On the brink of explosion? Identifying the source and potential spread of introduced *Zosterops* white-eyes in North America

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Abstract

Understanding the source of non-native introduced populations is crucial for forecasting geographic invasion potential and understanding the ecological consequences of potential establishment. Here we use genomics to identify the source populations and invasion dynamics of two non-native introduced populations from the iconic avian lineage of ‘great speciators’ known as white-eyes (genus *Zosterops*). We established confidently for the first time that introduced *Zosterops* populations in Hawaii and southern California are completely unrelated and derived from independent introductions of the species *Z. japonicus* and *Z. simplex*, respectively. We used descriptive population genetic statistics to identify a reduction in genetic diversity and increase in private alleles in the southern California population, supporting a recent, potentially ongoing genetic bottleneck in this population. In contrast, the introduced population in Hawaii showed no such characteristics, likely due to a larger founding population size and repeated introductions in this intentionally introduced population. Ecological niche modeling indicated that there is little environmentally suitable habitat for *Z. simplex* across the continent of North America, suggesting limited invasion potential, assuming niche conservatism. Yet, portions of the introduced *Z. simplex* population have already surpassed areas projected as suitable, likely because the urbanized environment of southern California offers biotic resources and microhabitats that are not captured by our model. Because it appears to have overcome the ‘invasion paradox’ of low founding genetic diversity and established despite relatively unfamiliar environmental conditions in southern California, we suggest *Z. simplex* may continue expanding in beyond our environmental niche model projections in other temperate, urban regions.

Introduction

Dispersal and colonization have long been recognized as crucial aspects in the speciation process, and more broadly the accumulation of biodiversity in ecosystems across the globe (Diamond et al., 1976; Mayr, 1942). Within the past few millennia, human-aided population dispersal, facilitated by the advent of tool-aided human movement across the globe (Wilson et al., 2009), has become increasingly recognized as a force shaping the ecology and evolution of every corner of the world (Hulme, 2009). Some of these introductions were intentional, for example as food sources for European colonists for the last five centuries (Crosby, 2004; Osborne, 2000), for cultural reasons associated with European colonialism (e.g., acclimatization), or more recently as attempted bio-control of native pest species (e.g., cane-toads; Mungomery, 1935). Other introductions were unintentional, when animals were moved unknowingly (e.g., rats on islands; Harper & Bunbury, 2015) or released accidentally (e.g., naturalized parrots across the globe; Pruett-Jones, 2021). Regardless of the original intentions of these human-mediated introductions, once a non-native species is introduced, it may become established and gain access to novel resources (Belnap et al., 2012), and enemy release (i.e., escape from predators and parasites limiting the species in its native range; Roy et al., 2011). These factors can facilitate rapid population expansion in the novel environment, with potential ecosystem-wide consequences for native species which may face increased competition (K. E. Barton & Fortunel, 2023), predation (Roemer et al., 2002), or toxicity (Okamiya et al., 2021), leading the introduced species to be dubbed as ‘invasive’ (Blackburn et al., 2014). Overall, invasive species have been implicated as one of the main factors threatening worldwide biodiversity (Clavero & García-Berthou, 2005; Roy et al., 2023).

In addition to these well-documented environmental consequences, there can also be economic costs associated with invasive species (Colautti et al., 2006; Linz et al., 2007). Therefore, both conservation and economic interests may align in efforts to identify introduced populations with invasion potential and mitigate their potential spread (Pimentel et al., 2005). Previous examples of this cooperation include efforts to eradicate: cane toads (*Rhinella marina*) in Australia (Greenlees et al., 2018), Burmese pythons (*Python bivittatus*) in Florida (Avery et al., 2014), and European Starlings (*Sturnus vulgaris*) at feed lots across the United States (Linz et al., 2018; Linz et al., 2007). This type of effort to mitigate the potential environmental and economic effects of an invasive population requires knowledge of the source of the introduction, in order to forecast the potential spread of the introduced population, and understand the potential ecological consequences of its establishment (Aagaard & Lockwood, 2016; Strayer et al., 2017). But, confidently identifying the source of an introduced population may be difficult in the absence of historical records, and these difficulties can be compounded if putative source populations have uncertain or confused taxonomic histories (Gotzek et al., 2012; Mazzamuto et al., 2016). In these cases, DNA sequencing can be used to confidently determine the genome-wide ancestry of individuals from the introduced population (Filipová et al., 2011; Mittan-Moreau et al., 2022; Sharaf et al., 2020). Only once the source of an introduced population is confidently identified can we begin to understand its genetic and ecological dynamics and the potential ecosystem-wide consequences of its establishment (Hudson et al., 2021; Kumar et al., 2015).

Abiotic and biotic factors limit the distributions of populations both in their native and introduced ranges. One particular approach, ecological niche modeling (ENM), has emerged as a useful tool in understanding distributional potential (Peterson et al., 2011). This approach works via characterizing associations between species' occurrences and variation in environmental parameters. It assumes that the actual distribution of a population is a function of three interacting constraints: the fundamental ecological niche, interactions with other organisms, and dispersal capability (Soberón & Peterson, 2005). Early explorations (Peterson, 2003; Peterson & Vieglais, 2001) illuminated the possibility that model transfers (Yates et al., 2018) from native to introduced areas could anticipate the geographic invasion potential of a population. Indeed, once source populations have been identified rigorously (Lee, 2002), ENM approaches can be used to assess the ecological niche and geographic potential of introduced populations on any novel landscape, subject of course to the degree of analogy in environmental characteristics between the landscapes in question (Owens et al., 2013).

An introduced population that has received limited attention is a population of birds in the genus *Zosterops* (colloquially known as 'white eyes'), which have recently become established in coastal southern California (Garrett, 2018). This is in contrast with the well-studied introduced population of *Z. japonicus* on the Hawaiian archipelago (Venkatraman et al., 2021). While the putative *Z. japonicus* population in Hawaii was intentionally introduced from Japan by the Hawaii Board of Agriculture and Forestry in 1929 (Caum, 1933), there have been no documented intentional introductions of *Zosterops* in southern California. In fact, there was a successful effort to eradicate an established population of *Z. palpebrosus* that likely escaped from the San Diego Zoo and began breeding in the southern California area in the early 1980s (Unitt & Klovstad, 2004). This history, plus the fact that *Zosterops* white-eyes are routinely identified as some of the most common species sold in the cagebird trade globally (Chng et al., 2018; Eaton et al., 2017), strongly suggests the pet trade, via escape or intentional release, as the likely origin of the current day southern California population.

The genus *Zosterops* is known for harboring one of the highest speciation rates of all birds, containing over 100 species found in Africa, Asia, and Australia, and all estimated to have diverged within the past 2 million years (Moyle et al., 2009). These birds are also known as exceptional dispersers, with apparent long-distance dispersal and colonization events to far flung islands throughout the Pacific within their recent evolutionary history (Estandía et al., 2023; Vinciguerra et al., 2023). Because of this explosive speciation history, evidence for interspecies gene flow (Gwee et al., 2020; Oatley et al., 2017), and the overall conserved morphology of the genus (i.e., over 100 species that all look roughly like a small gray, green, and yellow songbird), the taxonomy of the *Zosterops* white-eyes has been plagued by ongoing uncertainty and phylogenetic recalcitrance (Lim et al., 2019). For this reason, the exact species identity of the introduced *Zosterops* population in southern California remains uncertain, and the genomic ancestry of the putative *Z. japonicus* population in Hawaii has never been confirmed with comprehensive sampling of potential source populations.

Here, we use a reduced representation (i.e., RADseq) approach to sequence thousands of genomic loci from samples across the native ranges of six putative source species (*Z. japonicus*, *Z. simplex*, *Z. palpebrosus*, *Z. erythropleurus*, *Z. everetti*, and *Z. nigrorum*) from the ‘asiatic *Zosterops*’ clade (Vinciguerra et al., 2023) and individuals from the introduced populations in Hawaii and southern California, to facilitate the first confident determination of the genomic ancestry of these populations. This information will be a crucial resource for future studies attempting to study the eco-evolutionary consequences of translocation and establishment of introduced species (e.g., Gleditsch & Sperry, 2019; Mathys & Lockwood, 2011; Sendell-Price et al., 2020). Further, these genomic sequence data will allow us to search for signatures of genetic bottlenecks (e.g., reduced genetic diversity) and founder effects associated with the establishment of these introduced populations. Invasive populations regularly establish and spread despite limited genetic diversity (the ‘invasion paradox’; Estoup et al., 2016; Kolbe et al., 2004), and comparisons between two introduced populations of these ‘great speciators’ may help to understand whether and how the invasion paradox applies in rapidly evolving taxa (Diamond et al., 1976; Moyle et al., 2009). Finally, confident identification of the source of the introduced *Zosterops* population in southern California will allow us to use ecological niche modeling approaches to project the geographic invasion potential of this rapidly expanding population. The results of these investigations will have important implications for understanding both the invasion potential of Zosterops in North America and for understanding the eco-evolutionary consequences of the founding of novel populations more generally.

Methods

Visualizing genetic sampling and species distributions

We used the R (R Core Team, 2019) packages *sf* v1.0-12 (Yates et al., 2018) and *maps* v3.4.1 (Brownrigg, 2013) to visualize the distributions of all six focal *Zosterops* species (*Z. japonicus*, *Z. simplex*, *Z. palpebrosus*, *Z. erythropleurus*, *Z. everetti*, and *Z. nigrorum*) and the two focal introduced populations. Here we treat *Z. japonicus* as encompassing the contentious taxa *Z. montanus* and *Z. meyeni* (Lim et al. 2019) for the sake of taxonomic simplicity. Although we identify phylogeographic structure that generally corresponds with the proposed limits of *Z. montanus* and *Z. meyeni* within this broadly defined *Z. japonicus* clade, because it is not the focus of this paper, we do not address the phylogeographic and taxonomic implications of these results. Instead we refer to clades within *Z. japonicus* strictly by geography, to minimize taxonomic confusion.

