Analysis of Genomic Sequence Data Reveals the Origin and Evolutionary Separation of Hawaiian Hoary Bat Populations

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Data deposition: All sequencing data were deposited at NCBI SRA under accession PRJNA559902. Genome assembly and SNP data used in analysis are available at https://doi.org/10.5066/P9OJIVE6 (Pinzari et al. 2020).

Abstract

We examine the genetic history and population status of Hawaiian hoary bats (*Lasiurus semotus*), the most isolated bats on Earth, and their relationship to northern hoary bats (*Lasiurus cinereus*), through whole-genome analysis of single-nucleotide polymorphisms mapped to a de novo-assembled reference genome. Profiles of genomic diversity and divergence indicate that Hawaiian hoary bats are distinct from northern hoary bats, and form a monophyletic group, indicating a single ancestral colonization event 1.34 Ma, followed by substantial divergence between islands beginning 0.51 Ma. Phylogenetic analysis indicates Maui is central to the radiation across the archipelago, with the southward expansion to Hawai'i and westward to O'ahu and Kaua'i. Because this endangered species is of conservation concern, a clearer understanding of the population genetic structure of this bat in the Hawaiian Islands is of timely importance.

Key words: island colonization, genomic divergence, adaptation, bat conservation, bat genome assembly.

Introduction

The terrestrial biota of the Hawaiian archipelago offers numerous models of long-distance colonization and subsequent evolution on remote oceanic islands (Price and Clague 2002; Ziegler 2002; Holland and Hadfield 2004; Lerner et al. 2011). Forming over 70 Myr from a volcanic hot spot in the Earth's mantle, the Hawaiian Islands continue to rise above and subsequently sink below the ocean as the Pacific plate moves northwest across the hotspot (Wilson 1963). This archipelago is the most isolated large, linear island chain in the world, spanning 2,600 km across the North Pacific from Kure Atoll in the northwest to the rising volcanic seamount of Lō'ihi in the southeast. North America, the nearest continental source of biota, lies 3,600 km eastward.

Although successful colonization events for terrestrial fauna are rare, such events presage impressive radiations that are represented in Hawai'i by plants, insects, spiders, land snails, and birds (Ziegler 2002; Holland and Hadfield 2004; Lerner et al. 2011). Independent of human introductions, however, only two species of terrestrial mammals, both bats, successfully established in Hawai'i. These are the extinct lava tube bat, *Synemporion keana* (Ziegler et al. 2016), and

the northern hoary bat, *Lasiurus cinereus* (Baird et al. 2015; Russell et al. 2015). The geographic origin of the ancestor of *S. keana* is unknown (Ziegler et al. 2016), whereas that of the hoary bat diaspora clearly is North American (Russell et al. 2015; Baird et al. 2017). Bonaccorso and McGuire (2013) tested flight models based on empirical data from energetics, water balance, life history traits, and morphology of the northern hoary bat to hypothesize likely flight scenarios that facilitated hoary bats in reaching Hawai'i from the continent. At present, two phylogenetic studies have supported multiple colonization events by hoary bats to the Hawaiian archipelago (Russell et al. 2015; Baird et al. 2017). These events represent the longest successful trans-oceanic dispersal events known for terrestrial mammals (Bonaccorso and McGuire 2013). The exact timing for arrivals to the Hawaiian Islands remains questionable: Russell et al. (2015) found evidence for population expansions at ~10,000 and 800 years ago, whereas Baird et al. (2017) estimated the initial founding event occurred 1.35 Ma with a population expansion 20,000 years ago.

Here, we hypothesize that genetic divergence with distinct population structure and low rates of interisland gene flow exist among hoary bats across the Hawaiian Islands. The availability of insect food sources year-round on each island may have reduced gene flow between islands as there is likely little adaptive benefit from regular interisland movements despite the excellent flight range capability of hoary bats (Bonaccorso and McGuire 2013; Bonaccorso et al. 2015). The northern hoary bat, *L. cinereus*, is a solitary, foliage roosting, insectivorous bat that, in contrast to the Hawaiian hoary bat, *Lasiurus semotus*, undergoes large-scale annual migrations in gyres across large reaches of North America, likely in response to seasonal availability in food (Cryan 2003; Hayes et al. 2015). It is distributed as a very large panmictic population with virtually no distinct regional population structure (Korstian et al. 2015, Pylant et al. 2016). Migratory bats in general have high levels of gene flow and little population genetic structure (Burns and Broders 2014; Korstian et al. 2015; Vonhof and Russell 2015) compared with nonmigratory bats (Turmelle et al. 2011). Both Russell et al. (2015) and Baird et al. (2017) report unique mitochondrial haplotypes and nuclear gene variants found only in hoary bats inhabiting the Hawaiian Islands.

The systematics of lasurine (tree) bats using molecular techniques has been reviewed by others (Baird et al. 2015, 2017; Ziegler et al. 2016; Novaes et al. 2018). Although the systematic revision by Baird et al. (2017) placed the hoary bats in a new genus *Aeorestes*, as distinct from the genus *Lasiurus*, their revision is counter to that conservatively argued by Ziegler et al. (2016). Ziegler et al. (2016), Novaes et al. (2018), and Wilson and Mittermier (2019) advocated support of an alternative viewpoint that retains the genus name *Lasiurus*. Here, we will retain the use of *Lasiurus*, following the taxonomy of Ziegler et al. (2016), Novaes et al. (2018), and Wilson and Mittermier (2019). Recent examination of mitochondrial and nuclear DNA resulted in the recommendation that Hawaiian hoary bat receive full species status, and the additional proposal for the existence of two genetically distinct species of Hawaiian bats: *Aeorestes semotus* and *Aeorestes cinereus* (Baird et al. 2017). However, no identifiable morphological features have been published to distinguish between the proposed species; thus, we group all bats in the study together and refer to them as Hawaiian hoary bats.

