Journal of Heredity, 2023, **XX**, 1–12
https://doi.org/10.1093/jhered/esad044
Advance access publication 17 July 2023
Original Article

Original Article

Sampling affects population genetic inference: A case study of the Allen's (*Selasphorus sasin*) and rufous hummingbird (*Selasphorus rufus*)

Brian M. Myers[1], Kevin J. Burns[1], Christopher J. Clark[2] and Alan Brelsford[2]

[1]Department of Biological Sciences, San Diego State University, San Diego, CA, United States,
[2]Department of Evolution, Ecology, and Organismal Biology, Speith Hall, University of California, Riverside, CA, United States
[3]Present address: Department of Biology, California State Polytechnic University, Pomona, 3801 W Temple Ave, Pomona, CA 91768, United States.
Address correspondence to B.M. Myers at the address above, or e-mail: bmyers62622@gmail.com.

Corresponding Editor: Kira Delmore

Abstract

Gene flow can affect evolutionary inference when species are undersampled. Here, we evaluate the effects of gene flow and geographic sampling on demographic inference of 2 hummingbirds that hybridize, Allen's hummingbird (*Selasphorus sasin*) and rufous hummingbird (*Selasphorus rufus*). Using whole-genome data and extensive geographic sampling, we find widespread connectivity, with introgression far beyond the Allen's × rufous hybrid zone, although the Z chromosome resists introgression beyond the hybrid zone. We test alternative hypotheses of speciation history of Allen's, rufous, and Calliope (*S. calliope*) hummingbird and find that rufous hummingbird is the sister taxon to Allen's hummingbird, and Calliope hummingbird is the outgroup. A model treating the 2 subspecies of Allen's hummingbird as a single panmictic population fit observed genetic data better than models treating the subspecies as distinct populations, in contrast to morphological and behavioral differences and analyses of spatial population structure. With additional sampling, our study builds upon recent studies that came to conflicting conclusions regarding the evolutionary histories of these 2 species. Our results stress the importance of thorough geographic sampling when assessing demographic history in the presence of gene flow.

Key words: admixture, Allen's hummingbird, gene flow, introgression, rufous hummingbird, speciation

Introduction

Incomplete lineage sorting and gene flow affect biological interpretation of evolutionary history, as both phenomena can lead to individual gene trees that differ from the species tree (Hudson 1983; Tajima 1983; Slatkin and Maddison 1989; Rannala and Yang 2008; Leaché et al. 2014). Investigators can address incomplete lineage sorting with thorough sampling, increased sequencing effort, and more realistic phylogenetic models (Maddison and Knowles 2006; McCormack et al. 2009; Leaché and Rannala 2011). However, the effect of gene flow on inference of evolutionary relationships has been given less attention historically, even though gene flow may also compromise conclusions when species are undersampled (Slatkin and Maddison 1989; Leaché et al. 2014). When species hybridize, recent gene flow may obscure deeper evolutionary history, as introgression beyond known areas of hybridization affects inferences related to phylogeny (Leaché et al. 2014). When studies restrict sampling to populations with recent gene flow, historical events become obscured by current genomic similarity, leading to conclusions that may not accurately represent evolutionary relationships (Leaché et al. 2014).

Hybridization can influence populations beyond areas of overlap if genes introgress. These introgression events can be neutral, increase genetic diversity, or decrease genetic diversity of populations (Holderegger et al. 2006; Johnson et al. 2010; Roberts et al. 2010; Witte et al. 2013; Whiteley et al. 2015). Given the different potential outcomes of gene flow, analyses that identify the presence of introgression, and identify areas of the genome that freely introgress versus those which are selected against, are fundamental to population genetic and demographic inference.

Here, we study Allen's hummingbird (*Selasphorus sasin*), which breeds in a thin strip of habitat along the coast from southern coastal Oregon to northern Baja California (Erickson 2016; Myers et al. 2019). There are 2 subspecies of Allen's hummingbird, one migratory (*S. s. sasin*) and the other non-migratory (*S. s. sedentarius*). The migratory subspecies breeds in the northern part of the species range, from Ventura County, California, to Curry County, Oregon, and forms a hybrid zone with rufous hummingbird (*S. rufus*, Fig. 1) that is centered in Curry and Coos County, Oregon (Myers et al. 2019). Non-migratory Allen's hummingbird was endemic to the Channel Islands of southern California (Grinnell 1939; Grinnell and Miller 1944), but later colonized the

Received November 5, 2022; Accepted July 12, 2023

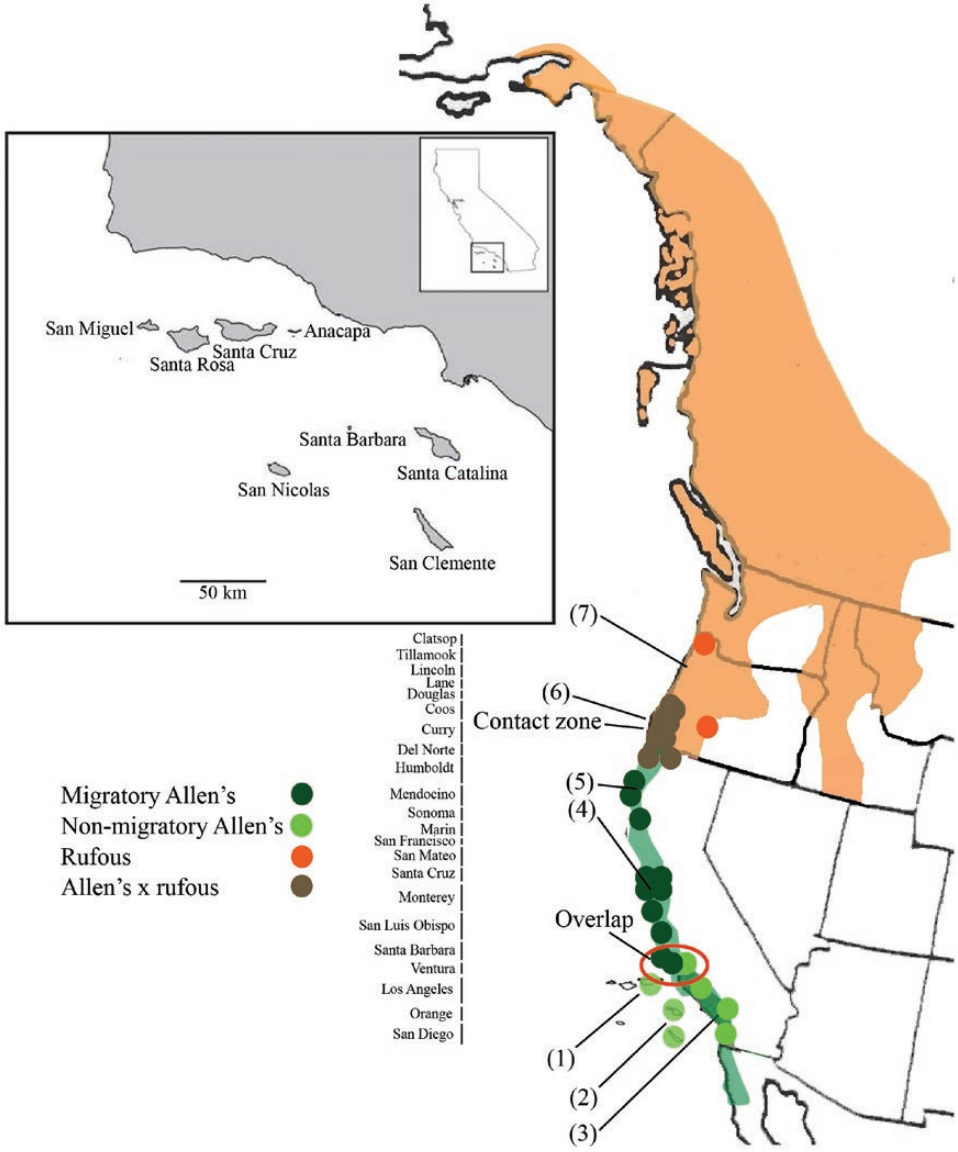

Fig. 1. Sampling locations and approximate breeding ranges of migratory Allen's, non-migratory Allen's, rufous, and Allen's × rufous hybrids (Myers et al. 2019). Numbers in parentheses denote sampling groups used for population genetic analyses. Red oval indicates the zone of intergradation between migratory and non-migratory Allen's hummingbird. Inset map shows the locations of the Channel Islands. Names are coastal counties.

mainland on the Palos Verdes Peninsula in the 1960s (Wells and Baptista 1979). Subsequently, this mainland population of non-migratory Allen's rapidly expanded its range on the mainland and now breeds as far south as Baja California, north to Santa Barbara County, and east to Riverside County (Wells and Baptista 1979; Unitt 2004; Erickson 2016; Clark 2017). As a result, the 2 subspecies of Allen's hummingbird have recently come into contact in Santa Barbara and/or Ventura County (Fig. 1; Godwin et al. 2020).

