SAN FRANCISCO ESTUARY & WATERSHED SCIENCE
Sponsored by the Delta Stewardship Council and UC Davis Institute of the Environment

RESEARCH

Combining Ecological and Genomic Diversity Surveys to Inform Conservation and Restoration of an Endangered Wetland Plant, Soft Salty Bird's-Beak (*Chloropyron molle* ssp. *molle*)

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ABSTRACT

Emergent tidal wetlands are declining globally as a result of sea level rise and land use change. This habitat loss can keenly affect rare plant species within wetlands, and may require restoration to meet species recovery goals related to retaining populations throughout species' ranges. Soft salty bird's-beak (*Chloropyron molle* ssp. *molle*) is a federally- and state-endangered hemi-parasitic plant that occurs at the upper marsh transition zone in the San Francisco Bay–Delta, California, USA. We combined field surveys to document habitat associations and trends in abundance with genomic surveys to understand patterns of genetic structure in this rare endemic. We found that *C. molle* ssp. *molle* persisted at nine previously occupied marsh sites, although four sites (Hill Slough, MOTCO East, Fagan Marsh, and Joice Island) were smaller in population size than when surveyed in the 1990s. Additionally, twelve sites contained plots with suitable but unoccupied habitat that could be further assessed for restoration. Genomic analysis of over 40,000 single-nucleotide polymorphisms (SNPs) and 253 individuals grouped *C. molle* ssp. *molle* into six to seven regional genetic clusters with isolation by distance, and confirmed that *C. molle* ssp. *molle* is genetically distinct from adjacent populations of its closest relative (*C. molle* ssp. *hispidum*). The western-most *C. molle* ssp. *molle* sites of Point Pinole and Fagan Marsh were the most genetically and geographically isolated and had the lowest genome-wide diversity. Heterozygosity in sets of genes associated with tidal elevation, salinity, and annual and summer precipitation varied independently across populations. Overall, these genomic patterns indicate that selecting donor sites with similar environmental conditions and utilizing composite seeding approaches from multiple sites could allow for local adaptation to a range of possible environmental conditions. This comprehensive survey of habitat and genomic patterns can allow for the development of restoration actions and build climate-adaptation planning to help prevent the loss of a rare plant.

SFEWS Volume 23 | Issue 2 | Article 5

https://doi.org/10.15447/sfews.2025v23iss2art5

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KEY WORDS

population structure, single nucleotide polymorphisms, adaptation, abundance, habitat suitability, annual plant, sea level rise, restoration

INTRODUCTION

Tidal wetlands occupy a unique landscape position between marine and terrestrial ecosystems, and include host plant communities that are influenced by both ocean waters and terrestrial inputs. Plant zonation patterns across tidal wetlands depend on elevation, hydrologic factors, soil conditions (salinity, redox state, texture, moisture content) and species interactions (Traut 2005). On the upslope boundary, tidal wetlands grade into an upper transition zone (ecotone) that is infrequently flooded by the tides but is still influenced by salinity and occasional flooding (Anderson et al. 2022). In some areas these transition zones are relatively small, given steep physical gradients or small watersheds (Wasson and Woolfolk 2011). This tidal wetland–upland transition zone has been substantially reduced in many areas as a result of coastal development, where it has been replaced with dikes or levees (Atwater and Hedel 1976).

Further, tidal wetlands are imperiled by climate change and rising sea levels (Schile et al. 2014; Thorne et al. 2018). Tidal wetlands will need to build elevation to outpace the rates of sea level rise to prevent submergence, or have space to migrate inland (Thorne et al. 2018; Osland et al. 2022). However, many estuaries have landscapes that have been altered, which will prevent inland migration of tidal wetlands, and in those cases "coastal squeeze" will occur (Osland et al. 2022). When a tidal wetland cannot migrate inland from sea level rise, increased flooding of the habitat will shift vegetation to more flood-tolerant plant species (Costa et al. 2003); therefore, species that reside within the upper transition zone may not persist over the long term if flooding in that zone increases (Kirwan and Guntenspergen 2015). To ensure habitats persist over the coming decades, managers are developing climate-adaptation strategies to reduce effects on tidal wetland and plant populations, (Kassakian et al. 2017). However, the fates of this transition zone and the specialized plant species that inhabit it are largely unknown.

Preserving tidal wetland plant species will rely on both protecting existing populations and active habitat restoration, such as transplantation and establishment of new populations (Grewell et al. 2013). Relocating species or populations at risk from climate change is an emerging concept, and for most species the baseline habitat and genomic information needed to make an informed decision is lacking (Rout et al. 2013 Hällfors et al. 2014; Butt et al. 2021). Genomic data can help assess population status, gene flow, and adaptive potential of affected tidal wetland species and inform their conservation management strategies (Ellstrand and Elam 1993; Frankham et al. 2002; Milano et al. 2020). Factors such as levels of diversity—particularly in adaptive genes—and genetic relatedness within and among sites can influence compatibility and survivorship of transplanted individuals or outcrossed offspring (McKay et al. 2005).

An archetype for upper-transition-zone specialists, soft salty bird's-beak, *Chloropyron molle* (A. Gray) A. Heller ssp. *molle* (hereafter *C. molle molle*) resides in tidal brackish wetlands in San Pablo Bay and Suisun Bay, California, USA (Figure 1) and was listed as a federally endangered subspecies in 1997 (USFWS 1997). *Chloropyron molle molle* is an annual hemi-parasite found in specific environmental conditions associated with the upper transition zone of intertidal wetlands (USFWS 2009, 2013). Major threats to the persistence of *C. molle molle* include habitat invasion by non-native vegetation, hydrologic alterations, and sea level rise with limited opportunities for landward migration of habitat (USFWS 2013). At the time of our study, *C. molle molle* was believed to be extant at nine geographically distinct wetlands (Table 1 in Grewell et al. 2013; USFWS 2013). These included wetlands in Napa, Solano, and Contra Costa counties, while those in Marin, Sonoma, and Sacramento counties were presumed extirpated; however, a full survey of abundance across all wetlands had not been completed in almost 3

decades. Although genomic analyses have been conducted on congeners (*C. palmatum* [Ferris] Tank & J. M. Egger, Ayres 2015; *C. martimum* (Benth.) A. Heller ssp. *maritimum*, Milano et al. 2020), no analyses explored levels of genomic variation and differentiation within and among populations of *C. molle molle*. In addition, the genetic relationship with geographically proximate populations of the other recognized subspecies, *C. molle* (A. Gray) A. Heller subsp. *hispidum* (Pennell) Tank & J. M. Egger (hereafter *C. molle hispidum*), had not been investigated, although these two taxa were found to be closely related in a broader molecular phylogenetic study (Gilman and Tank 2018).

In this study, we sought to document the current geographic distribution of *C. molle molle*, quantify suitable habitat conditions, and provide information on the distribution of genomic diversity across the extant range. We hypothesized that inundation and wetland inland migration from rising sea levels could lead to shifting distributions and lower abundance as a result of "coastal squeeze." We also expected that population structure would be localized, with genetic differentiation among sites, given a patchy distribution and large geographic distances among known remaining populations. Our results can help inform the status of the taxon and inform efforts to locate suitable habitat and appropriate source populations to help future restoration and re-establishment in response to sea level rise (Hughes et al. 2008; Engelhardt et al. 2014).

MATERIALS AND METHODS

Site Selection and Field Surveys

We conducted a range-wide survey to locate *C. molle molle* based on historical observations. We selected survey locations within the San Francisco Bay–Delta based on a literature review of all reported locations, known habitat preferences for the subspecies, and land access. In 2021, we visited 28 wetlands and from these, selected 25 for intensive *C. molle molle* surveys (Figure 1; Table A1). At nine of these sites, *C. molle molle* was extant as of 1993 (Ruygt 1994). We included the remaining sites because they contained presumed extirpated populations, restored populations (including a restoration at Spring Branch Creek in the Rush Ranch Open Space Preserve seeded in 2000; Figure A1), or potentially suitable habitat.

