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RESEARCH ARTICLE

Novel hybrid finds a peri-urban niche: Allen's Hummingbirds in southern California

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Abstract

Species range expansions and contractions can have ecological and genetic consequences, and thus are important areas of study for conservation. Hybridization and introgression are not uncommon in closely related populations that experience secondary contact during a range expansion. Allen's Hummingbird (*Selasphorus sasin*) in California comprises two subspecies: the migratory *S. s. sasin,* which winters in central Mexico and breeds in central and northern California, and the resident *S. s. sedentarius*, which lives and breeds year-round on several of the Channel Islands off the California coast. Within recent decades, Allen's Hummingbirds have been found living and breeding year-round in the southern California peri-urban mainland near Los Angeles. Ornithologists assumed that the L.A. birds were an expansion of the island subspecies, *S. s. sedentarius* due to similar but very subtle morphological characteristics. However, the genetic relationships among the three putative populations of Allen's hummingbird—migratory, southern California mainland, and island—are unknown. We investigated these relationships by analyzing variation of single nucleotide polymorphisms from the three geographic regions where *S. sasin* are present. Our population genomic analyses indicate that *S. sasin* hummingbirds inhabiting mainland southern California are a hybrid population resulting from admixture between *S. s. sasin* and *S. s. sedentarius*. From one perspective, these results may be interpreted as a positive development for *S. s. sasin* as the growing population represent an overall increase in the *S. sasin* population, and the expanding population contains a significant representation of *S. s. sasin* alleles.

Keywords Avian genetics · Conservation · Hybridization · Population genomics · Range expansion · *Selasphorus sasin* · Single-nucleotide polymorphism · Subspecies

Introduction

Unlike commonly known human-mediated hybridizations which often produce infertile offspring, many hybrids of wild species produce fertile offspring and those offspring

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may have high adaptive potential (Mallet 1995; Arnold 1997; Hamilton and Miller 2016). Hybridization (the interbreeding of individuals from two distinct populations or groups) and introgression (incorporation of alleles from one group of organisms into another) are not uncommon in closely related groups. Recent research has shown many evolutionary relationships to more closely resemble a "web of life" with frequent introgression and backcrossing between groups, rather than more isolated speciation events represented by a "tree of life" model (Arnold 2016). Genomic technology increasingly allows researchers to identify cryptic hybrid groups even though the current U.S. Endangered Species Act does not have clear language regarding hybrid populations (vonHoldt et al. 2018). Many hybrid zones are recent occurrences driven by climate and environmental change and other human involvements (Muhlfeld et al. 2014; Todesco et al. 2016).

A major driver of contemporary changes in environments is land conversion by humans (Hansen et al. 2001; McKinney 2002). Reconciliation ecology acknowledges the relevance of new and novel ecosystems, many of which may have been irreversibly changed by humans via modifications to abiotic conditions or biotic compositions (Fox 2007; Seastedt et al. 2008; Hobbs et al. 2009). Thus, the anthropogenic landscape impacts wildlife community composition and the phenology, abundances, and distributions of species (Walther et al. 2002; Tylianakis et al. 2008). In novel systems, expansion or retractions of species' ranges can have ecological and genetic consequences, and thus are important areas of study for ecology and conservation. Even closely related taxa may respond to environmental changes differently, depending on their niche and habitat type (Jetz et al. 2007). In particular, land-use and climate changes are causing previously isolated groups to come into contact (Allendorf et al. 2001; Bellard et al. 2013; Muhlfeld et al. 2014). When these taxa are difficult to differentiate based on phenotype, genomic tools can be useful to study how groups of organisms are interacting in novel landscapes and habitats.

The Allen's Hummingbird (*Selasphorus sasin*) is listed as a species of conservation concern because of decreasing habitat along its coastal range in California by the US Fish and Wildlife Service (USFWS 2008), National Audubon Society's Birds and Climate Change Report (2015), and Wilsey et al. (2019). The species comprises two subspecies: the migratory subspecies *S. s. sasin* that winters in Mexico and breeds along the central and northern coast of California, and the resident subspecies *S. s. sedentarius* that lives on five of the Channel Islands off the coast of southern California (Grinnell and Miller 1944; Clark and Mitchell 2013) (Fig. 1). The breeding range *of S. s. sasin* is almost entirely within the state of California, spanning the coast between Santa Barbara County north to the extreme southwest corner

