



Original Article

Expression of and choice for condition-dependent carotenoid-based color in an urbanizing context

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Urban environments create a unique suite of conditions, leading to changes in animal behavior, morphology, phenology, and physiology. Condition-dependent traits such as the carotenoid-based coloration offer a unique opportunity to assess the impacts of urbanization on organisms because they reflect the nutrition, health, or other resource-based attributes of their bearers and they play an essential role in intra and intersex interactions. To determine if and how the carotenoid-based coloration of male house finches (*Haemorhous mexicanus*) varies along a gradient of urbanization, we quantified the plumage coloration of more than 1000 individuals in urban, sub-urban, rural, and desert habitats over the course of 17 months. We also examined for the first time the preference of females for male plumage coloration across the urban–rural gradient, to test if and how female preferences varied relative to the plumage coloration displayed by males in their local population. We found that carotenoid-based coloration decreased along the gradient of urbanization, suggesting that the enzyme-driven metabolic conversion of dietary carotenoids into red carotenoids used to color plumage is sensitive to urban stressors. The stronger negative effect of urbanization on carotenoid-based plumage coloration during breeding than during molt and winter suggests that urbanization affects color fading rate, maybe through modifications of feather-degrading bacterial load. Finally, we have shown that urbanization influences female mate-choice behavior, suggesting that female color preferences may track the variation in male coloration across the gradient of urbanization.

Key words: Carotenoid, coloration, condition-dependent traits, mate choice, urbanization.

INTRODUCTION

Urbanization is an ongoing process that leads to a dramatic increase of the size of urban centers all around the world. Half of the human population now lives in cities and the UN World Urbanization Prospects report predicts that 60% (4.9 billion) will reside in urban environment by 2030 (UNPD 2005). The severe changes of land use associated with this process are expected to considerably affect ecosystems (Sauvajot et al. 1998; Marzluff 2001; Milesi et al. 2003). Thus, urbanization has become a topic of prime importance for conservation biologists and evolutionary ecologists, leading to the publication of an increasing number of papers on this topic over the last 15 years (Grimm et al. 2000; Marzluff 2001; Shochat et al. 2006). Among this literature, most

of the attention has been focused on urban animal communities, showing that urbanization leads to a decrease of biodiversity and an increase in the density of few species (Marzluff 2001; Shochat et al. 2004). Over these last few years, there has been a noticeable increase in the overall number of studies aiming at testing evolutionary divergence between urban and rural populations, and comparing urban and rural phenotypes (e.g. Alberti 2015; Alberti et al. 2017a, 2017b; Pelletier and Coltman 2018). These studies of intraspecific variation offer an opportunity to understand the novel challenges organisms face in urbanizing landscapes.

In cities, animals are exposed to many potential stressors such as an increase in noise levels (Slabbekoorn and Peet 2003), exposure to toxins (Vincent 2005; Swaileh & Sansur 2006), artificial lighting (Longcore and Rich 2004), warmer temperature (Luniak et al. 1990), and change of diet (Eeva et al. 1998). Besides these constraints, urban environments may also offer benefits such as a more predictable access to food and water and a reduced mortality by

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predation (Shochat et al. 2004). Thus, the urban environment creates a unique suite of conditions, leading to potential changes in the behavior (Beckmann and Berger 2003; Slabbekoorn and Peer 2003; Yeh et al. 2007), morphology (Horak et al. 2000; Beckmann and Berger 2003; Yeh 2004; Liker et al. 2008), phenology (Fleischer et al. 2003; Partecke et al. 2004; Yeh and Price 2004), or physiology (Partecke et al. 2005, 2006; Bonier et al. 2007) of wild animals.

Condition-dependent traits offer a unique opportunity to assess the impacts of human activities on the physiology of organisms because (1) they reflect the nutrition, health, or other resource-based attributes of their bearers (Kodric-Brown and Brown 1984; Hill 1995) and (2) they play an essential role in intra and intersex interactions (mate-choice and reproductive investment) which are key elements of evolutionary processes (Burley 1988; Hill 2006; Senar 2006). In birds, carotenoids are the second most prevalent pigments deposited in the integument where they produce yellow to red coloration (McGraw 2006). In many species, this coloration is a signal of sexual attractiveness and competitive ability. Due to the multiple functions of carotenoid molecules (antioxidants, immunostimulators, pigments) and the fact that they can be a limiting resource (animals must acquire them from food), these colors are considered as a classic example of a condition-dependent trait (Blount et al. 2003; McGraw and Ardia 2003; McGraw 2006). These attributes make carotenoid-based colors particularly useful indicators of the physiological challenges posed by urban environments.