We plotted occurrence localities on these maps according to sample size and species identity using *ggplot2* v3.4.1 (Wickham et al., 2020), using a colorblind-friendly palette that is maintained throughout the manuscript. We manually outlined the breeding distribution for each species to contextualize the geographic extent of our genetic sampling. We also accessed annual distribution maps for the introduced *Zosterops* population in southern California over the past 20 years using eBird (Sullivan et al., 2009; www.ebird.org). Code for mapping localities can be viewed here: https://devonderaad.github.io/zosterops.rad/zosterops.mapping.html.

DNA extraction, library preparation, and sequencing

We extracted DNA from 155 tissue/blood samples, including samples from the two introduced *Zosterops* populations in Hawaii (collected in 2002) and southern California (collected 2016-2022), plus comprehensive geographic sampling of potential source populations from across East and Southeast Asia and the Philippines. The University of Kansas Genomic Sequencing Core performed RADseq library preparation according to protocols outlined in Manthey and Moyle (2015). Our protocol involves digesting each DNA extract with the enzyme NdeI and performing size selection for fragments in the range of 495-605 base-pairs. The entire library prep protocol, including detailed results for this set of samples, is available here: https://github.com/DevonDeRaad/zosterops.rad/blob/main/lab.protocols/MSG-NdeI_2plates-150samples_DAD-Moyle_230530.doc. These pooled, barcoded libraries were then sequenced on an Illumina NextSeq2000 machine, using a P2 flow cell to generate 414,215,817 single-end 100 base-pair reads, i.e., ~41 giga-base-pairs of raw sequence data.

Read mapping, variant calling, and quality filtering

In general, we followed the RADseq mapping and filtering pipeline detailed in DeRaad et al., (2023). In specific, we demultiplexed the raw sequence data using the ‘process_radtags’ function from *Stacks* v2.41 (Rochette et al., 2019), removing low quality reads (any bases with phred score <10), and reads with uncalled bases. We then mapped the raw reads from each individual sample to a publicly available *Z. japonicus* reference genome assembly (Venkatraman et al., 2021; available at https://www.ncbi.nlm.nih.gov/assembly/GCA_017612475.1), using the command ‘mem’ from *BWA* v0.7.17 (Li & Durbin, 2009). We then converted each .sam file to a sorted .bam file using *SAMtools* v1.3.1 (Li et al., 2009). We used the *Stacks* module ‘gstacks’ to identify RAD loci using a sliding window algorithm and to call individual genotypes, and the *Stacks* module ‘populations’ to output an unfiltered set of sample genotypes as a variant call format (vcf) file. This generated an unfiltered SNP dataset containing 236,767 bi-allelic SNPs shared among 155 unique samples, with 65.5% missing genotypes.

We used the R packages *vcfR* v1.14.0 (Knaus & Grünwald, 2017) and *SNPfiltR* v1.0.1 (DeRaad, 2022) to interactively visualize key parameter distributions and implement optimized quality filters on this SNP dataset. First, we implemented a hard filter, recoding genotypes with a sequencing depth of <3 reads or genotype quality <30 as missing data. We then recoded heterozygous genotypes where the ratio of read counts between the two alleles was <0.1 or >0.9 as missing. We also removed SNPs with a mean genotype depth >250 from the dataset, as these SNPs likely have artificially inflated depth of coverage from the mapping of multiple, paralogous RAD loci to the same place in the reference genome. We then set a maximum threshold of 90% missing genotypes to allow a sample to be included in downstream analyses, resulting in removal of 25 samples from the dataset. Upon further investigation, we removed a further 6 samples that consistently displayed a high proportion of missing genotypes even after implementing exploratory per-SNP missing data filters.

For the remaining 124 samples (Table S1; Fig. 1), we constructed a phylogenetic network to visualize sample relatedness at a 99% per-SNP completeness threshold (789 retained SNPs). We compared the same phylogenetic network constructed under a 90% per-SNP completeness threshold and found similar inference of sample relatedness with no noticeable variation attributable to the inclusion of missing genotypes. We implemented this 90% per-SNP completeness threshold, which appeared to optimize the trade-off between genotype completeness and total number of SNPs retained, resulting in a filtered SNP dataset of 15,704 SNPs shared across 124 unique samples with 5.3% missing genotypes. Finally, we removed SNPs within a physical distance of <1,000 base-pairs, resulting in a filtered, unlinked SNP dataset comprising 1,554 SNPs shared across 124 unique samples with 5.5% missing genotypes. In both datasets, no individual sample retained for downstream analyses was missing >40% of genotype calls. The entire SNP filtering process along with exploratory data visualizations used to optimize these filtering thresholds can be followed in detail at https://devonderaad.github.io/zosterops.rad/zost.radseq.filtering.html.

Population genetic analysis

To visualize our SNP data as an unrooted phylogenetic network, we used our filtered SNP dataset (15,704 SNPs, 5.3% missing data) as input for the R package *StAMPP* v1.6.3 (Pembleton et al., 2013) and constructed a pairwise genetic distance (Nei's D; Nei, 1972) matrix among the 124 samples. We used this distance matrix as input in *SplitsTree4* v4.15.1 (Huson & Bryant, 2006), where we constructed a neighbor-net, with a single tip corresponding to each sample. This approach allows the simultaneous inference of distance-based phylogenetic relationships among clades and informative placement of admixed individuals with ancestry strongly affected by non-tree-like processes. We color-coded all tips according to species assignment and highlighted putative hybrid individuals. Code and resulting phylogenetic networks can be viewed at https://devonderaad.github.io/zosterops.rad/splitstree.html.

We next used our filtered, unlinked SNP dataset (1,554 SNPs, 5.5% missing data) as input for *ADMIXTURE* v.1.3.0 (Alexander et al., 2009) to assign all samples to bins of genomic ancestry using a model-based framework without the need for *a priori* sample assignments. We performed 10 successive *ADMIXTURE* runs, iteratively increasing the number of genomic ancestry bins (*K*) used to describe these 124 samples from 1-10. We specified the '--cv' flag to perform fivefold cross-validation across these iterations. We identified the optimal value for *K* as the value that resulted in the lowest cross-validation error out of our 10 runs.

Because of signals of hierarchical structure (i.e., samples from the sister species *Z. everetti* and *Z. nigrorum* consistently assigned to a single genomic ancestry bin despite visually apparent genetic structure in the phylogenetic network), we downsampled our unlinked filtered SNP dataset to retain only the 7 samples from the species *Z. everetti* and *Z. nigrorum*. We then removed SNPs that had become invariant owing to sample removal, resulting in 402 putatively unlinked SNPs with 4.0% missing data across the 7 samples. We performed an identical set of *ADMIXTURE* runs, as described above, using this downsampled SNP dataset as input, and plotted the $K = 2$ result to assess visually the degree of overlap in genomic ancestry between these species without the confounding effects of hierarchical structure present in the complete dataset. This entire *ADMIXTURE* procedure including all code necessary to reproduce these results is available at https://devonderaad.github.io/zosterops.rad/zosterops.admixture.html.

To quantify the degree of relative differentiation among our focal species, we calculated pairwise F_{ST} values for all possible among-species comparisons. We removed the two samples identified as putative hybrids in our *ADMIXTURE* investigation from our filtered SNP dataset (15,704 SNPs, 5.3% missing data), and then assigned the species identity of each of the remaining 122 samples based on ancestry assignments from *ADMIXTURE*. We used the *StAMPP* function ‘stamppFst’ to calculate pairwise F_{ST} values between these six species, and *ggplot2* to visualize the results as a heatmap. We also assigned samples within *Z. japonicus* into three separate geographically delimited clades following the *ADMIXTURE* ancestry assignments at $K = 6$ and calculated pairwise F_{ST} among these three groups using the same procedure. This entire process for calculating pairwise differentiation from an input vcf file can be viewed and reproduced at https://devonderaad.github.io/zosterops.rad/zosterops.pairwise.fst.html.

After confidently determining the species-level ancestry of these introduced populations (see Figures 1 and 2), we then performed detailed clustering analyses on subsets of the dataset to determine the exact sampling localities with the most genetic similarity to each introduced population using principal components analysis (PCA) via the R package *adegenet* 2.1.10 (Jombart, 2008). First, to investigate the source of the introduced population in Hawaii, we subset our unlinked filtered SNP dataset to only samples with >95% ancestry assigned to the northern *Z. japonicus* clade in the *ADMIXTURE* analysis. We then removed SNPs that became invariant and performed PCA retaining the first two axes of variation. We repeated this same procedure with individuals assigned >95% *simplex* ancestry to investigate the putative geographic source of the southern California introduction. For each of these subsets, we labeled each sample in the PCA based on sampling locality.

We then used each of these unique sampling localities described in the previous paragraph (22 for *Z. japonicus* and 13 for *Z. simplex*) to search for molecular signatures of ongoing population bottlenecks by comparing the genetic diversity of introduced populations to the diversity of conspecific populations sampled from the native range of each species. We used the *Stacks* 'populations' module to estimate per sample heterozygosity and per population nucleotide diversity (Pi) across all sites (including invariant sites) that mapped to the reference genome before applying any filtering criteria. We intentionally used this unfiltered dataset to calculate genetic diversity metrics to help to ameliorate the inherent biases introduced by the idiosyncratic process of filtering SNP datasets. Using an unfiltered dataset including all invariant sites is explicitly endorsed as resulting in more robust and comparable estimates of parameters describing the genetic diversity of natural populations (Shafer et al., 2017). We also visualized the number of unique (i.e., private) alleles present in each of the sampling localities, which is calculated as part of the standard suite of descriptive genetic diversity metrics by the *Stacks* 'populations' module. All of these analyses are available at https://devonderaad.github.io/zosterops.rad/investigate.introductions.html.

