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Inferring the timing of long-distance dispersal between Rail metapopulations using genetic and isotopic assignments

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Abstract. The stochastic and infrequent nature of long-distance dispersal often makes it difficult to detect. We quantified the frequency, distance, and timing of long-distance dispersal in a nonmigratory, secretive wetland bird, the California Black Rail (*Laterallus jamaicensis coturniculus*), between an inland and a coastal metapopulation separated by greater than 100 km. Using 15 microsatellites in conjunction with stable carbon, nitrogen, and sulfur isotopes, we classified Rails as residents of their capture population, recent migrants that dispersed to their capture population less than one year before capture, established migrants that dispersed to their capture population more than one year before capture, and seasonal migrants that dispersed away from their capture population to forage, but returned the next season. Most Rails (195 of 204, or 95.6%) were classified as residents, but we detected two established migrants that had moved >100 km more than a year before capture. Seven Rails appeared to be seasonal migrants, but comparisons of feather isotope values with isotope values from wetland soils indicated that the isotope values in the feathers of these Rails likely resulted from natural environmental variation (e.g., source element effects) rather than long-distance dispersal of individuals. Thus, these seven Rails were most likely misassigned by isotopic population assignments due to small-scale variation in the isoscape. Using genetic data in conjunction with isotopic data allowed us to not only infer the timing of long-distance dispersal events, but to successfully track long-distance movements of nonmigratory Rails between metapopulations even when environmental variation of isotopes occurred across small spatial scales.

Key words: connectivity; discriminant function analysis; dispersal; microsatellites; population assignments; stable isotopes.

Introduction

Despite the importance of dispersal in ecology (Sutherland et al. 2013), the stochastic and infrequent nature of long-distance dispersal makes it difficult to quantify the frequency, distance, and timing of dispersal between metapopulations (Koenig et al. 1996, Nathan et al. 2003). Genetic and isotopic markers offer a practical method for tracking long-distance movements. Genetic data can be used to estimate the frequency of dispersal and subsequent gene flow between metapopulations with measures of genetic differentiation (F_{ST}; Wright 1931), or by estimating recent migration rates (m; Wilson and Rannala 2003). Other genetic methods assign individuals to their populations of origin to measure the frequency and distance of dispersal events (Cornuet et al. 1999, Pritchard et al. 2000, Berry et al. 2004). Similarly, analysis of the stable isotope composition of animal tissues can detect a long-distance movement of an individual between its capture population and its most probable population of origin if populations occur at isotopically distinct locations (Hobson 1999, Rubenstein and Hobson 2004, Hobson and Wassenaar 2008). The combined use of genetic and isotopic data have contributed significantly to our understanding of annual movements of migratory species (Caccamise et al. 2000, Wennerberg et al. 2002, Kelly et al. 2005, Rundel et al. 2013). For nonmigratory species, however, population assignments from genetic and isotopic data could generate conflicting results due to the timing of dispersal events and spatial variation of isotopes across the landscape (i.e., "isoscape"; West et al. 2010).

The timing of dispersal events does not affect population assignments made with genetic data because the genetic signature of an individual is permanent, but can affect assignments made with isotopic data, because the isotopic signature of an individual can change over time depending on its diet and the turnover rate of the tissue being sampled (Hobson and Wassenaar 2008). Even isotopically inert tissues, such as feathers or hair, only retain an isotopic signature until the tissue is molted or shed (Hobson and Wassenaar 2008). Therefore, if an individual was captured soon after dispersing and before isotopic turnover had occurred, both the genetic and isotopic data should assign the individual to the same population of origin. However, if an individual was captured long enough after dispersing for isotopic turnover to have occurred, genetic data would assign the individual to its population of origin but isotopic data would assign it to

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TABLE 1. Classification of resident and migrant status using genetic population assignments in conjunction with isotopic assignments.

Classification	Population assignment		No. Rails
	Genetic	Isotopic	
Resident	capture population	capture population	195
Recent migrant	other population	other population	0
Established migrant	other population	capture population	2
Seasonal migrant	capture population	other population	7

Notes: Rails (n = 204) were assigned to their capture population or some other population of origin using genetic and isotopic data. For Rails classified as migrants, similarities or differences in assignments between data types were used to make inferences about the timing of dispersal. The number of Rails observed in each classification is given. Seven Rails were classified as seasonal migrants, but it is likely that these migrants were type I assignment errors resulting from isotopic variation within the isoscape.

the population where it was recently foraging. Another type of mismatch could occur if individuals disperse seasonally between breeding and foraging areas that are isotopically distinct. These mismatches in inference between genetic and isotopic data of nonmigratory species could be used to parse captured individuals into four groups (Table 1): (1) resident individuals that have genetic and isotopic signatures assigning them to the population in which they were captured, (2) recent migrants that have genetic and isotopic signatures assigning them to a population that differs from their capture population, (3) established migrants that have isotopic signatures assigning them to the population in which they were captured, but genetic signatures assigning them to a population that differs from their capture population, and (4) seasonal migrants that have genetic signatures assigning them to the population in which they were captured, but isotopic signatures assigning them to a population that differs from their capture population.

Errors in genetic and isotopic assignments could lead to false conclusions about the timing of dispersal events by either incorrectly identifying a resident as a migrant (type I error) or failing to identify a migrant (type II error). Accuracy of genetic assignments is affected by the level of genetic differentiation among populations, the quantity and variability of markers used, and the completeness of sampling across all possible source populations (Cornuet et al. 1999, Berry et al. 2004). Generally, assignment accuracy is greater for highly differentiated populations ($F_{ST} \geq 0.1$), and increases as the quantity and variability of markers increases, with highly accurate assignments achieved using 10 or more moderately variable (heterozygosity $H \approx 0.6$) loci (Cornuet et al. 1999). Further, simulations can be used to generate P values for assignments to control the type I error rate (Paetkau et al. 2004). Accuracy of isotopic assignments increases as the number of isotopic markers increases and as isotopic differences among capture populations increase (Hobson and Wassenaar 2008). Isotopic assignments should have the greatest accuracy when isotopic variation within populations is low (i.e., individuals captured in close proximity to one another are isotopically similar) and among populations is high (i.e., individuals captured in different populations are isotopically distinct). Small-scale, spatial variation in biogeochemical processes that effect environmental concentrations of isotopes could also lead to isotopic differences among individuals captured within a single population resulting in type I errors (Ben-David and Flaherty 2012, Gillies et al. 2012).

We quantified the frequency, distance, and timing of dispersal events in a threatened wetland bird, the California Black Rail (*Laterallus jamaicensis coturniculus*), between a coastal metapopulation of the San Francisco Bay Area (Bay Area) and an inland metapopulation of the Sierra Nevada Foothills (Foothills) separated by 100 km of unsuitable habitat (Richmond et al. 2008). The Black Rail is the smallest Rail in North America and rarely emerges from the dense vegetative cover it inhabits in shallow wetlands. It is currently a candidate for listing under the Endangered Species Act in the eastern United States and is state-listed as threated in California. Black Rails are nonmigratory, year-round residents of the Bay Area and Foothills metapopulations, and recaptures of banded Rails indicate that adults can occupy the same home ranges during consecutive breeding seasons (n = 4 birds; L. A. Hall and S. R. Beissinger, *unpublished data*). They eat seeds and small invertebrates (Eddleman et al. 1994). Adults undergo a definitive pre-basic molt on the breeding grounds between July and September, where all body plumage is replaced annually including synchronous replacement of primaries, secondaries, and rectrices (Flores and Eddleman 1991, Pyle 2008). Evidence suggests adults may undergo a partial pre-alternate molt of body feathers prior to breeding between February and April (Flores and Eddleman 1991, Pyle 2008).

