J Ornithol (2015) 156:143–151
DOI 10.1007/s10336-014-1130-0

ORIGINAL ARTICLE

Relationships between plumage coloration, diet diversity, and winter body condition in the Lesser Goldfinch

Melanie Frincke-Craig · Jessi L. Brown · Chris W. Briggs · Simon R. Poulson · Michael W. Collopy · Chris R. Feldman

Received: 9 January 2014 / Revised: 18 July 2014 / Accepted: 16 October 2014 / Published online: 8 November 2014

Abstract Bright and colorful plumage is thought to be an honest signal of individual quality in birds because consuming high-quality forage results in more colorful plumage. To gain insight into the ecological and evolutionary context of the relationship between color, body condition, and diet, we studied a mostly urban population of Lesser Goldfinches (*Spinus psaltria*). We collected body measurements and digital photographs of plumage, as well as feathers and blood of goldfinches during three winters (2009–2012) in northern Nevada, USA. We analyzed the body tissues (feathers and blood) for stable isotope values of carbon and nitrogen to infer the diets of individual goldfinches, and quantified CIELAB color space values of chroma, brightness, and hue of plumage from the digital images. We then examined the relationships between color values and body condition, and color and stable isotope values. We found that the brightness (L^* value) of the back plumage was correlated with both body condition and with stable isotope values of nitrogen ($\delta^{15}N$) in the winter diet. Furthermore, stable isotope analyses of both feathers and blood showed temporal differences in diet. However, hue and chroma, which are color values that are thought to more directly represent feather carotenoid content, were not related to body condition or diet. Our results suggest that the foraging ecology of Lesser Goldfinches changes over time, and that, in winter, plumage color values that are putatively indicative of carotenoid content do not seem to be an honest signal of individual quality as measured by body condition.

Keywords Body condition · Color · Diet · *Spinus psaltria* · Stable isotopes · Temporal variation

Communicated by C. Guglielmo.

Electronic supplementary material The online version of this article (doi:10.1007/s10336-014-1130-0) contains supplementary material, which is available to authorized users.

M. Frincke-Craig · C. R. Feldman
Department of Biology, University of Nevada, Reno, Reno, NV 89557, USA

J. L. Brown (✉) · C. W. Briggs
Program in Ecology, Evolution, and Conservation Biology, University of Nevada, Reno, Reno, NV 89557, USA
e-mail: jessilbrown@gmail.com

S. R. Poulson
Department of Geological Sciences and Engineering, University of Nevada, Reno, Reno, NV 89557, USA

M. W. Collopy
Academy for the Environment, University of Nevada, Reno, Reno, NV 89557, USA

Zusammenfassung

Beziehungen zwischen Gefiederfärbung, Nahrungsvielfalt und Körperkondition im Winter bei Mexikozeisigen *Spinus psaltria*

Glänzendes und buntes Gefieder bei Vögeln stellt wahrscheinlich ein ehrliches Signal individueller Qualität dar, da das Verzehren hochwertiger Nahrung in bunterem Gefieder resultiert. Um Einblicke in den ökologischen und evolutionären Kontext der Beziehung zwischen Farbe, Körperkondition und Ernährung zu gewinnen, haben wir eine größtenteils städtische Population des Mexikozeisigs (*Spinus psaltria*) untersucht. Über drei Winter (2009–2012) haben wir im Norden Nevadas (USA) Körpermaße ermittelt, Digitalfotos des Gefieders aufgenommen sowie Federn und Blut von Mexikozeisigen gesammelt. Wir haben die Körpergewebe (Federn und Blut) hinsichtlich stabiler Isotopenwerte von Kohlenstoff und Stickstoff analysiert,

um Rückschlüsse auf die Nahrung individueller Mexikozeisige zu ziehen, und haben anhand der Gefiederfotos Farbsättigung, Helligkeit und Farbton auf der Basis des CIELAN-Farbraums quantifiziert. Dann haben wir die Beziehungen zwischen diesen Farbwerten und der Körperkondition bzw. den stabilen Isotopenwerten untersucht. Wir fanden, dass die Helligkeit (L*-Wert) des Rückengefieders mit der Köperkondition sowie mit den Werten stabiler Stickstoffisotope (15 N) in der Winternahrung korreliert war. Des Weiteren zeigten die Isotopenanalysen von Federn sowie Blut zeitliche Unterschiede in der Nahrung auf. Farbton und Farbsättigung standen jedoch nicht in Bezug zur Körperkondition oder Ernährung, obwohl sie den Carotinoidgehalt von Federn wahrscheinlich unmittelbarer anzeigen. Unsere Ergebnisse deuten darauf hin, dass sich die Nahrungsökologie von Mexikozeisigen über die Zeit verändert und dass im Winter Gefiederfarbwerte, die vermeintlich den Carotinoidgehalt anzeigen, offenbar kein ehrliches Signal individueller Qualität (wie anhand der Köperkondition gemessen) darstellen.

Introduction

Sexual selection is driven by variation in the ability to attract and secure mates. As originally articulated by Darwin (1859, 1871), variation in sexual selection is considered one of the most important forces influencing phenotypic evolution. In many systems, females select males with specific traits, often promoting the evolution of dimorphic or exaggerated secondary sexual characteristics. Why females choose mates with specific traits is often debated (Emlen and Oring 1977; Greenwood 1980), but a leading hypothesis, the handicap principle (Zahavi 1977; Hamilton and Zuk 1982), suggests that secondary sexual characteristics are costly, allowing females to discern the most viable mates (strongest traits) from those of lesser quality (weaker traits). Under this scenario, secondary sexual characteristics must be honest signals, reflecting an individual's viability, genetic quality, or even capacity to rear offspring (Järvi et al. 1987; Searcy and Nowicki 2005; Emlen et al. 2012). If low-quality males are able to "cheat" and develop attractive sexual signals without handicap, then the value of such signals becomes unreliable (Olson and Owens 1998). Thus, major questions in the study of sexual selection are whether and how secondary sexual traits convey honest information between the sexes (Searcy and Nowicki 2005; Emlen et al. 2012).

