Received: 4 October 2022 | Revised: 28 February 2023 | Accepted: 8 March 2023

DOI: 10.1111/mec.16928

ORIGINAL ARTICLE

MOLECULAR ECOLOGY WILEY

Widespread gene flow following range expansion in Anna's Hummingbird

Nicole E. Adams[1] | Ruta R. Bandivadekar[2] | C. J. Battey[3] | Michael W. Clark[1] | Kevin Epperly[4,5] | Kristen Ruegg[6] | Lisa A. Tell[2] | Rachael A. Bay[1]

[1]Department of Evolution and Ecology, University of California, Davis, California, USA

[2]Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, California, USA

[3]Myriad Genetics, South San Francisco, California, USA

[4]Department of Biology, University of Washington, Seattle, Washington, USA

[5]Burke Museum of Natural History and Culture, Seattle, Washington, USA

[6]Department of Biology, Colorado State University, Fort Collins, Colorado, USA

Correspondence
Nicole E. Adams, Department of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor, MI, USA.
Email: nicoleadams.sci@gmail.com

Funding information
California Department of Transportation, Grant/Award Number: 69A3551747114; National Science Foundation, Grant/Award Number: 1837940

Handling Editor: Kimberly J. Gilbert

Abstract

Anthropogenic changes have altered the historical distributions of many North American taxa. As environments shift, ecological and evolutionary processes can combine in complex ways to either stimulate or inhibit range expansion. Here, we examined the role of evolution in a rapid range expansion whose ecological context has been well-documented, Anna's Hummingbird (*Calypte anna*). Previous studies have suggested that the *C. anna* range expansion is the result of an ecological release facilitated by human-mediated environmental changes, where access to new food sources have allowed further filling of the abiotic niche. We examined the role of gene flow and adaptation during range expansion from their native California breeding range, north into Canada and east into New Mexico and Texas, USA. Using low coverage whole genome sequencing we found high genetic diversity, low divergence, and little evidence of selection on the northern and eastern expansion fronts. Additionally, there are no clear barriers to gene flow across the native and expanded range. The lack of selective signals between core and expanded ranges could reflect (i) an absence of novel selection pressure in the expanded range (supporting the ecological release hypothesis), (ii) swamping of adaptive variation due to high gene flow, or (iii) limitations of genome scans for detecting small shifts in allele frequencies across many loci. Nevertheless, our results provide an example where strong selection is not apparent during a rapid, contemporary range shift.

KEYWORDS
adaptation, Anna's Hummingbird, genetic diversity, gene flow, range shift

1 | INTRODUCTION

The study of species' geographic limits encompasses some of the most fundamental processes in ecology and evolution including dispersal, gene flow, and adaptation. Species' ranges can be determined by abiotic factors such as precipitation, day length, and soil chemistry as well as by biotic factors such as population density, interspecific interactions, predator–prey relationships, and food resource availability (Cahill et al., 2014; Gaston, 2003; Louthan et al., 2015). Anthropogenic-induced changes in these factors can therefore result in rapid shifts in species abundance and distribution (Parmesan, 2006; Urban, 2015). For example, a staggering decline in North American bird abundance since 1970 has been documented and is potentially attributed to increased agriculture, urbanization, habitat loss, and climate change (Rosenberg et al., 2019). However, not all environmental shifts result in population decline — some

Lisa A. Tell and Rachael A. Bay contributed equally.

species have also been able to adapt to these changes (Hancock et al., 2011; Pespeni & Palumbi, 2013) or track optimal conditions, especially moving poleward and to higher elevation (Hitch & Leberg, 2007; La Sorte & Jetz, 2010). In fact, a number of North American birds appear to be shifting their ranges northward due in part to warmer temperatures and land use changes (Hitch & Leberg, 2007; Hovick et al., 2016; La Sorte & Thompson III, 2007; Princé & Zuckerberg, 2015). Contemporary shifts in species' ranges provide an opportunity to examine the factors defining range limits in real time.

While the ecological causes of range expansions are often well documented, evolutionary processes also contribute to changes in species' distributions. These processes include spatial sorting, natural selection, and genetic drift, which can act on species' ranges in intuitive ways. Spatial sorting followed by assortative mating among successful colonizers can shift phenotypic traits associated with expansion, such as dispersal abilities, which in turn can lead to further colonization (Cote et al., 2017; Travis et al., 2010; Weiss-Lehman et al., 2017). A good example of this is shown in multiple species of bush crickets, where frequencies of long-winged morphs, which have higher dispersal ability, are more common at the range front (Simmons & Thomas, 2004). Selection on life history traits that increase the reproductive rate can also promote range expansion, as can adaptation to novel environments (Andrade-Restrepo et al., 2019; Szűcs et al., 2017). Such is the case with the invasive shrub *Hypericum canariense*, which has evolved higher growth rates and local adaptation in flowering time (Dlugosch & Parker, 2008). Finally, strong genetic drift caused by small population sizes and serial bottlenecks at the range edge decreases genetic variation and limits the expansion potential (Excoffier, 2004; Excoffier et al., 2009).

While it is clear that spatial sorting, selection, and drift can each contribute to distributional limitations, theoretical and empirical literature also show us that these same evolutionary processes can have conflicting effects on range expansion outcomes depending on the context (Miller et al., 2020; Williams et al., 2019). For example, allele surfing, the fixation of alleles along an expansion front, can lead to greater expansion potential if the fixed alleles are beneficial, but the fixation of deleterious alleles can reduce fitness at the edge — a phenomenon known as expansion load — reducing the expansion potential (Klopfstein et al., 2006; Peischl et al., 2013; Peischl & Excoffier, 2015; Travis et al., 2007). Broadly, reduced gene flow (with gene flow defined as the movement of individuals and alleles) from the species' core to the range edge can decrease genetic diversity and thus adaptive potential. However, high gene flow can lead to either increased genetic diversity and higher evolutionary potential or a propagation of maladaptive alleles from the species core that can limit local adaptation at the edges (Bontrager & Angert, 2019; Eckert et al., 2008; Fedorka et al., 2012; García-Ramos & Kirkpatrick, 1997). Understanding and predicting the dynamics of range expansions therefore requires an understanding of the direction and magnitude of gene flow, levels of genetic diversity, and landscape of adaptive divergence across the species range paired with a knowledge of the ecological context in which the range expansion is occurring.

Foundational theoretical work has provided a framework for understanding the interplay between stochastic and deterministic forces in facilitating or hindering range expansion. In many cases, range expansions are expected to lead to higher divergence and lower genetic diversity at the expanding edge compared to the core due to small population sizes, serial bottlenecks, reduced gene flow, and selection pressures (Excoffier, 2004; Excoffier et al., 2009). This mixture of neutral and adaptive processes across the expansion axis often leads to spatial structuring and patterns of isolation by distance (Excoffier, 2004). Termed a "pulled wave", the founders at the range edge pull the expansion forward through increased dispersal and reproduction that stratifies demes (Miller et al., 2020). However, the opposite pattern of maintained/increased genetic variance at expansion fronts has also been reported in several empirical studies (Berthouly-Salazar et al., 2013; Bors et al., 2019; Vandepitte et al., 2017; Wang et al., 2017). Conceptual frameworks term these cases "pushed waves," where genetic variation is maintained at the range edge due to gene flow from the range core and potentially positive density dependence, novel interspecific competition, or environmental stress that result in less genetic sorting at the edge (Miller et al., 2020). These varying outcomes suggest that the interplay between neutral and selective evolutionary processes create variation in range expansion outcomes giving rise to more nuanced approaches to ecoevolutionary dynamics. (Miller et al., 2020; Williams et al., 2019).

A recent and dramatic range expansion in Anna's Hummingbird (*Calypte anna*) provides an ideal system to examine the evolutionary processes associated with rapid range expansion (Battey, 2019; Greig et al., 2017; Zimmerman, 1973). The historical breeding range of *C. anna* is central and southern California, USA and northwestern Mexico (Grinnell, 1915; Grinnell & Miller, 1944). By leveraging community science (Project FeederWatch, Christmas Bird Count) and museum data, previous studies showed a northern and eastern expansion of the breeding range starting around 1940 (Battey, 2019; Greig et al., 2017; Zimmerman, 1973). Currently, *C. anna* can be found breeding as far north as British Columbia, Canada, and southern Alaska, USA and as far east as Idaho, USA and western Texas, USA (Rudeen & Bassett, 2016; Zimmerman, 1973). Human habitation and climate change appear to be the drivers of the expansion. In the expanded ranges *C. anna* individuals were more likely to colonize areas with higher housing density and were more likely to visit bird feeders compared to those in the historical range (Greig et al., 2017). However, like many North American migratory birds, they may also experience mortality associated with urban settings such as window collisions and encounters with domesticated animals (Pandit et al., 2021). Increases in minimum winter temperatures were also shown to facilitate the expansion (Battey, 2019; Greig et al., 2017). However, Zimmerman (1973) suggested that the range expansion was largely driven by an "ecological release" facilitated by introduced plants and supplemental feeding and that *C. anna*'s climate niche had previously existed in the expanded ranges. Together the ecological evidence exposes open questions about whether populations at the expansion front are experiencing novel selection pressures.