In a variety of bird species, females have been shown to prefer males bearing the most elaborate and intensely pigmented carotenoid-based colors (Hill 2006; Blount and McGraw 2008). These preferences are shaped by a number of factors including heritable genetic determinants, developmental conditions, and current environmental conditions (Jennions and Petrie 1997; Ah-King and Gowaty 2016). Just as ornamental coloration of signalers varies among environments, the potential also exists for the color preferences to vary. For example, imprinting, mate-choice copying, and learning (Westneat et al. 2000; Badyaev and Hill 2002) may lead to preferences that converge toward the mean of ornament expression in the local population or shifts in the signal content of the ornament may favor specific localized preferences. The information content of ornaments may change in novel environmental contexts, as rapid ecological changes might degrade evolved links between signal producer viability, signal intensity, and the costs/benefits derived from choosing specific ornament types. The urban environment provides an opportunity to begin to parse these possibilities and investigate how both ornamental coloration and color preferences vary across an environmental gradient.

The house finch (*Haemorrhous mexicanus*) is a model system for the study of condition-dependent carotenoid-based coloration, honest signaling, and female choice (Hill 2002). In this species, males can vary from drab yellow to brilliant red depending upon their diet and health and females typically prefer the reddest males as mates (Hill 2002; Toomey and McGraw 2012). In the Phoenix, Arizona, USA metro area, the coloration of male house finches differs across the urban–rural gradient with individuals in urban habitats tending to have drabber plumage than their counterparts in more rural locations (Giraudeau et al. 2015; Hasegawa et al. 2014). Therefore, this population offers an opportunity to identify the specific attributes of the urbanizing landscape that are driving local variation in plumage coloration. To determine these attributes, we expanded the scope of our sampling, quantified the carotenoid-based plumage coloration of more than 1000 individuals in eight sites ranging from the urban Phoenix metro area to the surrounding rural Sonoran Desert over

the course of 17 months. For each of our study sites, we characterized and quantified 8 attributes of urbanization (e.g. population density, ground cover, disturbance) and used linear mixed models to examine these attributes as predictors of plumage coloration. In addition, we also measured the body mass of these birds in order to study how urbanization affects the individual's nutritional status and how this affects their carotenoid-based coloration.

The variation in male house finch plumage coloration across the urban–rural gradient also offers us an opportunity to examine if and how female preferences might be influenced by the available male phenotypes in the population and other local environmental conditions. Previous studies of female house finch preferences in the Phoenix metro area indicated strong preferences for saturated red plumage, but these studies were limited to a single local population (Toomey and McGraw 2012). To determine if and how female preferences vary among local populations; we conducted controlled choice trials to assess the plumage color preferences of females from three sites across the urban to rural gradient.

METHODS

Correlative study: carotenoid-based plumage coloration variation

Bird capture

Male house finches ($N = 1041$) were captured in 8 sites (see Giraudeau et al. 2014a, 2014b for a detailed description of the sites) from January 2011 to September 2011 using basket and potter traps baited with sunflower seeds; trapping sessions lasted from 0700 to 1200 h. At capture, each bird was leg banded with a numbered United States Fish and Wildlife Service metal tag for individual identification. We also weighed each bird to the nearest 0.1 g.

Habitat description

To assess relationships between finch coloration and characteristics of the built environment, we examined land-use and land-cover (LULC) features in 1-km radius around the 8 sites of capture. Features were derived from a LULC map of the greater Phoenix metropolitan area provided by the Central Arizona–Phoenix Long-Term Ecological Research (CAP LTER) project. Eight urbanization parameters were extracted: 1) human population density estimated from the 2010 US Census data; and 2) the percentage of land dedicated to 8 land use variables: human population within a 1-km radius, cultivated vegetation and cultivated grass, river gravel and water, vegetation, undisturbed, disturbed-commercial/industrial and asphalt, disturbed-compacted soil, and disturbed-mesic and xeric vegetation residential. We then generated urbanization scores using PCA for the 8 variables cited above. PC1 was loaded strongly and negatively with the amount of undisturbed land cover, and strongly and positively with the amount of disturbance caused by yards and industry. Our 2 desert sites (Estrella and South Mountain Regional Park) had the lowest PC1 scores whereas our 2 urban sites (Phoenix downtown and ASU campus) had the highest scores. PC2 was loaded strongly and positively with both the amount of compacted soil and vegetation. Our 2 urban sites had the lowest PC2 scores whereas our 2 suburban sites (Mesa and Chandler) had the highest scores (see Giraudeau et al. 2016 for a detailed description of the PC scores).

Plumage coloration

Plumage coloration was quantified using digital photography, following standard published methods for this species (Giraudeau et al.