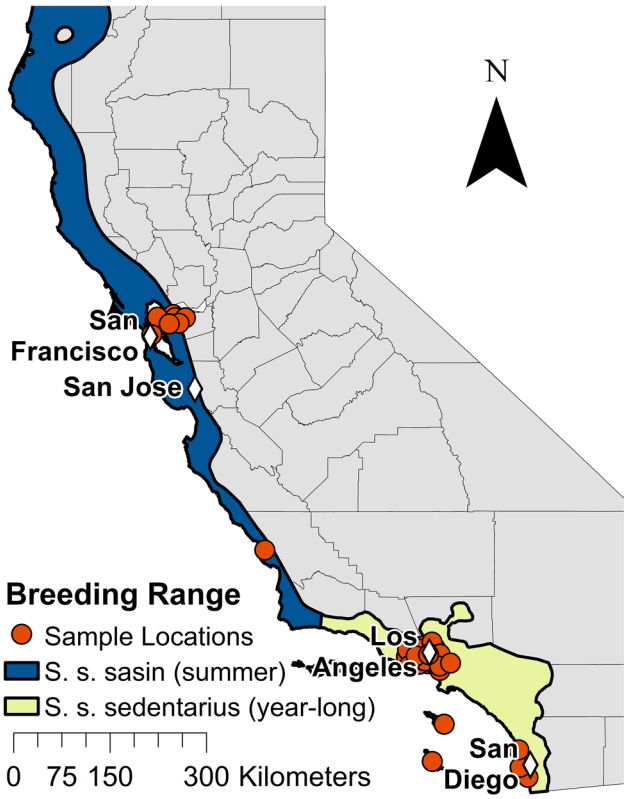

Fig. 1 Map of *Selasphorus sasin* range in California and locations of samples used in final population genomic analyses. Sample locations represent where both carcass and blood samples from live birds were collected. The range distribution for *S. s. sedentarius* is modified from Clark (2017).

of Oregon (Small 1994; Clark 2017). The first observations of Allen's Hummingbirds found that although migrants and vagrants were occasionally observed, there was no persistent population present in southern California (Grinnell and Miller 1944) and the authors know of no other observations or evidence to indicate the presence or absence of Allen's Hummingbirds in the region at any time before European colonization. An estimated time of divergence between the subspecies is also unknown. Many of the Channel Islands were connected to each other during the last glacial maximum, but the deep Santa Barbara Channel has prevented connection to mainland California (Rick et al. 2014).

In 1966, breeding Allen's Hummingbirds were reported on the Palos Verdes Peninsula in Los Angeles County, 36.7 km east of Santa Catalina Island (Wells and Baptista 1979). Banding studies, morphometric measurements, and observations of year-round breeding identified these birds as the resident subspecies *S. s. sedentarius* (Wells and Baptista 1979). Range expansion on the California mainland of resident *S. s. sedentarius* appears to have been rapid. Wells and Baptista (1979) reported a breeding pair of *S. s. sedentarius* in the Santa Monica Mountains, 67 km north of

the Palos Verdes Peninsula in 1966. Resident *S. s. sedentarius* established populations and expanded inland to the San Fernando and San Gabriel Valleys, and in 2005 were sighted in Riverside, CA (Clark 2017). As of 2017, resident *S. s. sedentarius* had an estimated range of over 26,000 km^2 between Santa Barbara (north), San Diego (south), and Riverside (east) in California (Fig. 1), where it was previously limited to 900 km^2 in the Channel Islands (Clark 2017). The rapid range expansion of resident *S. s. sedentarius* may have been facilitated by human activities including nectar provisioning via feeders and ornamental plants in gardens (DeSante and George 1994; Clark 2017) exploitable by these hummingbirds, which are considered an "urban-favoring" group (Cooper 2002).

Morphometric data and breeding behavior are compatible with the hypothesis that Allen's Hummingbirds breeding in southern California are resident *S. s. sedentarius* originating from the Channel Islands (Wells and Baptista 1979), but there is uncertainty. Consequently, there are three geographic groups of interest in our study (Fig. 1): (1) the Allen's Hummingbirds found within the range previously described for *S. s. sasin*, referred to herein as the "Northern California" (NC) group; (2) the Allen's Hummingbirds on the Channel Islands (ostensibly *S. s. sedentarius*, and the proposed source population for the southern mainland), and referred to herein as the "Channel Islands" (CI) group; and (3) the Allen's Hummingbirds in the greater Los Angeles region of mainland southern California (ostensibly *S. s. sedentarius*), referred to herein as the "Southern California" (SC) group.

