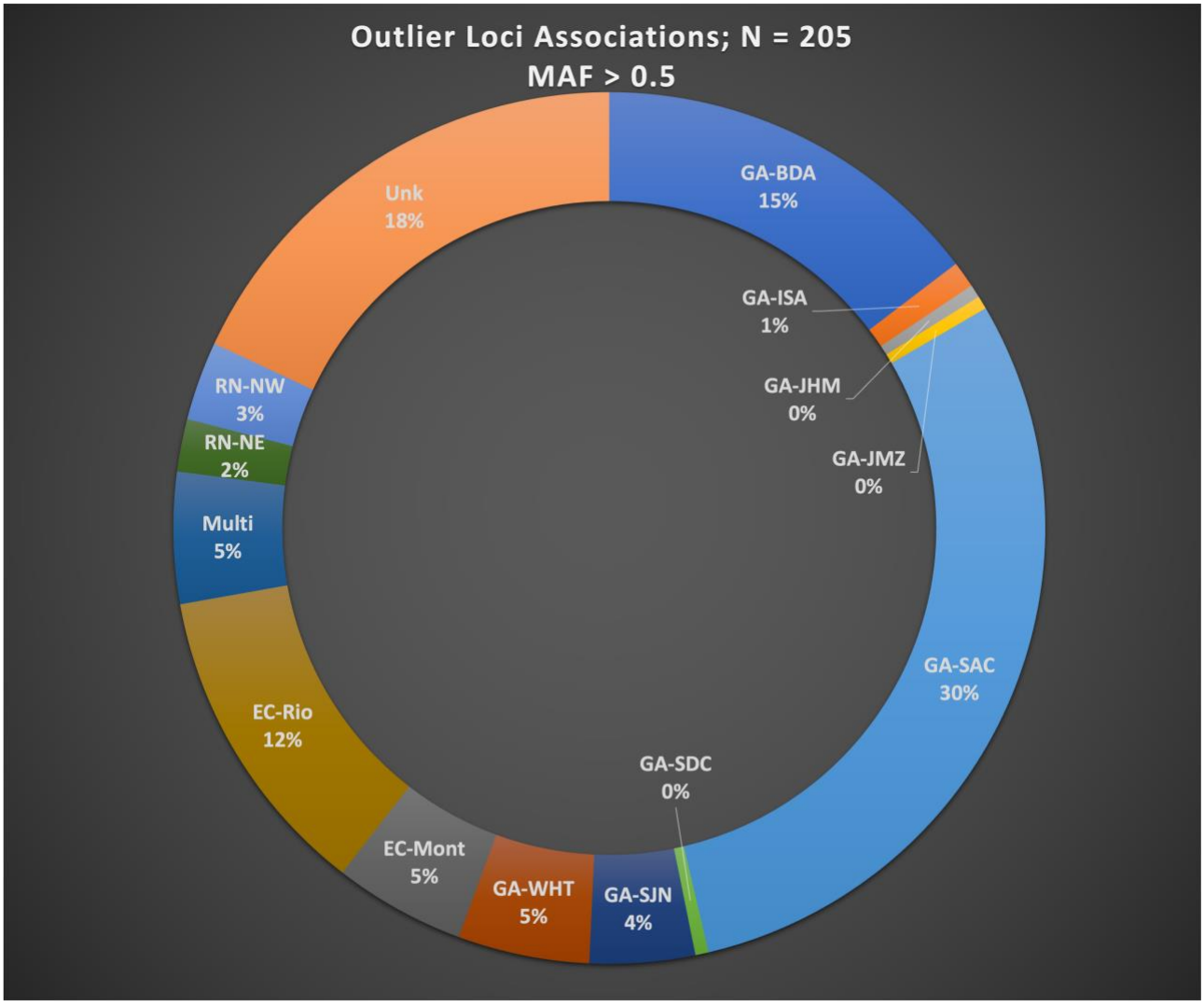
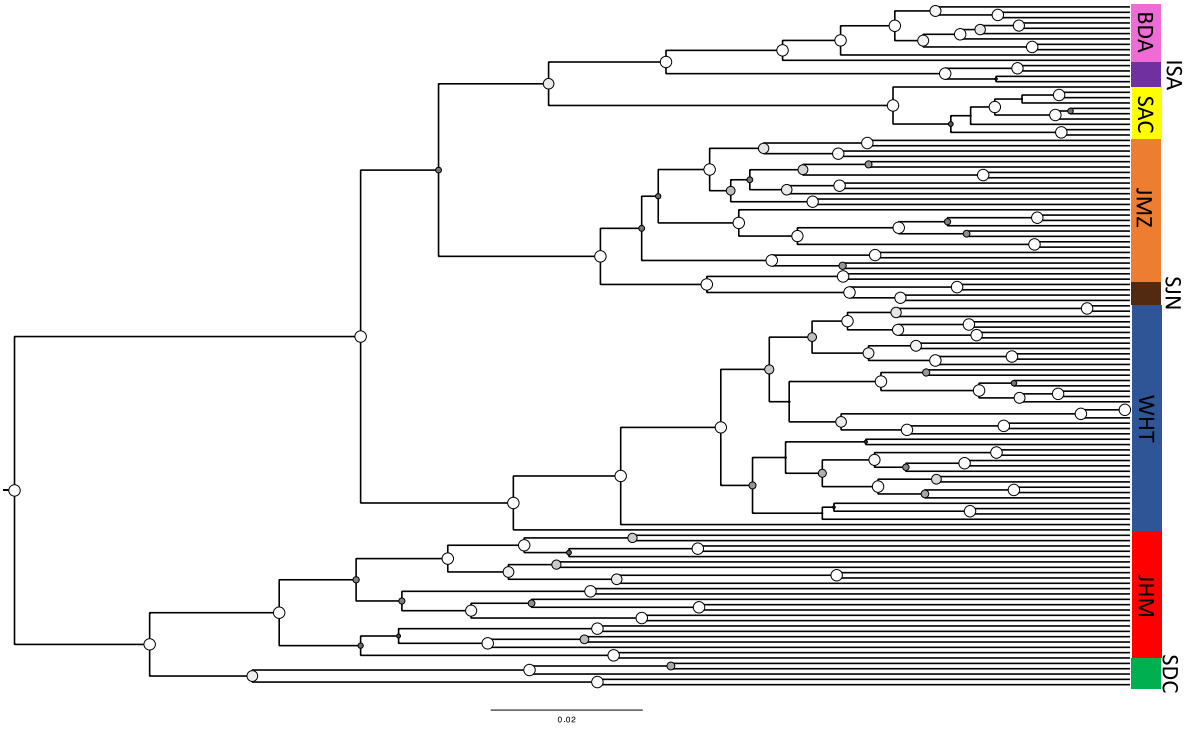

