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Prepared in cooperation with U.S. Fish and Wildlife Service, Carlsbad Fish and Wildlife Office

Genetic Structure and Diversity in Wild Populations of the Light-Footed Ridgway's Rail Reflect 20 Years of Augmentation Through Captive Breeding and Release

Open-File Report 2025–1011

U.S. Department of the Interior
U.S. Geological Survey

Cover. A Ridgway's Rail in pickleweed at Tijuana Slough National Wildlife Refuge. Photograph by Julia G. Smith, U.S. Geological Survey, June 21, 2021.

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Contents

Figures

Tables

Conversion Factors

International System of Units to U.S. customary units

Multiply	By	To obtain
	Length	
kilometer (km)	0.6214	mile (mi)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:

$$°F = (1.8 \times °C) + 32.$$

Datum

Horizontal coordinate information is referenced to the North American Datum of 1983 (NAD 83).

Supplemental Information

Concentrations are given in nanograms per microliter (ng/µL).

Abbreviations

>	greater than
<	less than
CI	confidence interval
DNA	deoxyribonucleic acid
F	inbreeding coefficient
F_{ST}	genetic differentiation
HWE	Hardy-Weinberg equilibrium
N_e	effective population size
NWR	National Wildlife Refuge
PCA	principal component analysis
PCR	polymerase chain reaction
USGS	U.S. Geological Survey
USFWS	U.S. Fish and Wildlife Service

Genetic Structure and Diversity in Wild Populations of the Light-Footed Ridgway's Rail Reflect 20 Years of Augmentation Through Captive Breeding and Release

By Amy G. Vandergast,[1] Julia G. Smith,[1] Anna Mitelberg,[1] Dustin A. Wood,[1] Kimberly A. Sawyer,[2] and Courtney J. Conway[1]

Abstract

Captive breeding and release programs aimed at recovery of rare species can be informed by genetic data to help select high-diversity source populations, make pairing decisions to minimize inbreeding, and manage release strategies. We developed a set of 54 microsatellite loci to assess genetic structure and diversity across the United States range of the Light-footed Ridgway's Rail (*Rallus obsoletus levipes*), a federally endangered marsh bird for which populations have been augmented by a captive breeding program annually since 2001. We identified three regional genetic clusters, with the highest genetic diversity reported in the central cluster, which included all sampled wetlands in north San Diego County. Recent (2019–24) captive-breeding adults all clustered within the northernmost cluster (Orange and Ventura Counties), which was expected given that this cluster included the source wetland for the captive breeding program. Gene flow rates, which approximate the proportions of individuals in a population originating from other populations, were relatively high among clusters (4–24 percent) and may have been enhanced through the release of captive-bred rails. Based on the genetic data analyzed in a genetic rescue decision framework, sourcing new breeding birds from the north San Diego County cluster could provide the greatest genetic diversity benefits. The northernmost cluster, which included Mugu Lagoon and all sampled Orange County wetlands, was considered the most in need of genetic rescue. Recent breeding pairs in the captive breeding program have comparatively low diversity and high interrelatedness. Sourcing birds from wetlands with high genetic diversity and population sizes, assessing genetic relatedness before pairing, and focusing releases in areas that have low estimates of genetic diversity could improve the distribution of genetic diversity across wild populations in the future.

Introduction

Genetic monitoring is frequently used along with ecological monitoring tools to assess and manage populations of endangered species (Schwartz and others, 2007; Antao and others, 2011). Genetic diversity data can be particularly informative for managing captive breeding and release programs aimed at restoring declining species. The maintenance of genetic diversity can reduce the potential for inbreeding depression and improve fitness in the short term (a few generations; Reed and Frankham, 2003; Spielman and others, 2004; Markert and others, 2010), and preserve adaptive potential in the long term (many generations; Kardos and others, 2021). For these reasons, measuring the amount and distribution of genetic diversity among wild populations can help to identify appropriate source populations and release sites to manage for diversity. In addition, genetic monitoring pre- and post-release can be used along with mark-recapture, telemetry, and other techniques to assess survival and integration of released individuals, and their genes, into wild populations (Bubac and others, 2019). Finally, genetic relatedness information can be incorporated into studbook management to help guide pairing decisions in captive settings to ensure that inbreeding is minimized and that multiple family lineages are consistently represented in captive populations (Ivy and Lacy, 2010).

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Light-footed Ridgway's Rails (*Rallus obsoletus levipes*; hereafter rails) are restricted to coastal wetlands within a small geographic range spanning from Ventura County, California, to Ensenada, Baja California, Mexico (fig. 1; Eddleman and Conway, 2020). The subspecies was listed as federally endangered in 1969 (Secretary of the Interior, 1969), state endangered in California in 1971, and was added to the official list of at-risk species in Mexico in 2002 (Secretaría del Medio Ambiente y Recursos Naturales, 2002). Annual call-broadcast surveys throughout the subspecies' U.S. range began in 1980 and have continued to the present (Zembal and others, 2024). During this period, total pair counts have fluctuated from year to year, but have increased slightly since range-wide counts began (U.S. Fish and Wildlife Service, 2020). These trends vary regionally, with an apparent increase in north San Diego County marshes but an apparent decline in Orange County (fig. 2); although changes in pair counts over time were not tested statistically (Zembal and others, 2024). In 1989, genetic samples were obtained from four consistently occupied populations (at that time) throughout the rails' range (Mugu Lagoon, Ventura County, Seal Beach and Newport Bay, Orange County, and Tijuana Slough National Wildlife Refuge (NWR), San Diego County; fig. 1). Genetic analyses of these samples (Fleischer and others, 1995; Nusser and others, 1996), reported low genetic diversity within populations and suggested that movement of individual rails from larger populations into smaller ones could be a possible management strategy.

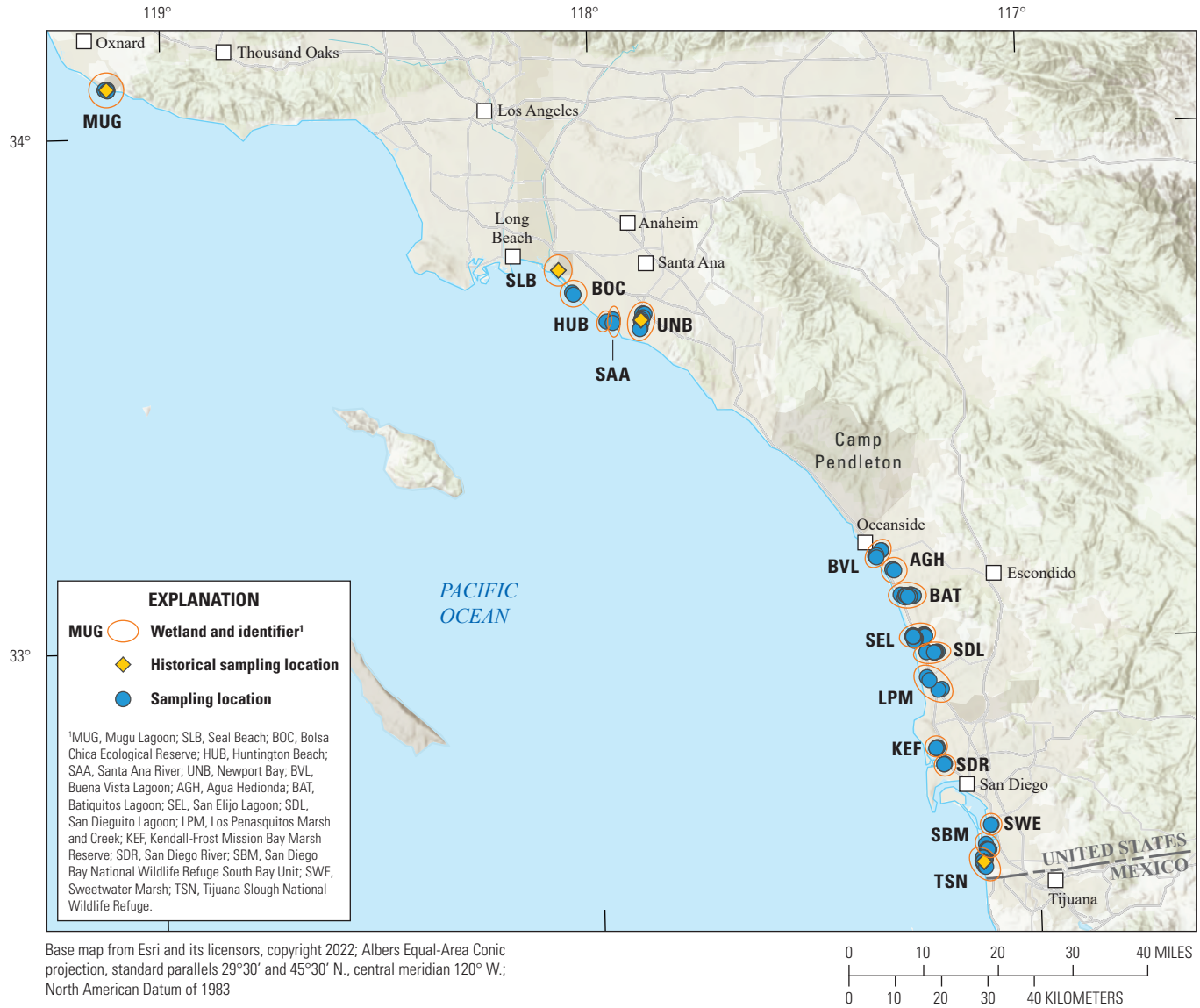


Figure 1. Locations of wetlands where Light-footed Ridgway's Rails (*Rallus obsoletus levipes*) were sampled in southern California for this study between 2020 and 2022 (blue points) and in 1989 (Historical sampling location; yellow points).

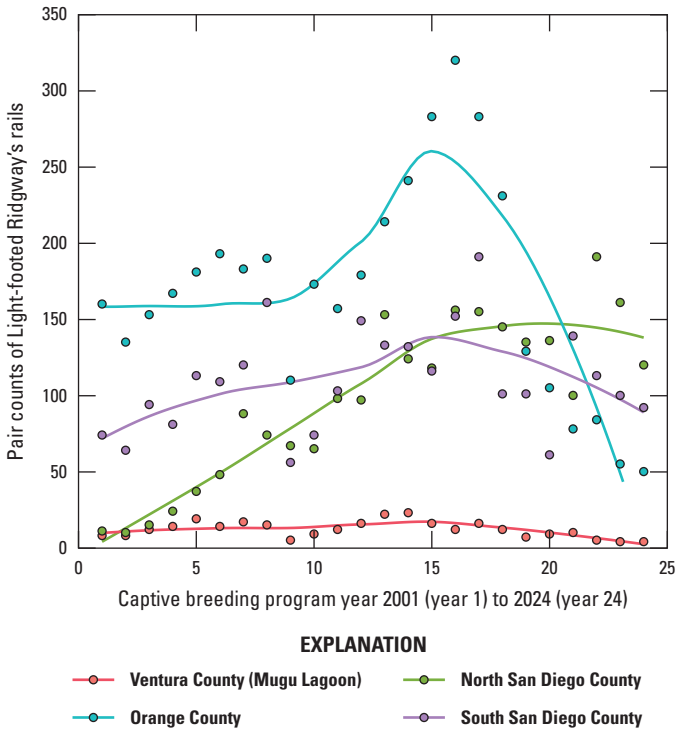


Figure 2. Annual pair counts of Light-footed Ridgway's Rails (*Rallus obsoletus levipes*) over time between 2001 (year 1) and 2024 (year 24) summed by region (data taken from Zembal and others, 2024). Points represent total pair counts, and lines are locally weighted (LOESS) smoothers. The Orange County region has declined, whereas north San Diego County has increased. Mugu Lagoon (the only population in Ventura County) has remained relatively low in comparison to all other regions.