Data Preparation for Ecological Niche Modeling

For occurrence data, we downloaded all data corresponding to *Z. simplex* in the GBIF biodiversity data portal (GBIF, 2023). Occurrence data were filtered to correspond only to the dates 15 May - 15 June in any year from 2000 to present, a conservative estimate of breeding occurrences. We removed one record under the name *Z. simplex salvadorii*, putative introduced records from the United States and Mexico, exact duplicate records, and records with no associated geographic coordinates or coordinate uncertainty >10,000 m. We manually checked the geographic distribution of occurrences against detailed descriptions in Avibase for each species (GBIF, 2023). We then assessed spatial clumping, as a way of reducing effects of spatial autocorrelation on model results, exploring records filtered to one per raster pixel, and records spatially filtered using distances of 10, 20, 50, and 100 km. We tested spatial autocorrelation via Moran's *I* for all sets of filtered records (Table S2). We kept the set of 119 records resulting from spatial thinning at 100 km considering that the effect of increasing distances did not change spatial autocorrelation substantially, but spatial clustering of points was not evident with this distance filter.

We summarized climatic landscapes via the MERRAClim dataset (Vega et al., 2017), downloading the most current version from https://datadryad.org/stash/dataset/doi:10.5061/dryad.s2v81. We used the 5' mean-value dataset for the 2000s (i.e., 2000-2010). Because MERRA data are not clipped to coasts, we used the China_ADM coverages from DivaGIS (https://www.diva-gis.org/) to identify records that show up in the ocean because of minor disagreements about coastline shape between data sources. We used these variables for M simulation analyses (see below). We performed a principal component analysis (PCA) with these variables to reduce dimensionality and avoid multicollinearity. The first five principal components (accounting for ~99% of the total variance; Tables S3-S4) were used for ecological niche modeling (see below).

Ecological Niche Model Development

A crucial initial step in developing ecological niche modeling is that of establishing an appropriate area for model calibration (i.e., 'M', the area accessible to the species; Owens et al., 2013). We approximated M using the spatially thinned records, the variables from the

MERRAClim database and the *grinnell* R package v0.0.21 (Machado-Stredel et al., 2021). We used a simulation extent of 10°S to 80°N latitude, and 50°E to 135°E longitude, masked with the GADM world country layer at the highest resolution. We parameterized the simulations as "normal" distribution of the dispersal kernel, with standard deviations of 0.5, 1.0, 3.0, 5.0, numbers of dispersal events of 75, 150, and 300, and a barrier corresponding to Wallace's Line. Our selected M area used a kernel SD of 3, 150 dispersal events, and the other features mentioned above. We created a buffer of 30 km around this final M and clipped it with the world country layer from GADM.

For model preparation, we masked each of the five PC variables to the extent of M. We assembled all possible combinations of $\geq$2 variables, for a total of 26 sets. We divided the 119 filtered occurrence points into two subsets: 70% for model calibration and 30% for testing and comparing candidate models. We assessed regularization multiplier values of 0.1, 0.3, 0.5, 0.7, 0.9, 1.0, 2.0, 3.0, 4.0, 5.0, and feature classes including "q," "p," "lq," "lp," "qp," and "lqp" (where l = linear models, q = quadratic models, and p = product-based models). The total number of candidate models was 1560. We evaluated performance of candidate models on statistical significance of predictions (partial ROC; Peterson et al., 2008), omission rate (allowing a 5% omission error; Anderson et al., 2003), and model fit and complexity (based on the Akaike information criterion corrected for small sample sizes; Warren & Seifert, 2011). We produced final models using the parameter settings of candidate models that performed the best, with 10 replicates, and cloglog outputs. We projected these models to both the native and introduced ranges of *Z. simplex*.

To understand where model outcome interpretations are risky due to the presence of conditions in transfer areas non-analogous to those in the calibration area, we used the MOP metric (Owens et al., 2013). We downloaded spatial data using the R package *geodata* v.0.5.8 (Hijmans et al., 2023) and all spatial analyses were done using the R package *terra* v1.7.29 (Hijmans, 2023). Ecological niche modeling routines were run using the package *kuenm* 1.1.10 (Cobos et al., 2019), using Maxent (Phillips et al., 2017) as the modeling algorithm. We performed the MOP analysis using the package *mop* 0.1.1 (Cobos et al., 2023). Commented code to perform data preparation and ecological niche modeling is available at: https://devonderaad.github.io/zosterops.rad/zosterops_ENM.html.

Results

Population genetics

A distance-based phylogenetic network describing relatedness among the 124 samples that passed filtering protocols revealed evidence for deep phylogenetic structure corresponding in largest part to the six focal species that we targeted with nearly comprehensive geographic sampling (Fig. 1A/B). The branching order in this network recapitulates known phylogenetic relationships (Gwee et al., 2020; Vinciguerra et al., 2023), including the sister relationship between *Z. everetti* and *Z. nigrorum*, and the sister relationship between *Z. simplex* and *Z. japonicus* (Fig. 1B). Within *Z. japonicus*, geographic substructuring was apparent, with a clear phylogeographic break separating populations in the Japanese archipelago and Korean peninsula from the rest of the clade.

Individuals sampled from the introduced southern California population formed a clade nested within a larger clade containing all *Z. simplex* samples. On the other hand, individuals sampled from the introduced population in Hawaii were scattered throughout the clade containing the Japanese and Korean *Z. japonicus* populations. Out of 124 samples, only two could not be assigned confidently to a clade corresponding to one of the six focal species; these two samples were considered as putative hybrids and were clearly identified as such in downstream analyses (see below).

