

Long-term changes of plumage between urban and rural populations of white-crowned sparrows (*Zonotrichia leucophrys*)

Shawn H. Smith ¹, Jesi Hessong-Brown,² Sara E. Lipshutz ³,
Jennifer N. Phillips,^{4,5} Catherine Rochefort,⁶ Elizabeth P. Derryberry⁷ and
David A. Luther ^{2,*}

¹Environmental Science and Policy Department, George Mason University, Fairfax, VA, USA, ²Biology Department, George Mason University, Fairfax, VA, USA, ³Department of Biology, Indiana University, Bloomington, IN, USA, ⁴Department of Biological Sciences, California Polytechnic State University, San Luis Obispo, CA, USA, ⁵Department of Life Sciences, Texas A&M University-San Antonio, San Antonio, TX, USA, ⁶Department of Psychology, Southern Methodist University, Dallas, TX, USA and ⁷Department of Ecology and Evolutionary Biology, University of Tennessee Knoxville, Knoxville, TN, USA

*Corresponding author: E-mail: dluther@gmu.edu

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Abstract

Urbanization is one of the most extreme forms of land transformation and results in changes to ecosystems and species compositions. As a result, there are strong directional selection pressures compared to nearby rural areas. Despite a surge in research on the different selection pressures on acoustic communication in urban and rural areas, there has been comparatively little investigation into traits involved with visual communication. We measured the plumage of museum specimens of white-crowned sparrows (*Zonotrichia leucophrys*) from urban and adjacent rural habitats in San Francisco, CA, to assess the effects of divergent habitats on plumage. We found significant differences in dorsal plumage, but not crown plumage, between urban and rural populations that have been diverging over the past 100 years. Urban birds have increasingly darker and duller dorsal plumage, whereas rural birds in adjacent areas have plumage with richer hues and more color complexity. Our findings suggest a newly observed adaptation to urban environments by native species and suggest that many traits, in addition to acoustic signals, may be changing in response to urban selection pressures. Additional collections in urban areas are needed to explore likely divergences in plumage coloration between urban and rural environments.

Key words: natural selection, plumage, anthropogenic, avian, urban

Introduction

Urbanization remains a rapidly growing phenomenon worldwide and is one of the most extreme forms of land transformation (Wu et al. 2011). Urbanization fragments and degrades

natural habitats, simplifies and homogenizes species composition (Alberti 2005; McKinney 2006; Grimm et al. 2008; Batáry et al. 2018; but see Aronson et al. 2014 for conflicting results), introduces novel predators (Fischer et al. 2012; Loss et al. 2013) and has contributed to the local extirpation of many species

(Clergeau et al. 2006; McKinney 2008; Evans et al. 2012). Populations of species that persist in urban areas likely face drastically different environmental pressures than nearby rural populations (Shochat et al. 2006; Swaddle et al. 2015). For example, available food (Seress et al. 2018), the effects of drought, pollution and heat (Paris 2016; Brans et al. 2018; Salmón et al. 2018), prevalence of disease (Costantini et al. 2014), anthropogenic noise (McKinney 2002; Shannon et al. 2016) and the structural form of cities (e.g. urban canyons, Warren et al. 2006) are quite different between urban and nearby rural habitats (Beissinger and Osborne 1982; Bonier 2012; Davies et al. 2016; Ditchkoff et al. 2006). These differences can lead to behavioral, physiological, genetic or morphological divergence between urban and rural populations (Rasner et al. 2004; Partecke et al. 2004; Partecke et al. 2005; Garroway and Sheldon 2013; Müller et al. 2013; Sparkman et al. 2018; Putman et al. 2019).