Field surveys were conducted during the expected peak blooming period to maximize identification of *C. molle molle* (June–September). Plants were identified to subspecies using morphological characters provided in the Jepson eFlora *Chloropyron* key to species (Jepson Flora Project 2021). Transect locations were selected throughout each site at ground surface elevations that corresponded to mean higher high water tidal datums plus or minus a buffer (San Francisco Bay: Buffington and Thorne 2018; Suisun Bay: Buffington et al. 2019). The number of transects varied by site size and area of target habitat. We selected sample plots along the transects at every vegetation transition, to capture community and soil changes along the elevation gradient from low marsh to upland. At each plot, we collected population, vegetation, elevation, and soil data and tissue samples of *C. molle molle,* when present, for genetic analysis. Additionally, we walked the transition zone of each wetland, spot-surveying for *C. molle molle* occurrences outside of the pre-selected transects.

Where we encountered *C. molle molle* within a site, we delineated the patch using Leica survey-grade Global Navigation Satellite System (GNSS) rovers (Viva GS15 and RX1250X models) with Global Positioning System (GPS) real-time kinematic (RTK) corrections (manufacturer-published horizontal precision ±1 cm and vertical precision ±4 cm; Leica Geosystems Inc., Norcross, Georgia, USA). Data corrections were streamed to the rover via an internet connection to GNSS base-station networks (Leica Smartnet, *www.smartnetna.com*), with the average measured vertical error within the ±2-cm error of the RTK at local benchmarks. *Chloropyron molle molle* patch elevation was combined with water levels from the nearest National Oceanic and Atmospheric Administration (NOAA) tide stations (Table A1) to calculate population-specific tidal datums and relative elevation position to mean higher

https://doi.org/10.15447/sfews.2025v23iss2art5

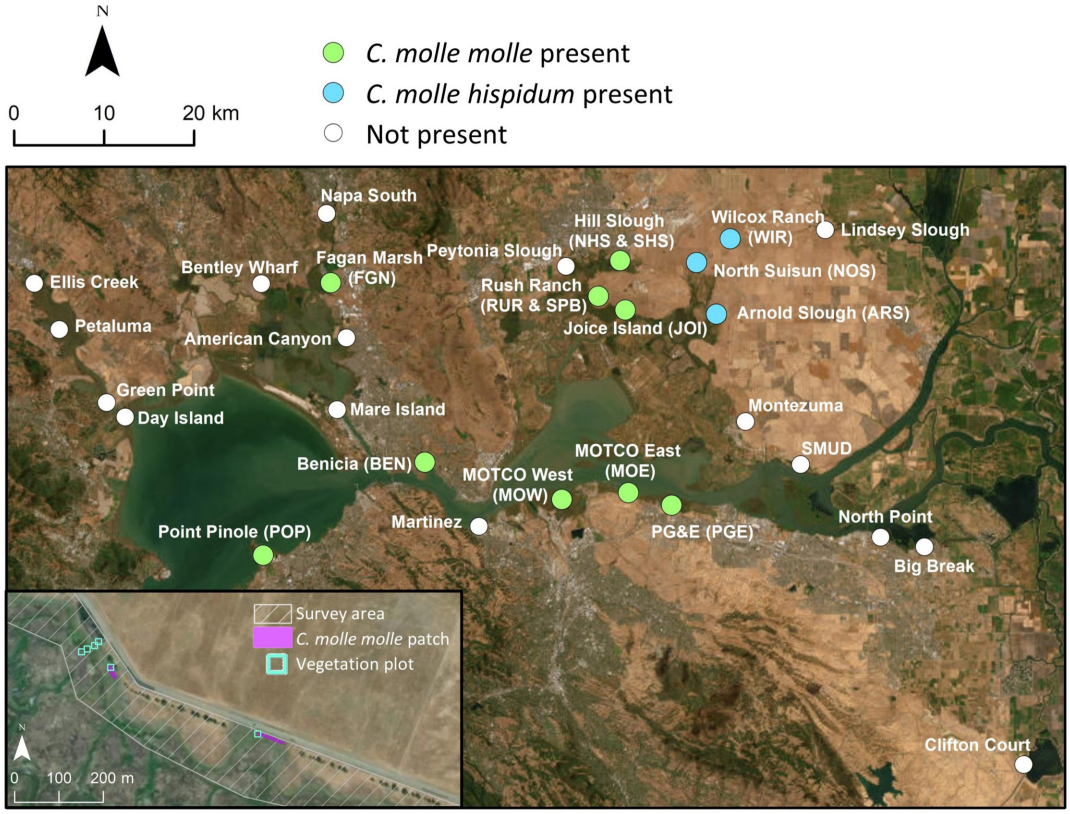

Figure 1 Site map of all locations surveyed intensively for *Chloropyron molle* ssp. *molle* (*C. molle molle*) and where tissue was collected for genetic analyses in the San Francisco Bay–Delta, California. *Colors* indicate the presence of *C. molle molle* (*green*), *Chloropyron molle* ssp. *hispidum* (*C. molle hispidum, blue*). *C. molle* sites include three-letter codes used in genetics analysis. **Inset map:** Example survey with approximate search area, *C. molle molle* patches, and vegetation plots as a transect through the transition zone and at *C. molle molle* patches.

high water (hereafter EMHHW). Each patch was assigned an abundance rank using a logarithmic scale from 1 to 5, where 1=1 to 10 individuals, 2=11 to 100 individuals, 3=101 to 1,000 individuals, 4=1,001 to 10,000 individuals, and 5=10,001 to 100,000 individuals (e.g., Grewell et al. 2003). We evaluated distribution and abundance changes over time by comparing patch-scale locations and site-scale abundances to those recorded in the last range-wide survey (Ruygt 1994). All sites were surveyed as described above, except for Benicia State Recreation Area, where access permissions for this project were limited to tissue collection for genetic analysis, given other ongoing population surveys.

We collected leaf tissue from up to 23 plants at each site where *C. molle molle* and *C. molle hispidum* were observed (Figure 1). We selected plants for genetic sampling that were dispersed throughout each patch, and prevented cross-contamination of samples using a bleach solution to wipe forceps between samples. Collected leaves were immediately placed in coin envelopes containing silica gel for rapid desiccation, and stored in a desiccant cabinet upon return from the field. The US Geological Survey (USGS) in San Diego, California, conducted the DNA extraction and analysis. Voucher specimens were collected when permit conditions were met, and were deposited in the University of California–Davis, Center for Plant Diversity (refer to Specimen ID numbers in Table A1).

Habitat Suitability

We quantified habitat characteristics at all sites regardless of *C. molle molle* presence. We conducted vegetation surveys to identify host

plant species where *C. molle molle* was present, and dominant plant cover at plots throughout the transition zone (Figure 1). All plants observed within 1-m^2 plots were recorded and identified with taxonomic keys to species (Baldwin et al. 2012). In addition, soil samples were collected from the top 10 cm of soil to characterize porewater salinity along the wetland-to-upland transition at all sites, including the specific zone where *C. molle molle* occurred, or, if absent, was expected to occur.

We used habitat characteristics measured within vegetation plots that contained *C. molle molle* to define suitable habitat. We analyzed the remaining plots to identify other areas with suitable habitat. We defined suitable habitat characteristics as values within the 95% range of elevation and salinity for *C. molle molle* plots and the presence of species commonly found within *C. molle molle* plots. Species were considered associated with *C. molle molle* if species were found at more than one-half of the plots that contained *C. molle molle* and had an average cover around 10% or greater. To determine suitable habitat, we used non-metric multi-dimensional scaling in the 'vegan' package (Oksanen et al. 2020) in R (R Core Team 2022) to compare plant communities at plots with and without *C. molle molle*.

Tissue Extractions and Genomic Data Collection

Dried leaf tissue was extracted using the E-Z 96 Plant DNA DS Kit (Omega Bio-Tek, Norcross, Georgia, USA) according to manufacturer's guidelines, with the following modifications: dry plant tissue was first pulverized using one 3.5-mm stainless steel bead and one 3.2-mm stainless steel bead in a Bullet Blender Storm 24 (Next Advance, Troy, New York, USA), and samples were eluted once with 100 µl of nuclease-free water.