Starting in 2001, a captive-release program was initiated with founders (birds and eggs) sourced from Newport Bay. Subsequently, eggs from Newport Bay have been brought in to replenish the breeding program (maintained at 3–6 pairs annually) about every 2–3 generations. Juvenile rails from this program have been released annually as part of species recovery efforts, with over 600 individuals released across southern California marshes between 2001 and 2023 (fig. 3). All breeding birds in the captive program were either taken from the Newport Bay wild population or from descendant captive offspring. All released birds can be traced to 76 wild founders through their pedigree between 2001 and 2023 (table 1.1).

Purpose and Scope

Although counts have been completed annually at most occupied wetlands since the 1980s, monitoring of movement and survivorship of released juvenile rails had not occurred until very recently (Zembal and others, 2017; Sawyer, 2024; Sawyer and Conway, in press). In addition, genetic monitoring of wild populations and genetic assessment of captive birds have been lacking until this study. Therefore, little is known about the cumulative effects of releases on population genetic structure and diversity of recipient populations. To address these uncertainties, we developed a set of microsatellite markers to allow for genetic monitoring of wild and captive rails. We evaluated the recent (2020–22) genetic population structure and diversity of rail populations throughout their U.S. range. We also compared the recent genetic structure to the pre-augmentation structure by comparing recent blood samples with blood samples available from the initial 1989 genetic surveys. Moreover, we examined genetic connectivity and diversity across the subspecies' U.S. range to identify extant populations with high genetic diversity that could be considered for future captive-rearing sources.

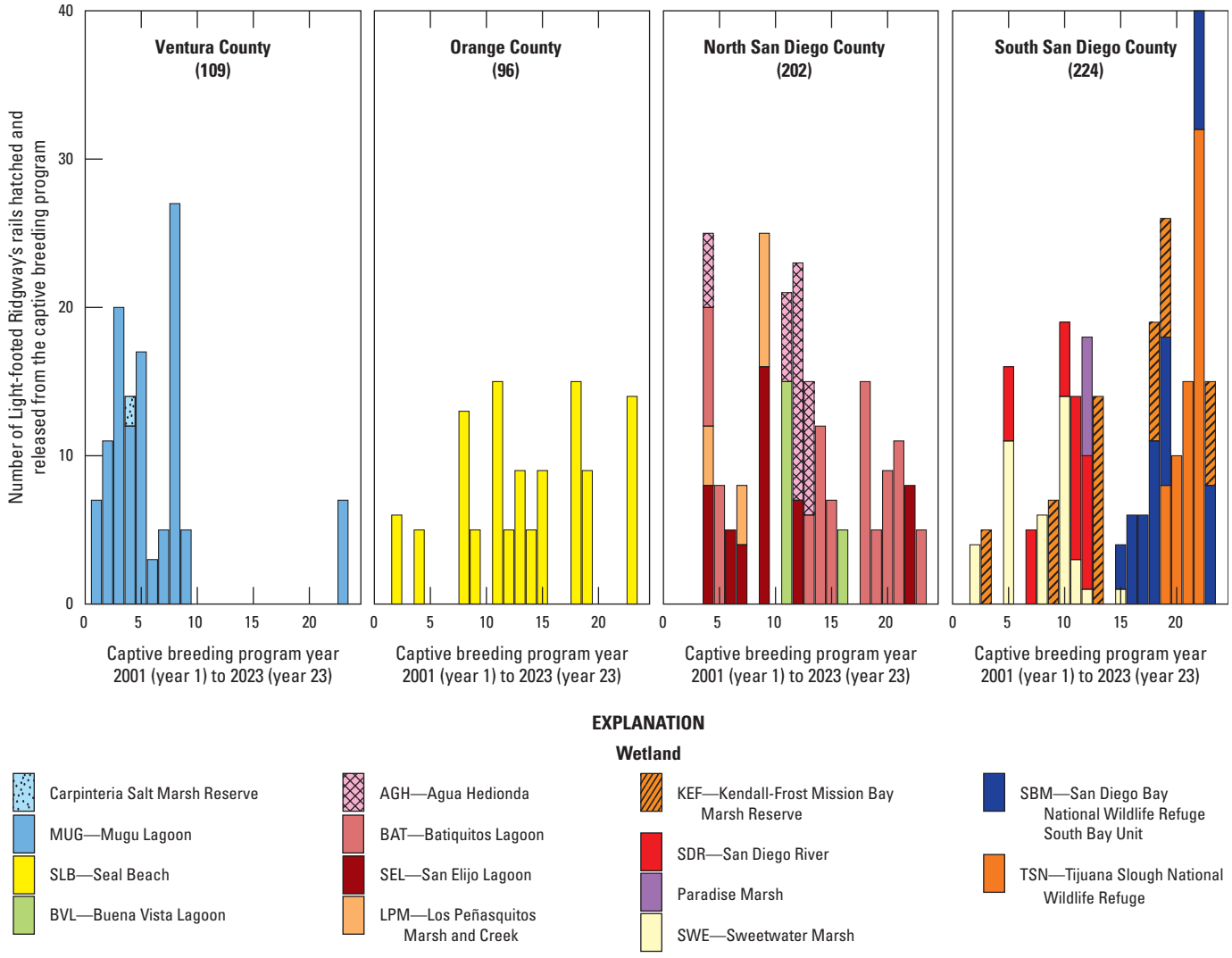


Figure 3. The number of Light-footed Ridgway's Rails (*Rallus obsoletus levipes*) hatched and released from the captive breeding program between 2001 (year 1) and 2023 (year 23) by wetland (colored bars) and grouped into geographic regions. Numbers in parentheses are the total number of releases in each region.

Methods

Field Sampling

We visited and captured wild individuals at 17 wetlands throughout the U.S. range for genetic sampling and banding between 2020 and 2022. Sites were visited during the breeding season, roughly between April and September of each year. We used carpet traps (Harrity and Conway, 2020) with a broadcast of Ridgway's Rail vocalizations to lure rails to the carpet traps (Pickens and King, 2013; Harrity and Conway, 2020). We removed rails from carpet traps immediately after capture, and we measured, weighed, photographed, and attached a federal leg band (Smith, 2013) to each rail. We collected blood samples from each captured rail via metatarsal venipuncture using a sterile, 26-gauge needle and transferred to a GenSaver 2.0 (AHLSTROM, Escondido, California, cat no. 8.566.0002.B-N) blood card with a non-heparinized capillary tube (Thermo Fisher Scientific, Waltham, Massachusetts, cat no. 22-260943). We then released rails at the capture location. All fieldwork was authorized following guidelines specified in Federal and State permits held by C. Conway (Federal Endangered Species Permit TE039466; Bird Banding Permit #22524; California Memorandum of Understanding (SCP-S-193610002-20008-001), and as approved by the University of Idaho Institutional Animal Care and Use Committee (2015-51).

Sampling of Captive-Bred Rails

Beginning in 2019, we collected blood samples from all captive-bred and released rails, and, when available, breeding adults. Blood samples were not regularly taken from captive rails before 2019. Rails were sampled before release, using metatarsal venipuncture, as described in the "Field Sampling" section; we attached a federal leg band (Smith, 2013) to each released bird.

1989 Baseline Samples

We received archived blood and genomic deoxyribonucleic acid (DNA) samples from the Smithsonian Museum which were used in previous population genetic analyses (Fleischer and others, 1995; Nusser and others, 1996). These samples were collected in the fall of 1989, before the start of the captive breeding program from four wetlands across the subspecies' U.S. range: Mugu Lagoon, Seal Beach, Newport Bay, and Tijuana Slough NWR (fig. 1; table 1.2). Although the number of available historical samples per wetland was small by contemporary standards, these samples represent the best available baseline dataset for comparison to recent genetic structure and diversity metrics. All samples were sent to the Western Ecological Research Center's San Diego Field Station genetic laboratory for extraction and amplification.

Marker Development

Microsatellite libraries were developed for *R. obsoletus* at Cornell University's Evolutionary Genetic Core Facility using genomic DNA extracted from four individuals. The Evolutionary Genetic Core Facility sequenced a tetrameric, enriched genomic library on an Illumina MiSeq with paired 250 base-pair reads (Nali and others, 2014), used SeqMan NGen (version 11, DNAStar, Madison, Wisconsin) to generate a de novo assembly from the paired fastq files (raw data), and used the program msatcommander 1.0.8_beta (Faircloth, 2008) to scan for candidate microsatellite loci and design primer pairs. To design a panel of highly multiplexed microsatellite markers, we randomly selected and evaluated approximately 500 candidate microsatellite loci (500 forward primers tagged at the 5-prime end with the sequence TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG, and 500 reverse primers, tagged at the 5' end with the sequence GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG) for multiplex polymerase chain reaction (PCR) suitability using Multiple Primer Analyzer (MPA; Thermo Fisher Scientific, Sunnyvale, California). We used the MPA output in the package igraph (Csárdi and Nepusz, 2006) to cluster the loci into an arrangement that would minimize primer-dimer formation. This process resulted in four multiplexes, composed of 30–40 loci each. These loci were individually amplified in two individuals, and only loci with successful amplification in both samples (as confirmed by gel electrophoresis) were retained in the final multiplexes. All samples were genotyped using the resulting panel of 108 loci (table 1.3) arranged into four multiplexes (Mpx1-4), as described in the following section.

DNA Extraction, Amplification and Sequencing

We extracted genomic DNA from blood cards or capillary tubes using the Puregene kit (QIAGEN, Germantown, Maryland) according to the manufacturer's protocol, with minor modifications including the addition of Proteinase K to cell lysis with an overnight incubation at 58 degrees Celsius (°C), and final resuspension in 100 microliter (µL) Tris Low ethylenediaminetetraacetic acid (EDTA; TLE) buffer (10 millimolar [mM] Tris, 0.1 mM EDTA, pH 8.0). We quantified extractions using Qubit Broad Range (Thermo Fisher Scientific) and standardized to 10–40 nanograms per microliter (ng/µL) before amplification with the Type-it Microsatellite PCR Kit (QIAGEN). We amplified loci by using four primer cocktails (Mpx1-4; table 1.3), with each primer at a 1.6 micromolar (µM) concentration in the primer cocktail. Each of four 10 µL PCR reactions contained 5 µL 2X Type-it Master Mix, 1 µL of Mpx1, Mpx2, Mpx3 or Mpx4, and 15–60 ng/µL DNA. Amplifications included 30 cycles of 95 °C for 5 minutes, 94 °C for 30 seconds, 56 °C for 1.5 minutes, 72 °C for 1.5 minutes, followed by a 12 °C hold. Upon completion, the four multiplexed PCRs per sample were pooled together, and the pooled PCR product was barcoded using Nextera N5/600 and N7/800 indexes to produce individual dual-indexed amplicon libraries for each sample. Individual sample libraries were then pooled together into one tube per 96-well plate and bead-cleaned to remove primer dimers. Pooled and bead-cleaned libraries from each plate of sample libraries were combined in equimolar proportions and sent for sequencing at MedGenome, Inc. (Foster City, California) on the NovaSeq 6000 (Illumina, San Diego, California), using the Illumina SP 300 cycle reagent kit v1.5.

