

Spatial variation in population genomic responses to over a century of anthropogenic change within a tidal marsh songbird

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Abstract

Combating the current biodiversity crisis requires the accurate documentation of population responses to human-induced ecological change. However, our ability to pinpoint population responses to human activities is often limited to the analysis of populations studied well after the fact. Museum collections preserve a record of population responses to anthropogenic change that can provide critical baseline data on patterns of genetic diversity, connectivity, and population structure prior to the onset of human perturbation. Here, we leverage a spatially replicated time series of specimens to document population genomic responses to the destruction of nearly 90% of coastal habitats occupied by the Savannah sparrow (*Passerculus sandwichensis*) in California. We sequenced 219 sparrows collected from 1889 to 2017 across the state of California using an exome capture approach. Spatial-temporal analyses of genetic diversity found that the amount of habitat lost was not predictive of genetic diversity loss. Sparrow populations from southern California historically exhibited lower levels of genetic diversity and experienced the most significant temporal declines in genetic diversity. Despite experiencing the greatest levels of habitat loss, we found that genetic diversity in the San Francisco Bay area remained relatively high. This was potentially related to an observed increase in gene flow into the Bay Area from other populations. While gene flow may have minimized genetic diversity declines, we also found that immigration from inland freshwater-adapted populations into tidal marsh populations led to the erosion of divergence at loci associated with tidal marsh adaptation. Shifting patterns of gene flow through time in response to habitat loss may thus contribute to negative fitness consequences and outbreeding depression. Together, our results underscore the importance of tracing the genomic trajectories of multiple populations over time to address issues of fundamental conservation concern.

KEY WORDS

conservation genomics, exome capture, habitat loss, museum collections, Passerellidae, tidal marshes

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1 | INTRODUCTION

Habitat loss is a primary driver of biodiversity loss (Brooks et al., 2002). At the genetic level, habitat-induced diversity loss can exacerbate extinction risk through increased mutational load and a reduced capacity to adapt to future environmental change (Forester et al., 2022; Frankham, 2005). Consequently, the preservation of genetic diversity is now recognized as a major priority for the conservation of global diversity (Díaz et al., 2020). A predictable relationship between habitat and genetic diversity loss exists that can inform how much of a species' range must be protected to maintain target levels of genetic diversity (Exposito-Alonso et al., 2022). However, across a species' distribution, local adaptation, population size, and gene flow will influence the rate and kinds (e.g., neutral vs. locally adapted variants) of genetic diversity being lost in response to habitat loss and degradation. For instance, habitat deterioration can turn populations into sinks with increased immigration from a source population needed to sustain the population. Changes in the rate of gene flow could lead to the evolutionary rescue of a small population (Whiteley et al., 2015) or could lead to outbreeding depression if immigrants from source populations are maladapted (Edmands, 2007). Despite mixed theoretical and empirical support for these different outcomes, a detailed understanding of how interactions among different ecological and demographic processes shape patterns of genetic variation in species threatened by habitat loss is lacking. Addressing this gap will be critical for guiding habitat protection and restoration efforts to conserve target levels of genetic diversity.

Looking at past population genetic responses to habitat loss will provide critical insights into the impacts of habitat degradation on genetic diversity. Natural history collections typically preserve specimens collected from the past 150–200 years, a period marked by dramatic human-induced landscape transformation, that can be used to reconstruct population genomic responses to past habitat loss and other human activities (Benham & Bowie, 2023; Billerman & Walsh, 2019; Holmes et al., 2016; Jensen et al., 2022; Lang et al., 2019). Sequencing of genome-scale data from historic specimens has revealed temporal declines in genetic diversity for a number of threatened and endangered species (Dussex et al., 2021; Feng et al., 2019; Sánchez-Barreiro et al., 2021; van der Valk et al., 2019). While these studies provide crucial insights into temporal changes in genetic diversity, few studies have compared temporal change across multiple populations to understand how environmental change may have disrupted historic patterns of gene flow, local adaptation, and drift within a species. To assess how human-induced changes in these meta-population dynamics may impact temporal patterns of genetic diversity, we leverage a spatially replicated time series of Savannah sparrow (*Passerculus sandwichensis*) specimens that were densely sampled from throughout the state of California over the past 128 years.

The Savannah sparrow is a widespread North American songbird, with 17 subspecies breeding across a range of open habitats from Alaska to Guatemala (Wheelwright & Rising, 2008). Within

California, four subspecies span a landscape that has experienced dramatic, but spatially variable, transformations due to human activity (Figure 1a). The coastal subspecies *P. s. alaudinus* and *P. s. beldingi* primarily occupy tidal marsh habitats in northern and southern California, respectively. These coastal populations exhibit local adaptation to high salinity and flooding in tidal marshes, with prior work documenting physiological, behavioral, and genomic divergence from other inland, freshwater-associated California populations of the species (Benham & Bowie, 2021; Benham & Cheviron, 2020; Cade & Bartholomew, 1959; Poulson & Bartholomew, 1962; Walsh et al., 2019). Coastal populations have experienced nearly 90% human-induced habitat loss in certain estuaries (Brophy et al., 2019; Marshall & Dedrick, 1994) due to agriculture, urbanization, and certain industries (e.g., salt evaporation mining) and are now listed as either state endangered species (*P. s. beldingi*; Zembal & Hoffmann, 2010) or bird species of special concern (*P. s. alaudinus*; Fitton, 2008). In contrast, *P. s. nevadensis* and *P. s. brooksi* of northern and eastern California are of least conservation concern, with habitats remaining relatively intact.

The contrasting histories of habitat loss experienced by different sparrow populations provide a natural experiment for exploring the temporal dynamics of genetic diversity change in response to human activities. We sequenced genomic data from 219 individuals sampled from 1889 to 2017, representing nine spatially replicated time series and all four subspecies. With this dataset, we asked: (1) How has habitat loss impacted patterns of population structure and gene flow among populations? (2) Does habitat loss predict genetic diversity loss? And (3), How have temporal changes in genetic diversity and gene flow among populations impacted loci associated with local adaptation to different habitats? Addressing these questions in concert with time-series data provides a novel framework for evaluating how human-induced ecological change can disrupt historic patterns of local adaptation, gene flow, and population size.

2 | MATERIALS AND METHODS

2.1 | Sample library prep, capture, and sequencing

Historical DNA was extracted from the toe pads of 155 museum specimens using a phenol-chloroform extraction protocol in a dedicated lab space for dealing with historic DNA. Prior to phenol-chloroform treatment, toe pads were minced and digested for ~24 h in a solution of 20 µL proteinase K, 20 µL DTT, and 300 µL cell lysis buffer in a rotisserie incubator set at 55°C. DNA was then added to a 2 mL phase lock tube. The sample was washed twice with 300 µL of phenol-chloroform and twice with 300 µL of chloroform, followed by a bead cleanup using a 2x volume of SeraMag beads. All historic samples were treated with the USER enzyme following the manufacturer's protocol (New England Biolabs) in order to cleave uracil nucleotides from historic DNA and eliminate erroneous C→T substitutions from historic samples (Briggs et al., 2009).