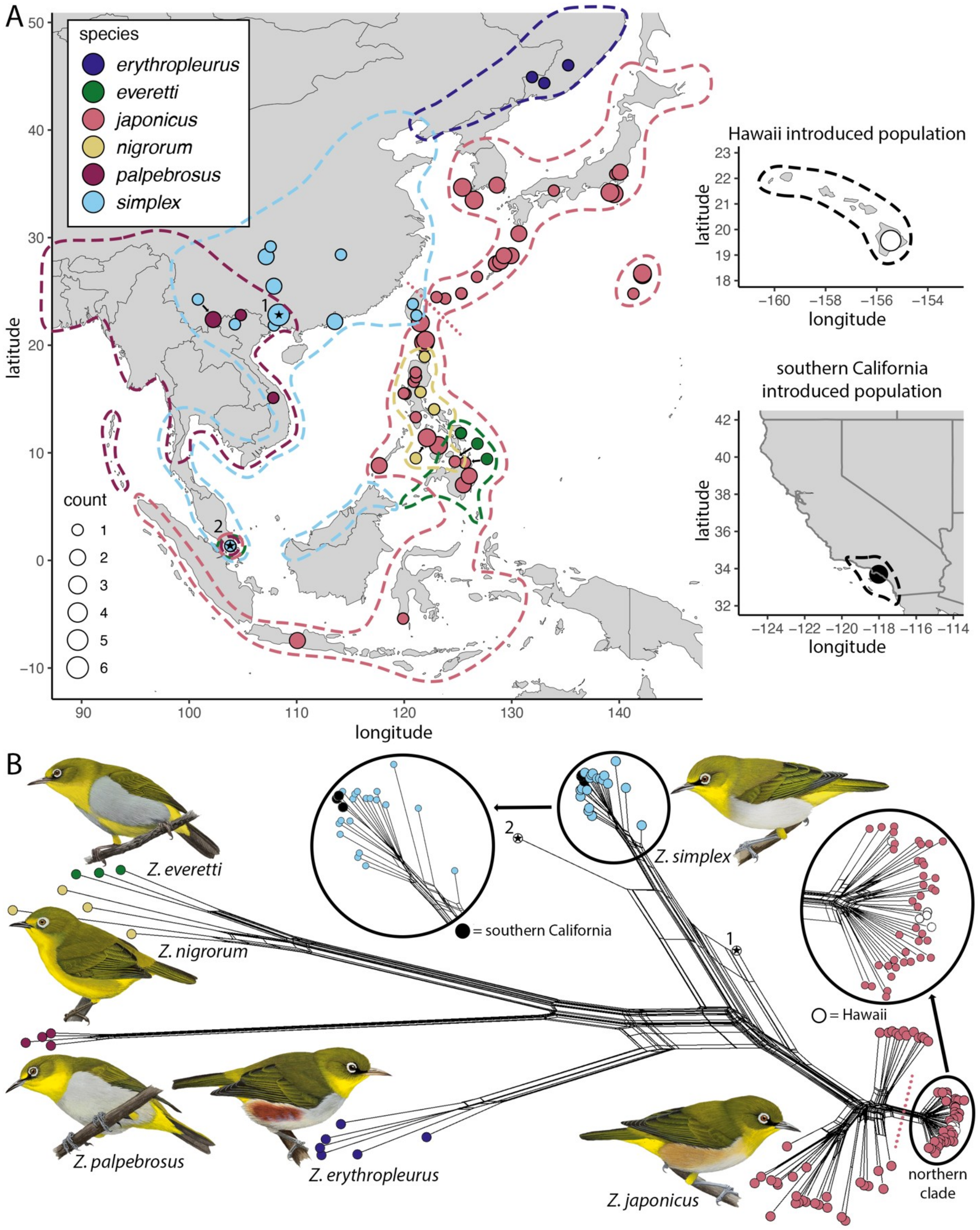


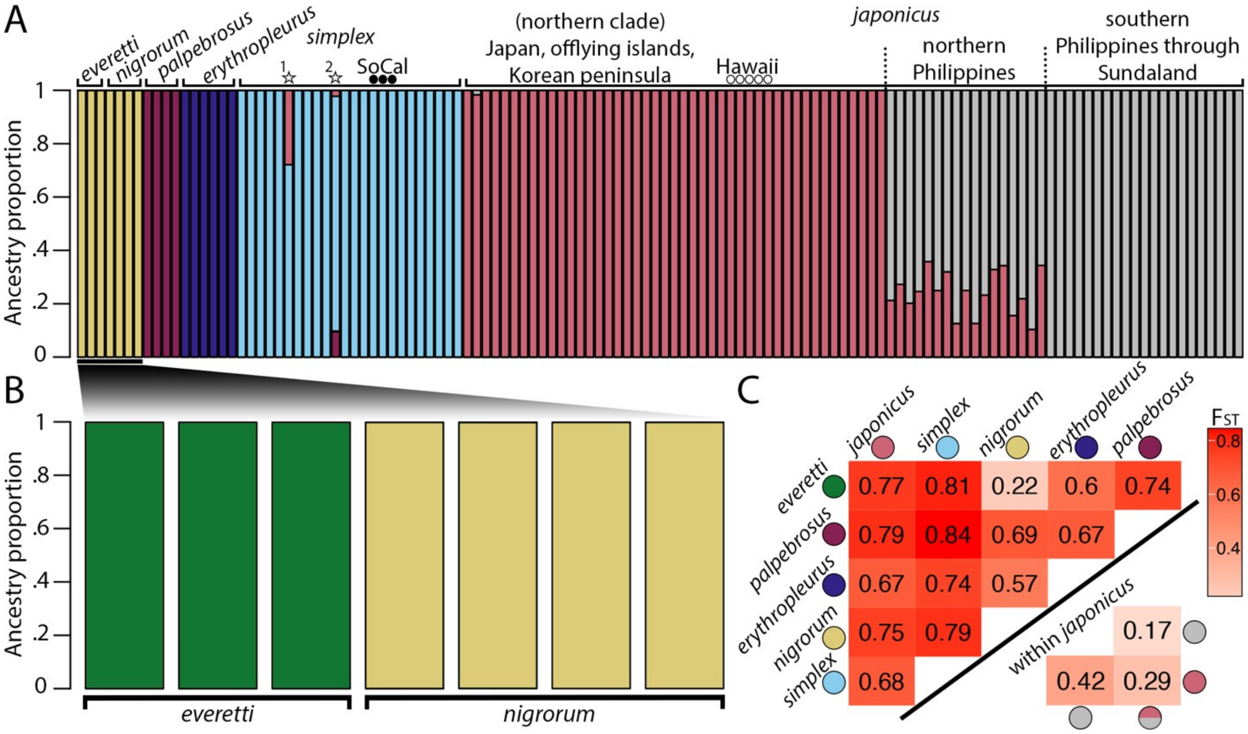
Figure 1. Connecting species distributions with genetic structure. (A) Map showing the sampling scheme for our RADseq dataset, in which each dot denotes species identity by dot color and sample size by dot size in the filtered SNP dataset (124 total samples). The breeding distribution

of each of the six focal species is enclosed by a color-coded dashed line. The breeding distribution of *Z. erythropleurus* continues slightly to the north, beyond the extent of this map, and the breeding distribution of *Z. palpebrosus* extends west across most of India. A single sampling locality for three *Z. erythropleurus* individuals collected from their wintering distribution in northern Vietnam is not shown for the sake of clarity. The introduced *Zosterops* populations of interest in Hawaii and southern California are each shown with distinct inset maps. The red dotted line between the southernmost islands of the Ryukyu archipelago and Taiwan indicates the location of the phylogeographic break identified within *Z. japonicus*. (B) Phylogenetic network showing relationships among all 124 individual samples passing filtering protocols. Individual tips are color-coded according to species identity. Two putative hybrid individuals that could not be confidently assigned to a specific clade are labeled with numbered stars corresponding to the sampling map.

Assignment of genetic background into the optimal number ($K = 6$) of predefined genomic ancestry bins using the program *ADMIXTURE* separated our six focal species, except that *Z. everetti* and *Z. nigrorum* were assigned to a single bin of genomic ancestry, while clades within *Z. japonicus* were assigned to two discrete bins of genomic ancestry. This result is corroborated by evidence that relative differentiation between clades within *Z. japonicus* (maximum pairwise $F_{ST} = 0.42$) is greater than relative differentiation between recognized species *Z. everetti* and *Z. nigrorum* (pairwise $F_{ST} = 0.22$; Fig. 2C). A subsequent *ADMIXTURE* analysis restricted to only *Z. everetti* and *Z. nigrorum* revealed no evidence for ancestry sharing among sampled individuals (Fig. 2B).

Of the 122 samples not identified as putative hybrids in the phylogenetic network, none were assigned >1% interspecies ancestry. This includes the samples from Hawaii and southern California, all of which were assigned >99% *Z. japonicus* and >99% *Z. simplex* ancestry, respectively (Fig. 2A). The putative hybrid sample from China was assigned 72.1% *simplex* ancestry and 27.9% *Z. japonicus* ancestry, whereas the putative hybrid sample from Singapore was assigned 88.0% *Z. simplex* ancestry, 9.4% *Z. palpebrosus* ancestry, and 2.2% *Z. japonicus* ancestry. All pairwise F_{ST} comparisons between recognized species (Fig. 2C), except the *Z.*

nigrorum / *Z. everetti* comparison, indicate high relative divergence (range 0.57 - 0.84). These results are concurrent with the well-described propensity for rapid differentiation and speciation among geographically isolated lineages of *Zosterops* white-eyes.



	japonicus	simplex	nigrorum	erythropleurus	palpebrosus
everetti	0.77	0.81	0.22	0.6	0.74
palpebrosus	0.79	0.84	0.69	0.67	
erythropleurus	0.67	0.74	0.57		
nigrorum	0.75	0.79			
simplex	0.68				

Figure 2. Population genetics of introduced and putative source Asiatic *Zosterops* populations. (A) Ancestry assignments from *ADMIXTURE* for an optimal $K = 6$. Individual vertical bars (i.e., samples) are labeled according to species identity, with introduced populations and putative hybrids labeled as in Figure 1. Southern California abbreviated as 'SoCal'. (B) *ADMIXTURE* ancestry assignments for only *Z. everetti* and *Z. nigrorum* samples reveal no ancestry sharing. (C) The upper diagonal shows pairwise F_{ST} (a measure of relative genetic divergence ranging from 0-1) comparisons among the six focal species (putative hybrid samples removed). Below the diagonal are pairwise F_{ST} comparisons among the three geographically structured *Z. japonicus* sub-populations identified in panel A. The red dot corresponds to the northern clade, the gray and red dot to the northern Philippines clade, and the gray dot to the southern Philippines through Sundaland clade.

Fine-scale structure and population dynamics

Principal components analysis revealed that individual birds from the introduced *Zosterops* population in Hawaii are genomically indistinguishable from *Z. japonicus* individuals living on mainland Japan and the Korean Peninsula (sampling sites 2-6, 9, and 10; Fig. 3A/B), which is consistent with the reported intentional introduction of birds from mainland Japan to the Hawaiian Islands (Caum, 1933). Samples from offshore islands east of 135° longitude (sites 7, 8, 15, 16, and 20) clustered discretely on PC1, while islands to the west of that line clustered separately on PC2 (except site 10). Meanwhile, measurements of heterozygosity and Pi indicated that the introduced Hawaii population contains comparable numbers of private (i.e., unique) alleles and levels of genetic diversity to populations from the native range of *Z. japonicus* (Fig. 3C/D). In sum, these metrics indicate little evidence for meaningfully different population dynamics between the introduced Hawaiian population and conspecific populations from the native range of *Z. japonicus*.