Little is known about genetic variation and population structure in *C. anna* beyond one study that showed low divergence between three California populations (Engeln, 2013) and observations of migration in some California populations, potentially tracking food resources, though these movements seem to be largely post-breeding (Clark & Russell, 2012). Further, the genetic makeup of populations in the expanded regions and whether they are adapting to the novel environments has not yet been explored. Here we present the first species-wide genomics study of *C. anna*, examining the distribution of genetic diversity across the native and expanded breeding range. First, we ask if there is evidence of population structure between the native and expanded breeding ranges. We would expect genetic divergence between native and expanded ranges if the expansions follow a stepping-stone model of colonization (Clegg et al., 2002; Le Corre & Kremer, 1998; Nei, 1972). We also test for genomic signals of selection between the native and expanded ranges. We expect to detect differing signals of selection between each leading edge and core populations due to unique combinations of selection pressures in the northern versus eastern range (Angert et al., 2020; Burton et al., 2010). Additionally, we may detect parallel selection signals at the range edges due to selection on traits associated with range expansion itself (Angert et al., 2020; Burton et al., 2010; Phillips et al., 2010). The degree to which adaptation occurs and can be detected in our data will depend on a number of factors, including gene flow across the range, the strength of selection, and the genetic architecture of the traits under selection. The two leading edges (northern and eastern) allow us to compare these expansions to answer questions about adaptation, gene flow, and genetic diversity across the native and expanded range, and broadly add to our understanding of ecoevolutionary dynamics in natural populations.

2 | MATERIALS AND METHODS

2.1 | Sampling

We collected blood ($N = 178$) and tissue ($N = 160$) samples from live hummingbirds and carcasses for a total of 338 unique hummingbirds across the historical Central and Southern California range and the expanded breeding ranges in Northern California, Washington, Arizona, and Nevada (Figure 1). Although *C. anna* can undergo post-breeding dispersal, we focused on sampling individuals in their breeding areas. Regions were categorized as "expanded" or "native" based on the predicted 1940 breeding range of *C. anna* as classified by Battey (2019). Although there are historical records of *C. anna* outside this range, the breeding range is most suitable for this classification in our study given that we are investigating genetic processes associated with range expansion and only during breeding time is genetic material exchanged. Hummingbirds were trapped using previously published methods (Russell & Russell, 2001) by a federally permitted hummingbird bander (Tell; US Geological Survey Bird Banding Laboratory Permit no. 23947). When possible, female birds were examined for evidence of an enlarged oviduct or the presence of an egg for inclusion in the study. Blood was collected (20–30 μL, <1% bodyweight) via a toenail clip ($N = 166$; (Tell et al., 2021)) and placed in Queen's lysis buffer (Seutin et al., 1991), or blood was collected on an FTA card ($N = 12$). All collection methods were approved by the University of California, Davis IACUC (F no. 20355). In addition to samples taken from live birds in the field, we added specimens from the Burke Museum collection at the University of Washington and carcasses from wildlife centers for nestlings or fledglings that did not survive the rehabilitation process (see Data S1, ANHU_metadata4ms.xlsx, for sample details). For native range samples, we used females and males collected between February and August when many of the birds that we sampled showed evidence of breeding. This also corresponds to the broad breeding season synthesized by nest reports (Battey, 2019). However due to low sample availability we used females and males collected throughout the year in the expanded regions. Sampling outside of the breeding season, however, did not appear to affect our results (see Section 3).

2.2 | DNA extraction and species identification

Whole genomic DNA was extracted from muscle tissue (for museum collection birds or carcasses from rehabilitation centres), 100–150 μL of blood stored in lysis buffer, or 2 to 3 blood spots from a blood collection card using the DNeasy Blood & Tissue Kit (Qiagen). The following modifications to the extraction protocol were used: samples were incubated overnight at 56°C, the sample was passed over the spin column twice prior to washing, an extra column drying step was taken (20,000*g* for 3 min), and DNA was eluted in 200 μL AE buffer heated to 56°C. Whole genomic DNA was quantified using a Qubit Fluorometer (Thermo Fisher Scientific) and the quality of DNA was assessed using a 2% agarose gel.

Because nestling and fledgling hummingbirds are difficult to identify to species, we used molecular methods to determine which of the juvenile samples represented *C. anna* and thus could be used in our study. To identify nestlings and fledglings from the wildlife centers as *C. anna* we used Sanger sequencing to sequence 32 unknown individuals and 30 known samples from Anna's (*C. anna*) and other hummingbird species likely to be collected in the region: Costa's (*C. costae*), Allen's (*Selasphorus sasin*), Calliope (*S. calliope*), and Rufous (*S. rufus*) hummingbirds. We amplified part of the NADH dehydrogenase subunit 2 (ND2) gene using H6313 and L5219 primers (Sorenson et al., 1999), cleaned the products using an ExoSAP protocol, then sequenced them at UCDNA Sequencing Facility at the University of California, Davis. We trimmed and aligned the resulting sequences and used the neighbour-joining method to build the tree in Geneious version 9.1.7 (http://www.geneious.com/). We visualized the phylogenetic tree with FigTree (http://tree.bio.ed.ac.uk/software/figtree/) using a Black-chinned Hummingbird sample to root the tree. Black-chinned Hummingbird was used because it was outside of the *Selasphorus* and *Calypte* subclades between which

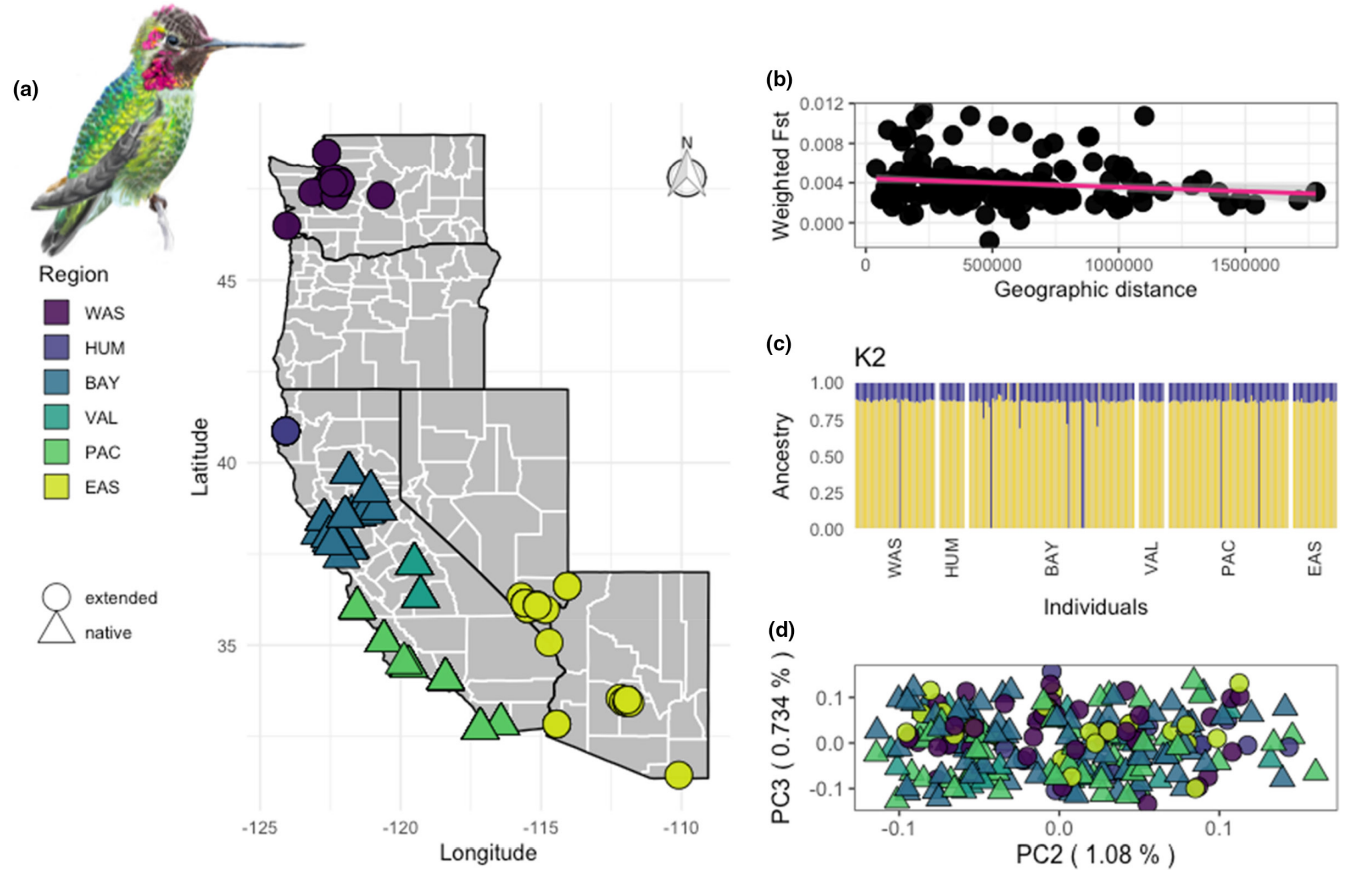

FIGURE 1 (a) Map of *C. anna* samples grouped by region with native (triangle) or expanded (circle) range indicated by shape. The regions are defined as follows: Washington state (WAS), Humboldt County (HUM), San Francisco Bay Area (BAY), California Central Valley (VAL), Pacific Coast (PAC), and the eastern expanded region (EAS). (b) Genetic divergence (weighted F_{ST}) showing little relationship with increasing pairwise county geographic distance ($y = 0.004 - 8.6E^{-10}x$). (c) Hierarchical clustering analysis for 2 ($K = 2$) ancestral groups, which was found to be the optimal number of groups. For additional ancestral groups ($K = 3$–5) see Figure S4. (d) The second and third components from the principal component analysis (PCA) in which samples are coloured by region (see [a]) and the shapes indicate if the samples are from the native (circle) or expanded (triangle) ranges. The first axis appeared to be driven by sequencing pool (see Figure S5A). [Colour figure can be viewed at wileyonlinelibrary.com]

we were identifying unknown samples. ND2 is a marker often used to create hummingbird phylogenies (Licona-Vera & Ornelas, 2017; McGuire et al., 2007, 2014), and in our study has consistently separated individuals that are *C. anna* from other hummingbird species found in California.

