

Differential Effects of Urbanization on Plumage and Morphometric Traits in Two Congeneric  
Desert Cardinal Species

**Abstract**

Urbanization has greatly affected wildlife and will continue to do so into the future, but we know little about whether traits of closely related species respond similarly to this environmental change. To address this gap, we tested the association between urbanization and morphological traits of two congeneric songbird species – northern cardinals (*Cardinalis cardinalis*) and pyrrhuloxia (*C. sinuatus*) – that largely share a niche in and around the Sonoran Desert city of Tucson, Arizona over 137 years. We measured museum and field-collected specimens and used novel hindcast models of urban areas to score urbanization over time. We found that urbanization reduced expression of carotenoid-based plumage coloration in males, but for different color traits in each species. Urbanization also reduced melanin coloration in breast plumage in female pyrrhuloxia. Both urbanization and time of sampling were associated with shifts in feather and skeletal traits that could allow for improved flight maneuverability, and time was associated with wider bills in males of both species, which may affect foraging, song, and heat tolerance. We demonstrate that the effects of urbanization can differ even in two closely related species, with complex effects on traits involved in visual signaling, heat tolerance, flight performance, and foraging.

## **Introduction**

Human activities have altered ecosystems globally, with only 23% of the terrestrial landscape (Watson et al. 2016) and 13% of the ocean still classified as wilderness (Jones et al. 2018), and with most wilderness areas under threat of global human impacts such as climate change, pollution, and habitat loss (Asamoah 2022). The impacts of urbanization on avian community structures have been extensively studied (Green 2003, Hostetler and Knowles-Yanex 2003, Litteral and Wu 2012, Hensley et al. 2019, Brown et al. 2022), but the effects of this ecological disruption on the phenotypic traits of bird species over time are less understood. Broad comparative studies at the community level can reveal overall trends in species abundance and distribution, such as the finding that bird species that persist in the city have a broader range of environmental tolerance than their rural congeners (Bonier et al. 2007, Palacio 2020, Tryjanowski et al. 2020). However, the fine-scale, fitness-relevant mechanisms underlying these differences have only occasionally been elucidated through studies of traits from different populations or between closely related species can reveal (e.g. Kern and Langerhans 2018, Winchell et al. 2018). This gap in our understanding highlights the need for evolutionary research on a diversity of morphological traits in response to urbanization.

Urban landscapes reshape avian selective pressures and environmental conditions in a number of ways (Badyaev et al. 2008, Brown and Brown 2013, Hutton and McGraw 2016, Giraudeau et al. 2014a,b, McNew et al. 2017), and urbanization often removes correlations between condition-dependent traits and fitness in birds (reviewed in Sepp et al. 2020). Urban stressors can be chemical pollution, noise, artificial light at night, human presence, novel patterns of resource distributions (reviewed in Isaksson 2018), and novel pathogens/parasites

(Giraudeau et al. 2014a, Jiménez-Peñuela et al. 2019). Phenotypic differences across urban gradients can indicate which of these stressors have affected species, and therefore indicate which stressors might limit or shape urban patterns of biodiversity (Isaksson 2018). For instance, urban decreases in carotenoid-based plumage color can result from chemical pollution (Isaksson et al. 2005) or an impaired diet (Isaksson and Andersson 2007), whereas urban increases in coloration can result from invasive food-plant species that are rich in carotenoids (Jones et al. 2010, Baldassarre et al. 2022). Also, bill shape can become longer and narrower due to reliance on anthropogenic food sources in bird feeders (Giraudeau et al. 2014b) or shorter and wider due to increased foraging competition and species interactions (Badyaev et al. 2008). Traits associated with maneuverability can be selected for in urban settings as well, due to increased need for quick, deft movements (i.e. longer tails and shorter wings) to navigate in a more complex and challenging vertical city infrastructure (e.g. buildings, moving automobiles; Brown and Brown 2013). Although studies of phenotypic change across an urban area can illuminate the particular stressors that affect free-ranging organisms, we do not know if the traits of closely related species, such as sympatric congeners, respond similarly to the same stressors in the same environment. Slight differences in congeneric species' responses to the same stressors could reveal intricate mechanisms of adaptation, acclimation, or extinction associated with urbanization.