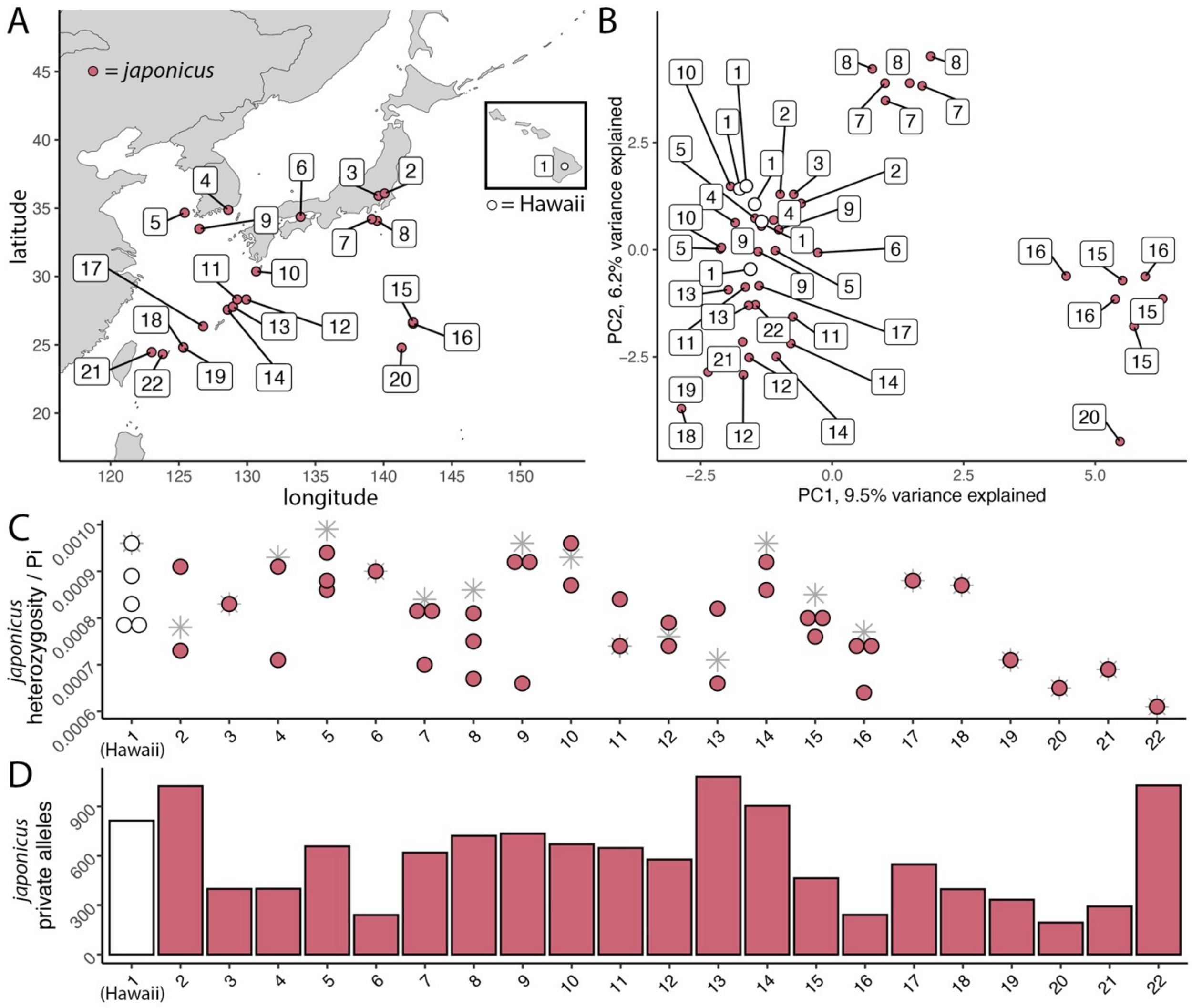


Figure 3. Comparing the genomes of introduced *Z. japonicus* in Hawaii with putative source populations from the species' native range. (A) Map showing all sampling localities for the northern *Z. japonicus* clade, with each sampling locality numbered arbitrarily but consistently across panels. (B) Principal components analysis (PCA) reveals fine-scale genomic structure within all sampled populations from the northern *Z. japonicus* clade. (C) Plots of heterozygosity per sample (colored dots) and overall nucleotide diversity per sampling locality (Pi; denoted with a gray asterisk) for the northern *Z. japonicus* clade, including the introduced population in Hawaii. (D) The number of private (i.e., unique) alleles identified in each sampling locality, calculated from all successfully mapped genomic loci.

In contrast, samples from the introduced southern California population form a distinct cluster in two-dimensional genomic space relative to all other sampled conspecific *Z. simplex* individuals (Fig. 4A/B), supported by a modest pairwise F_{ST} value of 0.083. The single sample from locality 13, located in the far southern extreme of the *Z. simplex* distribution, clustered discretely on PC1 (Fig. 4B). Further, samples from the introduced population in southern California rank among the lowest levels of genome-wide heterozygosity of all sampled *Z. simplex* individuals (Fig. 4C). Additionally, this introduced population had lower overall nucleotide diversity (Pi) than any locality sampled from the native range of *Z. simplex*. This introduced southern California population also possesses a greater number of private alleles than any native population of *Z. simplex* that we sampled (Fig. 4D).

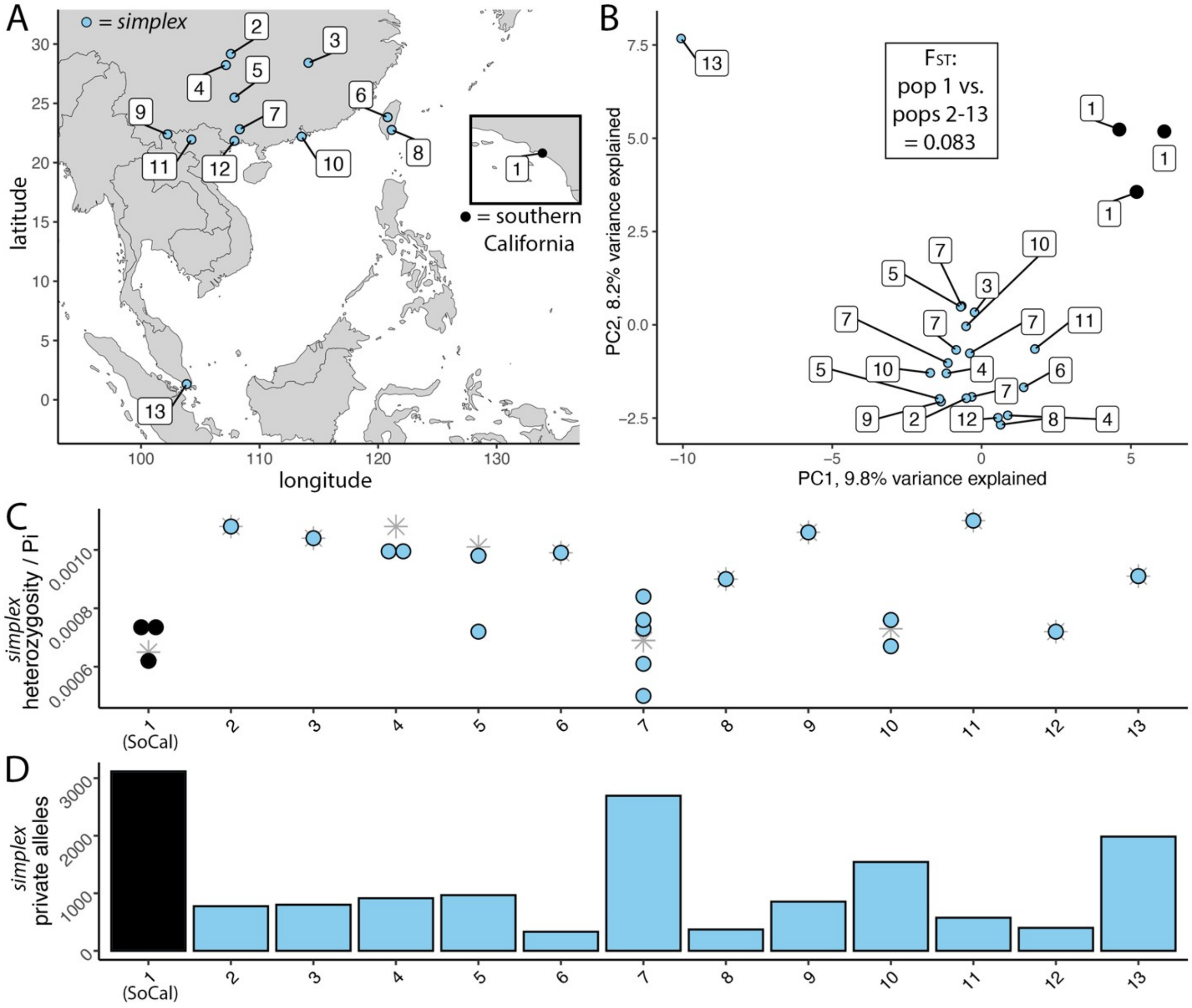


Figure 4. Comparing the genomes of introduced *Z. simplex* in southern California with putative source populations from the species' native range. (A) Map showing all sampling localities for *Z.*

simplex, with each sampling locality numbered arbitrarily but consistently across panels. (B) Principal components analysis (PCA) shows sample clustering among all sampled *Z. simplex* populations. (C) Plots of heterozygosity per sample (colored dots) and overall nucleotide diversity per sampling locality (Pi; denoted with a gray asterisk). The abbreviation 'SoCal' is used to denote the Z. *simplex* population established in southern California. (D) The number of private (i.e., unique) alleles identified in each sampling locality, calculated from all successfully mapped genomic loci.

Ecological dynamics of Zosterops *establishment in North America*

Observational data recorded by community scientists show the establishment and subsequent rapid geographic expansion of *Z. simplex* individuals across southern California over the past decade (Fig. 5). As recently as 2012-2013, this introduced population was restricted almost completely to a handful of locations along the Pacific coast in Orange County, California. Within a few years, however, the population expanded dramatically, reaching as far inland as San Bernardino, and radiating hundreds of kilometers north and south along the coastline. As of 2023, *Z. simplex* has now been documented from Santa Barbara, California, to Tijuana, Mexico, a total range spanning >300 km from end to end. Additionally, *Z. simplex* is now apparently established on the offshore islands of Catalina and San Clemente, underscoring the proclivity for overwater dispersal and island colonization in the *Zosterops* white-eyes. This clear evidence for rapid and ongoing geographic expansion within southern California raises the obvious question, what is the invasion potential of *Z. simplex* across North America?