2012; Lendvai et al. 2013) and others (e.g. McGraw et al. 2002). Because house finch plumage does not reflect significantly in the UV (McGraw and Hill 2000; Lendvai et al. 2013), techniques that rely on visible-light are sufficient to capture variation in bird-visible and carotenoid-relevant coloration. Using a Canon PowerShot SD1200S, we took 2 separate photographs of the head, breast, and rump of each bird against a gray-board, using identical distance from camera to object, shutter, exposure, and flash settings for each photograph and including a color/size standard in each photo to control for any slight variations in object illumination. Ambient lighting was kept relatively constant by photographing finches in the shade. Digital images (3648 × 2736 pixels) were imported into Adobe Photoshop to extract the plumage hue data. Values for the 2 pictures of each bird were averaged for statistical analyses given that hue measurement using this method has been shown to be highly repeatable in several studies published by our group (Giraudeau et al. 2012; Lendvai et al. 2013). Within each individual, hue was also averaged across all color patches. Hue was highly correlated between patches (crown-breast: $r = 0.904$, $t = 65.44$, $df = 949$, $P < 0.001$; breast-rump: $r = 0.885$, $t = 58.7$, $df = 948$, $P < 0.001$; crown-rump: $r = 0.840$, $t = 47.67$, $df = 946$, $P < 0.001$).

Avian Poxvirus infection

Pox infection was monitored by visual inspection of pox lesions on the head and legs (Thompson et al. 1997). Birds were either categorized as infected or noninfected.

Experimental study: mate choice

Bird catching and housing

To examine if and how the preferences of female house finches for male plumage coloration varied among sites with differing male coloration, we brought female house finches from 3 sites into captivity and assessed their behavior in response to males with artificially manipulated plumage coloration. From 11–24 January 2011, we captured 15 female finches from the ASU Campus, 13–25 January 2011, we captured 17 female finches from the Estrella Mountain Park, and 26 January to 3 February 2011, we captured 15 females from the Chandler, AZ using basket and potter traps as described above. These female birds were housed in pairs in wire cages (0.6 × 0.4 × 0.3 m) in a greenhouse aviary room with natural photoperiod and fed ZuPreem small bird maintenance diet (Premium Nutritional Products Inc. Mission, KS, USA). The male finches used in the mate-choice experiments were captured from the same 3 sites as the female birds earlier in the previous year (1 September to 3 October 2010). These birds were part of a separate study of nonvisual cues and food preference and details of their capture, diet, and housing conditions can be found in Giraudeau et al. (2012).

Mate-choice trials

To assess mate-choice behavior, we presented the female finches 4 males with plumage coloration that varied across the natural range. We captured these stimulus males prior to the molt period and maintained them in captivity on a low-carotenoid diet through, which resulted in the development of uniformly dull yellow plumage that did not differ by location of origin (mean hue = $28.33 \pm 1.07^\circ$; $F_{2,39} = 0.04$, $P = 0.96$). We then colored this uniformly drab plumage with Prismacolor® art markers (Newell Rubbermaid Office Products, Oak Brook, IL, USA) using various combinations of carmine red PM-4, yellowed orange PM-15, and canary yellow PM-19 as described in previous studies of color preference in the house finch (McGraw and Hill 2000; Toomey and McGraw 2012). We

quantified the coloration of the manipulated males using photography as described above. To test female choice across both wide and narrow ranges of male color variation, we carried out 2 separate experiments. In Experiment 1, we varied male plumage color over wide range and offered females the choice among 4 males with mean \pm SE hue of $8.5 \pm 1.0^\circ$, $20.9 \pm 1.0^\circ$, $28.9 \pm 0.6^\circ$, or $36.5 \pm 1.0^\circ$. This plumage color variation is much greater than is typical among the 3 sites (95% interquartile range: 0.833 to 26.98°, see Figure 3) and we were concerned that the inclusion of relatively rare drab males might confound our ability to detect fine variation in female preference. Therefore, we carried out a second experiment (Experiment 2) with a much narrowed range of manipulated male coloration with a mean \pm SE hue of $5.6 \pm 0.8^\circ$, $10.9 \pm 0.5^\circ$, $14.5 \pm 0.6^\circ$, or $18.4 \pm 1.0^\circ$ for the 4 males presented. For each trial, we randomly selected among 5 or more different males within each of the 4 color categories that originated from the same location as the female being tested (desert, suburban, or urban).