Generally, organisms foraging in marine ecosystems have more enriched stable isotope values than organisms foraging in freshwater ecosystems (Hobson and Wassenaar 2008). Thus, we predicted feathers of Rails from more saline, coastal wetlands in the Bay Area would be more enriched in the heavier isotopes of carbon (^{13}C), nitrogen (^{15}N), and sulfur (^{34}S) than feathers of Rails from inland, freshwater wetlands of the Foothills. Further, Black Rails in the Bay Area metapopulation are genetically differentiated from the metapopulation in the Foothills, and previous genetic assignments and migration rate estimates suggested these metapopulations are linked

by long-distance dispersal (Girard et al. 2010). We quantified the relative frequency, distance, and timing of long-distance dispersal events for Black Rails between the Bay Area and Foothills by estimating F_{ST} and recent migration rates with genetic data, and by analyzing genetic assignments in conjunction with isotopic assignments to classify migrants based on the timing of dispersal events (Table 1). In addition, we assessed spatial variation of isotope values in soils of wetlands in the Foothills, because preliminary analysis revealed a few Rails captured at the northern edge of the Foothills with highly enriched sulfur isotope values characteristic of the Bay Area.

Methods

Sample collection

Blood ($n = 336$) and feather ($n = 251$) samples were collected from Black Rails captured throughout the Bay Area and Foothills from 2004 to 2013 (Appendix S1: Fig. S1). Rails were captured during the day with mist nets and at night with spotlights and hand nets (Tsao et al. 2009, Girard et al. 2010). Blood was collected by venipuncture of a wing vein and stored in Longmire's solution (Longmire et al. 1988). One secondary feather from each wing and four to six breast feathers were collected and stored in paper envelopes. Only breast feathers were collected for birds molting their secondary feathers. No significant differences in $\delta^{13}C$, $\delta^{15}N$, or $\delta^{34}S$ have been detected between breast and secondary feathers of Rails (*unpublished data*). Age (after-hatch-year, AHY; hatch-year, HY) was determined using eye color and plumage characteristics (Pyle 2008), and sex was determined genetically with primers from Griffiths et al. (1998). Birds were banded with U.S. Geological Survey stainless steel bands, and morphometric measurements and molt were recorded.

Genetic data collection

DNA was extracted from blood using either phenol/chloroform (Sambrook et al. 1989) or a DNEasy Spin Column Kit (Qiagen, Valencia, CA, USA). Seventeen microsatellite loci developed by Girard et al. (Molecular Ecology Resources Primer Development Consortium 2009) were amplified in Black Rails by polymerase chain reaction (PCR; Appendix S2: Table S1). The experimental conditions were similar for all loci following Girard et al. (2010), with PCR annealing temperatures ranging from 52°C to 59°C (Appendix S2: Table S1). Microsatellites were labeled with fluorescent dye (HEX or FAM), run using POP buffer and LIZ 500 size standard on an ABI3730 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), and scored in GeneMapper ver. 5 (Applied Biosystems).

Isotopic data collection

Prior to isotopic analysis, feathers were soaked in a 2:1 chloroform:methanol solution for 24 h to remove dirt and oil, dried for 24 h, and then the vane was separated from the rachis and placed in tin capsules to obtain a mass ranging from 1.25 to 1.60 mg (Hobson and Wassenaar 2008). Feather samples were analyzed simultaneously for carbon, nitrogen, and sulfur stable isotope content (percent dry mass) and ratios at the Center for Stable Isotope Geochemistry at the University of California, Berkeley using a continuous flow CHNOS Elemental Analyzer (vario ISOTOPE cube; Elementar, Hanau, Germany) coupled with an IsoPrime100 IRMS (Isoprime, Cheadle, UK) following the methods of Mambelli et al. (2016). We included feather standards from Wild Turkey (*Meleagris gallopavo*), Common Murre (*Uria aalge*), and Pacific Loon (*Gavia pacifica*) with each batch of Rail samples to assess deviation among runs. Feather standards were prepared using a Wiley mill to create a fine, homogenous powder from a combination of breast, body, and flight feathers from each species. Deviation across runs was similar for all three species used as standards with the smallest deviation observed for $\delta^{15}N$, a moderate deviation observed for $\delta^{13}C$, and the greatest deviation observed for $\delta^{34}S$ (Appendix S3: Table S1). Isotope ratios are reported in parts per thousand (‰) using delta notation with $\delta^{h}N = (R_{sample}/R_{standard} - 1) \times 1000$, where R is the ratio of enriched to depleted isotopes for the sample and standard, N is the element of interest, and h is the mass of the enriched isotope. For $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values, Vienna Pee Dee Belemnite (VPDB), air (AIR), and Vienna Cañon Diablo Triolite (VCDT) were used as standards, respectively. Bovine liver, reference material NIST (National Institute of Standards and Technology, Gaithersburg, Maryland, USA) SMR 1547, was used as a calibration standard for $\delta^{15}N$ and $\delta^{13}C$. Two organic in-house standards with different S isotope composition, fish material ($\delta^{34}S$ value of 18.40‰) and algae material ($\delta^{34}S$ value of 1.98‰), were calibrated against IAEA sulfur sulfides and were used as a calibration standard for $\delta^{34}S$. The long-term analytical precision of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ are ±0.10‰, ±0.15‰, and ±0.40‰, respectively. A full discussion of the accuracy and precision of the simultaneous analysis of all three isotopes in a simple sample can be found in Mambelli et al. (2016).

Data analysis

Microsatellite genotypes were assessed for possible deviations from the expected Hardy-Weinberg equilibrium (HWE) and linkage equilibrium (LE) using exact tests with 20 batches of 10,000 iterations and a dememorization of 10,000 in GENEPOP ver. 4.2 (Raymond and Rousset 1995). We adjusted P values to account for multiple testing using a false discovery rate (FDR; Benjamini and Hochberg 1995) of 0.05. Loci that deviated significantly from expected HWE in either metapopulation were removed from the data set. We identified significant deviations from LE by splitting the data set for each metapopulation into two equally sized data sets containing

randomly assigned individuals. We then compared deviations from LE in the entire data set with the two smaller data sets. Pairs of loci that deviated from LE across all three data sets were considered linked, and one locus from each pair was removed from the data set (Waples 2015). We detected a marginally significant deviation from HWE at one microsatellite locus (C2) in the Foothills and one microsatellite locus in the Bay Area (D105) due to heterozygote deficiencies (Appendix S2: Table S1). In addition, locus C2 was part of a pair of loci that consistently yielded significant deviations from LE (Appendix S2: Table S1). Therefore, C2 and D105 were removed from subsequent analyses.