Two recent studies of the phylogeographic history of Allen's hummingbird came to conflicting conclusions about the status of mainland populations of Allen's hummingbird. Godwin et al. (2020) inferred that mainland populations in southern California form a hybrid swarm between the 2 subspecies, where all individuals are genetically admixed to varying degrees. In contrast, Battey (2020) investigated the evolutionary history of Allen's and rufous hummingbird using migratory Allen's hummingbird samples from the San Francisco Bay area and instead inferred that migratory Allen's hummingbird shares a hybrid ancestry with both non-migratory Allen's and rufous hummingbird. However, migratory Allen's hummingbird hybridizes with rufous hummingbird (Myers et al. 2019), and samples that Godwin et al. (2020) and Battey (2020) designated as parental migratory Allen's hummingbird may have actually been admixed with rufous hummingbird. Thus, gene flow from rufous hummingbird into the range of migratory Allen's hummingbird might have affected the inferences presented in these previous studies.

Both Battey (2020) and Godwin et al. (2020) had a similar sampling design with respect to Allen's hummingbird: they only sampled from a limited number of populations. Both Battey (2020) and Godwin et al. (2020) sampled migratory Allen's almost entirely from a single part of Allen's hummingbird's range, the San Francisco Bay area. Sampling of non-migratory Allen's hummingbird by Godwin et al. (2020) was restricted to the southern Channel Islands (e.g. Catalina Island) and the coast on the southern California

mainland. Godwin et al. (2020) did not include any samples from the northern Channel Islands, although previous studies across a variety of species, including non-migratory Allen's hummingbird (Myers et al. 2021), have found that the northern Channel Islands (e.g. Santa Cruz Island) have genetically distinct populations relative to the southern Channel Islands and mainland (Ashley and Willis 1987; Caballero and Ashley 2011; Sofaer et al. 2012; Walsh 2015; Wilson et al. 2015; Hanna et al. 2019). As Godwin et al. (2020) did not include samples from the northern Channel Islands, or further inland from the southern California coast, the inference of a hybrid swarm on mainland southern California needs to be addressed with additional data from the northern Channel Islands and inland southern California.

A recent phylogeny of North American hummingbirds reported unresolved relationships between Allen's, rufous, and Calliope hummingbird (*S. calliope*): Calliope hummingbird was embedded within the Allen's and rufous hummingbird (Licona-Vera and Ornelas 2017). Here, we sample Allen's hummingbird from throughout its breeding range, including populations not sampled by previous studies, and investigate the evolutionary relationships within Allen's hummingbird and between Allen's and rufous hummingbird. Furthermore, we evaluate the relationship of Calliope hummingbird relative to Allen's and rufous. Calliope hummingbird has historically been hypothesized to be the outgroup to Allen's and rufous hummingbird (see Battey 2020), although McGuire et al. (2014) identified rufous as the outgroup to a clade containing Calliope and Allen's hummingbird, with strong support for all nodes involved.

We evaluate the evolutionary history of migratory Allen's, non-migratory Allen's, rufous, and Calliope hummingbird by testing 4 scenarios. 1) Non-migratory and migratory Allen's hummingbird are each other's closest relative, with rufous hummingbird as the sister taxon, and Calliope hummingbird as the outgroup. 2) The result from Battey (2020), which suggests migratory Allen's hummingbird is a hybrid taxon of rufous and non-migratory Allen's hummingbird, with Calliope hummingbird as the outgroup. 3) The hypothesis that migratory and non-migratory Allen's hummingbird are each other's closest relative, Calliope hummingbird is their sister taxon, and rufous hummingbird is the outgroup, as reported by McGuire et al. (2014). 4) Migratory and non-migratory Allen's hummingbird form a single panmictic population, rufous hummingbird is the sister taxon to Allen's hummingbird, and Calliope hummingbird is the outgroup. We test for gene flow throughout the evolutionary history of these taxa, and estimate divergence dates, which have been variably estimated at 5,000 to 100,000 yr ago (Battey 2020; the divergence of Allen's and rufous hummingbird) and about 300,000 yr ago (McGuire et al. 2014; the divergence of Allen's and Calliope hummingbird). Finally, we evaluate whether a hybrid swarm between migratory and non-migratory Allen's hummingbird is present on the southern California mainland, as reported by Godwin et al. (2020).

Materials and methods

Sampling

We sampled tissue (N = 34; specimens deposited in the San Diego Natural History Museum and the SDSU Biodiversity Museum) and blood taken from a toenail clip (N = 74) based on the methodology provided by Tell et al. (2021) from 108 individuals along a north–south transect from northern Oregon to southern California (California: San Diego, Los Angeles, Santa Barbara, San Luis Obispo, Monterey, Mendocino, Humboldt, and Del Norte counties, Oregon: Curry, Coos, Douglas, Lane, and Clatsop counties), as well as inland along the Klamath River from Humboldt County and Siskiyou County, California. In southern California, we also sampled the Channel Islands and inland on the mainland, in Riverside County (Fig. 1; Supplementary Table S1). We supplemented the 108 samples gathered in the field with 25 tissues from museum collections; after filtering out individuals with a mean depth of less than 1 (see below), 118 individuals remained in the dataset (Fig. 1; Supplementary Table S1). Collection of samples in the field occurred during the breeding season, March through May (2014 to 2018) and museum specimens dated from March through May were used in our dataset. All sample collection was conducted in compliance with the IACUC at the University of California, Riverside (protocols 20130018 and 20160039), USFWS permit #MB087454-1, USGS Bird Banding Permit #23516, California Department of Fish and Wildlife permit #SC006598, California State Parks permit #17-820-01, Oregon Department of Fish and Wildlife permit #055-17, #049-16, and #103-14, and Oregon Parks and Recreation Department permit #011-14.

To address gene flow and the evolutionary history of Allen's and rufous hummingbird, we incorporated 9 rufous hummingbirds from northern Oregon (Clatsop County, approximately 300 km north of the hybrid zone) and 57 individuals from the phenotypic extent of the migratory Allen's × rufous hybrid zone from northern California and southern Oregon (Curry, Coos County, Oregon, and Humboldt, Del Norte, and Siskiyou County, California). Localities that included hybrids incorporated portions of the Allen's × rufous hybrid zone in which phenotypic data exhibited clinal variation between the 2 species, as described in Myers et al. (2019). For estimation of the evolutionary history between Allen's, rufous, and Calliope hummingbird, we included 7 Calliope hummingbird individuals sampled from Washington, New Mexico, and California and sequenced by Battey (2020).

Grinnell and Miller (1944) suggested that migratory Allen's hummingbird's breeding range extended as far south as the vicinity of the border between Ventura and Santa Barbara County. Non-migratory birds reached that area in roughly 2006, and there now appear to be continuous populations of migratory and non-migratory Allen's hummingbird along the coast. Clark (2017) speculated that there may be intergradation between the 2 subspecies, and birds we sampled in Santa Barbara County seemed to have similar morphology to non-migratory Allen's hummingbird based on measurements of exposed culmen, wing length, tail length, and tail rectrix 1 and 5 width (Stiles 1972; Pyle 1997). However, as Grinnell and Miller (1944) suggested that migratory Allen's extended through Santa Barbara, we did not a priori assume the Allen's hummingbird population in and north of Santa Barbara County are the non-migratory subspecies. Allen's hummingbird populations sampled in Los Angeles County and further south and inland were classified a priori as non-migratory Allen's hummingbird.

DNA extraction and whole-genome sequencing

We extracted genomic DNA from tissues and dried blood spots using a Qiagen DNeasy Blood and Tissue Kit following the recommendations of the manufacturer (Qiagen, Valencia,

California, USA). Library preparation was based on a modified Nextera protocol (Baym et al. 2015). We sequenced whole genomes of all individuals using an Illumina NextSeq 500 at the University of California, Riverside Genomics Core, HiSeq 4000 at the University of Berkeley Genomics Core, or Illumina HiSeq X at Novogene, Inc., with an average depth of 5.5× per sample (Supplementary Table S1). Additionally, low coverage reads of the 7 Calliope hummingbird samples sequenced to an average depth of 4× per sample on a Hiseq 3000 in Battey (2020) were acquired from the NCBI database for demographic analysis in Fastsimcoal2 (NCBI Resource Coordinators 2018). All samples were sequenced for paired-end 150 bp reads and were aligned to the Anna's hummingbird (*Calypte anna*) reference genome available on NCBI (accession number GCA_003957555.2; Rhie et al. 2021) using the software package BWA v0.7 (Burrows-Wheeler Aligner; Li and Durbin 2009; NCBI Resource Coordinators 2018). Allen's, rufous, and Calliope hummingbird are monophyletic (McGuire et al. 2014), thus these taxa are roughly equally distantly related to the Anna's hummingbird reference genome, making the alignment unlikely to bias our results in any way.

For demographic analysis in Fastsimcoal2 (see below), we called variants using SAMtools v1.9 and BCFtools v1.9 (Li et al. 2009; Danecek et al. 2021), while population structure and F_{ST} were estimated with ANGSD v0.941 (Korneliussen et al. 2014). We filtered and retained sites with a minimum depth of 2, that were successfully genotyped in at least 75% of individuals, had a minimum mapping quality score of 30, and a minimum minor allele frequency of 0.05 using VCFtools v1.16 for demographic analysis and ANGSD for all other analyses (Danecek et al. 2011). A minor allele frequency of 0 was used for demographic analysis in Fastsimcoal2 (see below). All individuals with a mean depth of less than 1 were removed from the dataset. After filtering, the dataset contained 1,415,936 single nucleotide polymorphisms (SNPs).