Population- and individual-level urban bird studies have largely focused on few species (e.g. house finches *Haemorrhous mexicanus*, great tits *Parus major*; Heinen-Kay et al. 2021), and sample birds over relatively short time frames, often less than 30 years (Fidino and Magel 2017). This limits our understanding of the consistency of urban-phenotypic trends across

species, and of the responses of species over long periods of urbanization. To develop a more comprehensive framework for the impacts of human activities on wildlife, new research methods that integrate species comparisons with sampling schemes across many decades are needed. Museum collections offer the ability to track phenotypes over time, as plumage and morphometric traits remain measurable across decades with proper methods (Burns et al. 2017). Despite this, preserved specimens have only rarely been used to analyze urban impacts on species over time, with only 12% of urban evolution studies accessing museum specimens (Shultz et al. 2021), and of those only one analyzed temporal changes in phenotype over time in both urban and rural populations of ten mammal species, rather than just in urban areas, and no such studies focused on bird species (Snell-Rood and Wick 2013).

Northern cardinals have recently emerged as a valuable study system for understanding effects of urbanization on phenotype in birds. Studies of the nominate subspecies (*Cardinalis cardinalis cardinalis*), which is distributed throughout the eastern United States, have shown effects of urbanization on plumage coloration and body condition (Baldassarre et al. 2022) as well as song (Narango and Rodewald 2016). Urbanization is also associated with larger bills in northern cardinals broadly across their range, with heat tolerance as a proposed explanatory factor (Miller et al. 2018). The northern cardinal is widespread throughout North America, with four genetic populations on the mainland of the continent that span urbanized areas (Smith et al. 2011). The second most predominant subspecies in the United States, *Cardinalis cardinalis igneus*, shares much of its range with its congener, pyrrhuloxia (*Cardinalis sinuatus*; Smith et al. 2011); both are found throughout the Sonoran Desert and in the greater metropolitan area of Tucson, Arizona.

The two species are easily distinguished by coloration (Figure 1), with male northern cardinals predominantly red with a black face mask and orange bill, male pyrrhuloxia predominantly gray with a red breast, face mask, crest, shoulder, and tail and orange bill, and females of both species similar to the coloration of the male pyrrhuloxia but with a buffy breast, black mask, and red-orange bill (female northern cardinal), or a gray breast and black mask (female pyrrhuloxia). The plumage of northern cardinals has been shown to have inter- and intra-sexual functions, although with variation among populations (Wolfenbarger 1999, Jawor et al. 2003, Jawor et al. 2004, Jawor and Brietwisch 2004, Jawor and Brietwisch 2006, Rodewald et al. 2011, Winters and Jawor 2017). Pyrrhuloxia also have a “parrotlike” curved bill, while northern cardinals have a more conical bill. Both species come to feeders in Tucson and generally occupy a similar niche, with no observed differences aside from a slight preference for proximity to water in northern cardinals (Gould 1961), and little is known about their diet in the Sonoran Desert population (although see McAtee 1908). Northern cardinals also occur more in the urban center of Tucson than Pyrrhuloxia ([Citation edited for double-blind review], unpublished manuscript). These slight differences in phenotype, but general similarities in ecology/distribution, allow for a unique comparative study on urban trait expression.

Phenotypic differences between these species in plumage, bill morphology, wing length, and tail length in relation to urbanization could reveal which environmental stressors limit pyrrhuloxia’s urban distribution more than that of northern cardinals and could highlight fine-scale differences in the mechanisms of urban adaptation between even closely related species.

We sought to determine if and how urbanization may similarly or differently affect the plumage and morphometric traits of male and female northern cardinals and pyrrhuloxia. We

present findings from morphological traits of northern cardinals and pyrrhuloxia measured across 137 years from urban, urban-outskirt, and rural areas across the state of Arizona. We model differences across urban areas to identify traits that have been impacted by human activities, as defined by proximity to urban areas. We expected to find that, if the effects of urbanization are consistent on similar species despite slight differences in ecological niche, the same traits would be impacted by urbanization in similar ways in the two species. However, if urbanization differentially impacts closely related species, either the same traits would be affected but in different ways, or different traits altogether would be impacted by urbanization.