To assess female mate choice, we presented the sets of 4 stimulus males to each female in a custom-built outdoor aviary and video recorded female and male behaviors for 1 h. Then 2 independent observers, blind to the female's site of origin, reviewed the videos, scored each trial and quantified the amount of time each female spent near (≤ 0.75 m distance) a given male. We then averaged these measures across observers for the statistical analyses. These trials replicated established methods from an early study and detailed descriptions of the mate-choice aviary and scoring procedures are available in Toomey and McGraw (2012). The choice trials were conducted from 0800 to 1300 h on 5–19 April 2011 (Experiment 1) and 25 April to 9 May 2011 (Experiment 2). In the wild, house finches begin pairing up in January–March. If unpaired, wild females will often seek to pair with a new mate (Oh and Badyaev 2006). Therefore, the timing of these mate-choice studies closely aligns with the natural seasonal phenology of house finches.

Statistical methods

Correlative study: carotenoid-based plumage coloration variation. Two linear mixed models were completed in the R statistical computing environment (R Core Team 2014) with α set to 0.05. The first model assessed whether PC1 and PC2 urbanization scores, season (molt: September–October, winter: November–February, breeding: March–August), pox presence (infected or not), and each of their 2-way interactions with both PCs influenced variation in body mass. Two-way interactions were included to test 1) for differences in the cost of being infected with pox on body mass along the gradient of urbanization and 2) for season specific effect of urbanization PCs on body mass. To account for interannual (this dataset include data from both 2010 and 2011) and intersite variation, we inserted both “year” and “site” as random factors. The second model tested for the effects of the same factors but on hue variation. Body mass was also included as an explanatory factor in this second model. Nonsignificant interactions ($P > 0.05$) were sequentially removed from the initial models (Zuur et al. 2009) to provide a final model containing all main fixed effects and significant interactions.

Experimental study: mate choice. To compare the plumage color preferences of female house finches among sites, we used a linear mixed-effects model (LMM) with total time each female spent near each male as the response variable, and site of origin, male plumage coloration, and the interaction of these terms as fixed effects. To account for the nonindependence of the 4 males

within each mate-choice trial, we included female identity as a random factor. The mixed-effects models were analyzed using the lme4 package (Bates et al. 2015) and *P* values for the fixed effects were estimated with Satterthwaite approximation implemented through the lmerTest package (Kuznetsova et al. 2017) in the R statistical computing environment (R Core Team 2014).

RESULTS

Urban-rural variation in ornamental hue

Body mass differed significantly between seasons (Table 1) and was highest during breeding, intermediate during winter, and lowest during molt. The variation in body mass was also explained by a significant interaction between PC2 (positively loaded for measures of soil compaction and vegetation cover) and season, which showed that only during molt was there a positive relationship between PC2 and body mass (molt: $\beta = 0.19 \pm 0.05$, $t = 3.53$, $P < 0.001$; winter: $\beta = -0.07 \pm 0.05$, $t = 1.31$, $P = 0.19$; breeding: $\beta = -0.02 \pm 0.04$, $t = 0.34$, $P = 0.73$) (Figure 1).

Ornamental hue was significantly related to several factors. First, we found that disturbance (PC1) was a strong predictor of hue, such that finches were redder in habitats with less disturbance by industrial sources. Additionally, this effect was dependent on season, as the relationship between hue and PC1 was more strongly positive during breeding than molt and winter (molt: $\beta = 0.57 \pm 0.19$, $t = 2.88$, $P = 0.004$; winter: $\beta = 1.35 \pm 0.29$, $t = 4.58$, $P < 0.001$; breeding: $\beta = 2.56$, ± 0.20 , $t = 12.48$, $P < 0.001$, Figure 2). The relationship between ornamental hue and PC2 varied significantly across seasons, however during no single season was there a significant relationship between these 2 variables (molt: $\beta = 0.44 \pm 0.35$, $t = 1.28$, $P = 0.20$; winter: $\beta = -0.19 \pm 0.47$, $t = 0.40$, $P = 0.90$; breeding: $\beta = 0.12 \pm 0.37$, $t = 0.30$, $P = 0.76$). Redder finches were

heavier overall, and finches also varied in hue over the year, as they were reddest during winter/breeding and yellowest during molt. Pox infection was not significantly associated with variation of body mass nor coloration (Table 1).