We quantified DNA on a Qubit fluorometer (Life Technologies, Waltham, Massachusetts, USA), and used 500 nanograms (ng) of genomic DNA for next-generation sequencing (NGS) library preparation. We followed the double-digest, restriction-associated DNA (ddRAD) sequencing protocol developed by Peterson et al. (2012) for NGS library preparation, modified to use separate indexing reads. We digested genomic DNAs using 20 units each of the restriction enzymes EcoRI and MseI (New England Biolabs, Ipswich, Massachusetts, USA). We used Solid Phase Reversible Immobilization (SPRI) beads (Beckman Coulter, Brea, California, USA) to purify the digestions before ligating 32 uniquely bar-coded adapters with T4 ligase (New England Biolabs). Ligation products were quantified on the Qubit fluorometer, pooled across index groups in equimolar concentrations, and then fragments between 250 and 400 base pairs (bps) were selected using a Pippin Prep-size fractionator (Sage Science, Beverly, Massachusetts, USA). We amplified the recovered fragments from each pool using 5 to 10 ng of the recovered DNA, Phusion High-Fidelity Taq (New England Biolabs), Illumina's common primer (Illumina, Inc., San Diego, California, USA) and 12 unique indexes. We then cleaned polymerase chain reaction (PCR) products with SPRI beads and quantified them using the Qubit fluorometer (Life Technologies) before pooling them for sequencing. MedGenome, Inc (Foster City, California, USA) sequenced 100 bp paired-ends on an Illumina Novaseq 6000 S4 lane.

Bioinformatics and Population Genomic Analyses

Raw sequence de-multiplexing, quality filtering, and genotyping were performed using Stacks v2.60 on the USGS Yeti High Performance Computing platform (Falgout and Gordon 2020). Clustering, assembly, and filtering parameters were optimized using a subset of individuals that followed the r80 method by Paris et al. (2017). This subset included 70 individuals that were distributed across collection locations and with read coverages that fell within one standard deviation of overall mean retained reads per sample. This subset was also used to create a locus catalog (cstacks) for the full Stacks genotyping pipeline. The following final parameters were used: minimum number of raw reads required to form a stack (putative allele), m = 3; maximum number of mismatches allowed when matching stacks (putative alleles within loci) within samples, M = 2; maximum number of mismatches between stacks (putative loci) of the samples and the catalog of loci among all samples, n = 1; minimum percentage

https://doi.org/10.15447/sfews.2025v23iss2art5

of individuals across populations required to process a locus, R = 80 (Catchen et al. 2013). We further filtered the number of samples and loci by requiring a minimum depth of coverage of 9x and less than 30% missing data per sample. One single-nucleotide polymorphism (SNP) was randomly chosen from each locus then rerun in the populations program in Stacks version 2.60 to calculate genetic differentiation among sampling locations (FST and ϕST). This dataset was exported in variant call format (.vcf) and ordinary PLINK (.ped) formatted files for downstream analyses, and contained all *C. molle molle* and *C. molle hispidum* that were retained after quality filtering.

For genetic analysis, Hill Slough was divided into North and South Hill Slough (NHS and SHS). The Spring Branch restoration site was seeded primarily using donor seeds from NHS and Joice Island (Grewell et al. 2003). Genetic clustering analyses were used to explore the pattern of genetic structure among all samples. We implemented a principal component analysis (PCA) in 'adegenet' version 2.1.3 (Jombart 2008), using R version 4.0.5 (R Core Team 2022), to visualize the genotypes in multi-dimensional space. We also applied the maximum-likelihood approach of ADMIXTURE (Alexander et al. 2009), using the PLINK file, to define the best supported number of genetic clusters (K) in the data fit to a population genetic model. We performed five replicate analyses to evaluate up to 14 ancestral populations (all sampled sites). To assess the best value of K, we performed 10-fold cross-validation, determined the K-values with the lowest cross-validation error, and examined the individual assignment plots. We visually examined PCA and ADMIXTURE plots by both subspecies and site groupings. To further assess spatial patterns in genetic structure among sites, we examined genetic isolation (FST) by geographic distance. We calculated geographic distances as Euclidean distances among sites, and used a Mantel test to examine correlation with FST among all pairs of sampled sites. We assessed isolation by distance among *C. molle molle* sites, and among all sites (both subspecies). We included an indicator matrix for subspecies designation, and—after controlling for geographic distance—used a partial Mantel test to determine whether subspecies were significantly differentiated (p-value ≤ 0.05).

Lastly, we examined gene-environment associations to identify subsets of loci associated with climatic and hydrological variation across sites. We used a redundancy analysis (RDA; van den Wollenberg 1977) to determine how groups of loci covary in response to climate. Redundancy analysis first uses multiple linear regression of response variables (genotypes) on predictor variables (environmental data). Then a matrix of the fitted values of all response values is subjected to PCA. Analyses were performed in 'vegan' version 2.5-6 (Oksanen et al. 2020) in R 4.2.1 (R Core Team 2022). Thirty-three bioclimatic variables for the climate normal 1991–2020 were downloaded from AdaptWest (*https://adaptwest.databasin.org/pages/adaptwest-climatena/*). We generated variables using ClimateNA version 7.3 at a 1-km^2 grid cell size. We extracted values for the centroids of all surveyed plots that contained *C. molle molle*. We also included EMHHW measured in plots that contained *C. molle molle*, and average porewater salinity (SAL) for each site (averaged across plots that contained *C. molle molle*). Both factors may limit the distribution of this mid- to high-marsh plant; tidal hydroperiod was found to be an important predictor of *C. molle molle* survival and reproductive success (Grewell et al. 2013). We found the bioclimatic variables to be highly cross-correlated, and so retained only two in analysis—mean annual precipitation (MAP) and summer precipitation (PPT_SM)—along with EMHHW and SAL. These four variables had correlation coefficients < 0.75 and variance inflation factors < 10 in the full RDA model. Variables were standardized and analyzed with individual genotypes. Missing data (7%) were imputed using the most common genotype at each SNP, following Forester et al. (2018). We performed a partial RDA, conditioned on X, Y coordinates, to control for inherent isolation by distance in the genetic data. Longitude was strongly negatively correlated with both mean annual precipitation (r = –0.89) and average porewater salinity (r = –0.87). We identified outlier