Outlier Loci Associations; N = 205
MAF > 0.5
GA-BDA
15%
GA-ISA
1%
GA-JHM
0%
GA-JMZ
0%
GA-SAC
30%
GA-SDC
0%
GA-SJN
4%
GA-WHT
5%
EC-Mont
5%
EC-Rio
12%
Multi
5%
RN-NE
2%
RN-NW
3%
Unk
18%

Figure 3. Outlier Loci Associations (potentially adaptive).

A. Time-calibrated tree (all samples)



B. Summary tree of multispecies coalescent-based model (putative OTUs)

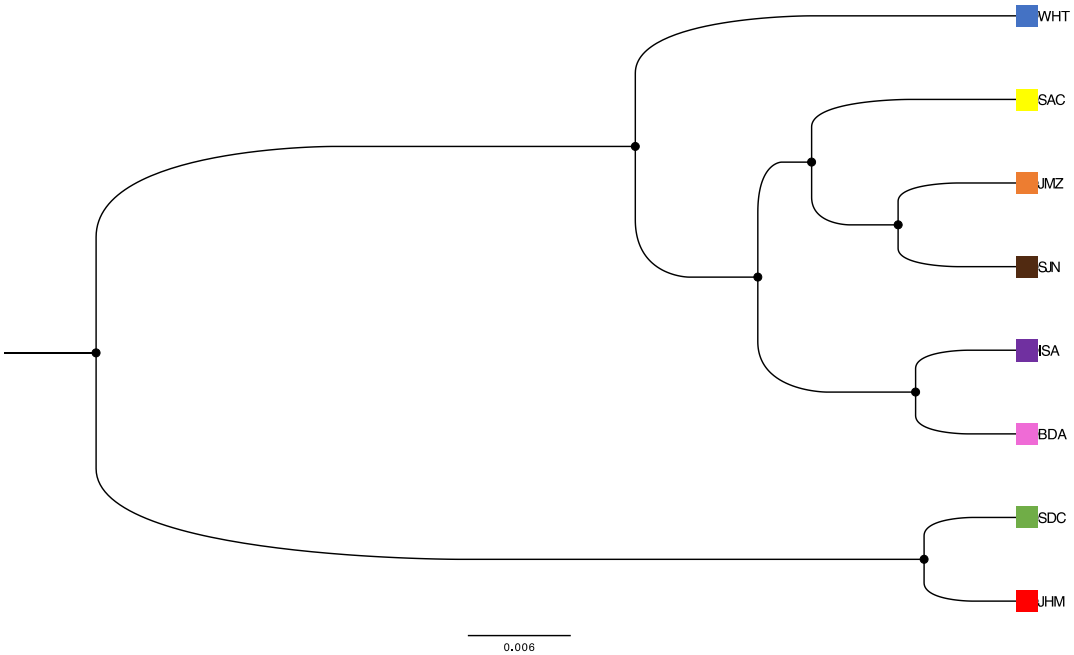


Figure 4. Evolutionary (phylogenomic) relationships and biogeography across *Zapus luteus luteus*.[i]

8. Tables

Table 1. The long-term isolation or vicariant hypothesis, the short term or disturbance hypothesis, and the combined hypothesis (a combination of the two first ones). The panmictic model is presented as the null hypothesis.