In this study, we determined the genetic relationships among three regional groups of Allen's Hummingbird in California and assessed the hypothesis that the SC population is composed solely of *S. s. sedentarius* individuals. There are several biologically plausible explanations for the appearance of *S. sasin* hummingbirds in southern California. If the SC hummingbirds are solely of CI origin, there should be two distinct genetic clusters among these three groups: one genetic cluster combining the CI group and the SC group and another cluster consisting of NC individuals. It is plausible that the SC group might have the lowest genetic diversity metrics of the three groups due to founder effects resulting from recent colonization from the Channel Islands. If the SC hummingbirds originate from migratory NC hummingbirds truncating their migration, we would expect that the SC hummingbirds cluster with the NC group and show similar genetic diversity, with founder effects unlikely due to potential continuous influx of new *S. s. sasin* individuals into the population. If the SC group is admixed between or otherwise genetically differentiated from the two subspecies, we would expect to see the SC group cluster between the CI and NC groups to varying degrees and to potentially have the highest nucleotide diversity due to the combination of alleles previously isolated within the parental groups (Hedrick 2013). To test these alternative predictions, we used restriction site associated DNA sequencing to sample single nucleotide polymorphisms (SNPs) throughout the genome.

Methods

Sample collection and selection

Samples for DNA analysis were collected from three regions of California between 2004 and 2016 (Fig. 1). Samples from southern California were screened by collection time. Due to migration patterns confounding the presence of hummingbirds we did not use samples collected between 01 January and 15 March, and between 01 June and 31 August.

Carcass and tissue specimens were acquired through donations from citizens, museums, and wildlife rehabilitation centers following mortality (primary cause of mortality was window strikes). From each carcass, approximately 120 mg of tissue was sampled from the pectoral muscle for DNA extraction. Blood samples were collected from live birds in the field during banding procedures and ecological studies at sites in Malibu and Catalina Island, California in March of 2016. To capture hummingbirds, modified Hall drop-net traps (Russell and Russell 2001) were placed around busy hummingbird feeders. Birds were examined to assess health, identified to species, age, and sex using morphological measurements described in Russell and Russell (2001), and fitted with a federal ID leg-band provided by the North American Bird Banding Laboratory. Approximately ~ 5–20 µl, (< 1% body weight) of blood was collected by clipping < 0.5 mm of a distal end of a toenail (Owen 2011). Blood was collected on Whatman FTA® preservation paper (GE Healthcare UK Limited, Buckinghamshire, UK), Nobuto sampling strips (Advantec®, Toyo Roshi, Ltd., Japan), or into EDTA capillary tubes placed into 1.5 mL centrifuge tubes filled with 100% ethanol. After blood collection, silver nitrate was applied to the toenails to prevent further bleeding and the hummingbirds were offered sugar water and monitored before release. Samples collected on Whatman FTA® and Nobuto® papers were stored in desiccant-filled coin envelopes at room temperature away from light, heat, and humidity (Dusek et al. 2011). Ethanol samples were stored at − 80 °C. Federal, state, and university permits and animal care and use protocols were maintained during sample collection.

DNA preparation and sequencing

Samples were extracted with Qiagen DNeasy® Blood and Tissue Extraction kits (Qiagen®, Hilden, Germany) using

modified protocols for tissues and blood. Capillary blood tubes were ethanol-evaporated and placed in extraction tubes for digestion. Approximately 60 mg of tissue was used for each tissue extraction. Extracted DNA was prepared for Illumina® genomic sequencing (Illumina, Inc., San Diego, CA) by following protocols modified from Parchman et al. (2012). Briefly, the extracted DNA was digested using two restriction enzymes (*EcoRI* and *MseI*) to generate DNA fragments. These enzymes were chosen after conducting digestion trials on hummingbird DNA with several combinations of enzymes. Adapters containing Illumina® primers and unique barcodes were ligated to the fragments allowing for identification of individuals during later analyses. These barcoded fragments were then amplified using polymerase chain reaction (PCR), all individuals were pooled together, and cleaned and concentrated using Agencourt® AMPure® XP magnetic beads (Beckman Coulter, Inc., Brea, CA). The pooled DNA was then size-selected (350–450 base-pair fragments) using a Pippin Prep™ (Sage Science, Beverly, MA). This barcoded, cleaned, and size-selected DNA was sequenced on single-end 150 base-pair runs on an Illumina HiSeq® 4000 by the Genomics Sequencing and Analysis Facility at the University of Texas, Austin. See Supplementary Materials for more in-depth methodology.