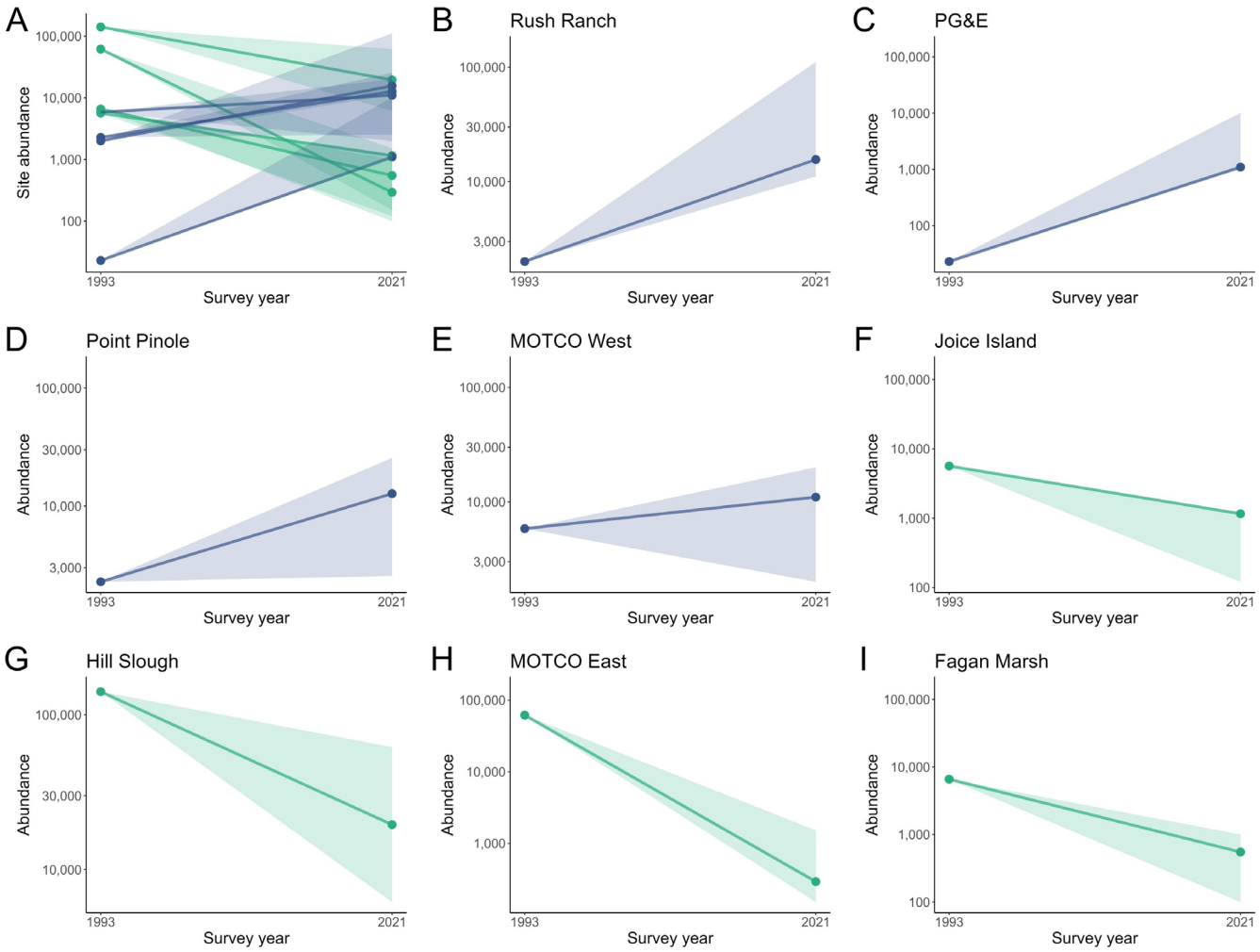

Figure 2 Change in abundance for wetlands with *Chloropyron molle* ssp. *molle* (*C. molle molle*). **Plot A** shows all site changes together, followed by individual site plots (**B–I**) all with logarithmic *y*-axes. Sites with upward abundance trends are in *blue*, and downward trends are in *green*. *Shaded areas* represent the range in abundance values. Most sites had minimal change in population estimates. Hill Slough and MOTCO East (MOE) had the two largest declines in abundance estimates between 1993 and 2021. Spring Branch is not included because it was a restoration site without *C. molle molle* presence in 1993.

loci greater than three standard deviations away from the mean RDA axis scores and examined their associations with each climate variable. We estimated genetic diversity in sets of climate-associated loci and the remaining (neutral) loci and compared these across sites. Diversity statistics were estimated with dartR version 2.0.4 (Mijangos et al. 2022). All genomic results are reported at the site level.

RESULTS

Distribution and Abundance

We found *C. molle molle* present at the same sites last observed in 1993–1994 (Table A1), plus at Spring Branch, a site restored within Rush Ranch after the 1993–1994 surveys. Four of the previous sites had population increases, while four sites had decreases (Table A1; Figure 2). The total abundance estimate from 1993 was about 236,680 individuals, and in 2021, we estimated 62,157, but with a range of 23,414 to 232,140. Although the range-wide abundance of the subspecies appeared lower in 2021 than in 1993–1994, we

https://doi.org/10.15447/sfews.2025v23iss2art5

Table 1 Habitat characteristics and community summaries at vegetation plots containing *Chloropyron molle* ssp. *molle* (n = 30 plots).

Environmental characteristics	Mean	SE	Range	95% Range
Elevation relative to mean higher high water (m)	0.13	0.01	−0.10–0.29	−0.03–0.25
Salinity (ppt)	30.19	3.60	6.51–74.00	7.59–72.05

Community cover	Frequency with *C. molle molle*	Mean Cover (%)	SE	Range	95% Range
Distichlis spicata	100%	44.10	4.78	1–80	3.91–80
Jaumea carnosa	70%	24.19	4.95	1–90	1–70
Bare ground	93%	18.79	3.47	1–60	1–56.63
Salicornia pacifica	100%	11.33	1.70	1–30	1–30
Cuscuta salina	53%	9.69	4.35	1–70	1–51.25

caution this may not indicate a decline because this annual plant can vary substantially in abundance from year to year.

Chloropyron molle molle patch locations in 2021 had varying degrees of overlap with patch locations from 1993 (Table A1). Given the locations of 1993 patches from hand-drawn maps, patches within the same vicinity between years—such as those at Hill Slough (NHS, SHS) and PG&E (PGE)—are likely related. However, there were clear losses of *C. molle molle* patches at some sites, such as Fagan Marsh (FGN; 13 patches in 1993 vs. one patch in 2021) and Joice Island (JOI; 22 patches in 1993 vs. three patches in 2021). We confidently re-surveyed all previous patches at Fagan Marsh because the lead biologist from the 1993 surveys was in attendance during the 2021 visit. MOTCO East and West (MOE and MOW) experienced near complete loss of 1993 patches and appearance of new 2021 patches hundreds of meters away (Table A1). Point Pinole and Rush Ranch had a mixture of patch overlap and recent gains in 2021.

Habitat Suitability

Out of 346 vegetation plots across all surveyed wetlands, 30 plots contained *C. molle molle*. Environmental measurements revealed site-specific narrow ranges in elevation (EMHHW; 0.13 ± 0.01 m) and porewater salinity (30.19 ± 3.60 ppt; Table 1; Figure A2). A total of 18 species were found to co-occur with *C. molle molle* during vegetation surveys (Table A2). Four species were found at more than half of the plots and had an average cover around 10% or greater: *Distichlis spicata* (saltgrass), *Jaumea carnosa* (marsh jaumea), *Salicornia pacifica* (pickleweed) and *Cuscuta salina* (saltmarsh dodder) (Table 1, Table A2). Suitable habitat characteristics were defined as values within the 95% range of elevation and salinity for *C. molle molle* plots and the presence of *Distichlis spicata* and *Salicornia pacifica* (which were both found in 100% of the *C. molle molle* plots; Table 1). Of the sites with existing *C. molle molle* patches, seven had additional vegetation plots with potentially suitable habitat (Figure A3). An additional five sites without existing *C. molle molle* populations had plots with potentially suitable habitat (Figure A3). These additional plots had community composition similar to existing C. *molle molle* plots (Figure 3).

Genomic Data Quality

In total, 279 samples were prepared and sequenced. Of 4.352 billion total reads, 99.6% were retained (mean = 15.5 million reads per sample). After final filtering, 253 samples were retained for population genetic analyses: 205 *C. molle molle* (missing data per sample ranged from 1% to 30% mean = 6.6%) and 48 *C. molle hispidum* (missing data per sample ranged from 3–29%, mean = 10%). The final dataset retained 40,707 loci (or 40,707 SNPs, using one SNP per locus). Average sequence coverage per sample across all loci was 23.2x (range 9.6x–81.9x).