	Null or panmictic model	**Long-term allopatric hypothesis**	**Recent disturbance hypothesis**	**Combined hypothesis**
Timeframe	Long-term	Assumes deeper time geologic and climatic disturbances (~20K years)	Assumes recent anthropogenic disturbance (~200 years)	~20 thousand years to present
Cause	No disturbance or isolation factors	Populations persistently restricted to separate mountain ranges or isolated riparian corridors for a long period of time (thousands of years) since warming temperatures after the LGM	Populations have been connected historically but grazing, cattle, logging, agriculture, roads, and other anthropogenic activities have decreased and impoverished suitable habitat leading to recent isolation	Populations largely isolated to mountain ranges since warming after the LGM, but minimal connectivity persisted until anthropogenic disturbances created isolation

Effective population size (N_E)	High and stable	If habitat persists through time, N_E will not be affected (stable N_E). Populations could be big or small, depending on the environment that has been available.	N_E was large historically but now we see signs of recent population decline – genetic drift	Low expected N_E sizes mostly due to recent disturbances causing genetic drift
Gene flow	Present among populations, as a panmictic population would behave	No expected recent gene flow at regional scales (among populations of different mountain ranges)	Gene flow has been interrupted both within and among populations (if they remained in contact)	Low historical gene flow due to the long-term isolation
Genetic structure	Low genetic structure among populations due to connectivity and gene exchange	High levels of geographic genetic structure between distant populations (populations in different mountain ranges particularly)	Low genetic structure among populations since they have very recently been separated	Moderate to high genetic structure among the entire distribution due to long-term low connectivity and genetic drift

Genetic variability	High genetic variability resulting in healthy populations	Expected high genetic variability if populations have remained intact since their long-term isolation	Low genetic variability due to decreased population sizes and lower connectivity among populations	Low genetic variability due to decreased population sizes, low gene flow and high population structure

Table 2a-c. Summary of population genomic statistics for eight geographic areas (GA) of *Zapus luteus luteus* across the American Southwest for the combined dataset (a. neutral + outlier), neutral only (b.) and outlier only (c.) loci. Measures include the number of individuals genotyped (N_G), mean allelic richness (A_R), observed heterozygosity (H_O), unbiased gene diversity (uH_E), inbreeding coefficient (F_{is}), Shannon's information index (I), Simpson's Index (l), mean minor allele frequency (MAF), number of private alleles (PA) and total alleles (A), and effective population size with 95% confidence intervals (N_E, with 95% CI). Infinity symbols (∞) indicate low power to make inferences about N_E, likely due to insufficient variation perhaps because of finite numbers of individuals or sampling error (Do et al. 2014). Dashes indicate insufficient samples for estimates and blanks represent uncalculated measure.

GAs include five mountain ranges and two river drainages.

BDA – Bosque del Apache (Lower Rio Grande), ISL – Isleta (Upper Rio Grande), SJN – San Juan tributaries

JHM – Johnson Mesa, JMZ – Jemez, SAC – Sacramento, SDC – Sangre de Cristo, WHT – White

Table 2a. combined loci (neutral + outlier; 8,849 loci; 17,411 alleles)

GAs	Management Units	N_G	A_R	H_O	uH_E	F_{is}	I	l	MAF	A	PA	N_E (95% CI)
JHM		24	1.301	0.1271	0.1444	0.1197	3.18	0.958	0.31	13,405	794	600.3 (448.1 – 902.6)
	Dorothey	13	1.124	0.1268	0.1446	0.1230	2.56	0.923				
	Fishers Peak	1	1.080	0.1267	–	–	–	–				
	Sugarite	10	1.120	0.1278	0.1414	0.0966	2.30	0.900				
SDC		5	1.300	0.1260	0.1545	0.1845	1.61	0.800	0.39	12,168	212	181.1 (98.5 – 933.5)
	Coyote	3	1.211	0.1237	0.1369	0.0966	1.10	0.667				
	Rayado	2	1.196	0.1281	0.1307	0.0197	0.69	0.500				
JMZ		27	1.176	0.0938	0.1046	0.1030	3.30	0.963	0.29	12,206	395	46.2 (43.8 – 48.9)
	Rio Cebolla	19	1.187	0.0924	0.1024	0.0973	2.94	0.947				

	Rio de las Vacas	5	1.173	0.1011	0.0919	-0.1001	1.61	0.800				
	San Antonio	3	1.160	0.9070	0.0988	0.0821	1.10	0.667				
SAC		10	1.085	0.0490	0.0592	0.1726	2.30	0.900	0.53	10,536	384	21.0 (18.2 – 24.6)
	Aqua Chiquita	1	1.022	0.0439	–	–	–	–				
	James Canyon	8	1.039	0.0487	0.0544	0.1049	2.08	0.875				
	Silver Springs	1	1.027	0.0505	–	–	–	–				
WHT		43	1.149	0.0773	0.0854	0.0949	3.76	0.977	0.21	12,929	1,135	52.9 (50.1 – 55.9)
	Black River	7	1.049	0.0819	0.0753	-0.0883	1.95	0.857				
	East Fork – Black R.	4	1.044	0.0718	0.0755	0.0485	1.39	0.750				
	West Fork – Black R.	23	1.058	0.0797	0.0847	0.0598	3.14	0.957				