Mate-choice experiments

We examined the color preferences of female house finches from 3 study sites that differed significantly in mean plumage coloration of the male population. Males at the Chandler site were significantly more yellow than males at ASU Campus or Estrella Mountain ($F_{2,464} = 109.6$, $P < 0.0001$, Tukey's post hoc comparisons all $P < 0.0001$; mean \pm SE male hue ($^{\circ}$): Estrella Mountain— 7.89 ± 0.59 , Chandler— 21.14 ± 0.64 , ASU Campus— 12.14 ± 0.47). Consistent with this variation in male plumage coloration, female preferences, as measured by association time, differed significantly among the 3 sites when we included Experiments 1 and 2 in the same analysis (site \times male hue— $F_{2,69.8} = 3.47$, $P = 0.036$; Figure 3, Supplementary Table S1). Females from the ASU campus and Estrella Mountain sites tended to spend more time associating with the reddest males, whereas females from the Chandler site displayed the opposite preference and tended to spend the majority of their time associating with the yellowest males in the experiment (Figure 3). The differences in the range of male plumage color variation between experiments one and two did not have a significant impact on female preferences (experiment \times male hue— $F_{2,332.0} = 1.66$, $P = 0.20$, Supplementary Table S1). However, when we analyzed these experiments separately we no longer detected a significant difference in female preferences among the study sites (Experiment 1—large color variation: site \times male hue— $F_{2,40.3} = 2.00$, $P = 0.15$; Experiment 2—narrow color variation: site \times male hue— $F_{2,54.3} = 0.40$, $P = 0.67$, Supplementary Tables S2–S4).

Table 1
Model outputs for 1) predictors of mass, and 2) hue with the Chandler site included

Response	Predictor	R^2_m	R^2_c	Reduced model			Full model		
				df	χ^2	<i>P</i> value	df	χ^2	<i>P</i> value
Mass	Season	0.0303	0.025	2	13.85	<0.0001	2	21.99	<0.0001
	PC1	0.0062	0.0095	1	0.21	0.63	1	1.15	0.28
	PC2	0	0.0091	1	6E-04	0.98	1	0.036	0.95
	Pox	0	0	1	1.45	0.22	1	0.057	0.81
	PC1*Season	0.00076	0	—	—	—	2	0.7	0.7
	PC2*Season	0.0064	0.0033	2	8.09	0.017	2	6.53	0.038
	PC1*Pox	0.00032	0	—	—	—	1	0.28	0.59
	PC2*Pox	0.000038	0	—	—	—	1	0.093	0.76
	Season*Pox	0.00041	0.0023	—	—	—	2	0.66	0.72
	Hue	Season	0.022	0.017	2	25.69	<0.0001	2	25.9
PC1		0.12	0.011	1	6.14	0.013	1	2.13	0.14
PC2		0	0.0303	1	0.1	0.75	1	0.42	0.52
Pox		0	0.0034	1	1.85	0.17	1	1.78	0.18
Mass		0.0087	0.0079	1	12.45	0.0004	1	12.2	0.0004
PC1*Season		0.0202	0.023	2	42.26	<0.0001	2	38.5	<0.0001
PC2*Season		0.0022	0.0059	2	6.26	0.043	2	4.91	0.085
PC1*Pox		0	0	—	—	—	1	0.0001	0.99
PC2*Pox		0.0028	0	—	—	—	1	2.63	0.1
PC1*Mass		0.00017	0	—	—	—	1	0.13	0.72
PC2*Mass		0.00027	0	—	—	—	1	0.38	0.54

Marginal (R^2_m , i.e. variation explained by fixed factors only) and conditional (R^2_c , i.e. variation explained by both fixed and random factors) R^2 values are shown for each fixed factor; these values were calculated by subtracting the corresponding value from a higher-order model (for interactions, the full model; for main effects, a model with all main effects and no interactions) with a model in which that fixed factor was removed. *P* values for significant factors ($\alpha < 0.05$) are shown in bold for both the full model and reduced (final) models.