## **Methods**

### **Data collection and processing**

For the field portion of this study, we captured and sampled 13 northern cardinals and 12 pyrrhuloxia at Tucson residences from March-May of 2021 and 2022 using a mist net at feeders. We also took measurements from birds of both species from the University of Arizona's Bird Collection (UAZ) and from the University of Washington's Burke Museum of Natural History and Culture (UWBM; Table S1), which together hold the majority of museum specimens collected in the state of Arizona and recorded in VertNet (Constable et al. 2010). We limited these to adult specimens collected in Arizona that were associated with reliable location data. We sampled 24 northern cardinal and 39 pyrrhuloxia skins from UAZ, and 11 northern cardinals and 5 pyrrhuloxia from UWBM, for a total of 48 northern cardinals (39 males and 9 females) and 56 pyrrhuloxia (34 males and 22 females) across both field and museum collections (Figure S1). One male northern cardinal had outlier values for bill width and length

and was the very first field specimen measured (NOCA 001), so we excluded it from all morphology analyses but included it in coloration analyses.

Integumentary coloration was quantified using standard digital-photography methods that have been validated in other carotenoid-colored passerine species (Giraudeau et al. 2012, Lendvai et al. 2013, McGraw et al. 2002). Our photographic methods did not capture variation in the ultraviolet (UV) portion of the spectrum, but UV and yellow-red reflectances are correlated in carotenoid-based plumages (Quesada and Senar 2006) and previous cardinal color studies have excluded UV quantification (e.g. Jawor and Breitwisch 2004). We took photographs with a Canon Rebel T3i and a Kodak color standard of two regions of each bird: their breast and their face in profile (Figure 1). We also photographed underwing plumage of the field specimens because this trait has been shown to vary with urbanization in female northern cardinals from New York (Baldassarre et al. 2022), but we could not evaluate underwing coloration of museum specimens due to the method of preservation (i.e. could not extend rigid, tucked wings without risking breaking them). All photographs are available in our Dryad repository ([reviewer sharing link](#)).

Field photographs were taken in the shade under diffuse sunlight conditions to minimize shadow and included an 8" Tiffen Color Separation Guide with Grey Scale. Museum photographs were taken in front of a window with color standards to best replicate the natural sunlight conditions of the field samples within the constraints in place by the museums to preserve the specimen. From all field and museum specimens, we also measured length of the crest, wing, and tail to the nearest 1 mm with a wing rule; specifically, we measured the longest erect crest feather and the middle of the tail. We also measured tarsus length as well as bill

length and width at the nares to the nearest 0.1 mm with analog calipers. All measurements of museum specimens were made prior to urban categorization of the specimens to prevent measurement bias, and a subset of museum specimens were measured by another researcher (Jason Lacson). Intraclass correlation coefficient (ICC) values were all significant ( $p$ -value  $< 0.05$ ), from smallest to largest, 0.651 for wing length, 0.671 for tarsus length, 0.690 for bill length, 0.782 for tail length, 0.793 for crest length, and 0.866 for bill width, with an overall mean value of 0.742.

We used Adobe Photoshop (v. 24.0.1) to score plumage coloration from photographs. We analyzed the crest and face mask of each bird from photographs of the bird's head in profile, and the breast from a separate photo of that region of the bird. We obtained hue, saturation, and brightness values from each patch and from the red square of the Tiffen Color Separation Guide following methods from Giraudeau et al. (2012), except for the mask which is a gray-black shade and thus lacks a spectral peak from which to quantify hue. To standardize our measurements across light environments, we subtracted the value obtained from the Tiffen Color Separation Guide red standard from the value of the trait of interest. All methods were repeated on a second photo of each bird; intraclass correlation coefficients (ICC) were calculated to assess repeatability, and then values were averaged between the two photographs. ICC values were all highly positive and significant, ranging from 0.79 to 1.00 and with all  $p$ -values  $< 0.05$  (mean = 0.91; Table S6).

Measuring degree of habitat urbanization



Dates for estimating degree of habitat urbanization for the capture locations of the cardinals ranged from 1885-2022, and the urban areas across Arizona changed drastically in size over those 137 years. To our knowledge, no studies have used long-term datasets to model urbanization of wild birds across more than a century of samples, so we developed a novel protocol to measure urbanization here. We used a long-term dataset of urban extent, which contains raster files of hindcast-modeled urban extents by decade (1880-1990), models from satellite nighttime light by year (1996-2009), and projected future urban sprawl (2020) (Li et al. 2021). The model for 2020 projected urban areas based on past data, rather than on data from 2020, and it modeled urban extents under five different human-development scenarios. Despite this, no difference was observed for our sampling locations between the different models. For each raster of urban extent, we generated a new raster file consisting of plots of the same resolution but where each cell represents the Euclidean distance (in m) of that cell to the nearest urban area in the original raster file using R Statistical Software (v4.2.2; R Core Team 2021). We then extracted this number from the cell at the same location as each bird-capture location within the raster file of the most recent year that predated the year of capture to generate a “Distance from Urban Area” score for each individual. If latitude and longitude data were not listed for certain museum specimens but there was a reliable description of capture location, i.e. “2.6 mi. E. of Arivaca, Pima Co., Arizona,” then estimates of latitude and longitude were obtained using Google Maps. This attributed an urbanization score ranging from 0 meters (most urban) to 191,634 meters (most rural) to each museum specimen and field-caught bird. Though this effectively captured variation in urbanization at the low end of the range (specimens at 0 meters were similarly all highly impacted by urbanization), cardinals