In this article, we examine the genetic history and population structure of Hawaiian hoary bats, and their relationship to the northern hoary bat *L. cinereus*, through novel analysis of single-nucleotide polymorphisms (SNPs) data. We also report levels of genomic diversity and divergence, population structure, and gene signatures of selective sweeps in populations on the individual islands of Hawai'i, Maui, O'ahu, and Kaua'i.

Materials and Methods

Tissue Sampling and DNA Extraction

Tissue samples from wings and muscles were collected during necropsies performed on 23 bat carcasses from Hawai'i ($n = 6$), Kaua'i ($n = 1$), O'ahu ($n = 8$), and Maui ($n = 8$) between 2009 and 2015 (fig. 1). These included eight males and 15 females. Carcasses were refrigerated or frozen upon discovery. Tissue samples were collected from pliable wing membranes, where possible, with a sterile 3-mm circular biopsy tool. When carcass condition was suitable, pectoral muscle tissue was collected with a sterile scalpel. All tissue samples were stored in 1.5-ml tubes containing a preservation solution of NaCl-saturated 20% DMSO and frozen at –80 °C until DNA extraction. DNA was isolated from bat tissues using a DNeasy Blood and Tissue Kit (Qiagen) according to the manufacturer's protocol for purification of total DNA from animal tissues. RNase A treatment was used to remove RNA contaminants. Where DNA concentration was lower than desired, a Genomic DNA Clean & Concentrator kit (Zymo Research) was used to purify samples. Genomic library preparation and sequencing was performed at the Virginia Bioinformatics Institute Genomic Core Lab. Tissue samples were authorized for collection under the following permits; State of Hawai'i Division of Forestry and Wildlife Protected Wildlife Permit WL 16-04; US Fish and Wildlife Service Threatened and Endangered Species Permit TE003484-31.

Sequencing

Sequencing was performed from a TruSeq paired-end library (2 × 150 bp) using HiSeq 2500 (Illumina). The sequencing depth for each individual Hawaiian hoary bat ranged from 3.55× (O23) to 6.85× (H16) with average of 5.63× (supplementary table S1, Supplementary Material online). For comparison, publicly available Illumina sequences from a single northern hoary bat (*L. cinereus*) sample from Maryland, USA, were used (Consortium et al. 2014).

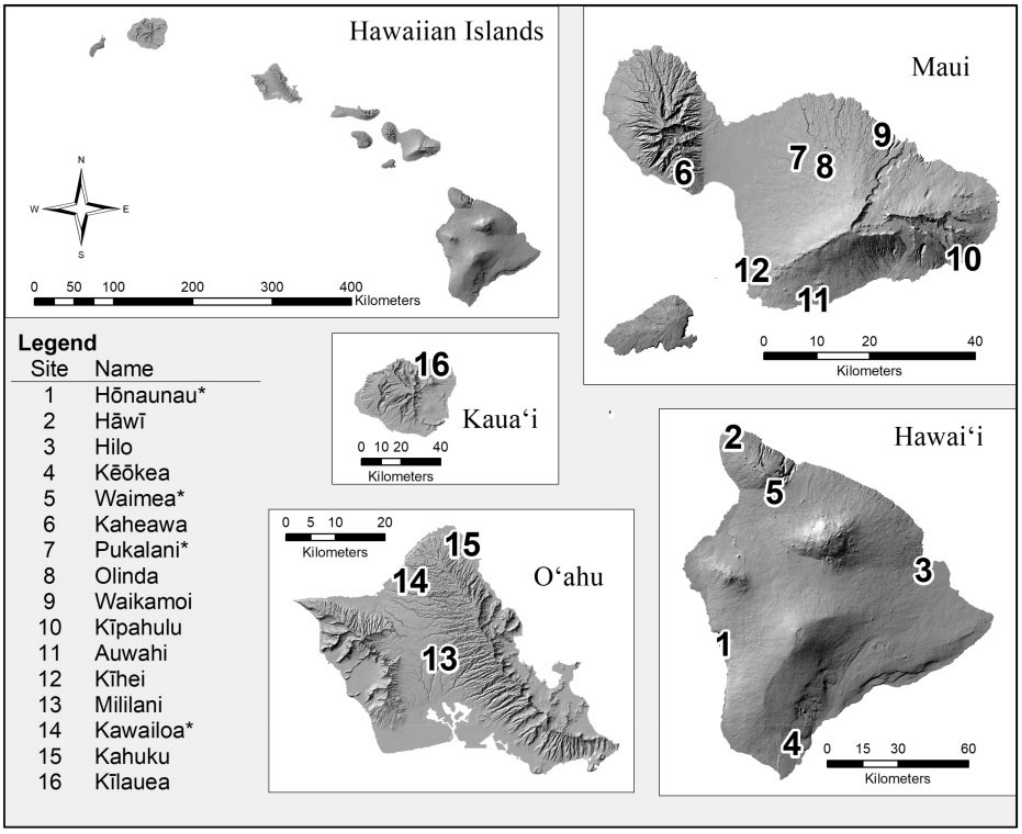

Fig. 1.—Map of the Hawaiian Islands with collection sites for Hawaiian hoary bat tissues used in this study. Sites with $n > 1$ are denoted with an asterisk.