We measured genetic differentiation between metapopulations in the Bay Area and Foothills by calculating F_{ST} with the R package PopGenReport (Adamack and Gruber 2014), and migration rates (m) were estimated from three independent runs in Bayesass (Wilson and Rannala 2003) with each run consisting of 10,000,000 iterations, sampled every 100 iterations, following a burn-in of 1,000,000 iterations. The Markov chain from each run was visually assessed for mixing using Tracer v. 1.6 (*available online*).[2] The number of genetic populations (K) was estimated in STRUCTURE (Pritchard et al. 2000) using the admixture model with the degree of admixture (α) inferred from the data, and the distribution of allele frequencies (λ) set to 1. For each value of K, ranging from one to four, we performed 10 runs of 100,000 iterations that were preceded by a burn-in of 1,000,000 iterations. The metapopulation where each Rail was captured was used to inform cluster assignments for individuals using the LOCPRIOR setting (Hubisz et al. 2009). First-generation migrants were identified in GENECLASS2 using λ, the ratio of the likelihood of drawing an individual's genotype from the population in which it was captured to the maximum likelihood observed for the individual's genotype in any population (Paetkau et al. 2004, Piry et al. 2004). Likelihoods were calculated from observed allele frequencies in each population using the Bayesian method of Rannala and Mountain (1997), and P values were calculated for each individual by comparing the observed λ to a distribution of λ values generated with 10,000 simulations (Paetkau et al. 2004). Individuals were identified as first generation migrants if their P value was less than 0.01.

Differences in $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values between metapopulations (Bay Area and Foothills), age classes (AHY and HY), and sexes (male and female) were assessed using generalized linear models implemented in program R (R Core Team 2013). Assignment probabilities used to identify migrants were generated using linear discriminant function analysis with cross validation of isotopic data in program R (R Core Team 2013). Individuals were classified as migrants if the assignment probability for the population in which they were captured was less than the assignment probability for the other population. We determined which isotopes contributed significantly to the discriminant function and calculated the percent contribution of each isotope using a Wilks' lambda test in R (R Core Team 2013). In addition, assignment accuracy was assessed by calculating the percentage of correct classifications from simulated isotopic signatures. The discriminant function, trained with our empirical data, was used to classify 2,000 (1,000 from each metapopulation) simulated isotopic signatures matching those observed in the Bay Area and Foothills.

Linear discriminant function analysis assumes covariances are equal among groups and that data are sampled from a multivariate normal distribution. We tested for equal covariances among groups using a Box's M test in the R package biotools (da Silva 2015) and found significant differences among isotopes (Box's $M\,\chi^2_{0.05,6} = 89.95$, $P < 0.001$). However, Box's M tests are highly sensitive to deviations from normality so the significant differences may have been caused by deviations from normality in the distributions of our isotope data (Appendix S3: Fig. S1). Distributions of $\delta^{13}C$ and $\delta^{15}N$ from feathers collected in the Bay Area exhibited multimodality because one collection site in the Bay Area was dominated by a C_4 plant (*Spartina foliosa*), whereas all other Bay Area collection sites were dominated by a C_3 plant (*Salicornia subterminalis*; Appendix S3: Fig. S1). It is not possible to test for multivariate normality, but if data are normally distributed within groups, it is reasonable to assume the data exhibit multivariate normality. Although the distributions of our isotope data deviated from normality because of differences in the photosynthetic pathways of dominant plant species in the Bay Area, linear discriminant function analysis is relatively robust to minor violations of the assumptions of equal covariances and multivariate normality (Williams 1983).

Environmental sampling and analysis of isoscape variation

To examine spatial variation of stable isotope values within and among Foothills wetlands, we collected topsoil (~6 cm) from 29 randomly selected locations within the home ranges (*unpublished data*) of Black Rails. Wetland soils were collected with a trowel and transported in plastic bags to the lab, where they were dried at 60°C in a drying oven for a minimum of six weeks (Yi-Balan et al. 2014). After drying, soils were homogenized with a mortar and pestle, and approximately 30–50 mg of each sample was weighed and packaged in a tin capsule for isotopic analysis. Simultaneous analysis of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ was performed using the same methodology as described for feathers. Soils that were highly enriched in ^{34}S were reanalyzed using approximately 5 mg of sample. The difference in $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values between soils collected at Bidwell Park and soils collected at other wetlands in the Foothills was assessed using a generalized linear model in R (R Core Team 2013).

[2] http://tree.bio.ed.ac.uk/software/tracer/

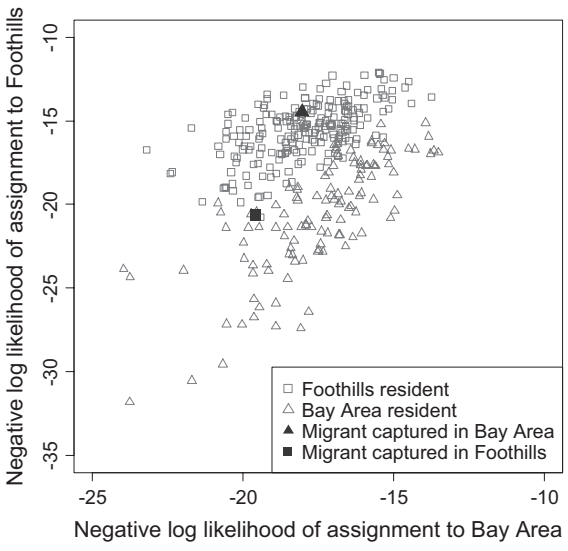

FIG. 1. Genetic population assignments of 336 California Black Rails (*Laterallus jamaicensis coturniculus*) to the San Francisco Bay Area (Bay Area; California, USA) or Sierra Foothills (Foothills). For each Rail, the negative log likelihood of assignment to the Bay Area is plotted on the *x*-axis, and the negative log likelihood of assignment to the Foothills is plotted on the *y*-axis. Rails captured in the Bay Area ($n = 123$) are plotted with triangles and Rails captured in the Foothills ($n = 213$) are plotted with squares. Most Rails (334 of 336) were assigned to the population in which they were captured and were classified as residents (gray), but two Rails were assigned to a population that differed from their capture location and were classified as migrants (black).

RESULTS

Genotypes clustered into two groups: Rails captured in the Bay Area were assigned to one group and Rails captured in the Foothills were assigned to a second group. Although we detected distinct genetic populations in STRUCTURE, gene flow between the Bay Area and Foothills was moderate ($F_{ST} = 0.018$), and recent migration rate estimates indicated a low level of gene flow between the metapopulations, with greater migration from the Foothills to the Bay Area (m [95% CI] = 0.075 [0.054, 0.096]) than from the Bay Area to the Foothills ($m = 0.016$ [0.009, 0.023]). Independent runs of Bayesass produced nearly identical migration rate estimates and the Markov chain for each run was well mixed. Genetic population assignments identified two individuals as first generation migrants: One Rail from the Foothills was assigned to the Bay Area ($P < 0.004$), and one Rail from the Bay Area was assigned to the Foothills ($P < 0.001$; Fig. 1). The migrant Rail captured in the Foothills would have dispersed a minimum of 110 km from the nearest known breeding location in the Bay Area, and the migrant Rail captured in the Bay Area dispersed a minimum of 160 km from the nearest known breeding location in the Foothills.