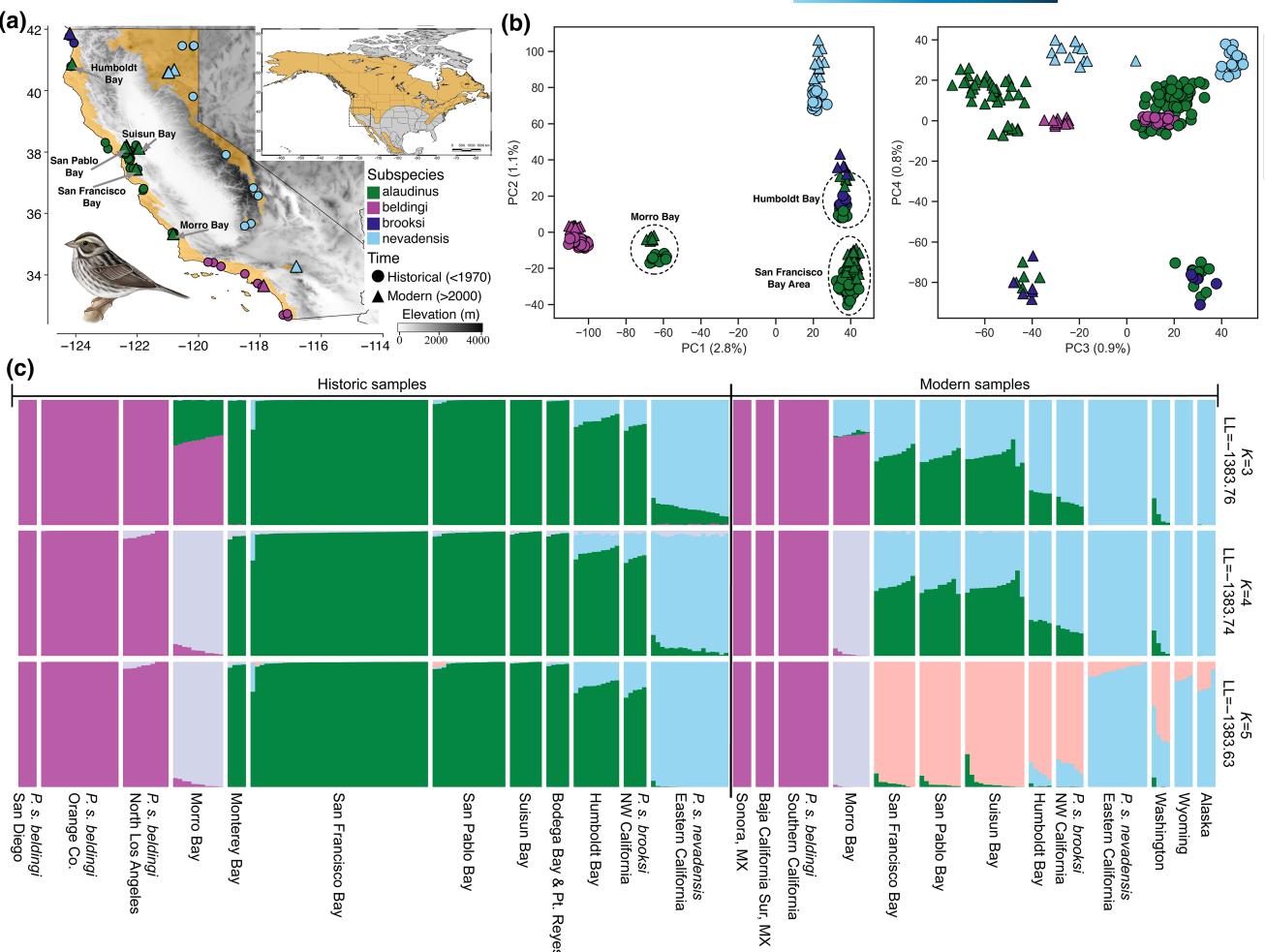


FIGURE 1 Spatial and temporal patterns of population structure within California populations of the Savannah sparrow (*Passerculus sandwichensis*). (a) California breeding distribution of the Savannah sparrow (ochre). Circles represent historical (pre-1970) sampling localities of specimens sequenced in the study, and triangles represent modern (post-2000) sampling localities. The inset shows the extent of the entire North American breeding distribution of the Savannah sparrow in ochre. (b) Principal component analysis of the full exome capture dataset (60,765 SNPs). The left panel shows PC1 (2.8% variance explained) versus PC2 (1.1% variance explained), and the right panel shows PC3 (0.9% variance explained) versus PC4 (0.8% variance explained). Colors correspond to different subspecies, as illustrated in Figure 1a. Dashed circles denote different clusters corresponding to different populations within *P. s. alaudinus*. (c) Results from DyStruct analysis. Runs with clusters specified as $K=3-5$ all exhibited very similar log-likelihoods and are illustrated here. Colors correspond to different K clusters. Artwork by *P. s. beldingi* individual courtesy of Jillian Nichol Ditner. Map lines delineate study areas and do not necessarily depict accepted national boundaries.

DNA for modern individuals was extracted from 94 fresh tissue and blood samples (74 from California and 20 from outside California) using the Qiagen DNeasy extraction kit and following the manufacturer's protocols (Valencia, California). Modern samples were randomly sheared using a sonication approach with a qSonica Q800R3 Sonicator (Newtown, CT). All samples were sonicated for a total of 12 min with an amplitude of 40% and a pulse of 15 s on/15 s off. We then performed a double-sided bead cleanup using SPRI low-ratio beads with 0.5x right-side selection and 0.65x left-side selection ratios in order to ensure that the DNA fragment distribution was in the 300–500 bp range. DNA from historic samples was not sonicated, given the small fragment size distribution generated by the passage of time.

Using a Kapa Illumina Hyper Prep kit, we prepared libraries of both historical and modern samples for capture and Illumina sequencing. In preparing these libraries, we employed a customized protocol using only a quarter volume and dual indexes, and we pooled the libraries to include 500 ng of DNA from each library. DNA from prepared libraries was captured using a custom-designed exome capture array (see supplemental methods for capture design). Despite the increasing feasibility of whole genome sequencing of degraded DNA, target capture approaches remain a highly targeted and cost-effective tool for analyzing temporal genetic change (Bi et al., 2019; Jones & Good, 2016). The pooled libraries were hybridized in solution with the SeqCap EZ probes, washed, and amplified following the manufacturer's

protocols (Roche Sequencing Solutions, Inc, Pleasanton, CA). Captured libraries were sequenced on two lanes of NovaSeq S4 and a single lane of NovaSeq SP at the University of California, Berkeley, Vincent J. Coates Genomics Sequencing Lab. Separate lanes of NovaSeq S4 included both modern and historic samples. The single lane of NovaSeq SP included only historic samples to increase the depth of coverage.

2.2 | Filtering, alignment, and variant calling

De-multiplexed reads were processed using the seqCapture pipeline (<https://github.com/CGRL-QB3-UCBerkeley/seqCapture/tree/master/scripts>). Briefly, Trimmomatic (Bolger et al., 2014) and Cutadapt (Martin, 2011) were used to remove low-quality bases (quality score <20) and adapter sequences from the raw reads. Only reads with a minimum length of 50 bp following trimming were retained. We removed PCR and optical duplicates using the hts_SuperDeduper function in HTStream (<https://s4hts.github.io/HTStream/>); overlapping paired end reads were merged using FLASH2 (Magoč & Salzberg, 2011); and we used Bowtie2 (Langmead & Salzberg, 2012) to map reads to the human and *E. coli* genomes and to remove any reads that mapped to these potential contamination sources. We then used the FastQC program (Andrews, 2010) to evaluate the overall quality of filtered reads and mapped the filtered reads to the white-throated sparrow (*Zonotrichia albicollis*; Tuttle et al., 2016) genome using BWA (Li & Durbin, 2009). Variants from the resulting bam files were identified and called using the "mpileup" and "call" functions in bcftools (Li, 2011). We also generated a dataset with similar mean coverage between historic and modern data by randomly down-sampling the number of reads included in bam files of the modern, higher-coverage samples using the DownsampleSam function in Picard tools (<https://broadinstitute.github.io/picard/>). Variants were called for historic and downsampled bam files using the same "mpileup" and "call" functions in bcftools as above.

VCF files of both the full data and the downsampled dataset were then filtered in VCFtools (Danecek et al., 2011) to remove indels and retain only biallelic sites. We removed from the datasets: all SNPs with missing data, with a minimum quality score less than 20, exceeding the Hardy-Weinberg threshold of 0.01, a minimum mean depth <1, and a minimum depth <5, individuals with mean coverage <4.5x, and individuals that appeared to be misidentified migrants based on preliminary principal components analyses (PCA). The final dataset included 196,151 SNPs from 145 historic and 94 modern individuals (see Appendix S1 for a list of final samples). We applied identical filters to the downsampled dataset and generated a final downsampled dataset with 219 individuals and 130,317 SNPs. Although we treated historic samples with the USER enzyme, sequencing errors could still be associated with certain sites in the genome. These include CpG sites or sites with C→T or G→A mutations (Bi et al., 2019; Briggs et al., 2009; van der Valk et al., 2019). We further filtered our datasets to remove all CpG SNPs from one

dataset and all C→T and G→A mutations from another dataset in order to evaluate the influence of these potential biases on our results.

2.3 | Population structure analyses

To test for temporal changes in population structure, we first performed a series of PCA on the full, exon, and non-genic datasets resulting from different filtering protocols. All PCAs were performed using the Python package scikit-allel v. 1.2.0 (Miles et al., 2019). For all SNP datasets, we removed singletons and pruned SNPs that were in linkage ($r=.25$) in 2500 bp blocks. To further test for temporal changes in population structure and admixture among populations, we analyzed a dataset of 65,583 unlinked SNPs and 239 individuals (an additional 20 from outside California) within the program DyStruct (Joseph & Pe'er, 2019). DyStruct resembles other model-based clustering programs (e.g., Admixture; Alexander et al., 2009), but explicitly accounts for the temporal dynamics of allele frequency change due to genetic drift in time-series data. The estimated effective population size (Ne) in DyStruct was set to 100,000 after preliminary analyses showed that results were robust to varying the Ne setting from the smaller (25,000) to larger population sizes (500,000) previously reported for this species (Benham & Cheviron, 2019). Generation time was set to 2.2 years (Bird et al., 2020), and we binned samples into eight 10-year windows spanning 48 generations from 1890 (generation 0) to 2017 (generation 48). We ran DyStruct three times independently for K=1 to 12, where K is the number of pre-defined population clusters. The most likely value of K was identified using a cross-validation, hold-out method within the program.