Genome Assembly

Adapters were first removed from raw sequencing reads, and low quality and duplicated reads were removed using FastqMcf v1.04.636 (Aronesty 2013). Post-quality control (QC) reads from all individuals were merged together and further duplication removal was conducted to accelerate the assembly process. To exclude possible genomic contamination, all reads were aligned to a bacterial database downloaded from NCBI (http://www.ncbi.nlm.nih.gov/), and only unmapped reads were used for the assembly. Processed reads were assembled with Spades v3.0.0 (Bankevich et al. 2012). Contigs in the final assembly with length <500 bp were discarded from further analyses. The final assembly was used as the reference for mapping and genotyping.

Gene Prediction and Annotation

Assembly sequences were first masked using RepeatMasker v4.0.3 (http://www.repeatmasker.org/) with parameters set to "-s -a -nolow" and using a customized repeat library. Protein-coding genes were predicted using MAKER2 v 2.31.8 (Holt and Yandell 2011), which used protein sequences that were downloaded from Ensembl (www.ensembl.org) and RefSeq (www.ncbi.nlm.nih.gov/refseq) as protein homology evidence and integrated with prediction methods including BlastX v2.2.28 (Altschul 1997), SNAP (Korf 2004), and Augustus v3.3 (Stanke and Waack 2003). The SNAP HMM file was generated by training with mammalian gene sequences. The Augustus model file was generated by training 3,026 core genes of vertebrates from a genome completeness assessment tool BUSCO v3.0 (Simão et al. 2015). Predicted genes were subsequently used as query sequences in a BlastX database search of NR database (the nonredundant database, http://www.ncbi.nlm.nih.gov/). BlastX alignments with *e*-value >1e-30 were discarded, and the top hit was used to annotate the query genes.

Genome Completeness

Two methods were used for genome completeness estimation. CEGMA v2.0 (Parra et al. 2007) examines the existence of 248 core eukaryotic genes in assembly. BUSCO v3.0 (Simão et al. 2015) was used to assess universal single-copy orthologs of vertebrates in the assembly.

Mapping and Genotyping

Post-QC reads from each individual were mapped to the reference assembly using BWA v0.7.12 (Li and Durbin 2009)

with default parameters. Genotypes for each sample were generated by using GATK v4.0.9 genotyping model (DePristo et al. 2011). Only sites with genotyping quality >30 and minimal depth 5 were kept, and a polymorphic site required at least two reads supporting the alternative allele. Population mutation rate θ (Watterson's estimator), nucleotide diversity (π), and fixation index (F_{ST}) were calculated based on a window size of 10 kb. For comparison, publicly available reads from *L. cinereus* (Consortium et al. 2014), *L. borealis* (Consortium et al. 2014), and *M. brandtii* (Seim et al. 2013) were downloaded, processed and mapped against the reference assembly as described above.

Population Genetic Structure

Sampled individuals were projected into a subspace spanned by the first principal components (PCs) using their genotypes as features. Top PCs reflect variation due to population structure in the sample, with individuals from the same population found to form a cluster in this subspace (Novembre and Stephens 2008; Ma and Amos 2012). EIGENSOFT v6.0.1 (Patterson et al. 2006; Price et al. 2006) was used for PCA. STRUCTURE v2.3.4 (Pritchard et al. 2000), which implements a model-based clustering method, was used for inferring population structure using genotype data. Individuals were assigned to populations, or jointly to two or more populations if their genotypes indicate that they were admixed. To minimize the effect of linkage and nonneutrality, the STRUCTURE analysis was based on a subset of all SNPs (a total of 199,921) where each contig contributes one random SNP from a noncoding region. STRUCTURE was run independently 20 times for each K value (range 1–10) using 250,000 iterations for burn-in and 1,000,000 iterations for MCMC (Markov chain Monte Carlo) with admixture model, and default values were used for other parameters. The delta K (the second order rate of change of the likelihood) method (Evanno et al. 2005) was also used to detect the number of clusters (K).

Phylogenetic Reconstruction

A coalescent analysis implemented in SNAPP (Bryant et al. 2012) module from BEAST package v2.6.0 (Bouckaert et al. 2019), was used to directly infer the species/population tree from unlinked biallelic markers. A total of 10,000 unlinked nonmissing SNPs was loaded to SNAPP analysis, whereas the northern hoary bat (*L. cinereus*) was used as an outgroup. The SNAPP run was conducted with 1,000,000 MCMC generations and 50,000 as the burn-in, sampled every 1,000 generations. A Gamma distribution was assigned to Lambda prior, with an Alpha of 1 and a Beta of 200. TreeAnnotator v2.6.0 was used to construct the "Maximum clade credibility" tree and annotate it with posterior probabilities. Tree sets were visualized in FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/) and DENSITREE v2.2.7 (Bouckaert 2010). The date of emergence of the island of Hawai'i (Fleischer et al. 1998; Lerner et al. 2011; Baird et al. 2017) was used (0.43 Ma) to infer the divergence dates, the node separating Hawai'i from the O'ahu/Kaua'i clade was set to 0.43 Ma.

Repeat Elements Analysis

A de novo repeat-family modeling package RepeatModeler v1.0.8 (http://www.repeatmasker.org) was used (with default parameters) to identify repeat elements. The output repeat library was combined with the mammalian repeat library from Repbase (http://www.girinst.org/repbase/) to form a customized repeat library. This library was used as the input of RepeatMasker v4.0.3 (-lib), in which RMBlast v2.2.28 was chosen as the sequence search engine (supplementary table S3, Supplementary Material online).

