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RESEARCH ARTICLE

Combining genomic and field analyses to reveal migratory status in a burrowing owl population

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Abstract

Partial migration, in which migrant and resident members of a species spend part of the annual cycle in the same habitat, is a widely-occurring strategy among animal species. However, studies of this behavior are impeded by problems such as distinguishing migrant from resident individuals and detecting resident-migrant hybridization. We used a combination of genomic sequencing and bird banding to determine the migratory status of individuals in a declining population of western burrowing owls (*Athene cunicularia hypugaea*) in northern California. We banded individuals for four consecutive years in winter and summer. Each summer we surveyed for birds we had banded during previous winters. Using genomic analysis, we analyzed feathers from birds found in winter and summer to assess migratory status and interactions between winter and summer owls. The data showed a pattern of migration in which long-distance migratory birds were found in areas outside the breeding sites and joined resident owls at the breeding sites in the winter, but disappeared from these areas by the next breeding season. These results fit a pattern of partial migration in which long-distance migrants join resident birds in the winter. Although during breeding seasons we never observed any migrants that we banded in winter either within or outside the breeding sites, genomic analysis showed that some migrants stayed into the summer and bred with resident owls to produce hybrid offspring. This interaction brings different genetic material into the small resident population, a contribution that may benefit this declining population. This work demonstrates the value of combining genomic assessments of migratory status with field data collection to better characterize population structure and inform conservation actions.

Keywords *Athene cunicularia* · Conservation · Hybrids · Partial migration · SNP

Introduction

The migratory strategy of a species can affect population trends (Gilroy et al. 2016), population dynamics (Adriaensen and Dhondt 1990; Griswold et al. 2011), and genetics (Macías-Duarte et al. 2020), and can even lead to speciation if migratory animals remain genetically distinct from isolated resident populations (Gómez-Bahamón et al. 2020).

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Partial migration, in which resident and migrant individuals "share a common site during one period of the annual cycle" (Griswold et al. 2011), is a widespread migration strategy (Chapman et al. 2011), especially in birds (Jahn et al. 2012; Hegemann et al. 2015). A commonly-documented form of partial migration is one in which some individuals from a breeding population migrate in the non-breeding season while others remain resident (Chapman et al. 2011). Another form of partial migration occurs when individuals breeding separately come together in the same habitat in the non-breeding season (Chapman et al. 2011; Griswold et al. 2011).

The extent to which resident (i.e., non-migratory) and migrant individuals interact when in proximity can provide insight into the effects of partial migration on population dynamics (Macías-Duarte et al. 2020). But, such interactions are often difficult to study (Pérez-Fris and Telleria 2002) because distinguishing migrants from residents and determining interbreeding, even if populations differ genetically,

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is challenging (Macías-Duarte and Conway 2021). To differentiate resident from migratory individuals, researchers have employed a range of techniques including stable isotope analysis (Hegemann et al. 2015; Dale et al. 2019; Macías-Duarte and Conway 2021), banding plus stable isotope analysis (Cardador et al. 2015), and population genetic analyses of microsatellite loci (Macías-Duarte et al. 2020). Townsend et al. (2018) compared three methods in their study of American crows (*Corvus brachyrhynchos*)—satellite telemetry, molecular markers (33 microsatellite loci) and stable isotope analysis—and found good congruence between the three methods in determining residents from long-distance migrants. However, no method to date has been able to also determine the degree to which resident and migrant individuals might interbreed, information which is critical to understanding current population dynamics and informing conservation actions.

Despite its importance, assessing the degree of hybridization between closely related groups is notoriously difficult due to the high degree of shared ancestral variation (Seehausen et al. 2014). However, advances in genomic sequencing have recently made it possible to rapidly and affordably scan entire genomes and detect a small number of genetic variants that can be used for distinguishing populations (Haasl and Payseur 2016). As a result, this subtle variation can be used to assess the degree of hybridization even between very closely related forms (Keller et al. 2013; Payseur and Riesberg 2016; Moran et al. 2021). Here, we harness the power of genomic sequencing technology to distinguish avian long-distance migrants from northern California residents in western burrowing owls (*Athene cunicularia hypugaea*), a subspecies previously known for its panmictic genetics (Korfanta et al. 2005; Macías-Duarte et al. 2020). We used the same genetic data to estimate the degree and timing of hybridization between the two migratory forms within a species. In combination with long term field observations, we demonstrate how genomic markers have the potential to inform current population dynamics, past patterns of gene flow, and conservation actions.

