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Achromatic plumage variation between and within hybridizing Black-capped and Mountain chickadees

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ABSTRACT. Feather coloration and patterning are major signals influencing mate choice within and between species. However, most studies of the role of plumage in mate choice have focused on colorful species with obvious sexual dichromatism. To better understand how achromatic plumage might influence hybridization, we quantified plumage variation between and within two achromatic songbirds that occasionally hybridize, Black-capped (*Poecile atricapillus*) and Mountain (*P. gambeli*) chickadees. We collected feathers from 43 live birds and photographed 155 prepared museum specimens to measure overall plumage color and the size of the throat and cheek patches. Using principal component analyses and generalized linear mixed models, we characterized plumage patterns within and between Black-capped and Mountain chickadees from Colorado to examine plumage color variation and differences in throat and cheek patch size. We found that Black-capped Chickadees (1) were less achromatic and had brighter plumage with more color contrast than Mountain Chickadees, (2) had smaller throat and cheek patches than Mountain Chickadees, and (3) were not sexually dimorphic. We also found that male Mountain Chickadee museum specimens had brighter plumage with more ultraviolet reflectance than female museum specimens (i.e., they are sexually dimorphic). However, we did not observe sexual dimorphism in live Mountain Chickadees, potentially because we did not sample the supercilium. In contrast to previous studies, we found that Black-capped Chickadees are not sexually dimorphic, potentially because plumage is not used in mating decisions for populations at lower latitudes. Between Black-capped and Mountain chickadees, differences in plumage color and patch sizes may influence hybridization if female Mountain Chickadees prefer the brighter plumage and greater color contrast of male Black-capped Chickadees. Our results will guide future work exploring the role plumage might play in hybridization between Black-capped and Mountain chickadees by informing plumage manipulation experiments investigating the influence of brighter plumage and color contrast in hybridization.

RESUMEN. **Variación acromática del plumaje inter- e intra-híbridos en los carboneros *Poecile atricapillus* y *P. gambeli***

La coloración y patrones de las plumas son señales mayores que influencian la selección de parejas intra- e interespecíficamente. Sin embargo, la mayoría de los estudios del papel del plumaje en la selección de pareja se han enfocado en especies coloridas con un evidente dicromatismo sexual. Para entender mejor cómo el plumaje acromático podría influenciar la hibridación, cuantificamos la variación del plumaje intra- e inter-específica de dos aves canoras que ocasionalmente se hibridan, los carboneros *Poecile atricapillus* y *P. gambeli*. Colectamos plumas de 43 aves vivas y fotografiamos 155 especímenes de museo para medir el color total del plumaje y el tamaño de los parches de garganta y mejillas. Usando un análisis de componentes principales y modelos lineales generalizados mixtos, caracterizamos los patrones del plumaje intra- e inter-específicos de estos carboneros de Colorado para examinar la variación y las diferencias en el tamaño de los parches de garganta y mejillas. Encontramos que *P. atricapillus* (1) fueron menos acromáticos y tuvieron un plumaje más brillante con más contraste de color que *P. gambelli*, (2) tenían parches de garganta y mejillas más pequeños que los de *P. gambelli* y (3) no son sexualmente dimórficos. También descubrimos que los especímenes de museo de machos de *P. gambelli* tenían un plumaje más brillante con mayor reflectancia ultravioleta que los especímenes de museo de hembras (i.e., son sexualmente dimórficos). Sin embargo, no observamos dimorfismo sexual en *P. gambelli* vivos, potencialmente porque no muestreamos el supercilium. En contraste con estudios previos, encontramos que *P. atricapillus* no son sexualmente dimórficos, potencialmente porque su plumaje no se usa en decisiones de apareamiento en poblaciones a latitudes más bajas. Entre *P. atricapillus* y *P. gambelli*, las diferencias en color del plumaje y los tamaños de parche podrían influenciar la hibridación si las hembras de *P. gambelli* prefieren el color más brillante y con mayor contraste de color de los machos de *P. atricapillus*. Nuestros resultados guiarán trabajo futuro para explorar el papel que el plumaje podría jugar en la hibridación entre estos carboneros, informando experimentos de manipulación de plumaje para investigar la influencia del brillo y el contraste de color del plumaje en la hibridación.

Key words: hybridization, plumage, sexually dimorphic, species recognition, tetrachromatic

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Species recognition is critical for preventing breeding mismatches and maintaining reproductive isolation between closely related species (Ficken and Ficken 1968, Alatalo et al. 1994, Sætre et al. 1997, Wirtz 1999, but see Mendelson and Shaw 2012). Plumage plays a role in regulating species recognition in birds, with differences in feather coloration and patterning influencing mating decisions both within and between (i.e., hybridization) species (Randler 2002, 2006, Bleiweiss 2004, Laaksonen et al. 2015, Bitton and Doucet 2016, López-Rull et al. 2016). Hybridization —the interbreeding of populations that differ by one or more heritable characters—is relatively common in birds, with ~10% of bird species documented to hybridize (Baker 2008). Hybridization between bird species can be the result of various factors, including population density (e.g., individuals mate with a common closely related species if conspecifics are rare), secondary contact after divergence in isolation (e.g., many of the Great Plains avian hybrid zones), female preferences for exaggerated signals (e.g., hybridization between male Blue-footed Boobies [*Sula nebouxii*] and female Peruvian Boobies [*S. variegata*]; Taylor et al. 2010), or male aggression (e.g., hybridization in many duck and albatross species; Mineau et al. 1983, Wirtz 1999). The outcomes of hybridization vary, but can have severe fitness consequences, including wasted reproductive effort if hybrid offspring are inviable or sterile (Harrison and Larson 2014).

The role of plumage in avian hybridization remains a topic of great interest. In many cases, females choose males with more colorful or elaborate ornaments when they hybridize (Randler 2002). Interestingly, Randler (2006) found that sexual dichromatism did not appear to influence the likelihood of birds hybridizing. However, the species included in this study were all categorized as sexually dichromatic from a human perspective—some birds (including songbirds) have an additional type of retinal cone that responds to ultraviolet wavelengths (Chen and Goldsmith 1986), potentially biasing the results of studies if birds that are sexually dichromatic from a songbird perspective were categorized as monochromatic. Further, many studies of the role of sexual dichromatism in hybridization have focused on species with colorful, chromatic plumage, such as the yellow collars of male Golden-collared Manakins (*Manacus vitellinus*) and the bright yellow plumage of Blue-winged Warblers (*Vermivora cyanoptera*) (Stein and Uy 2006, McKinnon and Robertson 2008). Less is known about how plumage influences species recognition among achromatic species.

In birds, achromatic plumage is correlated with components of fitness, suggesting that achromatic plumage may signal individual quality, potentially through color or patch size (Gladbach et al. 2011, Cantarero et al. 2017, Enbody et al. 2018, Taff et al. 2019). For example, higher testosterone levels of female White-shouldered Fairywrens (*Malurus alboscapulatus*) and female Pied Flycatchers (*Ficedula hypoleuca*) were found to be positively correlated with achromatic ornamentation and the size of white wing patches, respectively (Cantarero et al. 2017, Enbody et al. 2018). Male Upland Geese (*Chloephaga picta leucoptera*) in better body condition have darker specula and greater contrast between the black speculum and white coverts (Gladbach et al. 2011). Because achromatic plumage coloration can influence mate choice by signaling individual quality (Dunning et al. 2014, Cantarero et al. 2019), determining the possible role of achromatic plumage in intraspecific and interspecific communication is essential for understanding how plumage might influence the likelihood of hybridization. In contrast to studies of chromatic plumage, previous work on achromatic plumage highlights the importance of color contrast between neighboring patches and overall patch sizes (i.e., badges of status, where patch size may indicate aggressiveness; Johnstone and Norris 1993), in addition to wavelength reflectance (Mason and Bowie 2020). Therefore, characterizing both interspecific and intraspecific plumage variation that may be involved in species recognition and mate choice is important for understanding the possible role of plumage in mediating reproductive transactions within and between species. Few investigators have quantified plumage variation between closely related achromatic species with apparent sexual dimorphism (e.g., Enbody et al. 2017) and no one to date has examined variation in plumage color between hybridizing, achromatic species that appear sexually monochromatic to humans.