2.3 | Library preparation, and whole genome sequencing

We used a modified library preparation based on Illumina's Nextera protocol (Baym et al., 2015; Overgaard Therkildsen & Palumbi, 2017) to sequence whole genomes of 283 birds. To start, genomic DNA was standardized to 3 ng/μL then underwent a tagmentation step using TDE1 enzyme and buffer (Illumina). Dual combination Nextera indexes (Illumina) were then added to tagged DNA fragments followed by a booster PCR using the Kapa HiFi Kit (Kapa Biosystems). Libraries were then bead cleaned and single size selected to remove fragments <320 bp using AMPure XP Beads (Beckman Coulter) and quantified using a Qubit Fluorometer (Thermo Fisher Scientific). All libraries were pooled equimolarly then visualized with a Bioanalyser (Agilent). The pooled libraries were further size selected to 320–500 bp fragments using Ampure XP Beads (Beckman Coulter). A subset of samples ($N = 40$) was size selected using Blue Pippin (Sage Science; University of California Davis Genome Centre). The final libraries were sequenced on an Illumina HiSeq 4000 as 150 bp paired-end reads and the resulting sequences were demultiplexed by Novogene. The samples were sequenced across seven lanes to target 2.5× coverage.

2.4 | Data processing

Adapters and low-quality reads were trimmed using Trimmomatic (Bolger et al., 2014) or Trim Galore! (a wrapper around Cutadapt (Martin, 2011), accessible at http://www.bioinformatics.babraham.ac.uk/projects/trim_galore). Each sample was aligned to the *C. anna* reference genome, GCA_003957555.2 (Korlach et al., 2017)

using bwa mem (Li & Durbin, 2010) then sorted and indexed using Samtools (Li et al., 2009). For individuals sequenced across two lanes, bam files were merged using Samtools (Li et al., 2009). For all samples, duplicate reads were marked with MarkDuplicates from Picard Tools (http://broadinstitute.github.io/picard). For a subset of samples ($N = 40$), duplicate reads were removed using FastUniq (Xu et al., 2012) prior to mapping.

Single nucleotide polymorphisms (SNPs) were identified, and genotype likelihoods were estimated using the ANGSD tool (Korneliussen et al., 2014) accessed through ngsTools (Fumagalli et al., 2014). For the parameters used in ANGSD see Table S1. Potentially related samples were identified with NGSrelate (Korneliussen & Moltke, 2015), using the rab metric which calculates pairwise relatedness based on (Hedrick et al., 2015). For pairs of related samples (rab > 0.45), one individual of each pair was removed.

2.5 | Population structure

Population structure was analysed using principal components analysis as well as hierarchical clustering analysis. A covariance matrix was calculated using PCAngsd (Meisner & Albrechtsen, 2018) and then we used RStudio version 1.3.1093 (RStudio Team, 2018) with R version 3.6.0 (R Core Team, 2019) to conduct eigenvector decomposition and created plots comparing principal components (PCs). We used clustering in NGSadmix to infer the "best" number of populations and estimate ancestry proportions. We ran NGSadmix five times each with population numbers (i.e., K values) ranging from one to six. We used the Evanno method implemented in CLUMPAK (Kopelman et al., 2015) to determine the best fit K value (accessed at http://clumpak.tau.ac.il/bestK.html).

2.6 | Genetic variation and gene flow

To estimate nucleotide diversity, we first grouped samples into six regions (Figure 1a) based on geography and expansion history (Battey, 2019): Washington (WAS), Humboldt California (HUM), Bay Area California (BAY), Central Valley California (VAL), Pacific coast of southern California (PAC), and eastern expansion samples (EAS). Since the number of samples can affect estimates of genetic diversity, we downsampled each population to the lowest sample size ($N = 13$) by randomly selecting that number of individuals from each population for downstream calculations. A folded site frequency spectrum (SFS) was generated for each downsampled population by generating a site allele frequency file using ANGSD (for parameter details see Table S1) from which an SFS is estimated using realSFS -fold (Nielsen et al., 2012). Finally, each SFS was used as a prior (-pest) to estimate diversity statistics (-doTheta) in ANGSD. We estimated pairwise divergence between samples grouped by county for counties that had at least five individuals using ANGSD (for parameter details see Table S1) and realSFS (fst stats) on polymorphic sites. We estimated global heterozygosity per individual for 5–10 individuals per county (Table S2) using ANGSD (for parameter details see Table S1) and realSFS (parameters: -fold 1) to create site frequency spectra. Here we are using county as a proxy for geographic proximity. To assess the direction of gene flow among the defined populations we calculated a directionality index, ψ (Peter & Slatkin, 2013). First, we created pairwise 2D SFSs using the site allele frequency files created for each population SFS. Then we calculated ψ using equation 1b from Peter and Slatkin (2013), which detects mismatches between pairwise site frequency spectra indicative of successive founder events and thus identifies geographic origins and directionality of expansions.

2.7 | Selection

We tested for both local and species-wide genomic signals of selection associated with the recent range expansion in *C. anna*. We looked for potential genomic regions under selection in the expanded range using an F_{ST} outlier approach. F_{ST} outliers are a common metric for identifying selection. Peaks of significantly different allele frequencies between populations at close loci are often an indication of potential selection (Domyan et al., 2016; Vickrey et al., 2018). In this case, we compared the northern (WAS) and eastern (EAS) expansion regions to their nearest native range regions, Central California (BAY) and Southern California (PAC), respectively. We used the pFst tool (Kronenberg, 2014) in VCFlib (https://github.com/vcflib/vcflib) after creating a BCF file using ANGSD (–dobcf) and converting it to a VCF file with BCFtools accessed through Samtools. The pF_{ST} tool uses a likelihood ratio test to detect allele frequency differences between populations.

While the expectation for the magnitude and direction of gene flow is unknown in *C. anna*, largely due to enigmatic movement patterns, a previous study suggested high gene flow between three California populations (Engeln, 2013). Another California hummingbird, Allen's Hummingbird (*S. sasin*), was found to have high gene flow among the mainland populations (Myers et al., 2021), potentially indicative of high overall levels of mobility in hummingbirds. If gene flow in *C. anna* is extremely high, we might expect signatures of selection caused by exposure to novel selective agents during range expansion to be present across the entire species rather than divergent between populations. We used all samples to test for signatures of selective sweeps using SweeD version 3.2.1 (Pavlidis et al., 2013). Note that sweeps detected across the entire range could be the result of historical (pre-expansion) selection or recent selection. We first estimated minor allele frequencies at polymorphic sites using ANGSD (for parameter details see Table S1). We converted these into the required allele count input for SweeD by multiplying the minor allele frequency by the number of individuals sequenced for each site and rounding to the nearest integer. All sites were considered folded. We ran SweeD separately for each chromosome, with a grid equal to the length of the chromosome divided by 5000 (so that we tested every 5 kb).

3 | RESULTS

3.1 | Species identification

We used Sanger sequencing to identify *C. anna* from a set of 32 unknown nestlings and fledglings using 30 individuals of known species identity for comparison. We removed 14 of the 62 ND2 sequences due to low quality or short sequence length, including 10 samples of unknown species. Within known samples, *C. anna* samples formed a monophyletic group allowing us to reliably identify other *C. anna* in our unknown samples (Figure S1). Of the 22 remaining unknown hummingbird samples, 18 were identified as *C. anna* (Figure S1) and nine of those had location data and were therefore used for whole genome sequencing.

3.2 | Whole genome sequencing

We received no data for one sample and despite being sequenced across two lanes we received only one demultiplexed fastq file for two samples, both of which were in sequencing pool ANHU_003. Across 283 individuals, our sequencing runs produced 5.4 billion short reads with more than 99% of samples having 90% or more of reads with a quality score of Q >30. On average, 98.3% of the sequence reads mapped to the reference genome per individual, and individual coverage ranged from 0× to 4.7× with an average of 2.2×. We removed individuals ($N=35$) from the data set that exhibited any of the following: samples that failed to sequence, indicated by a very low number of raw reads (<1000), samples that mapped poorly to the reference genome (<50%), and samples that had low individual coverage (<1.0×). We also removed five outliers in an initial PCA, which we believe may have been misidentified based on a preliminary PCA that included other co-occurring hummingbird species (Figure S2). We removed samples that either grouped with the other species or fell in between two species in the preliminary PCA. We identified two pairs of potentially related individuals and removed one individual from each pair. The remaining 241 individuals had on average 98.7% of the sequence reads mapped to the reference genome, and their coverage ranged from 1.0× to 4.45× with an average of 2.5×. The number of loci used for analyses ranged from 22,902 for the PCA (SNPs present in all individuals) to 934,225,517 (all base pairs with sufficient coverage) to calculate theta (Table S3).

3.3 | High gene flow across the range

We found no evidence of barriers to gene flow across the native and expanded range of *C. anna*. Overall, genetic divergence was low species-wide, with pairwise county divergence (F_{ST}) ranging from 0–0.01 (Figure S3). We detected no pattern of isolation by distance in pairwise F_{ST} between counties (Figure 1b; Mantel test $p = .53$). The admixture analyses showed no population structure; inferred ancestral groups were evenly represented across geography. Although the optimal number of ancestral groups was $K=2$ (Figure 1c), there was no clear geographic structuring at either $K=2$ or higher values of K (Figure S4), suggesting only one major genetic group with no barriers to gene flow. We found no geographic signal based on the first three principal components axes although none of the PCs explained much more than 1% of the variance (Figure 1d). There was a correlation between sequencing pool and PC1 (Figure S5A), although it explained only a small amount of the variance similar to the other PCs, potentially highlighting the absence of other factors structuring genetic variation across the range but also reinforcing the need for consideration of sequencing artefacts in next-generation sequencing. To investigate if a larger SNP data set would clarify any population structure, we reran the PCA with more permissive filtering (-minInd 49, instead of -minInd 241 (all individuals)), which resulted in 9.5 million SNPs. The resulting PCA did not indicate spatial structuring, consistent with the result from the PCA with fewer SNPs (Figure S5B). We also conducted a PCA with only females collected between February and April ($N=72$, after PCA outliers were removed) to ensure only breeding birds were examined, which also showed no genetic structure (Figure S5C).