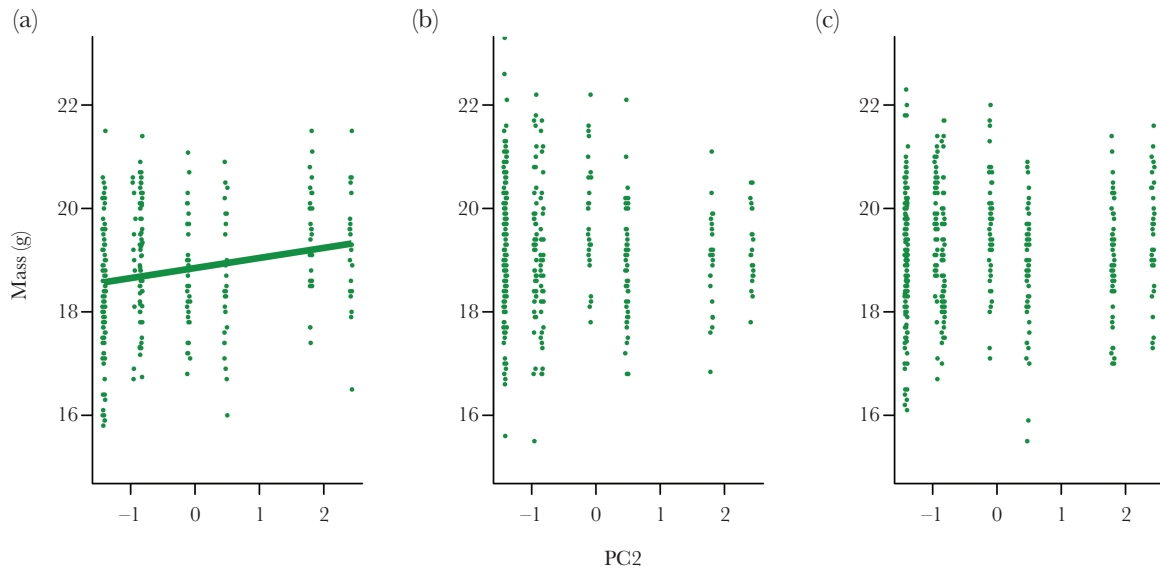


Figure 1

The relationship between mass and PC2 (compaction and vegetation) is dependent on season. During (a) molt there was a significant and positive relationship between mass and PC2, suggesting birds were in better condition during molt in less urbanized areas. However, during both (b) winter and (c) breeding, there was no relationship between mass and PC2. Points are slightly jittered along the x-axis to minimize overlap for purely graphical purposes.

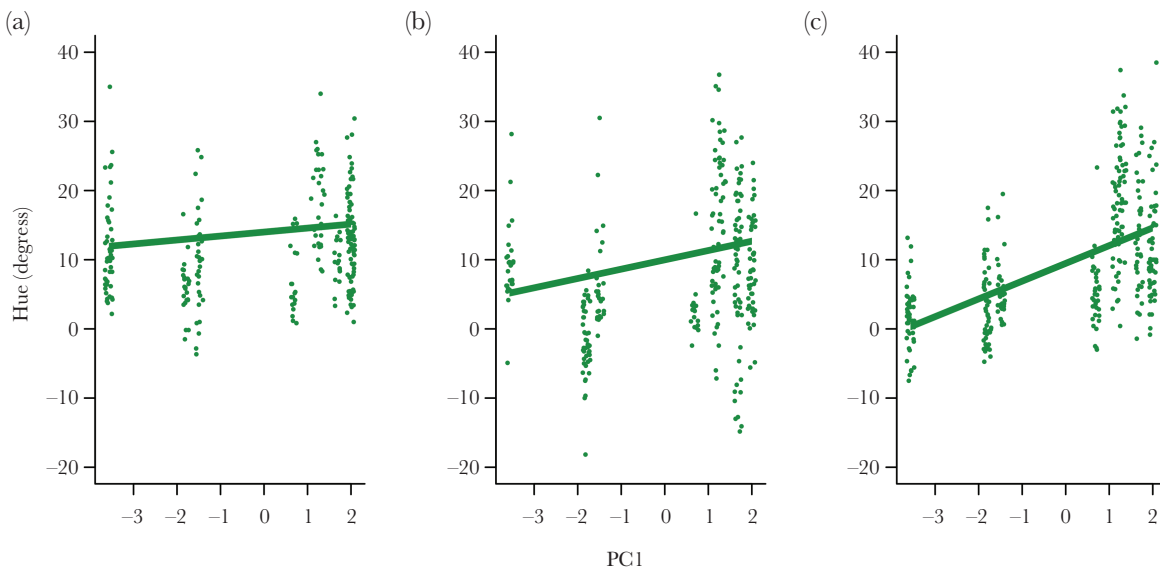


Figure 2

Relationship between hue and disturbance (PC1) is dependent on season. In all seasons there was a significant positive relationship between hue and PC1, indicating that more urbanized areas had yellower male finches. The strength of the positive relationship between hue and PC1 changed across seasons, with (a) molt and (b) winter having shallower positive slopes than (c) breeding. Lower hue values indicate relatively red plumage color, whereas higher hue values indicate relatively yellow plumage color. Points are slightly jittered along the x-axis to minimize overlap for purely graphical purposes.

DISCUSSION

This study provided 3 key results. First, we found a strong decrease of carotenoid-based coloration associated with an increase in levels of industrial and residential land use (PC1). In other words, birds were redder in rural areas and then became yellower toward the city centers. This relationship between hue and urbanization was most pronounced during the breeding season. Second, we found that variation in house finch body mass across a gradient of urbanization was positively related to our PC2 urbanization scores, specifically during the

molt period. Finally, we found that female color preferences tended to differ among sites suggesting that preferences may track the local variation in male coloration across the gradient of urbanization.