Black-capped (*Poecile atricapillus*) and Mountain (*P. gambeli*) chickadees are closely related, achromatic songbirds. Although ecologically segregated along mountain slopes, these species occasionally hybridize in sympatry (Hubbard 1978, Martin and Martin 1996, Grava et al. 2012), and hybridization typically occurs between female Mountain Chickadees and male Black-capped Chickadees (Grava et al. 2012). Our preliminary data indicate a rate of hybridization of ~6% (Grabenstein, unpubl. data) and that offspring are both viable and fertile (i.e., individuals with hybrid indexes indicative of backcrosses exist in our data), but hybrid offspring likely suffer fitness consequences (Grava et al. 2012). Given the importance of plumage in mate choice by Black-capped Chickadees, where females can identify dominant males by plumage alone (Woodcock et al. 2005), and hybridizing birds more broadly (Randler 2002, 2006), variation in plumage color and differences between the two species in the size of plumage patches might influence the likelihood of hybridization.

Previous studies have revealed differences in plumage color and patch sizes of Black-capped and Mountain chickadees (Mennill et al. 2003, Eaton 2007), but plumage characteristics of these hybridizing species from a region where both species occur have not been examined. Black-capped Chickadees are sexually dimorphic in Ontario, Canada, with male Black-capped Chickadees having brighter white plumage (e.g., cheek patch), greater plumage contrast, and larger black patches than females (Mennill et al. 2003, Eaton 2007). In addition, cheek patches of male and female Mountain Chickadee specimens in the Field Museum of Natural History and the American Museum of Natural History were found to be distinguishable (i.e., exceed the threshold discrimination value of 1 Just Noticeable Difference; Eaton 2007). Both Black-capped and Mountain chickadees have black heads and throats and white cheeks, but the size of these patches has not been compared despite their possible role in sexual signaling (Mason and Bowie 2020) and, potentially, in hybridization between the species.

We characterized plumage variation between and within Black-capped and Mountain chickadees using two complementary methods: digital photography of museum specimens and spectrophotometry of feathers from wild birds. Specifically, we measured (1) overall plumage color and the size of throat and cheek patches from photographs of museum specimens using both the Quantitative Colour and Pattern Analysis framework (QCPA; van den Berg et al. 2020) and manual patch tracing, and (2) plumage color using reflectance curves obtained using a spectrophotometer of feathers collected from live birds. Our objectives were to determine if Black-capped and Mountain chickadees in Colorado (1) differ in plumage coloration from a songbird perspective, (2) differ in the size of cheek and throat patches, and (3) are sexually dimorphic (i.e., plumage color and patch size) from a songbird perspective.

METHODS

Black-capped and Mountain chickadees are achromatic passerines that appear sexually monochromatic to humans. From a trichromatic human perspective, both species have black heads and throats and white cheek patches, but the cheek patches of the two species have qualitatively different coloration. In Colorado, the side patches of Mountain Chickadees (*P. gambeli gambeli*) appear uniformly gray whereas Black-capped Chickadees (*P. atricapillus septentrionalis*) have white side patches with tan flanks. The most noticeable difference between the two species is the white supercilium of Mountain Chickadees. Another difference is the distinct white wing bars of Black-capped Chickadees. We did not collect either supercilium or flight feathers from live birds in our study because the small areas they cover make these regions challenging to accurately measure with a spectrophotometer. In museum specimens, contour feathers and remiges (including the supercilium and white wing bars) were outlined in photographs and analyzed using the Quantitative Colour and Pattern Analysis framework (QCPA; van den Berg et al. 2020).

We used two independent methods to quantify plumage color variation between and within Black-capped and Mountain chickadees: digital photography of prepared museum specimens and spectrophotometry of feathers collected from live birds. We

measured four discrete plumage patches (head, throat, cheek, and side) using the feathers from live birds and museum specimens photographed from four different angles (dorsal, ventral, and left and right lateral) for QCPA in both species. Because color clusters generated using the QCPA framework did not coincide with the head, throat, cheek and side patches for every museum specimen, we were unable to analyze plumage color by distinct patches. As a result, we measured the bright region (i.e., 50% brightest color clusters), dark region (i.e., 50% dullest color clusters), and averages for the entire image weighted by the area of each color cluster. The following sections are organized by the methods used for data collection, including photographs of museum specimens and feathers collected from live birds, where photographs of museum specimens were used for QCPA and the manual tracing of throat and cheek patches, and feathers from live birds were measured using a spectrophotometer.

Photographs of museum specimens. We photographed 155 chickadees from Colorado, including 40 specimens from the University of Colorado, Boulder Research Collection, and 115 specimens from the Denver Museum of Nature and Science Collection (Fig. 1A). To examine plumage color and patch size variation in Black-capped and Mountain chickadees, we only photographed specimens with information on sex (44 male and 35 female Black-capped Chickadees; 38 male and 38 female Mountain Chickadees). Sex was determined by the gonads during specimen preparation.

To limit the possible effect of regional differences (e.g., subspecies variation) in feather color variation, we only photographed specimens collected in Colorado (*P. atricapillus septentrionalis* and *P. gambeli gambeli*). To control for additional variation due to differences in sampling localities, we divided Colorado into four quadrants and assigned a quadrant to each specimen based on the location collected. The eastern and western quadrants were divided at the ecotone where the Eastern plains meet the Colorado Rockies (~105°W), a change in habitat that may influence plumage. The northern and southern quadrants divide Colorado into halves (~39°N). Under these designations, 50 chickadees were collected from the northern montane quadrant, 53 from the northern plains, 33 from the southern plains, and 19 from the southern montane.