	Blue River – Gila	2	1.045	0.0748	0.0692	-0.0810	0.69	0.500				
	Little Colorado	3	1.054	0.0738	0.0855	0.1372	1.10	0.667				
	Nutriso	1	1.033	0.0656	–	–	–	–				
	San Francisco	3	1.043	0.0665	0.0742	0.1035	1.10	0.667				
BDA		11	1.117	0.0671	0.0731	0.0812	2.40	0.909	0.45	11,061	266	13.5 (12.5 – 14.9)
ISA		4	1.093	0.0832	0.0685	-0.2146	1.39	0.750	0.59	10,188	119	∞
SJN		4	1.114	0.0826	0.0895	0.0776	1.39	0.750	0.44	10,678	116	∞
	Florida	2	1.113	0.0808	0.0764	-0.0577	0.69	0.500				
	Sambrito	2	1.127	0.0847	0.0867	0.0228	0.69	0.500				
Total		128	1.944	0.0905	0.1581	0.4280	4.88	0.992		17,411		

Table 2b. neutral only (8,138 loci; 15,989 alleles)

GAs	N_G	A_R	H_O	uH_E	F_{is}	A	PA	N_E (95% CI)
JHM	24	1.301	0.1274	0.1450	0.1217	12,314	794	661.1 (468.3 – 1,111.6)
SDC	5	1.300	0.1254	0.1535	0.1823	11,171	212	142.6 (82.0 – 482.0)
JMZ	27	1.176	0.0927	0.1039	0.1079	11,181	394	47.7 (44.8 – 51.1)
SAC	10	1.085	0.0460	0.0557	0.1751	9,572	320	27.5 (22.2 – 35.1)
WHT	43	1.149	0.0775	0.0858	0.0970	11,849	1,131	51.7 (48.5 – 54.9)
BDA	11	1.117	0.0632	0.0693	0.0868	10,056	250	18.8 (16.8 – 21.0)
ISA	4	1.093	0.0773	0.0640	-0.2081	9,295	119	∞
SJN	4	1.114	0.0790	0.0856	0.0768	9,746	116	∞
Total	128		0.0861	0.0956	0.0998	15,989		

Table 2c. outlier only (711 loci; 1,422 alleles)

GAs	N_G	A_R	H_O	uH_E	F_{is}	A	PA
JHM	24	1.348	0.1243	0.1374	0.0951	1,091	0
SDC	5	1.337	0.1334	0.1684	0.2077	997	0
JMZ	27	1.265	0.1073	0.1131	0.0513	1,025	1
SAC	10	1.285	0.0839	0.0994	0.1555	964	64
WHT	43	1.183	0.0758	0.0814	0.0692	1,080	4
BDA	11	1.292	0.1115	0.1164	0.0418	1,005	16
ISA	4	1.236	0.1511	0.1203	-0.2553	893	0
SJN	4	1.265	0.1231	0.1346	0.0856	932	0
Total	128		0.1138	0.1204	0.0546	1,422	

Table 3a-b. Matrix of genomic differentiation (fixation) tests for eight geographic areas (GAs) of *Zapus luteus luteus* across the American Southwest for both neutral (a) and outlier loci (b). Pairwise F_{ST} and Jost's *D* values above and below the diagonal, respectively, in each. Pairwise indices (F_{ST} and *D*) detect genomic differentiation (below) and the fraction of allelic variation (above) among groups, respectively. Both metrics are represented by 0 when populations are identical or 1 when populations are completely distinct. Across mammals, values < 0.05 are often considered evidence of genomic differentiation between groups and represent a signal of low gene flow or unmixed genomes.

Note: Genomic differentiation (fixation) measures for GAs include five mountain ranges (SDC - Sangre de Cristo, JHM - Johnson Mesa, JMZ - Jemez, SAC - Sacramento, and WHT - White mountains) and two river drainages (ISA - Isleta, Upper Rio Grande, BDA - Bosque del Apache, Lower Rio Grande, and SJN - San Juan).