2.4 | Spatial and temporal patterns of genetic diversity

We next performed a series of analyses to determine how patterns of genetic diversity varied through time across the different Savannah sparrow populations. To begin, we compared historical and modern patterns of genetic diversity across California with estimated effective migration surfaces in the program EEMS (Petkova et al., 2016). The EEMS method models deviations from isolation by distance by estimating both a migration and diversity rate parameter. Effective migration rates are based on genetic dissimilarities among adjacent populations and are commonly used to visualize corridors and barriers to gene flow. The genetic diversity rate parameter estimates genetic dissimilarities between individuals within a population. We used this effective diversity rate parameter to produce spatially interpolated maps of genetic diversity among Savannah sparrow populations. For both the modern and historic datasets, input files were generated using the bed2diffs function in EEMS. We ran three independent runs of the MCMC chain. Each chain started from a



different random seed and ran for 2 million iterations of burn-in, followed by 5 million iterations. A population grid density of 250 demes was selected, and we adjusted the qEffectProposalS2 (0.01) and qSeedsProposalS2 (0.2) settings upwards to ensure a proposal acceptance rate between 10% and 40%. We used the R package rEEMSplots to assess model fit and convergence among the three independent chains.

Second, we estimated levels of genetic diversity in modern and historic populations using a number of different summary statistics. To match sequence depth between modern and historical samples, population genetic statistics were estimated using the most conservatively filtered dataset with downsampled coverage in modern samples and the removal of C → T and G → A mutations. We estimated Tajima's *D*, nucleotide diversity (π), and Watterson's Theta (θ) for each exon ($n=9971$ loci) and non-genic target region ($n=1024$ loci) in the dataset using scikit-allel. We also estimated these metrics across three and six time points in the Newport Bay (*P. s. beldingi*) and San Francisco Bay (*P. s. alaudinus*) populations, respectively. To estimate the magnitude of temporal change in each metric, we subtracted the historic value from the modern value. Within each population, we assessed whether significant differences existed between sampling points using *t*-tests or ANOVA, and post-hoc TukeyHSD tests, depending on the number of time points sampled. Finally, the amount of tidal marsh habitat lost is known to vary extensively across the six sampled estuaries. We performed a series of regression analyses to test whether the magnitude of tidal marsh loss explained the variation in levels of genetic diversity loss among the six tidal marsh populations of the Savannah sparrow. Additionally, we explored the relationship between the historic and modern extent of tidal marsh habitat on levels of genetic diversity. All statistical analyses were performed in R version 3.5.1 (<https://www.r-project.org/>).

2.5 | Temporal changes in demographic history

To quantify the extent of temporal change in genetic diversity and migration rates among populations, we next fit a series of demographic models to the three-dimensional site frequency spectrum (SFS) of eastern California (*P. s. nevadensis*), Bay Area (*P. s. alaudinus*), and Newport Bay (*P. s. beldingi*) birds in GADMA2 (Noskova et al., 2023). We ran separate analyses for a historic dataset and a modern dataset of these three populations. For separate historical and modern datasets, we generated the SFS from both exon and non-genic data; however, to restrict our analyses to putatively neutral loci, we eliminated regions of the genome found to be under selection by our analyses below. The final filtered vcf file was converted to a GADMA2 input format using a perl script from (https://github.com/wk8910/bio_tools/blob/master/01.dadi/00.convertWithFSC/convert_vcf_to_dadi_input.pl). The final datasets included 17 individuals of *P. s. nevadensis*, 37 *P. s. alaudinus*, and 10 *P. s. beldingi* in the historical dataset, and 13 *P. s. nevadensis*, 31 *P. s. alaudinus*, and 11 *P. s. beldingi* in the modern dataset.

Within GADMA2, these datasets were downprojected to include an equal number of samples for each population, with 11 for the modern population and 10 for the historic population. The final input datasets included 26,080 and 21,758 SNPs for historical and modern data, respectively.

Within GADMA2, we used *Moments* (Jouganous et al., 2017) as an engine for local optimization. We initialized the global search with a simple structured model (1,1,1) that allowed for one demographic event in between the divergence times of the three populations. We allowed for asymmetric migration among all populations and population size changes that were linear, exponential, or sudden. For each dataset, we performed 20 independent runs of the global search optimization in GADMA. Since all optimizations produced a model with the same number of parameters, the top models were identified based on their likelihood. Demographic parameter values were estimated from the value of theta ($4N_e\mu L$; where L is sequence length) based on a generation time of 2.2 years for the Savannah sparrow (Bird et al., 2020) and an estimated germline mutation rate of 4.6e-9 for another songbird species, *Ficedula albicollis* (Smeds et al., 2016). The total length of sequence (L) from which SNPs were called was estimated as: total sequence length × (SNPs in SFS/total SNPs pre-filtering). This resulted in a length estimate of 6.5 Mb for the historic dataset and 4.1 Mb for the modern dataset.

We next performed a series of coalescent simulations in msprime 1.2.0 (Baumdicker et al., 2022) to determine whether observed changes in gene flow patterns inferred from the DyStruct analyses could be generated via drift or other evolutionary processes. To parameterize these simulations, we used the historical best-fit demographic model from GADMA2. In total, we performed 25 separate simulations that included all combinations of five different timings of migration rate changes (5, 10, 15, 20, and 25 generations ago) and five different migration rates from eastern California to the Bay Area (0.00015, 0.0015, 0.015, 0.15, and 0.5). We ran a final simulation that involved no change in migration rate from the historical best-fit demographic model. For each simulation, we sampled 25 individuals from the Bay Area, Newport Bay, and eastern California at 50 generations before the present and another 25 individuals from each of the populations at the present time. This sampling scheme closely resembles the earliest and latest sampling times for DyStruct analyses performed on the empirical dataset. For each of the 26 simulations, we ran 60,000 replicates of the demographic model sampling from a sequence length of 650 to approximate the ascertainment scheme of our capture dataset. For each of the 60,000 replicated tree sequences, mutations were added using default parameters in msprime and a mutation rate of 4.6e-9 for passerine birds (Smeds et al., 2016). To generate a genotype matrix from these simulated mutations, we randomly selected a single variant from each of the 60,000 replicates that was present in two or more individuals. The resulting genotype matrix for each simulation was analyzed in DyStruct to test which parameter combinations most closely approximate observed changes in ancestry proportions. We ran DyStruct for each simulation with $k=3$ and the pop size parameter set to 70,000.

2.6 | Spatial and temporal selection analyses

Finally, we aimed to understand how any inferred changes in demographic history may have impacted divergence in loci putatively associated with local adaptation to tidal marsh habitats. Specifically, we explored how the observed changes in gene flow patterns from the above analyses impacted levels of divergence between tidal marsh and freshwater-adapted populations in outlier versus non-outlier loci. We identified putative outlier loci using the downsampled dataset with C→T and G→A sites removed, a maf filter of 0.05, and only retained sites with no missing data. We divided the dataset into five populations: (1) freshwater-adapted populations of eastern California (*P. s. nevadensis*); (2) Humboldt Bay estuary (*P. s. alaudinus*); (3) San Francisco Bay estuary (*P. s. alaudinus*); (4) Morro Bay estuary (*P. s. alaudinus*); and (5) Newport Bay estuary (*P. s. beldingi*). Each of these five populations was further divided into historic (sampled 1900–1920) and modern (sampled after 2000) datasets. We first compared genome-wide mean *Fst* divergence from eastern California in the four tidal marsh populations (Humboldt Bay, Bay Area, Morro Bay, and Newport Bay) for historic and modern datasets. Second, we estimated Hudson's *Fst* (Bhatia et al., 2013) and *Dxy* between each salt and freshwater-adapted population for 5 kb windows with a step size of 2.5 kb following approaches used to analyze other exome datasets (Schweizer et al., 2021). Third, we employed a Latent Factor Mixed Models approach (LFMM; Frichot et al., 2013) to identify SNPs with significant associations with mean salinity estimates from a previously published dataset (Benham & Bowie, 2021). Loci were designated as outliers if the 5 kb region was in the top 5% of *Fst* divergence, the top 5% of *Dxy* divergence, and contained a SNP with a *p*-value <.05 based on LFMM analyses for each of the four populations. To assess whether these outlier loci experienced more or less change in *Fst* relative to non-outlier loci, we compared patterns of ΔFst among tidal marsh populations. ΔFst measures the difference in *Fst* divergence from eastern California between the modern and historic datasets, with negative values signifying decreasing divergence through time. We compared temporal changes in *Fst* divergence for each 5 kb window by subtracting historical *Fst* from modern *Fst* divergence in each locus to estimate ΔFst . We used *t*-tests to compare whether outlier versus non-outlier regions exhibited significantly different changes in *Fst* through time.