Bioinformatics

We used the Python script amplicon (https://bitbucket.org/cornell_bioinformatics/amplicon) to extract reads from the Illumina runs and assign them to the appropriate locus and individual. Specifically, the script (1) trims adapters and low-quality reads, (2) creates contigs from overlapping reads (for paired-end sequencing), (3) identifies reads corresponding to each locus, (4) collapses identical reads for each individual, and (5) identifies the top two haplotypes for individuals at all loci (in other words, their diploid genotypes). We used the default options except for the following parameters: -c 1 (minimum number of samples per haplotype), -a 0.001 (minimum minor allele frequency), -l 75 (minimum haplotype length), -r 5 (maximum read count ratio between the two alleles in each sample). We then calculated the total number of reads per locus per individual, whether a locus was heterozygous or homozygous (and if heterozygous, the minimum number of minor allele reads per locus per individual). We scored loci as missing data if the total number of reads was less than (<) 200. Heterozygous loci were recoded as homozygous if the minor allele read count was low (<300), or if the total number of reads was low (<500). Before population genetic analyses, we used the R packages adegenet v. 2.1.10 (Jombart, 2008) and poppr v. 2.9.3 (Kamvar and others, 2014) to assess the quality of loci and samples using several filters. First, we removed any locus with greater than 10 percent missing data, and then removed any individual samples with greater than 10 percent missing data. Next, we applied a minor allele frequency cutoff (MAF=0.01) to identify monomorphic or uninformative loci. Once these loci and samples were removed, we used the R package genepop v. 1.2.2 (Rousset, 2008) to evaluate the dataset for linkage disequilibrium using the exact test for genotypic linkage disequilibrium with 10,000 dememorizations and 5,000 iterations; the significance of linkage disequilibrium was confirmed for loci with *p*-values below 0.0001. We also used the method described by Brookfield (1996) to estimate the frequency of null alleles for each locus with the R package popgenreport (Adamack and Gruber, 2014). We retained loci with null allele frequencies less than 0.2, following the recommendations of Dakin and Avise (2004).

Population Genetic Dataset

During field sampling, we captured and sampled hatch-year and adult rails. However, we removed hatch-year birds from the population structure and diversity analyses to avoid biases resulting from unequal sampling of family groups and to focus on the adult breeding populations present at the time of sampling. We included captive adults used in the breeding program to represent the captive "population" (hereafter "captive breeders"). We separated the captive breeders into two temporal groups: (1) parents of the captive offspring released before and during the wild sampling period (2019–21; group I), and (2) captive breeders held in the breeding program at the time of this report (2023–24; group II). Group I birds were included along with wild birds in structure and gene flow analyses to help evaluate the influence of the breeding program on genetic structure and diversity. Diversity metrics were calculated for groups I and II to provide information relevant to the breeders in captive breeding facilities at the time of this report. We analyzed the 1989 baseline samples separately from recent samples to compare population structure and diversity pre- and post-augmentation.

Loci were screened for deviations from Hardy-Weinberg equilibrium (HWE) at four wetland sites with greater than 20 samples (Newport Bay, Batiquitos Lagoon, San Elijo Lagoon, Tijuana Slough NWR) using an exact test based on 1,000 Monte Carlo permutations of alleles (Guo and Thompson, 1992) and applying the Benjamini and Yekutieli (2001) correction for multiple tests. We removed loci if they deviated significantly (corrected *p*-value less than 0.05) from HWE at three or more sites.

Population Structure and Gene Flow

We used multiple methods to assess population structure. First, we used STRUCTURE (Pritchard and others, 2000) to determine the supported number of genetic clusters (K) that conform to populations in genetic equilibrium. We specified a range for the maximum number of clusters that individuals could be assigned (K=1–10) and completed 10 replicate runs per K using 500,000 iterations of the Markov chain Monte Carlo algorithm following a burn-in of 500,000 iterations to verify consistency across chains. The optimal K was inferred by comparing the results from the maximum mean log-posterior probability for K estimated by STRUCTURE and the change in K (ΔK) criterion (Evanno and others, 2005). Second, we used principal component analysis (PCA) to visualize genotypes in multidimensional space with adegenet v2.1.10 (Jombart, 2008), in R v4.1.2 (R Core Team, 2018). We used the program PopCluster (Wang, 2022a) to estimate gene flow among populations. PopCluster provides estimates of recent gene flow rates (last 3 generations) from an admixture model. We first evaluated up to 10 clusters (K) with 20 replicate runs. After selecting the optimal K, we ran the PopCluster model with migration for 20 replicate runs to estimate gene flow rates among clusters from the individual admixture estimates.

We calculated allelic richness (*A*r), private allelic richness (*PA*r), observed heterozygosity (*Ho*) and unbiased expected heterozygosity (*H*e), and inbreeding coefficients across marsh sites, and clusters and groups of captive breeders. There was some geographic overlap between cluster assignments in Mission Bay (Kendall-Frost Mission Bay Marsh Reserve and San Diego River). For the purpose of reporting genetic diversity indices by cluster, we grouped these two wetlands in the south San Diego County cluster. The effective population size (N_e) was estimated in NeEstimator v2 (Do and others, 2014) for each cluster and period. We used the linkage disequilibrium method with monogamy and a minimum allele frequency of 0.02, and calculated 95-percent confidence intervals (CI) of point estimates by jackknifing across samples.

We compared genetic differentiation (F_{ST}), relatedness (*R*), allelic richness (*Ar*), and unbiased expected heterozygosity (*H*e) between the baseline and recent sample periods by using group comparisons in FSTAT v2.9.4 (Goudet, 2001), with *p*-values derived from 10,000 permutations. We restricted our analysis to the three wetlands that were sampled in both periods (Mugu Lagoon, Newport Bay, Tijuana Slough NWR). During the time of our field sampling, only a handful of birds were observed in Seal Beach; we did not pursue sampling there to avoid disturbing the remaining rails. We also ran a PCA across paired wetlands to visualize any changes in genetic clustering over time.

Decision Framework for Genetic Rescue

Following the decision framework presented in Frankham and others (2017), we assessed whether populations met certain criteria indicating genetic erosion and whether genetic rescue could improve genetic diversity in local populations. We calculated the mean inbreeding coefficient (*F*):

$$F = 1 - \frac{H\text{inbred}}{H\text{outbred}} \quad (1)$$

where

*H*inbred is the average heterozygosity of the receiver (inbred) population, and

*H*outbred is the average heterozygosity of the donor (outbred) population.

F values greater than 0.1 indicate that genetic diversity is sufficiently higher in donor population(s) to benefit the receiver population (Frankham and others, 2017). We used our estimates of expected heterozygosity to calculate *F* for each regional cluster in relation to the following donors: (1) captive breeders, (2) Orange County cluster, (3) north San Diego County cluster, and (4) south San Diego County cluster.

Comparing Coancestry and Inbreeding Coefficients from Studbook and Genetic Data

We calculated pedigree-based coancestry and inbreeding coefficients for captive breeders using the R package kinship2 (Sinnwell and others, 2014). We then estimated the genetic-based relatedness and inbreeding coefficients for captive breeders in the software EMIBD9 (Wang, 2022b). The EMIBD9 software implements a likelihood expectation maximization (EM) method, updating allele frequencies and identity-by-descent coefficients for each pair of sampled individuals until convergence. The EM method estimates relatedness and allele frequencies simultaneously from a small sample of genotypes, in contrast to traditional methods, which rely on unbiased allele frequencies obtained from a large sample of unrelated genotypes (for example, relatedness presented in table 1). We then compared the productivity, pedigree-based metrics and genetic-based metrics for recent breeding pairs.

Table 1. Number of Light-footed Ridgway's Rails (*Rallus obsoletus levipes*) sampled (*N*) per wetland and regional clusters and corresponding genetic diversity statistics including allelic richness (*Ar*) rarified to 10 gene copies, private allelic richness (*PAr*), rarified to 10 gene copies, observed heterozygosity (*Ho*), unbiased expected heterozygosity (*He*) and average pairwise relatedness among individuals (*R*; Lynch and Ritland, 1999).

[Diversity statistics were not calculated (NC) for wetlands with fewer than five individuals sampled. **Abbreviations**: ER, Ecological Reserve; NWR, National Wildlife Refuge; —, no samples were taken]

Cluster/wetland	*N* (current)	*Ar*	*PAr*	*Ho*	*He*	*R*	*N* (1989)	*Ar*	*PAr*	*Ho*	*He*	*R*
Ventura County	—	—	—	—	—	—	—	—	—	—	—	—
MUG, Mugu Lagoon	5	1.78	0.03	0.318	0.279	0.274	4	2.11	0.12	0.407	0.344	0.154
Orange County	**20**	**1.99**	**0.03**	**0.355**	**0.332**	**0.094**	**12**	**2.07**	**0.06**	**0.377**	**0.355**	**0.1**
SEB, Seal Beach NWR	—	—	—	—	—	—	4	NC	NC	NC	NC	NC
BOC, Bolsa Chica ER	2	NC	NC	NC	NC	NC	—	—	—	—	—	—
HUB, Huntington Beach	1	NC	NC	NC	NC	NC	—	—	—	—	—	—
SAA, Santa Ana River	2	NC	NC	NC	NC	NC	—	—	—	—	—	—
UNB, Newport Bay	15	1.95	0.01	0.351	0.323	0.102	8	2.02	0.02	0.345	0.338	0.124
North San Diego County	**69**	**2.28**	**0.22**	**0.394**	**0.389**	**0.028**	—	—	—	—	—	—
BVL, Buena Vista Lagoon	8	2.19	0.02	0.373	0.371	0.094	—	—	—	—	—	—
AGH, Agua Hedionda Lagoon	5	2.07	0.03	0.427	0.374	0.158	—	—	—	—	—	—
BAT, Batiquitos Lagoon	18	2.3	0.02	0.396	0.396	0.04	—	—	—	—	—	—
SEL, San Elijo Lagoon	23	2.24	0.05	0.39	0.385	0.033	—	—	—	—	—	—
SDL, San Dieguito Lagoon	11	2.3	0.04	0.407	0.378	0.011	—	—	—	—	—	—
LPM, Los Peñasquitos Marsh	4	NC	NC	NC	NC	NC	—	—	—	—	—	—
South San Diego County	**49**	**2.08**	**0.08**	**0.369**	**0.348**	**0.058**	**10**	**2.03**	**0.08**	**0.318**	**0.329**	**0.082**
KEF, Kendall-Frost Mission Bay Marsh Reserve	4	NC	NC	NC	NC	NC	—	—	—	—	—	—
SDR, San Diego River	3	NC	NC	NC	NC	NC	—	—	—	—	—	—
SWE, Sweetwater Estuary	2	NC	NC	NC	NC	NC	—	—	—	—	—	—
SBM, San Diego Bay NWR South Bay Unit	5	NC	NC	0.302	0.347	0.068	—	—	—	—	—	—
TSN, Tijuana Slough NWR	35	2.03	0.01	0.377	0.339	0.078	10	2.03	0.08	0.318	0.329	0.081
Captive Breeders Group I (2019–22)	**10**	**1.79**	**0**	**0.281**	**0.28**	**0.221**	—	—	—	—	—	—
Captive Breeders Group II (2023–24)	**10**	**1.8**	**0**	**0.308**	**0.302**	**0.209**	—	—	—	—	—	—

Results and Discussion

During locus and sample evaluations, we identified 17 loci with greater than 10 percent missing data and 24 additional loci that were monomorphic. We also identified nine loci that deviated significantly from HWE, four loci with significant linkage disequilibrium, and no loci with null allele frequencies greater than 0.2. These 54 loci were removed before further analysis. The final dataset retained the remaining 54 loci and included 143 wild adult birds sampled from 17 wetlands (table 1). We included 10 captive parents of the offspring released just before and during the sampling period (group 1; 2019–21) and 10 breeding adults in the captive breeding program at the time of this report (group II; 2023–24). We also included 27 baseline samples from four wetlands sampled in 1989, before augmentation efforts (table 1). Genotype data are available as a USGS data release in Mitelberg and others (2025).