Urban pressures have the potential to alter or even shape the evolution of communication signals. Whereas the adaptation of acoustic signals to urban landscapes has been studied extensively (Luther and Gentry 2013; Swaddle et al. 2015; Derryberry et al. 2016; Luther et al. 2016; Lipshutz et al. 2017; Kleist et al. 2018), visual signals have largely been overlooked in the literature. The visual environments of cities and adjacent rural areas tend to be different, with a larger percentage of gray coloration, and impervious and reflective surfaces in cities compared to rural areas (Warren et al. 2006; Dowling et al. 2012). In animals that rely extensively on visual signals and cues, differences in the visual environment between urban and rural areas may lead to divergences in color and behavior (Endler 1992), which will increase signal detection by intended receivers (such as mates or competitors) (Delhey and Peters 2017) and/or decrease detection by predators (Stevens and Merilaita 2009). One example of urbanization changing concealment behavior is the common rock agama (*Psammodromus dorsalis*), a lizard in India that selects different perches and has different escape strategies across an urban to rural gradient (Batabyal et al. 2017). Additionally, urban birds have altered their predator avoidance behaviors by flying shorter distances when escaping from predators as compared to members of the same species occupying rural areas (Møller 2008). These studies provide evidence for how urban landscapes shape predator avoidance behaviors, but less is known about differences in coloration and visual patterns between urban and adjacent rural populations of animals.

Over 2000 avian species occur in urban environments (Aronson et al. 2014), and avian plumage is an excellent trait to study regarding divergence between urban and rural populations. Plumage phenotypes are subject to both sexual and natural selection, sometimes in opposition of one another (Møller 1989; Olsen et al. 2010; Dunn et al. 2015; de Zwaan et al. 2019). For example, melanization in plumage can be used to signal aggressiveness between males (Gonzalez et al. 2001) and condition to potential mates (Badyaev et al. 2001) and predators (Huhta et al. 2003). Additionally, plumage on one portion of the body, such as the crown, may signal habitat quality and aggression (Jones et al. 2017), while other portions can aid in camouflage (Dunn et al. 2015). Thus plumage coloration is responsive to not only multiple factors such as sexual and natural selection but also changes in food nutritional values, fluctuating hormone levels and pollution in the local environment such as soot, (Chatelain et al. 2016; Corbel et al. 2016; Biard et al. 2017; Gryz and Krauze-Gryz 2018; Leveau 2019). All of these factors have been shown to differ between urban and rural areas.

Several features of urban environments, such as the aforementioned differences in pollutants, stress levels, food quality and availability, as well as background coloration in conjunction with mesopredators that target avian prey (Crooks and Soulé 1999), could select for different plumages in urban birds. Urban birds may have feathers that are darker (have higher absorbance and less reflectance) and duller (less color saturation) to better blend into the urban surroundings. Urban areas tend to have higher concentrations of environmental pollutants such as lead (Roux and Marra 2007; Kekkonen et al. 2012), which can result in increased melanin concentrations (Chatelain et al. 2014; Chatelain et al. 2016). In addition, urban living tends to increase corticosterone in birds, an indicator of stress, which can also increase melanin concentrations (Ruiz et al. 2002; Almasi et al. 2013; Beaugueard 2019; Ouyang et al. 2019). However, there have been contrasting results between stress and urbanization, and results may vary depending on species (Ibáñez-Álamo et al. 2020). The physical and visual characteristics of cities include large amounts of gray asphalt and reduced vegetation complexity (Warren et al. 2006; Dowling et al. 2012). Because the feathers on the back and head of ground feeding birds functions as camouflage against potential avian predators (Dunn et al. 2015), it is predicted that urban birds should have duller or darker plumage in urban areas that are relatively homogeneous and gray compared to rural landscapes (Farkas et al. 2013; Leveau 2019). Finally, although urban areas may provide increased quantities of food, the nutritional quality is reduced (Isaksson and Andersson 2007; Narango et al. 2017), although invasive plants and supplemental food may decrease this effect (Jones et al. 2010). Lower quality food can result in lower carotenoid levels and less bright plumage coloration (Jones et al. 2010; Biard et al. 2017). All of these potential differences between urban and rural areas lead to predictions of darker and duller dorsal plumage in birds that inhabit urban compared to rural areas.