To determine whether observed values of ΔFst differed from neutral expectations, we performed coalescent simulations in msprime 1.2.0 as above with the historic best-fit GADMA2 model used to parameterize simulations. We simulated a demographic history with no change in the demographic parameters over the past 100 years and one with an increase in migration rate to 0.15 from eastern California to the Bay Area at 15 generations ago. We simulated a sequence of length 100 Mb and estimated *Fst* divergence in sliding windows identically to the empirical dataset. Measures of historical *Fst* divergence and delta *Fst* were estimated for both the simulated Bay Area and Newport Bay populations. These simulated estimates were compared to observed patterns of *Fst* divergence using ANOVA and Tukey post-hoc tests in *r*.

Temporal increases in gene flow should also lead to the increased homogenization of allele frequencies among populations in the present. Further, if outlier loci associated with local adaptation to tidal marshes exhibit increased resilience to gene flow, then we would expect less homogenization of allele frequencies at these loci relative to non-outlier loci. We tested these predictions with allele frequencies estimated for each SNP in VCFtools and per SNP *Fst* divergence estimated in scikit-allel v. 1.2.0. We estimated the degree of correlation between historical deviation in allele frequency and change in allele frequency through time for each SNP following Gompert et al. (2021). In this analysis, stronger correlations will be indicative of greater homogenization due to higher levels of gene flow. For each of the four tidal marsh populations, we estimated the deviation from the mean allele frequency of the tidal marsh and eastern California populations as $\bar{p}_i - p_{ij}$. Where \bar{p}_i is the mean allele frequency of the *i*th SNP between inland eastern California and the *j*th tidal marsh population and p_{ij} is the allele frequency of the *i*th SNP in the *j*th tidal marsh population. The difference in allele frequency change through time was estimated for each SNP in each tidal marsh population as $p_{ij}^{\text{modern}} - p_{ij}^{\text{historical}}$. We used permutation tests to test for significant differences from zero (no correlation) as well as between outlier and non-outlier SNPs. We classified SNPs as outliers if they exceeded the 99th percentile of *Fst* divergence. We compared Pearson's correlation of the outlier SNPs to non-outlier SNPs by performing a permutation test where we randomly sampled an equal number of non-outlier to outlier SNPs and calculated correlations 1000 times. We then compared whether the non-outlier loci were both statistically different from 0 (no correlation) or from the correlation estimated for the outlier loci. We used changes in the proportion of eastern California ancestry found in each of the four coastal populations from the DyStruct analysis to approximate temporal increases in gene flow.

3 | RESULTS

3.1 | Spatial and temporal inference of population structure

We successfully sequenced exome data from 145 historical (sampled pre-1970) and 74 modern (post-2000) Savannah sparrows (see supplemental results, Figures S1–S3, and Table S1 for capture performance). The principal component analysis of all 219 individuals (60,765 SNPs) confirmed that modern and historical samples collected from the same locality clustered together (Figure 1b). The first principal component explained 2.8% of the variance in the data and separated out three clusters: (1) *Passerculus sandwichensis beldingi* subspecies from south coastal California; (2) birds from Morro Bay in San Luis Obispo County (*P. s. alaudinus*); and (3) birds from the rest of California (*P. s. alaudinus, brooksi*, and *nevadensis*). The second principal component explained 1.1% of the variance in the data and divided birds from the *P. s. nevadensis* subspecies in eastern California, birds in northwest California (*P. s. alaudinus*



and *P. s. brooksi*), and the remaining birds in the *P. s. alaudinus* subspecies from the Bay Area and central California coast. The third principal component (0.9% of variance) separated modern from historical samples, and the fourth component (0.8% of variance) split northwest California birds from other populations. PCA of other datasets (downsampled coverage, CpG sites removed, C → T and G → A sites removed) revealed similar patterns of population clustering (Figure S4).

The DyStruct analyses with the highest likelihoods were for $K=3$ to 5 (log-likelihood = -1383.63 to -1383.76; Figure 1c). All three values of K identified birds from south coastal California and Mexico as a distinct cluster, sparrows from the northern California coast as a second genetic cluster, and birds of eastern California, Wyoming, Alaska, and Washington State as forming a third cluster. The main difference between $K=3$ and 4 was that Morro Bay birds were recognized as a distinct cluster, and at $K=5$, modern birds from the northern California coast were identified as a distinct cluster. This analysis also revealed a key finding: temporal variation in population structure, with modern birds from the central and northern California coast exhibiting a 17% (Morro Bay) to 64% (Humboldt Bay) increase in the proportion of ancestry shared with inland eastern California birds. These temporal changes point to a recent increase in the level of gene flow from inland eastern California populations into coastal populations.

3.2 | Spatial and temporal patterns of genetic diversity

We next explored how variation in the scale of human-induced habitat loss influenced patterns of genetic diversity across sparrow populations. First, effective diversity surfaces estimated in EEMS showed similar patterns of genetic diversity variation among populations in both the historical (<1940) and modern (>2000) datasets (Figure 2a,b) with southern California birds consistently exhibiting lower effective diversity rates across time relative to northern California birds. Second, the southern California birds exhibited significantly lower levels of nucleotide diversity (π) and Watterson's theta (θ) compared to northern birds (Figure 2c; historic one-way ANOVA analysis [π : $F=58.2$, $df=7$, $p < .0001$; θ : $F=252.9$, $df=7$, $p < .0001$]; modern one-way ANOVA analysis [π : $F=101.9$, $df=7$, $p < .0001$; θ : $F=301.7$, $df=7$, $p < .0001$]).

California's coastal ecosystems have experienced more dramatic habitat loss over the past century relative to localities in eastern and northern California. Consistent with these differences in landscape change, we found significant increases in π and θ for eastern and northern California populations, but evidence for significant declines in genetic diversity for five out of six tidal marsh populations in coastal California (Figure 2c). Suisun Bay in the Bay-Delta region of central California was the only coastal population that exhibited significant increases in genetic diversity. Declines in genetic diversity were more evident in θ as opposed to π , which contributed to a significant increase in Tajima's D for all tidal marsh populations (except

Suisun Bay). This pattern is indicative of a population undergoing a contraction where rarer variants (as measured by θ) are eliminated before overall declines in heterozygosity (measured by π). Estimates of these parameters using datasets that were less conservatively filtered showed similar patterns (Figure S5). For Newport Bay and San Francisco Bay trends in genetic diversity metrics were also evaluated across samples taken from three and six time points, respectively. For Newport Bay birds, significant decreases in π and θ only occurred between the 1960s and 2010s (Figure S6). San Francisco Bay on the other hand, showed a long-term increase in Tajima's D from the early 1900s to the present, driven by decreasing θ through time (Figure S7).

We also noted significant differences in the magnitude of genetic diversity decline among tidal marsh populations (one-way ANOVA analysis; π : $F=294.7$, $df=5$, $p < .0001$; θ : $F=61.15$, $df=5$, $p < .0001$). To determine whether differences in the amount of habitat loss experienced by different tidal marsh populations explained this variation in genetic diversity loss, we compared estimates of percent marsh habitat lost for each estuary with temporal change in genetic diversity. The amount of tidal marsh loss varied from 2% to 87%, but this variation did not significantly explain the temporal change in π or θ based on simple linear regression models (Figure 2d). The relationship between temporal change in genetic diversity and total amount of tidal marsh area (log-transformed) in historic and modern times, and the relationship between contemporary extent of tidal marsh and genetic diversity were also non-significant (Table S2). The only significant predictor of modern levels of genetic diversity was the historic area of tidal marsh, which significantly predicted variation in contemporary θ ($R^2=.79$; $p=.01$) and the relationship with π was nearly significant ($R^2=.53$; $p=.06$; Figure 2e). These results indicate that despite widespread habitat loss across all coastal marshes, the populations from the historically largest marshes continue to maintain the highest levels of genetic diversity. This is true even though populations from some of the most expansive marshes (e.g., San Francisco Bay) experienced the greatest habitat loss and decline in θ , but even in modern times maintain levels of θ comparable to other northern California populations.

