Comprehensive transect sampling of the North American House Wren hybrid zone

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Co-investigators: Dr. Scott Taylor, Dr. Garth Spellman, Dr. Erik Funk

Project Goals: The House Wren (*Troglodytes aedon)* is a small, morphologically cryptic bird that occurs across North and South America. Two of the thirty-two recognized subspecies occur across most of the United States and Canada, *Troglodytes aedon aedon* in the east, and *Troglodytes aedon parkmanii* in the west, representing the only migratory House Wren subspecies. Previously, the east-west geographic transition from *aedon* to *parkmanii* was thought to be south of Lake Michigan along the Mississippi River, but recent work by myself and my colleagues, funded in part by the Denver Field Ornithologists, found that both subspecies are actually present in the state of Colorado, indicating that the hybrid zone between the subspecies is significantly further west than previously thought. We initially used restriction-site associated DNA sequencing (RADseq, a reduced-representation sequencing approach), to establish the presence, but not geographic context, of a hybrid zone between *aedon* and *parkmanii* along the Front Range of the Colorado Rockies. We then used whole genome sequencing (WGS), to examine the genetic basis of differentiation between the allopatric populations of *T. a. aedon* and *T. a. Parkmanii*. From the whole genome data, we found significant differentiation on the Z chromosome, one of the avian sex chromosomes, where a large chromosomal inversion contains >96% of the differences between northern House Wren populations, some of which are related to metabolic genes. These novel findings have contributed to our understanding of the contact zone between House Wrens and their genomic differentiation, but our understanding of the contact zone and hybridization between House Wren subspecies remains incomplete in the absence of genetic and morphological data from a robust and geographically extensive transect. Such additional sampling will allow us to precisely define where the transition from *aedon* to *parkmanii* occurs. We propose to sample House Wrens along a longitudinal transect to fully characterize the geographic extent of the House Wren contact zone in Colorado. This kind of sampling design is a crucial aspect of studying avian hybrid zones, as shown by previous work on Baltimore and Bullock's Orioles, Yellow-rumped Warblers, Hermit and Townsend's Warblers, Northern Flickers, and many other population pairs. We will obtain a blood sample and morphological measurements from each wren we capture, allowing us to finally define the geographic context of hybridization between the western and eastern House Wrens.

Locations: This work will take place along the I-70 corridor in the Rocky Mountains, the I-76 corridor in northeast Colorado along the South Platte River, and the U.S. 50 corridor in southeast Colorado along the Arkansas River. Specific sampling sites will be state wildlife areas along these routes, including but not limited to: Pony Express SWA, Prewitt Reservoir SWA, Brush SWA, Blue River SWA, Orchard Mesa SWA, Parachute Ponds SWA, Gypsum Ponds SWA, Lake Pueblo SWA, Holbrook Reservoir SWA, and Queens SWA. This work may also require expanding our transects into Nebraska and Kansas, though we will first attempt to obtain tissue samples from those locations from museum collections rather than collecting them ourselves. All DNA extractions will take place in the Taylor Lab, where we have experience using the wet lab techniques required for DNA extractions. Sequencing will occur at the Shared Genomics Core on The University of Colorado's Anschutz campus.

Timeline: This work will occur throughout 2024. We will sample wrens as soon as they return from the wintering grounds, in the middle of May 2024. I will extract and submit DNA for sequencing in July 2024. Following sequencing, bioinformatic analyses will be undertaken during the Fall of 2024, with a manuscript expected to be in review at a journal by January 1st, 2025.

Methods:

The first step of this project is to obtain ~80 individual House Wren blood samples from along our geographic transect. This will require approximately two weeks of fieldwork during the spring of 2024. Field collection of House Wrens entails first capturing them using a set of mist nets, followed by morphological measurements, the application of a USGS metal band, and sampling ~40ul of blood from the brachial vein. We will target five birds per sampling site across twenty sampling sites for a total of ~80 samples across our geographic transect. We have attached a map (Figure 1.) showing our potential sampling sites. We plan to only sample on state land, as the permission is easier to obtain. Sites within the Rocky Mountains were chosen at random, while sites on the eastern plains follow the two main river basins in eastern Colorado, as it is expected that the eastern forms of House Wren would follow the river basins west into Colorado. This pattern of eastern birds using the rivers as corridors into Colorado is seen in orioles and towhees. This sampling effort will require us to hire on an additional field technician for approximately two weeks to assist with capture and data collection efforts. Use of a site depends on access, condition of site during the sampling effort, and responsiveness of land managers. This sampling effort crosses ~6.2 degrees of longitude, and should encompass the entirety of the hybrid zone, however, if we find that we have not sampled the entirety of the eastern portion of the hybrid zone, we will augment our dataset with geographically relevant specimens from museums as we have done in previous work.