Differences in urban and rural landscapes may also affect plumage complexity, the variation in colors and patterns of plumage, in birds. Habitats with more complex vegetative habitats may select for increased plumage complexity (Hughes et al. 2019) as increased plumage complexity should reduce the silhouette allowing the bird to blend in with more visually complex backgrounds, which should decrease detection by predators. In habitats with relatively solid background coloration, less complex and more solid and single-hued plumage colors and shapes should increase camouflage and decrease detection from predators (Troschianko et al. 2013; Troschianko et al. 2016). Thus, it would be expected that birds in rural habitats would exhibit more complex dorsal plumage patterns while urban birds should have less complex dorsal plumage.

In this study, we compared the dorsal plumage and crown plumage of urban and adjacent rural populations of white-crowned sparrows (WCSP) (*Zonotrichia leucophrys*) over a 100-year time period to investigate if plumage coloration and complexity is different between urban and rural habitats. We hypothesized that dorsal plumage adapted to the local environment and differed between habitat types. We predicted that dorsal plumage would be duller or darker in color and less visually complex on birds in the urban habitat in comparison to the dorsal plumage of birds in nearby rural areas, which should have more reddish and rusty hues and complexity. We predicted that crown plumage, which would be under the same pressures of natural selection, but also used in sexual selection and social status in WCSP (Chilton et al. 1995), would also be duller or darker on urban birds than on rural birds. Testing these hypotheses provided insight into whether visual signals

in urban environments have diverged from visual signals in nearby rural areas.

Methods

Study system

WCSP are year-round residents in coastal central California with a long history of research (Chilton et al. 1995). They are commonly found in areas with limited tree cover such as chaparral habitat and grassy fields, but they also successfully inhabit and persist in both urban and rural areas (Derryberry 2009; Derryberry et al. 2016). The crown plumage of WCSPs is black and white and signals social status, while the plumage on the dorsal region has more grey, black and brown/rusty hues (Parsons and Baptista 1980; Fugle and Rothstein 1987; Laubach et al. 2013). The grey and black hues are melanin based, while the brown hues are thought to be carotenoid based (McGraw et al. 2004a) but could be melanin based (McGraw et al. 2004b). The crown plumage is sexually dimorphic, but the dorsal plumage is not (Chilton et al. 1995).

Data collection

We photographed all WCSP museum specimens at the California Academy of Sciences and the Museum of Vertebrate Zoology at UC Berkeley. All birds were collected in coastal California between 1895 and 1990 (Table 1). We measured specimens from San Francisco, CA (hereafter, 'urban') (20 males, 25 females) as well as the Pt. Reyes region of Marin County (14 males, 22 females) and the Pescadero region (both hereafter, 'rural') (7 males, 12 females), separated by approximately 40 km north and south of San Francisco, respectively (Fig. 1). It has been found that the color of museum specimens may fade over time (Hausmann et al. 2003; Doucet and Hill 2009). However, if specimens are maintained in insect- and light-free areas, plumage coloration is generally well preserved (Burns et al. 2017). Doucet and Hill (2009) found similar coloration values between museum and live specimens, but there was a loss in coloration values in museum specimens over time. In contrast, our results showed increases in values over time indicating that color loss in our specimens was negligible if even occurring.

Photographs were taken with a Canon EOS Rebel (XSi) with a 60 mm macro lens and an MR-14EX ring flash, set at 1/16 power, on a tripod, with a photo calibration gray-scale card. The focal distance of the lens was set at 0.44 m and the camera was set to manual mode and at ISO 100, F 11, 1/250 sec. We saved images as raw files for analysis with ImageJ (<http://rsbweb.nih.gov/ij/>). The gray standard, and the red, blue and green channels were standardized using the methods of Luttrell et al. (2015). The brightness was adjusted for the white square of the gray



Figure 1: Specimens were collected from three sites near San Francisco, California. Thirty-six were collected from the Point Reyes area 40 km north of San Francisco and 20 were collected from the Pescadero area 40 km south of San Francisco. Both sites were classified as rural. Forty-four were collected within the San Francisco city area and were classified as urban.

standard card at a luminance value of 230 (± 1.5) and the gray square a luminance value of 100 (± 1.5).