The burrowing owl (*Athene cunicularia*) is a species of conservation concern across its range as a result of wide-spread population declines (Macías-Duarte and Conway 2015). Western burrowing owl (*A. c. hypugaea*) populations, which occur from southern Canada to central Mexico and from the Great Plains states in the U.S. to the Pacific coast, display a range of migratory strategies. Breeding birds appear to be fully or nearly fully migratory in Canada (Holroyd et al. 2011), the Great Plains states of the U.S. (Haug et al. 1993; Poulin et al. 2020), eastern Oregon and Idaho (Navock et al. 2019), as well as locations in Texas (Woodin et al. 2007), Oklahoma (Butts 1976) and New Mexico (Martin 1973; Cruz-McDonnell and Wolf 2016). However, in other parts of New Mexico (Botelho and Arrowood 1998), southern Arizona (Ogonowski and Conway 2009) and southeast Washington (Conway et al. 2006) some breeding owls are year-round residents while others from the breeding population migrate in the winter. Alternatively, in some counties in Texas, resident and migrant owls in winter have been recorded, but the relationship between the two has not been described (McIntyre 2004).

Throughout California, breeding birds are considered resident (Coulombe 1971; Rosier et al. 2006; Harmon and Barclay 2007; Trulio and Chromczak 2007) but, in winter, burrowing owls occur in places where they are not detected during the breeding season (Harmon and Barclay 2007). The few band encounters and owls tracked in winter in California have detected birds from British Columbia, Canada, as well as Idaho, Washington and Oregon in the U.S. (Harmon and Barclay 2007; Holroyd et al. 2010; Holroyd and Trefey 2011). Beyond this, little is known about the relationship between the winter birds and resident owls or the migratory pattern (Harmon and Barclay 2007; Lincer et al. 2018). The resident breeding burrowing owl population in the urban South San Francisco Bay Area of northern California (Thomsen 1971; Trulio and Chromczak 2007) provided an opportunity to investigate the relationship between resident and winter birds, as owls appear every winter outside the sites where owls breed (pers. observation; Harmon and Barclay 2007; eBird 2023). This population is also worthy of study as it is at the far western edge of the western burrowing owl distribution in the continental U.S. and is declining (Townsend and Lenihan 2007; pers. observation) and may be more vulnerable to extirpation than larger, continental interior burrowing owl populations (Macías-Duarte et al. 2020).

In this study, we employed low coverage, whole genome resequencing and subsequent targeted genotyping to distinguish individuals in a northern California resident burrowing owl population from individuals belonging to long-distance migrant populations. Recent work on the genomics of western burrowing owls (Ruegg et al. 2020; Barr et al. in press) has shown that western burrowing owls that are migratory cannot be distinguished from each other. However, Ruegg et al. (2020) and Barr et al. (in press) have demonstrated that resident burrowing owl populations have genomic variation that distinguish each population and also differ from the genetic variation of the migrant burrowing owls—thus allowing migratory owls from outside resident populations to be distinguished from resident burrowing owls. We combined field data with genomic data developed using single nucleotide polymorphism (SNP) methodology to understand the migratory pattern of a declining avian population and to assess the extent to which residents and wintering birds were related.

Methods

Study population

We studied a population of western burrowing owls in Santa Clara County and adjacent southern Alameda County (the northern California population), CA for four winter-summer cycles from September 2014 to August 2018 (Fig. 1). Since 1998, we have banded the majority of the resident burrowing owls in summer at the known breeding sites in these counties (Trulio and Chromczak 2007) and, during this study, we banded approximately 86% of adults and 76% of juveniles each summer. The winter, non-breeding season was from September 1 to January 31 and the summer, breeding season was February 1 to August 31. Each non-breeding season, we located unbanded burrowing owls and banded as many as possible at known breeding sites and other locations in the study area (Fig. 1). During subsequent breeding seasons, we searched for the birds we had banded in winter at the sites where we had banded them, whether at known breeding sites or other sites.

Field methods for assessing migratory strategies

We located wintering owls based on information from biologists, resource managers, Christmas Bird Count data, previous reports, and personal observations. We conducted surveys following Trulio and Chromczak (2007) to detect owls at potential wintering locations. When we found and subsequently captured owls at a site, we revisited those locations in following winters, but we also added new locations based on observations and reports. In winter, we did not specifically survey to resight birds from previous winters, although encountering previously banded birds was possible. We also conducted winter surveys at the known breeding sites, which included extant sites occupied at the time of the study by nesting owls and within the last 15 years.