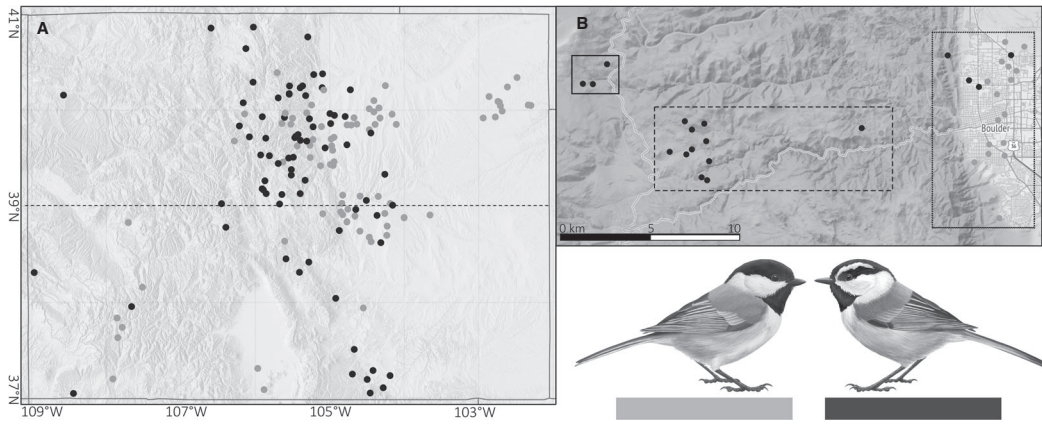

Fig. 1. Distribution of museum specimens (A) and live birds (B) sampled for this study. Museum specimens were sampled from across Colorado and live birds from in and around Boulder, CO. Dotted line in (A) designates the latitudinal divide between northern and southern quadrants (~39°N). Quadrants were divided longitudinally by the Front Range mountains (~105°W): rather than the longitudinal divide being linear, individuals were binned into quadrants based on geography (i.e., mountains or plains). In (B), live birds were collected from Boulder (dotted, right box), Sugarloaf Mountain (dashed, middle box) and the Mountain Research Station (solid, left box). Black-capped Chickadee sample locations are coral points and Mountain Chickadee sample locations are navy. Chickadee drawings by Jessica French. [Colour figure can be viewed at wileyonlinelibrary.com]

To quantify plumage color variation across the songbird visual spectrum (300–700 nm) from photographs of museum specimens, we followed the protocol outlined by Troscianko and Stevens (2015). Specifically, we captured both ultraviolet and visible photographs using a full-spectrum digital camera. For each individual, we captured three ultraviolet and three visible photographs of varying exposure for the dorsal, ventral, left and right lateral orientations for a total of 24 photos per individual. We retained eight photos (one ultraviolet and one visible photo of adequate exposure for each orientation) for color analysis for each individual.

Standard digital cameras are insensitive to ultraviolet wavelengths prior to modification. To capture ultraviolet photos, we removed the UV filter from the body of a Samsung NX2000 camera and fit it with a Nikon El-Nikkor 80-mm (1:5.6) lens and a M42-M42 Lens Adjustable Focusing Helicoid (25–55 mm) to allow UV light to enter the camera body. Images were photographed in RAW format with an ISO of 400, an aperture of f/5.6, exposure set to Aperture-Priority, and exposure bracketing of ±3. From each series of exposure bracketing, we selected photos of adequate exposure for downstream analyses (i.e., photos with greatest exposure without being over-exposed). To produce ultraviolet and visible photographs, we mounted external filters (UV filter: Baader Ultraviolet Venus Filter – 2 inch [5.1 cm], Visible filter: Baader UV-IR Cut Filter – 2 inch [5.1 cm]) on the camera lens using an Orion 6 slot 2-inch (5.1 cm) filter slider.

To emulate natural sunlight (i.e., produce a full spectrum of light), we photographed all specimens indoors under an Iwasaki eye-Colour MT70D E27 6500K lightbulb with the ultraviolet coating removed with a steel brush (ACE 3-inch [7.6 cm] wire wheel brush 1⁄4 inch [0.64 cm] stem). The lightbulb was wired to a ballast for use in the United States (Advance IMH-70-D-LFM 70 Watt Electronic Metal Halide Ballast). A PTFE (polytetrafluoroethylene) enclosure around specimens minimized shadows and ensured an even distribution of light on specimens. To standardize specimens photographed under inconsistent light conditions and varying distances from the camera, we included full-spectrum color standards in every photograph (Labsphere 99% and 20% standards) and placed a ruler (QP Card 101) next to the specimen.

Image analysis – color. We obtained feather color data from photographs using the Quantitative Colour and Pattern Analysis framework (QCPA; van den Berg et al. 2020) from the micaToolbox (V2 & QCPA; Troscianko and Stevens 2015) plugin in ImageJ (1.52o). For all orientations (dorsal, ventral, and left and right lateral) for each individual, we assembled a multispectral image stack using the ultraviolet and visible images of adequate exposure (one visible and one ultraviolet image per stack). This stack of images contained reflectance values from a trichromatic (e.g., human) perspective. To obtain tetrachromatic (e.g., songbird) data, we converted the multispectral stack using the micaToolbox Blue Tit (*Cyanistes caerulus*) cone catch. Blue Tits and chickadees are both in the family Paridae and likely have similar visual systems. By using the well-studied visual system of Blue Tits, we obtained objective color measurements from the perspective of chickadees. For each multispectral image stack, we outlined a region of the specimen to run through the QCPA framework; from the dorsal orientation, we outlined all contour feathers and remiges and, from the ventral and lateral orientations, we outlined all contour feathers and remiges cranial of the legs (Fig. 2 A). We ran the QCPA framework with a Gaussian acuity correction using the Carolina Chickadee (*Poecile carolinensis*) acuity value (5 cycles/degree; Moore et al. 2013) and a viewing distance of 0.5 m, a likely distance between individuals looking for a potential mate. Following acuity correction, we used a receptor noise limited (RNL) ranked filter, and an RNL clustering method (Color JND Threshold: 3; Luminance JND Threshold: 3). To quantify interspecific and intraspecific plumage color variation, we retained results for each color cluster (long-wave, medium-wave, short-wave, ultraviolet, and double-cone reflectance [i.e., luminance]) and the average value of the image weighted by the area of each color cluster (luminance, RNL saturation [i.e., distance a color is from the achromatic point], difference in luminance between color boundaries, and difference in RNL saturation between color boundaries) for each orientation for every individual.

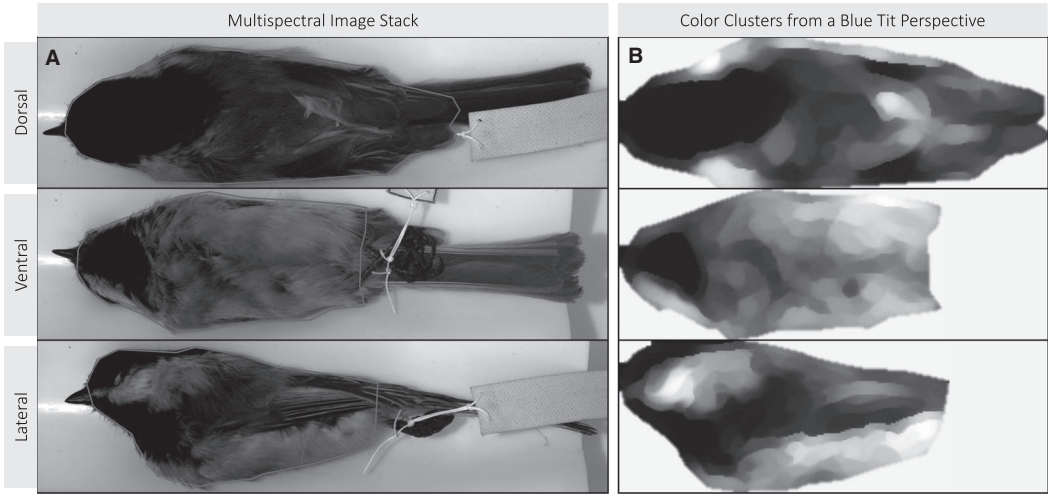

Fig. 2. Sample photographs of (A) the normalized visible red channel in a multispectral image stack and (B) clustered image from a Blue Tit perspective at 0.5 m. Orientations photographed include dorsal, ventral, and right and left lateral. Left and right lateral photographs were analyzed using QCPA and averaged to produce the lateral orientation. [Colour figure can be viewed at wileyonlinelibrary.com]