In many avian mating systems, females choose mates based on the brightness and color of the males' plumage. Vibrant males are thought have an advantage in mate acquisition over duller males because plumage traits may convey honest information about a potential male's condition or quality (Hill 2000). In particular, yellow, orange, and red feather colors are mostly derived from carotenoids—chemical compounds created by plants, algae, bacteria, and fungi that reflect both a high-quality diet and a vigorous immune response (Slagsvold and Lifjeld 1992; Blount 2004; McGraw et al. 2005). Because birds cannot create carotenoids, these pigments must be acquired through their diet (McGraw et al. 2001). Large amounts of carotenoids must be ingested to obtain a sufficient amount of pigments to dedicate to plumage coloration, and allocating dietary nutrients to feather coloration may directly limit the nutrients available to other physiological functions, especially immune defenses (Blount 2004). Thus, carotenoid acquisition and absorption into the blood stream is closely coupled with nutritional state and capacity to obtain high-quality forage (McGraw et al. 2005). Individual birds that can maximize their reproductive and survival success within the constraints of the tradeoffs will be evolutionarily favored (Blount 2004).

It is important to be able to accurately determine differences between the diets of individuals in order to understand how consumed resources affect plumage coloration. One method of quantifying an individual's diet is through the use of stable isotope analyses. Stable isotopes are naturally occurring elements that can be found in the tissues of both predators and prey, and the isotopic signatures of consumers will reflect the signatures of their prey. In the case of stable isotopes of nitrogen, consumer isotopic signatures typically shift to heavier values due to trophic enrichment (Boecklen et al. 2011). However, consumer carbon isotopic values are often, but not always, similar to prey carbon isotopic values (DeNiro and Epstein 1978; Podlesak et al. 2005; Caut et al. 2009). The time period over which the dietary information is integrated depends on the tissue analyzed: in small birds, stable isotope values from whole blood reflect the bird's diet for 24–30 days prior to sample collection, whereas stable isotopes in the feathers reflect what the bird ate during feather growth, thus allowing comparisons between diets over different time periods (Inger and Bearhop 2008). After analyzing tissue stable isotope values, mixing models that attempt to quantify the proportion of the diet that can be attributed to each potential prey item are often used to reconstruct animal diets (Semmens et al. 2009; Ward et al. 2011). However, alternative methods of exploring diet characteristics include comparing diet variability in multivariate diet isotopic space, or linear models that explore relationships between single isotopes and covariates (Beaulieu and Sockman 2012; Hsu et al. 2014).

Carotenoid-based plumage color appears to function as an honest signal of mate quality in the American Goldfinch (*Spinus tristis*; McGraw 2004). Its close relative, the Lesser Goldfinch (*Spinus psaltria*), is also sexually dimorphic in plumage, with male birds having bright yellow breasts and

black caps, whereas females have duller yellow coloration and lack contrasting caps. Thus, their carotenoid-dependent plumage coloration may provide honest sexual signals. However, the two color morphs of Lesser Goldfinch exhibit strikingly different molt strategies, with the eastern black-backed form molting twice a year, similar to the American Goldfinch, whereas the western green-backed form molts only once a year (Willoughby 2007). We focused on the potential relationship between plumage coloration and body condition and diet in Lesser Goldfinch males and females in northern Nevada, USA. In our study area, the resident morph is the western green-backed form, and thus likely molts only once a year in the summer or early autumn immediately following breeding or soon after fledging (Willoughby 2007). Thus, the feathers grown in summer and autumn are kept throughout the winter and following breeding season.

Our first goal was to better understand the relationship between color and body condition, to assess whether plumage traits may provide honest signals in this species. Our second goal was to assess how plumage coloration may be related to diet during two different seasons, as a potential causal mechanism of reliable signal production in these birds. We expected plumage traits to vary with both body condition and diet, providing females with an honest indication of male quality, as seen in the American Goldfinch (McGraw et al. 2002, 2005). Further, because of the annual production of feathers in later summer or early fall, we expected a robust relationship between plumage traits and summer diet.

Methods

Sample collection

We captured 433 Lesser Goldfinches using mist-nets and cage traps at 16 sites around Reno/Sparks and Carson City in northern Nevada, USA (center of study area, 39°25.731′N, 119°44.888′W; Fig. 1) over three winter seasons (October–February 2009–2012). After capture, we banded birds with unique combinations of three color bands and one aluminum USGS band. We measured the length of the bird's exposed culmen, tarsus, wing chord, extent of wing white patch, tail, and mass, and determined age and sex from plumage (Pyle 1997). We took digital photographs of each bird's breast and back with a Canon 30D SLR, including an IT8 color reference card in each image for future color calibration (example images in Online Supplement 1 of the Electronic supplementary material, ESM). We plucked 2–3 feathers from the breast of each bird and stored the feathers in paper envelopes. At a subset of five sites during the winter of 2011–2012, we took blood samples (200 µl) from the brachial vein with a 28-gauge needle and capillary tube and immediately placed blood samples in tin cups which were then allowed to air dry for ca. 6 weeks until the sample mass stabilized.