Population structure

We investigated population structure by implementing a principal component analysis (PCA). We performed a PCA, a model-free method based on variation in allele frequencies, to detect patterns of genetic structure using PCAngsd (Meisner and Albrechtsen 2018). To ensure the data input into the PCA was independent (there were no spurious correlations among genomic variants), we pruned the dataset of linked variants by setting an r^2 threshold of 0.1. Specifically, we pruned variants with an r^2 greater than 0.1 within 50-SNP windows to remove SNPs that were located close together on a given chromosome and in strong linkage disequilibrium. We extracted PC coordinates for each individual and plotted the results in the tidyverse package (Wickham et al. 2019) in R v3.5.2 (R Core Team 2018) and R Studio v1.2.5 (R Studio Team 2019).

To estimate potential admixture and further investigate population structure present within the dataset, we used NGSadmix (Meisner and Albrechtsen 2018). Battey (2020) and Henderson and Brelsford (2020) identified the Z chromosome (a sex chromosome in birds) as the most differentiated part of the genome. Thus, we analyzed the NGSadmix plot for the entire genome and compared this plot to the Z chromosome. We evaluated clusters of $K = 2$ to 5.

We organized individuals into 2 sets: a set of "parental" groups and a set of "admixed + parental" groups (see Fig. 1). The parental set included 3 groups of individuals representing: 1) the breeding range of non-migratory Allen's hummingbird (i.e. the northern and southern Channel Islands and mainland southern California south of Santa Barbara County), 2) migratory Allen's hummingbird, from Santa Barbara County, California, to Curry County, Oregon (the northern extent of migratory Allen's hummingbird is Curry County, Oregon; Myers et al. 2019), and 3) rufous hummingbird (samples from north of the hybrid zone, in Lincoln and Clatsop County, Oregon). These groups were used to evaluate evolutionary history. To achieve these groupings, we removed hybrids (individuals with admixture levels of over 5%, as determined by NGSadmix) from the dataset. Removal of admixed individuals trimmed each parental group size to the following: migratory Allen's (N = 12 individuals), non-migratory Allen's (N = 7 individuals), and rufous (N = 9 individuals) hummingbird. The second set, the admixed + parental set (N = 118 individuals), was used to evaluate population genetic dynamics between the Channel Islands and the mainland (within non-migratory Allen's), between non-migratory and migratory Allen's, and between migratory Allen's and rufous hummingbird. This set was divided into 7 a priori groups based on geography (labeled on Fig. 1): 1) non-migratory Allen's hummingbird on a northern Channel Island, Santa Cruz Island (N = 5 individuals), 2) non-migratory Allen's hummingbird on 2 southern Channel Islands (San Clemente, N = 1 individual, and Santa Catalina Island, N = 2 individuals), 3) mainland non-migratory Allen's hummingbird (San Diego County through Los Angeles County, California, N = 9 individuals), 4) the southern portion of the range of migratory Allen's hummingbird (Santa Barbara through Monterey County, California, N = 15 individuals), 5) the northern portion of the range of migratory Allen's hummingbird (San Francisco County through Mendocino County, California, N = 20 individuals), 6) the migratory Allen's × rufous hummingbird hybrid zone based on phenotypic data (N = 57 individuals), and 7) rufous hummingbird's historic range (N = 9 individuals; these are the same 9 individuals included in the rufous hummingbird parental group). The set of parental groups was embedded throughout the 7 admixed + parental groups: non-migratory Allen's hummingbird from parental group 1 was placed in admixed + parental groups 1 and 2, and individuals from parental group 1 from San Diego and Riverside County in admixed + parental group 3, migratory Allen's hummingbird from parental group 2 was placed in admixed + parental group 4, and rufous hummingbird from parental group 3 was placed in admixed + parental group 7 (Fig. 1).

Using the admixed + parental groups, we performed pairwise comparisons of F_{ST} in ANGSD (Korneliussen et al. 2014). We also investigated whether isolation-by-distance, where genomic differences between populations increase with geographic distance, is present across the dataset by calculating the Pearson coefficient of correlation (R^2) of the pairwise genetic distance (F_{ST}) between the 7 admixed + parental groups and their average geographic distances from each other using the *R* package ggpubr (Kassambara 2020) in R v3.5.2 (R Core Team 2018) and R Studio v1.2.5 (R Studio Team 2019). We also estimated F_{ST} between parental groups, which were comprised of non-admixed individuals belonging to non-migratory Allen's, migratory Allen's, and rufous hummingbird. Parental comparisons of F_{ST} were visualized on a Manhattan plot in 50-kb sliding windows, where outliers

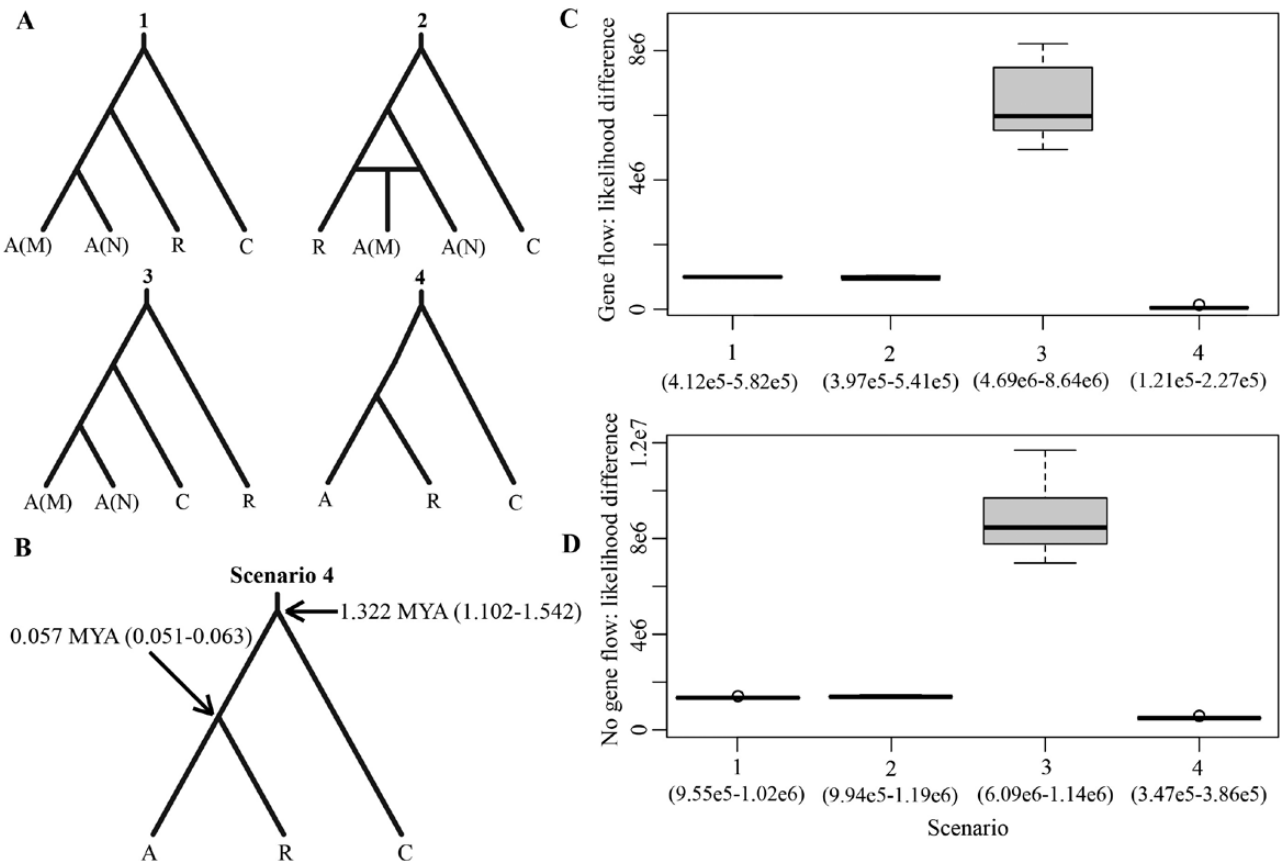

Fig. 2. Inference of the speciation history of migratory Allen's, A(M), non-migratory Allen's, A(N), rufous, R, and Calliope, C, hummingbird. For panels (C) and (D), confidence intervals of mean likelihood difference are included on the *x* axis below the name of each model. A) The 4 hypotheses of the speciation history of these taxa, which include 1) non-migratory and migratory Allen's hummingbird are each other's closest relative, with rufous hummingbird as the sister taxon, and Calliope hummingbird as the outgroup, 2) migratory Allen's hummingbird is a hybrid taxon of rufous and non-migratory Allen's hummingbird, with Calliope hummingbird as the outgroup, 3) migratory and non-migratory Allen's hummingbird are each other's closest relative, Calliope hummingbird is their sister taxon, and rufous hummingbird is the outgroup, and 4) no divergence between migratory and non-migratory Allen's hummingbird, rufous hummingbird is the sister taxon to Allen's hummingbird, and Calliope hummingbird is the outgroup. B) The best-supported scenario. C) The likelihood difference for each scenario, assuming gene flow between migratory and non-migratory Allen's and between migratory Allen's and rufous hummingbird. D) The likelihood difference for each scenario, assuming no gene flow between taxa.

were identified as the highest 1% of F_{ST} values for each pairwise comparison.