Statistical analysis – color. To analyze intraspecific and interspecific color variation in the museum specimens, we modeled feather color using digital photography data. Because each photograph contained a different number of color clusters (Fig. 2B), we ordered the color clusters in each image by double-cone reflectance (i.e., luminance) and averaged the brightest half to generate values for the "bright region" and the darkest half to generate values for the "dark region." This resulted in two averages (bright and dark) for each orientation for each individual for long-wave, medium-wave, short-wave, ultraviolet, and double-cone reflectance (i.e., luminance). This is an appropriate approach to measuring feather color because color clusters in each image were arbitrarily numbered and have quantitative values that are not consistent across photographs. Therefore, comparing color clusters across images if they occupy different regions of the specimen is not feasible. We found that ordering the color clusters by luminance and comparing the "brightest" and "darkest" was the most objective way to compare color across specimens. We had 32 plumage color variables for each orientation (Table 1), including bright region variables (e.g., long-wave mean and standard deviation for the brightest color clusters), dark region variables (e.g., long-wave mean and standard deviation for the darkest color clusters), and variables describing the entire image (e.g., mean, standard deviation, and coefficient of variation for weighted luminance). For variables representing an average for an entire image, six variables represent color contrast, where contrast is calculated as the average difference in luminance or RNL saturation between each set of adjacent color clusters in an image. Prior to analyses, we averaged the left and right lateral orientations for each individual for all plumage color variables.

To condense the variation among these color variables, we used a principal component analysis (PCA) and used the principal components as response variables for a linear mixed model (see Table S2 for proportion of variance and loadings). To generate PCAs, we used prcomp() in R v. 3.6.2, with both scale and center set as true. We ran linear mixed models using lm() from the stats package in R and obtained approximate *P* values from the lmerTest package (Kuznetsova et al. 2017). To examine interspecific and intraspecific variation in museum specimens, we generated PCAs that contained data for either both species (interspecific comparison) or one species (intraspecific comparison).

We constructed nine generalized linear mixed models using the first three principal components that accounted for >50% of the

Table 1. Plumage color variables produced by the Quantitative Colour and Pattern Analysis (QCPA) framework from photographs of museum specimens. "Plumage color" lists the plumage color variables. "Description" describes each plumage color variable and lists all sub-variables included in the principal component analysis. Orientations include dorsal, ventral and lateral sides. Left and right lateral photographs were analyzed using QCPA and averaged to produce the lateral orientation.

Plumage color	Description
Long-wave reflectance (%)	Average reflectance of the colors included in the dark and bright regions (600–700 nm). Variables include dark mean, dark standard deviation, bright mean, and bright standard deviation for all orientations.
Medium-wave reflectance (%)	Average reflectance of the colors included in the dark and bright regions (500–600 nm). Variables include dark mean, dark standard deviation, bright mean, and bright standard deviation for all orientations.
Short-wave reflectance (%)	Average reflectance of the colors included in the dark and bright regions (400–500 nm). Variables include dark mean, dark standard deviation, bright mean, and bright standard deviation for all orientations.
Ultraviolet reflectance (%)	Average reflectance of the colors included in the dark and bright regions (300–400 nm). Variables include dark mean, dark standard deviation, bright mean, and bright standard deviation for all orientations.
Double-cone reflectance (i.e., luminance)	Average luminance of the colors included in the dark and bright regions. Variables include dark mean, dark standard deviation, bright mean, and bright standard deviation for all orientations.
Weighted luminance	The luminance of the image weighted by the area of each color. Variables include the mean, standard deviation, and coefficient of variation for weighted luminance for all orientations.
Weighted RNL saturation	The RNL saturation of an image weighted by the area of each color. RNL saturation describes the distance (in receptor noise limited space [Vorobyev and Osorio 1998] each color is from gray, i.e., the achromatic point). Variables include the mean, standard deviation, and coefficient of variation for weighted RNL saturation for all orientations.
Weighted luminance boundary strength	The luminance difference of colors between boundaries across an image weighted by the relative frequency of each possible color combination transition. Variables include the mean, standard deviation, and coefficient of variation for weighted luminance boundary strength for all orientations.
Weighted RNL saturation boundary strength	The RNL saturation difference of colors between boundaries across an image weighted by the relative frequency of each possible color combination transition. Variables include the mean, standard deviation, and coefficient of variation for weighted RNL saturation boundary strength for all orientations.

variation in the data (Table S2). We included species and sex as fixed effects to describe interspecific or intraspecific variation, respectively. In the interspecific models, we used Akaike Information Criterion (AIC) to compare models with and without an interaction between species and sex (an interaction term determines if the effect of species depends on sex). Because the interspecific models with an interaction term were more parsimonious (Table S3), we included an interaction term between species and sex for all interspecific analyses. In the interspecific model, we included sex as a fixed effect to control for differences between males and females. In the interspecific and intraspecific models, we included ordinal date, year collected, quadrant, and museum as fixed effects to account

for variation not associated with species or sex. Because museum specimens were collected year-round, we included ordinal date (i.e., calendar date ranging from 1 to 366) to control for yearly, seasonal variation in plumage color. Likewise, we included the year collected to control for feather color variation between years and to account for specimen degradation (McNett and Marchetti 2005, Armenta et al. 2008). Although all specimens were collected in Colorado, we included quadrant to account for any variation due to differences by latitude and longitude in the state. To control for any differences in museum preservation, we also included museum as a fixed effect:

$$\begin{aligned}\mathrm{PC} = \; & \propto + \beta_1(\mathrm{Species})_1 + \beta_2(\mathrm{Sex})_2 \\ & + \beta_{12}(\mathrm{Species})_1(\mathrm{Sex})_2 + \beta_3(\mathrm{Ordinal\ date})_3 \\ & + \beta_4(\mathrm{Museum})_4 + \beta_5(\mathrm{Year\ collected})_5 \\ & + \beta_6(\mathrm{Quadrant})_6.\end{aligned}$$

Image analysis – patch size. Patch size was measured using only digital photographs of museum specimens. We measured the area of throat and cheek patches from photographs of museum specimens (Fig. S1). We measured patch area from the photographs with the greatest exposure to ensure that the outlines of each patch were distinct. To quantify patch area, we outlined each patch using the polygon tool in ImageJ (1.52o). Although outlining patches introduced subjectivity into the analysis, throat and cheek patches are discrete because they are surrounded by colors of qualitatively distinct brightness. Because brightness (i.e., full-spectrum reflectance) is largely affected by the visible spectrum in birds (Butler et al. 2011) and the patches are discrete in photographs, the throat and cheek patches are likely discrete from an songbird perspective. As confirmation, we qualitatively examined false color images (i.e., images from the perspective of a Blue Tit) produced in the micaToolbox (V2 & QPCA; Troscianko and Stevens (2015)). When photographing specimens, we placed a ruler (QP Card 101) next to the specimen to standardize length measurements and quantify area in the photos. To normalize patch area to specimen body size (e.g., to control for differences in museum preparation), we measured the length of the wing chord as a straight line from the top of the folded wing to the tip of the primaries and divided the patch area by the average of the left and right wing chord measurements. Because left and right lateral orientations both have a cheek patch, we averaged the area of the cheeks for statistical analyses.

Statistical analysis - patch size. To describe variation in patch size, we generated linear mixed models using throat and cheek data for interspecific and intraspecific comparisons. We used adjusted area (i.e., area normalized to wing chord) for each patch as the response variable for interspecific and intraspecific comparisons. For both patch size and plumage color analysis, we used the digital photography model discussed in *Statistical analysis - color*. Because the interspecific models with an interaction term were more parsimonious (Table S4), we included an interaction term between species and sex for all interspecific analyses.