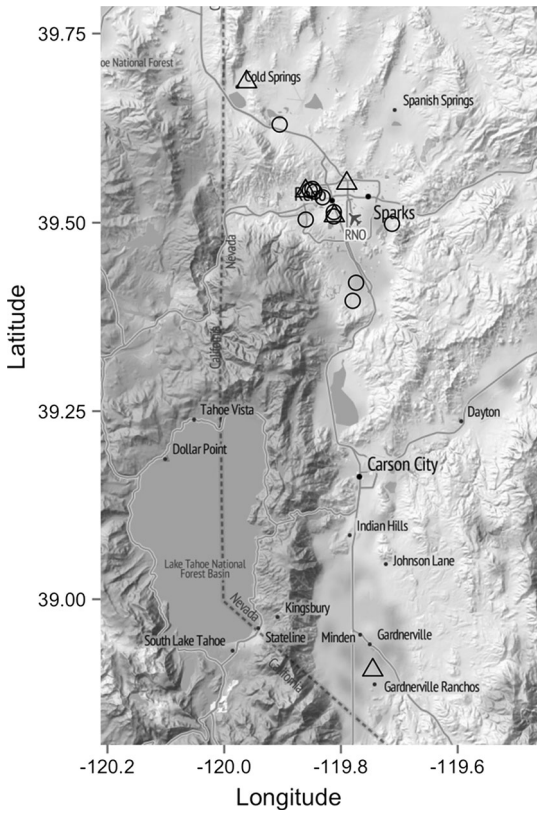

Fig. 1 Locations of banding and sampling sites of Lesser Goldfinches in northern Nevada (near Reno/Sparks and Carson City), USA. Sites where samples were collected for stable isotope analyses are indicated by *triangles*

Color quantification

Plumage color values, and hence the carotenoid content of feathers, may be measured by spectrophotometers, but the equipment can be difficult to use under field conditions (Saks et al. 2003; Lopez-Rull et al. 2010). Alternatively, color values may be quantified from digital images that have been rigorously calibrated (Stevens et al. 2007). One standard for color quantification is the CIE (International Commission on Illumination) 1976 L^*a^*b color space, or CIELAB. CIELAB is a derivation of the color plane where L^* corresponds to brightness, a^* to green to magenta, and b^* to blue to yellow. These values can then be used to calculate chroma and hue, which can be thought of as the purity or saturation of the color and the particular portion of the color spectrum, respectively, both of which have been linked to carotenoid content in feathers (Saks et al. 2003).

We calibrated digital images of adequate quality (both the bird and color reference card were visible and neither

under- nor overexposed) of 225 goldfinches to better indicate true color regardless of the light conditions at the time of photography. We generated a custom color profile (ICC, or International Color Consortium, profile) for the digital camera and lens with the IT8 reference color card and the open-source ICC profiling software ArgyllCMS v1.3.4 (accessed from http://www.argyllcms.com/). This custom ICC profile was then used to decode our raw format images into the CIE 1976 L^*a^*b color space using the program RawTherapee (accessed from http://rawtherapee.com). We then sampled the color three times in the brightest regions of the breast and back for each bird, and averaged the values of L^*, a^*, and b^* for our final color values. Chroma (C^*) and hue (h^*) were calculated by the equations $C^* = \sqrt{(a^{*2} + b^{*2})}$ and $h^* = \text{atan2}(b^*, a^*)$, respectively.

Diet quantification by stable isotope analysis

We analyzed feather and whole-blood samples for stable isotope values collected in the winter of 2011–2012 from 41 goldfinches captured in five sites (6–9 birds per site). The feather samples were washed using a two-step process to remove surface contaminants from the feathers (Paritte and Kelly 2009). We first washed the feathers in a 30:1 detergent/water solution then rinsed them three times in deionized water and left them to dry in a fume hood for 24 h. Next we washed the feathers in a 2:1 chloroform/methanol solution for 30 s and dried them overnight (24 h) at room temperature in a fume hood. After washing, the feathers were packed into tin cups, and tissues (blood and feathers) were analyzed for stable isotope values of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$). Pilot analyses showed consistently low C:N ratios (<4.0) for both tissues, indicating low lipid contents and therefore no need to extract lipids that may otherwise confound analyses (Post et al. 2007). Additionally, samples of several seeds that goldfinches were observed to consume were analyzed for stable isotope values: these were analyzed whole without washing or other treatment, as they were being examined as a pilot analysis. We used a Eurovector EA 3000 elemental analyzer interfaced to a Micromass Isoprime continuous flow isotope ratio mass spectrometer. The instrumentation was equipped with a helium diluter to allow for $\delta^{13}C$ and $\delta^{15}N$ analyses of each sample analysis, after the method described by Werner et al. (1999). Isotope results are reported in the usual δ notation in units of ‰ vs. the reference standards VPDB for $\delta^{13}C$, and vs. air for $\delta^{15}N$. Analytical error (one standard deviation) was estimated to be ±0.1 and ±0.2 ‰ for $\delta^{13}C$ and $\delta^{15}N$ measurements respectively. Isotope analyses were corrected to an acetanilide laboratory standard that had previously been calibrated using IAEA-CH-3, IAEA-CH-6, IAEA-N-1, and IAEA-N-2 standards. The acetanilide laboratory standard was analyzed repeatedly throughout the analytical series in order to correct for any instrumental drift.

Body condition

We evaluated the body conditions of Lesser Goldfinches by correcting mass for body size (Peig and Green 2009, 2010). We used wing chord as our measure of body size because this measurement is thought to be highly replicable under field conditions (Winker 1998) and exploratory plots showed a relationship between wing chord and mass. We performed, for each sex separately, standardized major axis (SMA) regressions of natural log-transformed wing chord on mass for goldfinches banded from 2009–2011 in R (v2.15.1) using the package "lmodel2" (Legendre and Legendre 1998; R Development Core Team 2010). The scaled mass index ($\widehat{M}_i$, hereafter "SMI") was calculated for each bird:

$$\widehat{M}_i = M_i \left[\frac{L_0}{L_i}\right]^{b_{\text{SMA}}}$$

where the measured mass and wing chord are M_i and L_i, the mean observed wing length is L_0, and the slope of the appropriate SMA regression line is the exponent b_{SMA} (Peig and Green 2009). Our final sample for SMI included 406 birds (247 males and 159 females) after removing mass outliers suggesting errors in measurement (mass <8 g or >13 g).