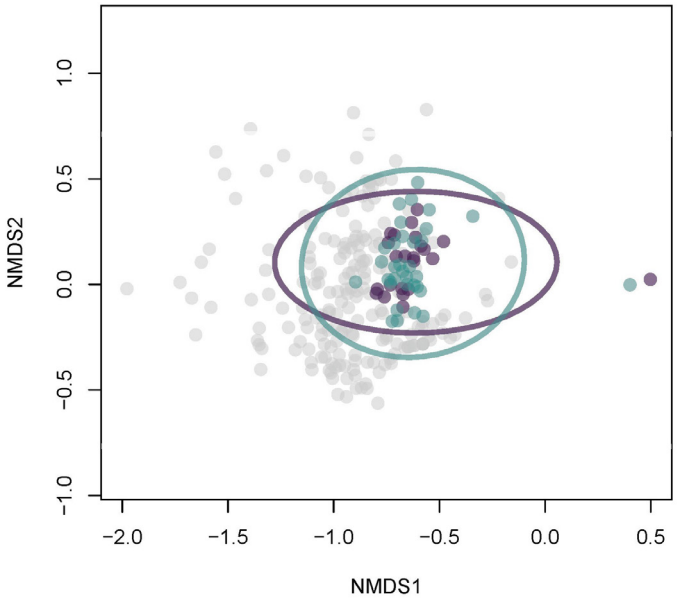

Figure 3 Non-metric multi-dimensional scaling (NMDS) plot showing similar communities between plots with *Chloropyron molle* ssp. *molle* (*C. molle molle*) (*purple*) and those with suitable habitat characteristics (*blue*). Additional plots without *C. molle molle* or suitable conditions are shown in *grey*. *Ellipses* represent 95% confidence intervals.

Genetic Structure and Diversity

The PCA separated *C. molle molle* and *C. molle hispidum* (Wilcox Ranch, North Suisun, and Arnold Slough; WIR, NOS, and ARS, respectively) along Axis 1, accounting for 9.78% of the variance (Figure 4). Axis 2 separated *C. molle molle* sites roughly along an east–west cline (5.17%; Figure 4A). Axis 3 (3.61%) loadings were less clear, but further separated the northeastern *C. molle molle* sites (Joice Island, North Hill Slough, Spring Branch, and Rush Ranch; JOI, NHS, SPB, RUR, respectively) from those to the south and west (Figure 4B).

The ADMIXTURE analysis indicated that the optimal number of genetic clusters (K) in the dataset was either eight or nine (Figure 5, Figure A4). At K=9, two clusters were composed of *C. molle hispidum* sites. The remaining seven clusters were composed of the 11 *C. molle molle* sites (Figure 5B). Sites Point Pinole (POP), Fagan (FGN), Benicia (BEN), and PGE each formed unique clusters (Figure 5C). Neighboring sites MOW and MOE clustered together, as did JOI and RUR. The Spring Branch (SPB) restoration site clustered with NHS and SHS consistent with known seeding history. Overall, more individuals with admixed ancestry were assigned within the northeastern clusters (SPB+NHS+SHS, JOI+RUR) than in the two sites farthest west (POP and FGN); Figure 5C. Point Pinole and Fagan also had the lowest within-site diversity metrics, measured across all loci (Table 2). When examined at K=2, the subspecies assigned separately as expected, with *C. molle hispidum* sites Wilcox Ranch, North Suisun, and Arnold Slough (WIR, NOS, and ARS) in one cluster and all *C. molle molle* sites in the second cluster (Figure A5). There was some evidence of low admixture proportions between the two subspecies localized in NOS and ARS and more proximate *C. molle molle* sites in the northeastern portion of the study area (Figure A5).

Pairwise genetic distances were greatest between sites of different subspecies (average pairwise FST=0.087, PHIST=0.137; Table A3). Among sites with *C. molle molle*, genetic differentiation was slightly lower (average pairwise FST=0.056, range 0.02–0.12; average pairwise ϕST=0.08, range=0.01–0.193; Table A3). Genetic distance and geographic distance were strongly correlated among *C. molle molle* sites (Mantel test r=0.608, $p \leq 0.001$), indicating a stepping-stone pattern of gene flow within the taxon. Genetic distance and geographic distance were also significantly correlated among all *C. molle* sites, including *C. molle hispidum* (Mantel test r=0.6765, $p \leq 0.001$). After controlling for geographic distance, the two subspecies were significantly different from one another genetically (partial Mantel test r=0.544, $p \leq 0.001$). This result indicates strong differentiation between the two taxa.

Total genomic diversity was relatively even across sites, but slightly lower in POP and FGN for most metrics (Table 2: % polymorphic loci, observed and expected heterozygosity and nucleotide diversity). Inbreeding coefficients were low in most sites, indicating *C. molle molle* is predominately outcrossing (Table 2: FIS; Table A4: neutral loci).

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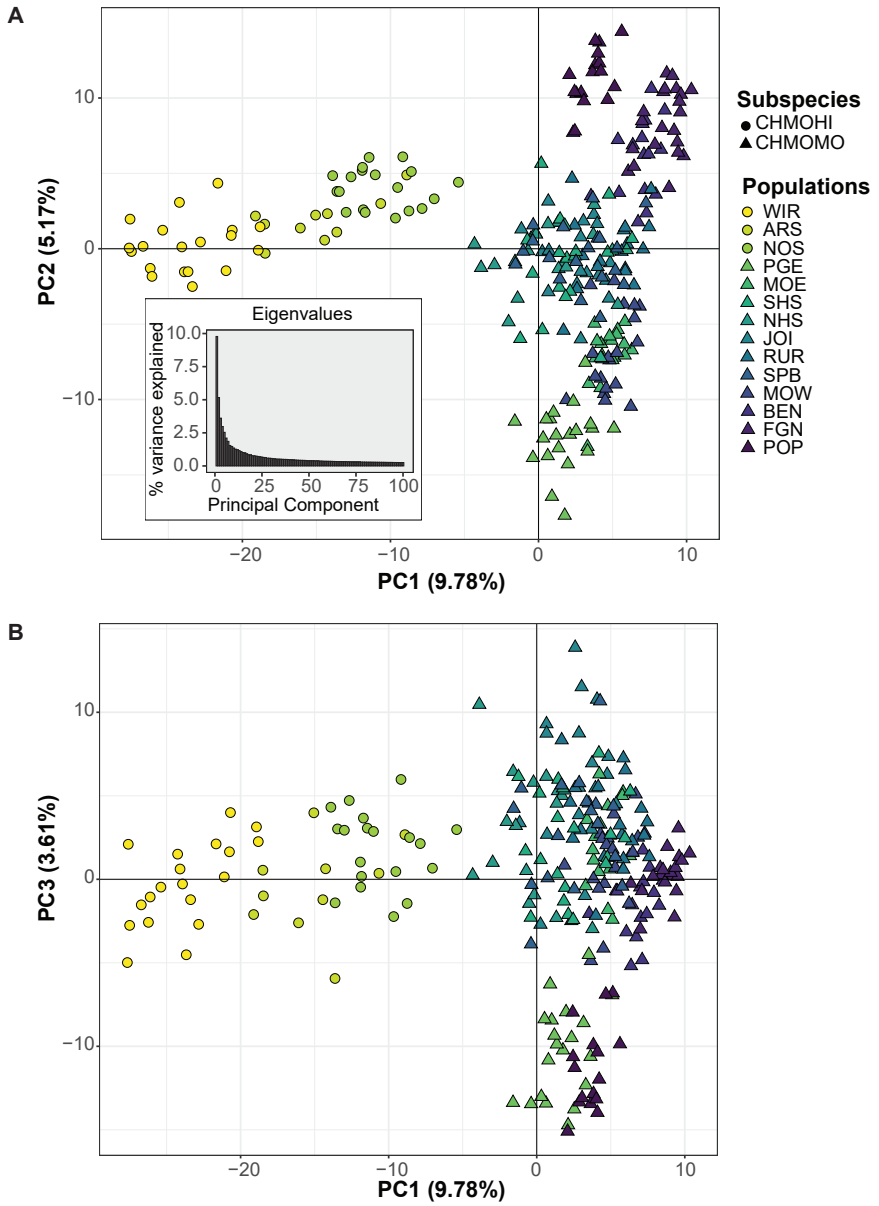

Figure 4 Plots of (**A**) PC1 vs. PC2 and (**B**) PC1 vs. PC3 for the principal component analysis (PCA) of genotypic data of *Chloropyron molle*. *Circles* represent *Chloropyron molle* ssp. *hispidum* individuals, and *triangles* are *C. molle* ssp. *molle* individuals. Populations are arranged in the color ramp from east (*yellow*) to west (*purple*). Refer to Table 2 for site codes.