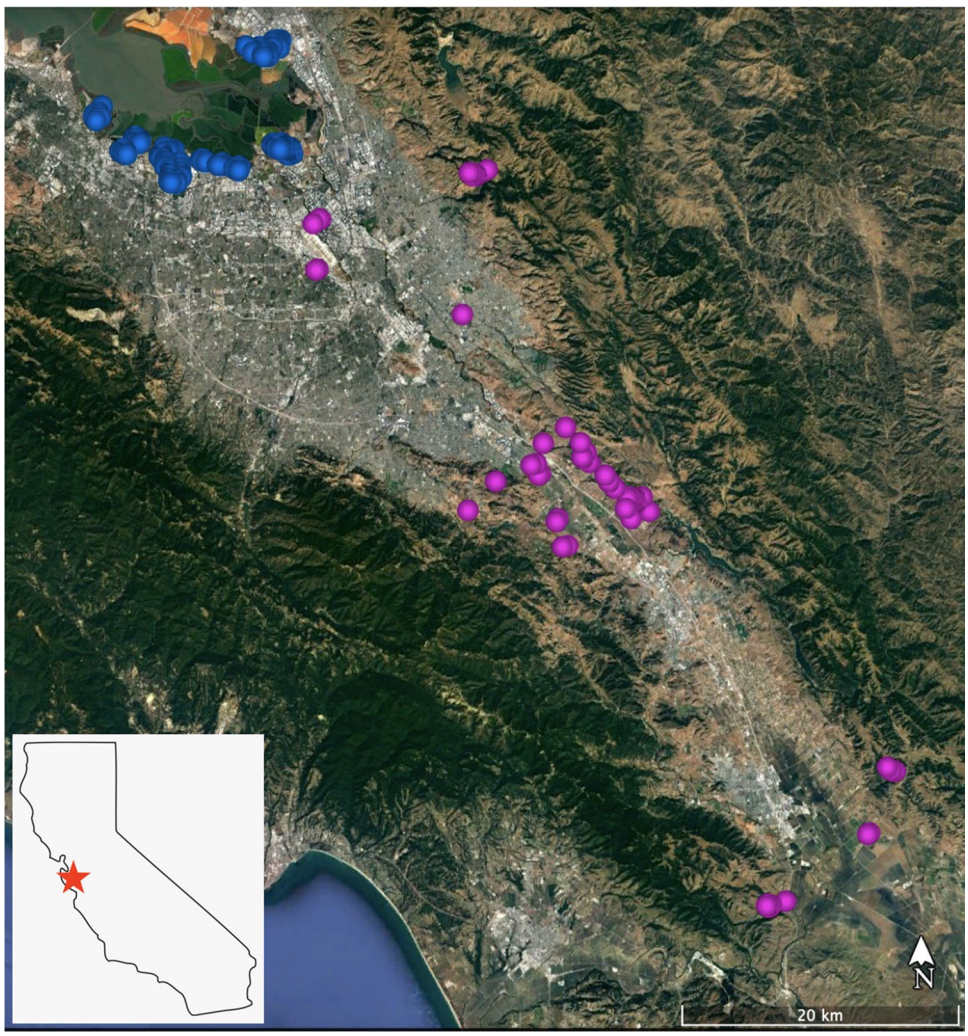

Fig. 1 Study area in northern California, USA, showing the locations of unbanded burrowing owls found in winter at known breeding sites (blue dots) and at foothill sites (magenta dots), 2014–2018 (satellite image created from Google Earth). Program used: PDF of a Microsoft Word doc; map image is from Google Earth Pro

Burrowing owls that were outside a burrow were captured using a spring trap (also called a "bownet") with an MP3 player and speaker broadcasting the male's primary call (call sources: American Bird Conservancy Library—https://abcbirds.org/bird/burrowing-owl/#sounds and Burrowing Owl Conservation Network—http://burrowingowlconservation.org/sounds/). We placed a small cage containing a live mouse as bait that was attached to the trigger of the trap. When an owl grabbed the cage with the mouse inside, the trap closed over the owl. Burrowing owls that were initially underground were captured using a one-way door placed in the burrow entrance and a bubble trap (a soft mesh dome secured to the ground) over the one-way door. We monitored all traps from a short distance away using a scope or binoculars. When we captured an owl, we quickly removed it from the trap, placed it in a sock to keep the owl contained and banded it with a metal bi-colored alphanumeric Acraft band (Acraft Sign and Nameplate Co., Ltd., Edmonton, AB, Canada) (left leg) and a metal U.S. Geological Survey band (right leg). Five breast feathers were collected from most owls captured during the study as well as from owls we captured in 2018–2020 during other research in the study area. We released each owl at its capture location the same evening captured. We recorded capture locations using a hand-held Garmin GPSMap 64 (Garmin, Ltd., Schaffhausen, Switzerland) and plotted coordinates in Google EarthPro, which we used to calculate the distance each owl traveled from previous non-breeding or breeding locations and to estimate elevations.

The following summer, we conducted walk-through transect surveys (Trulio and Chromczak 2007) at the known breeding sites and all the other sites where we had banded owls in winter to potentially resight owls we had previously banded. We identified previously banded owls by reading the unique alphanumeric band codes on the birds' legs using binoculars and spotting scopes. In summer, we captured and banded owls (although often without using callers) and recorded capture locations as per the methods described above.