Bioinformatics and data analysis

To identify SNPs from sequence reads, we followed methods modified from Parchman et al. (2012) and Mandeville et al. (2015). Briefly, we demultiplexed the file of sequence reads and identified individuals based on unique nucleotide barcodes from the adapters discussed above. Individuals with fewer than 10,000 sequence reads were excluded from further analyses. We used *dDocent* (Puritz et al. 2014) which utilizes CD-HIT (Li and Godzik 2006; Fu et al. 2012) and created a de novo reduced-representation reference genome using reads from the sequenced individuals (LaCava et al. 2019). We used *bwa* (Li and Durbin 2009) to align the individual reads to the reference genome. We identified single nucleotide polymorphisms and iteratively filtered SNP loci and individuals for quality, coverage, and percent of missing data, then exported the data for genomic analyses using SAMtools (Li et al. 2009) to choose the SNP dataset that maximized the number of SNPs and retained individuals. We calculated average read depth, or the number of times a specific locus appears, across all SNPs and individuals in the final dataset. All previous steps were conducted on the University of Wyoming's Teton Computing Environment (Advanced Research Computing Center 2018). Sequencing data is available at https://doi.org/10.5061/dryad.zgmsbcc84. For more details, see Supplementary Methods.

Population structure and diversity

We used a Principal Component Analysis (PCA) to visualize genetic clusters using a custom code (https://doi.org/10.5061/dryad.zgmsbcc84) in R (R Core Team 2017) that uses genetic covariance from genotype point estimates. This analysis outputs principal component vectors (PCs) that calculate variance among individuals.

We used STRUCTURE 2.3.4 with the admixture model (Pritchard et al. 2000) using StrAuto to parallelize the runs (Chhatre and Emerson 2017) to infer genetic population structure and genetic assignment. We ran a burn-in of 200,000 steps, followed by 1,000,000 Markov chain Monte Carlo (MCMC) steps to ensure model convergence (Pritchard et al. 2000). We ran 20 chains for each value of K = 1–5 (i.e., number of clusters). We used STRUCTURE HARVESTER 0.6.94 (Earl and vonHoldt 2012) and CLUMPAK (Kopelman et al. 2015) to compile the STRUCTURE results. We determined the most likely K by the Evanno method for delta K (ΔK) (Evanno et al. 2005) and the Pritchard maximum likelihood method (Pritchard et al. 2000).

Genetic distance between putative groups was assessed using a custom R script (https://doi.org/10.5061/dryad.zgmsbcc84) to calculate Hudson's F_{st} (Hudson et al. 1992) for all pairs of groups for all loci. Hudson's F_{st} provides a reliable measure of genetic distance and differentiation when analyzing groups with unequal sample sizes. We estimated nucleotide diversity for each group by calculating Watterson's theta (θ_w) (Watterson 1975) and theta pi (θ_π) (Tajima 1989) in the program ANGSD (Korneliussen et al. 2014). These estimates, respectively, use a standardized estimate of the number of segregating sites and the average number of heterozygous sites in a population sample.

Results

SNP dataset and genetic metrics

Our de novo genome combined 504,892 contigs from all sequenced individuals. The final filtered dataset retained 102 individuals (NC = 13, SC = 59, CI = 35). We identified 4,386 SNPs that met quality, coverage, missingness, outlier, and MAF criteria listed in the Supplemental Methods. All SNPs in the final dataset were present in at least 85% of the individuals, and each individual had information for at least 50% of SNPs. The average read depth across all individuals and all loci was 19.23.