3.3 | Temporal change in demography

For the historic dataset, the best-fit demographic model from GADMA2 analyses (Figure 3a; Table 1; Log-likelihood -1391.83) showed an initial divergence time between Newport Bay and the northern California populations of ~254 kya, followed by divergence between the Bay Area and Eastern California populations of ~32 kya. Following the initial split, birds from southern California (Newport Bay) grew from an effective population size (N_e) of 0.2 to 16,749 before contracting again to 6675. In contrast, the northern California population remained large with an N_e of 297,826 before splitting into an eastern California population that maintained a constant N_e of 68,438 and a Bay Area population that declined to an N_e

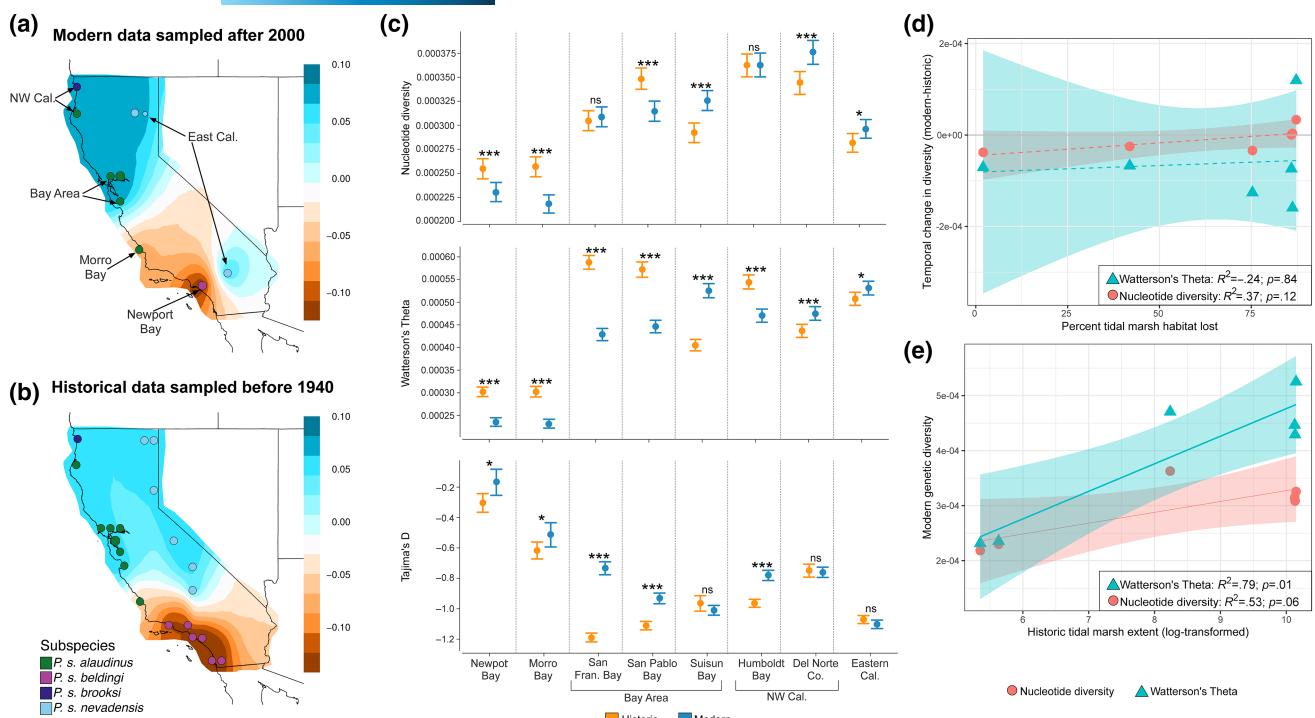


FIGURE 2 Spatial and temporal patterns of genetic diversity within California Savannah sparrows. (a) Modern variation in genetic diversity across California was estimated using the Estimated Effective Migration Surfaces (EEMS) program. Contour plots vary from low (dark brown) to high (dark blue) posterior mean diversity rates. (b) Historic variation in genetic diversity across California was estimated using EEMS. (c) Plots comparing historical (orange) and modern (blue) estimates of nucleotide diversity, Watterson's theta, and Tajima's D across all populations. For both (b) and (c) plots are based on a downsampled dataset with C \rightarrow T and G \rightarrow A mutations removed. Asterisks in (b) and (c) denote p -values based on t -tests between time groups (ns: non-significant; * p < .05; ** p < .01; *** p < .0001). (d, e) Relationship between the amount of tidal marsh habitat lost or available in each of the six estuaries and population genetic statistics. The values on the y-axis have all been scaled and centered to visualize on the same axis. (d) The amount of tidal marsh lost (%) over the past century is not significantly related to temporal changes in nucleotide diversity (red circle) or Watterson's theta (blue square). (e) Historically, the total amount of tidal marsh habitat present in each estuary significantly predicts modern levels of Watterson's theta, while nucleotide diversity is marginally non-significant. Contemporary levels of tidal marsh habitat did not predict levels of genetic diversity (Table S2). Map lines delineate study areas and do not necessarily depict accepted national boundaries.

of 16,755. Migration rates were roughly symmetrical from eastern California to the Bay Area (1.6×10^{-4}) and vice versa (1.3×10^{-4}), which translates to a rate of 8.9 migrants per generation (Nm) from the Bay Area to eastern California and $Nm = 2.7$ in the reverse direction. Overall, the top five of 20 models fit to the historic dataset show a similar demographic history and parameter estimates (Table 1).

The best-fit model for the modern dataset (Figure 3a; Table 1; Log-likelihood -1556.50) showed a similar initial divergence time of ~ 232 kya, but a much earlier divergence time between eastern California and the Bay Area (~ 125 kya). Newport Bay birds showed a similar history of expansion and decline to a smaller Ne of 4923. In contrast to the historic dataset, eastern California showed a smaller Ne (31,638) than Bay Area birds (57,309). This difference was consistent across four of the five top models estimated for the modern dataset. Migration rates were also inferred to be symmetrical between eastern California and the Bay Area (1.0×10^{-4} and 1.1×10^{-4}), with a slightly greater Nm per generation (5.73) from eastern California to the Bay Area than in the opposite direction ($Nm = 3.48$), a reversal from the historic dataset. Unlike the historic dataset, the top five models showed extensive variation in a number of

parameter estimates, notably related to divergence time between eastern California and the Bay Area (range 3.6–125 kya), Bay Area Ne (37,157–426,253), and Nm from eastern California to the Bay Area (0–63.94).

The reasons for the increased variation among models in the modern dataset were unclear; however, greater variation could relate to recent and unaccounted-for changes in demographic history due to population declines or shifts in migration rates that were not captured by the demographic model. The latter possibility was also suggested by DyStruct analyses, where a significant increase in eastern California ancestry was observed in modern-day Bay Area populations (Figure 1c). We tested this possibility with the analysis of 26 simulated SNP datasets in DyStruct, which showed that a shift to a migration rate of 0.15 from eastern California to the Bay Area 5–25 generations ago would be necessary to match observed proportions of eastern California ancestry in Bay Area birds (Figure 3c,d). Simulation results provide strong support for observed DyStruct patterns being the result of significant changes in the rate of gene flow from eastern California to the Bay Area as opposed to other processes such as genetic drift.