3.4 | Evidence for a species core

Despite the lack of population structure, we did find expected core-edge patterns of genetic diversity. We found higher nucleotide diversity (pairwise theta) in regions in the native range compared to the expanded ranges (Figure 3a), consistent with classic core-edge expectations, though the magnitude of these differences is small. The Pacific Coast region, in the native range, had the highest average nucleotide diversity at 1.88×10^{-3} while the eastern and Washington state expanded regions had the lowest values at 1.74×10^{-3} and 1.77×10^{-3}, respectively. In pairwise tests, all regions were significantly different (multiple test-corrected Kruskal-Wallis $p<.001$). Decreased diversity in the expanded regions did not appear to be driven by increased relatedness among sampled individuals (Figure S6A). However, some counties in the northern and eastern expanded regions appeared to have lower heterozygosity, which could contribute to the observed pattern (Figure S6B). The directionality index, ψ, was low (absolute value <0.05) in all pairwise comparisons across the six regions (Table S4). This result is far lower than the cutoff of $|\psi|>3$ used by Peter and Slatkin (2013, 2015) and those seen in other studies using this method (Bors et al., 2019; Puckett & Munshi-South, 2019; Streicher et al., 2016; Zhan et al., 2014). While the direction of ψ might suggest that the San Francisco Bay Area and the California Central Valley regions are sources of gene flow and Washington state in the expanded range is a majority sink of gene flow (Figure S7), the nonsignificant test statistics are consistent with our high levels of gene flow.

3.5 | No evidence of selection

We found no evidence for either local adaptation (comparing native and expanded ranges) or global selection (across all samples)

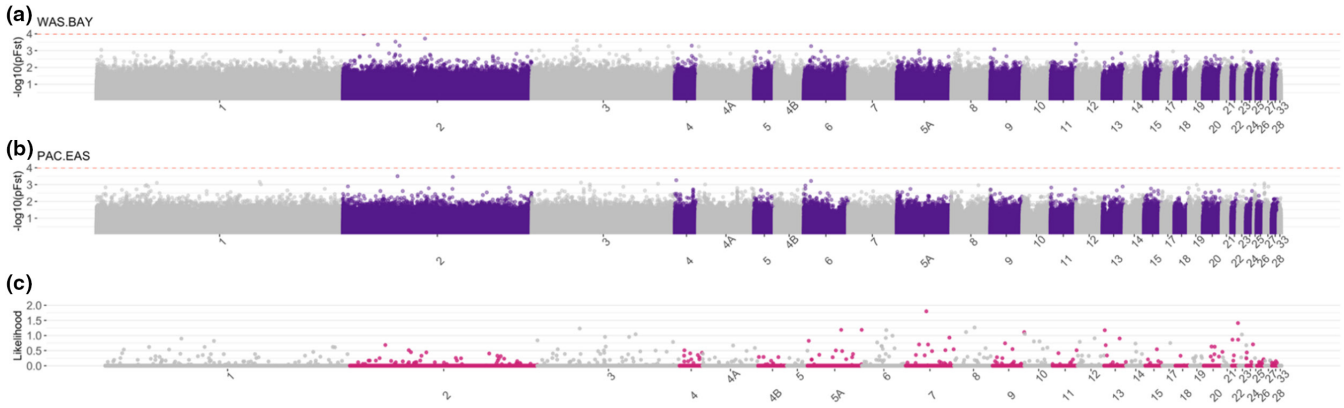

FIGURE 2 (a,b) Significance values (−log10 *p*-values) from the likelihood ratio test to detect allele frequency differences between WAS and BAY (a) and between PAC and EAS (b) regions plotted across the genome. The red dashed line is a 0.0001 significance threshold. There were no significant SNPs after Bonferroni or false discovery rate corrections. (c) Selection likelihood values resulting from the selective sweep analysis plotted across the genome. Chromosomes are numbered along the *x*-axis. [Colour figure can be viewed at wileyonlinelibrary.com]

that might be linked to the range expansion. For local adaptation we used F_{ST} to compare the expanded regions (Washington state and the eastern populations) to their nearest native range region (the Bay Area of California and the Pacific coast of southern California), but no obvious peaks stood out for either comparison (Figure 2a,b). Chromosomal mean pF_{STs} were $p = 0.7 \pm 0.2$ and no SNPs were significant after Bonferroni or false discovery rate corrections. Based on the most significant 1% of *p*-values from pF_{ST}, we found 6079 SNPs that were shared between the two expanded region comparisons, only slightly higher than the expected number of high F_{ST} shared SNPs (5599 SNPs). An alternative to divergence due to spatially varying selection is that with high gene flow homogenizing genetic diversity, selection in the expanded range would affect allele frequencies in the entire species. However, we also found no evidence of selective sweeps when analysing the site frequency spectrum generated from all samples (Figure 2c). In fact, the composite likelihood ratio (CLR) statistics representing the test for selective sweeps were all below 2.0, much lower than cutoffs found in many studies (Brand et al., 2020; Frantz et al., 2015; Jones et al., 2018; Laurent et al., 2016; Pavlidis et al., 2013).

4 | DISCUSSION

The redistribution of species globally has ignited interest in and urgency for understanding eco-evolutionary dynamics of range shifts (Miller et al., 2020; Parmesan, 2006; Sexton et al., 2009). In the well-documented contemporary range expansion in *C. anna*, we show patterns largely consistent with expectations of a "pushed wave" expansion; gene flow is high throughout the entire range, and we find no strong divergence in allele frequencies between the core of the range and expansion fronts. We do find reduced genetic diversity at expansion fronts, which is characteristic of pulled waves, but the magnitude of the reduction in genetic diversity is small. The lack of selective signals could support the previous hypothesis that the expanded range was within the historical climate niche, so adaptation was not required during expansion. Alternatively, the absence of significant selection could be the result of technical limitations, including limited samples sizes for genome scans and the recent nature of the expansion. Together our evidence highlights the complexity of rapid range shifts in natural populations and potential limitations of genomic data in investigating ecoevolutionary phenomena.

Evidenced by low species-wide genetic divergence and a lack of spatial structuring, we show that *C. anna* has few, if any, limits to gene flow (Figure 1; Figure S3). These results support a previous genetic study in *C. anna* that found no genetic structure among three California populations using mitochondrial DNA (Engeln, 2013). The preservation of genetic diversity across the expanded ranges is consistent with recent, rapid range expansions characterized by a short time frame, growing population sizes, and multiple independent expansion fronts, all of which characterize the *C. anna* expansion (Battey, 2019; Greig et al., 2017; Zimmerman, 1973). While the extent of seasonal movement varies by population (Clark & Russell, 2012), *C. anna* has a broad diet, relatively large territories, and some seasonal migration (Hazlehurst et al., 2021; Ortiz-Crespo, 1980; van Rossem, 1945; Yeaton & Laughrin, 1976), all of which could contribute to high gene flow in this system. Long-distance dispersal, especially from the core, has been shown to preserve genetic diversity in other taxa (Berthouly-Salazar et al., 2013). This result is often seen in highly mobile species and recent invasions. Examples of high gene flow within species in newly colonized territories include invasive Indo-Pacific lionfish (*Pterois volitans*) in the Caribbean (Bors et al., 2019) and European starlings (*Sturnus vulgaris*) in South Africa and North America (Berthouly-Salazar et al., 2013; Hofmeister et al., 2021). The similarities with colonizing species expansions (e.g., propagule and dispersal pressure, novel biotic and abiotic interactions) underscore the emerging work viewing range shifts and expansions of native species, especially those caused by climate change, through the lens of invasion biology (Wallingford et al., 2020).

Range expansions often expose species to novel environments containing new combinations of biotic and abiotic interactions that can coincide with niche shifts, expansions, or unfilling (Davies

et al., 2019; Strubbe et al., 2013). Previous modelling showed that the expanded regions fell within *C. anna's* fundamental climatic niche prior to the range expansion, suggesting that previous range limits were defined by the presence of resources (Battey, 2019). In this case, where the geographical limit of the fundamental niche is expanded by the addition of resources, selection would not necessarily be required during range expansion. In our data, we do not uncover signals of selection at the range fronts (Figure 2). Although it is possible that selection was overlooked due to the limitations of genome scans (see below), the lack of selective signatures between the core and expanded ranges identified here aligns with the previous hypothesis that the range expansion in *C. anna* could be the result of an ecological release facilitated by human-mediated landscapes. This hypothesis states that introduced plants and supplemental feeding have allowed *C. anna* to fill out its existing climate niche even in the expanded regions (Battey, 2019; Zimmerman, 1973). While other ecological factors induced by urbanization and climate change could also be aiding the expansion, a similar pattern of spatial expansion and ecological release associated with supplemental feeding has been documented in Eurasian Blackcap warbler (Plummer et al., 2015). Together, these studies provide evidence for the role of local anthropogenic alterations of the landscape shaping broadscale shifts in species' ranges.