Table 3a - F_{ST} and *D* of GAs using **neutral loci (8,138 loci)**

GA	BDA	ISA	JHM	JMZ	SAC	SDC	SJN	WHT
BDA		0.0309	0.0501	0.0314	0.0335	0.0516	0.0414	0.0332
ISA	0.4107		0.0534	0.0405	0.0421	0.0552	0.0510	0.0423
JHM	0.3752	0.3709		0.0504	0.0483	0.0157	0.0574	0.0852
JMZ	0.3549	0.3676	0.3487		0.0279	0.0470	0.0176	0.0287
SAC	0.5261	0.5753	0.3849	0.3619		0.0491	0.0337	0.0239
SDC	0.4417	0.4107	0.1013	0.3626	0.4629		0.0560	0.0484
SJN	0.4599	0.4996	0.3438	0.1977	0.4741	0.3686		0.0298
WHT	0.4132	0.4473	0.4153	0.3241	0.3581	0.4510	0.3497	

Table 3b - F_{ST} and *D* of GAs using **outlier loci (711 loci)**

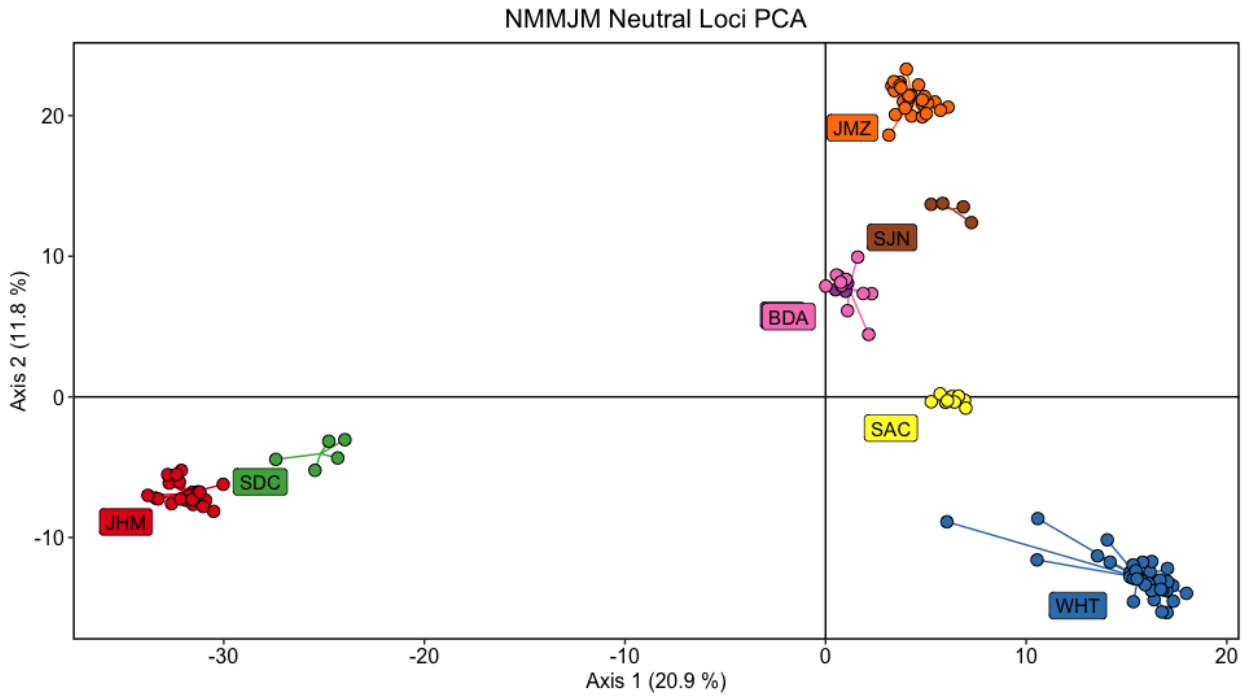
GA	BDA	ISA	JHM	JMZ	SAC	SDC	SJN	WHT
BDA		0.1020	0.3186	0.2986	0.6745	0.3099	0.3057	0.3877
ISA	0.4362		0.2453	0.2298	0.6164	0.2342	0.2331	0.3107
JHM	0.6826	0.6118		0.1793	0.4700	0.0253	0.1863	0.2614
JMZ	0.7008	0.6405	0.5600		0.5370	0.1567	0.0431	0.1847
SAC	0.8485	0.8387	0.7688	0.8145		0.4579	0.1616	0.5475
SDC	0.6724	0.5802	0.1268	0.5306	0.7667		0.5087	0.2457
SJN	0.6906	0.6170	0.5405	0.2463	0.8048	0.4698		0.1633
WHT	0.8011	0.7686	0.6996	0.6424	0.8553	0.7099	0.6336	