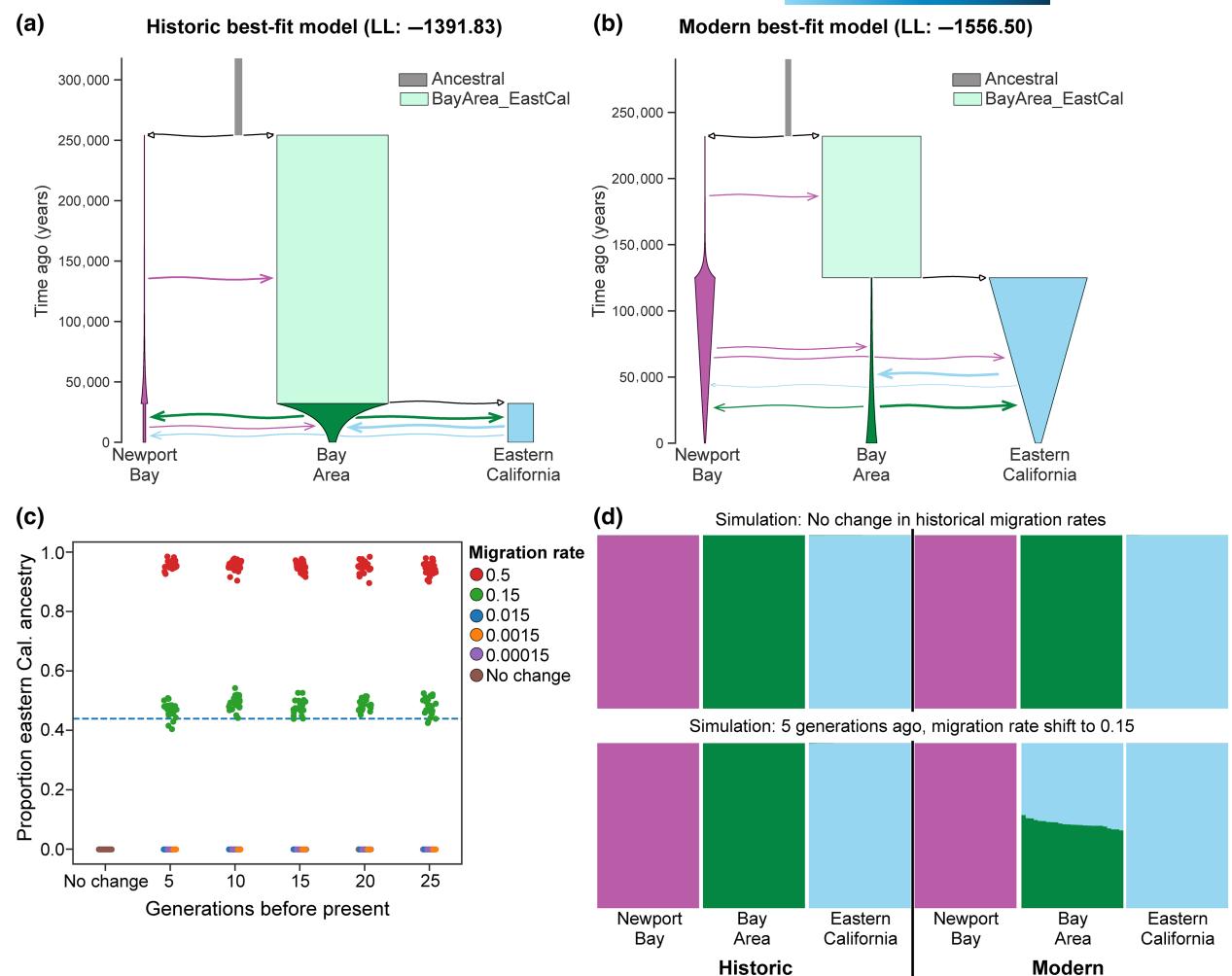


FIGURE 3 Temporal change in migration rates within California Savannah sparrows. (a) Historic best-fit model estimated with GADMA2. The y-axis shows the time of demographic events; the width of bars represents the effective population size for each population; and arrows signify migration rates from the source to the destination population forward in time. Arrows are colored by the source population and represent continuous migration. (b) Representation of the best-fit model to the modern dataset in GADMA2. (c) Coalescent simulation results where the historic best-fit model (Figure 3a) was used as the baseline demographic history with migration rates changing at varying generations before the present (x-axis) to different rates (colors in legend) from eastern California to the Bay Area. Simulated datasets were analyzed within Dystruct, and the proportion of eastern California ancestry in the modern Bay Area population was estimated (y-axis). The dashed blue line represents the average observed eastern California ancestry found in the modern Bay Area population (see Figure 1c). Migration rates <0.15 did not result in any changes to the proportion of eastern California ancestry. (d) Barplots showing ancestry proportions estimated for simulated datasets in Dystruct. The top plot shows the results based on a simulation of the historic demographic model (Figure 3a) with no change in migration rate. The bottom plot shows results from a simulated dataset where the migration rate shifted five generations ago from a proportion of 0.00015 immigrants per generation to a proportion of 0.15 immigrants per generation from eastern California into the Bay Area population.

3.4 | The influence of demographic change on locally adapted loci

Finally, we aimed to understand how the inferred changes in gene flow between tidal marsh and freshwater-adapted (eastern California) populations of the Savannah sparrow may have impacted divergence in loci putatively associated with local adaptation to tidal marsh habitats. Modern values of genome-wide *Fst* divergence showed increases in Morro Bay from 0.054 to 0.062, but a sharp decrease in mean *Fst* at Humboldt Bay from 0.014 to -0.002 and more modest changes in the other two populations (Table 2). We

next identified outlier loci that were in the 95th percentile of *Fst*, *Dxy*, and LFMM analyses. Across all four tidal marsh populations, 67 outliers were identified in the historic dataset (Figure 4a).

To assess whether these outlier loci experienced more or less change in *Fst* relative to non-outlier loci, we compared patterns of ΔFst among tidal marsh populations. This analysis revealed that all four tidal marsh populations have experienced significantly greater declines in *Fst* divergence within outlier loci relative to non-outlier loci (Figure 4b). Observed patterns of ΔFst in the Bay Area and Newport Bay significantly exceeded the declines predicted by simulations of a neutral demographic history with no demographic

TABLE 1 Parameter estimates for the top five best-fit models for the historic and modern datasets.

Historic					
LL	-1391.83	-1393.98	-1397.1	-1398.1	-1405.83
Tsp1 (years)	254,219	238,351	192,733	243,921	3525,73
Tsp2 (years)	32,164	48,431	69,673	51,533	18,578
NewportBay Ne	6675	9341	13,180	8878	6618
BayArea Ne	16,755	38,319	23,688	35,013	30,276
EastCal Ne	68,438	84,073	102,343	77,387	54,259
n → b	1.2E-05	2.6E-08	2.6E-05	3.3E-05	0
b → n	1.1E-04	8.5E-05	4.4E-05	2.8E-08	1.2E-04
n → e	0	7.5E-06	1.4E-05	0.0E+00	0
e → n	1.2E-05	0	2.7E-06	7.2E-05	0
e → b	1.6E-04	5.9E-05	9.8E-05	1.1E-04	1.1E-04
b → e	1.3E-04	1.1E-04	1.1E-04	1.1E-04	1.5E-04
b → e mig per gen	8.90	9.25	11.26	8.51	8.14
e → b mig per gen	2.68	2.28	2.32	3.85	3.33
Modern					
LL	-1556.5	-1583.13	-1585.11	-1585.43	-1595.09
Tsp1 (years)	232,053	276,701	310,210	313,395	343,840
Tsp2 (years)	125,040	31,727	5803	27,926	3694
NewportBay Ne	4923	3091	3290	3149	2379
BayArea Ne	57,309	41,061	37,157	426,253	54,707
EastCal Ne	31,638	212,932	37,029	17,969	34,174
n → b	4.2E-05	1.5E-05	5.2E-05	2.6E-05	7.2E-05
b → n	4.5E-05	8.4E-05	1.0E-04	6.1E-05	1.2E-04
n → e	2.6E-05	1.9E-20	0	5.9E-06	0
e → n	6.8E-09	2.6E-05	1.9E-05	7.7E-05	0
e → b	1.0E-04	1.0E-04	2.4E-08	1.5E-04	0
b → e	1.1E-04	4.0E-05	0	3.1E-05	0
b → e mig per gen	3.48	8.52	0.00	0.56	0.00
e → b mig per gen	5.73	4.11	0.0009	63.94	0.00

Note: Demographic models and parameters were optimized in GADMA2. We report parameters for only the most recent time slice. Models are ordered by likelihood from left to right. For both time periods, three populations were measured: Newport Bay (n), Bay Area (b), and East California (e).

TABLE 2 Mean Fst divergence between eastern California and each of the four tidal marsh populations of the Savannah sparrow in both the historic and modern datasets.

Population	Historic sample size	Modern sample size	Number SNPs	Historic mean Fst (SD)	Modern mean Fst (SD)
Newport Bay	10	11	4495	0.098 (0.008)	0.099 (0.009)
Morro Bay	11	8	5408	0.054 (0.003)	0.062 (0.006)
Bay Area	31	37	12,021	0.015 (0.001)	0.013 (0.001)
Humboldt Bay	10	5	6466	0.014 (0.001)	-0.002 (0.002)

changes over the past 100 years (Figure S8); however, ΔFst for Bay Area outlier loci remained significantly greater than ΔFst estimated from a simulated history of recent migration rate change (Figure S8).