Feathers from live birds. From May through July 2019, we collected feathers from 25 adult Black-capped and 18 adult Mountain chickadees along a nest box transect that extended from Boulder, CO (40°00′26.8″N 105°15′57.5″W) to the CU Boulder Mountain Research Station (MRS; 40°01′55.7″N 105°32′08.6″W). This transect consisted of 337 nest boxes, with 135 in Boulder (1457–1971 m), 102 along Sugarloaf Mountain Road (1946–2623 m), and 100 around the MRS (2837–3383 m). We captured 25 chickadees in Boulder (21 Black-capped and four Mountain chickadees), 15 in Sugarloaf (four Black-capped and 11 Mountain chickadees), and three around the MRS (three Mountain Chickadees; Fig. 1B).

Breeding chickadees were either hand-captured inside nest boxes or captured using mist-nets outside nest boxes or at feeders. Because we sampled both adults from numerous nest boxes, we included nest box ID as a random effect in downstream statistical analyses. Adult chickadees were banded with USGS metal bands and unique combinations of plastic color bands. We also collected > 10 feathers from each plumage patch, including the head, throat, cheek, and side patches of 15 male and 10 female Black-capped Chickadees and 11 male and seven female Mountain Chickadees. We did not collect feathers from the supercilium of Mountain

Chickadees because the supercilium patch was too small to collect feathers without altering the overall patch. Additionally, we did not collect primaries to measure the white wing bars of Black-capped Chickadees because we did not want to alter their flying abilities. Because feather color is indistinguishable between the sexes of each species in the field and we collected feathers during the breeding season, we determined sex using the cloacal protuberances of males and brood patches of females and did not include any ambiguous individuals in our study (Bronson et al. 2005, Bonderud et al. 2018).

Feather preparation and analysis. For each individual, we stacked 10–15 feathers from each patch on light-absorbing, non-adhesive flock paper based on recommendations by Quesada and Senar (2006) to best mimic how the feathers would lay on live birds. We analyzed feathers using an Ocean Optics USB 2000 spectrophotometer with a PX-2 pulsed xenon light source (220–750 nm) and an XSR fiber probe with BX jacketing (230-μm fiber core diameter). The spectrophotometer was calibrated using a Labsphere 99% white standard. We measured feather stacks using a continuous strobe with a 5000-μs strobe period, 20,000-μs integration time, and nonlinearity correction. Each feather stack (i.e., one stack per patch) was measured three times and averaged for analysis (see Table S1 for standard deviation of measurements). We held the probe at a fixed 17 mm from stacks using a reflection probe holder (Ocean Optics RPH-1). Between repeated measurements, we lifted the probe at least 1 cm from the sample.

Statistical analysis. To examine intraspecific and interspecific variation of live birds, we modeled feather color using reflectance curves. For each patch of every individual, we averaged reflectance values for every 10-nm interval in the songbird visual spectrum (300–700 nm) resulting in 160 color variables (Mennill et al. 2003). We quantified plumage variation using the entire visual spectrum because the reflectance curves showed qualitative interspecific variation from ~350 to 700 nm (Fig. 3). Additionally, we computed brightness as the average reflectance across the entire songbird spectrum for each patch for every individual, resulting in four additional color variables. As described in *Statistical analysis – color*, we used principal components from a principal component analysis as response variables for a linear mixed model (see Table S5 for proportion of variance and loadings). We ran linear mixed models using lmer() from the lme4 package in R (Bates et al. 2015) and obtained approximate *P* values from the lmerTest package (Kuznetsova et al. 2017). To examine interspecific and intraspecific variation in plumage patch color, we generated PCAs that either contained data for both species (interspecific comparison) or one species (intraspecific comparison) using the head, throat, cheek, and side color variables. Because Mennill et al. (2003) found significant differences in the cheek color of Black-capped Chickadees and our reflectance curves for the cheek patch showed qualitative differences between males and females (Fig. 3), we generated an additional PCA and modeled an intraspecific comparison for only Black-capped Chickadee cheek data.

To describe variation in plumage color, we constructed 12 generalized linear mixed models using the first three principal components that accounted for > 80% of the variation in the interspecific or intraspecific data (Table S5) and > 97% of the variation in the Black-capped Chickadee cheek data. We included species and sex as fixed effects to describe interspecific or intraspecific variation, respectively. In the interspecific models, we used Akaike Information Criterion (AIC) to compare the models with and without an interaction between species and sex. Because the interspecific models with an interaction were more parsimonious (Table S3), we included an interaction term between species and sex for all interspecific analyses. In the interspecific model, we included sex as a fixed effect to control for differences between males and females. In the interspecific and intraspecific models, we included ordinal date and location as fixed effects, and box number and number of patches as random effects to account for variation not associated with species or sex. We included ordinal date to control for seasonal variation in plumage color. Given that several birds formed pairs during the 2019 breeding season, we included box number as a random effect to control for mating bias because plumage is involved in mate choice (McDonald et al. 2001, Siefferman and Hill 2005). We included location as

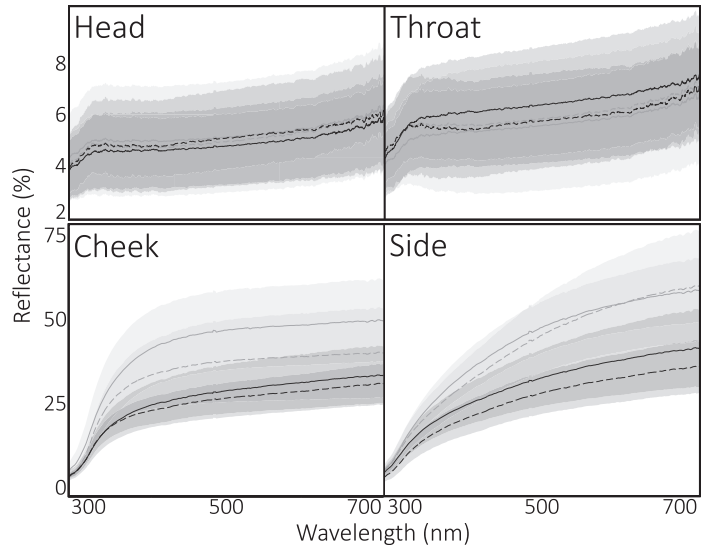

Fig. 3. Percent reflectance from 300 to 700 nm of the feathers collected from live birds for the head (top left), throat (top right), cheek (bottom left) and side (bottom right) patches. Black-capped Chickadees are represented in coral and Mountain Chickadees are represented in navy. Males are represented by solid lines and females by dashed lines. Lines represent the mean and shaded regions represent ±1 SD. [Colour figure can be viewed at wileyonlinelibrary.com]

a fixed effect because feathers were collected from individuals at different sites (Boulder, Sugarloaf, and MRS) and elevations, and plumage characteristics may vary along an elevational gradient (de Zwaan et al. 2017). Lastly, we included the number of patch feathers as a random effect to control for feather stacks with < 10 feathers. All individuals except one had at least one patch with < 10 feathers (Quesada and Senar 2006):

$$\begin{aligned}\mathrm{PC} = \; & \propto + \beta_1(\mathrm{Species})_1 + \beta_2(\mathrm{Sex})_2 \\ & + \beta_{12}(\mathrm{Species})_1(\mathrm{Sex})_2 \\ & + \beta_3(\mathrm{Ordinal\ date})_3 + \beta_4(\mathrm{Location})_4 \\ & + (f\mathrm{Box\_number}) \\ & + (f\mathrm{Number\ of\ patch\ feathers}).\end{aligned}$$