Feathers of Rails from the Bay Area had significantly ($P < 0.001$) enriched $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values compared with feathers of Rails from the Foothills (Tables 2 and 3; Appendix S3: Fig. S2). Delta values did not differ significantly between male and female birds for any of the isotopes analyzed, but $\delta^{13}C$ values were significantly different ($P = 0.011$) between the AHY and HY age classes (Table 3; Appendix S3: Fig. S2). Therefore, only AHY ($n = 204$) Rails were included in our discriminant function analysis.

TABLE 2. Delta values of stable carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), and sulfur ($\delta^{34}S$) isotopes in California Black Rail (*Laterallus jamaicensis coturniculus*) feathers from the San Francisco Bay Area (Bay Area) and Sierra Foothills (Foothills) used in linear discriminant function analysis.

Stable isotope	Bay area	Foothills	Contribution from Wilks' lambda (%)
$\delta^{13}C$	−20.93 ± 0.20	−23.15 ± 0.11	31
$\delta^{15}N$	15.14 ± 0.39	9.57 ± 0.15	50
$\delta^{34}S$	16.79 ± 0.34	5.61 ± 0.32	66

Notes: Values shown are mean ± SE. The percent contribution of each isotope to the discriminant function was calculated using a Wilks' lambda test. All isotopes contributed significantly to the discriminant function ($P < 0.001$).

Stable carbon, nitrogen, and sulfur isotopes all contributed significantly to the linear discriminant function ($P < 0.001$), with sulfur having the greatest percent contribution (66%), followed by nitrogen (50%), and carbon (31%; Table 2). Linear discriminant function analysis assigned 197 of 204 (96.6%) AHY Rails to their capture population (Fig. 2a, b). Of the seven Rails assigned to a population that differed from their capture population, four were captured in the Bay Area but were assigned to the Foothills, and three were captured in the Foothills but assigned to the Bay Area. Three observed heterozygosity Rails captured on the eastern fringe of the Bay Area metapopulation at wetlands in Lodi ($n = 1$) and Oakley ($n = 2$) had isotope values similar to Rails from freshwater wetlands in the Foothills. Another Rail captured in Martinez in the Bay Area had $\delta^{13}C$ and $\delta^{15}N$ values that were similar to other Rails captured in the Bay Area, but it was assigned to the Foothills because of its depleted $\delta^{34}S$ value. In addition, three Rails from Bidwell Park in the Foothills had enriched $\delta^{34}S$ values similar to Rails from marine wetlands in the Bay Area, but had $\delta^{13}C$ and $\delta^{15}N$ values similar to Rails captured in the Foothills. Assignments of simulated isotopic signatures using the discriminant function were highly accurate for the Bay Area (93.0%) and Foothills (97.8%).

When genetic assignments were used in conjunction with isotopic assignments, 195 of 204 (95.6%) Rails were classified as residents of the populations in which they were captured (Table 1). We did not detect any recent migrants for which both the genetic and isotopic data assigned an individual to a population that differed from its capture population. We did, however, detect two established migrants (one in the Foothills and one in the Bay Area) that were assigned to their capture population

TABLE 3. Generalized linear model results for individual and interaction effects of area (San Francisco Bay Area and Sierra Foothills), age (after-hatch-year and hatch-year), and sex (male and female) on delta values of carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), and sulfur ($\delta^{34}S$) isotopes from feathers of 251 California Black Rails (*Laterallus jamaicensis coturniculus*).

	$\delta^{13}C$			$\delta^{15}N$			$\delta^{34}S$		
	Estimate	SE	*P*	Estimate	SE	*P*	Estimate	SE	*P*
Intercept	−20.771	0.347	**<0.001**	15.494	0.594	**<0.001**	17.662	0.847	**<0.001**
Area	−1.991	0.447	**<0.001**	−6.030	0.765	**<0.001**	−11.568	1.090	**<0.001**
Age	−2.227	0.868	**0.011**	−2.161	1.486	0.147	0.468	2.117	0.825
Sex	−0.048	0.406	0.906	−0.211	0.695	0.762	−1.510	0.991	0.129
Area × Age	1.770	1.014	0.082	2.019	1.735	0.246	−0.522	2.472	0.833
Area × Sex	−0.329	0.527	0.533	0.040	0.901	0.965	1.136	1.284	0.377
Age × Sex	−0.270	0.699	0.700	0.037	1.195	0.975	−0.465	1.703	0.785
Area × Age × Sex	NA	NA	NA	NA	NA	NA	NA	NA	NA

Notes: Estimates of three-way interaction effects for area, age, and sex were not calculated because only one hatch year, male bird was captured in the San Francisco Bay Area. *P* values for statistically significant ($\alpha = 0.05$) effects are shown in boldface type.

using isotopic data, but were assigned to a population that differed from their capture population using genetic data (Table 1). In addition, seven Rails (three in the Foothills and four in the Bay Area) were assigned to their capture populations by genetic assignments, but were assigned to populations that differed from their capture populations by isotopic assignments (Table 1). These Rails may have been seasonal migrants that dispersed away from their natal populations, foraged in a population that differed from their natal population, and then later returned to their natal population. However, it is also possible that these seasonal migrants were assignment errors resulting from isotopic variation within the isoscape.

Stable isotope values of wetland soils in the Foothills ranged from −29.18‰ to −25.00‰ for $\delta^{13}C$, 1.97‰ to 7.17‰ for $\delta^{15}N$, and −3.69‰ to 22.35‰ for $\delta^{34}S$ (Fig. 3a). Values of $\delta^{13}C$ and $\delta^{15}N$ did not differ significantly ($P = 0.103$ and $P = 0.997$, respectively) between soils collected at Bidwell Park and soils collected at other wetlands in the Foothills. However, values of $\delta^{34}S$ from soils collected at Bidwell Park were significantly greater ($P < 0.001$) than values from soils collected at other wetland sites in the Foothills (Fig. 3a). The $\delta^{13}C$ and $\delta^{15}N$ values from wetland soils in Bidwell Park had smaller ranges (Appendix S4: Figs. S1 and S2) than $\delta^{34}S$ values, which were highly variable across tens of meters (Fig. 3b). The large range in $\delta^{34}S$ values from wetland soils in Bidwell Park mirrored the range we observed in $\delta^{34}S$ values from Rail feathers collected in the park. Therefore, it seems likely that the Rails classified as seasonal migrants represented type I assignment errors.