caught at sites without or far from any human development had wide-ranging scores. To account for this, we binned the urbanization scores into three categories: Urban (Distance from Urban Area = 0 meters), Urban Outskirts (0 meters < Distance from Urban Area < 12,000 meters), and Rural (12,000 meters < Distance from Urban Area). We chose 12,000 as the cutoff because this was a natural break in our dataset (Figure S2), with no specimen having a distance to an urban area between 11,655 and 32,597 meters, and because all samples in this range appear to fit within the assigned categories when plotted on a map (Figure S3). Analyses of females of both species lacked the sample size to retain these three urbanization categories, so for just the females of each species we lumped “Urban” with “Urban Outskirts” to create two categories: “Urban and Urban Outskirts” and “Rural.” Human activity has increased over time in all areas, not just in urban areas, and museum-specimen coloration might decay over time (Armenta et al. 2008). To account for this, we included the year of specimen collection in the model counting backwards with 2022 as year 0. For color traits, it is not possible to disentangle the effects of specimen decay from the effects of change over time on a population, but for morphological characteristics that are based on trait size (i.e. bill length), which should not decay, we inferred that the effects of year on that trait are a result of population change rather than specimen decay.

## Data analyses

Dataframes were manipulated prior to analyses in Python (vanRossum 1995) using the packages pandas and numpy, and spatial files were processed in R with the packages raster (Hijmans 2015), rgdal (Bivand et al. 2015), and terra (Hijmans et al. 2022). All analyses were run

in R with the packages afex (Singmann et al. 2015), car (Fox et al. 2012), effects (Fox and Weisberg 2018, 2019), emmeans (Lenth et al. 2018), FNN (Beygelzimer et al. 2015), Hmisc (Harrell and Harrell 2019), HSAUR (Everitt et al. 2017), interactions (Long 2019), jtools (Long and Long 2017), lme4 (Bates et al. 2015), lmerTest (Kuznetsova et al. 2017), MASS (Venables and Ripley 2002), and tidyr (Wickham and Wickham 2017). Figures were plotted in R with ggbiplot (Vu, n.d.) and ggplot2 (Wickham et al. 2016).

We tested each morphological variable for normality using a Shapiro-Wilk test, and many were significantly not normal, so we assessed non-parametric correlations between all morphological variables by calculating the Spearman correlation coefficient for each pair of variables and the associated p-value. A high number of variables were significantly correlated with each other (Tables S2-S5), but no obvious trends emerged in comparisons between sexes and species. Despite these intercorrelations, we chose to model each trait rather than, for example, the principal components of the traits, as an understanding of the effect of urbanization on each trait is biologically meaningful and statistically appropriate since we modeled each trait separately.

For all color analyses, we ran linear models with the color trait as the dependent variable and with urban category, year, and the interaction between year and urban category as fixed effects using the lm function from the R package “stats.” We assessed multicollinearity using variance inflation factors (VIF) on each sex of each species using 3 as a cutoff, and as a result dropped both year and the interaction between urban category and year from analyses of female northern cardinals. No predictors exhibited collinearity for color analyses of northern cardinal males, pyrrhuloxia males, or pyrrhuloxia females. We assessed significance with an

ANOVA on the fixed effects of the model using parametric bootstrapping with the Satterthwaite approximation for degrees of freedom applied to a restricted maximum likelihood (REML) fitted model (Luke 2017). We tested for differences in means following the ANOVA with Tukey post-hoc tests.

For all morphological analyses, we ran linear models with the morphological trait as the independent variable and with urban category, tarsus length, year, and the interaction between year and urban category as fixed effects using the `lm` function from the R package “stats.” Tarsus was included as a fixed effect to account for overall body size in each model. Significance was assessed using t-statistics output from the `lm` model. We again assessed multicollinearity using VIFs, and as a result dropped both year and the interaction between urban category and year from analyses of northern cardinal females. No predictors exhibited collinearity for color analyses of northern cardinal males, pyrrhuloxia males, or pyrrhuloxia females.