An alternative explanation for the lack of genetic divergence between the native and expanded ranges is that gene flow from the core is swamping genomic signals of adaptation. High gene flow can be maladaptive at the expansion edge (Haldane, 1956) and inhibited selection during pushed wave expansions often slows and/or prevents further range expansion (Barton, 2001; García-Ramos & Kirkpatrick, 1997; Miller et al., 2020). This pattern is seen in the southeastern invasion edge of cane toads in Australia where the range is thought to be limited by cold temperatures (Trumbo et al., 2016). Because we do see high gene flow between native and expanded range populations (Figure 1; Figure S3), it is possible that the influx of alleles from the range core inhibits adaptation at the range edge. However, observational data does not suggest that the range expansion in *C. anna* is slowing; new breeding populations in the north and west of the range have been observed in recent years. Recently, a growing breeding population of *C. anna* has been found in Idaho (Pollock et al., 2021), an area predicted to be suitable for *C. anna* (Battey, 2019). Southeast Washington state and sections of Utah are also predicted to have suitable habitat for *C. anna* (Battey, 2019), potentially suggesting that the expansion will continue in the coming decades. Our observations add to the growing literature suggesting that gene flow does not necessarily limit species ranges or their range expansions (Kottler et al., 2021).

Despite high gene flow and lack of genomic signatures of selection, we found very subtle evidence of classic core-edge patterns of genetic diversity. While we did not detect structure in the PCA or admixture analysis (Figure 1), we observed lower nucleotide diversity at both expansion fronts (Figure 3). This result could indicate that mating is not random across the range despite high gene flow (Jiang et al., 2013). Increased relatedness among individuals due to small populations sizes could drive the decrease; however, this does not appear to be the cause in *C. anna* (Figure S6). Alternatively, lower population-level genetic diversity (theta) could be a result of decreased observed individual heterozygosity, which is what we observed (Figure S6). The loss of heterozygosity at the expansion front could be caused by genetic drift, specifically in response to population bottlenecks or allele surfing (Goodsman et al., 2014) or by selective sweeps in the expanded ranges, although selective sweeps specific to the expansion front should cause allele frequency differences that could be detected by our F_{ST} scans. Relatedly, many of the northern expansion front samples were collected in earlier years (2000 vs. late 2010s) which may represent a time point closer to a founding bottleneck before more birds dispersed from the core, a pattern previously suggested in the invasive Indo-Pacific lionfish (Bors et al., 2019). However, this decrease of heterozygosity does not appear to be strong and consistent enough to result in signatures of divergence and selection between the range core and expansion fronts (Figure 2).

The seemingly contradicting observations of decreased genetic diversity in the absence of signals of selection or structure could have several biological or technical explanations. One possibility is that the expansion is too recent for the detection of significant divergence in range edge populations and more differentiation may develop over time. Our range edge samples were collected over the past 30 years, with the earliest samples taken approximately three decades after the breeding range reached Washington. We therefore cannot rule out that selection is happening at the range edges, but not enough time has passed to shift allele frequencies that would be detected in the current study, especially in the face of high gene flow. Additionally, the presence of potentially suitable natural habitat in southern Nevada and northern Arizona and limited historical records could blur the delineation of the eastern expansion (Phillips, 1947; van Rossem, 1945; Zimmerman, 1973). If

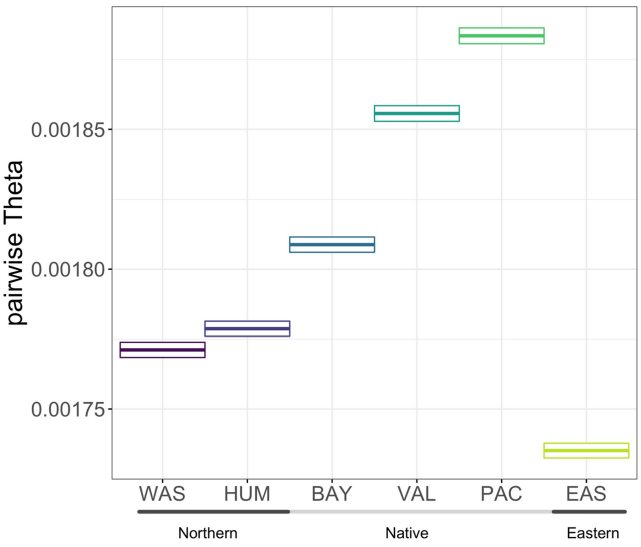

FIGURE 3 Mean (middle line) and standard error (box) of pairwise theta (a genetic diversity measure calculated as tP/nSites) for each region. [Colour figure can be viewed at wileyonlinelibrary.com]

these regions are at the range edge, they might confer less novel selection pressure; however, southeastern Arizona would remain a novel selection environment. Further, our low-coverage approach and moderate sample size may not have the power to detect multiple small shifts in allele frequencies across loci that could lead to adaptive evolution, a common issue with genome-wide scans for polygenic traits (Kemper et al., 2014; Pritchard et al., 2010). Reduced diversity in the expanded regions could therefore reflect this weak genome-wide selection that was not detected at any single SNP. Alternatively, environment-mediated trait differences may be plastic. For example, there is widespread use of torpor in Trochilidae and *C. anna* is no exception. In fact, *C. anna* were found to increase their use of torpor in cold temperatures (Spence et al., 2022; Spence & Tingley, 2021), which could conserve energy and aid in survival in the northern range expansion. However, we also know that cold snaps in the northern populations can still cause mortality so plastic modifications alone may not be enough to maintain populations, especially during extreme events. Further investigation of trait variation, both genetic and plastic, across the range could aid in our understanding of the mechanisms facilitating expansion.

Anthropogenic influences are changing the genetic landscape through shifting species ranges (Chen et al., 2011; Parmesan, 2006). Much of the recent focus has been on the role of climate change in facilitating range shifts and the likely ecoevolutionary dynamics of these phenomena (Miller et al., 2020; Sexton et al., 2009). However, our study demonstrates that not all expanding species respond in predicted ways, in fact, not all human-induced range expansions show obvious signatures of evolution. Further studies are needed to confirm these results and test the stability of our conclusions over time. For example, using museum specimens to understand the genetic landscape in *C. anna* before the expansions could confirm past gene flow or illuminate if increased urbanization is decreasing genetic diversity, increasing homogenization, or shifting the frequency of certain alleles (Bi et al., 2019). Additionally, while we focused on the northern and eastern expansions, sampling individuals from what might be the "trailing edge" in Mexico would further our understanding of whether climate or resources are defining species range limits in *C. anna*. This study contributes to the growing literature on the consequences of human-mediated range expansions by adding empirical evidence that eco-evolutionary dynamics are not one-size fits all.

AUTHOR CONTRIBUTIONS

Lisa A. Tell and Rachael A. Bay conceived of the study and secured funding. Ruta R. Bandivadekar, Lisa A. Tell, and Kevin Epperly supplied the samples. Michael W. Clark conducted the laboratory work and analysis for nestling identification. Nicole E. Adams conducted the DNA extractions, some of the library preparation, and the sequence processing. Kristen Ruegg supervised some of the library preparation. Nicole E. Adams and Rachael A. Bay conducted the analyses with input from C. J. Battey. Nicole E. Adams and Rachael A. Bay wrote the manuscript with contributions from all authors.

ACKNOWLEDGEMENTS

We thank Teia Schweizer for preparing libraries for sequencing, Christen Bossu for bioinformatic counsel, and Aya Brantley for the illustration of *C. anna* in Figure 1. Nestlings and fledglings that did not survive rehabilitation were provided by Liberty Wildlife (Arizona, USA), Wildlife Care Association of Sacramento (California, USA), Wetlands and Wildlife Care Centre (California, USA), Lindsay Wildlife Experience (California, USA), and Santa Barbara Wildlife Care Network (California, USA). Thanks to John Klicka and the Burke Museum for supplying samples from the northern expanded region. This project was supported by funding from CalTrans 65A0686 (USDOT 69A3551747114) to R.A.B and L.A.T, and NSF Rules of Life EAGER 1837940 to R.A.B and K.R. This study used the Extreme Science and Engineering Discovery Environment (XSEDE), which is supported by National Science Foundation grant no. ACI-1548562.

CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interests.

DATA AVAILABILITY STATEMENT

Raw ND2 gene sequences and metadata are available on Dryad (https://doi.org/10.25338/B8QM06). Raw whole genome sequence reads and metadata are available on NCBI's Sequence Read Archive digital repository (BioProject PRJNA946673), and R scripts are available on GitHub (https://github.com/NicoleAdams-sci/ANHU_geneflow).

REFERENCES

Andrade-Restrepo, M., Champagnat, N., & Ferrière, R. (2019). Local adaptation, dispersal evolution, and the spatial eco-evolutionary dynamics of invasion. *Ecology Letters*, *22*(5), 767–777. https://doi.org/10.1111/ELE.13234

Angert, A. L., Bontrager, M. G., & Aringgren, J. (2020). What do we really know about adaptation at range edges? *Annual Review of Ecology, Evolution, and Systematics*, *51*, 341–361. https://doi.org/10.1146/ANNUREV-ECOLSYS-012120-091002

Barton, N. H. (2001). Adaptation at the edge of a species' range. In J. Silvertown & J. Antonovics (Eds.), *Integrating ecology and evolution in a spatial context* (pp. 365–392). Blackwell.