Surprisingly, one band return from our study provided direct evidence of movement between the

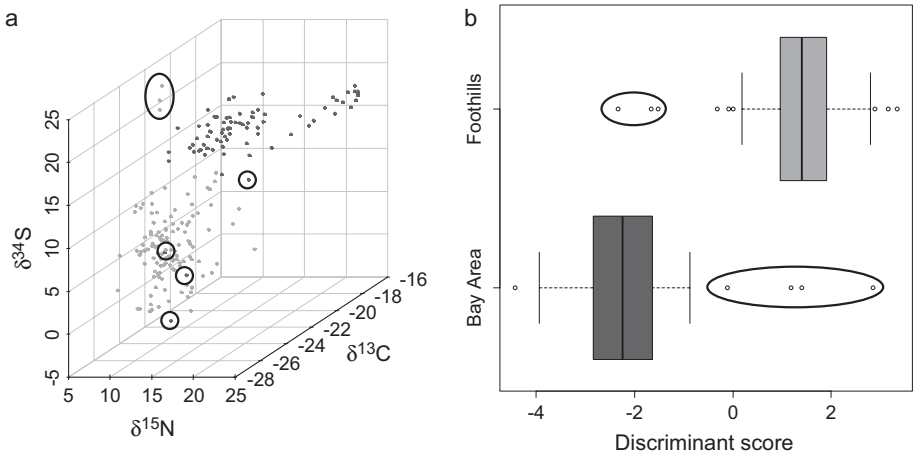

FIG. 2. (a) Delta values and (b) discriminant scores (box: median ± 1*interquartile range; whiskers: median ± 2*interquartile range) from linear discriminant function analysis of stable carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), and sulfur ($\delta^{34}S$) isotopes from feathers of 204 after-hatch-year California Black Rails (*Laterallus jamaicensis coturniculus*) captured in marine wetlands in the San Francisco Bay Area (Bay Area; dark gray) or freshwater wetlands in the Sierra Foothills (Foothills; light gray) of California. Three Rails from the Foothills and four Rails from the Bay Area were classified as migrants by the discriminant function analysis (circled in black). These same individuals were classified as residents by genetic population assignments. Therefore, they were not considered migrants, but yielded information about spatial variation of stable isotope values in the environment.

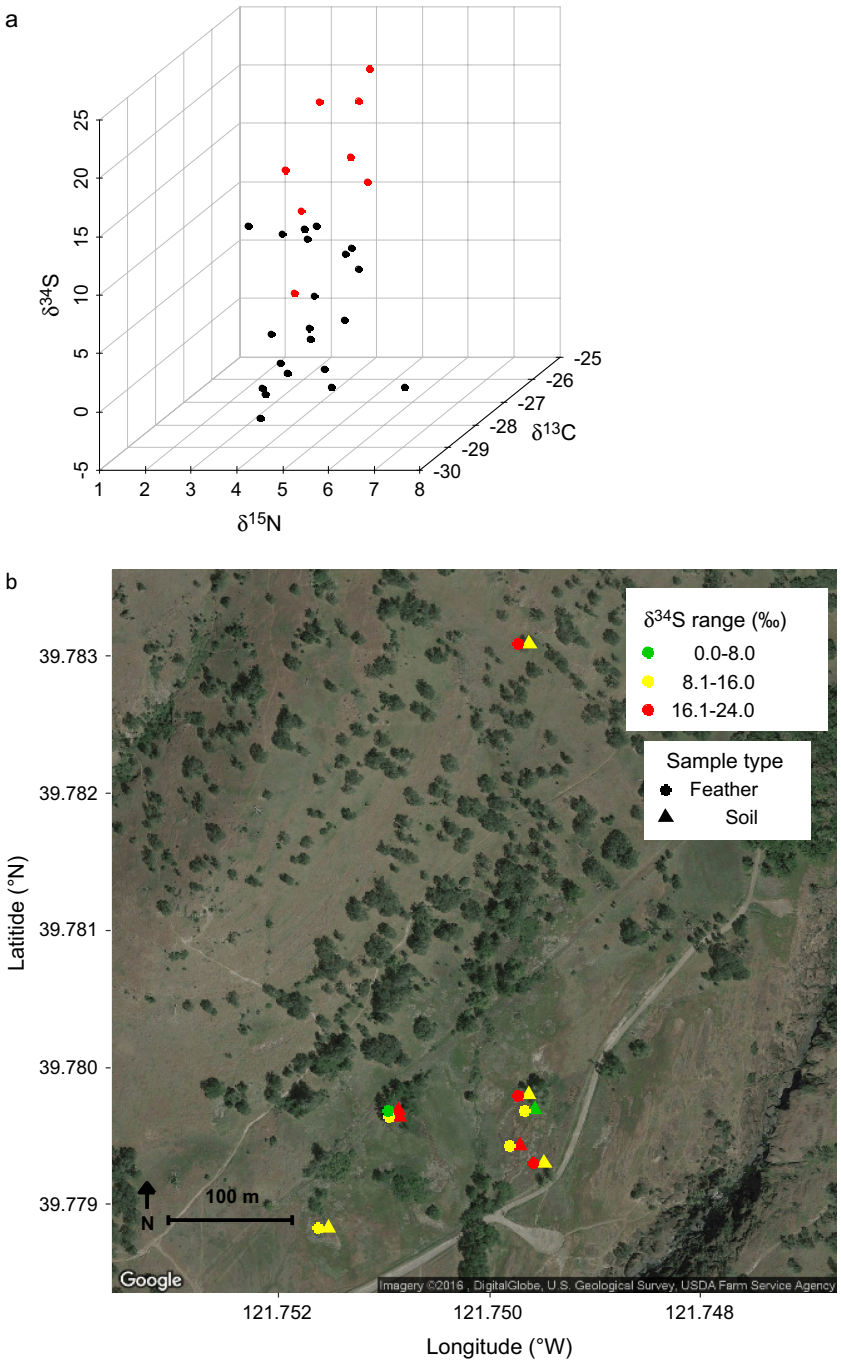

FIG. 3. (a) Delta values of stable carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), and sulfur ($\delta^{34}S$) isotopes from soils collected in the Sierra Foothills at Bidwell Park in Chico, California, USA ($n = 8$; red) and at 20 other wetland sites (black) occupied by California Black Rails (*Laterallus jamaicensis coturniculus*). (b) Collection locations of Rail feathers (circles) and soils (triangles) from Bidwell Park measured for $\delta^{34}S$ (reported as parts per thousand). Feathers and soils were collected at the same locations, but collection locations are jittered to show variation in $\delta^{34}S$. Delta sulfur isotopes were highly variable at small spatial scales (tens of meters) with some feathers and soils having highly enriched delta sulfur isotope values (red) and other feathers and soils having less enriched delta values (yellow and green).

Foothills and Bay Area metapopulations. An after-hatch-year, male Black Rail, banded on 12 June 2012 in southern Yuba County in the Foothills, was recovered 61 days later at a wind energy site in Solano County near the San Francisco Bay Delta, more than 128 km from its banding location. This Rail was assigned to its original capture population in the Foothills by both its genetic and isotopic signatures.

Discussion

Inferring the timing of dispersal for nonmigratory Rails

We used genetic assignments in conjunction with isotopic assignments to infer the timing of long-distance movements of nonmigratory Rails between metapopulations by classifying Rails as residents, recent migrants, established migrants, and seasonal migrants. Although most (95.6%) of the Rails sampled were classified as residents of the populations in which they were captured, our method allowed us to detect two established migrants that had dispersed between the Bay Area and Foothills metapopulations more than one year before being captured. In addition, seven Rails appeared to be seasonal migrants, because they were assigned by genetic signatures to the population in which they were captured and by isotopic signatures to a population that differed from their capture population. While it is possible that these Rails were seasonal migrants, it is more likely that these individuals were type I assignment errors caused by small-scale, spatial variation in the isoscape.