Laboratory methods for diagnosing migratory status

We created SNP type assays to differentiate the northern California resident burrowing owl population from all migratory populations of western burrowing owls. In previous population genomic analyses, Barr et al. (in press) conducted low coverage whole genome sequencing of 161 western burrowing owls from 15 populations (both resident and migratory) across their breeding range and demonstrated that population structure was limited to the resident populations. Specifically, northern California, Lake Havasu, AZ, Nevada and San Diego, CA residents were genetically distinct from each other and from all migratory populations, which clustered together in one panmictic genetic cluster (Barr et al. in press; Fig. S1A). The migratory populations investigated in Barr et al. (in press) were from Washington, Oregon, Idaho, South Dakota, Utah, Colorado and New Mexico. Here, we used ANGSD version 0.9.3 (Korneliussen et al. 2014) to identify candidate outlier loci that were diagnostic between northern California residents and migrants using low coverage whole genome resequencing of western burrowing owls. We first created site allele frequency files for a subset of unrelated owls sampled in Barr et al. (in press), specifically from the northern California resident population (n = 14), two migrant populations, Idaho (Rocky Mountain Arsenal National Wildlife Refuge, n = 12) and Colorado (Morley Nelson Snake River Birds of Prey National Conservation Area, n = 10), separately, and a pooled set of multiple migratory populations that cannot be differentiated from each other (n = 63). We then implemented *-realsfs* to calculate site-wise F_{ST} estimates between the northern California resident population and migratory groups, keeping only the most differentiated 396 variants for primer design. We created an initial PCA using PCAngsd (Meisner and Albrechtsen 2018) based on the genotype probabilities of the 396 variants using the—*sites* parameter to in ANGSD (Korneliussen et al. 2014) to validate this variant set's ability to distinguish northern California residents from all migratory individuals sampled in Barr et al. (in press) (Fig. S1B). From the highly differentiated list of 396 possible variants, we used custom R script to design primers for the 96 most informative variants, with an additional 21 variants to account for possible assay failure. We used the R package snps2assays (Anderson 2015) to evaluate which of the top-ranking SNPs would generate designable assays to diagnose northern California resident birds. We characterized primers as designable if GC content was less than 0.65, there were no insertions or deletions (indels) within 30 bp (base pairs), and there were no additional variants within 20 bp of the targeted variable site. Additionally, we aligned 25 bp surrounding the target variable site to the genome using *bwa* (Burrows-Wheeler Aligner) (Li and Durbin 2009) to determine whether the designable primers mapped uniquely to the burrowing owl reference genome (Barr et al. in press), and to filter out those that mapped to multiple locations. The resulting 104 designable and successful primers were validated using a principal component analysis in PCAngsd (Meisner and Albrechtsen 2018) including northern California residents and all migratory individuals sampled in Barr et al. (in press) (Fig. S1C), and were converted into SNP type Assays (Fluidigm Inc.; Table S1). These primers were used to screen breast feathers from 83 birds captured in winter and summer. An additional 19 known migrants and resident birds not part of the

original primer design were also genotyped to account for ascertainment bias of our assay panel and aid in determining resident from migrant birds sampled for the study.

Genotyping was performed on the FluidigmTM 96.96 IFC controller following amplification using the Juno GT Preamp Master Mix (Fluidigm, Item #100-8363). For each run, we screened 94 individuals and two non-template controls. We imaged the results on an EP1 Array Reader and called alleles using Fluidigm's automated Genotyping Analysis Software (Fluidigm Inc.) with a confidence threshold of 90%. In addition, we visually inspected all SNP calls and removed any calls that did not fall clearly into one of three clusters (heterozygote or either of the two homozygote clusters). As DNA quality can affect call accuracy, we employed a stringent quality filter and dropped variants with missing calls exceeding 10%.

Statistical and model analysis

To determine whether our 104 successful primers could differentiate northern California resident birds from migrants, our workflow included multiple sets of known individuals: (a) known residents and known migrants genotyped with Fluidigm assays, (b) called genotypes of known residents subset to the assay positions, and (c) simulated hybrids from genotypes of our known samples. First, we ran a principal component analysis (PCA) of all feather samples from birds captured during this study that were genotyped with Fluidigm assays, along with known migrants from Idaho and Colorado (n = 3 genotyped), and known northern California residents (3 genotyped). We additionally included called genotypes from 13 northern California resident owls that were sequenced at an average depth of 6X coverage in 2020 (see Supplementary methods). We then used *structure* (version 2.3.4; Pritchard et al. 2000) to assess the probability that individuals exhibited resident or migrant ancestry based on our primer set. Because SNPs targeted by our primer set were meant to distinguish a northern California resident from a migrant, we ran five iterations of the assumed two genetic clusters (K = 2), with a burn-in of 10,000 reps and a total run length of 50,000. We ran *structure* with an admixture model and a location prior, where known migrants, known residents from northern California and feathers from birds captured during the study were defined as different groups to assess how the genetic variation of study birds compared to the that of known residents and migrants. Finally, we summarized the posterior probability of group membership estimates from the best *structure* run.