Gene-Environment Associations

The conditioned RDA model explained 4.1% of the total observed genetic variation (adjusted $R^2 = 0.031$, $p \leq 0.001$). All four RDA axes ($p \leq 0.002$) and environmental variables were significant ($p \leq 0.001$). Variable loadings and explained variance are reported in Table A5, and loadings of individuals on RDA axes are displayed in Figure A6. The outlier analysis identified 1,026 SNPs that loaded ± 3 standard deviations from the mean on one or more of the RDA axes. Based on the strongest correlation between each outlier locus and climate variable, 446 loci were most strongly associated with EMHHW, 235 with summer precipitation, 173 with salinity, and 172 with mean annual precipitation. Patterns in heterozygosity in these sets of loci varied from one another and from the remaining presumed neutral loci (Figure 6; Table A4). Heterozygosity in loci associated with EMHHW was highest in the centrally located population of MOW, while heterozygosity in loci associated with summer precipitation was highest at northeastern sites of JOI, SPB, and RUR, respectively. Loci associated with salinity peaked in heterozygosity in NHS, while mean annual precipitation loci peaked in the far western site of POP. In contrast to these three groups, heterozygosity in the remaining

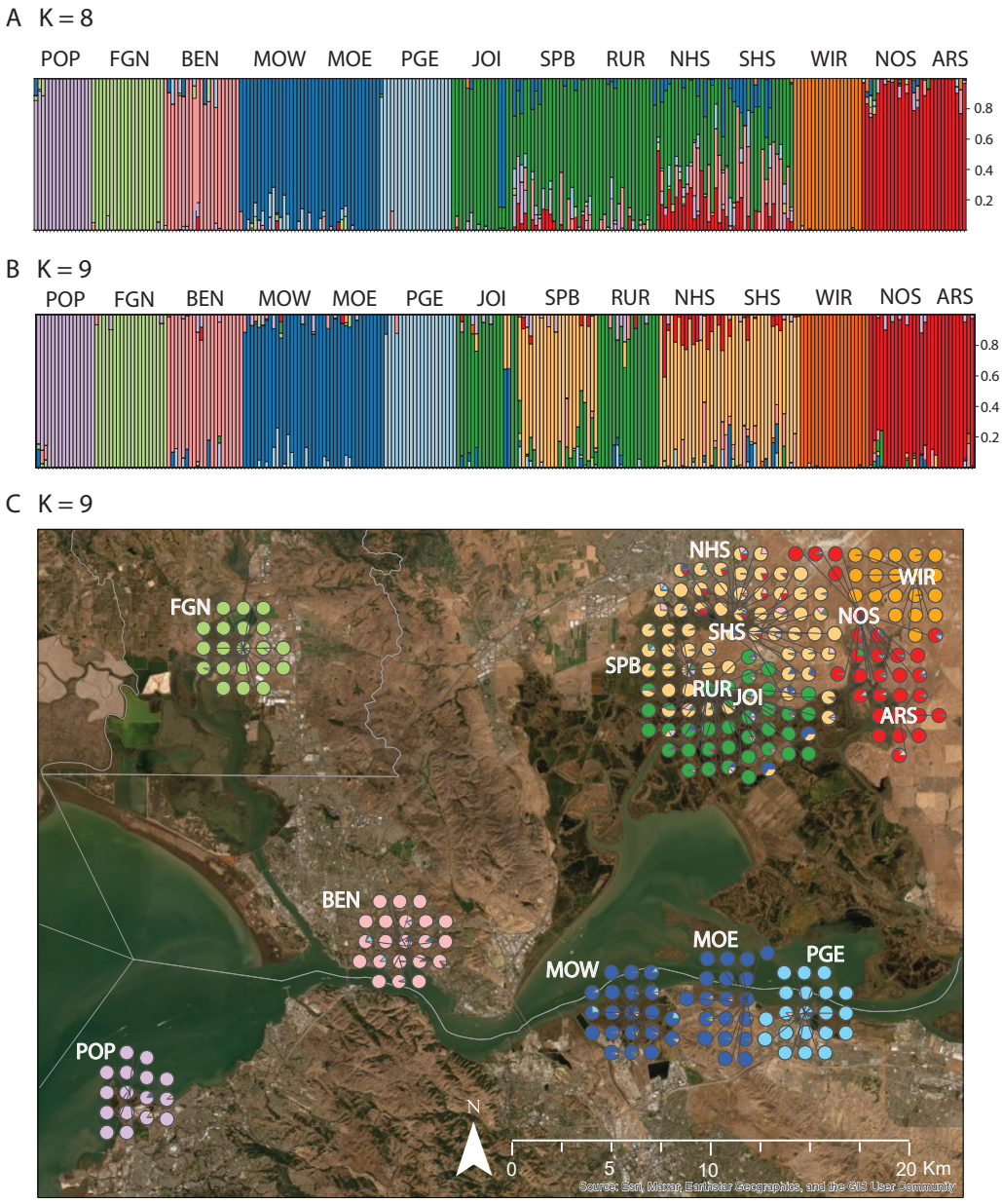

Figure 5 *Chloropyron molle* admixture results including (**A**) admixture assignment bar plot at genetic clusters (K) = 8, (**B**) admixture assignment bar plot at K = 9, and (**C**) pie charts of individual assignment proportions mapped to sampling locations at K = 9. Individual pie charts are expanded from location centroids for better visualization. Refer to *Table 2* for site codes. *Source: Basemap from ESRI and the GIS User Community.*

neutral SNPs was relatively equal across sites, with a slight increase in heterozygosity from west to east (Figure 6).

DISCUSSION

Shifts in Local Patch Locations and Abundance as Early Indicators of Habitat Change

Given continued human development and degradation in many areas and a changing climate, plant species can be early indicators for a changing environment. Tidal wetlands have declined substantially over the last century in the San Francisco Bay–Delta from habitat degradation and human development (Atwater and Hedel 1976). The upper ecotone has particularly been affected by loss from levee or dike construction, constraining this habitat to a narrow strip in many places. Endemic plants found in this upland ecotone can be affected by small changes in flooding and salinity (Kirwan and Guntenspergen 2015), with possible abrupt changes occurring in species composition and presence. Few studies have documented species distributions and

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Table 2 Genetic diversity across all loci for sampled sites of *Chloropyron molle* arranged from west to east. **Ns** is the total number of individuals sequenced, and **Nr** is the number individuals retained accounting for missing data. The percentage of polymorphic loci (**%P**), number of private alleles (**PA**), observed (**Ho**) and expected (*He*) heterozygosity, nucleotide diversity (**π**) and inbreeding coefficient (F_{IS}) were calculated across all variant and invariant sites.

Taxon	Site	Code	Ns	Nr	% P	PA	Ho	He	π	FIS
Chloropyron molle ssp. *molle*										
	Point Pinole	POP	20	13.6	0.18	971	0.00044	0.00049	0.00155	0.00024
	Fagan	FGN	19	18.1	0.2	1305	0.00059	0.00054	0.00169	−0.00005
	Benicia	BEN	21	18.2	0.27	1520	0.00061	0.00063	0.00197	0.00019
	MOTCO W	MOW	20	19.3	0.28	1389	0.00071	0.00069	0.00217	0.00009
	MOTCO E	MOE	20	18.2	0.26	1188	0.00062	0.00063	0.00198	0.00014
	PG&E	PGE	20	18.8	0.22	1076	0.00068	0.00063	0.00197	−0.00004
	Joice Island	JOI	20	15.8	0.31	1099	0.00069	0.00069	0.00218	0.00016
	Spring Branch	SPB	20	18.3	0.36	784	0.00078	0.00074	0.00231	0.00003
	Rush Ranch	RUR	20	15.5	0.28	859	0.00066	0.00064	0.00202	0.00008
	North Hill Slough	NHS	20	17.9	0.38	1084	0.00081	0.0008	0.00248	0.00013
	South Hill Slough	SHS	21	18.6	0.39	1219	0.00073	0.00074	0.00233	0.00024
Chloropyron molle ssp. *hispidum*										
	North Suisun	NOS	20	16.9	0.37	2108	0.00084	0.00082	0.00259	0.00008
	Arnold Slough	ARS	12	9.0	0.33	2265	0.00094	0.00089	0.00289	0.00006
	Wilcox Ranch	WIR	20	17.3	0.25	1661	0.00072	0.00071	0.00222	0.00007