Sweep Detection

Selective sweeps were identified with Pool-hmmv1.4.3 (Boitard et al. 2013), a hidden Markov model for detecting selective sweep based on Pool-Seq data. Only contigs with size >100 kb were included in this analysis (655 contigs with total size of 83.79 Mb).

Results

Reference Genome Assembly

We sequenced the individual genomes of 23 Hawaiian hoary bats (8 males and 15 females), including individuals from the islands of Hawai'i ($n = 6$), Kaua'i ($n = 1$), O'ahu ($n = 8$), and Maui ($n = 8$) collected between 2009 and 2015 (fig. 1). These samples were sequenced at low coverage (3.55–6.85×) using the HiSeq Illumina platform, followed by a de novo-assembly of a reference genome, and comparison of SNP polymorphisms across the 23 samples relative to publicly available sequences of a single northern hoary bat from Maryland, USA, obtained from Genomic Resources Development Consortium (Consortium et al. 2014). The total size of the reference assembly is ~2.07 Gb, with an average contig size of 9,556 bases (N50 = 23,695; supplementary table S1, Supplementary Material online). Even though the estimated genome completeness was low (37–55%, supplementary table S2, Supplementary Material online), we predicted 22,131 genes, 21,587 (97.54%) of which were annotated. Repeat elements constituted ~38% of the unmasked assembly, with LINE retroelements being most abundant (~20%, supplementary table S3, Supplementary Material online).

Sequence Polymorphism and Divergence

Mapping rates against the reference assembly ranged from 99.8% to 99.9% (supplementary table S4, Supplementary Material online). We found a total of 21,808,031 polymorphic sites, including 208,403 (0.01%) in coding sequences, among the 23 Hawaiian hoary bats. A total of 3,629 population-

Table 1

F_{ST} Estimates between island populations of Hawaiian hoary bats with Multiple Individual Samples (F_{ST} Based on a Single Polymorphic Site and the Average Was Taken)

	Hawai'i	Maui
Hawai'i	—	—
Maui	0.08508	—
O'ahu	0.11612	0.09199

unique SNPs was found, with 1,074, 717, and 1,838 SNPs fixed in each Hawai'i, Maui, and O'ahu populations, respectively, whereas the alternative allele fixed in the other two populations, under the sequencing depth exceeding 10 reads. As many as 42% (Hawai'i), 24% (Maui), and 48% (O'ahu) of all SNPs were fixed in one population, while being heterozygous in the rest. Heterozygosity varied between populations, ranging from 0.138 in O'ahu, 0.157 in Hawai'i, to 0.206 in Maui. All fixation index (F_{ST}) estimates between populations from different islands exceeded 0.08 (table 1), indicating a high degree of genetic differentiation among the islands' populations. The results based on principal components analysis (PCA) (Patterson et al. 2006; Price et al. 2006) (fig. 2) and STRUCTURE (Pritchard et al. 2000) (fig. 3) both corroborate this pattern. For the latter, we used the delta K method (Evanno et al. 2005) to determine the parameter K, which describes the number of clusters that make up the total population, and found that $K = 5$ has the highest support (fig. 3*A*). However, we notice after visual inspection that the $K = 3$ seems to make more biological sense, as all K clusters include different proportions of individuals from each sampling location, and all K clusters include at least some individuals who are strongly associated with that cluster (fig. 3*B*). Populations from Maui and Hawai'i were less distant from each other than either one from the O'ahu population, also consistent with our F_{ST} estimates. The single sample from Kaua'i clustered closely with the O'ahu population (fig. 2). The site frequency spectrum (SFS) analysis reveals a high density of minor alleles in the populations from Hawai'i, Maui, and O'ahu, which would be consistent with more recent expansions (supplementary fig. S1, Supplementary Material online). The estimates of population mutation rate θ (Watterson's estimator) range from 0.0010 to 0.0015, whereas nucleotide diversity (π) ranges from 0.0011 to 0.0016 (supplementary table S5, Supplementary Material online).

Phylogenetic Analysis and Divergence Dating

The SNAPP analysis yielded a tree topology with all ingroup nodes supported by the maximum posterior probability (fig. 4), which indicates an initial colonization of Maui, followed by migration to the other islands. Using the emergence of subaerial magma dates for Hawai'i (~0.43 Ma) as a calibration point (Fleischer et al. 1998, Lerner et al. 2011), we

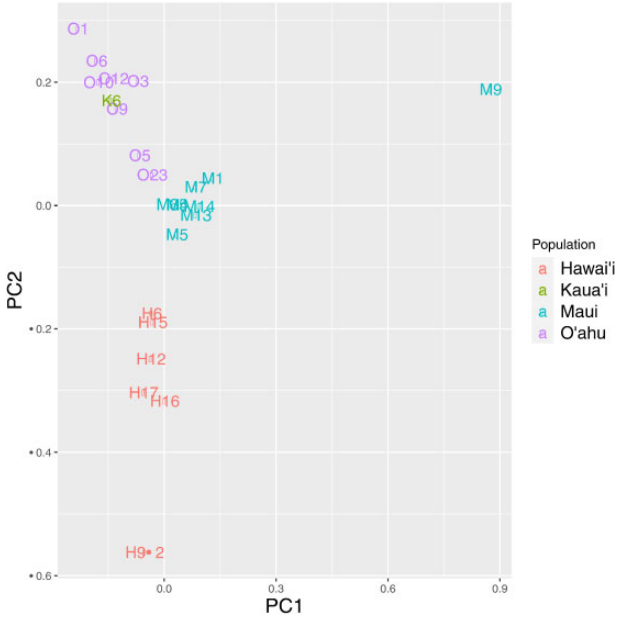

Fig. 2.—PCA result plot showing clustering of individual bats from four Hawaiian Islands using 21,808,031 SNPs. Sample information included in supplementary table S4, Supplementary Material online.