Recent Population Structure

Structure analyses best supported three genetic clusters across the range (fig. 4) that roughly corresponded to sampled regions ([1] Orange County plus Mugu Lagoon and the captive breeders, [2] north San Diego County plus San Diego River, and [3] Kendall-Frost Mission Bay Marsh Reserve and south San Diego County; fig. 5). Individuals of mixed assignment were reported in all clusters, indicating recent or ongoing dispersal and gene flow occur directly among wetlands, or that gene flow is facilitated through the efforts of the captive breeding program. Principal component analysis also grouped individuals into three regional clusters (fig. 6*A*) along axes 1 (7 percent of the total genetic variation) and 2 (6 percent of the variation), while axis 3 (5 percent of the variation) separated individuals within marsh sites, particularly within the Orange County cluster (fig. 6*B*). PopCluster also supported three clusters and estimated recent gene flow rates among clusters ranging from 4.1 percent (from north San Diego County to Orange County) up to 23.5 percent (from Orange County to north San Diego County; table 2). Recent gene flow estimates among clusters were high, especially into the north San Diego County cluster. However, natural versus augmented levels of gene flow are difficult to separate in this system given the approximately 20 years (10–20 generations) of captive breeding and releases before genetic monitoring efforts. Higher rates of recent gene flow (last 3 generations) from the Orange County cluster (the source of the captive program) into the other two clusters could reflect these captive release efforts. Recent telemetry data indicate rail movement is usually localized. In a group of transmittered wild (N=42) and captive released (N=46) hatch year rails, only one captive rail moved between wetlands (from Tijuana Slough NWR to the San Diego Bay NWR South Bay Unit, about 4 kilometers [km]); all other rails stayed close to the initial capture locations (Sawyer, 2024). Similar average distances are reported from earlier studies, although occasional long-distance movements of up to 258 km have been recorded (U.S. Fish and Wildlife Service, 2020). The structuring of individual wetlands into three broader genetic populations that appear to be connected by moderate levels of gene flow provides important context for population management, supporting an inclusive regional approach consistent with genetic structure, rather than focused on individual wetlands as independent populations.

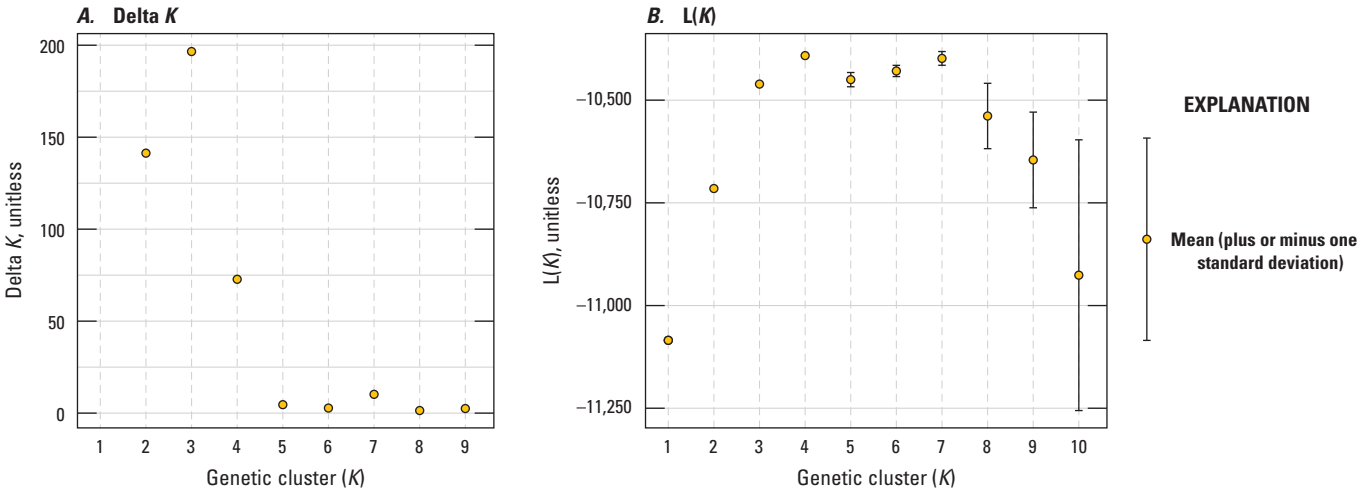
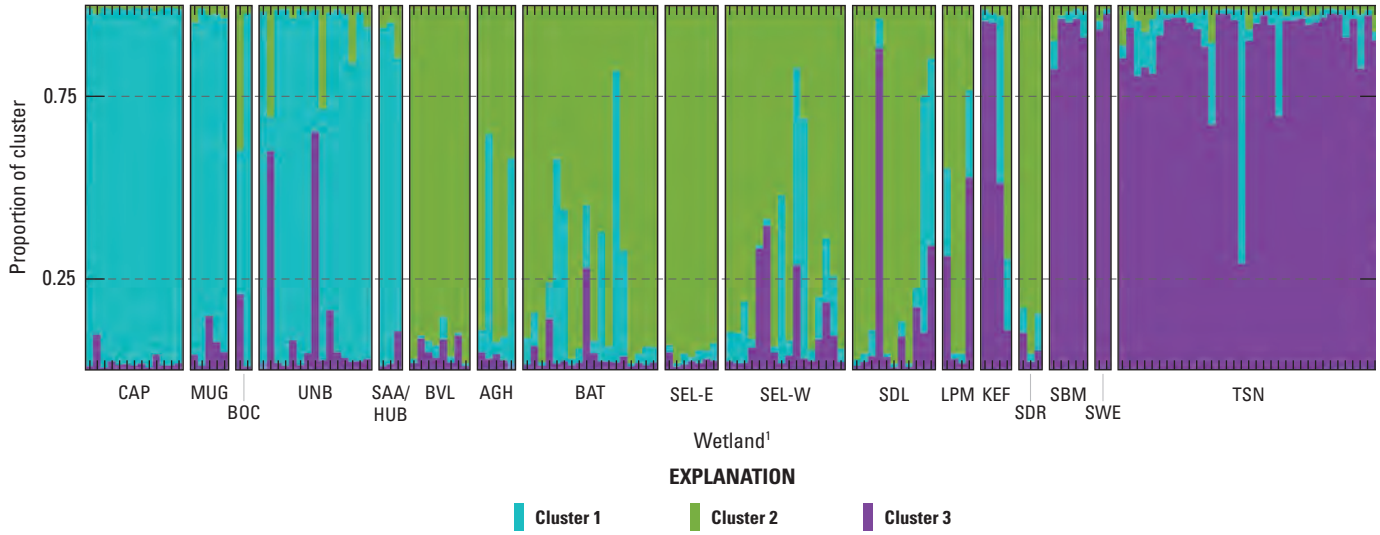


Figure 4. Results of STRUCTURE analyses of Light-footed Ridgway's Rails (*Rallus obsoletus levipes*) supporting three genetic clusters (K=3). *A*, Delta K (Evanno and others, 2005) for 1 to 9 clusters (K). *B*, mean log-posterior probability of K (L[K]) from STRUCTURE (Pritchard and others, 2000) for 1 to 10 clusters.



[1]CAP, captive breeders group I; MUG, Mugu Lagoon; BOC, Bolsa Chica Ecological Reserve; UNB, Newport Bay; SAA/HUB, Santa Ana River and Huntington Beach; BVL, Buena Vista Lagoon; AGH, Agua Hedionda; BAT, Batiquitos Lagoon; SEL-E, San Elijo Lagoon (east of I–5); SEL-W, San Elijo Lagoon (west of I–5); SDL, San Dieguito Lagoon; LPM, Los Peñasquitos Marsh and Creek; KEF, Kendall-Frost Mission Bay Marsh Reserve; SDR, San Diego River; SBM, San Diego Bay National Wildlife Refuge South Bay Unit; SWE, Sweetwater Marsh; TSN, Tijuana Slough National Wildlife Refuge.

Figure 5. Individual assignment plot for three clusters estimated with STRUCTURE. Light-footed Ridgway's Rails (*Rallus obsoletus levipes*) from Ventura County (Mugu Lagoon), Orange County, and the captive breeders were mainly assigned to Cluster 1 (blue). Cluster 2 (green) was mainly reported in north San Diego County wetlands. Birds from south San Diego County were mostly assigned to Cluster 3 (purple). Mixed assignments indicate genetic exchange across clusters, and the effect of the captive breeding program.