To assess the degree of hybridization between migrants and residents and assess the potential timing of gene flow events, we documented the frequency of different hybrid classes using the software program NewHybrids (Anderson and Thompson 2002). NewHybrids is a model-based method that estimates the posterior probability of individual assignment to six hybrid categories: pure migrant, pure resident, first generation hybrid (F1), second generation hybrid (F2) and backcross into either parental population (i.e., migrant or resident populations). For NewHybrids, five independent analyses were run on our data set with 104 loci and 105 individuals with an initial burn-in of 1000 replicates and 20,000 Markov Chain Monte Carlo (MCMC) sweeps afterward. We confirmed convergence of each NewHybrids run using hybriddetective (Wringe et al. 2017). Assignment efficiency, accuracy and overall performance were assessed by simulating all hybrid categories using the HybridLab algorithm (Nielsen et al. 2006). Using the genotype data of known resident individuals and known migrant individuals that had greater than 99% probability of being a migrant, we simulated 100 F1 individuals and 100 of each parental population (resident and migrant). We then used the simulated F1 hybrid genotypes to create 100 F2 individuals (F1 × F1), 100 backcross resident individuals (F1 × resident, BC resident) and 100 backcross migrant hybrids (F1 × migrant, BC migrant). These simulated individuals were assessed separately from the genotyped individuals in NewHybrids with a burn-in of 1000 replicates, 20,000 MCMC sweeps, and sample priors on 10 simulated pure migrants and 10 simulated pure residents. Again, convergence of each NewHybrids run was assessed in hybriddetective (Wringe et al. 2017) and we used custom scripts to determine the proportion of simulated hybrids that were assigned correctly to their hybrid category.

Results

Field results

We surveyed for owls at 17 and 18 sites, respectively, during the first two winters (2014–2015 and 2015–2016) and 25 and 24 sites in winters 2016–2017 and 2017–2018, respectively. In the intervening breeding seasons, we surveyed between 17 and 19 sites at known breeding and non-breeding sites. The sites outside the known breeding locations where we found wintering burrowing owls were at higher elevation than the breeding sites; we named these types of locations "foothill sites" (breeding site elevations = 1–23 m; foothill site elevations = 45–621 m above sea level).

We banded between 13 and 25 burrowing owls each winter for a total of 85 wintering owls at known breeding and foothill sites (Fig. 1). Over the four winters, we opportunistically resighted eight birds that had been banded the previous winter, as we surveyed to locate unbanded birds, and these resighted owls were resighted 0–145 m from the burrow they used the previous winter. None of these winter-banded birds were detected in an intervening breeding season and none of the banded owls we observed at the foothill sites in winter

had been banded at any of the breeding sites. However, at one foothill site, we detected one previously-banded owl—a bird that had been banded as a juvenile in British Columbia, Canada approximately 5 months earlier and an estimated 1368 km away. At the known breeding sites each winter, we observed a number of unbanded owls and a number of birds previously banded in summer, i.e., resident owls. None of the owls we observed at the breeding sites in winter had been banded previously at the foothill sites; all were banded at the breeding sites in summer.

Summer survey results showed: (1) No owls remained into summer at the foothill sites where we had banded or observed birds in previous winters; and (2) None of the owls we banded in winter at the breeding sites were found in the summer. Overall, the pattern from the 4 years of data showed burrowing owls present in winter at the foothill sites but absent from those sites in summer, whereas at known breeding sites resident owls were present year-round with an increase in the number of individuals during the winter (Fig. 2).

Laboratory results

We analyzed feathers from 83 owls captured during this study, 37 owls that were sampled during the breeding season and 46 owls that were sampled in the non-breeding season. The population structure analysis for migratory versus resident status clearly demonstrated the ability of the genetic primers to distinguish the migratory status of burrowing owls (Fig. S2). Known migrants from Idaho and Colorado and all birds sampled at the foothill wintering sites had a high probability of belonging to the migrant genetic cluster, whereas the known residents and owls sampled at the breeding sites in summer and winter showed a wide range of ancestry values (from 0.34 to 0.99 posterior probability of having resident ancestry); in other words, some owls had a high probability level of being residents or migrants, while others had intermediate probabilities between these two, indicating that hybridization between residents and migrants has occurred in this area (Fig. S2).