Battey, C. J. (2019). Ecological release of the Anna's Hummingbird during a Northern range expansion. *American Naturalist*, *194*(3), 306–315. https://doi.org/10.1086/704249

Baym, M., Kryazhimskiy, S., Lieberman, T. D., Chung, H., Desai, M. M., & Kishony, R. (2015). Inexpensive multiplexed library preparation for megabase-sized genomes. *PLOS ONE*, *10*(5), e0128036. https://doi.org/10.1371/journal.pone.0128036

Berthouly-Salazar, C., Hui, C., Blackburn, T. M., Gaboriaud, C., van Rensburg, B. J., van Vuuren, B. J., & le Roux, J. J. (2013). Long-distance dispersal maximizes evolutionary potential during rapid geographic range expansion. *Molecular Ecology*, *22*(23), 5793–5804. https://doi.org/10.1111/mec.12538

Bi, K., Linderoth, T., Singhal, S., Vanderpool, D., Patton, J. L., Nielsen, R., Moritz, C., & Good, J. M. (2019). Temporal genomic contrasts reveal rapid evolutionary responses in an alpine mammal during recent climate change. *PLOS Genetics*, *15*(5), e1008119. https://doi.org/10.1371/JOURNAL.PGEN.1008119

Bolger, A. M., Lohse, M., & Usadel, B. (2014). Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*, *30*(15), 2114–2120. https://doi.org/10.1093/bioinformatics/btu170

Bontrager, M., & Angert, A. L. (2019). Gene flow improves fitness at a range edge under climate change. *Evolution Letters*, *3*(1), 55–68. https://doi.org/10.1002/EVL3.91

Bors, E. K., Herrera, S., Morris, J. A., & Shank, T. M. (2019). Population genomics of rapidly invading lionfish in the Caribbean reveals signals of range expansion in the absence of spatial population structure. *Ecology and Evolution*, *9*(6), 3306–3320. https://doi.org/10.1002/ece3.4952

Brand, P., Hinojosa-Díaz, I. A., Ayala, R., Daigle, M., Yurrita Obiols, C. L., Eltz, T., & Ramírez, S. R. (2020). The evolution of sexual signaling is linked to odorant receptor tuning in perfume-collecting orchid bees. *Nature Communications*, *11*(1), 1–11. https://doi.org/10.1038/s41467-019-14,162-6

Burton, O. J., Phillips, B. L., & Travis, J. M. J. (2010). Trade-offs and the evolution of life-histories during range expansion. *Ecology Letters*, *13*(10), 1210–1220. https://doi.org/10.1111/J.1461-0248.2010.01505.X

Cahill, A. E., Aiello-Lammens, M. E., Fisher-Reid, M. C., Hua, X., Karanewsky, C. J., Ryu, H. Y., Sbeglia, G. C., Spagnolo, F., Waldron, J. B., & Wiens, J. J. (2014). Causes of warm-edge range limits: systematic review, proximate factors and implications for climate change. *Journal of Biogeography*, *41*(3), 429–442. https://doi.org/10.1111/jbi.12231

Chen, I. C., Hill, J. K., Ohlemüller, R., Roy, D. B., & Thomas, C. D. (2011). Rapid range shifts of species associated with high levels of climate warming. *Science*, *333*(6045), 1024–1026. https://doi.org/10.1126/SCIENCE.1206432

Clark, C. J., & Russell, S. M. (2012). Anna's Hummingbird (*Calypte anna*), version 2.0. In A. F. Poole (Ed.), *The Birds of North America*. Cornell Lab of Ornithology. https://doi.org/10.2173/bna.226

Clegg, S. M., Degnan, S. M., Kikkawa, J., & Owens, I. P. F. (2002). Genetic consequences of sequential founder events by an island-colonizing bird. *Proceedings of the National Academy of Sciences*, *99*(12), 8127–8132.

Cote, J., Bestion, E., Jacob, S., Travis, J., Legrand, D., & Baguette, M. (2017). Evolution of dispersal strategies and dispersal syndromes in fragmented landscapes. *Ecography*, *40*(1), 56–73. https://doi.org/10.1111/ECOG.02538

Davies, S. J., Hill, M. P., McGeoch, M. A., & Clusella-Trullas, S. (2019). Niche shift and resource supplementation facilitate an amphibian range expansion. *Diversity and Distributions*, *25*(1), 154–165. https://doi.org/10.1111/ddi.12841

Dlugosch, K. M., & Parker, I. M. (2008). Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Molecular Ecology*, *17*(1), 431–449. https://doi.org/10.1111/j.1365-294X.2007.03538.x

Domyan, E. T., Kronenberg, Z., Infante, C. R., Vickrey, A. I., Stringham, S. A., Bruders, R., Guernsey, M. W., Park, S., Payne, J., Beckstead, R. B., Kardon, G., Menke, D. B., Yandell, M. D., & Shapiro, M. D. (2016). Molecular shifts in limb identity underlie development of feathered feet in two domestic avian species. *eLife*, *5*, e12115. https://doi.org/10.7554/ELIFE.12115

Eckert, C. G., Samis, K. E., & Lougheed, S. C. (2008). Genetic variation across species' geographical ranges: the central–marginal hypothesis and beyond. *Molecular Ecology*, *17*(5), 1170–1188. https://doi.org/10.1111/J.1365-294X.2007.03659.X

Engeln, M. W. (2013). *Relationship between local vocal behavior and gene flow in the Anna's Hummingbird (Calypte anna)*. California State University.

Excoffier, L. (2004). Patterns of DNA sequence diversity and genetic structure after a range expansion: lessons from the infinite-island model. *Molecular Ecology*, *13*(4), 853–864. https://doi.org/10.1046/j.1365-294X.2003.02004.x

Excoffier, L., Foll, M., & Petit, R. J. (2009). Genetic consequences of range expansions. *Annual Review of Ecology, Evolution, and Systematics*, *40*, 481–501. https://doi.org/10.1146/annurev.ecolsys.39.110707.173414

Fedorka, K. M., Winterhalter, W. E., Shaw, K. L., Brogan, W. R., & Mousseau, T. A. (2012). The role of gene flow asymmetry along an environmental gradient in constraining local adaptation and range expansion. *Journal of Evolutionary Biology*, *25*(8), 1676–1685. https://doi.org/10.1111/j.1420-9101.2012.02552.x

Frantz, L. A. F., Schraiber, J. G., Madsen, O., Megens, H. J., Cagan, A., Bosse, M., Paudel, Y., Crooijmans, P. M. A., Larson, G., & Groenen, M. A. M. (2015). Evidence of long-term gene flow and selection during domestication from analyses of Eurasian wild and domestic pig genomes. *Nature Genetics*, *47*(10), 1141–1148. https://doi.org/10.1038/ng.3394

Fumagalli, M., Vieira, F. G., Linderoth, T., & Nielsen, R. (2014). ngsTools: methods for population genetics analyses from next-generation sequencing data. *Bioinformatics*, *30*(10), 1486–1487. https://doi.org/10.1093/bioinformatics/btu041

García-Ramos, G., & Kirkpatrick, M. (1997). Genetic models of adaptation and gene flow in peripheral populations. *Evolution*, *51*(1), 21–28. https://doi.org/10.1111/J.1558-5646.1997.TB02384.X

Gaston, K. J. (2003). *The stucture and dynamics of geographic ranges*. Oxford University Press on Demand.

Goodsman, D. W., Cooke, B., Coltman, D. W., & Lewis, M. A. (2014). The genetic signature of rapid range expansions: how dispersal, growth and invasion speed impact heterozygosity and allele surfing. *Theoretical Population Biology*, *98*, 1–10. https://doi.org/10.1016/j.tpb.2014.08.005

Greig, E. I., Wood, E. M., & Bonter, D. N. (2017). Winter range expansion of a hummingbird is associated with urbanization and supplementary feeding. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1852), 20170256. https://doi.org/10.1098/rspb.2017.0256

Grinnell, J. (1915). *A distributional list of the birds of California. Pacific Coast Avifauna 11*. Cooper Ornithological Club.

Grinnell, J., & Miller, A. H. (1944). *The distribution of the birds of California. Pacific Coast Avifauna 27*. Cooper Ornithological Club.

Haldane, J. B. (1956). The relation between density regulation and natural selection. *Proceedings of the Royal Society of London. B*, *145*(920), 306–308. https://doi.org/10.1098/rspb.1956.0039

Hancock, A. M., Brachi, B., Faure, N., Horton, M. W., Jarymowycz, L. B., Sperone, F. G., Toomajian, C., Roux, F., & Bergelson, J. (2011). Adaptation to climate across the *Arabidopsis thaliana* genome. *Science*, *334*(6052), 83–86. https://doi.org/10.1126/SCIENCE.1209244

Hazlehurst, J. A., Rankin, D. T., Clark, C. J., McFrederick, Q. S., & Wilson-Rankin, E. E. (2021). Macroecological patterns of resource use in resident and migratory hummingbirds. *Basic and Applied Ecology*, *51*, 71–82. https://doi.org/10.1016/j.baae.2021.01.003

Hedrick, P. P. W., Lacy, R. C., & Baker, C. S. (2015). Measuring relatedness between inbred individuals. *Journal of Heredity*, *106*(1), 20–25. https://doi.org/10.1093/JHERED/ESU072

Hitch, A. T., & Leberg, P. L. (2007). Breeding distributions of North American bird species moving north as a result of climate change. *Conservation Biology*, *21*(2), 534–539. https://doi.org/10.1111/J.1523-1739.2006.00609.X

Hofmeister, N. R., Werner, S. J., & Lovette, I. J. (2021). Environmental correlates of genetic variation in the invasive European starling in North America. *Molecular Ecology*, *30*(5), 1251–1263. https://doi.org/10.1111/mec.15806

Hovick, T. J., Allred, B. W., Mcgranahan, D. A., Palmer, M. W., Elmore, R. D., & Fuhlendorf, S. D. (2016). Informing conservation by identifying range shift patterns across breeding habitats and migration strategies. *Biodiversity and Conservation*, *25*, 345–356. https://doi.org/10.1007/s10531-016-1053-6

Jiang, Y., Bolnick, D. I., & Kirkpatrick, M. (2013). Assortative mating in animals. *American Naturalist*, *181*(6), E125–E138. https://doi.org/10.1086/670160