Small-scale variation in biogeochemical processes in the environment may cause variation in isotopic signatures measured in organisms (Ben-David and Flaherty 2012, Gillies et al. 2012). For example, differences in $\delta^{13}C$ values in wetland environments could be driven by different photosynthetic pathways or by differences between terrestrial and marine plant food sources, whereas differences in $\delta^{15}N$ and $\delta^{34}S$ values may be caused by variation in biogeochemical cycles as well as "source effects" (Hobson and Wassenaar 2008, Ben-David and Flaherty 2012, Mambelli et al. 2016). These processes may cause high variability among isotopic signatures of individuals captured at nearby locations, resulting in an increased number of assignment errors.

Of the seven Rails classified as seasonal migrants, four had depleted $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values compared to other Rails captured in the Bay Area (Fig. 2a). These Rails were captured in wetlands at the eastern edge of the San Francisco Bay Delta where salinity tends to be lower than in the western San Pablo and San Francisco Bays (Cloern et al. 2002). In fact, Cloern et al. (2002) observed less enriched $\delta^{13}C$ and $\delta^{15}N$ values in freshwater marsh plants and sediments collected in the San Francisco Bay Delta compared to marine marsh plants and sediments collected in the San Pablo and San Francisco Bays. The remaining three Rails were captured in the Foothills at Bidwell Park in Chico. These Rails had $\delta^{13}C$ and $\delta^{15}N$ values similar to other Rails captured in the Foothills, but had highly enriched $\delta^{34}S$ values similar to those of Rails captured in the Bay Area (Fig. 2a). However, wetland soils in Bidwell Park also had highly enriched $\delta^{34}S$ values that may have been caused by sulfate-reducing microorganisms or by the presence of volcanic sediments in the region from the adjacent Lassen Volcanic National Park (Thode 1991, Pester 2012). While fractionation of $\delta^{34}S$ has not been measured for Rail feathers, it is likely that the enriched $\delta^{34}S$ values observed in Rails from Bidwell Park were most likely caused by environmental variation in $\delta^{34}S$ values (Fig. 3a, b). If only isotopic data had been used for population assignments, the seven misclassified Rails would have been identified as migrants. However, by using genetic assignments in conjunction with isotopic assignments, we identified the type I errors and ascertained valuable information about small-scale variation in the Bay Area and Foothills isoscapes.

Accuracy of genetic and isotopic population assignments

The level of genetic and isotopic differentiation between Rail metapopulations allowed us to perform population assignments with high accuracy at smaller spatial scales than many previous studies (Wennerberg et al. 2002, Clegg et al. 2003, Kelly et al. 2005, Rundel et al. 2013). Black Rails from the Bay Area and Foothills metapopulations were only moderately genetically differentiated (F_{ST} = 0.018). However, we maximized our assignment accuracy by (1) using 15 polymorphic microsatellite loci (mean observed heterozygosity $H_o \approx 0.68$; Appendix S2: Table S1) and (2) controlling the type I error rate by comparing a *P* value, generated for each assignment using the simulation procedure of Paetkau et al. (2004), to a strict α value of 0.01. At this error rate, up to three type I errors could have occurred in our sample of 336 Rails. Therefore, there is a small probability that the two established migrants observed in our sample are assignment errors rather than true migrants.

To further minimize assignment errors, we collected samples from all known breeding areas of Black Rails in the region (Evens and Nur 2002, Richmond et al. 2008, Tsao et al. 2015). However, due to the secretive nature of this species, a small number of individuals in our sample may have originated from an unknown, and thus unsampled, breeding area. Breeding areas of Black Rails are poorly documented throughout the San Francisco Bay Delta and the coastal region northwest of San Francisco near Bolinas Lagoon and Tomales Bay (Evens et al. 1991, Evens and Nur 2002, Spautz et al. 2002, Tsao et al. 2015). In fact, one of the two established migrants detected by our population assignments was captured at Tomales Bay, the only known breeding area for Black Rails in the coastal region northwest of San Francisco. If Black Rails breed in other areas of this region, it is possible that this migrant originated from a closer breeding area rather than a more distant area in the Foothills.

Isotopic population assignments of Black Rails performed with linear discriminant function analysis were highly accurate for both the Bay Area and Foothills (93.0% and 97.8%, respectively) because $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values from feathers of Rails captured in marine wetlands of the Bay Area were significantly more enriched than from Rails captured at freshwater wetlands of the Foothills (Tables 2 and 3). Previous studies have reported a high contribution of sulfur isotopes for distinguishing between birds foraging in marine and freshwater environments (Caccamise et al. 2000, Lott et al. 2003), and we obtained similar results

with $\delta^{34}S$ having the greatest contribution (66%) to differentiation in the discriminant function (Table 2).

Connectivity of Rail metapopulations via long-distance dispersal

Black Rail metapopulations of the Bay Area and Foothills are genetically distinct, but appear to be connected by a moderate level of gene flow (F_{ST} = 0.018, Foothills to Bay Area m = 0.075, Bay Area to Foothills m = 0.016), suggesting that dispersal between metapopulations may occur infrequently. When genetic assignments were used in conjunction with isotopic assignments to infer the timing of dispersal, no recent migrants were detected and two established migrants were detected. One of the established migrants dispersed a minimum distance of 160 km from the Foothills to the Bay Area, and the other dispersed a minimum distance of 110 km from the Bay Area to the Foothills. These inferred dispersal distance estimates were similar in magnitude to the direct dispersal distance measurement of 128 km from a band return that occurred during this study.

Our estimates of gene flow were very similar to the migration rate estimates (Foothills to Bay Area m = 0.11, Bay Area to Foothills m = 0.02) and F_{ST} value (0.020) of a previous study of Black Rails in the Foothills and Bay Area by Girard et al. (2010). Although Girard et al. (2010) detected limited gene flow, they detected a high proportion of migrant individuals relative to their sample size (7 of 62, or 11%). In contrast, we detected a much lower proportion of migrants (2 of 336, or 0.6%). This difference likely resulted from differences in sample size and analytical methods between studies. First, the larger sample size of our study should improve the accuracy of allele frequency estimates, which in turn, increases the accuracy of population assignments (Paetkau et al. 2004, Hale et al. 2012). Second, unlike Girard et al. (2010), we used the capture location of individuals to inform their assignment probabilities in STRUCTURE because it has been shown to improve cluster assignments among populations with low genetic differentiation (Hubisz et al. 2009). Furthermore, because assignment accuracy declines with decreasing genetic differentiation, the moderate level of gene flow between the Foothills and Bay Area could affect the accuracy of population assignments. Therefore, we performed our population assignments in GENECLASS2 so that we could calculate P values for each assignment and control our type I error rate following Paetkau et al. (2004). Given the increased accuracy of our assignments, Black Rails probably disperse less frequently between the Foothills and Bay Area than suggested by Girard et al. (2010).

We report the first direct observation of dispersal between metapopulations of Black Rails, a distance of 128 km. Although Black Rails are secretive and rarely observed flying, the eastern subspecies, *L. j. jamaicensis*, is migratory across part of its range (Eddleman et al. 1994). Therefore, it is not surprising that Black Rails are capable of long-distance movements, even in California, where the subspecies is nonmigratory. Dispersal distance estimates for other species of Rails are similar in magnitude to those observed for Black Rails in our study. Takekawa et al. (2014) reported a Ridgway's Rail (*Rallus obsoletus*) in the Bay Area that dispersed 45 km in 43 d. In addition, a study by Adams and Quay (1958) of migratory Clapper Rails (*Rallus crepitans*) indicated Rails may travel greater than 480 km in three to four days during migration, and suggested that the total distance was probably covered by one or a few longer movements. Clearly, like other Rails, Black Rails are capable of dispersing long distances (>100 km), but determining whether long-distance dispersal occurs as the result of a single movement or multiple smaller movements has important management implications and requires further investigation.