estimated that the common ancestor of Hawaiian hoary bats arrived 1.34 Ma (95% confidence interval [CI] of 1.09–1.92) with a single dispersal to the archipelago, specifically to the island of Maui. Dispersal to the other islands from Maui may have begun as early as 0.51 Ma (95% CI of 0.46–0.58 Ma), with the Hawai'i population diverging 0.43 Ma (95% CI of 0.37–0.48), and the establishment of populations on O'ahu and Kaua'i 0.27 Ma (95% CI of 0.21–0.31 Ma). The initial founding date of ancestral populations, agrees with that proposed by Baird et al. (2017) however, dates of dispersal among islands are considerably older than previously reported dates of population expansions (Russell et al. 2015; Baird et al. 2017), revealing far longer periods of habitation on each of these three islands. Alternatively, 0.60 Ma has been indicated as potentially the earliest emergence date for Hawai'i Island, based on the emergences of Mahukona (now submerged) and Kohala volcanoes (Clague 1996); our estimates of bat population divergence times may actually be younger than they really are, if suitable habitat was available on Hawai'i Island prior to 0.43 Ma.

Selective Sweep Signatures

Given the prevalence of adaptive radiation among Hawaiian biota (Ziegler 2002; Holland and Hadfield 2004; Lerner et al. 2011), we set out to investigate adaptive changes in the bats at the genomic sequence level. To this end, we scanned the largest 655 contigs (>100 kb each), covering a total of 83.8 Mb of the assembly, for patterns of heterozygosity erosions corresponding to signatures of selective sweeps. Out of

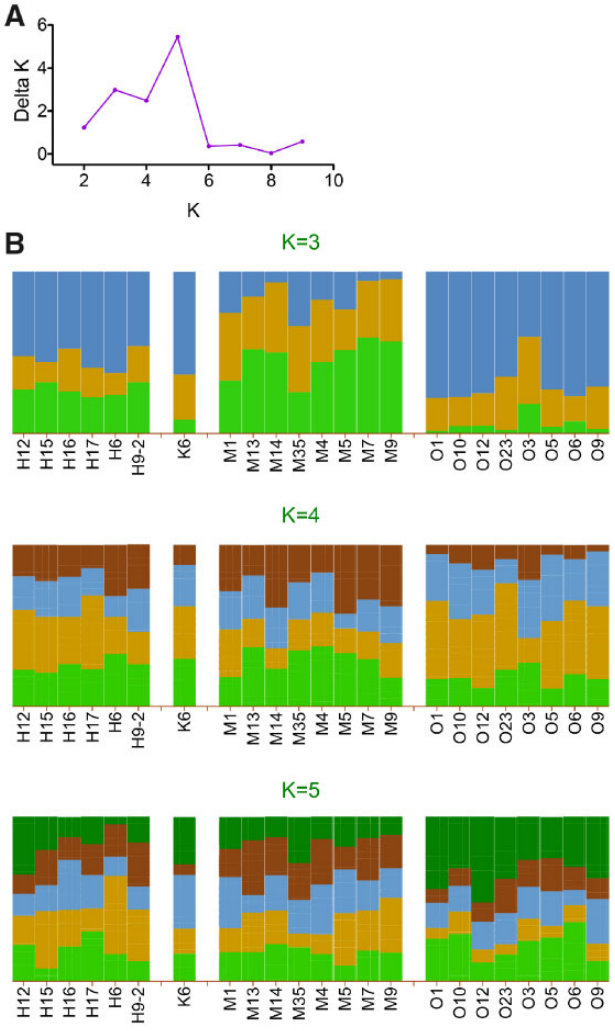

FIG. 3.—Population structure inference based on STRUCTURE analysis of 199,921 sites for individual bats from four Hawaiian Islands. (*A*) Ad hoc statistic delta K analysis indicates a peak at the $K = 5$; (*B*) STRUCTURE population inference with $K = 3$, 4, 5. Sample information included in supplementary table S4, Supplementary Material online.

the 655 contigs, 78, 159, and 311 harbored sweep signatures in Hawai'i, Maui, and O'ahu populations, respectively. The population in O'ahu had significantly more genomic regions affected by such selective sweeps than the other populations combined (Fisher's exact test $P = 1.24 \times 10^{-32}$; table 2). Notably, 73% of the signatures by size of sweep region in O'ahu were unique to that population, unlike those in Maui (45%) and Hawai'i (20%). These patterns can be confounded by global patterns of heterozygosity, which was lowest in O'ahu, and potentially related to demographic history rather than enhanced positive selection in one population relative to another. Nevertheless, there were 413 nonsynonymous and 343 synonymous SNPs within coding DNA of the putative regions of population-unique selective sweeps. The 413 nonsynonymous mutations were found within 99 genes (supplementary table S6, Supplementary Material online). Genes with the highest number (m) of nonsynonymous mutations were *Phosphatidylinositol-3,4,5-trisphosphate 5-phosphatase 1* ($m = 24$) fixed or near-fixed in O'ahu, *BAZ1A* (*Bromodomain adjacent to zinc finger domain protein 1A*, $m = 17$) fixed or near-fixed in O'ahu, *Laminin subunit gamma-1* ($m = 16$) fixed or near-fixed in O'ahu, *GRAM domain-containing protein 1B* ($m = 13$) fixed or high frequencies in Hawai'i and O'ahu, and *Glypican-1* ($m = 12$) fixed or high frequencies in O'ahu and Maui.