All measurements were based on the standardized images. The polygon tool in ImageJ outlined the dorsal area of each bird, beneath the nape but not including the wings (Fig. 2). We recorded the means and standard deviations from gray scale and color (red, green, blue channels) histograms as well as the total number of pixels in each bin of the gray scale histogram. Three statistics were used to characterize dorsal coloration: average luminance in the gray scale (indicating the brightness/lightness of the plumage); red dominance (red/(green + blue luminance)) (a measure of plumage rustiness); percent of black (the percentage of pixels with luminance values of 20 or less) (after Luttrell et al. 2015). The percent black measured the darkness of the dorsal region. The coefficient of variation of the luminance of the dorsal region was calculated based off of the luminance values from over 30 sites on each bird's dorsal region to measure the complexity of patterning of the dorsal plumage. Thus, higher values of the coefficient of variation of luminance resulted in more complexity, such that a low score indicated solid coloring and a high score indicated more contrasting patterns and complexity (Luttrell et al. 2015).

We also measured plumage of the white and black portion of the crown of the adult birds. Not all birds had suitable crown plumage due to damage to the study skins thus we only had 10 urban and 22 rural birds. In the white portion of the crown we assessed the luminance, as described above, and in the black

Table 1: The year in which each breeding season WCSP in San Francisco and nearby rural areas was collected

	1890	1900	1910	1920	1930	1940	1950	1960	1970	1980	1990	Total
Rural	1	2	14	2	7	1			1	1	1	30
Female	1	1	5	1	3				1			12
Male		1	9	1	4	1				1	1	18
Urban			5	1		1	1	2	1	6		17
Female			3			1	1		1	4		10
Male			2	1				2		2		7
Total	1	2	19	3	7	2	1	2	2	7	1	47



Figure 2: Dorsal and crown regions where plumage color values were taken from each photograph of WCSP museum specimens.

portion of the crown we assessed the darkness as the percent of black measured, as described above. We also measured the contrast between the white and the black portion of the crown.

All plumage traits were normally distributed, except the percent of black on the dorsal region for which we applied an arc sign transformation. Due to the correlative nature of the luminance, rustiness, and percent of black in the feathers as shown by Luttrell et al. (2015) we conducted a Bartlett's Test of Sphericity for the correlation between these variables ($\chi^2 = 136.95$, $DF = 3.71$, $P = 0.0001$), which indicated that the variables were highly correlated. Thus, we conducted a principal components analysis using the dorsal plumage values. Of which, only one principal component (PC1) had an eigenvalue >1 . It loaded positively with gray luminance and red dominance, and negatively with the percent of black on the dorsal region (Table 2).

Prior to analysis, young birds (determined based on brown rather than black crown feathers), and specimens with undetermined sex were removed, which left 98 individuals for analysis. In preliminary analyses, dorsal plumage PC1 did not differ based on season (breeding April to August or non-breeding) ($F_{Ratio_{1,97}} = 0.8$, $P = 0.78$); however, nonbreeding season birds that were collected could have been migrant birds from other urban or rural regions. Thus, to avoid potentially having migrant birds in the analysis, we did not include any nonbreeding season birds which left us with 47 birds (30 rural and 17 urban birds) (see Table 2).

To examine how plumage varied between urban and rural populations we conducted an analyses of variance (ANOVA) with environment (urban/rural) as the independent variables and dorsal plumage PC1 [a combination of luminescence, red dominance (an indicator of complexity), and percent of black in the feather] as the dependent variable. We also conducted an ANOVA in which we assessed how plumage changed over time for urban and rural birds with environment (urban/rural), year, and an interaction term of environment and year as independent variables and dorsal plumage PC1 [a combination of luminescence, red dominance (an indicator of complexity), and percent of black in the feather] as the dependent variable. Date was coded as a fractional year. We excluded one outlier from the dataset as it was more than 2 standard deviations from all others in the sample (CAS 40236 1). ANOVAs were used to assess differences in crown coloration between urban and rural populations as well as between males and females of each population. All statistical analyses were conducted using R 4.0.3 (R-Core-Team 2020) and data is available on Dryad.