Following blood sampling, we will extract DNA from our blood samples using a salt extraction protocol that I have previously used successfully in our lab. Library prep and whole genome sequencing will be performed at the Shared Genomics Core located on the University of Colorado, Anschutz campus. Following sequencing, reads will be aligned to a high quality *Certhia americana* genome available on NCBI. This reference has been used successfully by our lab for prior House Wren studies and has been found to be sufficient. We will then filter for depth and quality, resulting in a final high quality whole genome dataset to use for downstream analyses.

The main goal of this project is to place the House Wren hybrid zone within its geographic context using extensive geographic sampling combined with whole genome data. From our whole genome data, we will first use the program gghybrid to generate hybrid indices for each individual that we have sampled. Hybrid indices are calculated between two distinct populations on a scale from 0-1, where a hybrid index of 0 is entirely one parental population and a hybrid index of 1 is entirely the other parental population. A first-generation hybrid, F1, would then be assigned a hybrid index of 0.5. Importantly, these analyses require samples from allopatry, deep within the core range of each population. We currently have 10 allopatric House Wren whole genomes, 5 from each subspecies, that we will use to polarize the transect data and generate hybrid indices. Once we have generated hybrid indices for each individual, we will plot those by longitude to observe any changes in genomic ancestry by geographic location. We expect to see a smooth cline from a hybrid index of 0 in the west to a hybrid index of 1 in the east, with the individuals from the intermediate longitudes falling somewhere in between the allopatric samples. We will also generate genomic clines for individual single nucleotide polymorphisms (SNPs) to observe if there are any patterns of selection across the hybrid zone. We can observe the change in allele frequencies across geographic space and make inferences about how selection is acting on the SNP. If some SNPs change rapidly across geographic space, they may be under stronger selection. Those regions may be involved in contemporary reproductive isolation. Identification of those regions can help to further our understanding of how populations differentiate, the process that leads to speciation.

Budget:

Funding Agency	Item	Cost per Unit	Total Cost	Amount Requested	Status
Denver Field Ornithologists	**Field Assistant salary**	**$15.00 per hour**	**$15.00 x 80 = $1200.00**	**$1,200.00**	**Pending**
Denver Field Ornithologists	**Transportation**	**$0.655 dollars per mile**	**$0.655 x 992= $649.76**	**$649.76**	**Pending**
CU Boulder SEED Grant	Whole Genome Sequencing	$287.00	$287.00 x 80 = $22,960.00	$22,960.00	Pending
CU Boulder SEED Grant	Lab Consumables	-	$500	$500	Pending

Budget Justification: Funds provided by the Denver Field Ornithologists will be used for two purposes. First, I will need to hire a field technician to assist with mist net set up, break down, bird capture, and data collection. This position will be provided to an individual with minimal experience in the field, providing valuable field experience and resume building for someone trying to expand their experiences in field ornithology. We seek to provide them with 80 hours of pay at CU Boulder's minimum wage of 15$ an hour, for a total of $1,200.00 over the course of two work weeks. The second item that we are requesting DFO fund is the mileage reimbursement so that we can afford to drive the transect. We seek to reimburse for mileage at the federal rate of $0.655 dollars per mile, with an expected transect length of 992 miles, totally ~$650.00. This comes to a total of $1,850.00 that we are requesting from the Denver Field Ornithologists. Other costs involved in this project include the cost of sequencing, which we are requesting from the University of Colorado's SEED grant, as well as the consumable products for extraction of DNA, which is also included in the CU Boulder SEED Grant budget.