Statistical analyses

We used digital images of 225 goldfinches of known sex and age class, calibrated to extract $L^*a^*b^*$ color values, for the analysis of color and SMI by general linear models. Most of the color values were highly correlated with each other (Pearson's correlation coefficient, $r > 0.6$, $P < 0.05$), so we chose to consider the uncorrelated variables of chroma (C^*) and hue (h^*) of the breast and brightness (L^*) of the back. Our other variables for the color and SMI analysis were sex and age class (either "Older," combining after hatch year [AHY] and after second year [ASY] birds, or "Young," combining hatch year [HY] or second year [SY] birds). For each analysis, we assessed sets of a priori models by Akaike's information criterion scores corrected for small sample size (AIC_c; Burnham and Anderson 2002).

The stable isotope values of various tissues from the same organism will likely vary due to different fractionation rates for each tissue, so rather than analyze the raw

Table 1 Comparison of linear models arranged in order of AIC_c score showing the relationships between standardized mass index (SMI) and plumage color, sex, and age class in Lesser Goldfinches ($n = 225$) captured in northern Nevada, USA, during winter 2009–2012

Model parameters	K	AIC_c	ΔAIC_c	W_i
Brightness (back) + sex	4	429.83	0	0.26
Brightness (back) + hue (breast) + sex	5	431.14	1.31	0.13
Brightness (back) + chroma (breast) + sex	5	431.64	1.81	0.10
Brightness (back) + sex + age class	5	431.85	2.02	0.09
Brightness (back) + chroma (breast) + hue (breast) + sex	6	432.90	3.07	0.06
Brightness (back) + hue (breast) + sex + age class	6	433.03	3.20	0.05
Sex	3	433.32	3.49	0.05
Brightness (back) + chroma (breast) + sex + age class	6	433.74	3.91	0.04
Hue (breast) + sex	4	434.08	4.25	0.03
Brightness (back)	3	434.25	4.42	0.03
Null	2	437.78	7.95	0

Age class is either "adult" (AHY or ASY) or "young" (HY or SY); chroma of the breast plumage was quantified by the C^* color value, hue of breast plumage was represented by the h^* color value, and brightness of the back plumage by the L^* value. K is the number of model parameters, and w_i is the AIC_c model weight. Only the models with AIC_c <5.0 and the null model (intercept only) are shown

data from the stable isotope analyses, we reconstructed the stable isotope values of the original diet by correcting the raw values with experimentally determined fractionation values for the Song Sparrow (*Melospiza melodia*), another seed-eating passerine (Kempster et al. 2007). Thus, reconstructed stable isotope values of diet in winter were estimated as $\delta^{13}C$ of whole blood +1.55 and $\delta^{15}N$ of whole blood −2.15, and values of diet in summer were estimated via $\delta^{13}C$ of feathers −0.25 and $\delta^{15}N$ of feathers −2.85. We then determined the relationships between color and diet using general linear models for univariate analyses of stable isotope values. Because our sample size in the stable isotope data set was relatively small, we minimized the number of potential predictor variables by considering only sex in addition to the color values. We further analyzed the stable isotope data using bivariate stable isotope Bayesian standard ellipses and stable isotope "population metrics" with the R package "SIAR" to illustrate any differences in diet across time (Layman et al. 2007; Parnell et al. 2010; Jackson et al. 2011). We compared metrics summarizing the trophic structure inferred by each tissue analysis. We calculated the ranges of nitrogen $\delta^{15}N$ (NR) and carbon $\delta^{13}C$ (CR) observed; the mean distance to centroid (CD), which represents the population trophic diversity; and the standard deviation of the nearest neighbor distance (SDNND), which indicates the evenness of the distribution of trophic niches (Layman et al. 2007) for each seasonal diet. We also compared diet composition and breadth by comparing the locations and overlaps in bivariate isotope space of standard ellipses corrected for small sample sizes (SEA_C) and Bayesian standard ellipses (SEA_B) estimated separately for diets in summer and in winter.

Results

Body condition

The standardized mass index for female goldfinches was calculated by the equation $M_i \times (61.579/L_i)^{1.912}$, and for male goldfinches, $M_i \times (63.271/L_i)^{1.794}$. The best predictors of SMI were L^*, or brightness, of the back plumage and sex (Table 1; sum of Akaike model weights, $\Sigma w_i = 0.84$ and 0.90, respectively). SMI increased as the brightness of the back increased (Fig. 2a, model-averaged beta parameter 0.00753, 95 % CI 0.0011–0.014). Absolute L^* values (intercept of regression line) differed between the sexes, with males tending to be brighter, but the relationship between L^* and SMI (slope of regression line) was similar for both sexes (Fig. 2a). We found little support for influences of chroma or hue of the breast plumage, or age class, on SMI ($\Sigma w_i = 0.29$, 0.36, and 0.27, respectively).

Diet

In our univariate analyses, we found that the brightness of the back plumage (L^*) predicted $\delta^{15}N$ of the winter diet (Table 2; $\Sigma w_i = 0.87$). Nitrogen isotope ratios in the winter diet became enriched as L^* of back plumage increased (Fig. 2b; model-averaged beta parameter 0.0166, 95 % CI 0.00343–0.0297). We found no relationships between any of our variables and $\delta^{15}N$ in the summer diet, or $\delta^{13}C$ during either season (lowest AIC_c scores for null models and no variables with large sums of Akaike weight).

