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Exploring the geographic consistency of the genetic basis of spatial cognition in mountain chickadees

Introduction The mountain chickadee (*Poecile gambeli*, MOCH) is a widespread, gregarious nonmigratory passerine common to the high elevation coniferous forests of Western North America. Phylogeographic studies based on genetic data have described two distinct populations east and west of the Sierra Nevada Mountains between which there is limited gene flow. These populations have likely been isolated for between 610,000 and 1,530,000 years and beyond genetic variation, morphological and plumage differences corroborate the hypothesis of long term isolation of MOCH in the Rocky and Sierra Nevada Mountains (Spellman et al. 2007). Montane bird species are particularly vulnerable to climate change, primarily due to changes in habitat suitability and upslope movement which can lead to species extirpation (Şekercioğlu et al. 2012). In Colorado, MOCH are likely to experience increased pressures ranging from loss of suitable habitat to encroachment of competing species, particularly the Black-capped chickadee, as climate change accelerates (Grabenstein et al. 2022). Importantly, understanding how potentially adaptive alleles, like those involved in MOCH spatial cognition (how they acquire information about their environment and use it to determine where they are, and how to obtain resources), are distributed can help predict future species responses to changing conditions (Bay et al. 2017). A better understanding of intraspecific variation in the genetic basis of spatial cognition in MOCH, will allow us to determine how Colorado chickadees might adapt as our climate changes. Mountain chickadees rely on their spatial cognitive ability to survive the harsh winter. Specifically, they cache seeds when resources are plentiful in the fall, and recover them in the difficult winter months. Some MOCH at high elevations, in harsher environments, have been shown to have enhanced spatial learning; and have larger hippocampi, the region of the brain associated with memory (Sonnenberg et al. 2019). Further, natural selection acts on spatial cognition in MOCH at high elevations in the Sierra Nevada Mountains, resulting in birds at higher elevations generally exhibiting enhanced spatial cognition (Sonnenberg et al. 2019). Finally, an examination of whole genome variation between the best and worst performing MOCH from the Sierra Nevada Mountains revealed significant differences in genes involved in hippocampal development and function, as well as neurogenesis (Branch et al. 2022). Thus far, the genetic basis of spatial cognition in MOCH has only been examined in a single geographic region where only one subspecies of MOCH occurs. Here I seek to expand our understanding and determine whether the genetic basis of spatial cognition in MOCH is geographically consistent between populations in the Colorado Rocky Mountains (*P.g. gambeli*) and the California Sierra Nevada Mountains (*P.g. inyoensis*). Answering this question will provide a unique opportunity to evaluate genetic parallelism in adaptive regions of the genome and help us understand the adaptive capacity of spatial cognition in MOCH in Colorado

Methods This project will build upon the well-established Boulder Chickadee Study at the University of Colorado Boulder Mountain Research Station (MRS) in Nederland, Colorado and will be conducted in collaboration with my colleagues, Sara Padula and Nicole Addison. Starting in the spring of 2023, we will capture birds by monitoring nest boxes, and using basket traps at two sites at the MRS (~2900 m). To ensure that we capture MOCH across the spectrum of spatial cognitive function, which is better on average at higher elevations, we will sample as many individuals as possible and select 50 that span the range of our performance metric. We will draw blood for DNA samples, band each bird with a Passive Integrated Transponder (PIT) tag and color band at least 50 adult and juvenile MOCH, following established methods (Branch et al. 2022). We will install two identical radio frequency

identification (RFID) feeder arrays at the MRS far enough apart that they should draw different foraging flocks of chickadees. These arrays consist of eight identical feeders, which use radio frequency antennae and PIT tag bands to identify and open for specific individuals (Figure 1); allowing us to determine key behavioral characteristics, like error rate and serial reversal learning, which will be used to assess the cognitive performance of wild birds in situ during winter 2023 and 2024 (Branch et al. 2022). Using DNA extracted from the blood samples, we will sequence whole genomes of each individual, and call Single Nucleotide Polymorphisms (SNPs) following the Genome Analysis Toolkit (GATK) best practices. We will then assign each chickadee a spatial cognition performance score, making sure to include individuals spanning the range of trait variation, following the criteria outlined in previous work (Branch et al. 2022). We will then conduct a genome-wide association study using GEMMA and a high quality annotated MOCH genome already produced by my lab. We will further investigate patterns in the SNPs, genes, and gene ontology between both populations using PANTHER Classification System (Mi et al. 2020), to characterize similarities and differences in the genetic architecture of spatial cognition between the Sierra Nevada and Rocky Mountain MOCH.