

Further Exploration of a Front Range House Wren Contact Zone

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Project Goals: Population divergence and speciation is a complex, and often non-linear, process influenced by many factors including physical isolation, drift, selection, and gene flow. In North America, the contemporary distributions of many species reflect expansion from the refugia they existed in during the last ice age. This is particularly obvious in North American birds, which often exist as eastern and western species or subspecies (e.g., flickers, orioles, buntings, and towhees), the majority of which are well-studied (Sibley and Short 1959; Walsh et al. 2020; Lovell et al. 2021). Examining the underlying processes that lead to genetic divergence and, ultimately, reproductive isolation is key to understanding the process of speciation and the generation of biodiversity. Hybrid zones provide unique opportunities to study the maintenance of species boundaries, and the evolutionary outcomes of genetic exchange between divergent lineages, and the study of North American avian hybrid zones has provided many important insights. Hybrid zones, often described as windows into the evolutionary process, provide a unique opportunity to understand the generation, or maintenance, of reproductive barriers. Individuals with a mix of genomic backgrounds in hybrid zones can be used to quantify the consequences of gene flow between divergent lineages: the extent of admixture can reveal negative or neutral costs to hybridization and can highlight genomic regions that are resistant to gene flow that may play an important role in the maintenance of species boundaries. I propose to use whole genome data to explore the House Wren contact zone, a previously uncharacterized North American avian contact zone, to better understand genetic variation between house wren subspecies in North America, and more thoroughly understand the dynamics of gene flow within the region of hybridization along the Front Range.

The House Wren (*Troglodytes aedon*) is an incredibly diverse species, with 32 identified subspecies and the largest breeding range of any songbird in the New World (Johnson 2020). Across the continental United States, there are two distinct House Wren mitochondrial clades, the eastern and western forms. These two forms exhibit 4% mitochondrial divergence, which is comparable to other species-level pairs (e.g. Black-capped and Carolina Chickadees, 5% divergent, (Reudink 2007)). Work conducted by me and my collaborators has recently provided more information about this system. We have established that both mitochondrial types are present along the Front Range of the Colorado Rocky Mountains, but our results from examining 88,826 single nucleotide polymorphisms (SNPs) show only weak divergence in the nuclear genome between eastern and western forms and the existence of a large region of potential admixture along the Front Range (Figure 1). To better characterize the region of contact between eastern and western house wrens I seek to expand our study and the resolution of our genetic sampling. Unpublished work on House Wrens has demonstrated that the Western and Eastern forms are not sister taxa according to mitochondrial data but are sister according to nuclear data. Broadly, these results highlight there is a deep mitochondrial divergence within House Wrens (Chavez et al. in revision), but that there appears to be a discordant pattern of differentiation in the nuclear genome as has been found in other avian systems (e.g., golden-winged and blue-winged warblers; Toews et al. 2016)). I propose to generate higher resolution whole genome data from house wrens spanning their zone of contact in the Rocky Mountains. Only higher resolution genomic data will allow us to understand the dynamics of gene flow in this region of contact and potentially identify regions of the genome involved in reproductive isolation.

I propose to sequence approximately ninety-six House Wrens sampled from our 400 nest box transect using a whole genome approach to fully characterize nuclear differentiation between these two mitochondrial clades. As part of ongoing research in our lab (The Boulder Chickadee Study), we have

developed a large elevational transect of nest boxes extending from the city of Boulder Colorado, west to the University of Colorado mountain Research Station. Each year, approximately 65 of these boxes are occupied by House Wrens, providing access to dense, population level sampling within the hybrid zone. Importantly, both mitochondrial types are present along our nest box transect. Additionally, we will sequence whole genome samples from outside the region of hybridization to characterize allele frequencies in the parental western and eastern House Wren forms. These parental samples will allow us to polarize our sequence data, and more accurately characterize admixture along the Front Range. We have already obtained parental samples from our museum partners, locally in Denver from the Denver Museum of Nature and Science, and from the Burke Museum at University of Washington and Louisiana State University's Museum of Natural Science. In order to first characterize population structure, we will construct a principal component analysis using the R package SNPRelate v1.12.2 (Zheng et al. 2012) and quantify ancestry proportions using the program ADMIXTURE v1.3.0 (Alexander et al. 2009). These clustering approaches will provide a broad estimate of the total genomic variation among these House Wren clades, and the degree to which intermediate individuals exist. To further quantify the width and center of the hybrid zone, we will calculate hybrid indices using gghybrid v.1.0.0 (Bailey 2020) and plot geographic clines to identify potential correlates between the landscape and the transition between eastern and western forms. We will also examine genomic clines to identify regions of the genome that may be incompatible with alternative genomic backgrounds or that are differentially introgressing between eastern and western House Wren lineages.