Bivariate analyses of stable isotopes indicated temporal differences in diet. Comparison of isotope niche metrics for each season's diet suggested that diets were less diverse during the winter than the summer. The ranges of both $\delta^{15}N$ and $\delta^{13}C$ were lower in the winter diet (2.8 and 1.9, respectively) than in the summer diet (5.6 and 4.4, respectively). Similarly, overall population trophic diversity, as measured by CD, and population trophic evenness, or SDNND, were less variable during the winter (for CD, 0.7 in winter versus 1.2 in summer; for SDNND, 0.1 versus 0.4). We found that both the standard ellipses of isotopes and the Bayesian standard ellipses differed in size depending on the season, again suggesting that the Lesser

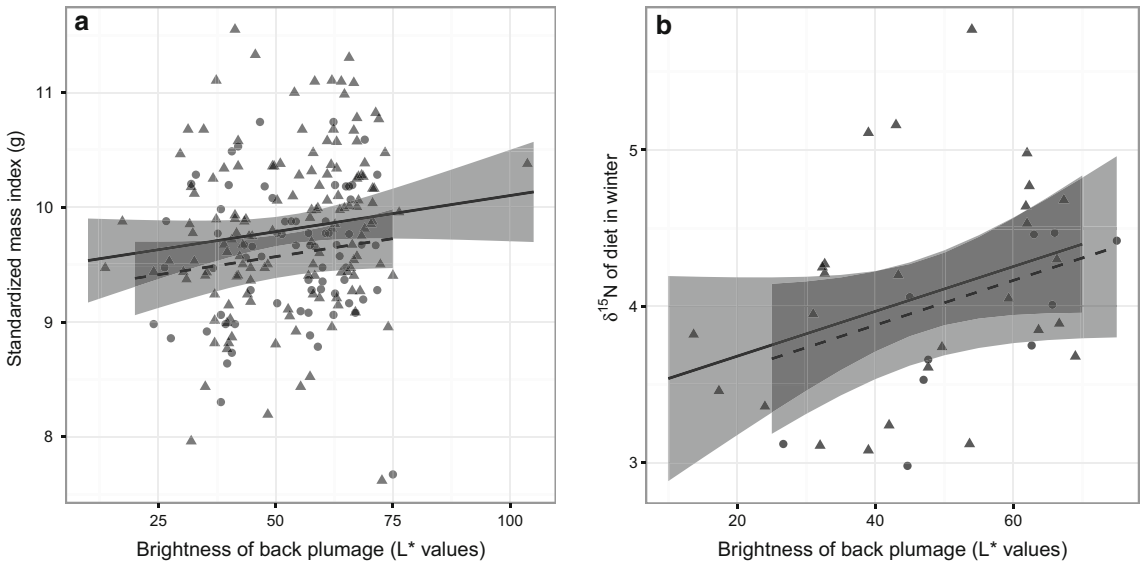

Fig. 2 Relationships between (**a**) standardized mass index, or SMI, and brightness of the back plumage, or L^*back; and (**b**) $\delta^{15}N$ of the reconstructed winter diet and L^*back for Lesser Goldfinches sampled in northern Nevada, USA, during winter months. *Triangles* represent values for male birds and *circles* are data points for females. *Solid lines* and *dashed lines* are model-averaged parameter estimates for males and females, respectively, and *shading* indicates 95 % CI

Table 2 Comparison of linear models arranged in order of AIC_c score, showing the relationships between $\delta^{15}N$ of winter diet (as estimated by stable isotope analysis of whole blood) and plumage color and sex in Lesser Goldfinches ($n = 37$) captured in northern Nevada, USA, during winter

Model parameters	K	AIC_c	ΔAIC_c	w_i
Brightness (back) + hue (breast)	4	73.33	0	0.25
Brightness (back) + sex	4	74.13	0.79	0.17
Brightness (back)	3	74.56	1.22	0.14
Brightness (back) + hue (breast) + sex	5	75.06	1.73	0.11
Brightness (back) + chroma (breast) + hue (breast)	5	75.95	2.61	0.07
Brightness (back) + chroma (breast) + sex	5	76.22	2.89	0.06
Brightness (back) + chroma (breast)	4	77.04	3.71	0.04
Hue (breast)	3	77.24	3.91	0.04
Brightness (back) + chroma (breast) + hue (breast) + sex	6	77.28	3.95	0.03
Null	2	77.38	4.05	0.03

Chroma of the breast plumage was quantified by the C^* color value, hue of breast plumage was represented by the h^* color value, and brightness of the back plumage by the L^* value. K is the number of model parameters and w_i is the AIC_c model weight. Only the models with $AIC_c < 4.0$ and the null model (intercept only) are shown

Goldfinch's diet was more diverse during the summer than the winter (Fig. 3a, b). Winter diet appeared to be nested within summer diet, as 93 % of the standard ellipse for winter diet was overlapped by that of summer diet (Fig. 3a). We did not analyze a full representation of potential goldfinch diet items, but our pilot analyses revealed marked variability between our limited samples. We observed relative enrichment of $\delta^{15}N$ in locally grown sunflower seeds (*Helianthus annus*; mean $\delta^{15}N = 9.8 \pm 0.4$ SD, $n = 3$) as compared to several other seeds collected from local unidentified plants ($\delta^{15}N = 4.18 \pm 0.3$, $n = 2$; $\delta^{15}N = -1.7 \pm 0$, $n = 2$) as well as human-provided millet (*Panicum miliaceum*; $\delta^{15}N = 2.7 \pm 0.9$, $n = 4$) and nyjer (*Guizotia abyssinica*; $\delta^{15}N = 4.3 \pm 0.3$, $n = 3$).

