

Across a wide range of taxa, animals use visual signals to communicate their quality as a potential mate. The majority of research regarding the signal function of visual traits has concerned color or patches of color. This focus on charismatic coloration has left unexplored the potential signaling function of achromatic patterns. Some patterns, such as the white check patch of great tits (*Parus major*) or the black facial spots of female paper wasps (*Polistes dominula*), have been suggested to function as assessment signals¹. Yet, there is little empirical evidence that females consider patterns when selecting a mate. In the songbird family Estrildidae, patterns or certain features of patterns may be dimorphic, which suggests that these may be sexually selected traits. This idea is only beginning to be explored, with some studies linking estrildid patterns to individual quality^{2,3}. Little is known, however, about how females perceive these signals, including the extent to which features that stand out to human observers draw the attention of the birds themselves.

The zebra finch (*Taeniopygia guttata*) is an ideal species in which to study the role of achromatic patterns in estrildids. This species exhibits four notable sexually dichromatic traits, two being related to color and two being related to pattern. These include red beak color, which is more pronounced in males than females, and an orange cheek patch, barred bib, and white flank spots that are only exhibited by males. Although the function of male beak color has been well studied, little attention has been paid to the other three dimorphisms, aside from one study showing that female zebra finches prefer males with more symmetrical barred bibs⁵. There also is little known about how these dichromatic patterns have evolved, and to what extent these patterns vary in wild populations. Furthermore, zebra finches come from the subfamily Poephilinae⁶, which exhibit a wide range of chromatic and achromatic patterns, allowing for a tractable phylogenetic comparison of perceptual abilities. **I hypothesize that dimorphic achromatic patterns function as signals of quality independent of color, and that species with these patterns are able to perceive and assess variation in this signal better than those without.**

Aim 1a: Quantify the range of natural variation of zebra finch patterns. Using museum specimens from the University of Michigan's ornithology collection, I propose to measure the degree of variation of plumage patterns within zebra finches. After photographing the ventral and lateral sides of specimens, I will use ImageJ software to determine the degree of variation in male bar and spot pattern. This is an important analysis because in order for sexual selection to operate, there must be existing variation for it to act upon. After determining the range of this variation, I will categorize the types of plumage variation that may exist among males. Likely, these will include differences in regularity, contrast level, and density of patterns, though other features may also differ. Finally, I will test if certain aspects of these patterns covary with body size, beak color, or other indicators of individual quality.

Aim 1b: Determine the extent to which females can discriminate natural pattern variation. Using methods developed to test for categorical color perception in this species (i.e. training birds to flip discs of different colors for a food reward)⁷⁻⁹, I propose to ask how female zebra finches perceive variation in patterns of bars and spots. To do this, I will make discs using paper printed with images of male chest or flank plumage as determined in Aim 1a. I will place these discs on wells in which a food reward is found and train birds to select the pattern that varies the most from the others. Using the range of variation determined in Aim 1a, I will test for the extent to which two varied patterns are indistinguishable to zebra finches. Given that zebra finches court at close range, this approach matches how close females would be to these patterns when assessing males. Additionally, I will test for sex differences in contrast perception to ask whether dichromatic bar/spot patterns could function in female choice, male competition, or both. **If dichromatic patterns serve as signals of quality, I predict that female zebra finches will have the visual acuity necessary to discern variation in this signal.**

Aim 2: Test female preference for pattern quality. I will test for female preference of male pattern variants using digitally manipulated images of male conspecifics. Using a television screen separated by a partition (hereafter "Bird TV"), I will observe how long females spend near one of two videos of male zebra finches as evidence of their mate preference. I will first record videos of male zebra finches and then digitally alter certain aspects of their achromatic patterning that have been positively correlated with body condition and dominance hierarchy in other estrildids: regularity of barred plumage² and number of white spots¹⁰, respectively. Bird TV, a novel method for mate choice experiments, is currently being developed

by my advisor, Dr. Steve Nowicki, to test female preference for male beak color, which is a well-supported preference. After this approach is validated, Bird TV will be used in my proposed experiment. **I predict that females will prefer the aforementioned pattern variants that have been previously suggested to serve as signals of quality (regularity of barred plumage and large spot size), but have not been explicitly tested.**