Genetic variation and structure

Principal Components Analysis of pairwise genetic distances among individuals showed that 49.6% of the variation

in the SNPs could be explained with the first two principal components (Fig. 2). The three sampling regions show distinct separation from each other, with the CI group being approximately intermediate between the NC and southern SC groups along the first axis. The second axis showed less distinct separation among groups. These results are in accordance with the Structure K = 3 model.

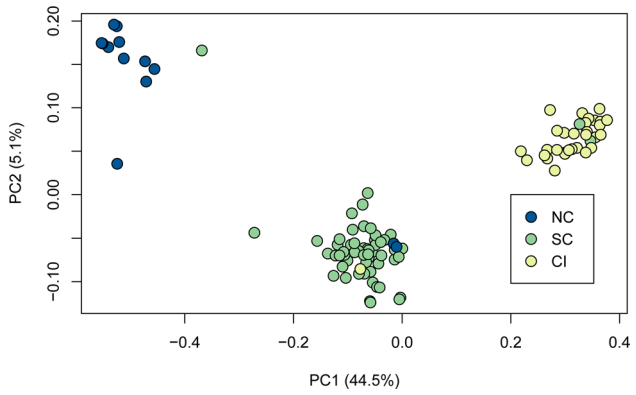

Fig. 2 Principal component analysis for 4836 SNPs from 102 *Selasphorus sasin* individuals collected from three different regions in California. Axis labels show percentage of variation explained. The southern California mainland group (SC) shows the greatest separation from the Channel Islands (CI) and the northern California (NC) groups along the first principal component axis. The position of the SC group suggests that the group is admixed between the other two groups (Patterson et al. 2006)

The results of Structure with admixture indicated that the most likely number of populations was 3 according to ΔK (Fig. 3). For the Ln(K) method (Pritchard et al. 2000), K values of 2, 3, and 4 had similar likelihoods with a K of 2 having marginally higher support. Structure plots for K = 2 show NC and CI predominately assigning to different clusters and SC is admixed or otherwise genetically intermediate between the two groups. At K = 3 and K = 4 all but three individuals sampled in SC predominately assign to a unique genetic cluster from NC and CI (Fig. 3). However, the evidence from K = 4 indicates that SC is a unique genetic cluster largely distinct from NC and CI (Fig. 3). Not all individuals clustered clearly with groups that reflect their sampling location in the Structure or pca results. These individuals are marked with asterisks in the Structure plots and are colored by sampling region in the PCA (Fig. 3).

The greatest genetic distance was between the CI and NC groups, with the SC group being of intermediate genetic distance between the other two (Table 1), which is consistent with the K = 2 Structure model (i.e., SC has on average ~ 50% assignment to each of the K = 2 genetic clusters). Nucleotide diversity, as measured by Watterson's theta (θ_w) (Watterson 1975) and theta pi (θ_π) (Tajima 1989), for the sampling regions was low in all groups (Table 2). In both analyses, the CI group showed the lowest diversity with 6×10^{-4} (θ_w) and 8×10^{-4} (θ_π) and the SC group showed the highest θ_w diversity with 2×10^{-3}. Diversity for NC and SC as measured by θ_π was approximately equal at 1×10^{-3}.