No genes were found to be outliers in all four coastal populations, but two genes were outliers in three populations (Figure 4a;

Table S2). This includes the estrogen receptor 1 gene (ESR1), which was an outlier in all populations except Humboldt Bay. Moreover, a T → C mutation in the 3' UTR region in the final exon of ESR1 exhibited the greatest per SNP Fst divergence in both Newport Bay (Figure 4c; $Fst=0.73$) and the Bay Area (Figure 4d; $Fst=0.41$). This

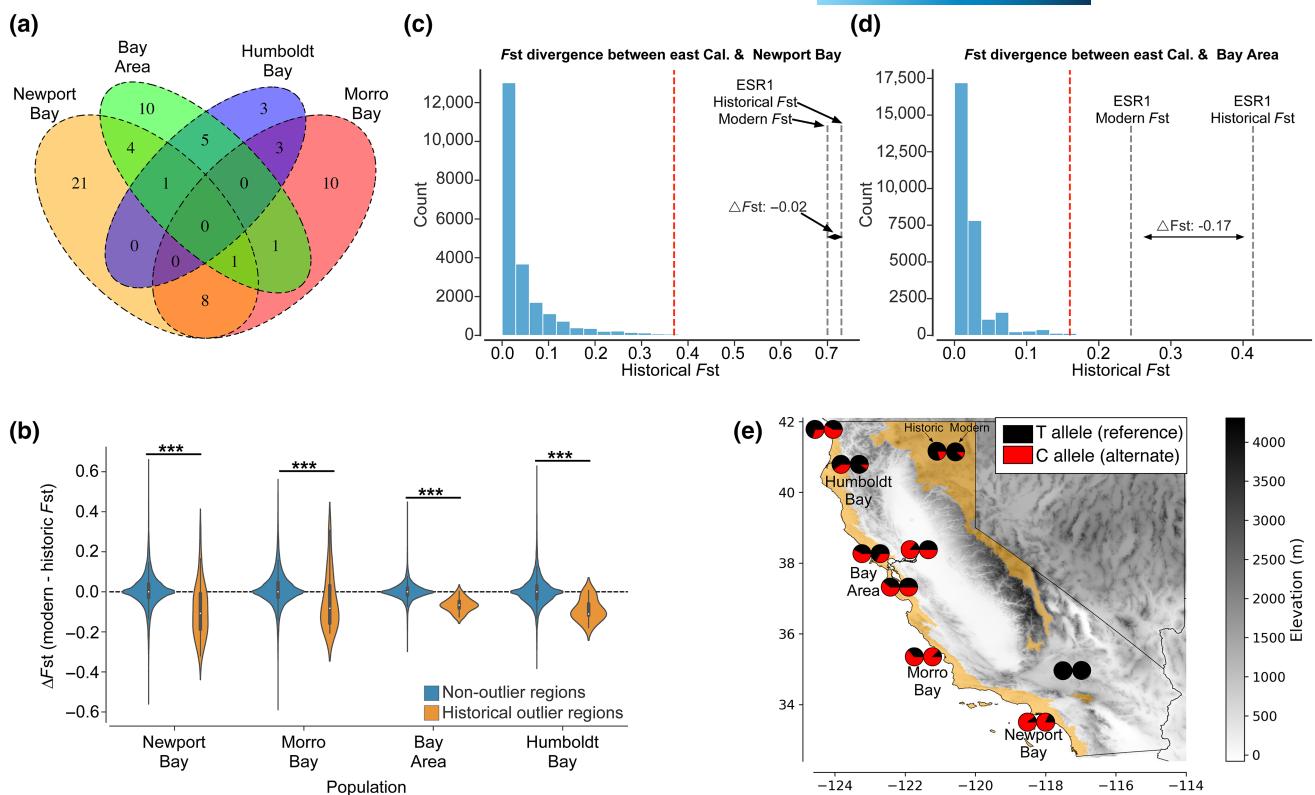


FIGURE 4 Temporal variation in F_{ST} divergence estimated for genes found to be outliers in F_{ST} , D_{XY} , and LFMM analyses. (a) Venn diagram showing overlap in genes with outlier SNPs across the four tidal marsh populations. (b) Change in F_{ST} divergence between eastern California and four tidal marsh populations of Savannah sparrow. ΔF_{ST} for each locus was estimated as the difference between historic and modern F_{ST} divergence, with positive values indicating increasing F_{ST} divergence through time. Asterisks denote p -values from t -tests of ΔF_{ST} between outlier and non-outlier SNPs (ns: non-significant; * $p < .05$; ** $p < .01$; *** $p < .0001$). (c, d) Distribution of per SNP F_{ST} estimates for historical comparison between Newport Bay (*P. s. beldingi*) and Bay Area (*P. s. alaudinus*) with eastern California (*P. s. nevadensis*). The red dashed line denotes the mean 99th percentile threshold for calling outlier SNPs. The gray dashed lines represent modern and historic F_{ST} estimates for a C → T mutation in the 3' UTR region of the final exon of the estrogen receptor 1 gene (ESR1). (e) Spatial variation in allele frequencies for the ESR1 T → C SNP across nine sampling localities with paired modern and historic sampling. Pie charts on the left illustrate historical allele frequencies, and pie charts on the right illustrate modern allele frequencies. Map lines delineate study areas and do not necessarily depict accepted national boundaries.

SNP exhibited large temporal declines in the Bay Area ($\Delta F_{ST} = -0.17$), which was likely due to decreasing frequencies of the derived C allele of this SNP in the Bay Area (change in frequency -0.24 ; Figure 4e) as opposed to allele frequency change in the ancestral T allele that occurs at higher frequency in eastern California. Again, patterns at the ESR1 locus are consistent with temporal changes in gene flow eroding divergence at potentially locally adapted loci.

Finally, we explored whether increased gene flow into coastal marsh populations led to increased homogenization of allele frequencies through time. These analyses showed that correlations between historic deviation and temporal change in allele frequency were significantly different from zero for both the Bay Area (mean correlation: 0.17; p -value = .015) and Humboldt Bay tidal marsh populations (mean: 0.30; p -value = .001), but not significant for Morro Bay (mean: 0.16; p -value = .08) or Newport Bay (mean: 0.05; p -value: .33; Figure 4a). This indicates that populations shown to experience increased levels of gene flow through time have become more similar to the eastern California population across all SNPs. Second, we found that outlier loci exhibit significantly more positive

correlations relative to non-outlier loci across all four coastal populations (Figure 4a), suggesting that these loci are not more resilient to the homogenizing influence of gene flow. Finally, the degree of correlation varied significantly with the extent to which inland eastern California ancestry increased in each tidal marsh population through time ($r^2 = .92$; p -value = .03; Figure 4b). This result remained regardless of the threshold used to identify outlier SNPs (thresholds: 0.9, 0.95, and 0.99th percentile of F_{ST} divergence). In sum, ΔF_{ST} and temporal allele frequency change results both indicate that the inferred increase in gene flow levels from the freshwater-adapted, eastern California population into tidal marsh populations eroded divergence at loci potentially associated with adaptation to a high salinity environment.

4 | DISCUSSION

There is a pressing need to understand the ecological and demographic factors that influence population genetic responses

to habitat loss and environmental change. Here, we leveraged a spatially replicated time series of specimens to document the impacts of habitat loss on tidal marsh populations of the Savannah sparrow in California. We found that five of six tidal marsh populations exhibit evidence for declines in genetic diversity, in line with expectations that coastal populations would experience the greatest overall loss. However, the amount of tidal marsh habitat lost was not linked to patterns of genetic diversity declines (Figure 2d). Rather, we showed that: (1) historic levels of genetic diversity in southern California were lower than in northern California (Figure 2a–c); (2) modern levels of genetic diversity were correlated with the historic extent of tidal marsh habitat in each estuary (Figure 2e); and southern populations have likely remained small since divergence from northern populations ~250 kya (Figure 3a). Further, despite experiencing nearly 90% tidal marsh habitat loss, populations from the Bay Area (San Francisco, San Pablo, and Suisun Bays) still exhibit similar levels of genetic diversity to other northern California populations (Figure 2c). While habitat loss and fragmentation likely played a primary role in observed declines of genetic diversity, our analyses point to more complex demographic dynamics contributing to overall patterns of changing genetic diversity among these populations.

In other species, a disconnect between habitat loss and genetic diversity declines has been attributed to a range of different demographic, life history, ecological, and behavioral factors (Lino et al., 2019; Welch et al., 2012). Many of these factors are unlikely to contribute to the observed differences in genetic diversity between southern and northern tidal marshes, as generation time, body size, and migratory behavior are all similar across coastal populations. Differences in the degree of specialization in tidal marshes could contribute to declines in genetic diversity. Tidal marsh habitats are the stronghold for *P. s. alaudinus* populations in northern California, but this subspecies can regularly be found breeding in coastal grasslands (Grinnell & Miller, 1944). This greater niche breadth may buffer them from tidal marsh habitat losses. Indeed, populations from Humboldt Bay shifted from tidal marsh into pasture habitats as pasture land expanded at the expense of tidal marsh habitat (Fitton, 2008).