Discussion

We sought to determine the potential relationships between body condition, plumage traits, and diet in the Lesser Goldfinch. We found relationships between plumage brightness (L^*) and both body condition and $\delta^{15}N$ in the winter diet in Lesser Goldfinches. Unexpectedly, our study did not suggest any strong relationships between putative carotenoid-based coloration (hue and chroma) and condition or diet, although in other passerines carotenoid-based coloration is associated with many costs and trade-offs (Hill 2000). Any possible relationship between carotenoid-based coloration and our variables of interest, especially body condition, should have been detected because of our robust sample size of birds captured across three consecutive winters ($n = 225$). Because we did not directly measure feather carotenoid content, nor assess its relationship to color values in this species, it is possible that our color variables did not adequately capture feather

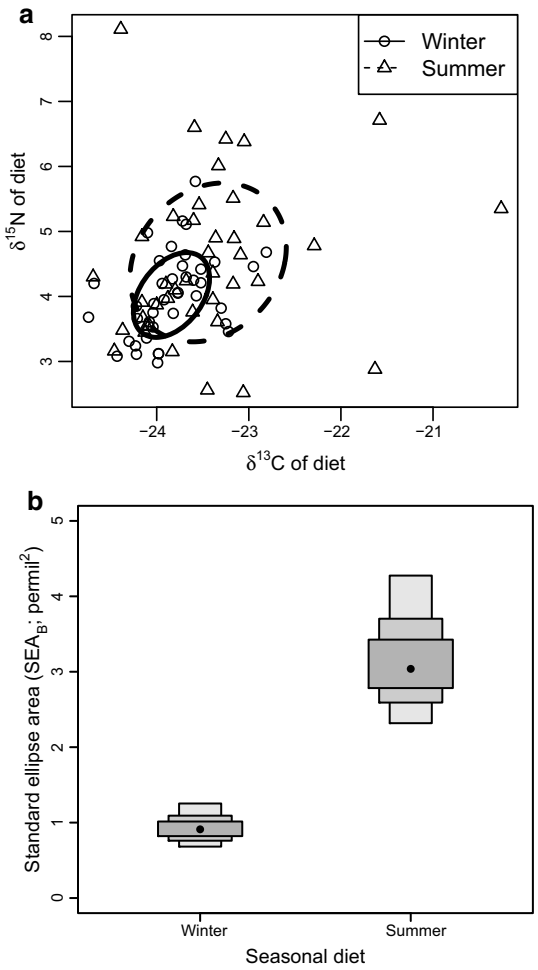

Fig. 3 Summaries of Lesser Goldfinch diets in northern Nevada as estimated by stable isotope analysis of whole-blood and feathers. Whole-blood analyses integrate dietary information over the previous 24–30 days, or from November–December 2011, whereas feather analyses represent diet during feather growth, which was likely from July–September 2011. In the isotope biplot (**a**), *symbols* represent individual birds (*triangles* males, *circles* females), and *lines* indicate the standard ellipse areas (SEA_C) for each season. Density plot of Bayesian standard ellipse areas (SEA_B) for stable isotopes (**b**) implies a less diverse diet during the winter of 2011 than the summer or fall. *Dots* indicate means, and *boxes* show 95, 75, and 50 % CI

carotenoid content. However, a strong correlation between saturation and feather carotenoid content is well established in passerines (Gray 1996), and has been shown in multiple studies of the American Goldfinch (McGraw et al. 2002, 2005).

Plumage brightness can be influenced by melanin, which sometimes serves as the pigment responsible for sexual ornaments (McGraw 2008; Guindre-Parker and Love 2013). However, the brightness of the back plumage of the birds in our study was not likely to be related to melanin, although we did not measure melanin content directly. The brightness of the back plumage did not correlate to the C/N ratio in the feathers, nor was there depletion in feather $\delta^{13}C$ for darker feathers, which would be expected if there was a large difference in melanin content in the darker feathers (Michalik et al. 2010). Plumage coloration, as measured by brightness, saturation, or chroma, can also be affected by ectoparasites, feather damage, cleanliness, and cosmetics (Delhey et al. 2007; Surmacki and Nowakowski 2007; Lopez-Rull et al. 2010; Pérez-Rodríguez et al. 2011). Because feathers are nonliving, the only way that birds can maintain optimal attractiveness is through the removal of soils and application of preening oils or other cosmetic enhancements (Delhey et al. 2007). Whether such cosmetics or more well-groomed feathers provide an additional form of honest signaling remains uncertain; perhaps males that preen more diligently are less prone to disease and parasitism, so they not only represent more viable mates but they are also less likely to transmit disease to potential mates and offspring, and they may teach beneficial grooming behaviors to offspring (Walther and Clayton 2005; Lenouvel et al. 2009).

We also found that goldfinches with winter diets enriched in $\delta^{15}N$ had brighter backs. Typically, $\delta^{15}N$ values are thought to reflect the trophic level of food consumed (Inger and Bearhop 2008); however, Lesser Goldfinches are known to eat primarily seeds and other plant products, so the observed differences in isotope values are likely related to the choice of plant species consumed (Watt and Willoughby 1999). Our pilot analyses indicated that there was marked variability in $\delta^{15}N$ values among the diet samples. However, stomach content analysis and foraging observations of birds in other populations suggest that goldfinches consume a broad range of available plants, >50 species in California (Linsdale 1957). Because we opportunistically collected samples from only five plant species, we assumed that we did not sample an adequately broad representation of potential Lesser Goldfinch diet items in northern Nevada to definitively suggest the sources of enriched dietary nitrogen.

Even without knowing the exact composition of the diet, stable isotope analyses can successfully demonstrate the variation among both natural and artificial food choices (Robb et al. 2011), and suggest the locations of foraging sites as well as the shift of the foraging niche over time (Podlesak et al. 2005; Darimont et al. 2007; Hammerschlag-Peyer et al. 2011). We detected temporal shifts in the diet of Lesser Goldfinches indicating that the birds generally consumed less diverse food items during the winter (Fig. 3). Even with less diet diversity during the entire winter, we found evidence that diets shifted during the winter season. Diets may have changed simply due to differences in food availability: fresh plant tissues are of course mostly unavailable during the winter, and much of the aboveground plant biomass present during summer is either buried under snow, incorporated into litter, or standing dried. However, a diet difference may

alternatively arise from movements of goldfinches into the city during the winter, where there may be fewer food sources available or greater reliance on food from anthropogenic sources. Further studies could explore whether survival differs for birds with different diets, or between those that tend to use urban and rural sites within the study area. Determining if and how demographic parameters differ between urban and rural locations, and how these might relate to diet, will help to explain the significance of subtle plumage variation between seasons.