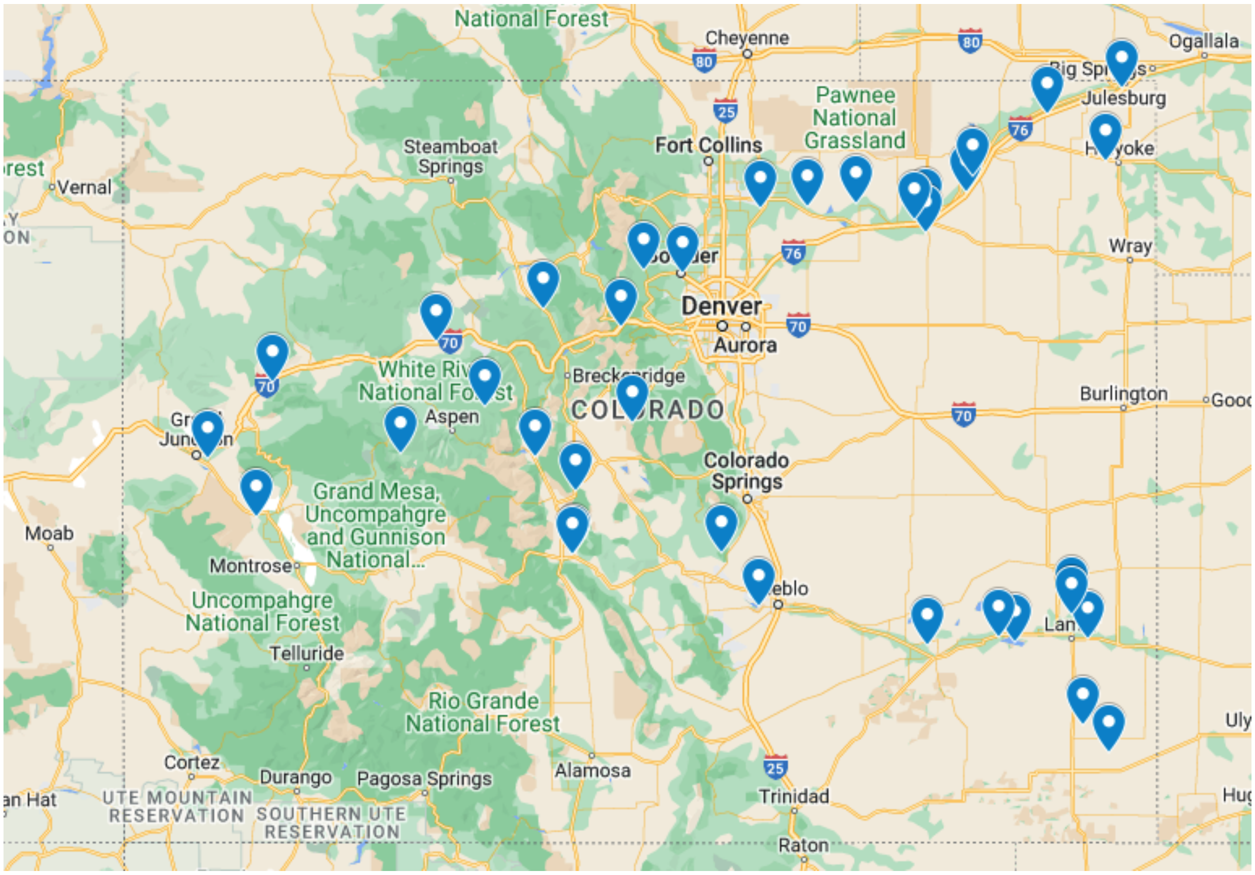

Figure 1: This figure shows all the potential locations for sampling House Wrens. They are all state wildlife areas, maintained by the Colorado Parks and Wildlife. The transect to the northeast of Denver in the North follows the South Platte River, while the transect in the southeast of Denver follows the Arkansas River.

Prior Publications:

Anderson, W.C., et. al., 2024 (in review) Exploration of a novel avian hybrid zone along the Colorado Rocky Mountains. (Funded in part by the DFO)

Anderson, W.C., et. al., 2024 (in prep) Characterization of genomic divergence between migratory North American subspecies of the House Wren (*Troglodytes aedon*)

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January 25, 2024

Dear Denver Field Ornithologists Research Grant Selection Committee,

Please accept my highest and most enthusiastic recommendation for awarding William Anderson a DFO Research Grant. I know Will as both a student in a classroom setting and as a mentee in a research setting and have known him for 6 years. I met Will when he was a first-year undergraduate student in my General Biology class and have since had him as a student in both Population Genetics and Ornithology. It is hard to stand out in a class of 400 but Will did just that. He is an engaged and enthusiastic person and a joy to work with. Beginning spring 2021, Will joined my lab as an undergraduate research assistant and throughout the summers of 2021and 2022 he was a member of the Boulder Chickadee Study field crew. Will completed his undergraduate Honors thesis project in my lab and is now completing a PhD in my lab. Throughout the time that I have known him, Will has demonstrated an incredible passion for birds, a strong self-motivated drive to better understand their ecology and evolution, and the skills necessary to investigate high-level research questions. His honors thesis work was exceptional and will soon be turned into a publication describing a novel avian hybrid zone in North America—the first novel hybrid zone described in North America in decades.

From the time Will approached our lab to begin conducting research, it was clear he possessed one of the strongest passions for birds of anyone I have ever known. It was also clear that this passion had developed long before coming to the University of Colorado. In this time, Will has accumulated an incredible knowledge of birds and their ecologies, and he now applies this knowledge as a lens through which to examine critical questions in ecology and evolutionary biology. Specifically, Will is very interested in combining his passion for birds with his growing love of evolutionary biology to better understand how biodiversity is generated and maintained. In sharing this passion with others, Will has demonstrated incredible leadership and community outreach by, not only volunteering time to lead field trips for various societies, but also as the president and founder of the University of Colorado Birding Club. In this role, Will has conducted outreach, and obtained critical funding for his organization.