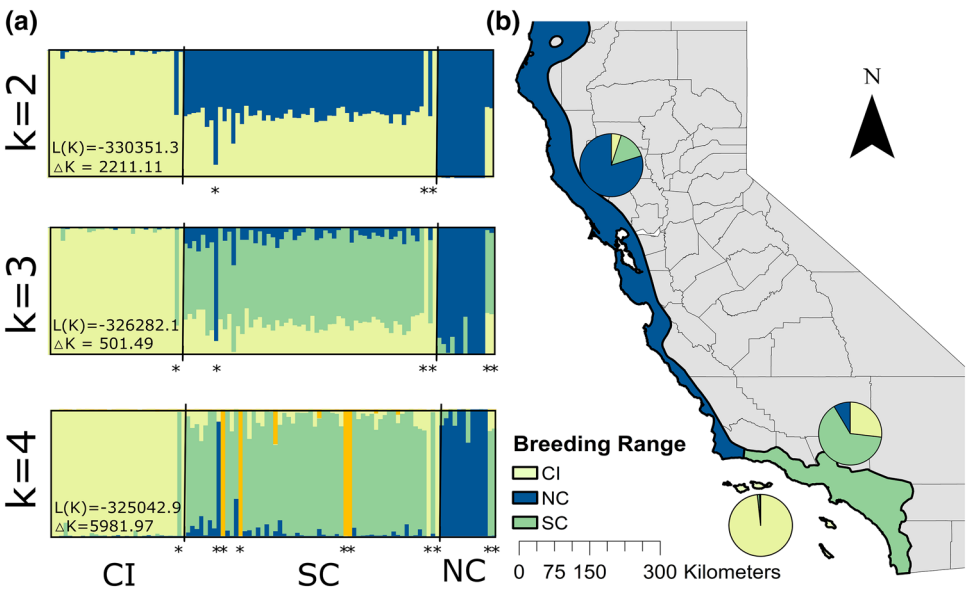

Fig. 3 **a** Structure results organized by sample location (Channel Islands, southern California mainland, and northern California) for K = 2–4. Individuals which cluster with a group outside of their sampling region are noted with an asterisk (*). **b** Range of map of *Selasphorus sasin* in California with proportional genomic composition based on Structure results. Likelihood analysis gives highest support for K = 2, though only slightly. Analysis of ΔK suggests K = 3. Ranges and genomic proportions are divided and colored to reflect clustering from the K = 3 Structure model in order to illustrate how the southern California mainland group is distinct from the others

Table 1 F_{st} estimates for the three *Selasphorus sasin* groups studied (Channel Islands = CI, southern California mainland = SC, northern California = NC)

	CI	SC
CI		
SC	0.06	
NC	0.14	0.07

The greatest F_{st} value is between the CI group and the NC group. The SC group shows lower and approximately equal F_{st} values to the other two groups

Table 2 Nucleotide diversity values for the three *Selasphorus sasin* groups studied (Channel Islands = CI, southern California mainland = SC, northern California = NC)

	θ_W	θ_π
CI	0.00058	0.00076
SC	0.00149	0.00111
NC	0.00127	0.00113

The CI group shows the lowest nucleotide diversity in both Watterson's theta (θ_W) and theta pi (θ_π) estimators. The SC groups shows the highest nucleotide diversity according to θ_W

Discussion

Multiple lines of evidence indicate that *S. sasin* individuals in the southern California mainland are a distinct genetic cluster, possibly resulting from admixture between northern California mainland and the Channel Islands groups. Although the Southern California group appears to share morphometric and behavioral traits with *S. s. sedentarius* (Wells and Baptista 1979; Clark 2017) and has been putatively classified as this subspecies, our population genomic analyses show that the sampled SC individuals do not cluster with CI individuals. If the SC individuals were an expanding subset of the CI population, we would have expected the SC and CI individuals to cluster together.

Based on the observed genetic structure this system could be interpreted reasonably in two ways. Either NC and CI are distinct genetic clusters and SC is a unique or third genetic cluster (K = 3) (Evanno et al. 2005), or that NC and CI are distinct genetic clusters with the third SC cluster arising from admixture (K = 2 with admixture). The NC and CI genetic clusters likely represent the published subspecies *S. s. sasin* and *S. s. sedentarius,* respectively. In PCAs, admixed individuals are often dispersed along a principal component axis and at intermediate distances between parentals groups (Ma and Amos 2012). Such dispersion along a principal component axis between groups is often interpreted as a measure of admixture (Patterson et al. 2006; Ma and Amos 2012). If we assume this, then SC individuals show a level of admixture between the NC and CI groups that creates a grouping separate but intermediate between them on PC1 where the majority of variation is explained (44.5%). That the SC individuals are clustered rather than scattered continuously throughout the parental NC and CI groups is indicative of an established hybrid population. Hudson's F_{st} (Table 1) showed the largest genetic distance between the CI and NC groups. The SC group had the highest nucleotide diversity as measured by Watterson's theta (θ_W) and theta pi (θ_π) (Table 2). This pattern differs from what we would expect from an expanding founder population with a single origin as described by Peter and Slatkin (2015). An expanding founder population with a single point of origin would likely exhibit lower nucleotide diversity than the parent population (Peter and Slatkin 2015; Shultz et al. 2016), in this case CI. Because SC instead has the highest nucleotide diversity, we infer that the SC population is a result of admixture between two previously isolated and differentiated groups (De La Torre et al. 2014; Bradburd et al. 2016; Hohenlohe et al. 2011; Twyford and Ennos 2012).