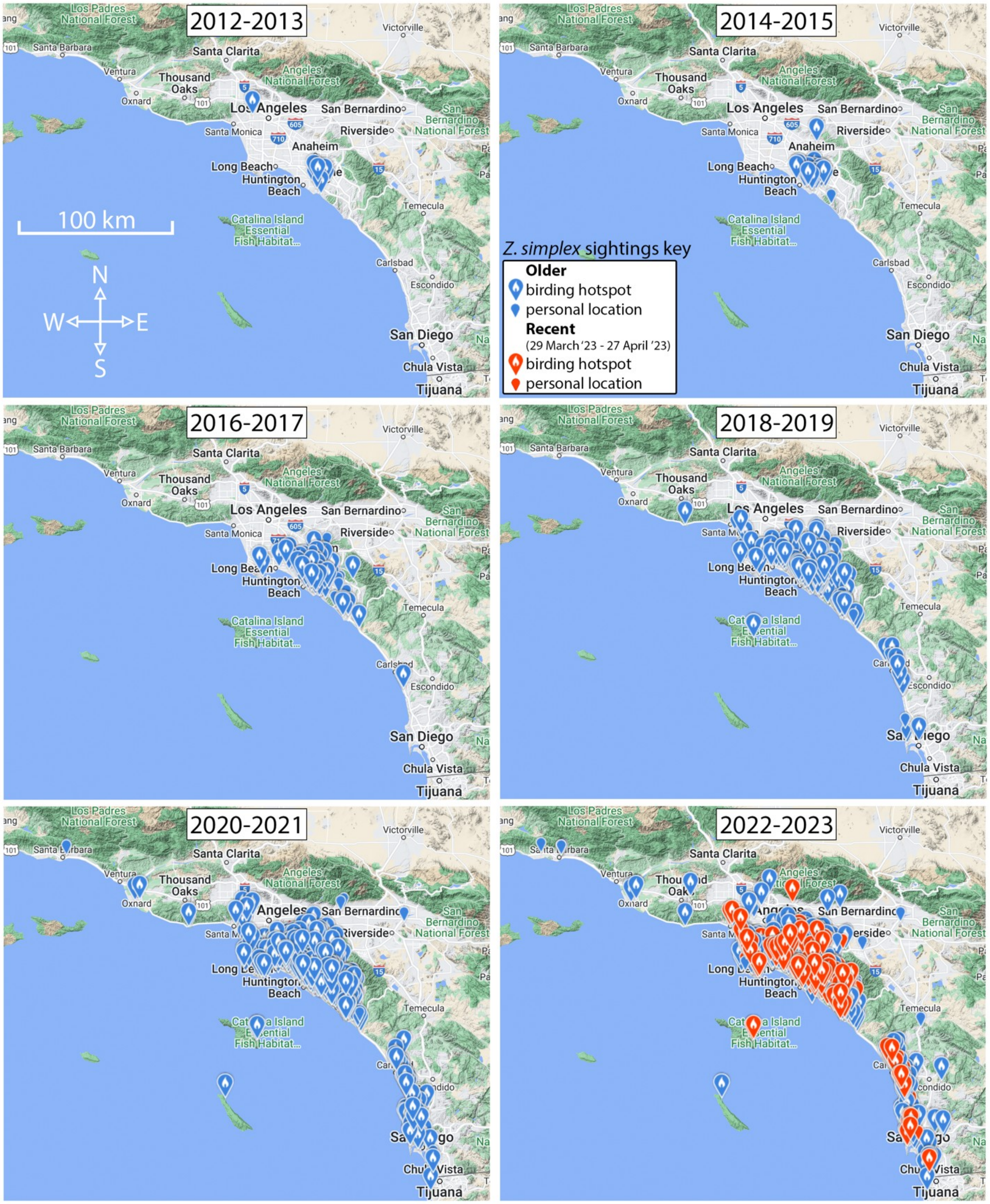


Figure 5. Maps showing the rapid expansion and establishment of introduced *Zosterops simplex* in southern California over the last 12 years, as tracked by community scientists in the eBird database. Images provided by eBird (www.ebird.org), created 27 April 2023. Each bubble

corresponds to a single geographic location where a sighting was reported. Refer to the inset key for detailed locality information.

To address this question, we used ecological niche modeling to explore the distributional potential of the species under a variety of scenarios. We used breeding-season occurrence data from across the native range of *Z. simplex* to train correlative models of the fundamental niche of the species, which we then transferred onto North American climatic landscapes. 1,549 out of 1,560 candidate models generated statistically significant predictions of independent subsets of the available occurrence data. Of those models, 487 also had sufficiently low omission rates, and AICc scores identified four individual models that had an optimal combination of good predictive ability while utilizing the fewest numbers of total input parameters. All selected models used four predictors, linear, quadratic, and product responses, and similar regularization multipliers (i.e., PC1, PC2, PC4, and PC5; see Table S5 for more details). On average, PC5 contributed the most to our four models ~43%, followed by PC1 with ~22%; whereas, the average permutation importance was similar among the four predictors (i.e., 24.43-25.85; Table S6).

The consensus of these four best models is highly predictive of the overall distribution of *Z. simplex* across eastern China and Southeast Asia (Fig. 6). Suitability for the species is lower at the geographic extremes of the distribution in northeastern China, and on the Malay Peninsula and northern Borneo. Mean response curves for the species' distribution with respect to environmental dimensions were truncated only for PC5 (Fig. S1). For other predictors, response curves had a bell shape, which indicates relatively safe model extrapolations. Transferring this 'best model consensus' to the species introduced range reveals a relatively low degree of environmental suitability across most of North America (Fig. 6), assuming a model of niche conservatism from the native range of *Z. simplex*. However, the southern extremes of the United States and much of Mexico appear to closely match the abiotic environmental conditions of the species' breeding range, as indicated by high degrees of suitability. Zooming in on southern California, the current introduced *Z. simplex* population appears to inhabit a broad range of suitability values. The putative introduction site, near Huntington Beach (Fig. 5), is recovered as moderately suitable. Meanwhile individuals on the northern edge of the expansion front (Fig. 6) appear to inhabit areas of low environmental suitability, outside of the range experienced in the native distribution of *Z. simplex*.

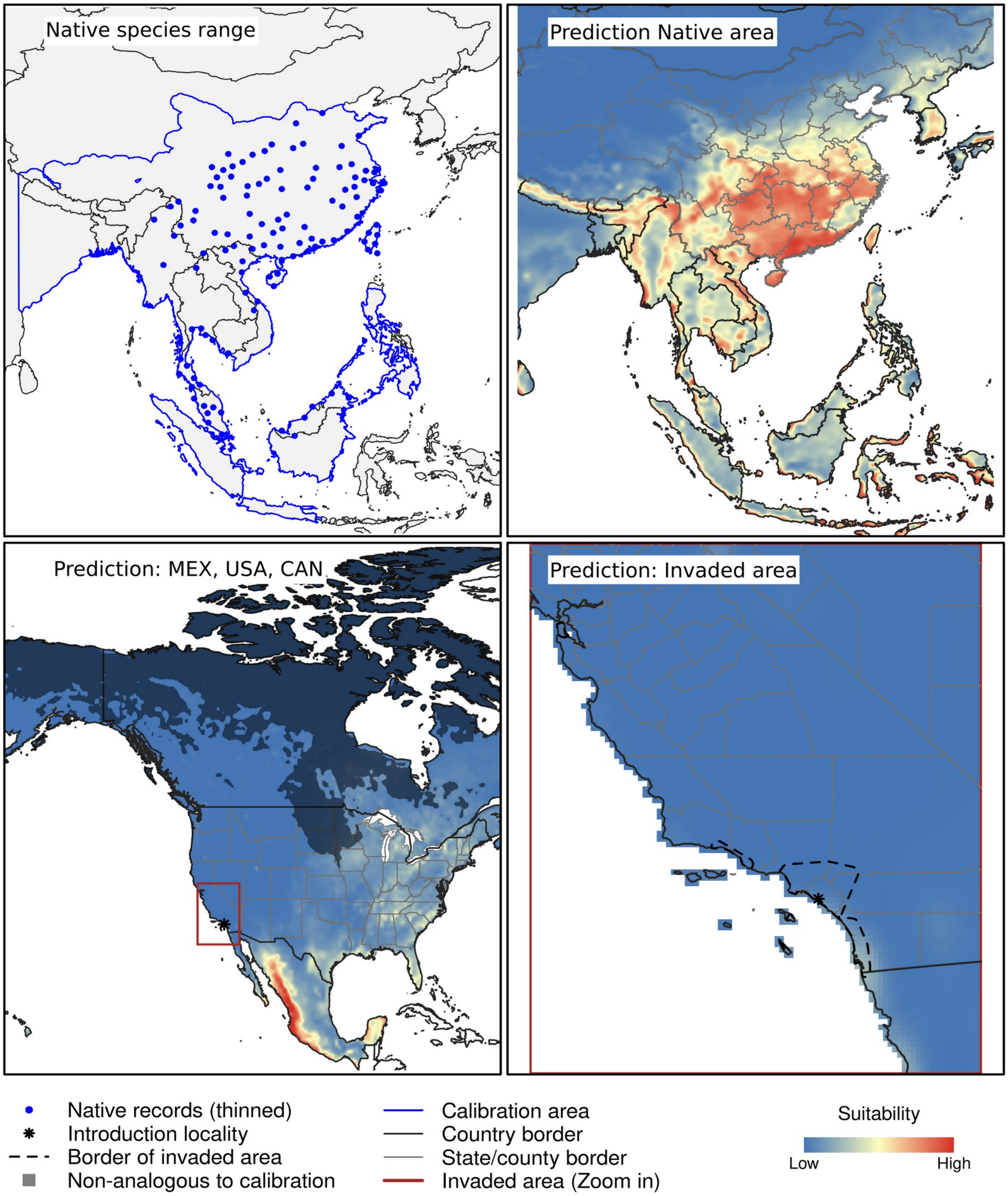


Figure 6. Results from ecological niche modeling for *Zosterops simplex* across Mexico, United States, and Canada. Top panels show all distance-thinned breeding season occurrence observations used to build the ecological niche model, and the subsequent model projected onto the native range of the species. The bottom panels show the model projected onto North America, with a specific focus on the current introduced range in southern California. The border of species range in southern California is represented as a convex hull produced from clusters of invasive records, with a ~10 km buffer.