Aim 3: Compare the perceptual abilities of closely related estrildids: The zebra finch is a unique estrildid in that it simultaneously exhibits dimorphic carotenoid coloration, barred plumage, and spotted plumage. As a result, it is possible that zebra finches are adept at both color and contrast perception. Other closely related species, such as the long-tailed finch (*Poephila acuticauda*) or double-barred finch (*Taeniopygia bichenovii*), exhibit fewer dimorphic traits. Therefore, I will compare the visual abilities of zebra finches to its closest relatives to see if there are trade-offs associated with color and contrast perception. For example, the double-barred finch does not exhibit carotenoid coloration, but both males and females have bars and spots, and so may have been selected to specialize in perceiving patterns. In contrast, the long-tailed finch exhibits carotenoid coloration in both male and female beaks, but lacks bars or spots, suggesting that it may have been selected to specialize in color perception. **I hypothesize that there is a trade-off between color and contrast perception—specifically, that exclusively colorful birds are better at color perception, and that exclusively patterned birds are better at contrast perception.** I propose to test the color and contrast perception of the long-tailed finch and double-barred finch using the methods from Aim 1, then test female choice using methods from Aim 2, and compare these results to those of the zebra finch. An inconspicuously colored and more distant relative of the zebra finch, the Bengalese finch (*Lonchura striata domestica*), was recently shown to discriminate colors differently than zebra finches¹¹. My work will follow up on this investigation to test the effect of phylogenetic relatedness on color and pattern perceptive abilities in Estrildids.

Intellectual Merit: In studying the potential signaling function of certain visual traits, it is critically important to first understand how these signals are perceived. If such signals are not being perceived or differentiated at all, then we need to re-evaluate what other function they could possibly serve apart from intraspecific communication. While patterned plumage has been previously proposed to function in intraspecific signaling, no study has actually examined how adept animals are at perceiving variation in these patterns. Therefore, my work will be a necessary first step in determining if patterned plumage should continue to be explored as a signal of quality.

Broader Impacts: As an NSF fellow, I will ensure that my research, at every stage, contributes to my personal goal of retaining as many students as possible in the sciences. At Duke, I will invite undergraduates to not only assist in collecting data, but also in contributing intellectually so that they may share authorship on future publications. During the summer, I will train undergraduates through Duke's paid Biological Sciences Undergraduate Research Fellowship. This program not only introduces students to biological research, but also provides professional development opportunities and a campus-wide showcase to present their research. I benefited greatly from summer research programs like this, particularly the NSF REU, so I know firsthand the impact they can have. I want to help Duke undergraduates feel comfortable in a research environment through close mentorship and reassurance that questions and mistakes are part of the learning process. Beyond Duke, I plan to share my research with scientists and science enthusiasts in the broader "Triangle" (Durham, Raleigh, and Cary, NC) area. The North Carolina Museum of Natural Sciences in nearby Raleigh hosts opportunities for science communication and outreach that I plan to fully engage in. For one, they host the Scientific Research and Education Network (SciREN) Networking Event, which gives an opportunity for scientists to adapt their research into K-12 lesson plans. They also host a Darwin Day event, in which people of all ages are invited to learn about Darwin and his legacy. I will share my findings at these events, and adapt them so that they are appropriate and accessible to all ages and backgrounds. Finally, I will participate in Skype-a-Scientist to connect with students globally.

¹Pérez-Rodríguez et al. 2017. *Proc. Royal Soc. B.* ²Marques et al. 2016. *Roy. Soc. Open Sci.* ³Soma & Garamszegi 2018. *Behav. Ecol.* ⁵Swaddle & Cuthill 1994. *Proc. R. Soc. Lond. B.* ⁶Olsson & Alström 2020. *Mol. Phylogenet. Evol.* ⁷Caves et al. 2018. *Nature* ⁸Zippel et al. 2019. *Proc. Royal Soc. B.* ⁹Caves et al. 2020. *Behav. Ecol. Sociobiol.* ¹⁰Crowhurst et al. 2012. *Ethology* ¹¹Caves et al. (in press) *Am. Nat.*