Using genetic and isotopic markers in conjunction to infer timing of long-distance dispersal

While previous studies have used genetic and isotopic assignments to quantify the distance and frequency of movements between an individual's capture population and its probable population of origin (Caccamise et al. 2000, Kelly et al. 2005, Cook et al. 2007), to our knowledge, this is the first study to use these approaches in conjunction to also deduce the timing of long-distance dispersal (Table 1). We inferred timing of dispersal using mismatches in the population of origin assigned by genetic data, which is a permanent marker, and the origin assigned by isotopic data, which is a temporary marker that changes according to an organism's diet and the turnover rate of the tissue sampled (Hobson and Wassenaar 2008). A mismatch between the genetically assigned population of origin and the isotopically assigned population of origin occurs when an individual moves to a new population and is sampled after isotopic turnover has begun in the sampled tissue. If isotopic turnover is likely to occur before sample collection, genetic data should be used in conjunction with isotopic data (Table 1). If, however, isotopic turnover is unlikely to occur before sampling, our method should not be used, and it is more appropriate to analyze genetic and isotopic data simultaneously with a spatially continuous assignment method that has greater assignment accuracy than using either data type alone (Rundel et al. 2013).

Temporal inference of dispersal events is determined by the turnover rate of the tissue sampled for stable isotope analysis (Table 1). We used secondary feathers, which are molted annually on the breeding ground in Rails (Pyle 2008), to differentiate between residents, recent migrants that had immigrated less than one year before their capture, and established migrants that had immigrated more than one year before capture. However, movement could be inferred at a different timescale by sampling a tissue with a different turnover rate. Tissues with faster turnover, such as plasma and organs, have been used to infer dispersal on a shorter timescale than those with slower turnover, such as muscle or blood (Vander Zanden et al. 2015).

Our approach could be applied to infer the timing of dispersal in species characterized by low to moderate gene flow and that forage in habitats with distinct isotopic signatures. Species with low to moderate gene flow are likely to exhibit genetic differentiation on a spatial scale that is small enough to accurately assign individuals to a population of origin (Berry et al. 2004). Differences in stable isotope signatures among ecosystems can also be exploited to ensure accurate assignments. For example, $\delta^{34}S$ has been used to assign geese to isotopically distinct marine or freshwater ecosystems (e.g., Caccamise et al. 2000), and hummingbirds have been tracked along an altitudinal gradient using differences in deuterium (δD; e.g., Hobson et al. 2003).

In conclusion, our study shows how the use of genetic data in conjunction with isotopic data obtained from a single capture event can offer a practical method to ascertain valuable information about the frequency and timing of long-distance dispersal of both juveniles and adults. Although this approach is unable to uncover the detailed movement patterns that telemetry or mark–recapture studies can reveal, the latter methods are usually more expensive to implement, are often limited to small sample sizes, require extensive field work, and may fail to detect long-distance movements (Koenig et al. 1996, Nathan et al. 2003). Combining approaches is, perhaps, the best way forward; telemetry can be used to quantify dispersal patterns and behavior at smaller spatial scales, and genetic and isotope markers can be used in conjunction to infer the frequency and timing of long-distance movements.

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Literature Cited

Adamack, A. T., and B. Gruber. 2014. PopGenReport: simplifying basic population genetic analyses in R. Methods in Ecology and Evolution 5:384–387.

Adams, D. A., and T. L. Quay. 1958. Ecology of the clapper rail in southeastern North Carolina. Journal of Wildlife Management 22:149–156.

Ben-David, M., and E. A. Flaherty. 2012. Stable isotopes in mammalian research: a beginner's guide. Journal of Mammalogy 93:312–328.

Benjamini, Y., and Y. Hochberg. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society Series B 57:289–300.

Berry, O., M. D. Tocher, and S. D. Sarre. 2004. Can assignment tests measure dispersal? Molecular Ecology 13:551–561.

Caccamise, D. F., L. M. Reed, P. M. Castelli, S. Wainright, and T. C. Nichols. 2000. Distinguishing migratory and resident Canada geese using stable isotope analysis. Journal of Wildlife Management 64:1084–1091.

Clegg, S. M., J. F. Kelly, M. Kimura, and T. B. Smith. 2003. Combining genetic markers and stable isotopes to reveal population connectivity and migration patterns in a Neotropical migrant, Wilson's Warbler (*Wilsonia pusilla*). Molecular Ecology 12:819–830.

Cloern, J. E., E. A. Canuel, and D. Harris. 2002. Stable carbon and nitrogen isotope composition of aquatic and terrestrial plants of the San Francisco Bay estuarine system. Limnology and Oceanography 47:713–729.

Cook, B. D., S. E. Bunn, and J. M. Hughes. 2007. Molecular genetic and stable isotope signatures reveal complementary patterns of population connectivity in the regionally vulnerable southern pygmy perch (*Nannoperca australis*). Biological Conservation 138:60–72.

Cornuet, J., S. Piry, G. Luikart, A. Estoup, and M. Solignac. 1999. New methods employing multilocus genotypes to select or exclude populations as origins of individuals. Genetics 153:1989–2000.

da Silva, A. R. 2015. Biotools: tools for biometry and applied statistics in agricultural science. R package version 2.0.

Eddleman, W. R., R. E. Flores, and M. Legare. 1994. Black rail (*Laterallus jamaicensis*). *In* A. Poole, editor. The Birds of North America Online. Cornell Lab of Ornithology, Ithaca, New York, USA. http://bna.birds.cornell.edu/bna/species/123

Evens, J. G., and N. Nur. 2002. California black rails in the San Francisco Bay region: spatial and temporal variation in distribution and abundance. Bird Populations 6:1–12.

Evens, J. G., G. W. Page, S. A. Laymon, and R. W. Stallcup. 1991. Distribution, relative abundance and status of the California black rail in western North America. Condor 93:952–966.

Flores, R. E., and W. R. Eddleman. 1991. Ecology of the California black rail in southwestern Arizona. Final Technical Report, Arizona Game and Fish Department, Phoenix, Arizona, USA.

Gillies, C. L., J. S. Stark, and S. D. A. Smith. 2012. Research article: small-scale spatial variation of δ13C and δ15N isotopes in Antarctic carbon sources and consumers. Polar Biology 35:813–827.

Girard, P., J. Y. Takekawa, and S. R. Beissinger. 2010. Uncloaking a cryptic, threatened rail with molecular markers: origins, connectivity and demography of a recently-discovered population. Conservation Genetics 11:2409–2418.

Griffiths, R., M. C. Double, K. Orr, and R. J. Dawson. 1998. A DNA test to sex most birds. Molecular Ecology 7:1071–1075.

Hale, M. L., T. M. Burg, and T. E. Steeves. 2012. Sampling for microsatellite-based population genetic studies: 25 to 30 individuals per population is enough to accurately estimate allele frequencies. PLoS ONE 7:e45170.