Our results indicate that carotenoid-based winter plumage coloration is not a reliable indicator of winter body condition, and thus might not be an honest signal of mate quality in Lesser Goldfinches. This is in contrast to the closely related American Goldfinch, in which carotenoid-based plumage color relates to individual condition and health (McGraw and Hill 2000), and brightly colored males are preferred by females (Johnson et al. 1993). Furthermore, in the American Goldfinch, the reduced ornamentation of winter plumage seems likely to continue to function as a signal of status, as it is related to summer breeding plumage (McGraw 2004). Although wintering plumage hue or chroma may not be a signal of an index of condition in the Lesser Goldfinch, honest signaling of body condition via plumage may well only occur during the breeding season, as changes in coloration are known to occur in this species that are not related to molting. For example, the black cap may be a possible signal of quality in males during the breeding season, as the black color intensifies pre-breeding due to the wearing away of olive feather tips (Willoughby 2007). As discussed above, changes in plumage maintenance behavior during pair formation or breeding may well affect plumage hue, chroma, or brightness to act as a signal during that period. Comparing the summer plumage with the winter plumage would provide insight into the consistency of signaling throughout the seasons and the potential of carotenoid-derived plumage coloration to act as an honest signal in Lesser Goldfinches.

Acknowledgments We are grateful to landowners who generously provided access to their bird feeders, to the citizen scientists that reported bird sightings, and student volunteers that aided in fieldwork. We thank the UNR Honors Undergraduate Research Award to MFC for financial support. We appreciate useful feedback from the UNR Evol Doers and two anonymous reviewers. All research protocols were conducted under appropriate federal and state permits, and reviewed and approved by the Institutional Animal Care and Use Committee at the University of Nevada, Reno (protocol no. 2008-0383).

References

Beaulieu M, Sockman KW (2012) One meadow for two sparrows: resource partitioning in a high elevation habitat. Oecologia 170:529–540

Blount JD (2004) Carotenoids and life-history evolution in animals. Arch Biochem Biophys 430:10–15

Boecklen WJ, Yarnes CT, Cook BA, James AC (2011) On the use of stable isotopes in trophic ecology. Annu Rev Ecol Evol Syst 42:411–440

Burnham KP, Anderson DR (2002) Model selection and inference: a practical information-theoretic approach. Springer, New York

Caut S, Angulo E, Courchamp F (2009) Variation in discrimination factors (δ^{15}N and δ^{13}C): the effect of diet isotopic values and applications for diet reconstruction. J Appl Ecol 46:443–453

Darimont CT, Paquet PC, Reimchen TE (2007) Stable isotopic niche predicts fitness of prey in a wolf–deer system. Biol J Linn Soc 90:125–137

Darwin C (1859) On the origin of species. John Murray, London

Darwin C (1871) The descent of man, and selection in relation to sex. John Murray, London

Delhey K, Peters A, Kempenaers B (2007) Cosmetic coloration in birds: occurrence, function, and evolution. Am Nat 169:S145–S158

DeNiro M, Epstein S (1978) Influence of diet on distribution of carbon isotopes in animals. Geochim Cosmochim Acta 42:495–506

Emlen ST, Oring LW (1977) Ecology, sexual selection, and the evolution of mating systems. Science 197:215–223

Emlen DJ, Warren IA, Johns A, Dworkin U, Lavine LC (2012) A mechanism of extreme growth and reliable signaling in sexually selected ornaments and weapons. Science 337:860–864

Gray DA (1996) Carotenoids and sexual dichromatism in North American passerine birds. Am Nat 148:453–480

Greenwood PJ (1980) Mating systems, philopatry and dispersal in birds and mammals. Anim Behav 28:1140–1162

Guindre-Parker S, Love OP (2013) Revisiting the condition-dependence of melanin-based plumage. J Avian Biol 44:1–5

Hamilton WD, Zuk M (1982) Heritable true fitness and bright birds: a role for parasites? Science 218:384–387

Hammerschlag-Peyer CM, Yeager LA, Araújo MS, Layman CA (2011) A hypothesis-testing framework for studies investigating ontogenetic niche shifts using stable isotope ratios. PLoS One 6:e27104

Hill GE (2000) Energetic constraints on expression of carotenoid-based plumage coloration. J Avian Biol 31:559–566

Hsu Y-C, Shaner P-J, Chang C-I, Ke L, Kao S-J (2014) Trophic niche width increases with bill-size variation in a generalist passerine: a test of niche variation hypothesis. J Anim Ecol 83:450–459

Inger R, Bearhop S (2008) Applications of stable isotope analyses to avian ecology. Ibis 150:447–461

Jackson AL, Inger R, Parnell AC, Bearhop S (2011) Comparing isotopic niche widths among and within communities: SIBER—stable isotope Bayesian ellipses in R. J Anim Ecol 80:595–602

Järvi T, Røskaft E, Bakken M, Zumsteg B (1987) Evolution of variation of male secondary sexual characteristics. Behav Ecol Sociobiol 20:161–169

Johnson K, Rosetta D, Burley DN (1993) Preferences of female American Goldfinches (*Carduelis tristis*) for natural and artificial male traits. Behav Ecol 4:138–143

Kempster B, Zanette L, Longstaffe FJ, MacDougall-Shackleton SA, Wingfield JC, Clinchy M (2007) Do stable isotopes reflect nutritional stress? Results from a laboratory experiment on song sparrows. Oecologia 151:365–371

Layman CA, Arrington DA, Montaña CG, Post DM (2007) Can stable isotope ratios provide for community-wide measures of trophic structure? Ecology 88:42–48