Speciation history

We inferred the speciation history of non-migratory Allen's, migratory Allen's, rufous, and Calliope hummingbird (using the parental groups) in Fastsimcoal2, a coalescent-based simulation program that estimates demographic parameters of complex models from the site frequency spectrum (SFS) under a maximum-likelihood framework (Excoffier et al. 2021). In Fastsimcoal2, explicit models are specified by the user to evaluate competing hypotheses of the evolutionary history of a group of taxa. Fastsimcoal2 enables comparison of competing historical/demographic scenarios and accounts for historic hybridization between taxa, compared with traditional phylogenetic methods, which give confounding results due to hybridization (McDade 1992; McVay et al. 2017).

We used easySFS to generate the site frequency spectrum for use in Fastsimcoal2 (https://github.com/isaacovercast/easySFS). First, the "--preview" option was used to calculate the optimal number of individuals to include in each population to minimize the amount of missing data. Next, the --proj command was used to generate the site frequency spectrum.

In Fastsimcoal2, we tested the 4 scenarios of evolution for non-migratory Allen's, migratory Allen's, rufous, and Calliope hummingbird outlined in the introduction (Fig. 2A). To explicitly test for gene flow within the species complex, we ran Fastsimcoal2 for each scenario twice: once assuming gene flow between migratory Allen's and non-migratory Allen's, and between migratory Allen's and rufous hummingbird, and once assuming no gene flow. For Scenario 4, we only assumed gene flow between Allen's and rufous hummingbird.

For each model, we initially performed 50 iterations of Fastsimcoal2, running 1,000,000 coalescent simulations and 100 optimization cycles for each iteration. We generated boxplots for each model based on the difference between the likelihood estimated within each iteration and the best possible likelihood to choose the best-fit model. The model with the smallest difference between the observed likelihood and best possible likelihood implies the best fit. Next, for the best-fit model, we performed block bootstrapping to generate confidence intervals for divergence dates within the species complex. We split the original genomic data into 50 different 200 kbp blocks, and, for each bootstrap, randomly sampled the 50 blocks with replacement until the length of the original genome was reached. We then recalculated the SFS for each

bootstrap replicate and ran Fastsimcoal2 50 times on each bootstrapped SFS. Finally, we computed a confidence interval of the parameter estimates using the highest likelihood run from each bootstrap replicate using a custom R script.

To estimate divergence times in Fastsimcoal2, we first converted generations to years using an average estimated generation time of 2.75 yr, the average of generation times proposed for other hummingbird species, based on the observation that maturity begins 1 yr after hatching and the average assumed survival rates of 4 hummingbird species, which range from 0.30 to 0.52 (Hilton and Miller 2003; Ruiz-Gutiérrez et al. 2012; Da Cruz Rodrigues et al. 2013; Ornelas et al. 2016). Average generation time can be estimated as $(T) = a + [s/(1 - s)]$, where a is the time to maturity and s is the adult annual survival rate (Lande et al. 2003). Based on this, estimates for T range from 2.43 to 3.08 yr, with an average of 2.75 yr. We assumed a mutation rate of 4.6×10^{-9} based on a pedigreed population of the collared flycatcher (*Ficedula albicollis*) as reported by Smeds et al. (2016).

Results

Population structure

NGSadmix results showed that $K = 3$ was consistent with the approximate historic described range of migratory Allen's, non-migratory Allen's, and rufous hummingbird (Fig. 3). $K = 2$, $K = 4$, and $K = 5$ showed less correspondence to known phenotypic groups (Supplementary Fig. S1). $K = 2$ clustered migratory Allen's and rufous hummingbird together, separate from non-migratory Allen's, $K = 4$ separated migratory Allen's hummingbird into 2 separate, highly admixed clusters, and separate clusters for non-migratory Allen's and rufous hummingbird, while $K = 5$ split non-migratory Allen's hummingbird into a single cluster, followed by a southern migratory Allen's hummingbird cluster, followed by 2 separate (highly admixed) clusters of northern migratory Allen's hummingbird, and a rufous hummingbird cluster. For $K = 3$, there was a genomic signature of hybridization throughout the extent of the migratory Allen's × rufous hummingbird hybrid zone from Coos County, Oregon, to Humboldt County, California, with a long tail of introgression of rufous hummingbird alleles present in Allen's hummingbird populations south of Humboldt County, far south of the point at which the birds have phenotypes of Allen's hummingbird (Fig. 3A). There was 1 exception to this: the Z chromosome (see below).

In southern California, introgression appears to be occurring both up and down the coast. The most intermediate genetic intergrades of migratory and non-migratory Allen's hummingbird were located in Los Angeles and Santa Barbara counties (Fig. 3A), a distance of 54.5 km south and 39.7 km north, respectively, of the border between Santa Barbara and Ventura counties. Assuming intergradation began in 2006, the center of this intergradation zone, where we observe the most admixture, is potentially expanding down the coast, into Los Angeles County, at an average rate of 5.0 km/yr and up the coast, into Santa Barbara County, at an average rate of 3.6 km/yr. We observed clinal variation across southern California, and identified geographic areas comprised predominantly of effectively unadmixed, parental individuals (for example, on the southern Channel Islands, in San Diego County, and in Riverside County), but also identified areas where most individuals were admixed (Santa Cruz Island and most of the southern California mainland, especially along the coast). Thus, our results suggest a zone of intergradation between migratory and non-migratory Allen's hummingbird, although our results do not corroborate the findings of Godwin et al. (2020), which suggested that southern California is entirely comprised of admixed migratory and non-migratory Allen's hummingbird (Fig. 3A).

NGSadmix analysis of the Z chromosome, isolated from the rest of the genome, showed patterns different from the entire genome. Introgression of rufous hummingbird was mostly restricted to the phenotypic hybrid zone between rufous and Allen's hummingbird and did not extend into the breeding range of migratory Allen's hummingbird (Fig. 3B). Introgression was present, but more limited on the Z chromosome relative to the autosomes, between migratory and non-migratory Allen's hummingbird (Fig. 3B). Furthermore, including autosomes and the Z chromosome, we identified non-admixed, parental migratory Allen's hummingbird individuals in San Luis Obispo County and Monterey County, California. The presence of these individuals in our dataset refutes the hypothesis that migratory Allen's hummingbird is a hybrid taxon between non-migratory Allen's and rufous hummingbird.

PCA revealed additional fine-scale population structure (Fig. 4). PC1 (14.5% of the variation) separated 4 groups: 1) a group containing rufous, migratory Allen's hummingbird from northern California, and rufous × migratory Allen's hummingbird hybrids, 2) migratory Allen's hummingbird from central California, 3) a group containing non-migratory Allen's hummingbird from Santa Cruz Island and the mainland, and 4) a group containing 3 non-migratory Allen's hummingbirds from the southern Channel Islands (San Clemente and Santa Catalina islands). PC1 and PC2 (7.8% of the variation) were able to partially separate rufous and migratory Allen's hummingbird from northern California, and rufous × migratory Allen's hummingbird hybrids. These groups did not form separate clusters. Instead, PC1 and PC2 organized rufous, hybrids, and migratory Allen's hummingbird along a continuum across the principal component space (Fig. 4). The 4 individuals from mainland Santa Barbara County marked as migratory Allen's hummingbird a priori also clustered closer to non-migratory Allen's than migratory Allen's hummingbird on PC1 and PC2 (Fig. 4). These individuals were all intergrades between migratory and non-migratory Allen's hummingbird (Fig. 3). PC3 (6.4% of the variation) strongly differentiated Santa Cruz Island from all other populations.

Between parental groups, genome-wide F_{ST} was higher between migratory and non-migratory Allen's hummingbird ($F_{ST} = 0.12$) than between migratory Allen's and rufous hummingbird ($F_{ST} = 0.08$). Between migratory and non-migratory Allen's hummingbird, F_{ST} was 0.11 on the autosomes and 0.17 on the Z chromosome, while between migratory Allen's and rufous hummingbird, F_{ST} was 0.07 on the autosomes and 0.18 on the Z chromosome. Two-thirds of the highest 1% of F_{ST} values between migratory Allen's and rufous hummingbird were on the Z chromosome, while over half of the highest 1% of F_{ST} values between migratory and non-migratory Allen's were on the Z chromosome (Fig. 5 and Table 1).