Discussion

Understanding the source of these introduced *Zosterops* populations is an important step toward understanding their invasion potential and more broadly, understanding the ability of these 'great speciators' (Moyle et al., 2009) to repeatedly overcome the genetic bottlenecks associated with founding new populations (i.e., the 'invasion paradox'; Estoup et al., 2016; Kolbe et al., 2004). Here we confirm for the first time with genomic data that the recently introduced *Zosterops* white-eye population in southern California (~17 years; Cook, 2006) represents a distinct introduction of *Z. simplex* individuals with no apparent relation to the long established (~93 years; Scott et al., 1986) introduced *Z. japonicus* population in Hawaii. We find no evidence for shared ancestry between these introduced populations and other closely related Asiatic *Zosterops* species. Further, we find limited evidence for hybridization (2/124 samples) across the native range of these Asiatic *Zosterops* species, which occur in various stages of secondary sympatry, suggesting the extraordinarily rapid evolution of reproductive isolating barriers among these natural populations (estimated *Zosterops* crown age = 1.6 – 2.5 MYA; Vinciguerra et al., 2023). These results are largely consistent with a recent detailed genomic investigation into the northern Melanesian *Zosterops* radiation, which found deep phylogenetic divergence between described taxa and no evidence of recent hybridization, despite statistical signatures of gene flow, suggesting incomplete reproductive isolation (Manthey et al., 2020). Our detailed genomic investigation revealed evidence for a population bottleneck associated with the founding of the introduced population in southern California, but not Hawaii, concordant with assumed differences in founding population size and ongoing propagule pressure between these populations. Finally, ecological niche models identified relatively limited geographic areas across North America with suitable environmental conditions for *Z. simplex*. Intriguingly, community science observations from the northern edge of the expanding population front in southern California record *Z. simplex* individuals outside of suitable habitat identified by our model, suggesting that the geographic invasion potential of this population may prove to be broader than our ecological niche model suggests. Together, our parallel genomic and ecological investigations offer practical insights into the invasion potential of *Zosterops* across North America, and a window into the general ability of *Zosterops* lineages to rapidly overcome the invasion paradox (Estoup et al., 2016).

The evolutionary history of native Zosterops *populations*

Accurately resolving the ancestry of the introduced Zosterops populations studied here presents a unique challenge because the taxonomy and phylogenetic relationships of the genus have long been plagued by polytomies and a general lack of resolution (Guest, 1973; Moyle et al., 2009; Vinciguerra et al., 2023). The extraordinarily rapid radiation of the 111 (Gill et al., 2023) currently recognized *Zosterops* species within the last ~2.1 million years (estimated crown age 1.6 – 2.5 MYA; Vinciguerra et al., 2023), has made the phylogenetic backbone of this clade extremely challenging to resolve, with startlingly short internode distances suggesting very little time for lineage sorting to occur (Maddison & Knowles, 2006). Among our small focal subclade, the ‘Asiatic *Zosterops*’ (Gwee et al., 2020; Vinciguerra et al., 2023), we did not attempt comprehensive phylogenetic sampling, instead focusing on just six geographically proximate and phenotypically similar species that could have plausibly been involved in founding the introduced *Zosterops* populations in Hawaii and southern California (Fig. 1).

Because the introduced Zosterops populations we investigate here likely contain some pet trade influence (Chng et al., 2018), we suspected that ongoing hybridization might be an important force in shaping patterns of relatedness among the samples we sequenced. Yet, we identified only two putatively admixed individuals using thousands of genomic loci (Fig. 1), neither of which were sampled from an introduced population. One of these admixed individuals came from Singapore, and was assigned > 2% ancestry from *Z. simplex*, *Z. palpebrosus*, and *Z. japonicus*, suggesting potential complex multispecies hybridization. Despite the rarity of documented tri-species hybridization events in birds (except see: Natola et al., 2022), this uncommonly complicated evolutionary history is especially plausible in Singapore, where the native *Zosterops* population was reported to have been locally extirpated by poaching for the pet trade in the 1970s, being subsequently replaced by a flock of feral white-eyes derived largely from captive escapees, including *Z. simplex*, *Z. palpebrosus*, and *Z. japonicus* individuals (Eaton et al., 2017; Ng & Wee, 1994). Lim et al. (2019) identified only *Z. simplex* haplotypes among the mitochondrial DNA of 17 birds from Singapore, but suggested that nuclear DNA sequence data would be needed to rule out hybridization. Despite our highly limited sample size (two samples from Singapore), our results suggest that the ancestry of Singapore's white eye population is derived from at least three *Zosterops* species commonly found in the pet trade. Future work with greater sample size will be essential for understanding the extent of hybridization, and the taxonomic status of the *Zosterops* population in Singapore.

Regarding the overall evolutionary history of the Asiatic *Zosterops*, we find support for rapid branching and strong yet imperfect (Manthey et al. 2020) reproductive isolation. Because of the lack of strong support for a single set of bifurcating phylogenetic relationships among taxa and statistical signatures of excess allele sharing between non-sister lineages, previous studies have concluded that gene flow is rampant among naturally distributed *Zosterops* lineages (Gwee et al., 2020). In contrast, our genomic data suggests that ongoing hybridization among 'Asiatic *Zosterops*' species is quite rare (2/124 individuals with detectably admixed genomic backgrounds). We suggest that rampant significant ABBA/BABA tests between *Zosterops* taxa (Gwee et al., 2020) could be largely explained by rapid trifurcations and polytomies which make correct topology specification nearly impossible, and the well-documented ability of substitution rate variation to create false positive ABBA/BABA results (Frankel & Ané, 2023), rather than extensive ongoing hybridization. The exact nature of reproductive barriers (e.g., intrinsic versus extrinsic; Christie & Strauss, 2019) between *Zosterops* species deserves further study. Regardless, we suggest that the 'great speciator' status of the genus *Zosterops* (i.e., the ability to repeatedly colonize new locales and rapidly speciate; Moyle et al., 2009) is not only a result of exceptional dispersal capability, but also of this propensity for exceptionally rapid evolution of reproductive isolating barriers.

The genomic signatures of Zosterops *introductions*

Upon establishment, a novel population must overcome the genetic bottleneck and founder effect resulting from an inherently limited founding population size (i.e., invasion paradox; Estoup et al., 2016; Halliburton, 2004; Kolbe et al., 2004). Here we searched for signatures of population bottlenecks, which reduce genome-wide diversity, in the introduced *Zosterops* populations in Hawaii and southern California. We found reduced genetic diversity in the introduced *Z. simplex* population in southern California, but not in the introduced *Z. japonicus* population from Hawaii, indicating that the southern California population is experiencing an ongoing genetic bottleneck, while the Hawaii population is not. Further, we find evidence for both genetic divergence from the putative source population and an increase in private alleles in the southern California population, but not in the Hawaiian population. These results indicate that unlike the Hawaiian population, the southern California population is genetically differentiated from any sampled source population. This could be explained by genomic adaptation to a novel environment in southern California (Lee, 2002, 2016), exacerbated by strong founder effects (Jamieson, 2011) and increased genetic drift during a genetic bottleneck (N. H. Barton, 1997; Sendell-Price et al., 2021), or a lack of genetic sampling from the closest source population. It is important to note that we did not sample any *Z. simplex* individuals directly from the pet trade, and it is possible that a series of genetic bottlenecks and novel selection pressures associated with a transition to captivity had already shaped the demographic history of the putative pet-trade escapees (see detailed discussion below) that founded the southern California population.

One obvious explanation for the difference in genomic diversity between these two introduced populations is a fundamental difference in their founding. Because the *Z. japonicus* population in Hawaii was initially founded by the Hawaii Board of Agriculture and Forestry on the island of Oahu in 1929 with the intention of establishing a viable population, the effort likely involved the release of dozens to hundreds of individuals (although no exact numbers of individuals are reported; Caum, 1933). Further, it was documented that in 1937, an additional 252 *Z. japonicus* individuals were introduced to the big island of Hawaii with the goal of controlling insect populations (Guest, 1973). In contrast, as there is no record of *Z. simplex* introduction in southern California, and this population is likely the result of the release/escape of a small number of captive birds, meaning this population may have been founded by only a handful of individuals. In fact, only four birds were seen when the population was first documented in 2006 (Cook, 2006), and no one subsequently documented a group of white-eyes containing more than four individuals in southern California for more than five years following this initial sighting (Daniels, 2011). Therefore, our genomic results indicating a reduction in genetic diversity and increase in private alleles in the southern California population, but not the Hawaii population, are concordant with expectations derived from population genetic theory given the number of individuals we think contributed to the founding of each population (Nei et al., 1975).

An important caveat to these interpretations is the availability of genetic samples. The genetic divergence between the southern California population and all putative source populations may indicate divergence between *Z. simplex* individuals found in the pet-trade versus the wild. It is notable that we find no evidence for genetic divergence between Z. japonicus from Hawaii and the native range, considering the documented evidence for significant morphological changes between these populations (Gleditsch & Sperry, 2019). This could indicate that morphological traits in Z. simplex have significant lability and are strongly affected by environmental conditions, or that we have simply missed the regions of the genome that encode these morphological differences in our RADseq dataset which covers <1% of the total genome. Additionally, in the Hawaiian archipelago, it is worth noting that all our samples from the Hawaii population come from the Big Island, Hawaii, the site of at least one intentional introduction effort. Based on this sampling, we cannot determine whether populations located on further outlying islands (e.g., Oahu or Kauai) may suffer detectable genetic bottlenecks or founder effects following repeated dispersal and colonization events across the Hawaiian island archipelago (Clegg et al., 2002; Shultz et al., 2016). Detailed genomic investigation of the varying strength of population bottlenecks across the Hawaiian archipelago offers yet another potentially fruitful avenue for future genomic research.