Results

Urban birds had significantly different dorsal plumage PC1 than rural birds ($F_{Ratio_{1,45}} = 19.93$, $P < 0.001$). Plumage values were compared between the two rural sites and no significant

difference was found ($P = 0.53$). There was no significant difference in dorsal plumage between male and female birds ($P = 0.39$) in urban and rural environments. The interaction of environment and year was significant in our models but year itself was not significant ($F_{Ratio_{1,45}} = 19.93$, $P < 0.0001$) with PC1 values increasing with time in urban areas while decreasing in rural areas (Fig. 3).

Crown plumage was not significantly different between urban and rural populations (white $P = 0.23$; black $P = 0.86$; contrast $P = 0.28$). Brightness of white-crown plumage and the contrast between white- and black-crown plumage was significantly different between male and female birds, regardless of environment (white $F_{Ratio_{1,30}} = 0.02$; contrast $F_{Ratio_{1,30}} = 0.02$), but the darkness of the black-crown plumage was not different between males and females ($P = 0.58$). Males had brighter white ($M = 118.05 \pm 4.3$; $F = 102.03 \pm 4.9$) and greater contrast between the white and black stripes on their heads than females ($M = 111.9 \pm 4.6$; $F = 94.86 \pm 5.2$).

Discussion

Our results provide evidence of differences in dorsal plumage coloration between urban and rural populations of birds and that the plumage changed during the 20th century. The plumage of urban birds became darker, duller and less complex than that of rural birds, consistent with our predictions. These changes over time in plumage coloration and color complexity are consistent with our predictions. Unexpectedly, we found that birds in urbanized populations were lighter than rural birds historically, such that urban and rural populations appear to have initially been divergent, but over time, have converged and look to be diverging in opposite directions in dorsal plumage coloration. In contrast, we found no difference in bird crown plumage between urban and rural sites, but we did find differences between the sexes.

While we document differences in plumage between urban and rural populations on the dorsal but not crown region of WCSP, and that they appear to be changing over time, we cannot identify a specific cause of the differences in plumage. The differences and temporal changes in dorsal plumage, but not crown plumage, could be a result of natural selection for camouflage, a result of pollution, or dietary differences between urban and rural populations. Here, we address each of these hypotheses and how they might explain our results.

In urban WCSP populations, plumage melanism increased over time. Although much of San Francisco burnt down in the 1906 earthquake, rebuilding and expansion was achieved by 1915 (Richards 2007). Given that WCSP have an average lifespan of 1.5 years (Chilton et al. 1995), this would allow for an increase in melanism in roughly 40 generations, suggesting strong directional selection for increased melanism in the city. Over the same time period, plumage melanism decreased in rural WCSP populations. In Marin and San Mateo counties (just north and south of San Francisco, respectively), visual landscapes are also changing, which could contribute to changes in plumage as well. As sheep and cattle grazing have reduced in these locations in recent decades, shrubs and Douglas fir (*Pseudotsuga menziesii*) have encroached on the previously open grasslands (Chase et al. 2005) and the change in vegetative complexity from grazed open grassland to shrub vegetation. The lighter but more complex plumage of rural birds would allow them to better blend into this new landscape. In addition, the amount of fog, and associated cloud cover, in coastal California has reduced in the past century (Johnstone and Dawson 2010), which

Table 2: Factor loadings for the first principal component derived from color spectra on the dorsal region of urban and rural WCSP

Response variables	PC1	Mean $\pm$ SD	
		Rural	Urban
Percent black on dorsal	-0.68	47.98 $\pm$ 19.56	54.12 $\pm$ 19.32
Mean gray on dorsal	0.62	66.90 $\pm$ 4.12	57.49 $\pm$ 1.37
Red Dominance on dorsal	0.38	0.825 $\pm$ 0.074	0.85 $\pm$ 0.08
Eigenvalues	1.97		
Cumulative percent	66%		