Between admixed + parental groups, F_{ST} generally increased with geographic distance, although the coefficient of correlation of genetic and geographic distance across groups was

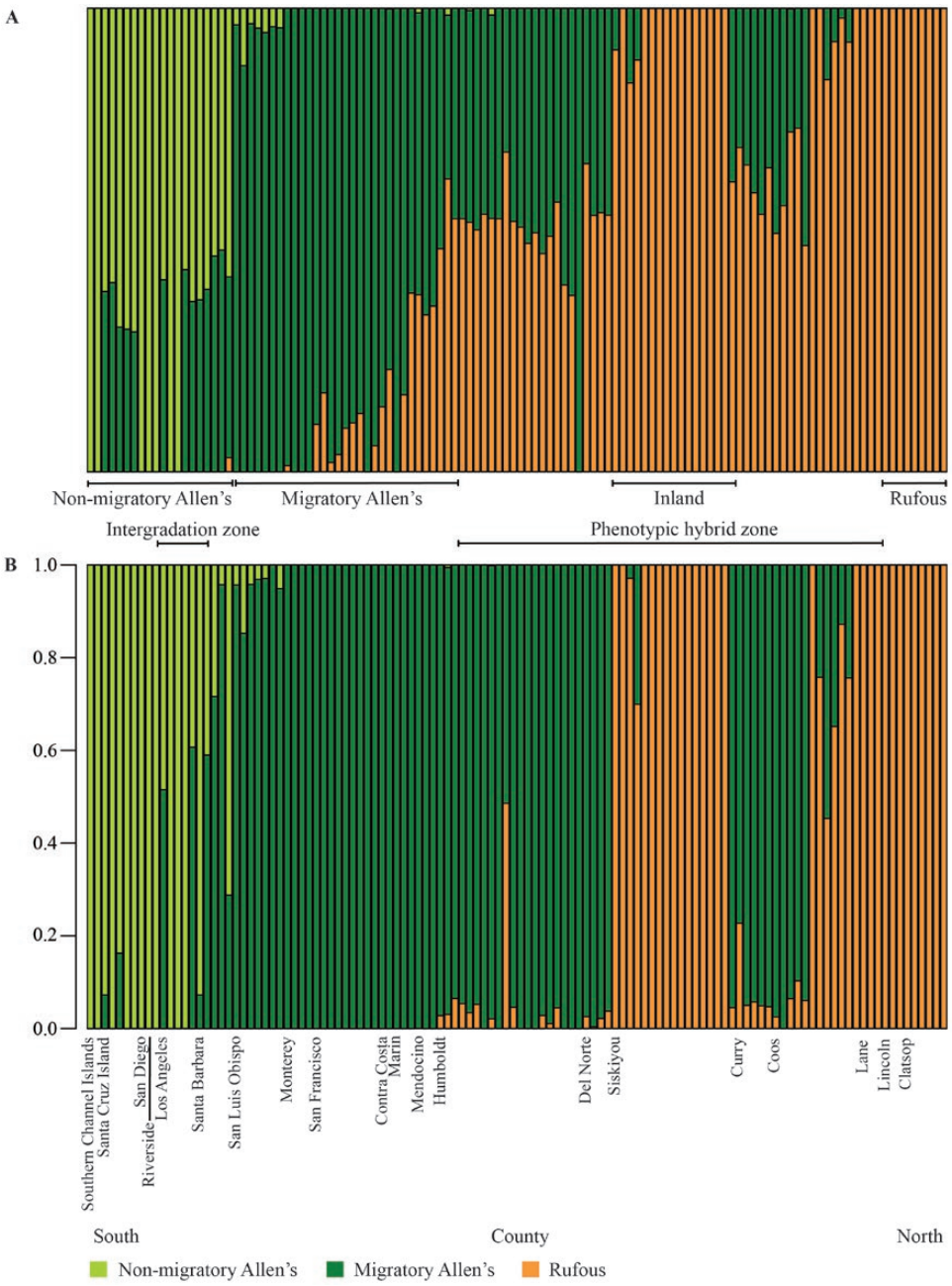

Fig. 3. NGSadmix plot for $K = 3$ groups A) across the whole genome and B) for the Z chromosome. "Inland" indicates individuals sampled from an inland transect of the hybrid zone in Siskiyou County, south of Curry and east of Del Norte County. There was introgression by rufous hummingbird into the range of migratory Allen's hummingbird, beyond the phenotypic hybrid zone described in Myers et al. (2019) for the whole genome as far south as the San Francisco Bay Area. Introgression was more limited on the Z chromosome and was restricted to extreme northern California. There was also evidence of intergradation between non-migratory and migratory Allen's centered in Santa Barbara and Los Angeles County, with low to absent levels of admixture south to San Diego County and inland to Riverside County.

weak and insignificant ($R^2 = 0.10$; $P > 0.05$, Fig. 6). F_{ST} between southern migratory and mainland non-migratory Allen's hummingbird was 0.06, while northern migratory and mainland non-migratory Allen's hummingbird was 0.07. Among the 3 non-migratory groups of Allen's hummingbird, Santa Cruz Island was the most isolated. F_{ST} values comparing the mainland ($F_{ST} = 0.07$) and the southern Channel Islands ($F_{ST} = 0.11$, Table 2) to Santa Cruz Island were both relatively high. The F_{ST} of southern migratory Allen's and rufous hummingbird ($F_{ST} = 0.09$,) was higher than the F_{ST} of the northern migratory Allen's and rufous hummingbird groups ($F_{ST} = 0.06$). The highest F_{ST} estimates were between Santa Cruz Island and the southern Channel Islands ($F_{ST} = 0.15$) and between Santa Cruz Island and rufous hummingbird ($F_{ST} = 0.17$, Table 2).

Speciation history

All models with gene flow received more support than models that assumed no gene flow. Across all scenarios, Scenario 3 was least supported, and Scenarios 1 (migratory and non-migratory Allen's are sister taxon, rufous is most closely related to migratory and non-migratory Allen's, and Calliope

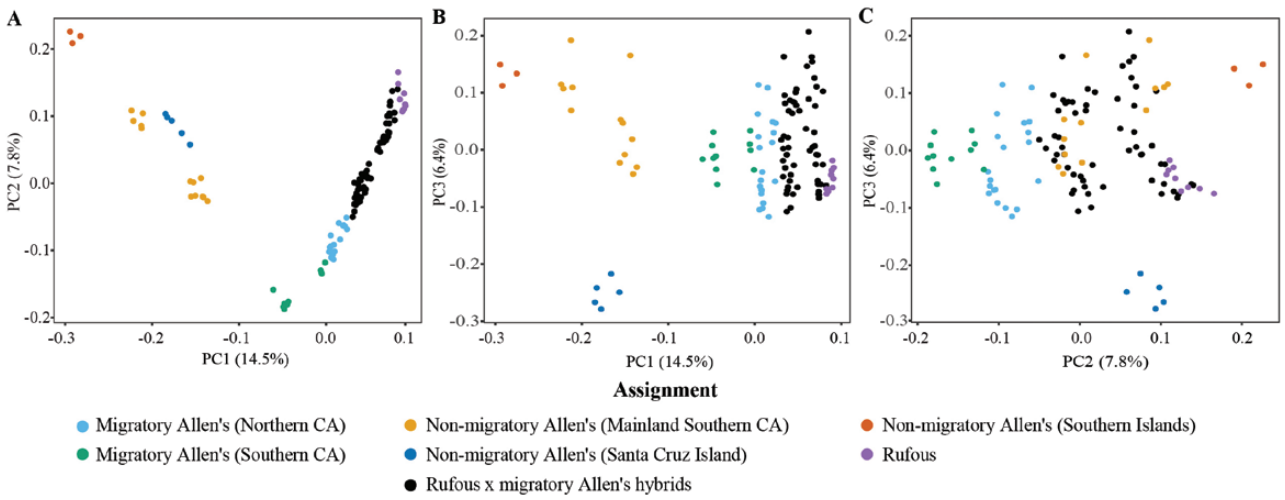

Fig. 4. PCA of the 7 admixed + parental groups across the first 3 principal components. A) Three main clusters, which were mostly separated by PC1 and PC2 (14.5% and 7.8% of the variation, respectively), where there is clinal variation through the migratory Allen's × rufous hybrid zone from Rufous (left) to migratory Allen's (right). B) Four main clusters, which were mostly separated by PC1 and PC3 (14.5% and 6.4% of the variation, respectively). C) Three main clusters, separated by PC2 and PC3 (7.8% and 6.4% of the variation, respectively).

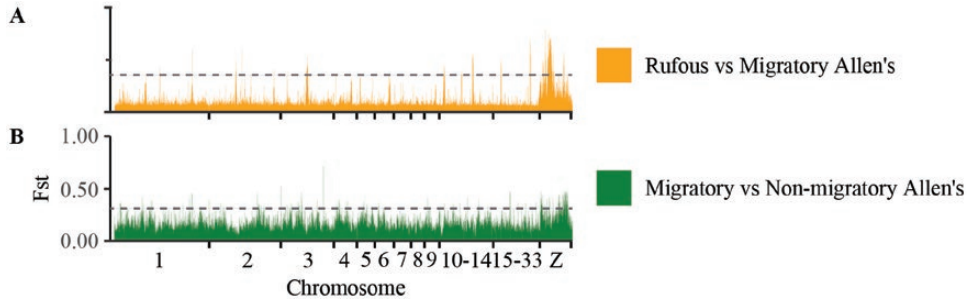

Fig. 5. Manhattan plot of pairwise F_{ST} between A) migratory Allen's and rufous and B) migratory and non-migratory Allen's hummingbird, plotted in 50 kb windows. Points above the dotted line are the highest (top 1%) of all values of F_{ST}. Most outliers were on the Z chromosome between both migratory Allen's and rufous hummingbird, and migratory and non-migratory Allen's hummingbird.