Stemming from his passion for birds and their natural histories, Will is now applying his knowledge to ask important questions in the field of evolutionary biology. He approached our lab with novel, self-motivated research questions, and has shown a dedicated drive to answering them. To this end, he has acquired over 3000USD in independent funding to support his work. Over the past 3 years, Will has excelled in courses in Genomics, and Population Genetics, gathering skills in data analysis and interpretation while also generating and analyzing his own dataset. Specifically, I have worked closely with Will as his instructor for Population Genetics and subsequently as his mentor as he generated and analyzed both a double-digest restriction-associated (ddRAD) reduced representation genomic dataset and a smaller whole genome dataset to explore whether there is a house wren hybrid zone along the Front Range of the Rocky Mountains. Throughout his time in the lab Will has demonstrated a thorough understanding of theoretical frameworks, and a mastery of the complex analyses that are required to be successful in the big data and genomics era of scientific research. For his PhD work Will is using whole genome data and broad geogrpahic sampling to better characterize the house wren hybrid zone in North America as detailed in his research proposal.

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I have never met anyone with Will's ability to inspire others to pay attention to, and care about, the natural world. He has an infectious personality and is always optimistic and kind. At the same time, Will is acutely aware that his background and privilege in STEM have led, at least in part, to his current position. He is one of the rare members of the majority who are really committed to being change makers, to listening to the voices of historically underrepresented people, and to using his position to address equity, inclusion and diversity in STEM. The ease with which Will relates to others, combined with his passion for the natural world and his knowledge of inequity in STEM, poise him to be a leader in the field as he continues on his academic journey. Speaking from my own experience, the burden of addressing equity diversity, and inclusion in STEM often falls on members of historically underserved groups. This burden is unfair, and Will is passionate about helping change this pattern. I have every confidence that this motivation will continue, and that Will has the potential to be a force for good in a field that struggles with a lack of kindness and abuse of power by those in the majority. Maybe it seems weird to include this in a letter of support for a research grant, but I really feel that we should be supporting those who will help advance our field both scientifically and from an inclusivity perspective.

Overall, Will possesses not just the skills necessary to ensure that he accomplishes his proposed work, but the motivation, character, and excitement that makes for a great scientist who will inspire and care for others. Will's love of birds, and eagerness to always learn more about them, has sparked a drive that propels Will's research and has made him a leader in his community. He is also an exceptional teacher and someone who cares deeply about fair treatment and effective communication For this and the other reasons I have mentioned, Will is an excellent candidate for a DFO Research Grant and I strongly urge you to consider him.

Sincerely,

Scott A. Taylor
Associate Professor, Dept. of Ecology and Evolutionary Biology, University of Colorado Boulder
Director, Mountain Research Station

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17 January 2024

To Whom It May Concern:

I am writing to support Will Anderson's application for funding from Denver Field Ornithologists for his project on mapping the House Wren subspecies hybrid zone in Colorado.

I first met Will in fall 2019 when he walked into my office at the University of Colorado, Boulder, and asked me to be the faculty advisor for the student birding club he was in the process of starting. Will is going to make a terrific professor of biology someday – not only because of his deep love of birds, his sharp intellect, and his curiosity about their evolution, but because he loves teaching and sharing his knowledge. He comes to me frequently with questions about how to interpret student performance on the activities and assessments he's written, showing a deep engagement with whether or not his students are understanding the material. I've also been impressed by how much he enjoys the technical aspects of genomic analysis, taxonomy, and population biology. His academic writing is clear and his thinking high-level. He is highly dedicated to his work and will back out of social commitments without hesitation if he feels he's in danger of falling behind. His personal and professional standards are high.

As is essential for a graduate student, he takes the initiative. Since he didn't find a birding club at CU when he arrived, he founded one. Just as essentially, he remains flexible. He initially proposed an ambitious undergraduate honors thesis on raven hybridization, driven by his own curiosity to pursue avenues not currently being studied by other researchers, but when the raven idea ran into insurmountable logistical roadblocks, he readily switched his focus to House Wrens.

His proposed project is scientifically valuable, as House Wrens are simultaneously well studied and understudied. They are easy to work with, as they are abundant and readily nest in artificial boxes, and yet they are complicated. Scott Taylor's lab has begun using them as a valuable model organism to test theories about what happens when separate populations that have begun the process of speciation come back into contact before speciation is complete. The potential results have much to teach us about avian hybrid zones, species boundaries, and genetic divergence. The involvement of Scott Taylor, Garth Spellman, and Erik Funk in this work guarantee that it is on strong theoretical and logistical footing.

I believe Will's proposal is an excellent fit for this grant. If I can provide you with any additional information, please do not hesitate to contact me.

Best,

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