In both the STRUCTURE analysis and the PCA, several individuals cluster with groups outside of their sampling region (see "Results"). These individuals were not from outlier locations, but near geographic centers of the sampling regions. A few individuals clustering with groups outside of their sampling region is not surprising in an avian (highly vagile) study system. There is a report of an individual banded on Catalina Island and later found on the California mainland in the SC region *(unpublished data, H. Ernest)*. Two individuals in the K = 3 STRUCTURE model, and one of the same individuals in the PCA, sampled in the SC region were strongly clustered with the NC group. All individuals were collected outside of described migratory periods defined in the Methods to minimize sampling NC individuals migrating through the SC region. However, it is likely that these individuals were migratory *S. s. sasin* traveling south from the NC region outside of the typical migration seasons. Interestingly, two individuals collected in NC showed strong clustering with the SC group. It is possible that these individuals are back-crossed individuals with strong genetic assignment to the SC group, but have retained the migratory behavior of typical *S. s. sasin*. It is also possible that some individuals in the largely residential SC group are expanding farther north.

The likelihood analysis of the STRUCTURE results (Pritchard et al. 2000) indicates more support for K = 2 than K = 3 or 4 (Fig. 2a). However, the K = 4 model presents interesting results. In this model, the SC group is more distinctly differentiated and unique than in other models, and four Allen's Hummingbirds sampled in the SC region were differentiated enough to constitute the fourth cluster. A careful morphological review of specimens indicated that

they were not misidentified as the morphologically similar Rufous Hummingbirds (*S. rufus*). Given the support for K = 3 from the Evanno ΔK method, the PCA showing three distinct groups, and the similar results in likelihoods among K = 2, 3, and 4, we conclude that K = 2, with a third admixed group, is the most biologically plausible result. However, the four individuals in the fourth cluster in K = 4 are of interest and may justify future genomic investigation in this system.

The Allen's Hummingbird system in California is an informative example of newly arising hybrid zones (in this case beginning in the 1960′s). This study highlights the power of genomics to assess interbreeding between taxa that are cryptic or otherwise difficult to distinguish. Climate change and habitat disruption are causing shifts in the ranges of populations and new contact zones where hybridization can occur (Garroway et al. 2011; Muhlfeld et al. 2014). New hybrid zones have the potential for significant conservation importance for many species. For *S. sasin*, both subspecies and the admixed SC group likely perform similar ecological roles as pollinators, as implied by similar size and morphology (López-Segoviano et al. 2018). However, the expansion of the SC group is likely facilitated by anthropogenic land-use change where resources are provided by both native and non-native plants in gardens and artificial hummingbird feeders (Clark 2017).

This study illustrates that geographic location or morphology are imperfect indicators of genetic relatedness. The morphology of Allen's Hummingbirds in SC has been described as matching *S. s. sedentarius* rather than *S. s. sasin*, and the non-migratory behavior of SC individuals also has supported their being classified as *S. s. sedentarius* (Phillips 1975; Wells and Baptista 1979). However, the SC hummingbirds exhibit a distinct genomic signature, which does not support their assignment solely to the S. *s. sedentarius* subspecies. These results are an important reminder that migratory behavior in avian species is plastic and influenced by multiple factors beyond genetics and should not be a primary factor in identification (Zink 2011; Charmantier and Gienapp 2014; Van Doren et al. 2017). Similar results were found in the dusky seaside sparrow (*Ammodramus maritimus*), where researchers discovered that the most closely related groups were neither geographically closest nor most morphologically similar (Avise and Nelson 1989; Woltmann et al. 2014).