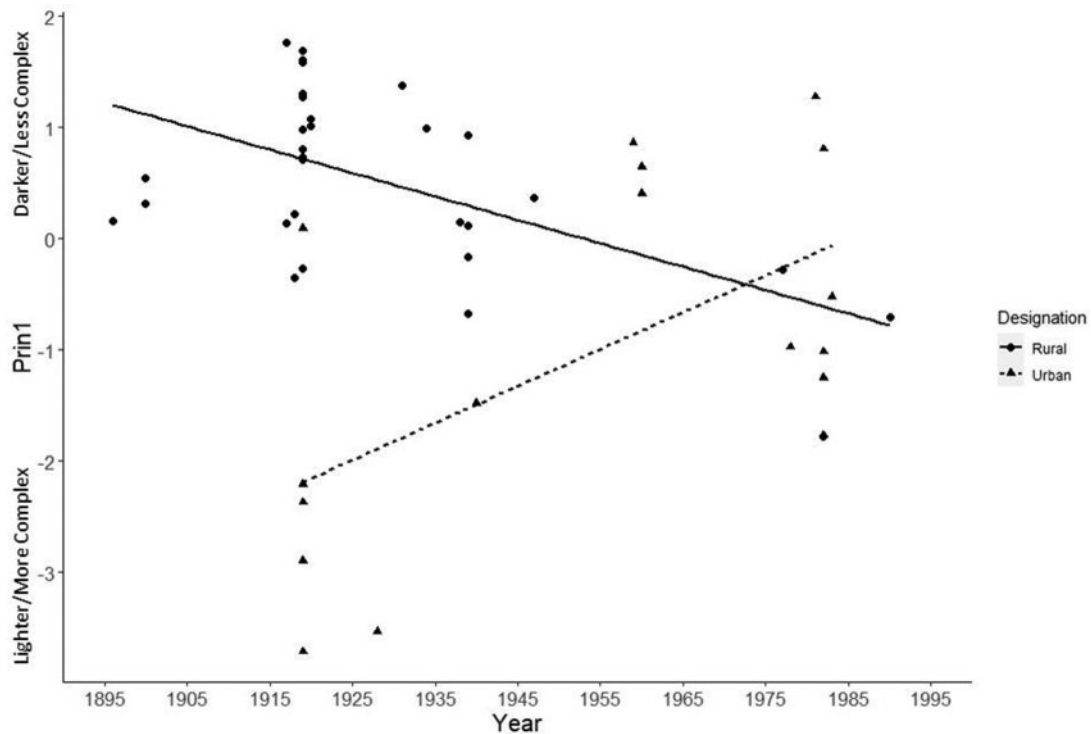


Figure 3: The change in dorsal plumage during the 20th century in urban and rural birds. PC1 represents the plumage color with higher values indicating brighter plumage with richer colors and lower values indicating darker and duller color values. Plumage is getting duller and darker on urban birds and richer and lighter in color on rural birds. The darker line and circles represent the rural birds, while the triangles and dashed line represent the urban birds.

leads to increased sunlight, less shade, greater drought stress and drying of vegetation (Fischer et al. 2009). The removal of large predators and the subsequent release of mesopredators that target avian prey items may also select for increased camouflage in both habitat types (Crooks and Soulé 1999). Thus, the plumage of both urban and rural birds is changing in a manner that could increase their camouflage in their respective environments over a relatively short period of time. Eventually these changes caused plumage characteristics to converge, but the ever-changing landscape in both areas is causing them to continue to diverge.

During the early 20th century, there was a rise in the production of black carbon from factory output and increased residual amounts of carbon were found on bird feathers until the carbon output peaked in the middle of the 20th century (DuBay and Fuldner 2017). While our results of dorsal plumage of urban birds are consistent with increased urban pollution, the crown plumage did not vary between the urban and rural populations. Thus, pollution, at least from factory soot, is not a likely explanation for the differences in dorsal plumage in each environment as pollution would not be likely to target a specific portion of bird plumage. However, studies have identified duller plumage in urban birds, which is consistent with our results, attributed to reduced oxidative balance from urban diets and pollutants (Isaksson et al. 2005; Giraudeau et al. 2015; Chatelain et al. 2016).