Echolocation-Related Genes

Two genes, *Cadherin 23* (*Cdh23*) and *protocadherin 15* (*Pcdh15*), related to echolocation in bats and toothed whales (Shen et al. 2012) were found in the annotated reference assembly (supplementary table S7, Supplementary Material online). *Cdh23* had 10 polymorphic sites, nine of which were in introns and one in the coding sequence (position 83690, a nonsynonymous change of C into A leading to Leu→Met), with the latter polymorphic only in O'ahu (minor allele A frequency 13%). *Pcdh15* showed 86 polymorphic sites, 82 intronic and four exonic, including one synonymous (position 15801), and three nonsynonymous sites. The first nonsynonymous site (position 28075, ATT→ACT; Ile→Thr) was polymorphic only in Hawai'i (minor allele C frequency 8%). Strikingly, polymorphism in the second nonsense site (position 39029) produced a stop codon (TTA→TAA; Leu→STOP), with significant higher frequency in Hawai'i (65%) and Maui (64%) samples than in O'ahu samples (0%; Fisher's exact test $P = 1.96 \times 10^{-5}$), whereas the only sample from Kaua'i was heterozygous. If translated, this variant would lead to a substantial truncation of the polypeptide product from 437 to 251 amino acids. We also failed to find such an allele in genomes of three other species, namely the northern hoary bat *L. cinereus*, eastern red bat, *L. borealis* (Consortium et al. 2014), and Brandt's bat, *M. brandtii* (Seim et al. 2013). The third *Pcdh15* nonsynonymous change (GTG→GGG; Val→Gly) occurred in position 47807, and was highly polymorphic in all the Hawaiian populations, including the Kaua'i sample; however, the North American individual was homozygous for the major allele.

Discussion

Based on the 23 bat genomes sequenced, we found that Hawaiian hoary bats are distinct from northern hoary bats (*L. cinereus*), and likely form a monophyletic group, given that the continental population is near-panmictic (Korstian et al. 2015). This supports previous conclusions on the matter of a distinct bat species in Hawaii, diverged from a North American founder to the island of Maui (Baird et al. 2015, 2017). However, we did not find evidence to support Baird

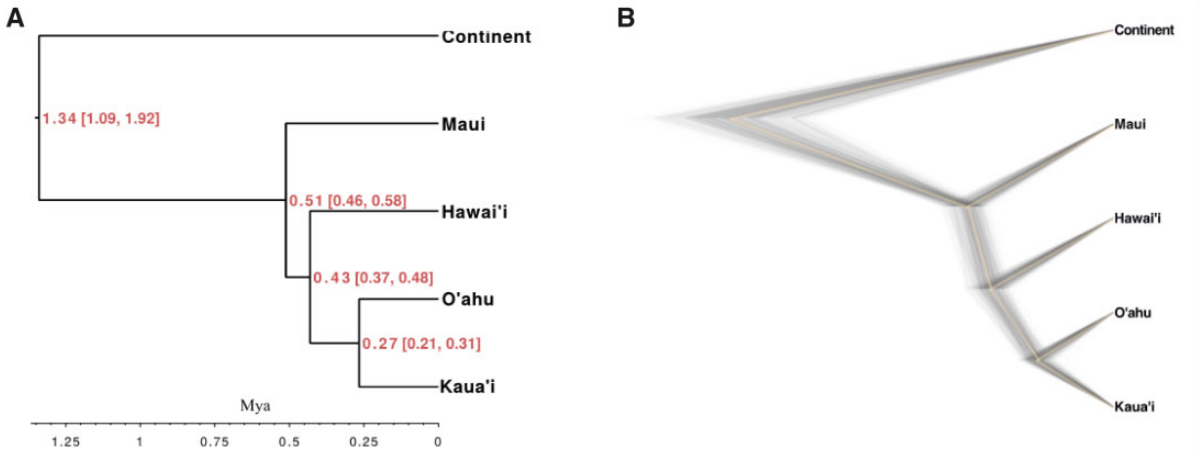

Fig. 4.—SNAPP-based phylogenetic tree inference. (*A*) The maximum clade credibility or consensus tree, showing approximate divergence of hoary bats across the Hawaiian archipelago. The axis on the bottom of the figure corresponds to million years before present (Ma), using the emergence of Hawai'i (~0.43 Ma) as a calibration point (95% confidence intervals were given in square brackets). (*B*) The drawing of all sampled trees showing all ingroup nodes were supported by maximum posterior probabilities (1.00).

Table 2

Putative Selective Sweep Signatures in three island populations of Hawaiian hoary bats.