Jones, M. R., Scott Mills, L., Alves, P. C., Callahan, C. M., Alves, J. M., Lafferty, D. J. R., Jiggins, F. M., Jensen, J. D., Melo-Ferreira, J., & Good, J. M. (2018). Adaptive introgression underlies polymorphic seasonal camouflage in snowshoe hares. *Science*, *360*(6395), 1355–1358. https://doi.org/10.1126/science.aar5273

Kemper, K. E., Saxton, S. J., Bolormaa, S., Hayes, B. J., & Goddard, M. E. (2014). Selection for complex traits leaves little or no classic signatures of selection. *BMC Genomics*, *15*(1), 1–14. https://doi.org/10.1186/1471-2164-15-246/TABLES/6

Klopfstein, S., Currat, M., & Excoffier, L. (2006). The fate of mutations surfing on the wave of a range expansion. *Molecular Biology and Evolution*, *23*(3), 482–490. https://doi.org/10.1093/MOLBEV/MSJ057

Kopelman, N. M., Mayzel, J., Jakobsson, M., Rosenberg, N. A., & Mayrose, I. (2015). Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. *Molecular Ecology Resources*, *15*(5), 1179–1191. https://doi.org/10.1111/1755-0998.12387

Korlach, J., Gedman, G., Kingan, S. B., Chin, C. S., Howard, J. T., Audet, J. N., Cantin, L., & Jarvis, E. D. (2017). De novo PacBio long-read and phased avian genome assemblies correct and add to reference genes generated with intermediate and short reads. *GigaScience*, *6*(10), 1–16. https://doi.org/10.1093/gigascience/gix085

Korneliussen, T. S., Albrechtsen, A., & Nielsen, R. (2014). ANGSD: analysis of next generation sequencing data. *BMC Bioinformatics*, *15*(1), 356. https://doi.org/10.1186/s12859-014-0356-4

Korneliussen, T. S., & Moltke, I. (2015). NgsRelate: a software tool for estimating pairwise relatedness from next-generation sequencing data. *Bioinformatics*, *31*(24), btv509. https://doi.org/10.1093/bioinformatics/btv509

Kottler, E. J., Dickman, E. E., Sexton, J. P., Emery, N. C., & Franks, S. J. (2021). Draining the swamping hypothesis: little evidence that gene flow reduces fitness at range edges. *Trends in Ecology and Evolution*, *36*(6), 533–544. https://doi.org/10.1016/j.tree.2021.02.004

Kronenberg, Z. (2014). *Association testing with GPAT*. https://github.com/zeeev/vcflib/wiki/Association-testing-with-GPAT

La Sorte, F. A., & Jetz, W. (2010). Avian distributions under climate change: towards improved projections. *Journal of Experimental Biology*, *213*(6), 862–869. https://doi.org/10.1242/JEB.038356

La Sorte, F. A., & Thompson, F. R., III. (2007). Poleward shifts in winter ranges of North American birds. *Ecology*, *88*(7), 1803–1812. https://doi.org/10.1890/06-1072.1

Laurent, S., Pfeifer, S. P., Settles, M. L., Hunter, S. S., Hardwick, K. M., Ormond, L., Sousa, V. C., Jensen, J. D., & Rosenblum, E. B. (2016). The population genomics of rapid adaptation: disentangling signatures of selection and demography in white sands lizards. *Molecular Ecology*, *25*(1), 306–323. https://doi.org/10.1111/mec.13385

Le Corre, V., & Kremer, A. (1998). Cumulative effects of founding events during colonisation on genetic diversity and differentiation in an island and stepping-stone model. *Journal of Evolutionary Biology*, *11*(4), 495–512.

Li, H., & Durbin, R. (2010). Fast and accurate long-read alignment with Burrows-Wheeler transform. *Bioinformatics*, *26*(5), 589–595. https://doi.org/10.1093/bioinformatics/btp698

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R., & 1000 Genome Project Data Processing Subgroup. (2009). The sequence alignment/map format and SAMtools. *Bioinformatics*, *25*(16), 2078–2079. https://doi.org/10.1093/bioinformatics/btp352

Licona-Vera, Y., & Ornelas, J. F. (2017). The conquering of North America: dated phylogenetic and biogeographic inference of migratory behavior in bee hummingbirds. *BMC Evolutionary Biology*, *17*(1), 1–17. https://doi.org/10.1186/s12862-017-0980-5

Louthan, A. M., Doak, D. F., & Angert, A. L. (2015). Where and when do species interactions set range limits? *Trends in Ecology & Evolution*, *30*(12), 780–792. https://doi.org/10.1016/J.TREE.2015.09.011

Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.Journal*, *17*(1), 10. https://doi.org/10.14806/ej.17.1.200

McGuire, J. A., Witt, C. C., Altshuler, D. L., & Remsen, J. V. (2007). Phylogenetic systematics and biogeography of hummingbirds: Bayesian and maximum likelihood analyses of partitioned data and selection of an appropriate partitioning strategy. *Systematic Biology*, *56*(5), 837–856. https://doi.org/10.1080/10635150701656360

McGuire, J. A., Witt, C. C., Remsen Jr, J. V., Corl, A., Rabosky, D. L., Altshuler, D. L., & Dudley, R. (2014). Molecular phylogenetics and the diversification of hummingbirds. *Current Biology*, *24*(8), 910–916. https://doi.org/10.1016/j.cub.2014.03.016

Meisner, J., & Albrechtsen, A. (2018). Inferring population structure and admixture proportions in low-depth NGS data. *Genetics*, *210*(2), 719–731. https://doi.org/10.1534/genetics.118.301336

Miller, T. E. X., Angert, A. L., Brown, C. D., Lee-Yaw, J. A., Lewis, M., Lutscher, F., Marculis, N. G., Melbourne, B. A., Shaw, A. K., Szucs, M., Tabares, O., Usui, T., Weiss-Lehman, C., & Williams, J. L. (2020). Eco-evolutionary dynamics of range expansion. *Ecology*, *101*(10), e03139. https://doi.org/10.1002/ecy.3139

Myers, B. M., Burns, K. J., Clark, C. J., & Brelsford, A. (2021). The population genetics of nonmigratory Allen's Hummingbird (*Selasphorus sasin sedentarius*) following a recent mainland colonization. *Ecology and Evolution*, *11*(4), 1850–1865. https://doi.org/10.1002/ECE3.7174

Nei, M. (1972). Genetic distance between populations. *The American Naturalist*, *106*(949), 283–292.

Nielsen, R., Korneliussen, T., Albrechtsen, A., Li, Y., & Wang, J. (2012). SNP calling, genotype calling, and sample allele frequency estimation from new-generation sequencing data. *PLoS ONE*, *7*(7), e37558. https://doi.org/10.1371/journal.pone.0037558

Ortiz-Crespo, F. I. (1980). *Agonistic and foraging behavior of hummingbirds co-occurring in central coastal California*. University of California.

Overgaard Therkildsen, N., & Palumbi, S. R. (2017). Practical low-coverage genomewide sequencing of hundreds of individually barcoded samples for population and evolutionary genomics in nonmodel species. *Molecular Ecology Resources*, *17*(2), 194–208. https://doi.org/10.1111/1755-0998.12593

Pandit, P. S., Bandivadekar, R. R., Johnson, C. K., Mikoni, N., Mah, M., Purdin, G., Ibarra, E., Tom, D., Daugherty, A., Lipman, M. W., Woo, K., & Tell, L. A. (2021). Retrospective study on admission trends of Californian hummingbirds found in urban habitats (1991–2016). *PeerJ*, *9*, e11131. https://doi.org/10.7717/peerj.11131

Parmesan, C. (2006). Ecological and evolutionary responses to recent climate change. *Annual Review of Ecology, Evolution, and Systematics*, *37*, 637–669. https://doi.org/10.1146/annurev.ecolsys.37.091305.110100

Pavlidis, P., Živković, D., Stamatakis, A., & Alachiotis, N. (2013). SweeD: likelihood-based detection of selective sweeps in thousands of genomes. *Molecular Biology and Evolution*, *30*(9), 2224–2234. https://doi.org/10.1093/molbev/mst112

Peischl, S., Dupanloup, I., Kirkpatrick, M., & Excoffier, L. (2013). On the accumulation of deleterious mutations during range expansions. *Molecular Ecology*, *22*(24), 5972–5982. https://doi.org/10.1111/MEC.12524

Peischl, S., & Excoffier, L. (2015). Expansion load: recessive mutations and the role of standing genetic variation. *Molecular Ecology*, *24*(9), 2084–2094. https://doi.org/10.1111/mec.13154

Pespeni, M. H., & Palumbi, S. R. (2013). Signals of selection in outlier loci in a widely dispersing species across an environmental mosaic.

Molecular Ecology, *22*(13), 3580–3597. https://doi.org/10.1111/MEC.12337

Peter, B. M., & Slatkin, M. (2013). Detecting range expansions from genetic data. *Evolution*, *67*(11), 3274–3289. https://doi.org/10.1111/evo.12202

Peter, B. M., & Slatkin, M. (2015). The effective founder effect in a spatially expanding population. *Evolution*, *69*(3), 721–734. https://doi.org/10.1111/EVO.12609

Phillips, A. R. (1947). Status of the Anna Hummingbird in southern Arizona. *The Wilson Bulletin*, *59*(2), 111–113.

Phillips, B. L., Brown, G. P., & Shine, R. (2010). Life-history evolution in range-shifting populations. *Ecology*, *91*(6), 1617–1627.