More in-depth analyses into the hybridization dynamics between migratory and resident burrowing owls via hybrid category assignment revealed distinctly different patterns depending upon the sampling location and time of year (Fig. 3). Specifically, 10 of the 37 birds captured during the breeding season at breeding sites were classified as pure residents, 16 were classified as second-generation hybrids (F2), six classified as later generation backcrosses to resident breeders, and three could not be diagnosed to any hybrid category, thus classified as not certain (Table 1). Surprisingly, two birds sampled during the breeding season were classified as migrants, suggesting two migrant individuals did not migrate in spring, but instead stayed to potentially breed with resident owls. During the winter, non-breeding season, all owls sampled from foothill sites were classified as pure migrants (25 of 25) as well as 17 of 21 birds at the known breeding sites (Table 1). Of the non-migrants caught in winter, two were F2 hybrids, which we expect were birds we had not captured in summer. The accuracy of our assignment based on the simulated hybrid categories supports the highest accuracy of assignment to pure resident and migrant categories (93 and 98% accuracy, respectively) with lesser

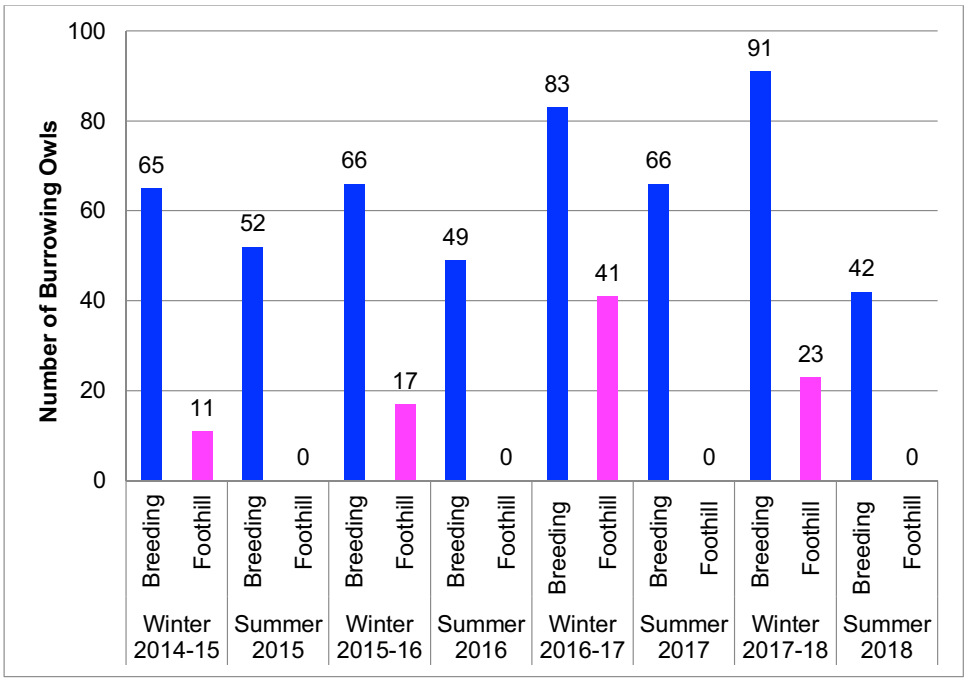

Fig. 2 Numbers of burrowing owls captured, resighted (previously banded), or observed (unbanded or banding status unknown) during four winter-summer cycles from 2014 to 2018 at known breeding sites (breeding sites in blue) and sites outside the known breeding sites (i.e., foothill sites in magenta). Program used: Microsoft Excel

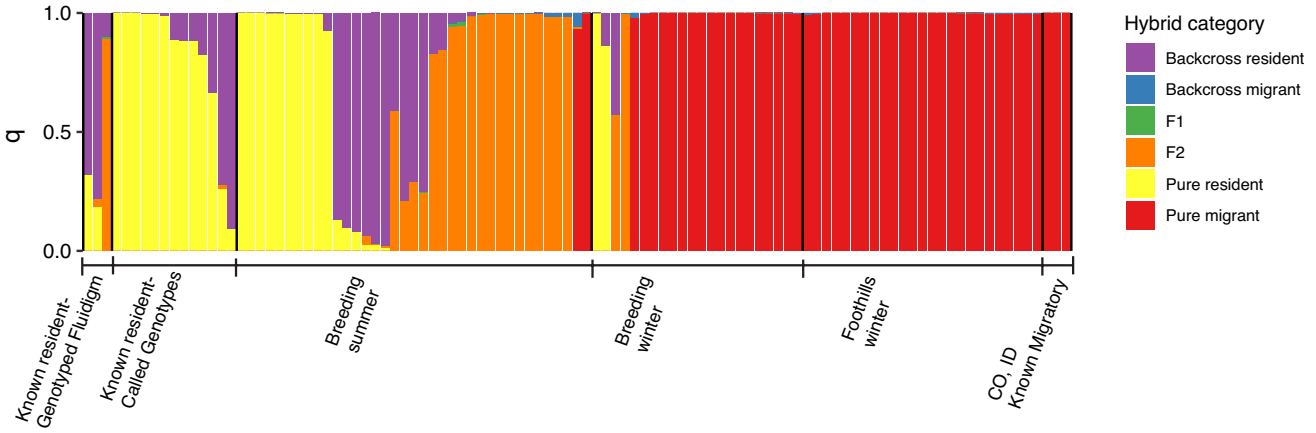

Fig. 3 Posterior probability of assignment to six hybrid categories of 83 unknown individuals collected for the study at the known breeding sites during the breeding season (breeding summer: n = 37), during the winter season (breeding winter: n = 21), and at the foothill sites in winter (foothills winter: n = 25). Also included are known residents genotyped with Fluidigm assays (n = 3), known residents with called genotypes from whole genome sequencing (n = 13) and migratory birds sampled from Colorado (CO) and Idaho (ID) genotyped with Fluidigm assays (n = 3). Program used: R program pophelper