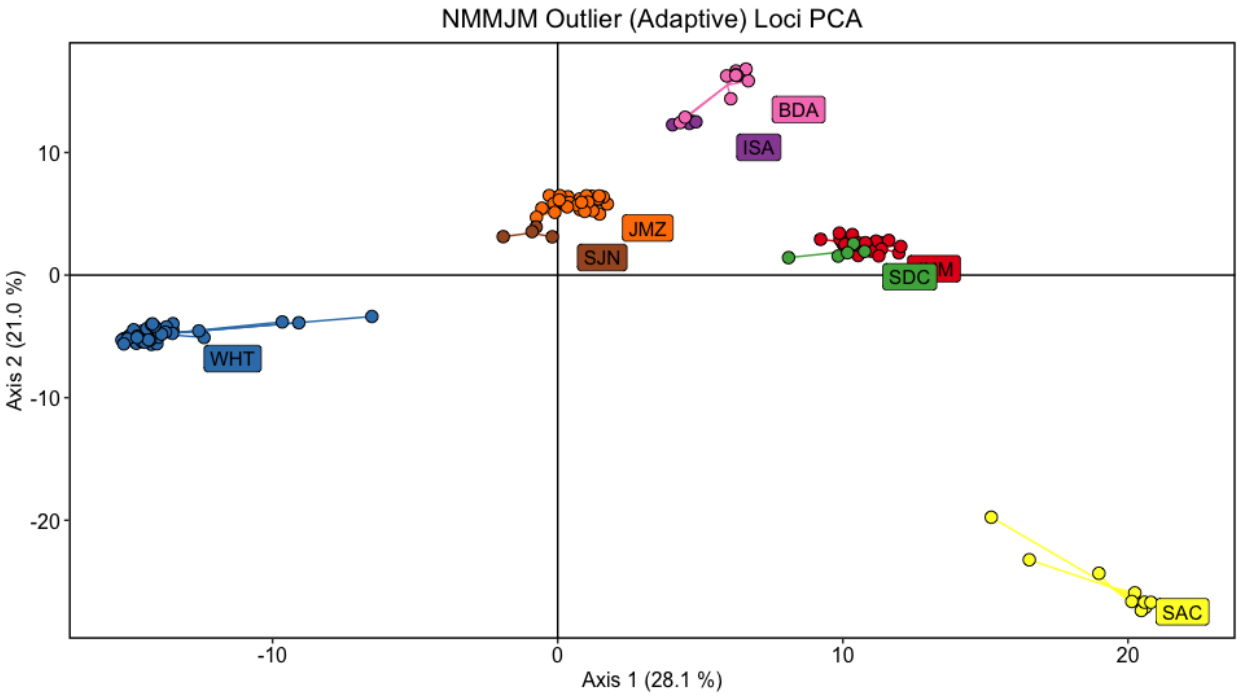
Table 4. Genomic differentiation indices for comparing *Z. luteus luteus* to other subspecies. Number of alleles (# alleles) represents the genomic diversity sampled with ddRADSeq approaches and the number and proportion (%) of private alleles (PA) per subspecies. Pairwise indices (F_{ST} and Jost *D*) detect the genomic differentiation (below) and the fraction of allelic variation (above) among groups, respectively, and both are 0 when populations are identical or 1 when populations are completely distinct. Across mammals, values <0.05 are often considered evidence of genomic differentiation between groups due to low levels of gene flow.

	Z. luteus luteus	*Z. luteus pallidus*	*Z. hudsonius campestris*	*Z. princeps princeps*
	N = 37	N = 4	N = 15	N = 27
# alleles	10,928	9,466	9,240	9,146
PA (%)	989 (9.0%)	552 (5.8%)	727 (7.9%)	802 (8.8%)
Pairwise index	F_{ST} below	*D* above		
Z. l. luteus		0.040228	0.148145	0.457344
Z. l. pallidus	0.181920		0.104194	0.456541
Z. h. campestris	0.522336	0.380195		0.456674
Z. p. princeps	0.808905	0.766067	0.823903	

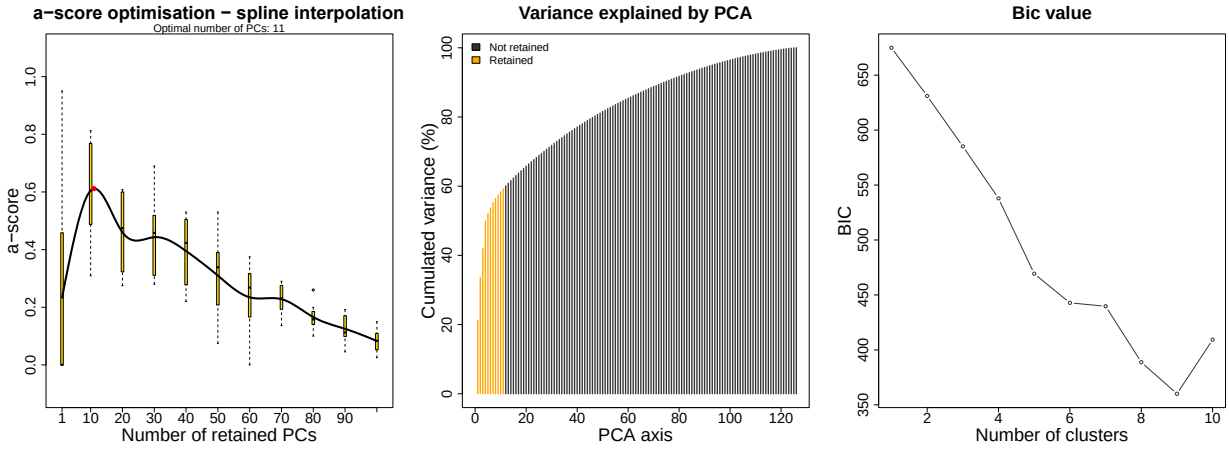
9. Supplementary Figures

Supplementary Figure 1. Principal Component Analysis of neutral (n = 8,138) and outlier (adaptive, n = 711) loci depicting genomic differentiation of eight geographic areas (GAs – colors). Points represent individuals and colors represents lineages. For both analyses, two PCs (PCA eigenvalues) were sufficient to capture most of the observed genomic variation.