Population	No. of Sweep Regions	Size of Sweep Region[a]
Hawai'i (H)	78	4,125,849
Maui (M)	159	8,407,768
O'ahu (O)	311	20,339,802
H unique[b]	57	814,416
M unique[b]	133	3,799,547
O unique[b]	308	14,883,156
H & M[c]	46	2,064,665
H & O[c]	57	2,913,091
M & O[c]	90	4,209,878
H & M & O[c]	38	1,666,329

[a]Total size of all contigs (>100 K, 655 contigs) in sweep detection is 83.8 Mb (83,790,696).
[b]Sweep exists in one population but not in the other two populations.
[c]Overlap between population.

et al. (2017) that two extant bat species (*A. semotus* and *A. cinereus*) occur within the Hawaiian Islands, nor evidence that there were multiple waves of colonization in the founding history of hoary bat populations in Hawaii. This discrepancy is likely due to the fact that previous results were based on a small number of mitochondrial and nuclear markers, prone to effects of incomplete lineage assortment. Individuals sampled in our study included bats from both mitochondrial lineages on O'ahu and Maui described in prior genetic studies (Russell et al. 2015; Baird et al. 2017). Our SNP data indicate Hawaiian hoary bats have shared ancestry with a North American continental relative, but are more clearly genetically distinct by island rather than maternal lineage or clade, and likely represent the singular unique Hawaiian hoary bat species, *L. semotus*.

Our phylogeny estimates an initial founding event of a common ancestor to Hawaiian hoary bats shortly after 1 Ma, then divergence and dispersal to other islands from Maui beginning ~0.51 Ma. The potential for dispersal from Maui to Hawai'i Island at this time, follows findings of divergence times to Hawai'i Island for endemic free flying birds, such as the Hawaiian nēnē Goose (*Branta sandvicensis*), the extinct giant Hawaii Goose (*Branta rhuax*) (Paxinos et al. 2002), and Hawaiian honeycreepers, specifically the Hawai'i 'amakihi (*Chlorodrepanis virens*) (Lerner et al 2011; Campana et al. 2019). Observational records from the Krakatau Islands, show that insectivorous bats along with other complex flora and fauna recolonized the islands as little as 100 years after a volcanic eruption destroyed all life (Rawlinson et al 1992). After the emergence of Hawai'i, a few decades to a few hundred years would likely produce an environment suitable for insectivorous bats to survive, especially with Maui remaining a close, continuous source of plant and animal diaspora. A relatively quick colonization of Hawai'i Island would be possible for this bat species, given their flight ability, and foraging behavior of exploiting moth fauna associated with newly formed and vegetated lava landscapes (Bonaccorso et al. 2016; Howarth et al. 2020). Population expansion signals in Hawaiian hoary bats described by prior studies (Russell et al. 2015; Baird et al. 2017) may instead correspond to growth in population sizes in response to changes in island biogeographic features. The Maui Nui island complex experienced re-emergence during low sea levels ~0.02 Ma, whereas the end of glacial period on Hawai'i Island occurred ~0.01 Ma (Price and Elliott-Fisk 2004). During this time of climate warming, vegetation zones expanded upwards in elevation on volcanic slopes, creating new habitat. Initial timing of Polynesian settlement of the Hawaiian Islands is believed to range from AD 1,000 to 1,200 (Kirch 2011), the most recent expansion

signal appearing ~800 years ago may indicate a response in bat populations to land use modification by Polynesian settlers (Olson and James 1982). Recent population expansions on the islands are supported by the excess of low frequency variants in the SFS (Lapierre et al. 2017). Allele frequency differences between island populations could be due to genetic drift during bottleneck events or "allele surfing" during a population expansion (Excoffier and Ray 2008).

Although the number of bats per island sampled in our study is smaller than the studies of Baird et al. (2017) and Russell et al. (2015), that used fewer markers, our conservative 3,629 single nucleotide polymorphic loci give more comprehensive estimates of genetic diversity and population structure. Heterozygosity values, an indicator of genetic diversity, in Hawaiian hoary bats on O'ahu (0.138) and Hawai'i (0.157) islands are lower than documented in northern hoary bats *L. cinereus* (0.182) by Sovic et al. (2016), yet Maui island is curiously higher (0.206). Our results also find that populations of the Hawaiian hoary bat on Maui, O'ahu, and Hawai'i are differentiated by large F_{ST} values. The greatest difference in population structure is between Hawai'i and O'ahu, whereas those of Maui and Hawai'i are more similar. A population structure with unique subpopulations on each of these islands also is supported by separation into distinct clusters from our PCA (fig. 2). The placement of two individuals, H9-2 and M9, are distinct from the main clusters on their respective islands; however, increased sampling from these locations would better illustrate potential substructure within Hawai'i and Maui islands. The topographic variability on larger, recently emerged islands, such as Hawai'i and Maui, could maintain separation and show low gene flow due to stable climatic conditions and year-round presence of insect prey. Cold winters limit prey availability on the North American continent, where hoary bats there undergo large-scale seasonal migrations to reach breeding grounds and winter foraging areas (Cryan 2003). Continental panmixia occurs in several species of lasiurine bats. Northern hoary bat (*L. cinereus*), silver-haired bat (*Lasionycteris noctivagans*), and eastern red bat (*L. borealis*) are genetically diverse across large regions of the continent but lack strong population structure (Korstian et al. 2015; Russell et al. 2015; Vonhof and Russell 2015; Pylant et al. 2016). Although the distances between adjacent Hawaiian Islands are relatively small, there may be no strong incentive for bats to move between islands to access resources for breeding and foraging, and bat genetic structure may mimic that of both endemic and introduced island birds. Several endemic Hawaiian honeycreeper genera have island specific populations, including the 'amakihi and nukupu'u (*Hemignathus* spp.), 'akepa (*Loxops* spp.), and creepers (*Oreomystis* spp.) (Lerner et al. 2011). Shultz et al. (2016) characterized genomic signatures using SNPs in the house finch, and found that Hawaiian populations introduced in the 1870s currently show signs of significant population structure by island. Genetic isolation of insular populations has been found in wide-ranging continental molossid and vespertillonid bat species, with open-water crossings between islands being barriers to gene flow (Salgueiro et al. 2008; Biollaz et al. 2010; Weyeneth et al. 2011; Speer et al. 2017).