Table 1. The highest 1% of F_{ST} values and their chromosomal locations.

A		B			
Chromosome	Top 1% outliers	Chromosome	Top 1% outliers	Chromosome	Top 1% outliers
1	27	1	100	17	4
2	45	2	74	20	18
3	67	3	84	21	1
4	3	4	48	22	5
6	2	5	32	23	1
10	30	6	9	24	4
12	20	7	3	27	1
13	58	9	7	28	1
18	37	11	5	Z	602
26	43	12	15		
27	6	13	5		
Z	697	14	16		

Pairwise comparisons are between the parental groups of (A) migratory Allen's hummingbird and rufous hummingbird, where 67% of outliers are on the Z chromosome, and (B) migratory and non-migratory Allen's hummingbird, where 58% of outliers are on the Z chromosome.

hummingbird is the outgroup) and 2 (where Allen's hummingbird is a hybrid taxon of rufous and non-migratory Allen's hummingbird, and Calliope hummingbird is the outgroup) were supported more than Scenario 3. Of the 4 models of speciation we tested, Scenario 4, where migratory and non-migratory Allen's form a single panmictic population, rufous

hummingbird is Allen's hummingbird's sister taxon, and Calliope hummingbird is the outgroup, received the most support (Fig. 2B). There was no significant difference between the likelihoods of Scenarios 1 and 2, which was likely due to the small number of differences across the genomes of migratory and non-migratory Allen's hummingbird identified here (Fig. 2C). In Scenario 4, the ancestor of Allen's and rufous hummingbird and the ancestor of Calliope hummingbird split from each other 1.32 million years ago, and Allen's diverged from rufous hummingbird 57,000 yr ago. Based on this model, non-migratory and migratory Allen's hummingbird form a single panmictic population (Fig. 2B).

Discussion

Demography and widespread gene flow

Simulations in Fastsimcoal2 from the best-fit model that incorporated non-admixed migratory Allen's hummingbird identified migratory and non-migratory Allen's hummingbird as a single panmictic population, with rufous hummingbird as the sister taxon, and Calliope hummingbird as the outgroup (Scenario 4; Fig. 2B). Furthermore, across all models, a model of gene flow throughout the evolutionary histories of Allen's and rufous hummingbird was best supported. We also identified populations of parental migratory Allen's hummingbird in San Luis Obispo County and Monterey County that had no introgression with rufous (Fig. 3). Thus, increased sampling in the current study clarified previously conflicting phylogenetic relationships by leveraging increased and more widespread sampling. Our results suggest that broad-scale phylogenetic studies may lead to incongruent results when few individuals and/or loci per taxon are sampled (McGuire et al. 2014; Licona-Vera and Ornelas 2017; Battey 2020; Godwin et al. 2020).

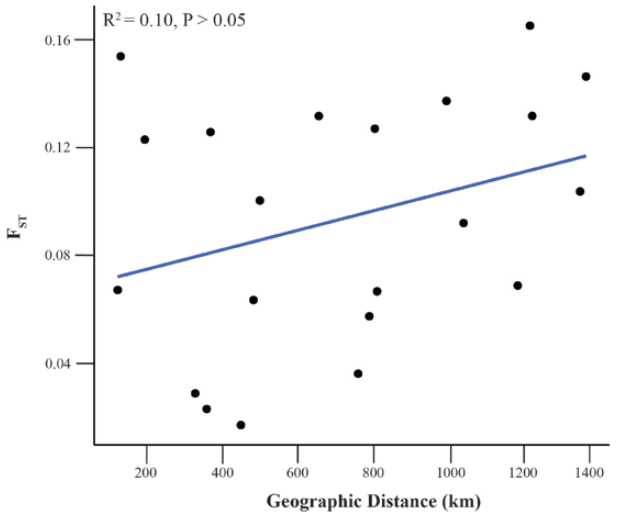

Fig. 6. The pairwise relationship between genetic distance (F_{ST}) of the 7 admixed + parental groups and geographic distance shows isolation by distance. Pairwise distances of the 7 admixed + parental groups were plotted based on average distances of the sampled localities within 1 group from the average distances of the sampled localities in the other. Generally, F_{ST} increased with geographic distance, although the association was weak and non-significant ($R^2 = 0.10$, $P > 0.05$).

Because hybridization affects phylogenetic inference, sampling schemes for studies investigating evolutionary history should sample a variety of populations within a given species to best account for gene flow before making evolutionary inference (McDade 1992; Leaché et al. 2014; McVay et al. 2017). Our results highlight that differential sampling could be an overlooked source of topological incongruence among phylogenetic studies with conflicting results. Because of our more extensive sampling, we posit that the most accurate result to date describing the phylogenetic relationships of Allen's, rufous, and Calliope hummingbird is from our demographic analysis that incorporated parental individuals that indicated no recent admixture, where rufous hummingbird is the sister taxon to Allen's hummingbird, and Calliope hummingbird is the outgroup (Scenario 4). It is likely that widespread, recent gene flow and inadvertent sampling of a recently admixed migratory Allen's population from the San Francisco Bay area in previous work, which our results indicate have up to 40% admixture with rufous hummingbird (Fig. 3), led to the discrepancies observed in the current study (Fig. 2; McGuire et al. 2014; Battey 2020; Godwin et al. 2020).

The best-fit scenario, Scenario 4, did conflict with the population structure we observed. Migratory and non-migratory Allen's hummingbird formed distinct clusters at $K = 3$, while the best-fit model in Fastsimcoal2 supported migratory and non-migratory Allen's hummingbird as a single panmictic population. Population structure analyses can detect weak genetic structure that may not be detectable using phylogenetic and/or demographic inference (see Themudo et al. 2020 for an example). Thus, Fastsimcoal2 may have less power to detect the same fine-scale differences between the 2 subspecies of Allen's hummingbird that are found in PCA and NGSadmix in this study. Alternatively, the true evolutionary

Table 2. F_{ST} values (below diagonal) average geographic distance (km; above diagonal), between groups in the admixed + parental group.

	1	2	3	4	5	6	7
1		122	187	364	656	1,000	1,225
2	0.153		114	497	807	1,231	1,376
3	0.113	0.067		480	813	1,192	1,360
4	0.126	0.100	0.064		323	762	1,046
5	0.132	0.127	0.067	0.029		446	792
6	0.137	0.132	0.069	0.036	0.017		354
7	0.165	0.146	0.104	0.092	0.058	0.023	

Average distance was calculated based on the average distance of the sampled localities between 2 groups. (1) northern Santa Cruz Island non-migratory Allen's, (2) Southern Channel Island non-migratory Allen's, (3) mainland non-migratory Allen's, (4) southern migratory Allen's, (5) northern migratory Allen's, (6) individuals in the migratory Allen's × rufous hybrid zone, and (7) rufous hummingbird.

history of Allen's hummingbird may be so complex that it is not accurately explained by any of the 4 models we tested in Fastsimcoal2, although Scenario 4 was best-fit model among the models we did test.

Genomic variation exhibited a clinal pattern (Fig. 3A). Samples from northern California (including the San Francisco Bay area) showed extensive introgression from rufous, samples from Monterey County showed no introgression with rufous, and samples from San Luis Obispo County showed no introgression from rufous, with minimal admixture from non-migratory Allen's hummingbird. Populations north of San Luis Obispo County exhibited admixture levels from non-migratory Allen's hummingbird at 0% (Fig. 3A). Thus, the observed clinal variation showed that the signature of non-migratory Allen's and rufous alleles on the genome of migratory Allen's varied by geography.

The Z chromosome resisted much of the clinal variation exhibited by the rest of the genome in migratory Allen's hummingbird (Fig. 3B). Furthermore, most F_{ST} outliers between Allen's and rufous hummingbird resided on the Z chromosome, indicating this sex chromosome is an important contributor to reproductive isolation between these 2 species (Fig. 5 and Table 1). The Z chromosome may also be an important contributor to reproductive isolation between migratory and non-migratory Allen's hummingbird, as more than half of F_{ST} outliers between them were also on the Z chromosome, and population structure was stronger between migratory and non-migratory Allen's hummingbird on the Z chromosome (Fig. 5 and Table 1). When interspecific hybridization occurs, loci associated with reproductive isolation do not tend to pass beyond regions of hybridization (Cortés-Ortiz et al. 2019). We observed a similar pattern here: within migratory Allen's and rufous hummingbird (and migratory and non-migratory Allen's hummingbird), the observed patterns suggested that the Z chromosome makes a disproportionate contribution to reproductive isolation between these species (Battey 2020; Henderson and Brelsford 2020).

Intergradation within Allen's hummingbird

Between migratory and non-migratory Allen's hummingbird, the inclusion of widespread sampling identified fine-scale patterns across the landscape rather than the presence of a hybrid swarm (Godwin et al. 2020). We detected a zone of intergradation between migratory and non-migratory Allen's hummingbird in southern California, but we did not find evidence of a hybrid swarm (Fig. 3). Sampling across the geographic ranges of both subspecies of Allen's hummingbird showed clinal variation in admixture between non-migratory and migratory Allen's hummingbird on the southern California mainland, with less admixture or a lack of admixture present in San Diego and Riverside County individuals, the sampling localities furthest from the range of migratory Allen's hummingbird. We also report lower overall levels of admixture overall on the southern California mainland than prior work (Godwin et al. 2020; Fig. 3).