Table 1 Assignment of 83 burrowing owls into six hybrid categories by location and season sampled

Location—season	Pure resident	Pure migrant	F2	Backcross resident	Not certain	Total
Breeding—summer	10	2	16	6	3	37
Breeding—winter	1*	17	2	0	1	21
Foothills—winter	0	25	0	0	0	25

All birds sampled in winter were previously unbanded except one, indicated with an asterisk

but still good accuracy of assignment to later stage hybrids (Fig. S3; Table 2). In combination with our banding records, we determined that eight birds classified as hybrids were 1 year old, two were 2 years old, and one was a 3-year old bird, indicating that hybridization had been occurring over the course of at least 3 years.

Discussion

Distinguishing long-distance migrants from resident individuals is a challenge and a number of methods have been used to make this determination. However, previous methods have not provided the resolution necessary to determine the extent of gene flow between migrant and resident individuals in partially migratory populations, a key source of information that can provide insight into migration ecology, evolution, and conservation. This study used new genomic methods for assigning individuals to hybrid classes in order to characterize the migratory status of burrowing owls and determine the extent of gene flow between wintering and resident individuals. Our combined genomic and field methods allowed us, for the first time, to characterize the population dynamics of this declining avian population, lending valuable insight into conservation actions.

The migratory pattern our field and genomic data revealed was one in which burrowing owls from other populations arrived in the region in the fall and winter—both within and outside the known breeding sites—and then left by the next breeding season. Our findings supporting this migratory pattern included: (1) Owls banded during winter that were resighted were seen only during subsequent winters and often were found in close proximity to where they were banded; (2) Genomic data showed that all the owls captured in winter outside the breeding sites exhibited genetic variation consistent with migratory burrowing owls, as did 17 out of 20 unbanded birds found at the breeding sites in winter; capture of a bird banded in British Columbia supported the genomic data; (3) No owls banded in summer were found outside their breeding sites in winter; and (4) The numbers of owls on the breeding sites increased each winter from summer numbers and then decreased the following summer, as expected for this form of partial migration (Chapman et al. 2011). These findings were not consistent with the type of partial migration researchers have previously described in burrowing owl populations, in which some breeding birds migrated in winter while others remained resident (Botelho

Table 2 Validating the explanatory power of the panel to diagnosing migratory status using known and simulated data

Sample type	Structure K2				NewHybrids hybrid category							
	K1 genetic resident (prob > 0.7)	K2 genetic migrant (prob > 0.7)	Not certain	Accuracy	Pure resident	Pure migrant	F1	F2	Backcross resident	Backcross migrant	Not certain	Total
Known												
Resident- Fluidigm genotyped	**2**	0	1	66.67	0	0	0	0	**2**	0	1	3
Resident- WG called genotype	**13**	0	0	100	**11**	0	0	0	**1**	0	1	13
Migrant- Fluidigm genotype	**0**	**3**	0	100	0	**3**	0	0	0	0	0	3
Simulated Hybrids												
Pure resident	–	–	–	–	**93**	0	0	0	2	0	5	100
Pure migrant	–	–	–	–	0	**98**	0	0	0	1	1	100
F1	–	–	–	–	0	0	**88**	3	1	0	8	100
F2	–	–	–	–	0	0	1	**83**	2	1	13	100
Backcross resident	–	–	–	–	4	0	0	0	**88**	0	8	100
Backcross migrant	–	–	–	–	0	0	2	0	0	**89**	9	100

Bolded numbers represent correct assignment to a specific structure or NewHybrid genetic category

and Arrowood 1998; Conway et al. 2006; Ogonowski and Conway 2009). Partial migration, in which migrants from other breeding populations join residents in winter, has been documented in marsh harriers (*Circus aeruginosus*) (Cardador et al. 2015) and American dippers (*Cinclus mexicanus*) (Gillis et al. 2008). This study is the first to clearly characterize, in burrowing owls, a relationship between residents and long-distance winter migrants occupying the same area.