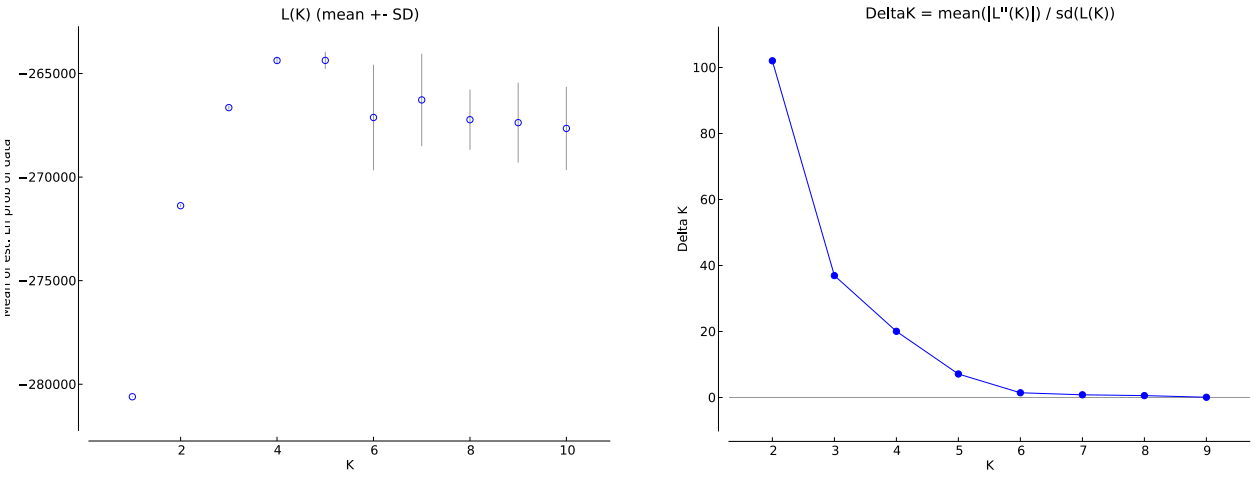




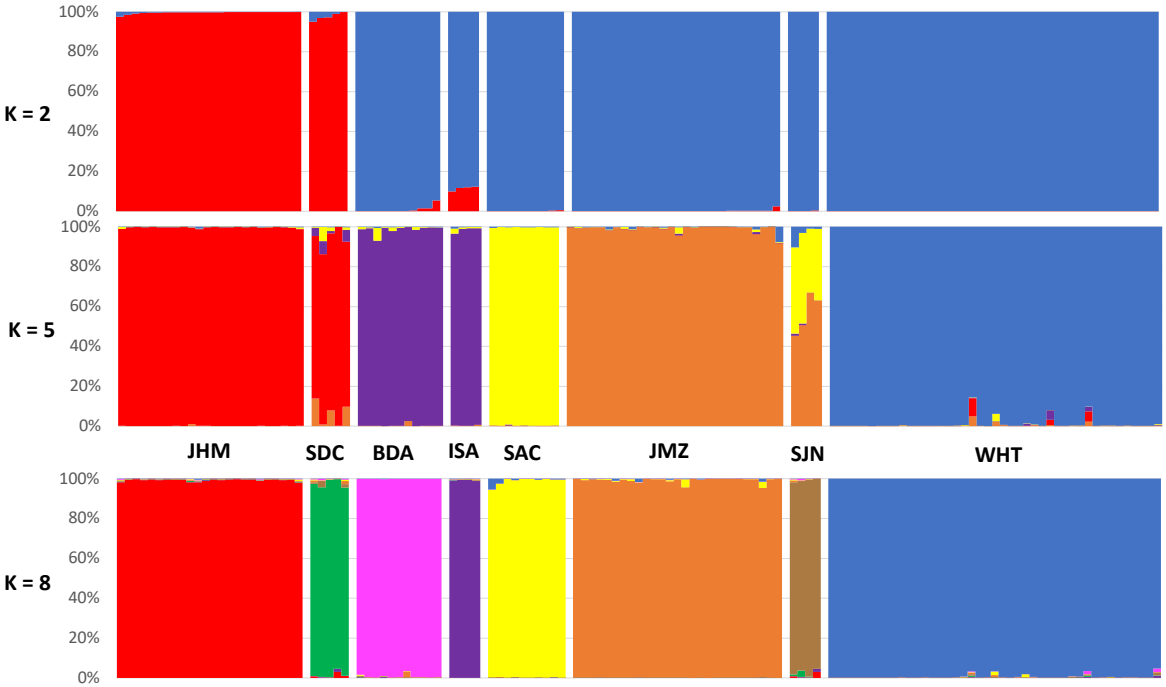
Supplementary Figure 2. DAPC optimization, variance explained, and BIC for determining optimal clusters