Reduced genetic diversity declines in the Bay Area may also be due to temporal changes in gene flow rates among populations. Population structure (Figure 1c), demographic (Figure 3; Table 1), and simulation analyses (Figure 3c,d) all support a recent shift to greater immigration from eastern California into the Bay Area. These results support the theoretical work that habitat loss and subsequent population declines can leave populations more susceptible to hybridization and gene flow (Todesco et al., 2016). Further, differences in N_e between populations will influence the direction of gene flow, with greater gene flow expected from larger into smaller populations (Currat et al., 2008). Finally, theoretical work suggests that as local population growth declines, regular migration from source populations will be needed to maintain diversity within a sink population (Dias, 1996; Gaggiotti & Smouse, 1996). Our empirical results and theoretical work together suggest that habitat loss may have led to declining local recruitment and population growth within

the Bay Area, resulting in the Bay Area becoming a sink population, sustained by migration from eastern California.

The impact of gene flow on the persistence of natural populations is hotly debated in the conservation genetics literature (Bell et al., 2019; Frankham et al., 2011; Tallmon et al., 2004). Gene flow can be crucial for preventing or reversing inbreeding depression (Fitzpatrick et al., 2020; Hogg et al., 2006; Westemeier et al., 1998). Gene flow can also contribute critical standing variation for adaptation and may be important for future responses to climate change (Taylor & Larson, 2019). However, gene flow could also play a role in eliminating divergence between populations and may lead to outbreeding depression (Edmands, 2007). Our data show that coastal populations experiencing higher levels of gene flow do exhibit reduced declines in π (but not Watterson's θ) over time. However, we also show that increased immigration into the Bay Area through time likely contributed to the homogenization of outlier regions associated with tidal marsh adaptation (Figures 4 and 5). Our findings contrast with a recent study on Trinidadian guppies, which found that allele frequency differences in outlier loci were more resistant to experimentally induced gene flow than non-outlier loci (Fitzpatrick et al., 2020). This study also showed that increasing gene flow from main-stream environments led to increased fitness in headwater guppy populations, but the study was performed only for a limited time frame of ~6 generations. It will be critical for the future management of the tidal marsh sparrow populations to determine whether increased gene flow contributes to reduced fitness and outbreeding depression. However, the current study remains an important natural replication of the guppy experiment, showing that shifting gene flow rates in response to human-induced habitat degradation can lead to the greater erosion of locally adapted loci.

Linking outlier loci from genome scans to performance and fitness is a persistent challenge in studies of non-model organisms (Storz & Cheviron, 2021). Despite this caveat, over 40% of identified outlier loci may contribute to increased osmoregulatory performance in high-salinity tidal marsh environments via their known roles in kidney development, function, and pathology (Table S3). Only the estrogen receptor 1 (ESR1) and IgGFc-binding protein-like (FCGBP-like) genes were found to be outliers across multiple populations. The ESR1 gene in birds has primarily been linked to differences in aggressive behavior (Merritt et al., 2020; Tuttle et al., 2016). However, ESR1 is also widely expressed in the mammalian kidney (Jelinsky et al., 2003) and contributes to the generation of important osmoregulatory phenotypes matching those found in tidal marsh sparrows (Benham & Cheviron, 2020; Goldstein, 2006). These include larger kidneys (Lovegrove et al., 2004) and the production of a more concentrated urine through the inhibition of Aquaporin-2 expression in the collecting duct of rodent kidneys (Cheema et al., 2015). Salinity and temperature are expected to increase in northern California in the near future and could exacerbate the osmoregulatory stress experienced by these sparrows (Callaway et al., 2007). If the ESR1 and other candidate genes identified in this study contribute to important osmoregulatory functions, declining allele frequencies due to gene flow may reduce the adaptive capacity of these Bay Area birds.

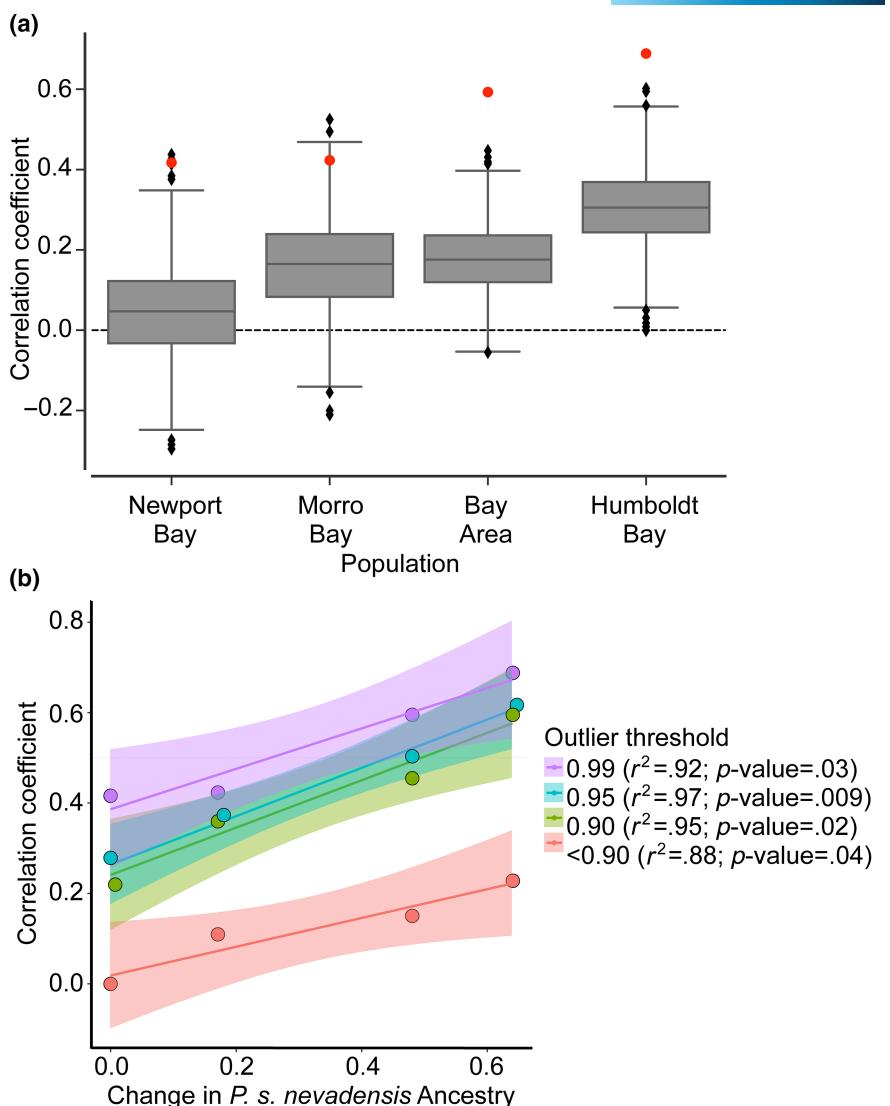


FIGURE 5 Temporal patterns of allele frequency change across four tidal marsh Savannah Sparrow populations. (a) Correlations between the historical deviation from mean allele frequency and direction of temporal change in each tidal marsh population. More positive correlations indicate greater changes in allele frequency toward the mean due to higher levels of gene flow. Red circles show the observed correlation for outlier SNPs, and gray box plots indicate the null distribution from 1000 permutations of non-outlier SNPs. (b) Regression analyses showing relationship between the change in the proportion of ancestry from *P. s. nevadensis* populations in each tidal marsh population and the correlation coefficient for outlier SNPs shown in (a). Ancestry proportions from DyStruct analysis (Figure 1c). Different colors represent SNPs inferred to be outliers from different percentile thresholds, with red points showing data from non-outlier SNPs.

4.1 | Conclusions

Research on geographic variation has long motivated the growth of natural history collections. Consequently, museum collections now provide abundant material for documenting the impact of human-induced ecological change on temporal changes in migration, population size, and selection. Taking full advantage of this unique strength of collections across broad taxonomic scales will be essential for developing a more robust understanding of plant and animal responses to anthropogenic change and translating this knowledge into improved management strategies. Our work demonstrates the power of analyzing these replicate time series of specimens to reconstruct how habitat loss and demography

have interacted over the past century to shape population genetic change. Specifically, we provide novel evidence for human-induced habitat loss transforming a locally adapted population from a source population to a sink with associated declines in the divergence of locally adapted loci.

AUTHOR CONTRIBUTIONS

Phred M. Benham: Conceptualization; data curation; formal analysis; funding acquisition; investigation; resources; writing – original draft; writing – review and editing. **Jennifer Walsh:** Conceptualization; resources; writing – review and editing. **Rauri C. K. Bowie:** Conceptualization; funding acquisition; resources; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Sample information and meta-data can be found in Appendix S1. The code for all analyses performed in this paper can be found on Github at: https://github.com/phbenham/SAVS_temporal_genomic_change and <https://doi.org/10.5281/zenodo.10386033>. Finally, raw sequence data for all individuals has been submitted to GenBank under BioProject PRJNA1043693 (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1043693>).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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