RESULTS

Feather color and patch size – museum specimens. Feather color in museum specimen photographs differed between species, with Black-capped Chickadees having greater luminance, greater color contrast, more reflectance in the brightest region, less reflectance in the darkest region, and being less achromatic (i.e., greater RNL saturation; Table 1) than Mountain Chickadees (PC1: -5.87 ± 1.31, $P < 0.001$; PC2: -5.20 ± 0.60, $P < 0.001$; PC3: -0.92 ± 0.67, $P = 0.17$; Tables 2 and S2, Fig. S2). Differences in plumage between species were driven by PC1, with Black-capped Chickadees having greater visible-spectrum reflectance (i.e., long-wave, medium-wave, and short-wave reflectance) in the brightest region and greater luminance (i.e., double-cone reflectance), and PC2, with Black-capped Chickadees having greater luminance (i.e., weighted luminance), greater weighted RNL saturation, and greater color contrast (i.e., weighted luminance boundary strength and weighted RNL boundary strength). Mountain Chickadees had greater visible-spectrum reflectance (i.e., long-wave, medium-wave, and short-wave reflectance) in the darkest region for all orientations. In the interspecific model, we found a significant interaction between species and sex for PC1 (4.17 ± 1.61, $P = 0.011$), but not for either PC2 (0.0079 ± 0.73, $P = 0.99$) or PC3 (-1.08 ± 0.82, $P = 0.19$; Table 2).

Within species, male Mountain Chickadees had greater full-spectrum reflectance in the

Table 2. Museum specimen color results, including the estimate, standard error (SE), *t* value, and *P* value, for the interspecific, intraspecific Black-capped Chickadee (BCCH), and intraspecific Mountain Chickadee (MOCH) models for Principal Components 1, 2, and 3. Fixed effects include species, sex, ordinal date, museum, year collected, and quadrant, where species was only a fixed effect in the interspecific model. Species:Sex represents the interaction between species and sex in the interspecific model. Explained variance for each principal component can be found in Table S2.

		Interspecific				Intraspecific BCCH		Intraspecific MOCH	
		Intercept	Species	Sex	Species:Sex	Intercept	Sex	Intercept	Sex
PC1	Estimate	5.987	−5.865	−2.243	4.166	−5.797	1.283	6.587	−1.521
	SE	4.570	1.311	1.131	1.608	4.295	0.869	5.268	1.147
	t	1.3	−4.5	−2.0	2.6	−1.4	1.5	1.3	−1.3
	P	0.19	**< 0.001**	0.050	**0.011**	0.19	0.15	0.22	0.19
PC2	Estimate	6.380	−5.201	0.543	0.008	1.342	0.160	0.942	0.618
	SE	2.085	0.598	0.516	0.734	4.141	0.838	3.524	0.767
	t	3.10	−8.7	1.1	0.01	0.3	0.2	0.3	0.8
	P	**0.003**	**< 0.001**	0.30	0.99	0.75	0.85	0.79	0.43
PC3	Estimate	−0.314	−0.917	0.922	−1.076	−4.538	0.318	2.879	−1.730
	SE	2.335	0.670	0.578	0.822	2.287	0.463	3.501	0.762
	t	−0.1	−1.4	1.6	−1.3	−2.0	0.69	0.82	−2.3
	P	0.89	0.17	0.11	0.19	0.055	0.50	0.42	**0.029**

Bold values indicate significance where $P < 0.05$.

brightest and darkest regions for the ventral orientation, greater weighted luminance, and greater ultraviolet reflectance (i.e., compared to other wavelengths) than females for all orientations (PC1: -1.52 ± 1.15, $P = 0.19$; PC2: 0.62 ± 0.77, $P = 0.43$; PC3: -1.73 ± 0.76, $P = 0.029$; Tables 2 and S2). For Black-capped Chickadees, we found no differences between the sexes for the first three principal components (PC1: 1.28 ± 0.87, $P = 0.15$; PC2: 0.16 ± 0.84, $P = 0.85$; PC3: 0.32 ± 0.46, $P = 0.50$; Tables 2 and S2).

Mountain Chickadees had larger throat and cheek patches than Black-capped Chickadees (throat: 32.92 ± 11.24, $P = 0.0043$; cheek: 35.28 ± 9.27, $P < 0.001$; Table 3). In the interspecific model, we found no significant interaction between species and sex for the size of throat and cheek patches (throat: -21.61 ± 13.79, $P = 0.12$; cheek: -13.79 ± 11.37, $P = 0.23$; Table 3). The size of throat and cheek patches did not differ between the sexes for either Black-capped (throat: 9.19 ± 8.45, $P = 0.28$; cheek: 1.71 ± 7.12, $P = 0.81$) or Mountain (throat: -11.25 ± 13.10, $P = 0.40$; cheek: -17.07 ± 9.16, $P = 0.07$; Table 3) chickadees.

Feather color – live birds. The cheek and side patches of Black-capped Chickadees had greater reflectance than those of Mountain Chickadees across the entire songbird spectrum. These differences were primarily driven by greater short-wave reflectance (400–500 nm) for Black-capped Chickadees (PC1: -12.49 ± 2.51, $P < 0.001$; PC2: 2.15 ± 3.09, $P = 0.49$; PC3: -2.92 ± 2.92, $P = 0.32$; Tables 4 and S5, Fig. 3). In the interspecific model, we found no interaction between species and sex for the first three principal components (PC1: -0.057 ± 2.94, $P = 0.99$; PC2: 3.99 ± 3.56, $P = 0.27$; PC3: 3.01 ± 3.41, $P = 0.39$; Table 4). We also found that feather color did not differ between the sexes for either Black-capped (PC1: 3.53 ± 2.46, $P = 0.17$; PC2: 1.55 ± 2.81, $P = 0.59$; PC3: -2.05 ± 1.81, $P = 0.29$; Tables 4 and S5) or Mountain (PC1: -4.42 ± 2.93, $P = 0.16$; PC2: -4.77 ± 3.57, $P = 0.21$; PC3: -4.97 ± 2.69, $P = 0.089$; Tables 4 and S5) chickadees. Using only the cheek patch, we found no differences between male and

Table 3. Museum specimen area results, including the estimate, standard error (SE), *t* value, and *P* value, for the interspecific, intraspecific Black-capped Chickadee (BCCH), and intraspecific Mountain Chickadee (MOCH) models for throat and cheek patches. Fixed effects included species, sex, ordinal date, museum, year collected and quadrant, where species was only a fixed effect in the interspecific model. Species:Sex represents the interaction between species and sex in the interspecific model.

		Interspecific				Intraspecific BCCH		Intraspecific MOCH	
		Intercept	Species	Sex	Species:Sex	Intercept	Sex	Intercept	Sex
Throat	Estimate	204.147	32.923	11.329	−21.605	197.803	9.186	184.344	−11.247
	SE	39.183	11.244	9.697	13.790	41.775	8.452	60.139	13.095
	t	5.2	2.9	1.2	−1.6	4.7	1.1	3.1	−0.9
	P	**< 0.001**	**0.004**	0.25	0.12	**< 0.001**	0.28	**0.004**	0.40
Cheek	Estimate	91.159	35.275	0.545	−13.785	106.303	1.711	145.554	−17.070
	SE	32.301	9.270	7.994	11.368	35.179	7.117	42.087	9.164
	t	2.8	3.8	0.1	−1.2	3.0	0.2	3.5	−1.9
	P	**0.006**	**< 0.001**	0.95	0.23	**0.005**	0.81	**0.001**	0.07

Bold values indicate significance where $P < 0.05$.

female Black-capped Chickadees for the first three principal components (PC1: -2.55 ± 2.12, $P = 0.24$; PC2: 0.013 ± 0.40, $P = 0.97$; PC3: -0.19 ± 0.12, $P = 0.11$; Table 4), which accounted for 99.93% of the variation (Table S5).