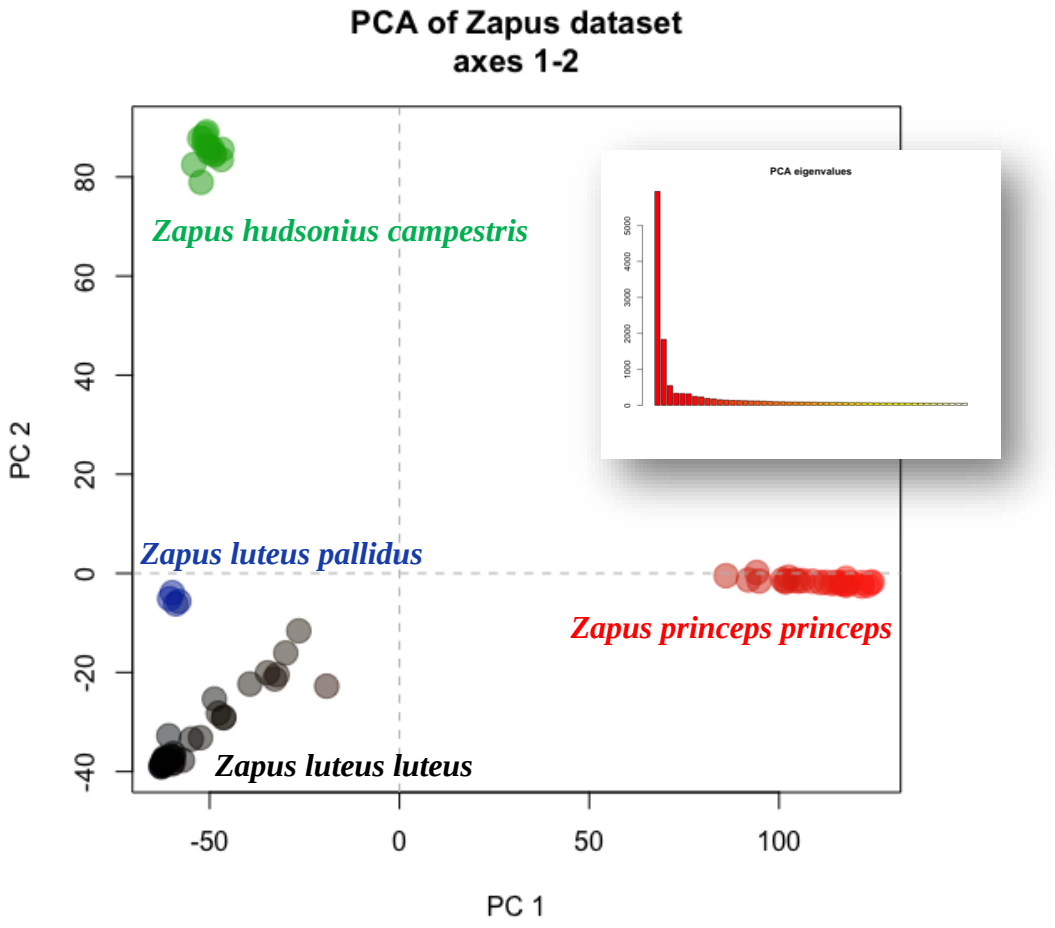
Supplementary Figure 3. Structure Plots of New Mexico meadow jumping mouse (*Zapus luteus luteus*). Above the mean of estimated likelihood probability for each K and the DeltaK value determined using the Evanno et al. (2005) method. Below the optimal K = 2 structure plot with subsequent hierarchical variation.



Structure Plots for K = 2, K = 5, and K = 8



Supplementary Figure 3. Principal Component Analysis of genomic differentiation of closely related jumping mice subspecies (*Zapus luteus luteus*, *Z. luteus pallidus*, *Z. hudsonius campestris*, and *Z. princeps princeps*). The PCA indicates that subspecies are well differentiated without any samples showing mixed ancestry (*i.e.*, hybridization or introgression). Two PCs (PCA eigenvalues – inset) were sufficient to capture most of the genomic variation.



Supplementary Figure 4. Discriminant Analysis of Principal Components (DAPC; left) and Assignment Test (right) for closely related jumping mice taxa (Zapus luteus luteus, Z. luteus pallidus, Z. hudsonius campestris, and Z. princeps princeps). Taken together, tests show that putative taxa are genomically independent. For DAPC, 3 PCA eigenvalues and 2 DAs eigenvalues (insets) were used to identify and describe genomic clusters. For assignment tests, individual samples were randomly removed to determine posterior membership probability. These analyses indicate the proximity of samples to different clusters and measures of potential admixture between groups, which was zero.