Ghost admixture, where the genetic signature of an unsampled taxon is present within a dataset, likely influenced the results of Godwin et al. (2020) because rufous hummingbird was not included in their analyses (Lawson et al. 2018; Garcia-Erill and Albrechtsen 2020). Thus, the population structure Godwin et al. (2020) reported may have been affected: a cluster of northern California migratory Allen's, a cluster in southern California comprised of entirely non-migratory × migratory Allen's individuals, and a cluster of southern Channel Island individuals. Their reported northern California population presumably would have clustered with rufous hummingbird, had samples from rufous hummingbird been included in their study.

Inference into the intergradation zone

Clark (2017) hypothesized that, as the non-migratory Allen's hummingbird range expanded, it came into contact with the migratory Allen's hummingbird at the historic southern range limit of migratory Allen's hummingbird, which Grinnell and Miller (1944) thought was somewhere in the vicinity of the border between Santa Barbara and Ventura counties. The data presented here support this hypothesis, where intergradation likely initiated in Santa Barbara and Ventura counties (Fig. 3A). It appears that this zone of intergradation has expanded west along the coast, throughout Santa Barbara County, and south, into Los Angeles County (Fig. 3A). Given the rapid, recent range expansion of non-migratory Allen's hummingbird, and an average rate of expansion of the intergradation zone 3.6 km/yr up the coast and 5.0 km/yr down the coast, we predict that the zone of intergradation will expand further north, possibly into San Luis Obispo County, and inland, into Riverside County, in the near future.

In species with genetically distinct island and mainland populations, dispersal can occur in either direction (Sofaer et al. 2012; Mason et al. 2014; Hanna et al. 2019). Furthermore, when a mainland colonization is recent, as is the case with non-migratory Allen's hummingbird, individuals on the mainland are expected to be genetically similar to island populations. Consistent with the findings of Myers et al. (2021), despite evidence of recent admixture with migratory Allen's hummingbird, we found that Santa Cruz Island is the most genetically isolated from all other non-migratory Allen's hummingbird groups (Fig. 4 and Table 1), in congruence with other bird studies on the Channel Islands (Ashley and Willis 1987; Caballero and Ashley 2011; Sofaer et al. 2012; Walsh 2015; Wilson et al. 2015; Hanna et al. 2019). Furthermore, the relative similarity of the southern Channel Islands and the mainland may be an artifact of the founding mainland population. Because the mainland population was recently colonized by a population from the southern Channel Islands, the genomic signature of the southern islands remains on the mainland.

Conclusion

We demonstrate the importance of sampling design and accounting for hybridization when performing evolutionary inference. Accounting for gene flow across the study system, demographic analyses led to the result that migratory and non-migratory Allen's hummingbird form a single panmictic population, that rufous hummingbird is Allen's hummingbird's sister taxon, and Calliope hummingbird is the outgroup. We also identify a zone of intergradation (rather than a hybrid swarm) between migratory and non-migratory Allen's hummingbird in southern California (Fig. 3). Here, we come to different conclusions than a prior study of non-migratory Allen's and migratory Allen's hummingbird (Godwin et al. 2020), and previous work on the evolutionary relationships of Allen's and rufous hummingbird (McGuire et al. 2014;

Battey 2020), likely because these previous studies did not sample critical populations.

Supplementary material

Supplementary material is available at *Journal of Heredity* online.

Acknowledgments

We are grateful to Zachary Williams, Lisa Tell, CJ Ralph, and David Rankin for assistance in the field, Elisa Henderson, Nadje Najar, Dylan Zubieta, and Lily Hollingsworth for their contributions in preparing the dataset, and Elizabeth Scordato and Nicholas Mason for helpful insight in evaluating historical hypotheses with this complex dataset. We thank the curators and collectors at the institutions that provided tissues, in particular Philip Unitt at the San Diego Natural History Museum, Mike Van Patten at the San Diego State University Museum of Biodiversity, Rauri Bowie and Carla Cicero at the Museum of Vertebrate Zoology, John Klicka at the Burke Museum, Christopher Witt at the Museum of Southwestern Biology, and Jack Dumbacher and Moe Flannery at the California Academy of Sciences. We thank David Payne and Sam Cuenca, Bree Yednock and Angela Doroff at the South Slough National Estuarine Research Reserve, Sara Griffith at Oregon State Parks, California State Parks, Nita Barve, Amber Transou, and Carol Wilson at California State Parks, the California Department of Fish and Wildlife, the Oregon Department of Fish and Wildlife, Randi Lisle, and the United States Fish and Wildlife Service.

Funding

Funding was provided by the Society of Integrative and Comparative Biology, the American Ornithological Society, the Los Angeles Audubon Society, the Pasadena Audubon Society, the American Museum of Natural History, and the National Science Foundation (IOS-1656867 and IOS-1656708). We also thank the California Doctoral Incentive Program, the Crouch Scholarship for Avian Behavior, the James and Mary Crouch Memorial Scholarship, and the San Diego State University Graduate Fellowship.

Conflict of interest

None declared.

Author contributions

B.M.M. collected and analyzed data, designed and performed the research, obtained funding, and wrote the paper. C.J.C. obtained funding, collected data, supervised research, and contributed to writing. K.J.B. obtained funding, designed the research, supervised research, and contributed to writing. A.B. obtained funding, collected and analyzed data, designed and performed research, and supervised research.

Data availability

Location data where each sample was collected and voucher numbers for samples donated by museums are available in the supplemental material. The Anna's hummingbird reference genome is available on the NCBI database (accession number GCA_003957555.2). All original genomic data used in this study are available on Dryad.

References

Ashley M, Willis C. Analysis of mitochondrial DNA polymorphisms among Channel Island deer mice. *Evolution*. 1987;41(4):854–863.

Battey CJ. Evidence of linked selection on the Z chromosome of hybridizing hummingbirds. *Evolution*. 2020;74(4):725–739. doi:10.1111/evo.13888

Baym M, Kryazhimskiy S, Lieberman TD, Chung H, Desai MM, Kishony R. Inexpensive multiplexed library preparation for megabase-sized genomes. *PLoS One*. 2015;10(6):e0128036.

Caballero IC, Ashley MV. Genetic analysis of the endemic island loggerhead shrike, *Lanius ludovicianus anthonyi*. *Genetics*. 2011;12:1485–1493.

Clark CJ. eBird records show substantial growth of the Allen's hummingbird (*Selasphorus sasin sedentarius*) population in urban Southern California. *Condor*. 2017;119(1):122–130.

Cortés-Ortiz L, Baiz MD, Hermida-Lagunes J, García-Orduña F, Rangel-Negrín A, Kitchen J, Bergman TJ, Dias PAD, Canales-Espinosa D. Reduced introgression of sex chromosome markers in the Mexican howler monkey (*Alouatta palliata* × *A. pigra*) hybrid zone. *Int J Primatol*. 2019;40:114–131.

Da Cruz Rodrigues L, Ibanez Martins F, Rodrigues M. Survival of a mountaintop hummingbird, the hyacinth visorbearer, *Augastes scutatus*, in southeastern Brazil. *Acta Ornithol*. 2013;48(2):211–219.

Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo E, Handsaker RE, Lunter G, Marth GT, Sherry ST, et al. The variant call format and VCFtools. *Bioinformatics*. 2011;27(15):2156–2158.

Danecek P, Bonfield JK, Liddle J, Marshall J, Ohan V, Pollard MO, Whitwham A, Keane T, McCarthy SA, Davies RM, et al. Twelve years of SAMtools and BCFtools. *GigaScience*. 2021;10(2):1–4.

Erickson RA. Allen's hummingbird nests in Mexico: expansion of *Selasphorus sasin sedentarius* into Baja California. *West Birds*. 2016;47:161–166.

Excoffier LG, Marchi N, Marques DA, Matthew-Doret R, Gouy A, Sousa VC. Fastsimcoal2: demographic inference under complex evolutionary scenarios. *Bioinformatics*. 2021;37(24):4882–4885.

Garcia-Erill G, Albrechtsen A. Evaluation of model fit of inferred admixture proportions. *Mol Ecol Resour*. 2020;20(4):936–949.

Godwin BL, LaCava MEF, Mendelsohn B, Gagne RB, Gustafson KD, Love-Stowell SM, Engilis J, Tell LA, Ernest HB. Novel hybrid finds a peri-urban niche: Allen's hummingbirds in southern California. *Conserv Genet*. 2020;21:989–998.

Grinnell J. Bibliography of California ornithology. Third installment. *Pac Coast Avifauna*. 1939;26. https://sora.unm.edu/sites/default/files/journals/pca/pca_026.pdf

Grinnell J, Miller AH. The distribution of the birds of California. *Pac Coast Avifauna*. 1944;27. https://sora.unm.edu/sites/default/files/journals/pca/pca_027.pdf

Hanna ZR, Cicero C, Bowie R. Molecular evidence that the Channel Islands populations of the orange-crowned warbler (*Oreothlypis celata*; Aves: Passeriformes: Parulidae) represent a distinct evolutionary lineage. *PeerJ*. 2019;7:e7388. doi:10.7717/peerj.7388

Henderson EC, Brelsford A. Genomic differentiation across the speciation continuum in three hummingbird species pairs. *BMC Evol Biol*. 2020;20(1):1–11.

