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**Title: The effects of color pattern differences on between-species aggressive interactions in a Colorado Front Range avian hybrid zone**

Background: Signals like color patterns and vocalizations mediate interactions among animals that live together and are thus important for reducing costly encounters, such as misguided fights or mating attempts [1]. This is largely due to the role that signals play in species recognition, enabling individuals to distinguish members of their own species (conspecifics) from other species (heterospecifics), with whom mating attempts may be unsuccessful and aggressive interactions may be unnecessary. In birds, species that live together are more divergent in color pattern than those that live apart [2], suggesting that signal divergence is important for coexistence; the influence that color pattern divergence has on between-species interactions, however, remains poorly understood. Study species: I will conduct this work on Black-capped (*Poecile atricapillus*) and Mountain Chickadees (*P. gambeli*) in the Front Range of the Colorado Rocky Mountains, where they occasionally interbreed [3]. Across an elevational gradient, mixed *Poecile* populations transition from consisting of mostly Black-capped Chickadee individuals to mostly Mountain Chickadee individuals [3]. While fairly ecologically similar, Black-capped and Mountain Chickadees exhibit some striking differences in color pattern (Fig. 1; note, especially, the Mountain Chickadee white eyebrow stripe). Experiments involving naïve chickadees from areas where the two species do not co-occur suggest that these color pattern differences can reduce mistakes in mate choice [4], but do not influence levels of aggression [5]. Some evidence, however, suggests that experience with heterospecifics may influence chickadee between-species aggression in areas of co-occurrence [6], but we do not yet know whether differences in color pattern guide these responses.

Objectives: Here, I aim to understand whether the color pattern differences among chickadee species influence aggressive interactions between them when they live together. I will integrate results from robust field experiments, population surveys, and cutting-edge genomic analyses from Colorado Front Range populations of Black-capped and Mountain Chickadees. The presence of individuals with varying genetic ancestries, trait combinations, and social experiences, make this contact zone an excellent setting in which to examine the interplay between responses to divergent signals and experience with heterospecifics on ecological and evolutionary timescales. Specifically, I will conduct behavioural experiments across the hybrid zone to test one hypothesis explaining the importance of signal divergence for species coexistence: if signal divergence is favoured by selection against heterospecific aggression, I predict that individuals will be less likely to engage in aggressive encounters with divergently colored heterospecifics. I will further test whether responses vary with experience with heterospecifics or genetic ancestry

Relevance to DFO grant program mission: Through this project I aim to study between-species behavioral interactions and the traits that influence them in the Front Range of the Colorado Rocky Mountains. The occasional hybridization observed between Black-capped and Mountain Chickadees in the Front Range is thought to be facilitated by increased contact between these species caused by urbanization [3]. While both the local distributions of these species and the frequency of hybridization between them are becoming increasingly well-understood [3], the between-species behavioral interactions that occur due to recent habitat changes that increase contact between Black-capped and Mountain Chickadees remain less-explored. This work will investigate whether the striking color pattern differences between these birds influence species recognition in aggressive interactions, which have important implications for resource (e.g., food, territory, nest cavity) and mate acquisition [1].

Learning about the traits that mediate between species behavioral interactions in this unique Front Range contact zone will help us to better understand the impact that human disturbance has on birds locally, and beyond.

**Research methods:** This project will be carried out by Haley Kenyon, a Postdoctoral Associate in Ecology and Evolutionary Biology at University of Colorado Boulder, under the mentorship of Dr. Scott Taylor. This work will be located along an elevational gradient in the Front Range of the Rocky Mountains between Boulder, CO and the CU Boulder Mountain Research Station near Ward, CO.

**Behavioral experiments:** I will present paired 3D printed models painted to match the color patterns of focal *Poecile* species (Fig. 1) to hybrid zone individuals [4, 5]. To examine whether color pattern differences decrease aggression among species, I will compare how frequently territorial males attack heterospecific models with divergent vs. similar color patterns (n=40); fewer attacks on divergent models will indicate reduced heterospecific aggression based on differences in color pattern. I will compare these responses to previously measured responses of naïve Blackcapped Chickadees living far from the contact zone [5] to understand the influence of experience with heterospecifics on color pattern-based species recognition.

**Covariates:** To understand the genetic ancestry of each experimental individual, I will collect blood samples, extract DNA, and construct whole-genome libraries to calculate hybrid index using diagnostic genomic variants (i.e., SNPs [3]). I will test whether hybrid index predicts responses to varying color patterns. To further explore the influence of learned experience with co-occurring chickadee species on aggressive responses to divergent color patterns, I will survey the *Poecile* population near each experimental individual and incorporate focal bird age to understand how likely focal birds are to have encountered divergent color Fig. 1: Chickadee species that live together differ more in color pattern than those that live apart. Black-capped Chickadees co-occur with Mountain Chickadees in part of their range (e.g., Boulder County, CO) but never co-occur with the equally closely related Mexican Chickadee [7]. Mountain Chickadees differ more in color pattern from Blackcapped Chickadees (especially the white eyebrow) than Mexican Chickadees do [5], despite being equally closely related. Illustrations: [8]. patterns; young birds their first breeding season (second year birds) that live in areas with only members of their own species are unlikely to have learned to respond differently to divergent *Poecile* color patterns. I expect that if learned experience with heterospecifics influences species recognition in aggressive contexts, then birds' responses to divergent color patterns will be influenced by the frequency of their interactions with differently colored *Poecile* individuals.