Secondary contact between two diverged taxa in nature without direct facilitation by humans, as appears to be the case in Allen's Hummingbirds, is known as "natural hybridization" (Allendorf et al. 2001; Genovart 2009). Hybridizations may conflict with some definitions of species (Mayr 1942), but natural hybridization is more common than is sometimes supposed (Mallet 2005) and plays an important role in the evolution and adaptation of species (Barton and Hewitt 1989; Seehausen 2004; Pfennig et al. 2016). Hybridization occurs in approximately a quarter of plant species and 10% of animal species and is especially common in more recently diverged taxa (Arnold 1997). In vertebrates, natural hybridization seems to be most common in birds and their frequent hybridization has historically fascinated naturalists (Grant and Grant 1992; McCarthy 2006; Ottenburghs et al. 2015). Mallards (*Anas platyrhynchos)* and the Ring-necked Pheasant (*Phasianus colchicus*), in particular, are known to hybridize with multiple other species (Wells et al. 2019; Ottenburghs 2019). Apodiformes (the family including hummingbirds and swifts) are also known to frequently hybridize, with approximately 19% of species having known hybridization events (Grant and Grant 1992). In mammals, a well-known example of natural hybridization is the expansion of coyotes (*Canis latrans*) out of the eastern United States in the last 100 years and subsequent hybridization with wolves (*C. lupus*), introducing wolf alleles into coyote populations throughout their historic range (vonHoldt et al. 2016; Hody and Kays 2018; Hinton et al. 2019). Natural hybridization creates some difficulties for conservation and management decisions because it complicates fundamental units commonly used in conservation law and practices.

We suggest future research to investigate potential genetic influences of migratory behavior in *S. sasin*. Several studies have begun to identify these genes in other avian species (Contina et al. 2018; Ralston et al. 2019), however migration behavior is the result of complex interactions involving physiological, behavioral, and genetic processes (Bowlin et al. 2010). If the migratory behavior is heavily influenced by genetics, then this hybrid complex could provide an informative example of hybridization causing rapid genetic and behavioral change allowing exploitation of a new anthropogenic habitat. Similarly, Eurasian blackcap hybrids gained some migratory behaviors (Berthold et al. 1990) after controlled hybridization. However, given the complex nature of migration it is likely that other factors, such as resource availability in southern California previously discussed, are also important in the behavior of these hummingbirds.

Our results could inform future conservation decisions because *S. s. sasin* is currently listed as a species of concern by the USFWS (2008) and habitat change in California from human population growth and climate change is likely to continue (Wilson et al. 2016). Additionally, increased wildfires along the California coastlands (Keeley and Syphard 2019) may cause changes to *S. s. sasin* habitat. The goal of conservation is to preserve biodiversity and the evolutionary processes that support it. Thus, hybridization stemming from anthropogenic causes (in this case land-use change) is often viewed as a threat to the conservation of species through genetic swamping and the extinction of pure parental genomes leading to the loss of local adaptations (Simberloff 1996; Allendorf et al. 2001; Todesco et al. 2016). This concern may be elevated as *S. s. sasin* has recently been

shown to be hybridizing with the sister species *S. rufus* at the northern edge of their range (Myers et al. 2019). Conversely, hybridization may provide increased adaptive potential (Seehausen 2004, 2013; Becker et al. 2013). Arguments have been made for attempting to preserve demographics rather than genetics (Lande 1988; Pimm et al. 2006). Because of the SC group, the overall population of *S. sasin* is increasing (Clark 2017) and this population possesses alleles of *S. s. sasin*. The southern California hybrid zone could act as a conservation reservoir for *S. s. sasin* alleles in the face of potentially declining abundance and potential maladaptive alleles introduced by S. rufus or as a beneficial introduction of new alleles from *S. s. sedentarius* to potentially help the declining *S. s. sasin* subspecies. The expanding population of Allen's Hummingbirds in southern California could be interpreted as a positive development as the overall population of the species appears to be increasing (Clark 2017) and alleles specific to *S. s. sasin* are remaining in the subspecies complex.

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Author contributions BLG and HBE developed the hypothesis and design and collected samples. LAT, AE, and HBE assisted in sample collection and provided expertise. HBE supervised the research. BLG, MEFL, BM, RBG, KDG, SMLS, AE, LAT, and HBE wrote or substantially contributed to editing the paper or specific analyses. BLG analyzed the data with assistance and/or guidance from all other authors.

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Data and code availability The datasets generated during this study will be available in Dryad data repository: https://doi.org/10.5061/dryad.zgmsbcc84.

Compliance with Ethical Standards

Conflict of interest Not applicable.

Ethical approval All procedures conformed to the animal care and use protocols approved by the University of Wyoming, the University of California, Davis, state and federal permitting requirements. University of California protocols for animal use and care: 15387, 16977, 18605. University of Wyoming protocol for animal use and care: 20150716HE00183. Federal Bird Banding permit: 23765.

Consent for publication All authors consent.

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