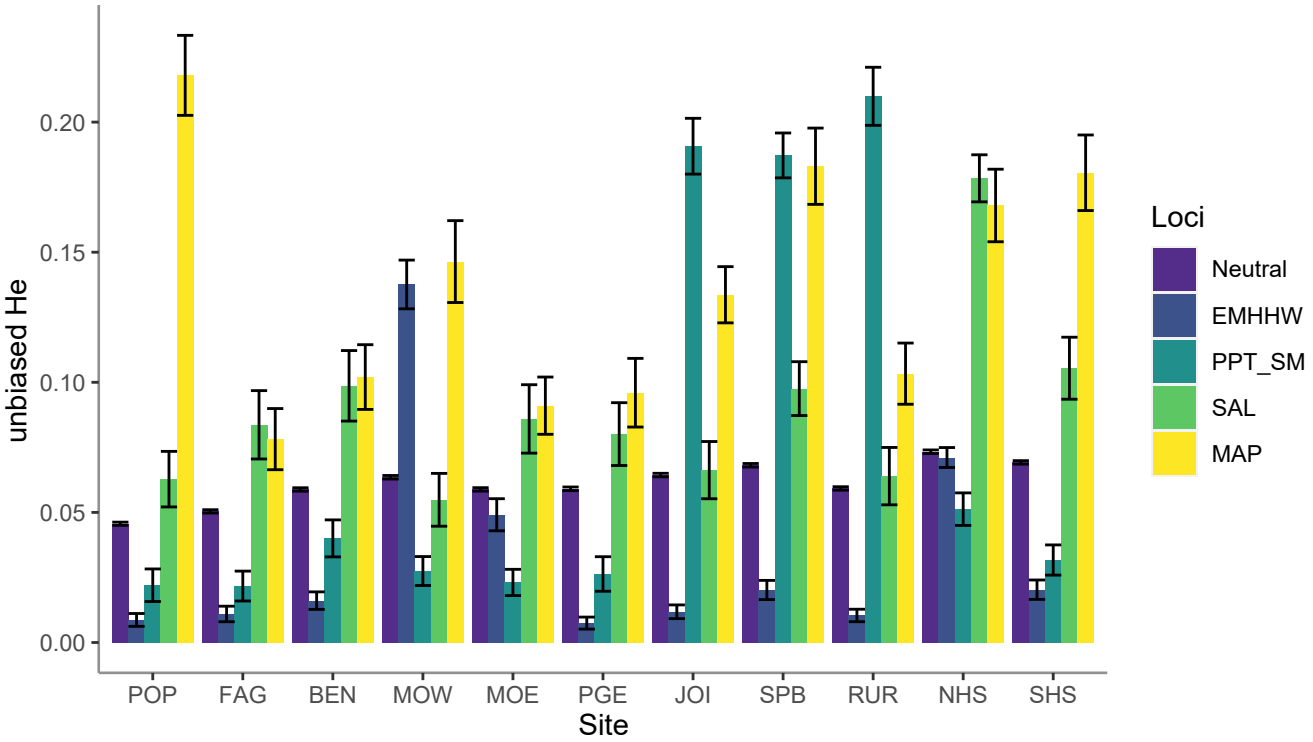

Figure 6 *Chloropyron molle* ssp. *molle* unbiased expected heterozygosity (**He**) with standard error by site for neutral (*purple*), and putatively adaptive single nucleotide polymorphisms (**SNPs**) associated with habitat and climate variables [Elevation relative to Mean Higher High Water (**EMHHW**) = *dark blue*; Summer Precipitation (**PPT_SM**) = *turquoise*; Salinity (**SAL**) = *green*; Mean Annual Precipitation (**MAP**) = *yellow*]. Sites are arranged from west to east. Refer to Table 2 for site codes.

changes in this upland ecotone (e.g., James and Zedler 2000; Traut 2005). Here, we determined the habitat suitability for *C. molle molle* and showed a contemporaneous decline in species abundance.

Decisions regarding the management and conservation of rare endemic plants under climate change can be fraught with uncertainty and complexity. Using a combination of field surveys and genomic analyses could be beneficial to inform decision-making regarding species recovery and climate adaptation. This study represents the first range-wide abundance survey in 27 years and the first population genomic survey of *C. molle molle*. Our surveys confirmed that *C. molle molle* was present at all previously known sites last surveyed in 1993, but some patches within sites have shifted in location. Current abundance estimates indicate an overall loss in the number of individuals, driven by two sites, Hill Slough (comprising NHS and SHS) and MOTCO East, with much lower comparative abundance.

In an annual plant such as *C. molle molle*, abundance estimates from a single season represent a snapshot of aboveground productivity during the sampling year. The summer of 2021 temperature ranged from 85 to 102 °F (29 to 39 °C) and 2021 was the second driest year on record in California, due to extreme heat and lack of rain and snow (CDWR 2022). Little is known about the inter-annual variation in abundance and correlations with weather conditions, although *C. molle molle* could undergo wide swings in local abundance across years. Long-term monitoring of populations of the related southern California salt marsh endemic *Chloropyron maritimum* ssp. *maritimum* found that large changes in population size were linked to cyclical variation in tidal amplitude, spring rainfall and maximum spring temperatures (Noe et al. 2019). Shifts in abundance and patch locations in *C. molle molle* could also be related to inter-annual variation, drought conditions, or indicate long-term directional responses to climate change. More frequent and regular range-wide surveys could help determine whether these changes are indicative of upslope range shifts, cyclical weather conditions, or other factors (Grewell et al. 2003).

Genetic Structure

We found that *C. molle molle* sites were genetically differentiated across the range, forming six or seven distinct regional genetic clusters. Evidence of mixed assignment in some individuals and significant isolation by distance indicate a low level of gene flow, mostly in a stepping-stone pattern among wetlands. *Chloropyron molle molle* has previously been described as dispersal limited (Grewell et al. 2013), which is consistent with these genetic patterns. Gene flow may occur through movement of pollinators and cross-pollination or movement of seeds among locations. Generalist pollinators have been noted visiting flowers, including bumblebees (*Bombus californicus, Bombus vosnesenskii*), leaf cutter bees (*Anthidium edwardsii*), sweat bees (*Halictus tripartitus, Lasioglossum* sp.) solitary bees (*Melissodes*: Anthophoridae) and bee flies (Diptera: Bombyliidae; Grewell et al. 2003; Ruygt 1994). Genetic connectivity may be related to these pollinators' foraging distances (Brunet et al. 2019). Although we are unaware of any studies of pollinator foraging distances in these specific wetlands, pollinator movements are likely localized. Generally, pollinator foraging distance increases with body size (Greenleaf et al. 2007). In upland chaparral habitats in Napa County, California, foraging distances of one of the larger observed species (*Bombus vosnesenskii*) ranged between 300 m and just over 3 km and were negatively correlated with flower cover (Pope and Jha 2018). In addition, seeds of *Chloropyron* are buoyant and could be dispersed among patches by tidal flows. Other published genetic studies of related species, the palmate-bracted bird's-beak, *Chloropyron palmatum,* and salt marsh bird's-beak, *Chloropyron maritimum* ssp. *maritimum,* also reported strong genetic differentiation across their respective ranges, where specific marsh habitat was patchy (Ayres 2015) or where wetlands were separated by large distances and not connected by tidal flows (Milano et al. 2020).