Another, potentially complementary, explanation for the genomic differences we observe between these populations is the difference in timing since introduction (~17 years in southern California, Cook, 2006; versus ~94 years in Hawaii, Scott et al., 1986). Population genetic theory has shown that even a severe reduction in genetic diversity from a population bottleneck can be quickly erased if it is followed by a rapid increase in population size (Nei et al., 1975). Based on the documented rapid expansion of the Hawaiian *Zosterops* population (estimated 245,000 *Z. japonicus* individuals in just the Kaʻū Region of Hawaii, as of 2007; Gorresen et al., 2007), it is plausible that the Hawaii population has already overcome any initial reduction in genetic diversity associated with establishment and returned to stable levels of genetic diversity seen in putative source populations, with limited long term evolutionary cost (James et al., 2016). In comparison, the house finch (*Haemorhous mexicanus*) population on the Hawaiian islands was founded at a similar time and also grew rapidly, yet still shows a detectable reduction in genetic diversity, suggesting a more severe bottleneck, lower propagule pressure, a lower growth rate, or a combination of these factors (Shultz et al., 2016).

This ability to rapidly overcome relatively small founding population sizes via explosive population growth could be a key life history strategy that has facilitated the repeated colonization and speciation of *Zosterops* species throughout the Pacific (Gwee et al., 2020; Moyle et al., 2009; Sendell-Price et al., 2021; Sendell-Price, Ruegg, Anderson, et al., 2020). This would suggest that the continued, rapid expansion of the *Z. simplex* population in southern California may be erasing signatures of low genetic diversity in real time. It is worth noting that while effective population sizes can be tracked backwards through time using genomic information from even a single individual (Li & Durbin, 2011), these approaches are notoriously sensitive to population structure and gene flow (Heller et al., 2013; Shchur et al., 2022) and unreliable on recent time scales (Liu & Hansen, 2017), which led us to forego this approach for the specific question of comparing recent genetic bottlenecks. Future investigations using whole genome sequencing, along with nuanced and careful approaches to demographic modeling, will be necessary to effectively reconstruct population dynamics through time in these introduced populations (e.g., Hewett et al., 2023).

Forecasting the establishment of Zosterops *in North America*

Forecasting the potential establishment and spread of introduced species is an important exercise because of the environmental (Clavero & García-Berthou, 2005) and economic (Linz et al., 2018) costs associated with ongoing biological invasions across the globe. Here, we use an ecological niche modeling approach to quantify the multidimensional environmental space inhabited by *Z. simplex* (Sóberon & Peterson, 2005) and project these environmental conditions across North America, effectively forecasting areas at the greatest risk for potential invasion and establishment (Peterson, 2003; Peterson & Vieglais, 2001). It is important to note that this exercise relies on the assumption of niche conservatism, or the idea that novel introduced populations will be limited by the abiotic conditions inhabited in the native range of the species (Losos, 2008; Peterson et al., 1999; Pyron et al., 2015). This assumption can be violated in cases where the inhabited niche is only a subset of the fundamental niche, meaning that the species is physiologically capable of inhabiting a broader suite of environmental conditions than it does in its native range (Sóberon & Peterson, 2005). For the rapidly expanding *Z. simplex* population in southern California, we discuss the implications of projected patterns of environmental suitability across North America, and highlight necessary caveats for interpreting forecasted habitat suitability.

Based on our forecast, the environmental conditions inhabited by *Z. simplex* across its native range cover only a small portion of North American geography, with the largest swath of contiguous habitat found on the Pacific coast of Mexico. If *Z. simplex* is limited to the projected suitable habitat, then we can expect the southern California population to continue expanding south in coastal Baja California, where the warm, temperate environment is similar to the subtropical conditions of the species' native range. If *Z. simplex* can disperse across barriers of unsuitable habitat, the species would find highly suitable conditions along the Pacific coast of Mexico, and moderately suitable conditions near the Atlantic gulf coast, throughout the southeastern United States. The main barrier to accessing these swaths of suitable habitat is their discontinuous nature, with the deserts of southwestern North America and the Sierra Madre Oriental mountain range presenting apparent barriers to the continuous establishment of *Z. simplex* across low latitude habitats in North America. It is worth noting that rapid warming of the global climate caused by massive anthropogenic release of greenhouse gases could bring the subtropical environmental conditions favored by *Z. simplex* in its native range further north, creating more continuous swaths of habitat (Anderson et al., 2012). Future work should explore the interaction between this rapidly adapting *Z. simplex* population and its rapidly changing climate.

Surprisingly, the main stronghold of this introduced population, coastal southern California, between San Diego and Los Angeles (Fig. 5), displayed low to moderate environmental suitability for *Z. simplex* according to our model. In fact, the northernmost front of the expanding population appears to be completely outside of the environmental conditions inhabited by *Z. simplex* in the species native range (Fig. 6). These results suggest that the model of niche conservatism assumed by this approach may be not be fully appropriate for projecting the invasion potential of *Z. simplex* across North America. This could suggest that *Z. simplex* has a broader fundamental niche than is captured by occurrence points from the native range (Jiménez et al., 2019), due to interactions with other species (i.e., biotic conditions), or geographic barriers which prevent dispersal into other suitable habitats (Feeley & Silman, 2010; Fell et al., 2022). Under these circumstances, the geographic invasion potential of this population may prove to be broader than our ecological niche model suggests. Alternatively, populations on the leading expansion edge of the introduced population may prove ephemeral, leading to an eventual range contraction into a core distribution of suitable habitat in southern California.

Meanwhile, there are additional possibilities that could explain the observed colonization of areas predicted unsuitable by our model even if the fundamental niche of *Z. simplex* is perfectly captured by our native range occurrence points, such as the ability to exploit novel anthropogenic resources not included in our model variables, or rapid genetic adaptation to novel environmental conditions. For instance, *Z. simplex* has been documented using novel biotic resources such as ornamental flowers and fruit trees as food resources in the urban environment of southern California (e.g., Garrett, 1997). Additional resources facilitating establishment could include supplementary food sources such as hummingbird feeders (Battey, 2019), or warmer microhabitats offered by the urban built environment on cold nights (Wang & Chu, 2021). In addition, genetic adaptations could already be facilitating inter-generational adaptation to novel environmental conditions (Hofmeister et al., 2021; Lee, 2002, 2016; Stuart et al., 2023), although large evolutionary niche shifts have rarely been documented in vertebrate populations (Peterson, 2011). Nonetheless, shifts in the frequencies of many alleles, each contributing weakly to highly polygenic traits such as body size, metabolism, or rate of shivering (e.g., Stager et al., 2021), may have already begun to expand the physiological tolerance range of this introduced population. Ultimately, further study in the coming decades will be necessary to understand the nature of the mechanisms involved in adaptation to the novel environment of urban southern California, as well as the factors (i.e., biotic versus abiotic) that will eventually act to limit the geographic spread and establishment of this introduced population.

Conclusions

This work contributes to a growing body of literature focused on projecting the invasion potential of introduced species in novel environments (Dong et al., 2020; Early et al., 2018; Mutamiswa et al., 2021; Nuñez-Penichet et al., 2021; Vega et al., 2021). Ours is one of the few investigations to date that has attempted to jointly assess the genetic effects of introduction and the suitability of environments across geographic space for a novel introduced population (e.g., Banerjee et al., 2019; Estoup et al., 2010). While we identify a reduction in genetic diversity associated with the introduction of *Z. simplex* into southern California, the population is expanding rapidly in this novel environment. We suggest that this ability to overcome the invasion paradox (Estoup et al., 2016) via rapid population expansion could be a key factor in helping the *Zosterops* white-eyes earn their "great speciator" reputation (Diamond et al., 1976; Lee, 2002). Ecological niche modeling results suggest that broad-scale invasion of *Z. simplex* throughout the western United States is unlikely considering low suitability levels. Yet, the rapid expansion of *Z. simplex* throughout Los Angeles suggests that urban environments may offer biotic resources and suitable microhabitats that are not incorporated into our ecological niche model, and could aid in facilitating the expansion of this introduced population beyond the limits suggested by our forecast. If the introduced *Z. simplex* population is not limited by genetic bottlenecks or unfamiliar environmental conditions, only time will reveal what forces will eventually act to limit its geographic spread in North America.

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Data availability

All code needed to reproduce the results of this study can be found by following the links on the homepage of the dedicated GitHub repository for this project at: https://github.com/DevonDeRaad/zosterops.rad. Upon manuscript acceptance, this entire repository will be archived permanently with a stable DOI via Zenodo, and all raw sequence data will be deposited on NCBI's Sequence Read Archive.

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Statements and Declarations

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Competing interests

The authors have no competing interests to disclose at this time.

Author contributions

DAD and AJS conceptualized and designed the study. DAD performed molecular lab work, genomic data analysis, and wrote the first draft of the manuscript. MEC and ATP performed ecological niche modeling analyses. RGM, AJS, KLG, IN, BM, FSZ, KK, CHK, RSY, CTY, and HLMJ all contributed crucial specimens and tissue samples to the project. RGM secured funding for genomic sequencing. All authors contributed substantial feedback on the analysis and interpretation of data across multiple drafts of this manuscript, and approved the final submitted version of the manuscript.