More melanistic feathers, as we found in the dorsal plumage in cities, degrade at slower rates than lighter colored feathers (Goldstein et al. 2004). This could be valuable in the city, especially with higher levels of pollution that could introduce more wear and tear on feathers (Giraudeau et al. 2015). Melanin pigments bind metal ions, which are abundant in urban areas, thereby potentially sequestering them in inert body parts such

as feathers and facilitating body detoxification of these trace metals (Chatelain et al. 2014; Chatelain et al. 2016). Thus, more melanistic plumage could give an advantage to animals in areas with relatively higher amounts of pollution. In addition, a different suite of bacterial fauna in urban and rural environments could impose divergent selection on feather melanism so that the feathers are more resistant to degradation from bacteria in one environment over the other (Leclaire et al. 2014).

Melanin is seen as an honest signal that reflects the condition of an individual. The increase in food availability in urban environments could be allowing for less risky access to foods containing melanin (Jawor and Breitwisch 2003; Parker et al. 2003). Urban environments can have more reliable food sources available due to anthropogenic feedings (Evans et al. 2009) and larger quantities of some natural food sources (Isaksson and Andersson 2007). However, food quantity is not equal to food quality and in urban areas the available food can lack needed nutrients, such as carotenoids when compared with rural food sources (Isaksson and Andersson 2007; Narango et al. 2017). Potentially, rural areas have food richer in carotenoids, which is associated with redder plumage (Inouye et al. 2001), as observed in our rural birds with a higher red dominance than the urban birds. Similarly, rural great tit (*Parus major*) chicks had higher yellow carotenoid-based pigments compared with their urban counterparts (Biard et al. 2017).

Stress can also influence the expression of carotenoid-based colors (Isaksson et al. 2005), where higher stress levels lead to duller plumage (Giraudeau et al. 2018). Urban male WCSP tend to have higher stress levels than rural male WCSP (Bonier et al. 2007), which could lead to duller plumage in urban males. Thus, higher stress levels among urban males could help to explain why urban males had duller plumage than rural males.

Contrary to our predictions, we found no difference in the white or black, feathers on the crown of WCSP in urban and rural populations. Potentially the lack of divergence is due to strong sexual selection effects of the crown of both urban and rural WCSP populations (Chilton et al. 1995), whereas dorsal plumage would be more susceptible to natural selection pressures. There is no reason to believe that pollution, stress or diet would disproportionately affect feathers in a certain region of the body, such as dorsal compared with other regions, such as the crown, although to our knowledge there has been no investigation into this possibility.

In summary, we found distinct dorsal plumage, but not crown plumage, between urban and rural birds, and evidence that plumage in both habitats changed over time. There are multiple explanations for the observed divergence, such as camouflage, pollution, hormone response, diet or interactions between several of these factors. We encourage researchers to investigate each of these factors to disentangle them. The museum specimens used for this study were collected over a 100-year span, but the most recent specimens were collected roughly 20 years before this study took place. Additional collections are needed to explore more contemporary changes and determine the potential factors driving changes in plumage coloration in urban areas.

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Availability of data and material

We would like for it to say “All data is available on the Dataverse Network and is available at <https://doi.org/10.13021/orc2020/84KXPS>.”

Author contributions

DAL conceived the idea and design of experiment, SHS, JB, DAL, SEL and EPD wrote the manuscript. JB and DAL developed the methods, SEL, JNP and CR photographed the specimens. JB, EPD and DAL analyzed the data and contributed substantial materials and funding.

Ethics statement

No live animals were used thus no IACUC protocols were needed. All skin handling procedures of the museum collections were used according to the two different participating institutions requirements and no specimen was damaged during the study.

Conflict of interest statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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