DISCUSSION

We found that Black-capped Chickadees were less achromatic and had brighter plumage with more color contrast than Mountain Chickadees for all orientations, but they were not sexually dimorphic. However, in support of previous findings (Eaton 2007), Mountain Chickadees in our study were sexually dimorphic, with museum specimen males having brighter plumage, particularly from the ventral orientation, and greater ultraviolet reflectance than museum specimen females. Possibly because we did not sample feathers from the supercilium (i.e., the supercilium was included in the museum specimen photographs), we did not observe sexual dimorphism in live Mountain Chickadees. In contrast, Mennill et al. (2003) found that male Black-capped Chickadees had greater color contrast and brighter white plumage than females. Although widespread and common, Black-capped Chickadees exhibit considerable population genetic structure across their range in North America (Adams and Burg 2015, Hindley et al. 2018). The populations sampled by Mennill et al. (2003) belong to a different genetic cluster than those in our study, are located 4.5° north of Colorado, and at an elevation 1000 m lower (Adams and Burg 2015, Hindley et al. 2018). Within species, plumage can vary with latitude and elevation (de Zwaan et al. 2017) and our results suggest that the degree of sexual dimorphism exhibited by Black-capped Chickadees varies across their range. The extent of sexual dimorphism may vary with elevation and latitude in Black-capped Chickadees because different populations are adapted to various ecological drivers (e.g., UV radiation, lower temperatures, and increased precipitation; Margalida et al. 2008), which might result in differences in female preferences (Bastianelli et al. 2015, de Zwaan et al. 2017). Hamilton (1961) found that warblers (Parulidae) and orioles (Icteridae) at lower latitudes were less sexually dimorphic than relatives at higher latitudes, potentially because decreasing female coloration and increasing sexual dimorphism at higher latitudes may reduce intraspecific aggression during pair formation and facilitate mate recognition, respectively. Limited aggression and facilitated mate recognition may be important for quickly finding a mate during the shorter breeding seasons at higher latitudes (Badyaev and Hill 2003).

Table 4. Live bird color results, including the estimate, standard error (SE), *t* value and *P* value, the interspecific, intraspecific Black-capped Chickadee (BCCH), intraspecific Mountain Chickadee (MOCH), and intraspecific Black-capped Chickadee (BCCH) cheek models for Principal Components 1, 2 and 3. Fixed effects include species, sex, ordinal date, and location, where species was only a fixed effect in the interspecific model. Random effects include box number and number of patch feathers (i.e., head, throat, cheek, and side). Species:Sex represents the interaction between species and sex in the interspecific model. Explained variance for each principal component can be found in Table S5.

		Interspecific				Intraspecific BCCH		Intraspecific MOCH		Intraspecific BCCH Cheek	
		Intercept	Species	Sex	Species:Sex	Intercept	Sex	Intercept	Sex	Intercept	Sex
PC1	Estimate	35.318	−12.492	3.432	−0.057	−1.133	3.528	−94.241	−4.416	−27.035	−2.545
	SE	11.071	2.507	1.905	2.939	16.882	2.462	27.202	2.925	14.051	2.115
	t	3.2	−5.0	1.8	−0.02	−0.07	1.4	−3.5	−1.5	−1.9	−1.2
	P	**0.003**	**< 0.001**	0.08	0.99	0.95	0.17	**0.006**	0.16	0.068	0.24
PC2	Estimate	35.611	2.146	−1.670	3.986	−6.678	1.554	−36.624	−4.767	0.204	0.013
	SE	13.561	3.088	2.300	3.559	18.597	2.814	33.992	3.568	2.662	0.400
	t	2.6	0.7	−0.7	1.1	−0.4	0.6	−1.1	−1.3	0.1	0.03
	P	**0.013**	0.49	0.47	0.27	0.72	0.59	0.30	0.21	0.94	0.97
PC3	Estimate	−4.106	−2.922	−0.727	3.006	−36.455	−2.048	22.765	−4.966	1.774	−0.192
	SE	13.487	2.916	2.382	3.409	16.754	1.805	26.727	2.685	0.790	0.115
	t	−0.3	−1.0	−0.3	0.9	−2.2	−1.1	0.9	−1.8	2.2	−1.7
	P	0.76	0.32	0.76	0.39	0.15	0.29	0.43	0.09	**0.036**	0.11

Bold values indicate significance where $P < 0.05$.

Among tits and chickadees, ultraviolet reflectance frequently contributes to sexual dimorphism and potentially provides information important in inter- and intrasexual interactions. For example, the blue-black crown of male Great Tits (*Parus major*) reflects more ultraviolet light than those of females (Hegyi et al. 2007) and blue-black crowns with more ultraviolet reflectance and shorter wavelength hues are associated with males better at learning (Cauchard et al. 2017). In our study, male Mountain Chickadee museum specimens had greater ultraviolet reflectance than female museum specimens for all orientations, potentially because ultraviolet reflectance conveys individual quality, which is important information for mate selection. Among Black-capped Chickadees, the brightness of the white cheek and ultraviolet-chroma of dark body regions is greater in more dominant (i.e., preferred) males (Woodcock et al. 2005). White cheek brightness and ultraviolet reflectance may signal dominance in Black-capped Chickadees. Because Mountain Chickadee museum specimens were sexually dimorphic with males being brighter and having greater ultraviolet reflectance than females, brightness and ultraviolet reflectance could potentially be involved in similar signaling. Further research is needed to determine if brightness and ultraviolet reflectance are used for social signaling and serve as badges of dominance status or indicators of fitness for Mountain Chickadees.

Differences in plumage color and patch sizes between Black-capped and Mountain chickadees may influence the likelihood of hybridization. In northern British Columbia, Grava et al. (2012) found that all hybrid offspring resulted from extrapair copulations between female Mountain and male Black-capped chickadees, and we found that Black-capped Chickadees had greater overall plumage contrast than Mountain Chickadees. Hybridization between these two species represents a breakdown of reproductive isolation, where sex-specific characters of a heterospecific may be more attractive than that of a conspecific (Wirtz 1999, Stein and Uy 2006). Color contrast is associated with male quality in several species of birds (Loyau et al. 2007, Gladbach et al. 2011). Male Black-capped Chickadees have more color contrast than male Mountain Chickadees and this may influence the purported unidirectional hybridization observed between these two species (Grava et al. 2012).

In the hybrid zone of Black-capped and Carolina chickadees, male Carolina Chickadees are dominant to male Black-capped Chickadees and females prefer dominant males regardless of species (Bronson et al. 2003), suggesting that hybridization may primarily occur between female Black-capped Chickadees and male Carolina Chickadees. Likewise, offspring produced by Black-capped and Carolina chickadee hybridization are primarily male, suggesting selection against female hybrids (which follows Haldane's Rule), further influencing hybridization in this system (Bronson et al. 2005). However, little is known about the extent to which Black-capped and Mountain chickadee hybrids are selected against or how this selection influences reproductive isolation. Because Gladbach et al. (2011) reported a correlation between greater color contrast with metrics of condition and fitness, future work in this system is needed to explore the influence of color contrast on hybridization in chickadees (e.g., unidirectional hybridization or selection against hybrids), ideally through plumage manipulation experiments.