Hilton B, Miller MW. Annual survival and recruitment in a ruby-throated hummingbird population, excluding the effect of transient individuals. *Condor*. 2003;105(1):54–62.

Holderegger R, Kamm U, Gugerli F. Adaptive vs. neutral genetic diversity: implications for landscape genetics. *Landsc Ecol*. 2006;21:797–807.

Hudson RR. Testing the constant-rate neutral allele model with protein sequence data. *Evolution*. 1983;37:203–217.
Johnson WE, Onorato DP, Roelke ME, Land ED, Cunningham M, Belden RC, McBride R, Jansen D, Lotz M, Shindle D, et al. Genetic restoration of the Florida panther. *Science*. 2010;329(5999):1641–1645.
Kassambara A. Ggpubr: 'ggplot2'-based publication-ready plots. R package version 0.2.5. 2020. https://cran.r-project.org/web/packages/ggpubr/index.html [accessed 2023 Jun 30].
Korneliussen TS, Albrechtsen A, Nielsen R. ANGSD: analysis of next generation sequencing data. *BMC Bioinformatics*. 2014;15(1):1–13.
Lande R, Engen S, Saether BE. *Stochastic population dynamics in ecology and conservation*. Oxford (UK): Oxford University Press; 2003.
Lawson DJ, van Dorp L, Falush D. A tutorial on how not to over-interpret STRUCTURE and ADMIXTURE bar plots. *Nat Commun*. 2018;9(1):1–11.
Leaché AD, Harris RB, Rannala B, Yang Z. The influence of gene flow on species tree estimation: a simulation study. *Syst Biol*. 2014;63(1):17–30.
Leaché AD, Rannala B. The accuracy of species tree estimation under simulation: a comparison of methods. *Syst Biol*. 2011;60(2):126–137.
Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*. 2009;25(14):1754–1760.
Li H, Handsaker B, Wysoker A, Fennel T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R, 1000 Genome Project Data Processing Subgroup. The sequence alignment/map format and SAMtools. *Bioinformatics*. 2009;25(16):2078–2079.
Licona-Vera Y, Ornelas JF. The conquering of North America: dated phylogenetic and biogeographic inference of migratory behavior in bee hummingbirds. *BMC Evol Biol*. 2017;17(1):126–142.
Maddison WP, Knowles LL. Inferring phylogeny despite incomplete lineage sorting. *Syst Biol*. 2006;55(1):21–30.
Mason NA, Title PO, Cicero C, Burns KJ, Bowie RCK. Genetic variation among western populations of the horned lark (*Eremophila alpestris*) indicates recent colonization of the Channel Islands off southern California, mainland-bound dispersal, and postglacial range shifts. *Auk*. 2014;131(2):162–174.
McCormack JE, Huang H, Knowles LL. Maximum likelihood estimates of species trees: how accuracy of phylogenetic inference depends upon the divergence history and sampling design. *Syst Biol*. 2009;58(5):501–508.
McDade LA. Hybrids and phylogenetic systematics II. The impact of hybrids on cladistic analysis. *Evolution*. 1992;46(5):1329–1346.
McGuire JA, Witt CC, Remsen JV, Corl A, Rabosky DL, Altshuler DL, Dudley R. Molecular phylogenetics and the diversification of hummingbirds. *Curr Biol*. 2014;24(8):910–916.
McVay JD, Hipp AL, Manos PS. A genetic legacy of introgression confounds phylogeny and biogeography in oaks. *Proc R Soc B*. 2017;284(1854):20170300.
Meisner J, Albrechtsen A. Inferring population structure and admixture proportions in low-depth NGS data. *Genetics*. 2018;210(2):719–731.
Myers BM, Burns KJ, Clark CJ, Brelsford A. The population genetics of non-migratory Allen's Hummingbird (*Selasphorus sasin sedentarius*) following a recent mainland colonization. *Ecol Evol*. 2021;11(4):1850–1865.
Myers BM, Rankin DT, Burns KJ, Clark CJ. Behavioral and morphological evidence of an Allen's × rufous hummingbird (*Selasphorus sasin* × *S. rufus*) hybrid zone in southern Oregon and northern California. *Auk*. 2019;136(4):1–24. doi: 10.1093/auk/ukz049
NCBI Resource Coordinators. Database resources of the National Center for Biotechnology Information. *Nucleic Acids Res*. 2018;46:D8–D13.
Ornelas JF, Gonzalez C, Hernandez-Banos BE, Garcia-Moreno J. Molecular and iridescent feather reflectance data reveal recent genetic diversification and phenotypic differentiation in a cloud forest hummingbird. *Ecol Evol*. 2016;6(4):1194–1127.
Pyle P. *Identification guide to North American Birds, Part I: Columbidae to Ploceidae*. Bolinas (CA): Slate Creek Press; 1997.
R Core Team. R: a language and environment for statistical computing. Vienna (Austria): R Foundation for Statistical Computing; 2018. http://www.R-project.org/ [accessed 2023 Jun 30].
R Studio Team. RStudio: integrated development for R. Boston (MA): RStudio, Inc.; 2019. http://www.rstudio.com/ [accessed 2023 Jun 30].
Rannala B, Yang Z. Phylogenetic inference using whole genomes. *Ann Rev Genom Hum Genet*. 2008;9:217–231.
Rhie A, McCarthy SA, Fedrigo O, Damas J, Formenti G, Koren S, Uliano-Silva M, Chow W, Fungtammasan A, Kim J, et al. Towards complete and error-free genome assemblies of all vertebrate species. *Nature*. 2021;592:737–746.
Roberts DG, Gray CA, West RJ, Ayre DJ. Marine genetic swamping: hybrids replace an obligately estuarine fish. *Mol Ecol*. 2010;19(3):508–520.
Ruiz-Gutiérrez V, Doherty PF, Santana E, Contreras-Martínez S, Schondube J, Verdugo-Munguía H. Survival of resident neotropical birds: considerations for sampling and analysis based on 20 years of bird-banding efforts in Mexico. *Auk*. 2012;129(3):500–509.
Slatkin M, Maddison WP. A cladistic measure of gene flow inferred from the phylogeny of alleles. *Genetics*. 1989;123(3):603–613.
Smeds L, Qvarnström A, Ellegren H. Direct estimate of the rate of germline mutation in a bird. *Genome Res*. 2016;26(9):1211–1218.
Sofaer HR, Sillett TS, Peluc SL, Morrison SA, Ghalambor CK. Differential effects of food availability and nest predation risk on avian reproductive strategies. *Behav Ecol*. 2012;24(3):698–707.
Stiles FG. Age and sex determination in rufous and Allen hummingbirds. *Condor*. 1972;74(1):25–32.
Tajima F. Evolutionary relationships of DNA sequences in finite populations. *Genetics*. 1983;105(2):437–460.
Tell LA, Hazlehurst J, Bandivadekar RR, Brown J, Spence A, Powers DR, Agnew DW, Woods LW, Engilis A. *Welfare conscious study techniques for live hummingbirds and processing of hummingbird specimens*, Vol. 76. Lubbock (TX): Texas Tech University Natural Science Research Laboratory; 2021. p. 1–84.
Themudo GE, Rey-Iglesia A, Tascón LR, Jensen AB, da Fonseca RR, Campos PF. Declining genetic diversity of European honeybees along the twentieth century. *Sci Rep*. 2020;10(1):10520.
Unitt P. *San Diego county bird atlas*. San Diego (CA): Proceedings of the San Diego Society of Natural History, Vol. 39; 2004.
Walsh SE. *Genetic and phenotypic divergence of the Spotted Towhee (Pipilo maculatus) on the California Channel Islands* [master's thesis]. San Diego State University. San Diego (CA): Montezuma Publishing; 2015.
Wells S, Baptista LF. Breeding of Allen's hummingbird (*Selasphorus sasin sedentarius*) on the southern California mainland. *West Birds*. 1979;10:83–85.
Whiteley AR, Fitzpatrick SW, Funk WC, Tallmon DA. Genetic rescue to the rescue. *Trends Ecol Evol*. 2015;30(1):42–49.
Wickham H, Averick M, Bryan J, Chang W, McGowan LD, Francois R, Grolemund G, Hayes A, Henry L, Hester J. Welcome to the tidyverse. *J Open Source Softw*. 2019;4(43):1686–1691.
Wilson A, Chan Y, Taylor SS, Arcese P. Genetic divergence of an avian endemic on the Californian Channel Islands. *PLoS One*. 2015;10(8):p.e0134471. doi:10.1371/journal.pone.0134471
Witte F, Seehausen O, Wanink JH, Kishe-Machumu MA, Rensing M, Goldschmidt T. Cichlid species diversity in naturally and anthropogenically turbid habitats of Lake Victoria, East Africa. *Aquat Sci*. 2013;75:169–183.