Legendre P, Legendre L (1998) Numerical ecology. Elsevier, New York

Lenouvel P, Gomez D, Théry M, Kreutzer M (2009) Do grooming behaviours affect visual properties of feathers in male domestic canaries, *Serinus canaria*? Anim Behav 77:1253–1260

Linsdale JM (1957) Goldfinches on the Hastings natural history reservation. Am Midl Nat 57:1–119
Lopez-Rull I, Pagan I, Macias Garcia C (2010) Cosmetic enhancement of signal coloration: experimental evidence in the house finch. Behav Ecol 21:781–787
McGraw KJ (2004) Winter plumage coloration in male American Goldfinches: do reduced ornaments serve signaling functions in the non-breeding season? Ethology 110:707–715
McGraw KJ (2008) An update on the honesty of melanin-based color signals in birds. Pigment Cell Melanoma Res 21:133–138
McGraw KJ, Hill GE (2000) Differential effects of endoparasitism on the expression of carotenoid- and melanin-based ornamental coloration. Proc R Soc B Biol Sci 267:1525–1531
McGraw KJ, Hill GE, Stradi R, Parker RS (2001) The influence of carotenoid acquisition and utilization on the maintenance of species-typical plumage pigmentation in male American Goldfinches (*Carduelis tristis*) and Northern Cardinals (*Cardinalis cardinalis*). Physiol Biochem Zool 74:843–852
McGraw KJ, Hill GE, Stradi R, Parker RS (2002) The effect of dietary carotenoid access on sexual dichromatism and plumage pigment composition in the American Goldfinch. Comp Biochem Physiol B Biochem Mol Biol 131:261–269
McGraw KJ, Hill GE, Parker RS (2005) The physiological costs of being colourful: nutritional control of carotenoid utilization in the American Goldfinch, *Carduelis tristis*. Anim Behav 69:653–660
Michalik A, McGill RAR, Furness RW, Eggers T, van Noordwijk HJ, Quillfeldt P (2010) Black and white—does melanin change the bulk carbon and nitrogen isotope values of feathers? Rapid Commun Mass Spectrom 24:875–878
Olson VA, Owens IPF (1998) Costly sexual signals: are carotenoids rare, risky or required? Trends Ecol Evol 13:510–514
Paritte JM, Kelly JF (2009) Effect of cleaning regime on stable-isotope ratios of feathers in Japanese Quail (*Coturnix japonica*). Auk 126:165–174
Parnell AC, Inger R, Bearhop S, Jackson AL (2010) Source partitioning using stable isotopes: coping with too much variation. PLoS One 5:e9672
Peig J, Green AJ (2009) New perspectives for estimating body condition from mass/length data: the scaled mass index as an alternative method. Oikos 118:1883–1891
Peig J, Green AJ (2010) The paradigm of body condition: a critical reappraisal of current methods based on mass and length. Funct Ecol 24:1323–1332
Pérez-Rodríguez L, Mougeot F, Bortolotti GR (2011) The effects of preen oils and soiling on the UV–visible reflectance of carotenoid-pigmented feathers. Behav Ecol Sociobiol 65:1425–1435
Podlesak DW, McWilliams SR, Hatch KA (2005) Stable isotopes in breath, blood, feces, and feathers can indicate intra-individual changes in the diet of migratory songbirds. Oecologia 142:501–510
Post DM, Layman CA, Arrington DA, Takimoto G, Quattrochi J, Montaña CG (2007) Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152:179–189
Pyle P (1997) Identification guide to North American birds. Part 1. Columbidae to Ploceidae. Slate Creek, Bolinas
R Development Core Team (2010) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. http://www.R-project.org.
Robb GN, McDonald RA, Inger R, Reynolds SJ, Newton J, McGill RAR, Chamberlain DE, Harrison TJE, Bearhop S (2011) Using stable-isotope analysis as a technique for determining consumption of supplementary foods by individual birds. Condor 113:475–482
Saks L, McGraw K, Hõrak P (2003) How feather colour reflects its carotenoid content. Funct Ecol 17:555–561
Searcy WA, Nowicki S (2005) The evolution of communication: reliability and deception in animal signaling systems. Princeton University Press, Princeton
Semmens BX, Ward EJ, Moore JW, Darimont CT (2009) Quantifying inter- and intra-population niche variability using hierarchical Bayesian stable isotope mixing models. PLoS One 4:e6187
Slagsvold T, Lifjeld JT (1992) Plumage color is a condition-dependent sexual trait in male pied flycatchers. Evolution (N Y) 46:825–828
Stevens M, Parraga CA, Cuthill IC, Partridge JC, Troscianko TS (2007) Using digital photography to study animal coloration. Biol J Linn Soc 90:211–237
Surmacki A, Nowakowski JK (2007) Soil and preen waxes influence the expression of carotenoid-based plumage coloration. Naturwissenschaften 94:829–835
Walther BA, Clayton DH (2005) Elaborate ornaments are costly to maintain: evidence for high maintenance handicaps. Behav Ecol 16:89–95
Ward EJ, Semmens BX, Phillips DL, Moore JW, Bouwes N (2011) A quantitative approach to combine sources in stable isotope mixing models. Ecosphere 2:art19
Watt DJ, Willoughby EJ (1999) Lesser Goldfinch (*Spinus psaltria*). In: Poole A (ed) The birds of North America online. Cornell Lab of Ornithology, Ithaca
Werner RA, Bruch BA, Brand WA (1999) Conflo III—an interface for high precision $\delta^{13}C$ and $\delta^{15}C$ analysis with an extended dynamic range. Rapid Commun Mass Spectrom 13:1237–1241
Willoughby EJ (2007) Geographic variation in color, measurements, and molt of the Lesser Goldfinch in North America does not support subspecific designation. Condor 109:419–436
Winker K (1998) Suggestions for measuring external characters of birds. Ornitol Neotrop 9:23–30
Zahavi A (1977) The cost of honesty: further remarks on the handicap principle. J Theor Biol 67:603–605