Chapman et al. (2011) noted that this partial migration strategy separates breeding populations, allowing for genetic divergence of the populations. However, bringing individuals together from these different breeding populations may result in interbreeding if migrants switch to becoming residents. Although we never resighted any banded migratory burrowing owls in the following breeding seasons, the genomic results from the birds we found in summer at the breeding sites revealed that 2 of 37 birds were migrants, and another 22 had genomic signatures intermediate between resident and migrant genomes—indicating that these were the likely offspring of resident-migrant breeding pairs. Thus, some migratory owls stayed and bred with the local population, a finding that has proven difficult to confirm in burrowing owls (Holroyd et al. 2011; Macías-Duarte and Conway 2021). In addition, we found resident-migrant offspring that were between 1 and 3-years old, showing that migrant-resident crosses had been occurring for some time. While we found multiple hybrid categories in the population, we did not detect first-generation birds breeding with migrants (the backcross migrant category); we found only first-generation hybrid owls that bred with residents. Thus, at least some hybrids were staying and mating, rather than migrating to breed elsewhere.

Theory suggests that residents may experience greater fitness benefits over migratory birds, indicating that individuals should seek to be residents when possible (Adriaensen and Dhondt 1990; Pérez-Tris and Telleria 2002; Buchan et al. 2019). Studies have shown individuals can change migratory strategy, typically from migrant to resident (Sutherland 1998; Ogonowski and Conway 2009; Gilbert et al. 2016; Grist et al. 2017; Dale et al. 2019; although see Hegemann et al. 2015), and that migratory status could be flexible, depending on conditions at wintering and breeding sites (Griswold et al. 2011). The use of full genome sequencing and subsequent targeting SNP genotyping allowed us to definitively show that long-distance migrants stayed to breed with resident birds and we were able to identify the distribution of admixed individuals within the population, something previously-used methods for distinguishing residents from migrants had not accomplished. These results were possible even though the population structure within burrowing owls generally is very low (Barr et al. in press). Overall, this work highlights the utility of genomic tools for identifying migrant

classes and the extent and timing of hybridization even in species where gene flow is high and population structure is low.

A potential source of genetic variation that we did not explore here is immigration from local resident populations (Macías-Duarte and Conway 2021), as our primers were designed to specifically to detect the northern California resident population and the migrant genome cluster. Whether or not such immigration might occur in this population does not impact our findings that long-distance migrants bred with a resident population and produced hybrid offspring. In the future, the potential for local immigration could be studied using the genomic methods we have described here.

The results of this combined field and genomic study have direct management implications for burrowing owls. In general, we found that open grassland habitat in the area of northern California region we studied supported a large number of migratory burrowing owls that breed in other parts of the range—thereby, providing valuable wintering habitat for western burrowing owls. Protected winter habitat is an urgent need for all species with migratory populations (Faaborg et al. 2010); thus, identifying and protecting habitat in perpetuity that supports wintering burrowing owls is critical for this species. These results also suggest that other resident burrowing owl populations receiving long-distance migrants in winter could experience an input of genetic material from migrants, which could influence population genetic diversity, and putatively decrease elevated inbreeding levels exhibited by resident populations (Barr et al. in press).

Specific to the study population, our work has demonstrated that natural hybridization between residents of this northern California population and long-distance migrant individuals has been occurring for several generations. This hybridization may potentially increase numbers of breeding birds and promote genetic variation, to the extent that this small population may be isolated from other immigration. Despite the introduction of long-distance migrants, this population has experienced significant population declines in the recent decades (Townsend and Lenihan 2007), likely due to large-scale factors such as habitat loss and degradation due to urbanization (Trulio and Chromczak 2007), drought in the western US (Cruz-McDonnell and Wolf 2016), and the location of this population on the far western edge of the species' continental distribution (Macías-Duarte et al. 2020). The combination of field and genomic techniques we employed in this study may be used in the future to track the population dynamics of this declining population, but also offer an approach that could be applied to investigate genetic exchange in other populations.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10592-023-01578-3.

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Author contributions The field study was designed and implemented by the team of LAT, DAC, PGH and SM. DAC compiled the field data for analysis. LAT led the manuscript writing with substantial contributions from KR, CMB, and DAC. Genomic analyses and interpretation were conducted by KR and CMB.

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Data availability The genotype data can be accessed by reviewers temporarily using the following link: https://datadryad.org/stash/share/qe5MUuYovbnd7aRszdUHl7SZK0Uz42Dt4KoIHAtSr-Q. The raw genotype files used for analyses in this manuscript can be found on the dryad repository (https://doi.org/10.5068/D1CT14). Field data are available from the corresponding author upon request.

Declarations

Competing interests The authors declare no competing interests.

Ethical approval Trapping, banding, feather and blood collection were sub-permitted under two United States Geological Survey Federal Bird Banding Permits, one from the Institute for Bird Populations in Point Reyes Station, California (Permit number 22423) for 2014–2015 and one from the San Francisco Bay Bird Observatory Coyote Creek Field Station in Milpitas, California (Permit number 22109) for 2015–2018. The field research for this study was conducted under California Department of Fish and Wildlife Scientific Collecting Permit number SC-7012 and San José State University IACUC Permit number 1013. Feathers were transported under US Department of Agriculture transport Permit #138961.

Consent for publication All authors consent to the submission of this article to Conservation Genetics.

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