The plumage differences we found between sexes and species align with well-established dominance hierarchies between and within Black-capped and Mountain chickadees, given that plumage brightness (i.e., luminance) may be associated with male quality in these two species (Grava et al. 2012). Male Mountain Chickadee museum specimens had greater luminance than female museum specimens and Black-capped Chickadees were brighter than Mountain Chickadees. Similarly, Grava et al. (2012) found that males were most dominant in single-species flocks, and Black-capped Chickadees were dominant to Mountain Chickadees (Grava et al. 2012). Male Black-capped Chickadees may be perceived as being of higher quality by female Mountain Chickadees than male Mountain Chickadees because they are dominant and have greater overall brightness (i.e., brightness may signal dominance; see Woodcock et al. 2005), potentially explaining why female Mountain Chickadees may choose male Black-capped Chickadees as mates (Wirtz 1999, Randler 2006, Grava et al. 2012).

Finally, we found that Mountain Chickadees had darker, more achromatic (i.e., less RNL saturation) plumage than Black-capped Chickadees, and the results of previous studies have also revealed that darker, more achromatic species typically live in wetter and colder regions (Roulin et al. 2011, Delhey 2018), such as the high alpine environments preferred by Mountain Chickadees. For example, owls with more reddish pheomelanin-based coloration live at warmer latitudes (Roulin et al. 2011) and Australian land birds in colder, wetter environments have darker, more achromatic plumage (Delhey 2018). Species living at higher elevations endure wetter and colder environments than those at lower elevations, even after controlling for latitude (Theurillat and Guisan 2001). For Black-capped and Mountain chickadees, this relationship between plumage color and elevation could be due to the fitness costs associated with melanin. Melanin comes in two forms: eumelanin and pheomelanin. Eumelanin produces dark colors, such as the head and throat plumage of chickadees (D'Alba et al. 2014), whereas pheomelanin is responsible for reddish/chestnut colors, such as the sides of Black-capped Chickadees (Galván and Møller 2013). Although pheomelanin-based coloration may evolve through sexual selection (Arai et al. 2015), the pigment is costly because it consumes glutathione, an important antioxidant (Galván and Møller 2013, but see López-Arrabé et al. 2014). Therefore, at higher elevations with harsher conditions, producing pheomelanin could be costly for Mountain Chickadees, resulting in the greater production of eumelanin. At the lower elevations with milder climates occupied by Black-capped Chickadees, pheomelanin production may be less costly.

In summary, Black-capped and Mountain chickadees are chromatically distinct species. Black-capped Chickadees are less achromatic and have brighter plumage with more color contrast than Mountain Chickadees. In addition, male Mountain Chickadee museum specimens have brighter plumage (i.e., from the ventral orientation) with more ultraviolet reflectance than female Mountain Chickadee museum specimens. However, we found no such differences between the sexes of live Mountain Chickadees. In Black-capped Chickadees from Colorado, we found no differences in the plumage color of males and females. Where Black-capped and Mountain chickadees hybridize in sympatry, plumage manipulation experiments may provide additional insight into the role of the brighter white cheeks and side patches, and greater color contrast of male Black-capped Chickadees.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website.

Fig. S1. Sample photographs for area measurement of museum specimens. Photos represent the orientations photographed (i.e., ventral, and left and right lateral) and the patches measured (i.e., throat and cheek). The yellow outline indicates the region measured for area analysis. The long, blue line in the *Lateral* photo is the wing chord measurement. The area of each patch was divided by the length of the wing chord for statistical analyses.

Fig. S2. Eigenvectors for the interspecific, museum specimen model (i.e., the only model with > 1 significant principal components). (A) Biplot comparing principal component 1 (-5.87 ± 1.31, $P < 0.001$) and principal component 2 (-5.20 ± 0.60, $P < 0.001$). Arrows represent eigenvectors and the length of each arrow indicates the eigenvalue (actual values can be found in Table S2). The color of each arrow indicates whether the color variable is associated with reflectance values in the bright region (light orange; B), reflectance values in the dark region (dark orange; C), average luminance or RNL saturation for the entire image (light blue; D) or the contrast between color clusters across the entire image (dark blue; E). Boxes highlight eigenvectors for each group and follow the same color schematic. A closer view of each box can be found in B, C, D and E. Black-capped Chickadees are represented by coral points and Mountain Chickadees are represented by navy points. (B) Reflectance color variables for the bright region. (C) Reflectance color variables for the dark region. (D) Average color variables (i.e., luminance and RNL saturation) for the entire image. (E) The contrast between color clusters across the entire image for luminance and RNL saturation. For B, C, D, and E, the properties of the color variables can be found in F (i.e., types of reflectance or color variable, and values and regions photographed).

Table S1. Repeatability of spectrophotometer measurements—for each wavelength measured using a spectrophotometer, mean and standard deviation for repeated measurements. We measured each patch (i.e., head, throat, cheek and side) for each individual three times. We averaged and computed standard deviation of measurements for each individual and then averaged these values for all individuals for Black–capped and Mountain chickadees. Wavelength[1] includes measurements from 300–700 nm.

Table S2 Principal component explained variance and loadings for the interspecific, intraspecific Black-capped Chickadee (BCCH) and intraspecific Mountain Chickadee (MOCH) museum specimen models. Columns describing the color variables include Orientation[1], Region[2], Wavelength[3] and Variable[4]. Orientation[1] values include Dorsal (D), Ventral (V) and Lateral (L). Region[2] values include Dark (D) and Bright (B). Wavelength[3] values include Longwave (LW), Mediumwave (MW), Shortwave (SW), Ultraviolet (UV) and Double-cone (DBL) Reflectance. Variable[4] values include Mean (M), Standard Deviation (SD) and Coefficient of Variation (CoV). For entire image variables, Region[2] and Wavelength[3] describe the variables: Weighted Luminance (Luminance), Weighted RNL Saturation (RNL Sat.), Weighted Luminance Boundary Strength (Lum. BS) and Weighted RNL Saturation Boundary Strength (RNL BS). The value next to the label for each PC indicates the explained variance.

Table S3. Comparing interspecific models with and without an interaction between species and sex for the museum specimen and live bird color models. We compared models with and without an interaction using AIC—where models with an interaction were more

parsimonious. Entire model results (i.e., models with an interaction) can be found in Tables 2 (museum specimen color) and 4 (live bird color).

Table S4. Comparing interspecific models with and without an interaction between species and sex for the museum specimen area model. We compared models with and without an interaction using AIC—where models with an interaction were more parsimonious. Entire model results (i.e., models with an interaction) can be found in Table 3 (museum specimen area).

Table S5. Principal component explained variance and loadings for the interspecific, intraspecific Black-capped Chickadee (BCCH), intraspecific Mountain Chickadee (MOCH) and intraspecific Black-capped Chickadee (BCCH) cheek live bird models. Columns describing the color variables include Patch[1] and Wavelength[2]. Patch[1] values include Head (H), Throat (T), Cheek (C) and Side (S). Wavelength[2] values include 10 nm wavelength ranges from 300–700 nm and overall brightness (bright) for each patch. The value under the label for each PC indicates the explained variance.