A decreased cost of molt in suburban birds

Numerous studies have been published over the last decade assessing variations in body mass and body condition in birds along the rural-to-urban gradient but, so far, no clear pattern has emerged (Ruiz et al. 2002; Auman et al. 2008; Liker et al. 2008; Evans et al.

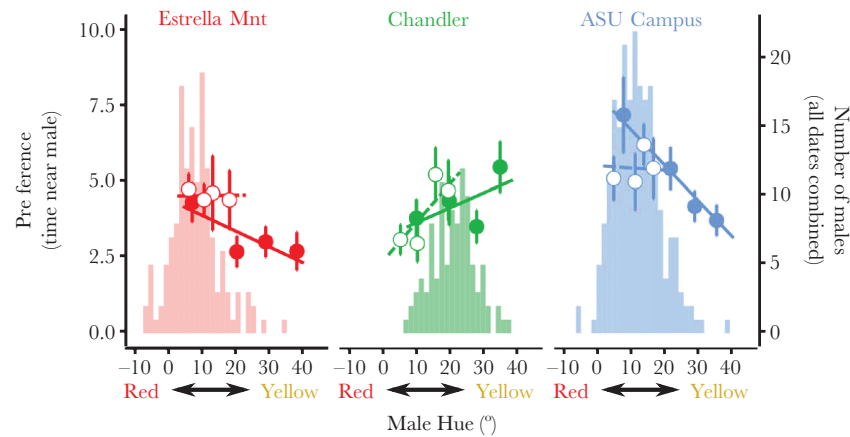


Figure 3

The color preferences of female house finches varied among study sites. The points depict the color preferences of female house finches from the Estrella Mountain, Chandler and ASU Campus sites, as measured by mean \pm s.e. association time with males of each color on four-way choice trials (y-axis to the left). The results of experiment one that included males with a wide range of coloration are shown in the solid points and lines. The results of experiment two that involved a narrower range of male color variation are given in the open points and dashed lines. Overlaid on the preference results are histograms of showing the abundance of males (y-axis to the right) of the varying coloration (mean plumage hue ($^{\circ}$)) observed at each of the sites across the entire sampling period.

2009; Fokidis et al. 2011; Seress et al. 2011; Davies et al. 2013; Giraudeau and McGraw 2014; Giraudeau et al. 2015; Meillère et al. 2015; Audet et al. 2016). Here, we did not find any effects of our urbanization PC scores on body mass during the breeding and winter periods but we found that the decrease of body mass during molt was less pronounced in suburban birds (highest PC2 scores) compared with rural and urban birds (lowest PC2 scores). Our study is, to the best of our knowledge, the first one to measure seasonal effects on this trait in an urbanizing context; showing that the positive effect of suburban conditions on body mass might only be detectable during the stressful period when birds must regrow their plumage. Potentially, the increased availability of food resources provided by the extensive network of bird feeders and other resources in suburban areas of desert cities in Arizona (natural and cultivated vegetation, Giraudeau et al. 2014) might buffer seasonal variation in body mass.

Decrease of carotenoid-based coloration along the gradient of urbanization

We found that the expression of carotenoid-based plumage in the house finch decreases over a gradient of urbanization for the 3 seasons considered. The fact that this pattern was already detected immediately after molting suggests that urbanization affects carotenoid pigment deposition. Our result also adds to a growing body of evidence that carotenoid-based plumage coloration varies between individuals living in cities and those living in natural surroundings (Hörak et al. 2000, 2001; Isaksson et al. 2005; Giraudeau et al. 2015) and with the general trend showing that animals have reduced signal quality in urban settings (Hutton and McGraw 2016). We previously found no urban–rural differences in the levels of circulating dietary carotenoids during the molt period, suggesting that the availability of these pigments cannot explain the decrease of coloration observed here. However, in the same study, the degree of urbanization was negatively associated with circulating levels of 3-hydroxy-echinone (3HE, Giraudeau and McGraw 2014), the most abundant red pigment in the plumage of male house finches (Inouye et al. 2001). This red ketocarotenoid is produced through an oxidation

reaction of other dietary yellow forms of carotenoids (Inouye et al. 2001) likely catalyzed by the action of the recently discovered CYP2J19 ketolase (a cytochrome P450 enzyme, Lopes et al. 2016). This suggests that urban stressors are directly affecting this enzyme-driven metabolic conversion of dietary carotenoids into 3HE. Future studies now need to test this hypothesis by 1) quantifying potential difference in CYP2J19 expression between urban and rural birds during the molt season and 2) assessing experimentally the effects of urban stressors on CYP2J19 expression and function.