Variation in echolocation-related genes in Hawaiian hoary bats raise questions of evolutionary interest based on our current findings. It is possible that shifts in allele frequency are due to natural selection when bats colonized Hawai'i, as they encountered new vegetation, insect prey, climatic conditions, and reduced competition from only one other sympatric bat species. We found interesting gene ontology during our analyses of selective sweeps that may support phenotypic differences and adaptive evolution in Hawaiian bat populations. Alterations in mandibular morphology have been documented in mice with mutations in *Glypican-1* (Mian et al. 2017). Meanwhile, *Laminin subunit gamma-1* was a gene found to be associated with vision in a genome-wide study of echolocating animals including bats (Parker et al. 2013). Two genes associated with echolocation in bats, *Cadherin 23* (*Cdh23*) and *protocadherin 15* (*Pcdh15*), have varying levels of polymorphism and striking protein-coding differences in our island populations, presenting an interesting topic for further study on the evolution of echolocation in Hawaiian hoary bats.

The overall genomic changes and specific changes at important loci identified in this study may be, in part, associated with the substantial behavioral, ecological, and morphological differences between Hawaiian and northern hoary bats. Hawaiian hoary bats are ~45% smaller in body mass and wing size than northern hoary bats and may have become more generalist in their foraging and habitat preferences (Jacobs 1996). Several studies have compared echolocation characteristics and variation in northern and Hawaiian hoary bat populations (Barclay et al. 1999), as well as foraging selectivity and interspecific competition (Belwood and Fullard 1984; Poe 2007) and use of vision while hunting (Barclay et al. 1999). Hawaiian hoary bats use a large range of echolocation calls and can utilize higher frequencies than northern hoary bats, possibly as a consequence of their smaller body size. Flight activity studies on Hawaiian hoary bats, coupling videography and acoustic recordings, indicate this species flies silently without using echolocation in some contexts (Gorresen et al. 2017). Jacobs (1996) hypothesized that Hawaiian hoary bats experienced a "character release" upon arrival in the Hawaiian islands, with no resource competition offered by conspecific bat species. Thus, hoary bats arriving in Hawai'i could expand their foraging behavior to a broader range of insect prey and habitats. However, *S. keana*, the lava tube bat, occurred on all main Hawaiian Islands prior to 0.32 Ma (Ziegler et al. 2016). Our findings indicate potential overlap of *L. semotus* ancestors with the now extinct *S. keana*, was longer than estimated. It is unlikely that sympatry between only two bat species would have resulted in

heavy competition, if insect prey and roost availability were not limiting factors (Salinas-Ramos et al. 2020). Hawaiian habitats likely provided year-round prey resources, and roost preferences differed between bats, with *S. keana* inhabiting lava tubes (Ziegler et al. 2016) whereas *L. semotus* utilized trees and foliage. Jacobs also measured divergence in cranial morphology, finding that Hawaiian hoary bats have proportionally longer skulls, higher coronoid processes, increased gape, and larger masseter muscles. This may have been an adaptive response to the inclusion of a wider range of larger insect prey beyond moths, including hard-bodied beetles.

Fatal collisions with wind turbines are a threat to population viability for migratory tree bats, including hoary bats (Frick et al. 2017), but population-level effects are unknown for the endemic Hawaiian hoary bat. Managing anthropogenic threats and aiding the recovery of this endangered species across the island state of Hawai'i requires information about genetic variation, population size, and structure. Our study demonstrates that among the islands of Hawai'i, Maui, and O'ahu, bat populations are genetically distinct and may warrant classification into separate evolutionary significant units (ESU). The bat population on O'ahu can be noted as having the highest number of unique, fixed SNPs, and lowest heterozygosity. The O'ahu population also shows genomic regions affected by putative selective sweeps, specifically nonsynonymous mutations in genes that may be under positive natural selection.

The data presented here may not reflect recent changes in Hawaiian hoary bat population genetic diversity due to human effects; however, this type of data is a valuable starting point for future genetic monitoring of allelic diversity and population trends (Schwartz et al. 2007; Allendorf et al. 2008). Discerning whether genotypic changes are due to natural selection or due to drift associated with founder events may not be possible when limited to contemporary samples from a single time period (Shultz et al. 2016). We caution the use of diversity measures provided here to answer population level questions, as the sample sizes of <10 bats per island are insufficient for population size analyses. Ultimately, continued sampling is needed across multiple individuals and time periods to address such questions about Hawaiian hoary bat effective population sizes on islands, their evolutionary past, and population trajectories.

Supplementary Material

Supplementary data are available at *Genome Biology and Evolution* online.

Acknowledgments

We thank T. Anderson, R. Breeden, M. Craig, F. Duvall, M. Hayes, R. McGuire, B. Okimoto, M. VanZandt, and T. Work for facilitating our tissue collections. Laboratory support for DNA extraction was provided by the University of Hawai'i at Hilo Core Genomics Facility with assistance from A. Veillet. Logistical and financial support for our research was provided by the US Geological Survey, Pacific Island Ecosystems Research Center. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the US Government.

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Associate editor: Adam Eyre-Walker