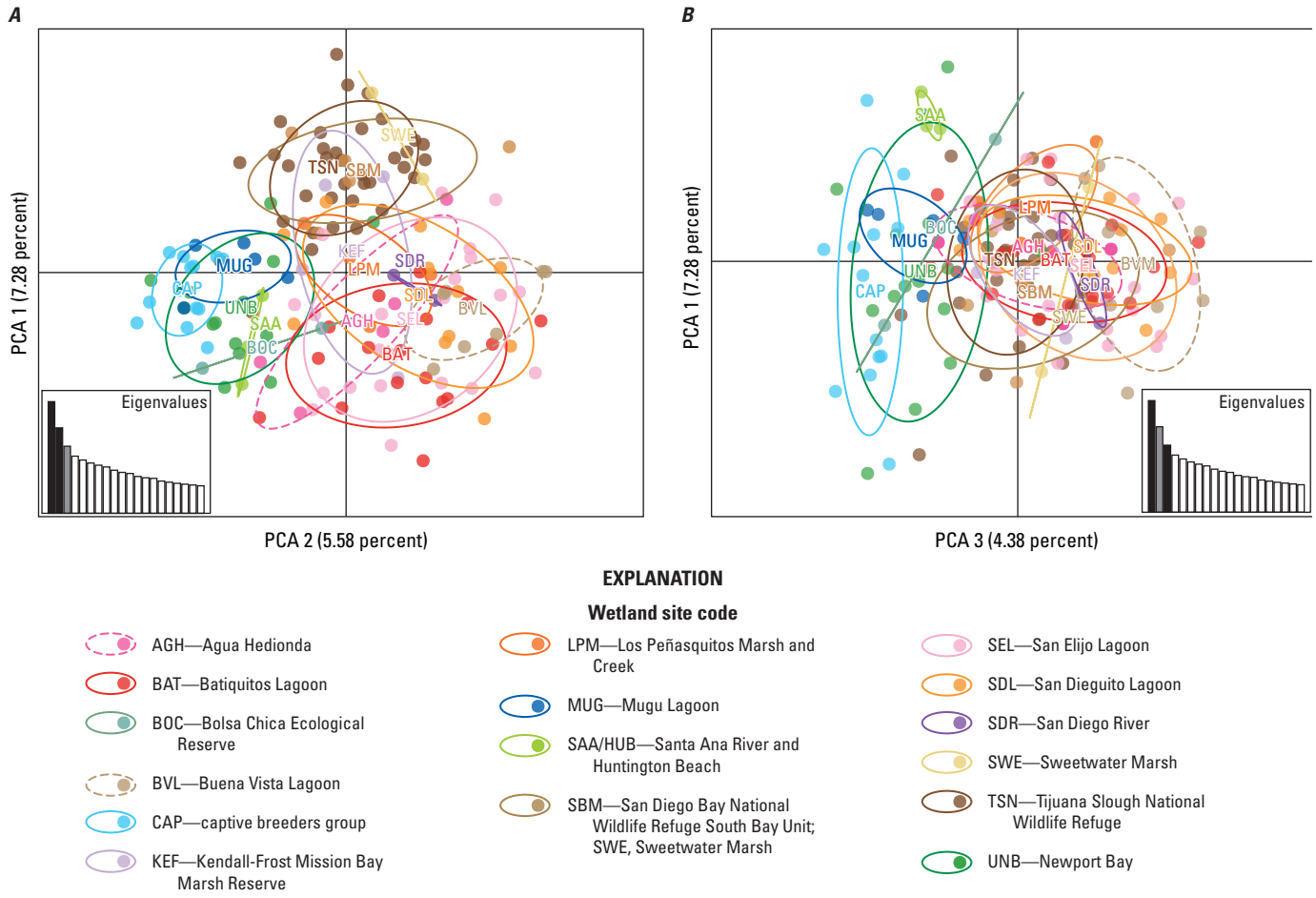


Figure 6. Principal component analysis (PCA) plots of major axes of all contemporary sampled Light-footed Ridgway's Rails (*Rallus obsoletus levipes*). Points representing individuals are colored by wetland with standard ellipses around wetlands. *A,* PCA axes 1 and 2 roughly group wetlands into three overlapping regional clusters (Orange County plus Mugu Lagoon and the captive breeders; north San Diego County; south San Diego County). Inset histograms show the proportions of variance explained by each vector, with plotted vectors shaded black; *B*, PCA axis 3 (plotted with PCA axis 1) appears less geographically informative, and further separates some individuals, particularly in the Orange County cluster.

Table 2. Estimated gene flow rates among regional populations of Light-footed Ridgway's Rails (*Rallus obsoletus levipes*).

[Columns denote the source populations and rows the receiver populations. Proportions to and from the same populations represent non-migrant sources. Sums greater than 1 indicate overall source populations]

To	From Orange County	From north San Diego County	From south San Diego County
Orange County[1]	0.865	0.041	0.094
North San Diego County	0.235	0.656	0.11
South San Diego County	0.11	0.083	0.807
Sum	**1.21**	**0.78**	**1.011**

[1]Includes all birds sampled within Orange County and all birds sampled at Mugu Lagoon in Ventura County.

Comparisons with Historical Samples

Comparing our recent samples to the 1989 baseline samples across the same set of wetlands suggests that genetic differentiation has declined slightly, relatedness has increased, and allelic richness has decreased over time (table 3). Although none of these changes were statistically significant, the direction of these respective measures is consistent with a small decline in overall genetic diversity in rail populations, which is noteworthy given the increase in sample size in two of three wetlands. The slight decline in F_{ST} may reflect the effect of the captive-bred individuals being sourced from a single site (Newport Bay) and released throughout the range or could reflect an increase in naturally occurring dispersal and gene flow among regions, facilitated by the increased population sizes in the center of the range. The PCA of baseline and contemporary samples separated sites spatially along the primary axis (fig. 7*A*). The second axis separated the temporal sampling periods at Tijuana Slough NWR and the third separated the temporal sampling periods within Mugu Lagoon and Newport Bay (figs. 7*A*, *B*). Mugu Lagoon seems to be the most distinctive over time, with non-overlapping point clouds (fig. 7*B*). Genetic differentiation over time is consistent with genetic drift (loss of genetic diversity over time), which is more extreme in smaller and more isolated populations.

Genetic Diversity and Effective Population Size

By all measures, north San Diego County has the highest genetic diversity of all sampled regions, followed by south San Diego County, Orange County and, having the lowest genetic diversity, Mugu Lagoon (table 1). Low genetic diversity at Mugu Lagoon could reflect its position at the northern range edge and consistently low survey numbers. Despite augmentation attempts with more than 100 captive-reared birds between 2001 and 2009, the maximum number of pairs observed at Mugu Lagoon during the last two decades was in the low 20s, and only a handful of pairs were observed during the past few years (fig. 2; Zembal and others, 2024). Newport Bay also appears to have relatively low levels of genetic diversity compared with its baseline sample. Counts during annual surveys have rapidly declined since 2017 in Newport Bay and in surrounding wetlands in Orange County. Declining numbers here have been attributed to increasing tidal inundation (Zembal and others, 2024). We could not estimate effective population size (N_e) at Mugu Lagoon due to low sample size. Among the other three regions, the contemporary N_e point estimate was lowest in Orange County and highest in north San Diego County (table 4), which is consistent with all other diversity metrics. Contemporary N_e point estimates were lower than baseline samples, although CIs overlapped (table 4). General guidelines suggest N_e should be greater than 50–100 to avoid inbreeding, and greater than 500–1,000 to preserve allelic richness and long-term adaptive potential (Frankham and others, 2014). Orange County may be at or below these lower thresholds (upper 95-percent CI=113), whereas north San Diego (upper 95-percent CI=486) and south San Diego County (upper 95-percent CI=559) may be at or below the upper thresholds.

Table 3. Tests for differences in genetic differentiation (F_{ST}), relatedness (*R*), allelic richness (*Ar*) and unbiased expected heterozygosity (*He*) in populations of Light-footed Ridgway's Rails (*Rallus obsoletus levipes*) by period.

[*P*-values were all greater than 0.1 and were considered not statistically significant. **Abbreviations**: MUG, Mugu Lagoon; TSN, Tijuana Slough National Wildlife Refuge; UNB, Newport Bay]

Group	F_{ST}	*R*	*Ar*	*He*
Baseline (MUG, UNB, TSN)	0.081	0.053	1.834	0.335
Current (MUG, UNB, TSN)	0.071	0.196	1.756	0.329
P-value	0.366	0.118	0.125	0.331

Genetic Rescue

Frankham and others (2017) provides decision tables for determining whether a population could benefit from genetic rescue and state that appropriate source populations should have higher heterozygosity than the receiver population (F>0.1). Because it was estimated to have greater heterozygosity than the other genetic clusters, the north San Diego County cluster could be a genetically beneficial source for all other regions examined, producing F>0.1 in the receiver populations (table 5). Mugu Lagoon, with the lowest heterozygosity of any site, could benefit from genetic rescue from any other source (table 5). Finally, because the captive breeders have low diversity when compared to wild populations, augmentation results in negative F for all sites except for Mugu Lagoon (table 5). New source populations could improve diversity in the captive breeding program (see the "Managing Genetic Diversity in the Captive Program" section).

Whether or not wild regional clusters could benefit from genetic rescue can also be assessed with information about population size and isolation. Although Mugu Lagoon and the Orange County cluster have low or declining survey numbers, low effective population sizes, and are geographically more isolated, the north and south San Diego County clusters have larger survey numbers based on recent call-broadcast surveys (fig. 3) and higher effective population sizes. Although gene flow estimates among clusters were high, augmentation through the captive release program could account for some of this, and rates were lowest into the Orange County cluster.

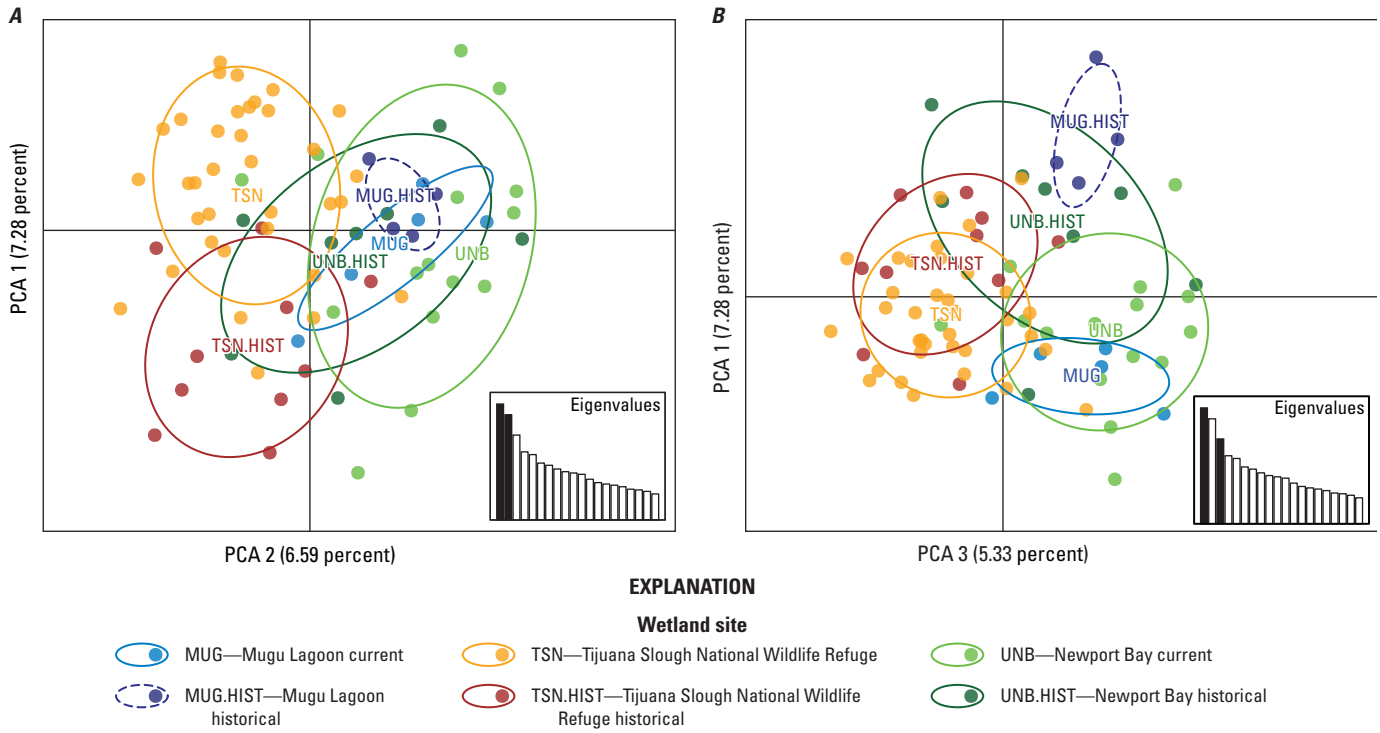


Figure 7. Principal component analysis (PCA) plots of historical baseline and recent samples of Light-footed Ridgway's Rails (*Rallus obsoletus levipes*) colored by wetland. *A*, PCA axes 1 and 2 separate the northern and southern sites and historical and contemporary samples in Tijuana Slough National Wildlife Refuge (TSN and TSN.HIST); *B*, PCA axis 3 separates baseline and recent samples from Mugu Lagoon and Newport Bay, respectively.