Chloropyron molle molle populations were genetically distinctive from adjacent *C. molle*

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hispidum, which is found in non-tidal (vernal pool) habitat. Small amounts of mixed assignment in some individuals could be indicative of shared ancestry or limited ongoing gene flow. Two *C. molle hispidum* sites, Arnold Slough and North Suisan, were historically tidal marsh (and more typical of *C. molle molle* habitat) but have since been diked and now serve functionally as vernal pool habitat, potentially allowing *C. molle hispidum* to colonize or emerge from the seed bank. These two subspecies may tolerate different habitat conditions, which may reinforce genetic differentiation despite geographic proximity. Common garden or greenhouse studies may be beneficial to better understand the extent to which these two subspecies can interbreed, the viability of hybrids, and their tolerances for different abiotic and biotic conditions. The range of *C. molle hispidum* is much broader than sampled here. It is patchy throughout the perimeters of the Central Valley, where it occurs in isolated pockets to the northeast of our study area in Placer County and to the southeast in Merced and Kern Counties (Calfora 2024). Further sampling across the full range could better elucidate the evolutionary history of this taxon and its distinctiveness from *C. molle molle* and other *Choropyron* taxa.

Across *C. molle molle* occurrences, strong differences in heterozygosity associated with different environmental conditions indicate that local adaptation shapes genetic structure across the range. Genomic diversity provides the raw material for selection and diversification and can influence persistence and adaptive potential (Hoffmann et al. 2017). Populations with high diversity may provide reservoirs that allow for flexibility to adapt to future climate conditions, a key component of "representation" in conservation and recovery (Funk et al. 2019). In *C. molle molle*, average heterozygosity varied among sites and between putatively adaptive and neutral loci. Heterozygosity in neutral loci was relatively evenly distributed across sites compared to climate associated loci, although lowest in the two westernmost sites (Fagan Marsh and Point Pinole). In contrast, diversity associated with an elevation-tidal gradient was highest in the middle of the range in MOTCO West, loci associated with soil salinity and summer precipitation had highest diversity in the north-eastern sites, and loci associated with mean annual precipitation had highest heterozygosity in Point Pinole. These differing patterns emphasize that preserving sites across the range and across genetic clusters would help maximize the preservation of putatively adaptive genetic diversity in *C. molle molle*. This finding supports the current recovery recommendations of the US Fish and Wildlife Service (USFWS 2009, 2013), which include maintaining well-distributed populations throughout the geographic range that encompass the full range of microhabitats and environmental conditions in which the taxon historically occurred, and maintaining the range of genetic variation to minimize the risk of inbreeding depression and to allow for future evolution and resilience to environmental change.

Potential for Restoration

Ecological restoration is a tool for species recovery (Grewell et al. 2013) and can build climate resilience into a population (Simonson et al. 2021). Given ongoing sea level rise and expected upslope migration of tidal wetland habitats with climate change (Osland et al. 2022), restoration of upslope habitat and seeding could become more important strategies to preserve *C. molle molle* (USFWS 2013), especially given apparent declines in abundance of some populations. Successful restoration depends on both identifying suitable sites and identifying suitable source populations. As expected, *C. molle molle* was found in relatively narrow ranges of elevation and salinity, with similar community structures that always included two species (*Distichlis spicata* and *Salicornia pacifica*). Potentially suitable habitat was identified at sites currently occupied by *C. molle molle* within vegetation plots outside of existing patches and at five additional wetlands without *C. molle molle*, providing potential locations for future restoration.

Best practices for selecting source populations include evaluating the ecological similarity, geographic proximity, and size or genetic diversity of potential source populations (Maschinski and

Albrecht 2017). Although across the range of *C. molle molle* more proximate populations share more genetic similarities than those farther way, different spatial patterns in diversity of putatively adaptive loci indicate that using a composite seeding approach (e.g., using seeds from multiple sites with similar environmental conditions, rather than from the single most proximate source) could enhance adaptive potential to different combinations of local environmental conditions (Broadhurst et al. 2015; Bucharova et al. 2019). Indeed, the Spring Branch restoration site, established from wild seeds sourced from both Joice Island and Hill Slough, indicates that establishing new populations of *C. molle molle* is possible with multiple seed sources (Grewell et al. 2003). Not only has the success of the seeded restoration at Spring Branch increased the overall number of extant populations, but this site has retained relatively high genetic diversity and plants with admixed ancestry, indicating compatible inter-breeding among founding plants. In future restoration efforts, sites with similar tidal, salinity, and rainfall regimes could be prioritized as seed sources. Further, given concerns pertaining to tidal wetland loss from sea level rise (Buffington et al. 2021), restoration efforts could also match donor and recipient sites taking into account the expected future habitat conditions.

CONCLUSIONS

Habitat fragmentation and degradation have been the primary threats to the survival of endemic wetland plant species, but climate change will interact with these anthropogenic drivers creating unprecedented challenges for managers. Restoration opportunities are key components towards building climate resilience for rare species. Efforts to establish new populations will likely be most successful in areas with similar environmental conditions and community composition. Site selection could include further consideration of other factors, such as land conservation and management status (Falk et al. 1996), availability of upslope habitat for marsh movement (Osland et al. 2022), potential for connectivity through pollination and seed movement with other occupied sites, and ability and commitment to perform ongoing habitat management activities, particularly control of invasive species (Grewell et al. 2013). Despite having a narrow suitable habitat range and restricted distribution, *C. molle molle* exhibited genetic structuring with differences in putatively adaptive genetic diversity across occurrences that may be important to consider when determining appropriate seed sources for restoration. In conclusion, this study can provide a model for integrating genetic and ecological studies to inform rare plant restoration.

ACKNOWLEDGMENTS

This work was supported by an Interagency Agreement between US Fish and Wildlife Service (USFWS), San Francisco Bay Delta Fish and Wildlife Office and the US Geological Survey (USGS) Western Ecological Research Center, and permitted under USFWS Recovery Permit ESPER0002932; California Endangered Species Act Scientific, Educational, or Management Permit 2081(a)-20-019-RP; and Plant Voucher Collecting Permit 2081(a)-21-025-V. We thank Anne Mankowski and Steven Detwiler (USFWS) for support and assistance in developing the project. We also thank Anna Kennedy, Alexander Ryktarsyk, and Audrey Colley from USGS for logistical support and field survey assistance. Julia Smith, Anna Mitelberg (USGS San Diego Field Station), and Elizabeth Milano (US Department of Agriculture Forest Service) assisted with tissue extractions, sequencing preparations, and sample organization. For help with site access or general information, we thank D. Rogers from Center for Natural Lands Management; K. Hiatt and W. Schroeder from City of Woodland; G. Smick and R. Scampavia from WRA Environmental Consultants; J. Westbrook and D. Wirtz from Solano Land Trust; S. Martinelli, K. Taylor, O. Rocha, S. Estrella, and R. Carrothers from California Department of Fish and Wildlife; E. Jeu, P. Good, A. Candela–Cooney, and T. Schaffer from California Department of Water Resources; M. Hammond, R. Deikman, D. Kendall, and C. Lare–Masters from East Bay Regional Park District; M. Vasey and M. Ferner from San

Francisco State University; C. Lew from California State Parks; J. Schweitzer and C. Pinnell from Vollmar Natural Lands Consulting; J. Collins from San Francisco Estuary Institute; S. Volk from USAG Military Ocean Terminal Concord; B. Gray and J. Wolf from Pacific Gas and Electric Company; C. Thompson and M. Dabner from City of Petaluma; E. Handford from City of Vallejo; J. Sarrow from Napa County Flood Control and Water Conservation District; R. Donovan and G. Hildebrand from Sacramento Municipal Utility District; T. Wong from Resource Environmental Solutions; M. Snyder at Solano County Water District; L. Fitch and N. Schofield from Storie and Severson; and J. Ruygt from Napa County California Native Plant Society. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the US Government.

DATA AVAILABILITY

Raw sequence data are accessible as an NCBI Sequence Read Archive (BioProject PRJNA1123295). Single nucleotide polymorphisms (SNP) genotypes and sample data are available as a USGS data release (Vandergast et al. 2025). Raw field data are available on request from US Fish and Wildlife Service.

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