We also found that the relation between PC1 scores and plumage coloration was strongly influenced by the season at which the birds were trapped, with a stronger effect of urban conditions on plumage coloration during breeding compared with any other seasons. Plumage color is known to fade over the breeding season in several species including the house finch (Ornborg et al. 2002; Senar 2004; McGraw and Hill 2004) and our result showing a significant decrease of coloration from the winter months to the breeding stage confirms that plumage coloration is a dynamic trait. Given the increased effect of urban conditions on plumage coloration at the breeding stage, it suggests that urban birds fade more rapidly than rural birds. This difference in the color-fading rate might be explained by the abundance of feather-degrading bacteria that is, on average, 3 times higher on the plumage of urban house finches compared with rural individuals (Giraudeau et al. 2016). Feather-degrading bacteria have the capacity to alter plumage integrity through their ability to degrade β -keratin, a protein which composes more than 90% of feather mass (Gunderson 2008). Most of the plumage colors, including the carotenoid-based coloration, result from the combination of a keratin-based background structure that reflects light and pigments that selectively absorb light (Jacot et al. 2010). Several *in vitro* studies have now shown that, through their deterioration of the feather keratin structure, these bacteria can alter plumage coloration and be an essential actor of the plumage color-fading process (Onifade et al. 1998; Shawkey et al. 2007, 2009; Gunderson 2008). Thus, a higher rate of feather degradation by bacteria in urban environments compared with rural ones might influence the rate of plumage coloration

deterioration in urban birds and the inter and intrasexual interactions in the urban environment.

Mate-choice preferences

Female preferences for male coloration varied in a pattern that tracked variation in male coloration among the study sites. Females from the Chandler site where males had drab yellow plumage tended to prefer yellow males, which contrasts with females from Estrella Mountain and ASU campus that had a more typical preference for intense red coloration (Hill 2002; Toomey and McGraw 2012). This result is intriguing, however it should be interpreted with caution. Although we observed a significant effect of the interaction between site and male coloration on female preference when we considered both experiments in a single analysis, this effect was not significant when we analyzed the experiments separately. The sample size and power of the individual experiments are insufficient to detect this effect suggesting that the strength of the site effect may be limited. Additionally, our sample was limited to just 3 sites and it is possible that these patterns of choice are idiosyncratic to these sites rather than a general feature of house finch biology. Moving forward it will be important to replicate these studies to include more individuals from a wider range of sites.

The variation we have observed in female preference contrasts with previous studies that have shown consistent preferences for elaborate red plumage in female house finches from geographically distant populations that differed significantly in male plumage coloration (Hill 1994). However, our study differed methodologically in several ways and may have revealed different aspects of female choice. Hill (1994) used individuals from a single population (Michigan) as stimulus males to access the preference of females from 4 different locations, whereas we matched the source locations of our test females and stimulus males. It is possible that females rely primarily on color when choosing among males that are unfamiliar but that this preference can be modulated by other locality specific cues like song that were present in our experiments (Giraudeau et al. 2014). It is notable that in organisms, like house finches, with multiple modes of sexual signaling (i.e. song and plumage color), the signals may be evaluated sequentially (Candolin 2003). Females may locate males via their songs over long distances, then closely inspect plumage color as the primary condition-dependent trait. Here, females were always near the available males, which may emphasize choice based on close-distance signaling.

Elaborate red plumage coloration in male house finches is associated with individual diet and health (Hill 2002); however, these are not the only aspects of male quality that may be important for choosing females. In challenging environments, the contributions of males to offspring care may substantially influence a female's reproductive success. In some house finch populations (e.g. Montana, USA), nestling provisioning by males is negatively associated with plumage redness and older experienced females tend to pair with drabber males (Badyaev and Hill 2002; Duckworth et al. 2003). It is possible that local patterns of female preference we have observed could be driven by differences in female survival, age, and breeding experience. However, the age and breeding history of the females we tested in this study were not known. Clearly, to parse the causes and consequences of this preference variation across the urban–rural gradient will require studies of a broader range of sites with careful tracking of the ontogeny, plasticity, and heritability of preference.

To conclude, we have shown that carotenoid-based coloration decreased along the gradient of urbanization in house finches, suggesting that the enzyme-driven metabolic conversion of dietary

carotenoids into red carotenoids used to color plumage is sensitive to urban stressors. The difference in male coloration was stronger during the breeding than during molting and winter suggesting differences in the rate of color fading probably associated with feather-degrading bacterial load (Giraudeau et al. 2016). Finally, we have shown for the first time that urbanization might influence female mate-choice behavior, suggesting that female color preferences may track the variation in male coloration across the gradient of urbanization.

SUPPLEMENTARY MATERIAL

Supplementary data are available at *Behavioral Ecology* online.

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