Table 4. Linkage disequilibrium estimates of genetic effective population size (N_e) of Light-footed Ridgway's Rails (*Rallus obsoletus levipes*) populations assuming a monogamous breeding system and using alleles with a frequency of greater than 1 percent.

[Corresponding 95-percent confidence intervals (CI) were jackknifed across samples. Estimates for Mugu Lagoon could not be calculated (NC) because of low sample size. An upper CI of infinity (INF) indicates that there is not enough information in the dataset to estimate the upper bound. This can occur when sample sizes are small or when N_e is large. **Abbreviation**: —, no data]

Region	Current N_e (95-percent CI)	Baseline N_e (95-percent CI)
Ventura County (Mugu)	NC	NC
Orange County	45 (25–113)	140 (49–INF)
North San Diego County	235 (148–486)	—
South San Diego County	115 (55–559)	915 (32–INF)

Table 5. Genetic rescue decision table for source populations of Light-footed Ridgway's Rails (*Rallus obsoletus levipes*).

[In all cases, birds sourced from the north San Diego County Cluster could provide the greatest potential improvements to genetic diversity. **Abbreviations**: *F*, inbreeding coefficient; *He*, unbiased expected heterozygosity]

Region	*He*	*F* by Source				Is the population isolated (no or low gene flow)?	Is the population very small or small for multiple generations?
		Captive	Orange County	North San Diego County	South San Diego County		
Ventura (Mugu Lagoon)	0.279	0.004	[1]0.16	[1]0.285	[1]0.2	Yes	Yes
Orange County	0.332	−0.185	0	[1]0.16	0.047	Yes	Yes
North San Diego County	0.389	−0.391	−0.173	0	−0.118	No	No
South San Diego County	0.348	−0.348	−0.05	[1]0.106	0	No	No

[1]Combinations of source and receiver populations with *F*-values greater than 0.1 indicate an improvement in genetic diversity.

Managing Genetic Diversity in the Captive Program

Adjustments to captive rearing source populations and release strategies, informed by new empirical estimates of population genetic diversity and structure, could help preserve genetic diversity. The pool of captive breeders has lower genetic diversity than all wild genetic clusters except for Mugu Lagoon. Therefore, the recently released hatch year birds likely added little or no genetic diversity benefit to the receiver populations into which they have been released (table 5). Two factors may contribute to this. First, breeding birds for the captive program have been consistently sourced from one wetland across the range (Newport Bay). However, this wetland has recently declined in size and has low genetic diversity and low effective population size, suggesting it may benefit from genetic rescue itself (tables 4, 5). Second, the captive breeding program is small, composed of up to six pairs annually. Some of the recent breeding birds have high inbreeding coefficients and some pairs have elevated (non-zero) genetic relatedness, despite efforts to minimize pairings between known relatives based on the pedigree (table 6). These genetic estimates could indicate non-zero relatedness among the wild ancestors. Given the small number of breeding birds in the captive program at any one time, efforts to rotate in wild birds more frequently could help to incorporate new genetic diversity. Retaining later generations of offspring in the breeding program could increase relatedness, depending on pairings. Large differences in productivity among breeding pairs may also skew the genetic makeup of captive-released cohorts. This could be reduced by limiting the number of clutches produced by each captive pair each season. Limiting breeding windows, especially to the beginning of the season, may also help increase the probability of survival for captive-released juvenile rails. Analysis of telemetry data indicated that captive rails released early in the summer had higher survival rates than those released later (Sawyer, 2024; Sawyer and Conway, in press). Finally, ensuring receiver sites receive a mix of clutches produced by unrelated pairs could decrease the overall relatedness of birds released at a single site and season.

Wetlands in north San Diego County have the highest heterozygosity, allelic richness, and private allelic richness across all surveyed regions and the lowest relatedness. Sourcing birds or eggs from the larger wetlands within the north San Diego County cluster could provide the greatest increase to the genetic diversity and representation within the captive breeding population for future population augmentation (table 4). Because relatedness values were generally higher within than among wetlands even within the same regional clusters, pairing birds sourced from different wetlands instead of a single wetland could also help reduce the chances of including closely related birds in the captive program. Finally, genotyping all candidate parents could directly estimate genetic relatedness and suggest pairings to minimize inbreeding.

Wetland Restoration

Given that wetlands in north San Diego County appeared largely unoccupied before the mid-2000s, it could be possible that a combination of habitat restoration coupled with captive releases (fig. 3) are responsible for the increase in numbers of pairs and high genetic diversity in north San Diego County. In addition, given estimated gene flow rates of 8–11 percent between south and north San Diego County genetic clusters, it is possible that natural dispersal of wild birds may be sufficiently high to maintain genetic diversity and connectivity across this part of the subspecies' range.

Although opportunities to restore wetlands may be rare throughout the northern part of the subspecies' range, restored wetlands could provide more stepping stones for increased connectivity. In the more immediate time frame, our genetic analyses suggest that Orange County and Mugu Lagoon clusters could benefit from augmentation and genetic rescue from a higher-diversity source population.

Another important factor in maintaining high diversity is retaining large populations to minimize the erosion of local genetic diversity. Habitat management and restoration can assist in maintaining large populations and could become even more critical given predicted sea-level rise, which may threaten wetland habitat in areas without sufficient upland habitat for marsh retreat (Osland and others, 2022), and may already be affecting the population at Newport Bay (Zembal and others, 2024). Models of California wetland vulnerabilities to sea-level rise, including three marshes occupied by rails (Newport Bay, Sweetwater, and Tijuana Slough NWR) predicted significant loss of high and middle marsh habitat by 2050 and between 50- and 100-percent conversion to bare mudflats by 2100 under moderate to high sea-level rise scenarios (Thorne and others, 2018). Survival of juvenile rails is affected by elevation, and the timing and water level at high tide (Sawyer, 2024; Sawyer and Conway, in press). The abundance of raptors may also have a negative effect on survival, especially for captive-released rails (Sawyer, 2024; Sawyer and Conway, in press). A recent 5-year study of mortality in California Ridgway's Rails in San Francisco Bay indicated that avian predators accounted for most of the observed mortalities (Casazza and others, 2016).

Table 6. Recent breeding pairs in the captive breeding program of Light-footed Ridgway's Rails (*Rallus obsoletus levipes*), including hatch years, number of offspring produced, pedigree and genetic-based coancestry/relatedness and individual inbreeding coefficients (±standard deviations).

[Pedigree-based statistics were calculated in kinship2 and genetic statistics were calculated in EMIBD9. Superscripts following sire and dam studbook numbers denote the maximum number of generations the individual is removed from wild ancestors. **Abbreviations**: ±, plus or minus; NC, not calculated because a genetic sample was not available]

Breeding pair studbook numbers (sire and dam)	Offspring hatch years	Number of offspring produced	Pedigree-based coancestry of breeding pair	Genetic relatedness of breeding pair	Pedigree-based inbreeding coefficient of sire	Mean genetic inbreeding coefficient of sire	Pedigree-based inbreeding coefficient of dam	Mean genetic inbreeding coefficient of dam
678[1], 681[1]	2018–20	57	0	NC	0	0.0683±0.0175	NC	NC
675[1], 679[1]	2018–19	32	0	NC	0	0.0534±0.0130	NC	NC
680[1], 676[1]	2018–19	4	0	NC	0	NC	0	0.0403±0.0148
751[1], 761[2]	2020–22	19	0	NC	0	NC	0	0.0207±0.0076
812[2], 822[2]	2023	1	0	0.0433	0	0.0792±0.0237	0	0.1370±0.0170
817[2], 848[2]	2021–22	19	0.125	0.0481	0	0.0287±0.0141	0	0.0234±0.0159
832[2], 839[2]	2021–22	30	0	0.0074	0	0.0283±0.0099	0	0.1360±0.0252
838[3], 825[1]	2022–23	41	0	0.0061	0	0.1169±0.0169	0	0.3799±0.0182
890[3], 839[2]	2023	16	0	0.0032	0.125	0.0171±0.0085	0	0.1360±0.0252
794[1], 926[3]	In progress	In progress	0.03125	0.0742	0	0.0036±0.0036	0	0.0498±0.0120
893[3], 907[5]	In progress	In progress	0	0.0286	0.125	0.0701±0.0242	0	0.0438±0.0125

Preliminary Conclusions and Future Research Objectives

In collaboration with researchers from Mexico, USGS has received samples from the southernmost part of the range in Ensenada, Baja California, Mexico (Estero de Punta Banda and Bahía de San Quintín). Genetic and genomic analyses of these samples can help characterize genetic diversity across the full subspecies range. A larger, genome-wide set of single nucleotide polymorphisms (Peterson and others, 2012) may provide greater sensitivity to discern any additional structure among sampled wetlands, could help assess genomic diversity, and may better resolve effective population sizes given small sample sizes (Andrews and others, 2016). Nevertheless, results to date suggest that the microsatellite loci described and analyzed here identified regional patterns in genetic diversity in wild populations and estimates of genetic relatedness and inbreeding of captive rails. These markers could provide a cost-effective tool to monitor genetic diversity in the breeding program moving forward.

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Appendix 1. Supplementary Tables

Table 1.1. Founder contribution to the release program by region and overall, expressed as percentage of the 655 Light-footed Ridgway's Rails (*Rallus obsoletus levipes*) released in southern California wetlands between 2001 and 2024.