Plummer, K. E., Siriwardena, G. M., Conway, G. J., Risely, K., & Toms, M. P. (2015). Is supplementary feeding in gardens a driver of evolutionary change in a migratory bird species? *Global Change Biology*, *21*(12), 4353–4363. https://doi.org/10.1111/GCB.13070

Pollock, J. J., Ware Carlisle, H., Hayes, H. M., & Robinson, B. W. (2021). Monitoring through community science: Anna's Hummingbird winter range expansion into Idaho. *Western Birds*, *52*, 58–67. https://doi.org/10.21199/WB52.1.4

Princé, K., & Zuckerberg, B. (2015). Climate change in our backyards: the reshuffling of North America's winter bird communities. *Global Change Biology*, *21*(2), 572–585. https://doi.org/10.1111/GCB.12740

Pritchard, J. K., Pickrell, J. K., & Coop, G. (2010). The genetics of human adaptation: hard sweeps, soft sweeps, and polygenic adaptation. *Current Biology*, *20*(4), R208–R215. https://doi.org/10.1016/J.CUB.2009.11.055

Puckett, E. E., & Munshi-South, J. (2019). Brown rat demography reveals pre-commensal structure in eastern Asia before expansion into Southeast Asia. *Genome Research*, *29*(5), 762–770. https://doi.org/10.1101/GR.235754.118

Rosenberg, K. V., Dokter, A. M., Blancher, P. J., Sauer, J. R., Smith, A. C., Smith, P. A., Stanton, J. C., Panjabi, A., Helft, L., Parr, M., & Marra, P. P. (2019). Decline of the North American avifauna. *Science*, *366*(6461), 120–124. https://doi.org/10.1126/SCIENCE.AAW1313

R Core Team. (2019). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/

RStudio Team. (2018). *RStudio: Integrated development environment for R*. RStudio, PBC. http://www.rstudio.com/

Rudeen, C., & Bassett, F. (2016). Apparent breeding by Anna's Hummingbird in Idaho. *Western Birds*, *47*, 237–241. https://doi.org/10.21199/WB47.3.6

Russell, S. M., & Russell, R. O. (2001). *The North American banders' manual for banding hummingbirds*. Point Reyes Station, CA.

Seutin, G., White, B. N., & Boag, P. T. (1991). Preservation of avian blood and tissue samples for DNA analyses. *Canadian Journal of Zoology*, *69*, 82–90. https://doi.org/10.1139/z91-013

Sexton, J. P., McIntyre, P. J., Angert, A. L., & Rice, K. J. (2009). Evolution and ecology of species range limits. *Annual Review of Ecology, Evolution, and Systematics*, *40*, 415–436. https://doi.org/10.1146/annurev.ecolsys.110308.120317

Simmons, A. D., & Thomas, C. D. (2004). Changes in dispersal during species' range expansions. *The American Naturalist*, *164*(3), 378–395.

Sorenson, M. D., Ast, J. C., Dimcheff, D. E., Yuri, T., & Mindell, D. P. (1999). Primers for a PCR-based approach to mitochondrial genome sequencing in birds and other vertebrates. *Molecular Phylogenetics and Evolution*, *12*(2), 105–114. https://doi.org/10.1006/mpev.1998.0602

Spence, A. R., LeWinter, H., & Tingley, M. W. (2022). Anna's hummingbird (*Calypte anna*) physiological response to novel thermal and hypoxic conditions at high elevations. *Journal of Experimental Biology*, *225*(10), jeb243294. https://doi.org/10.1242/JEB.243294

Spence, A. R., & Tingley, M. W. (2021). Body size and environment influence both intraspecific and interspecific variation in daily torpor use across hummingbirds. *Functional Ecology*, *35*, 870–883. https://doi.org/10.1111/1365-2435.13782

Streicher, J. W., McEntee, J. P., Drzich, L. C., Card, D. C., Schield, D. R., Smart, U., Parkinson, C. L., Jezkova, T., Smith, E. N., & Castoe, T. A. (2016). Genetic surfing, not allopatric divergence, explains spatial sorting of mitochondrial haplotypes in venomous coralsnakes. *Evolution*, *70*(7), 1435–1449. https://doi.org/10.1111/EVO.12967

Strubbe, D., Broennimann, O., Chiron, F., & Matthysen, E. (2013). Niche conservatism in non-native birds in Europe: niche unfilling rather than niche expansion. *Global Ecology and Biogeography*, *22*(8), 962–970. https://doi.org/10.1111/geb.12050

Szűcs, M., Vahsen, M. L., Melbourne, B. A., Hoover, C., Weiss-Lehman, C., & Hufbauer, R. A. (2017). Rapid adaptive evolution in novel environments acts as an architect of population range expansion. *Proceedings of the National Academy of Sciences*, *114*(51), 13,501–13,506. https://doi.org/10.1073/PNAS.1712934114

Tell, L. A., Hazlehurst, J. A., Bandivadekar, R. R., Brown, J. C., Spence, A. R., Powers, D. R., Agnew, D. W., Woods, L. W., & Engilis, A. (2021). Hummingbird (family Trochilidae) research: welfare-conscious study techniques for live hummingbirds and processing of hummingbird specimens. In *Museum of Texas Tech University Special Publication* (Vol. *76*). Texas Tech University Natural Science Research Laboratory. https://doi.org/10.1109/mspec.1976.6367467

Travis, J. M. J., Münkemüller, T., & Burton, O. J. (2010). Mutation surfing and the evolution of dispersal during range expansions. *Journal of Evolutionary Biology*, *23*(12), 2656–2667. https://doi.org/10.1111/J.1420-9101.2010.02123.X

Travis, J. M. J., Münkemüller, T., Burton, O. J., Best, A., Dytham, C., & Johst, K. (2007). Deleterious mutations can surf to high densities on the wave front of an expanding population. *Molecular Biology and Evolution*, *24*(10), 2334–2343. https://doi.org/10.1093/MOLBEV/MSM167

Trumbo, D. R., Epstein, B., Hohenlohe, P. A., Alford, R. A., Schwarzkopf, L., & Storfer, A. (2016). Mixed population genomics support for the central marginal hypothesis across the invasive range of the cane toad (*Rhinella marina*) in Australia. *Molecular Ecology*, *25*(17), 4161–4176. https://doi.org/10.1111/MEC.13754

Urban, M. C. (2015). Accelerating extinction risk from climate change. *Science*, *348*(6234), 571–573. https://doi.org/10.1126/SCIENCE.AAA4984

van Rossem, A. J. (1945). The eastern distributional limits of the Anna Hummingbird in winter. *The Condor*, *47*(2), 79–80.

Vandepitte, K., Helsen, K., van Acker, K., Mergeay, J., & Honnay, O. (2017). Retention of gene diversity during the spread of a non-native plant species. *Molecular Ecology*, *26*(12), 3141–3150. https://doi.org/10.1111/mec.14119

Vickrey, A. I., Bruders, R., Kronenberg, Z., Mackey, E., Bohlender, R. J., Maclary, E. T., Maynez, R., Osborne, E. J., Johnson, K. P., Huff, C. D., Yandell, M., & Shapiro, M. D. (2018). Introgression of regulatory alleles and a missense coding mutation drive plumage pattern diversity in the rock pigeon. *eLife*, *7*, e34803. https://doi.org/10.7554/ELIFE.34803

Wallingford, P. D., Morelli, T. L., Allen, J. M., Beaury, E. M., Blumenthal, D. M., Bradley, B. A., Dukes, J. S., Early, R., Fusco, E. J., Goldberg, D. E., Ibanez, I., Laginhas, B. B., Vila, M., & Sorte, C. J. B. (2020). Adjusting the lens of invasion biology to focus on the impacts of climate-driven range shifts. *Nature Climate Change*, *10*(5), 398–405. https://doi.org/10.1038/s41558-020-0768-2

Wang, E., van Wijk, R. E., Braun, M. S., & Wink, M. (2017). Gene flow and genetic drift contribute to high genetic diversity with low phylogeographical structure in European hoopoes (*Upupa epops*). *Molecular Phylogenetics and Evolution*, *113*, 113–125. https://doi.org/10.1016/j.ympev.2017.05.018

Weiss-Lehman, C., Hufbauer, R. A., & Melbourne, B. A. (2017). Rapid trait evolution drives increased speed and variance in experimental

range expansions. *Nature Communications*, *8*, 14303. https://doi.org/10.1038/ncomms14303

Williams, J. L., Hufbauer, R. A., & Miller, T. E. X. (2019). How evolution modifies the variability of range expansion. *Trends in Ecology and Evolution*, *34*, 903–913. https://doi.org/10.1016/j.tree.2019.05.012

Xu, H., Luo, X., Qian, J., Pang, X., Song, J., Qian, G., Chen, J., & Chen, S. (2012). FastUniq: a fast de novo duplicates removal tool for paired short reads. *PLoS ONE*, *7*(12), e52249. https://doi.org/10.1371/journal.pone.0052249

Yeaton, R. I., & Laughrin, L. (1976). Fall resource division in Santa Cruz Island hummingbirds. *The Wilson Bulletin*, *88*(2), 272–279.

Zhan, S., Zhang, W., Niitepõld, K., Hsu, J., Haeger, J. F., Zalucki, M. P., Altizer, S., de Roode, J. C., Reppert, S. M., & Kronforst, M. R. (2014). The genetics of monarch butterfly migration and warning colouration. *Nature*, *514*(7522), 317–321. https://doi.org/10.1038/nature13812

Zimmerman, D. A. (1973). Range expansion of Anna's Hummingbird an inquiry into the extent, chronology, and possible causes of a striking recent phenomenon in distribution. *American Birds*, *27*(5), 827–835.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Adams, N. E., Bandivadekar, R. R., Battey, C. J., Clark, M. W., Epperly, K., Ruegg, K., Tell, L. A., & Bay, R. A. (2023). Widespread gene flow following range expansion in Anna's Hummingbird. *Molecular Ecology*, *32*, 3089–3101. https://doi.org/10.1111/mec.16928