Hobson, K. A. 1999. Tracing origins and migration of wildlife using stable isotopes: a review. Oecologia 120:314–326.

Hobson, K. A., and L. I. Wassenaar, editors. 2008. Tracking animal migration with stable isotopes. Academic Press, San Diego, California, USA.

Hobson, K. A., L. I. Wassenaar, B. Mila, I. Lovette, C. Dingle, and T. B. Smith. 2003. Stable isotopes as indicators of altitudinal distributions and movements in an Ecuadorean hummingbird community. Oecologia 136:302–308.

Hubisz, M. J., D. Falush, M. Stephens, and J. K. Pritchard. 2009. Inferring weak population structure with the assistance of sample group information. Molecular Ecology Resources 9:1322–1332.

Kelly, J. F., K. C. Ruegg, and T. B. Smith. 2005. Combining isotopic and genetic markers to identify breeding origins of migrant birds. Ecological Applications 15:1487–1494.

Koenig, W. D., D. Van Vuren, and P. N. Hooge. 1996. Detectability, philopatry, and the distribution of dispersal distances in vertebrates. Trends in Ecology & Evolution 11: 514–517.

Longmire, J. L., et al. 1988. Isolation and molecular characterization of a highly polymorphic centromeric tandem repeat in the family Falconidae. Genomics 2:14–24.

Lott, C. A., T. D. Meehan, and J. A. Heath. 2003. Estimating the latitudinal origins of migratory birds using hydrogen and sulfur stable isotopes in feathers: influence of marine prey base. Oecologia 134:505–510.

Mambelli, S., P. D. Brooks, R. Sutka, S. Hughes, K. Finstad, J. P. Nelson, and T. E. Dawson. 2016. High throughput method for simultaneous analysis of N, C and S stable isotopes and concentrations in organics and soils. Rapid Communications in Mass Spectrometry 30:1743–1753.

Molecular Ecology Resources Primer Development Consortium. 2009. Permanent genetic resources added to Molecular Ecology Resources Database 1 May 2009–31 July 2009. Molecular Ecology Resources 9:1460–1559.

Nathan, R., G. Perry, J. T. Cronin, A. E. Strand, and M. L. Cain. 2003. Methods for estimating long-distance dispersal. Oikos 103:261–273.

Paetkau, D., R. Slade, M. Burden, and A. Estoup. 2004. Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. Molecular Ecology 13:55–65.

Pester, M. 2012. Sulfate-reducing microorganisms in wetlands—fameless actors in carbon cycling and climate change. Frontiers in Microbiology 3:1–19.

Piry, S., A. Alapetite, J. M. Cornuet, D. Paetkau, L. Baudouin, and A. Estoup. 2004. GENECLASS2: a software for genetic assignment and first-generation migrant detection. Journal of Heredity 95:536–539.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155:945–959.

Pyle, P. 2008. Identification guide to North American birds: part II Anatidae to Alcidae. Slate Creek Press, Point Reyes Station, California, USA.

R Core Team. 2013. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.r-project.org/

Rannala, B., and J. L. Mountain. 1997. Detecting immigration by using multilocus genotypes. Proceedings of the National Academy of Sciences USA 94:9197–9201.

Raymond, M., and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity 86:248–249.

Richmond, O. M., J. Tecklin, and S. R. Beissinger. 2008. Distribution of California black rails in the Sierra Nevada foothills. Journal of Field Ornithology 79:381–390.

Rubenstein, D. R., and K. A. Hobson. 2004. From birds to butterflies: animal movement patterns and stable isotopes. Trends in Ecology & Evolution 19:256–263.

Rundel, C. W., et al. 2013. Novel statistical methods for integrating genetic and stable isotope data to infer individual-level migratory connectivity. Molecular Ecology 22:4163–4176.

Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning: a laboratory manual, Second edition. Cold Spring Harbor Laboratory Press, New York, New York, USA.

Spautz, H., N. Nur, D. Stralberg, C. J. Ralph, and T. D. Rich. 2002. California black rail (*Laterallus jamaicensis coturniculus*) distribution and abundance in relation to habitat and landscape features in the San Francisco Bay Estuary. Pages 20–24 *in* J. C. Ralph and T. D. Rich, editors. Bird conservation implementation and integration in the Americas: Proceedings of the Third International Partners in Flight Conference. http://www.treesearch.fs.fed.us/pubs/31463

Sutherland, W. J., et al. 2013. Identification of 100 fundamental ecological questions. Journal of Ecology 101:58–67.

Takekawa, J. Y., M. Casazza, C. Overton, T. Bui, A. G. Vandergast, D. A. Wood, and S. Estrella. 2014. Applied studies on California clapper rail dynamics in support of comprehensive multispecies tidal marsh recovery. Unpublished Data Summary Report. U.S. Geological Survey, Western Ecological Research Center, Vallejo, Dixon, and San Diego, California, USA.

Thode, H. G. 1991. Sulfur isotopes in nature and the environment: an overview. Pages 1–26 *in* H. R. Krouse and V. A. Grinenko, editors. Stable isotopes: natural and anthropogenic sulfur in the environment. John Wiley & Sons, Chichester, United Kingdom.

Tsao, D. C., R. E. Melcer Jr., and M. Bradbury. 2015. Distribution and habitat associations of California black rail (*Laterallus jamaicensis coturniculus*) in the Sacramento–San Joaquin Delta. San Francisco Estuary and Watershed Science 13, Article 4.

Tsao, D. C., J. Y. Takekawa, I. Woo, J. L. Yee, and J. G. Evens. 2009. Home range, habitat selection, and movements of California black rails at tidal marshes at San Francisco Bay, California. Condor 111:599–610.

Vander Zanden, M. J., M. K. Clayton, E. K. Moody, C. T. Solomon, and B. C. Weidel. 2015. Stable isotope turnover and half-life in animal tissues: a literature synthesis. PLoS ONE 10:e0116182.

Waples, R. S. 2015. Testing for Hardy-Weinberg proportions: Have we lost the plot? Journal of Heredity 106:1–19.

Wennerberg, L., M. Klaassen, and A. Lindstrom. 2002. Geographical variation and population structure in the white-rumped sandpiper *Calidris fuscicollis* as shown by morphology, mitochondrial DNA and carbon isotope ratios. Oecologia 131:380–390.

West, J. B., G. J. Bowen, T. E. Dawson, and K. P. Tu, editors. 2010. Isoscapes: understanding movement, pattern, and process on Earth through isotope mapping. Springer, New York, New York, USA.

Williams, B. K. 1983. Some observations of the use of discriminant analysis in ecology. Ecology 64:1283–1291.

Wilson, G. A., and B. Rannala. 2003. Bayesian inference of recent migration rates using multilocus genotypes. Genetics 163:1177–1191.

Wright, S. 1931. Evolution in Mendelian populations. Genetics 16:97–159.

Yi-Balan, S. A., R. Amundson, and H. L. Buss. 2014. Decoupling of sulfur and nitrogen cycling due to biotic processes in a tropical rainforest. Geochimica et Cosmochimica Acta 142:411–428.

Supporting Information

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/eap.1432/full

Data Availability

Data associated with this paper are available in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.27mk2