Founder	Year of first offspring released	Year of last offspring released	Mugu Lagoon	Orange County	North San Diego County	South San Diego County	Percent total contribution
WILD8	2001	2005	0.88	0.38	0.88	0.53	2.67
WILD7	2001	2005	0.88	0.38	0.88	0.53	2.67
WILD1	2001	2005	0.88	0.38	0.88	0.46	2.60
WILD2	2001	2005	0.88	0.38	0.88	0.46	2.60
WILD3	2001	2006	1.35	0.11	0.50	0.39	2.35
WILD5	2001	2006	1.35	0.11	0.50	0.39	2.35
WILD4	2001	2006	1.35	0.11	0.50	0.39	2.35
WILD6	2001	2006	1.35	0.11	0.50	0.39	2.35
WILDSB2	2003	2003	0.00	0.53	0.00	0.00	0.53
WILDSB1	2003	2003	0.00	0.53	0.00	0.00	0.53
WILD9	2003	2012	1.35	0.15	0.73	0.28	2.51
WILD10	2003	2012	1.35	0.15	0.73	0.28	2.51
WILD14	2004	2008	0.11	0.00	0.00	0.00	0.11
WILD13	2004	2008	0.11	0.00	0.00	0.00	0.11
WILD11	2004	2010	0.08	0.19	0.23	0.34	0.84
WILD12	2004	2010	0.08	0.19	0.23	0.34	0.84
WILD18	2005	2007	0.08	0.00	0.04	0.19	0.31
WILD17	2005	2007	0.08	0.00	0.04	0.19	0.31
WILD24	2006	2013	0.84	0.36	0.88	0.43	2.51
WILD23	2006	2013	0.84	0.36	0.88	0.43	2.51
WILD27	2006	2014	0.23	0.61	0.97	1.11	2.91
WILD28	2006	2014	0.23	0.61	0.97	1.11	2.91
WILD19	2007	2009	0.11	0.00	0.11	0.08	0.31
WILD20	2007	2009	0.11	0.00	0.11	0.08	0.31
WILD22	2007	2012	0.27	0.19	0.34	0.31	1.11
WILD21	2007	2012	0.27	0.19	0.34	0.31	1.11
WILD25	2007	2012	0.11	0.00	0.11	0.08	0.31
WILD26	2007	2012	0.11	0.00	0.11	0.08	0.31
WILD15	2008	2009	0.04	0.08	0.00	0.00	0.11
WILD16	2008	2009	0.04	0.08	0.00	0.00	0.11
WILD32	2008	2012	0.27	0.19	0.31	0.11	0.88
WILD31	2008	2012	0.27	0.19	0.31	0.11	0.88
WILD30	2008	2018	0.15	1.11	2.18	2.30	5.74
WILD29	2008	2018	0.15	1.11	2.18	2.30	5.74
WILD36	2009	2012	0.00	0.19	0.00	0.08	0.27
WILD35	2009	2012	0.00	0.19	0.00	0.08	0.27

Table 1.1. Founder contribution to the release program by region and overall, expressed as percentage of the 655 Light-footed Ridgway's Rails (*Rallus obsoletus levipes*) released in southern California wetlands between 2001 and 2024.—Continued

Founder	Year of first offspring released	Year of last offspring released	Mugu Lagoon	Orange County	North San Diego County	South San Diego County	Percent total contribution
WILD33	2009	2018	0.00	0.31	0.98	1.01	2.29
WILD34	2009	2018	0.00	0.31	0.98	1.01	2.29
WILD39	2010	2010	0.00	0.00	0.00	0.08	0.08
WILD40	2010	2010	0.00	0.00	0.00	0.08	0.08
WILD37	2010	2014	0.00	0.03	0.05	0.18	0.25
WILD38	2010	2014	0.00	0.03	0.05	0.18	0.25
WILD47	2011	2011	0.00	0.08	0.08	0.00	0.15
WILD48	2011	2011	0.00	0.08	0.08	0.00	0.15
WILD51	2011	2012	0.00	0.19	0.08	0.00	0.27
WILD52	2011	2012	0.00	0.19	0.08	0.00	0.27
WILD49	2012	2012	0.00	0.08	0.00	0.00	0.08
WILD50	2012	2012	0.00	0.08	0.00	0.00	0.08
WILD41	2012	2015	0.00	0.08	0.31	0.08	0.46
WILD42	2012	2015	0.00	0.08	0.31	0.08	0.46
WILD_L	2012	2016	0.00	0.04	0.69	0.11	0.84
WILD_K	2012	2016	0.00	0.04	0.69	0.11	0.84
WILD46	2012	2016	0.00	0.04	0.69	0.11	0.84
WILD45	2012	2016	0.00	0.04	0.69	0.11	0.84
WILD56	2014	2014	0.00	0.00	0.08	0.00	0.08
WILD54	2014	2014	0.00	0.00	0.08	0.00	0.08
WILD55	2014	2014	0.00	0.00	0.08	0.00	0.08
WILD53	2014	2014	0.00	0.00	0.08	0.00	0.08
WILD57	2014	2019	0.00	0.00	0.15	0.00	0.15
WILD58	2014	2019	0.00	0.00	0.15	0.00	0.15
WILD_I	2018	2019	0.00	0.00	0.18	0.20	0.38
WILD_J	2018	2019	0.00	0.00	0.18	0.20	0.38
WILD66	2018	2023	0.03	0.34	0.94	2.39	3.71
WILD65	2018	2023	0.03	0.34	0.94	2.39	3.71
WILD64	2018	2023	0.03	0.34	0.86	2.39	3.63
WILD63	2018	2023	0.03	0.34	0.86	2.39	3.63
WILD69	2018	2024	0.13	0.76	0.64	1.34	2.87
WILD68	2018	2024	0.13	0.84	0.56	1.34	2.87
WILD67	2018	2024	0.13	0.84	0.56	1.34	2.87
WILD70	2018	2024	0.13	0.76	0.64	1.34	2.87
UNK_B	2019	2019	0.00	0.00	0.00	0.08	0.08
UNK_C	2019	2019	0.00	0.00	0.00	0.08	0.08
WILD_A	2020	2024	0.13	0.27	0.41	0.94	1.75
WILD_B	2020	2024	0.13	0.27	0.41	0.94	1.75
WILD_H	2022	2024	0.10	0.27	0.10	0.29	0.74
WILD_G	2022	2024	0.10	0.27	0.10	0.29	0.74
All founders	2001	2024	17.25	16.49	30.99	35.27	100.00

Table 1.2. Baseline blood and DNA samples of Light-footed Ridgway's Rails (*Rallus obsoletus levipes*) collected in 1989 and provided by R. Fleischer, Smithsonian Institution.

[DNA, deoxyribonucleic acid; ID, identification; NWR, National Wildlife Refuge]

Sample ID this study	Smithsonian sample ID	Sample type	Site	County
TSN_001	486	Blood (capillary)	Tijuana Slough NWR	San Diego
TSN_002	487	Blood (capillary)	Tijuana Slough NWR	San Diego
TSN_003	488	Blood (capillary)	Tijuana Slough NWR	San Diego
TSN_004	490	Blood (capillary)	Tijuana Slough NWR	San Diego
TSN_005	491	Blood (capillary)	Tijuana Slough NWR	San Diego
TSN_006	492	Blood (capillary)	Tijuana Slough NWR	San Diego
TSN_007	493	Blood (capillary)	Tijuana Slough NWR	San Diego
TSN_008	494	Blood (capillary)	Tijuana Slough NWR	San Diego
TSN_009	495	Blood (capillary)	Tijuana Slough NWR	San Diego
TSN_082	498	Blood (capillary)	Tijuana Slough NWR	San Diego
UNB_001	496	Extracted DNA	Newport Bay	Orange
MUG_001	497	Extracted DNA	Mugu Lagoon	Ventura
UNB_002	601	Extracted DNA	Newport Bay	Orange
UNB_003	602	Extracted DNA	Newport Bay	Orange
UNB_004	605	Extracted DNA	Newport Bay	Orange
UNB_005	606	Extracted DNA	Newport Bay	Orange
UNB_006	607	Extracted DNA	Newport Bay	Orange
UNB_007	608	Extracted DNA	Newport Bay	Orange
MUG_002	609	Extracted DNA	Mugu Lagoon	Ventura
MUG_003	613	Extracted DNA	Mugu Lagoon	Ventura
MUG_004	614	Extracted DNA	Mugu Lagoon	Ventura
UNB_008	616	Extracted DNA	Newport Bay	Orange
SEB_001	622	Extracted DNA	Seal Beach	Orange
SEB_002	623	Extracted DNA	Seal Beach	Orange
SEB_003	624	Extracted DNA	Seal Beach	Orange
SEB_004	626	Extracted DNA	Seal Beach	Orange

Table 1.3. Microsatellite primers and multiplex mixes designed for Light-footed Ridgway's Rails (*Rallus obsoletus levipes*). All primer sequences are presented 5′ to 3′.

Locus	Multiplex	Forward primer sequence	Reverse primer sequence
RAOB_12508	Mpx1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGAAGGCCCATAATGCTTTGAAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGCCCATCATCAGGGTCTG
RAOB_1332	Mpx1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGACTTTACTCTTCCTCGTTTC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTATTGCTGGTACTGGTCACATG
RAOB_14777	Mpx1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGTCTTCTCCCATCACCTCTG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTGTCTTCTTGGAAAGGCAAATC
RAOB_17999	Mpx1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTACTTCACACCCAGAATCAACC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTTCACAGGCTGTTCTATCACTG
RAOB_2350	Mpx1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGCCAGAATTTCGATACACATGG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCGTTCGGTTTAGAAGAATCATC
RAOB_23910	Mpx1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTGATTCATTCCCACACCTACAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAGCAGCATGAACTTTAAGGTTTG
RAOB_25163	Mpx1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGTCCTCCTGTTACTAGGAAGG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTGCTTTACCATCTTGCACTCC
RAOB_2774	Mpx1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTTGCATGAGTGTTGGATACC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCTGCAAACCATCAATGTTTAC
RAOB_3020	Mpx1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGAGCAAAGAAAGTGAGGGCATC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTTTCTGCTTCTGGTTTGGTTTG
RAOB_32277	Mpx1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGTCCAGGTGAGCTGAGTTAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTTGACCAGATGATTACAGAGGG
RAOB_34562	Mpx1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTACTCTGGTTCTTGAGTGTGG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCAAGGACAGGCAGCAC
RAOB_36470	Mpx1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTGCAGAAATCCATAGAGAAGCC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAGGGCTCCTCTAGAAGTATTCC
RAOB_4239	Mpx1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTCCTGCAGAGCGTGTTTG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTGCAGCTCAGAGGTATCAC
RAOB_4254	Mpx1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTTTGTGAGGATGTGAATGAGGG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTTAACCAAGGGCACAGG
RAOB_4555	Mpx1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCACTTGGCCAAACAAATGTG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTACCACCAACAGAAGA-CAATTG
RAOB_4746	Mpx1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTATGACTAGGAAGCTGGGAGTC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCTTAGTGCTTGGAGTTCTTTG
RAOB_4853	Mpx1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTTTCAGAGAAGCACATGGAAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCACAATGCCACTTTCTTGAGAC
RAOB_5114	Mpx1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGCTTTGCCTTTGATCTCCAGAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTTGCCGCTTCCATCCATC
RAOB_5737	Mpx1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGAGGGAGTAGGTTGTGTATTGG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCTTGAGTTTGGAAGGTTTGGG
RAOB_6796	Mpx1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGCTCATATGTAGAGAAAGTCCC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGATGCAATTTACACTTGCTCAAC
RAOB_7107	Mpx1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGACTAGTACAGTCTGCCCTTAGG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAAGAGCTCTGACATTAACTGGG
RAOB_7389	Mpx1	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGAAACTGTTGCGTTCGCTG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGAAGGAGGGAGACACTACAG
RAOB_10511	Mpx2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGATGCCCTGCTCATATTGTCAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCAGAATTCAAGGTGGGTGCC
RAOB_1164	Mpx2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCAGCAAGATCTTACCAGTCCTG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTGAGAAATGAGGATCAGCTTGG
RAOB_1329	Mpx2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGACACAAATCTTTAAAGCTGGGC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCTATTCCACTATTCCAGCAAG
RAOB_13848	Mpx2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGAGATGTGCCTTTGTTACATGC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTAGATGAGCGGGTACTTAGAGG
RAOB_13860	Mpx2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTCATCAGTGCAGTGTTCAGAAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTTTGTTCATTGCTTTCACCACC
RAOB_1556	Mpx2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGACAGTCTGGACCATGGATG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTTACTTGCAAACTCCCACCTAC
RAOB_1639	Mpx2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGAACCTTGACACTGAGATAGCC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCCAGGGAGAGCCTTATATTGC
RAOB_20271	Mpx2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTGTCTGTGCACTTGTTCTCTAC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTTATCCAGGTCATTCATCCACC
RAOB_22344	Mpx2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTACTTGCCATACTTCAGAAGCG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTTTGCACACATGAAACTGGAAC
RAOB_22510	Mpx2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGTGTGATGGTGTGATGCC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGATGGGTGCTTGGAGGTG
RAOB_2335	Mpx2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTTACACTAGGAAGGCTGCTTG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGATCTAGCCATCAATTCCCAGTG
RAOB_24304	Mpx2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTGCTGCCATGGAAGGATG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCTTGGTCTTCTTGTTACCAGC
RAOB_2524	Mpx2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGATAAGAAGCATGGAGGAAGG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCAGGTTTAGGAGGTGTTTAGGG
RAOB_25393	Mpx2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTGTTTGGCTCATCTCTGTAGG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTAGGAGAGGACCCATGAAAGAG
RAOB_2565	Mpx2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTCCAAGACTCAGTGACATC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTACAAATCCCACTACCAGCAG
RAOB_2664	Mpx2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTAGGCAATGCTTCTTTCTGC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTGGGCAGATCTTAGGAAACAC

Table 1.3. Microsatellite primers and multiplex mixes designed for Light-footed Ridgway's Rails (*Rallus obsoletus levipes*). All primer sequences are presented 5′ to 3′.—Continued

Locus	Multiplex	Forward primer sequence	Reverse primer sequence
RAOB_3316	Mpx2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGAGGTCAGGGTGTTACTTCTTG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGAAAGTGTAGTGATTGCATGGC
RAOB_3540	Mpx2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGACAGTAATGCAGGAAGTCATGG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAAGAGCAAGAGTAGGTTTGGTC
RAOB_3642	Mpx2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCGGCCTACAAATGGGTCAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGACAGTGCAGGGAAACATCATC
RAOB_3754	Mpx2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCAGTGTTCTTGCTTACTTGTG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCAAATGATGAAGCAGTAGGGC
RAOB_4634	Mpx2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGATGGAGGAGTTACTAGGGTTGG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAGCAGGACTATGTTGTACAGTC
RAOB_4709	Mpx2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTAGGTCCATATGCAGACAAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTGGAGGTATCAGTATTGTGGC
RAOB_4768	Mpx2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTTGGAGATATGGTTTAGCAGCG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTGGTCATGTTTGTATGCTCTCC
RAOB_5228	Mpx2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTTTCGGTGTTGTGGGTTGG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCAAGTGGAGCTGTTGTGC
RAOB_5271	Mpx2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCCTTGTGTTTGTTCTTCCC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGTGGAAGCCTGTGAATCAAC
RAOB_5797	Mpx2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGATGAAAGCTGTGAGAAAGGAGG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAATCATGGGTCAAGGTAAAGGC
RAOB_6334	Mpx2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGAAGAGAGAGTTGTTGGTAGGG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTGAGACCTGTGACTTGTTCC
RAOB_6838	Mpx2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGATTGTGTCTGGGAGATGCTAAC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGATAGCTATTCATTCCACAGTTA-AAC
RAOB_6945	Mpx2	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGCTGGAAACCACCCTCC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAGCTTGATCGTTATGGGAAAGG
RAOB_13269	Mpx3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGAACCTTACCCATGAGCATTG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCCACAGAGGGATGGCAAC
RAOB_13477	Mpx3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGCTTCACGTGGCAAGTCAC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGATGAGATGCACAGAGAAGAC
RAOB_1383	Mpx3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGCCACTCTCCTTCACAATATC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAGTTCTGGTTAGCTGCTGTTAG
RAOB_1475	Mpx3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGCAAATGGAGGTTCAACTG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCAGGAATAGCAAGGTCTGGATC
RAOB_15280	Mpx3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGACAGAGGAAGGGAAGGGAAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTCTTCTTAGCATTTCTGAGGC
RAOB_15740	Mpx3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTTTCTCCTCCAAGTCTGTTTGC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTCTGTGTAAGGCTGTAAGGG
RAOB_1743	Mpx3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTTGAGTTGGGAAGTAGGGAAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGAAAGGCTCGAGATAAGGACAG
RAOB_1786	Mpx3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTGTGTAGTTATCAAGCCAGGTC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGTTGAAATGGAATGGAAATT-GAC
RAOB_2253	Mpx3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTTCAGGATGTGGATTTGTAGGC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTGCAAAGGACAAGTTAGGAGTC
RAOB_2268	Mpx3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCACAGATGCAAAGACAGGC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTTCTCCATCTAGCCCTCAAAC
RAOB_24572	Mpx3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTAGGTGGAAAGGTCAGATGTG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAAACCCACAGAACCAAGGAAG
RAOB_2982	Mpx3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGACTCATCAGGGCTTCTTTGTTG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGATGTTTAGAAGGGTGATTGCC
RAOB_3322	Mpx3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTCAGACCCACACAAATGAAGAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGAACTTCAGGGAACGAGGTATC
RAOB_3577	Mpx3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGAGAGGGCTGATAGATGATGG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGAAACCTTCAGCTCCTCCAG
RAOB_35986	Mpx3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTTTATGGCACTGGGAAGGAAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTTGCTGGTGAAGACTGAGTC
RAOB_3965	Mpx3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCGCCAAATGACAACTGAAAGG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTTTGCTCCTGGGTTTAGCTCTC
RAOB_45183	Mpx3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTTATGAAGAAAGGATGGGTGGG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTGACGACTATTTGTGCAAGACC
RAOB_4686	Mpx3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGTTGAGGCTTTGACAGGTTTG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTCACAACAGGGTCACATGAATC
RAOB_4758	Mpx3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTAACATGGGAATTCAGCTGGTG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAGGGTTGCAGCACATTCAG
RAOB_4933	Mpx3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGATGGAAAGTCTGGTGATCTC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAGCGTAGTTACAAAGACAGAGC
RAOB_5623	Mpx3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCACTTGTCTCGCTTTGATGTTG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTTGTTAACCAAGCAACTGGG
RAOB_5805	Mpx3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGAAATGGAGTCTTTGGCTCTAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTGTGACAATGGCAAAGTGTTTC
RAOB_6407	Mpx3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGCAACACAAAGCAGCTGTAAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGACCGTGAGTTACCCAAAGATTG
RAOB_7609	Mpx3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTTTCAGTGATGAGTAGGTGGC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTGGTCTCTTGGTGTATTATGGC

Table 1.3. Microsatellite primers and multiplex mixes designed for Light-footed Ridgway's Rails (*Rallus obsoletus levipes*). All primer sequences are presented 5′ to 3′.—Continued

Locus	Multiplex	Forward primer sequence	Reverse primer sequence
RAOB_8569	Mpx3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGCGTAGTTACAAAGACAGAGC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGATGGAAAGTCTGGTGATCTC
RAOB_9050	Mpx3	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTGGATGCAGTCGTGGC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTTGCTGCTTGTCGAGGTAC
RAOB_11545	Mpx4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTCAAATCCCTCCTGAATGCTG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGCAGAGGAAGAGAAGGGC
RAOB_1299	Mpx4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTTGAGTGGGTTAGGCTCTG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTTTCCACCCATCCACTTGTC
RAOB_1423	Mpx4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGTGAAGAAGGAGGCAGAGC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAGTGCTTGCTCCTCTCCAG
RAOB_1474	Mpx4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGACAGGAAGGTGCAGCAGG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAAAGCATCCAATACATCCAGGG
RAOB_1492	Mpx4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTAAGCAGATGAGACCAGACCAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGACAGGTAAGAAGCAAAGAAATGAC
RAOB_1552	Mpx4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTACATCTTCCTGGAGTTCACC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAGAGCACGTAAACAACAGAAGG
RAOB_1748	Mpx4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGAAGGCAGAATAGGGCAGAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTTACTCTTCCCTCAACCACTC
RAOB_1837	Mpx4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTTTCCTTCCTCCTCAACAATGC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTAAAGCCAGAGGAAATAGGTGG
RAOB_1950	Mpx4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCAGGAGTTGGACTTGATGATCC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCATGGGCAAAGTTTGGTAGATG
RAOB_19980	Mpx4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGAAGAAGTAACGTGTAGGTCC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTTCAGCATCTTCATAGCCCTG
RAOB_2122	Mpx4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTTACAGGAAGACAAAGGAGCAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTAGCCCAAAGTCTCAATGGAAG
RAOB_2211	Mpx4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTTCTGGGCTGAGATTTCCTAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTGGGCACAATTCCACAAGTAAC
RAOB_2246	Mpx4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGACACTCAAGCAACAATAAACCTG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGACATTTGGAGGTGAAGAGCTAG
RAOB_2325	Mpx4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGCAGTAGTAGAAAGCCAGACTC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAACGTTATCAGACATACCAGGC
RAOB_26068	Mpx4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGCCTGAATTACCCTCTTCCAC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCCCATGCCTCCTTCTAATAAAG
RAOB_28243	Mpx4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAAAGCCGCCGTCAATCAC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCATCTGTGCCATGGACAGG
RAOB_28586	Mpx4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCATACAGATTGATGGCATCTTGG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGATACAGGGCTTTGGTGGG
RAOB_29614	Mpx4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTATCCTCTTACATTTGGCCACC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTGCCTATCAGTGCATTATGTCC
RAOB_3740	Mpx4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGCTTCCAGATTCCCAACTC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAGAACTAAAGGACAAAGGAGCC
RAOB_40351	Mpx4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTGCAACAGAGGGTCTAATCAC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTGAGTTGAATCACTCGCTATCC
RAOB_4473	Mpx4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGACTGCTCCTTCTAATTTGCCTG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTGCCATTTGTGGTCATGTTAC
RAOB_554	Mpx4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTCAGATGTGAGGTTTGCAGC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGTGTTAGGCATGCATTCATTC
RAOB_653	Mpx4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCCAGGTGTTCTTAGTTCTGAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGATTTGGATCCAGTTGCCTAGTG
RAOB_6556	Mpx4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGACTGAGAGATTTCCTGAGGGC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCAAATTCAAGAGGTCATGTGG
RAOB_6812	Mpx4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCAGCATTGTCTGTCCTGGG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGAGCTGCAGTCTATTCAGAGAG
RAOB_7093	Mpx4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGAGAACAAGCAGGATGACC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGTGTGTGTTGGAGCAGAG
RAOB_746	Mpx4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTTTGGAGATTCTTGTGTTGGC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGATCCATCCTTCTGTCCTCCC
RAOB_807	Mpx4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGAGAAGAAAGCCATATTTAGAAGGTG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCACCAGGTTCCCAGCTTATC
RAOB_854	Mpx4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTAGGCTGCATGGAGTTCAG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAAAGGAACAAGAGCAGTTGGAG
RAOB_8994	Mpx4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTTCCTACCTCCACATTGTAAGG	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGACAGCGACTACCCTGAAGATAG
RAOB_9014	Mpx4	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGTCCTTTGCAGAGTCACAAGTC	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTCACCTGGTCATAGAAAGACC

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