Title: Quantifying the Effects of Geographic Isolation on Song Variation Between Populations of Black-Capped Chickadees Objective: This project seeks to investigate bird song in geographically isolated populations of a common songbird species, the black-capped chickadee, to further our understanding of how isolation influences song evolution. Introduction: Bird song is critical for conspecific recognition, territory defense, and assortative mating (i.e., preferential mating based on phenotypic similarity). Much like human language, bird song is learned at a young age through exposure to parents or other neighboring adults (Lynch 1996). It is likely due to this learned quality that cultural evolution in song has been observed across multiple species, resulting in a range of geographically associated "dialects" (Lynch 1996; Krebs & Kroodsma 1980). This cultural evolution has been proposed to operate under many of the same phenomena as biological evolution: mutation, selection, flow, drift, etc. (Lynch 1996). Songs of geographically isolated populations may be prone to cultural divergence due to limited contact with individuals from outside populations, which reduces "flow" and enables variation (Lang & Barlow 1997; Kroodsma et al. 1999). It has been proposed that, because such populations tend to be smaller, there may be decreased selective pressure on individuals to sing their established species song (Gammon et al. 2005). Consequently, "errors" in an individual's song may also be more common in isolated populations. These errors can subsequently be transmitted to future generations and cause further divergence. Most previous literature on bird song evolution has shown that a species'song can display significant variation between continuous mainland and isolated island populations (Lang & Barlow 1997; Parker et al. 2012; Kroodsma et al. 1999). However, song variation may also occur at a smaller scale. For example, one study showed that populations of ruddy-capped nightingale thrushes (Catharus frantzii) isolated by montane barriers exhibited variation in their songs (Ortiz-Ramírez et al. 2016). My project aims to explore the potential for song dialect evolution given the presence of smaller-scale natural geographic barriers that exist for a common Colorado songbird species, the black-capped chickadee. I will examine the effects of canyons, barriers unique in that they are located intimately within surrounding continuous habitats, as isolating mechanisms that drive song evolution. I hypothesize that, due to a lack of exposure to outside intraspecific individuals and reduced cultural flow, the songs of canyon-isolated populations will diverge from each other and from the songs of continuous populations. Furthermore, I hypothesize that, given the existence of geographically associated song dialects, song divergence will increase with distance between canyon-isolated populations.

Study System: The black-capped chickadee (Poecile atricapillus; BCCH) is a familiar, non-migratory songbird found in riparian habitats throughout North America (Sullivan et al. 2009). In Colorado, they typically occur at lower elevations, but are occasionally observed in more montane habitats (Grava et al. 2012). The BCCH's notorious fee-bee song is learned in juveniles via exposure (Kroodsma et al. 1995), and in laboratory settings, juveniles of the species have exhibited the ability to learn and perform a great variety of songs (Kroodsma et al. 1995). These two findings would suggest that this song experiences a wide range of variation in correlation with geographic separation, but the fee-bee song is standard in most observed populations— a 1999 study surveyed the fee-bee song across North America and found it to be largely uniform (Kroodsma et al. 1999). However, populations sampled in the same study from three islands off the coast of Massachusetts each displayed an array of unusual songs that strayed from continental conformity in frequency and amplitude (Kroodsma et al. 1999). Another study reported that song diverged more from the continental standard in isolated "habitat islands" than in continuous populations established along the Poudre River. It is worth noting, though, that these habitat islands were suspected to be sink populations, relying mostly on dispersing juveniles (Gammon et al. 2005). Nevertheless, these studies provide evidence that, despite its widespread stereotypy, the fee-bee song is susceptible to variation in not just completely, but perhaps even moderately isolated populations. Canyons provide ideal structures for studying moderately isolated BCCH populations due to their inherent riparian habitats and the dispersal patterns of BCCHs. BCCHs disperse from their natal populations as juveniles, and typically relocate to a site approximately 1.1km

away, where they live for the remainder of their lives (Weise & Meyer 1979). Because of their relatively small average dispersal distance, individuals from populations that have established themselves within canyons would be unlikely and perhaps unable to disperse to areas outside of the canyon. Additionally, the regions within the canyons at which BCCHs are most likely to occur exist at lower elevations than surrounding areas. This paired with the species' elevational preferences would further limit the mobilization of a canyon-dwelling individual, thus maintaining the isolation of such populations. These two observations, along with the existence of canyon-dwelling populations, suggest that such populations are lasting and stable, potentially unlike the isolated populations measured in the Fort Collins study. My study will take place in Boulder County, CO, and nearby areas, where there are both a number of BCCH canyon populations and BCCH continuous populations in urban areas.
Field Methods: I will sample chickadees in five Boulder County canyons (i.e., isolated populations) and two canyons outside of Boulder for outgroups (Figure 1). Sample sizes will differ according to the length of each canyon (Table 1) . My anticipated sampled sizes are calculated based on the total canyon length, and the fact that I will sample no more than one bird per 500 meters in order to avoid sampling the same bird twice. All of these canyons have similar starting elevations and elevational ranges, so it is unlikely for elevation to be a confounding variable, but I will be recording GPS coordinates at the location of each bird sampled, and include elevation as a random effect for my statistical analyses. I will compare song variation between my proposed isolated canyon populations and two continuous populations. Sampling in continuous populations will occur in the cities of Boulder and Denver, and will consist of 25 birds in each city. I will record chickadees using a Sennheiser ME-66 unidirectional microphone and Marantz digital recorder. Data Analysis: I will analyze recorded songs with the bioacoustics software Raven Pro 1.6, and use its automatic spectrogram measurement features. The song characteristics that I will measure will be total duration, frequency ratio between the two notes, and, for each note, relative duration, glissando, starting frequency, and number of amplitude breaks. The selection of these characteristics are informed by prior literature on BCCH song analysis (Kroodsma et al. 1999; Hahn et al. 2016). I will use these song features for downstream statistical analyses with the following aims: (1) determine how much song varies between canyons, (2) test whether canyon populations have more distinct songs compared to continuous populations, and (3) determine whether song variation occurs between continuous populations. To compare song variation between sampling populations I will run an analysis of variance (ANOVAs) for each song feature. I will further test the ability of each sampling population to predict differences in song features using generalized linear mixed models (GLMs) where I will control for elevation. Lastly, to visualize song variation between sampling populations, I will run a principal component analysis (PCA). I will use the program R to conduct all statistical analyses.