## **Results**

### **Color traits**

#### **Northern Cardinal Males**

Birds from different urbanization categories differed significantly in breast brightness ( $F_2 = 4.056$ ,  $p = 0.027$ ; Figure 2, Table S7). Urban birds had darker carotenoid-based breast plumage than rural birds ( $df = 31$ ,  $p = 0.024$ ,  $CI = [11.59, 0.721]$ ).

Year had a significant effect on crest brightness ( $F_1 = 6.382$ ,  $p = 0.017$ ) and face-mask brightness ( $F_1 = 13.391$ ,  $p = 0.001$ ), with older specimens having darker crests and brighter face masks (Table S7).

The interaction between year and urbanization category had a significant effect on face-mask saturation ( $F_2 = 8.040$ ,  $p = 0.002$ ), with more recent rural birds exhibiting higher saturation than older rural birds, but more recent urban birds exhibiting a lower saturation than older urban birds, and no change over time for the urban-outskirt birds (Figure S4, Table S7).

#### Northern Cardinal Females

We found no significant effect of urbanization category on the evaluated color traits for northern cardinal females (Table S8).

#### Pyrrhuloxia Males

Urbanization category had a significant effect on crest brightness ( $F_2 = 5.220$ ,  $p = 0.013$ ; Figure 2, Table S9). Crests of urban male pyrrhuloxia were darker than those of rural males ( $df = 24$ ,  $p = 0.022$ ,  $CI = [-14.461, -1.03]$ ). Year had a significant effect on breast hue ( $F_1 = 4.498$ ,  $p = 0.043$ ) and breast brightness ( $F_1 = 6.364$ ,  $p = 0.018$ ), with older specimens having less red and brighter breasts.

#### Pyrrhuloxia Females

Urbanization category had a significant effect on breast saturation of female pyrrhuloxia ( $F_1 = 4.448$ ,  $p = 0.050$ ), with rural birds being more saturated than urban and urban-outskirt birds ( $df = 17$ ,  $p = 0.012$ ,  $CI = [2.28, 16.2]$ ) (Figure 2, Table S10). Year had a significant effect on face brightness ( $F_1 = 4.802$ ,  $p = 0.043$ ), breast hue ( $F_1 = 15.643$ ,  $p = 0.001$ ), and breast brightness

( $F_1 = 10.674$ ,  $p = 0.005$ ), with older specimens having brighter faces as well as brighter and less red breasts.

#### Size traits

For male northern cardinals, we found a significant effect of urbanization on tail length ( $F_2 = 13.440$ ,  $p = <0.001$ ; Figure 3, Table S11). Urban birds had a longer tail than both urban-outskirt and rural birds ( $p < 0.001$  and  $p = 0.016$  respectively). Year had a significant effect on bill length ( $F_1 = 5.71$ ,  $p = 0.023$ ), bill width ( $F_1 = 9.81$ ,  $p = 0.004$ ), and tail length ( $F_1 = 21.974$ ,  $p = <0.001$ ); over time, bills have gotten longer ( $\beta = -0.014$ ) and wider ( $\beta = -0.009$ ), and tails have gotten longer ( $\beta = -0.139$ ).

As with plumage, we found no effect of urbanization category on morphological traits of female northern cardinals (Table S12).

For male pyrrhuloxia, urbanization category had a significant effect on bill width ( $F_2 = 3.376$ ,  $p = 0.049$ ), with urban males having a wider bill than urban-outskirt males ( $p = 0.036$ , Figure 3, Table S13). Year had a significant effect on bill width ( $F_1 = 8.949$ ,  $p = 0.006$ ), and tail length ( $F_1 = 9.238$ ,  $p = 0.005$ ); over time, bills have gotten wider ( $\beta = -0.011$ ) and tails have gotten longer ( $\beta = -0.068$ ).

For female pyrrhuloxia, there were no effects of urbanization on morphological traits (Table S14), but year had a significant effect on wing length ( $F_1 = 5.481$ ,  $p = 0.032$ ), with older specimens having longer wings ( $\beta = 0.024$ ; Figure 3, Table S14).

#### Discussion

We observed that, for plumage and size traits, urbanization was not always associated with changes in the same traits between the species, but the effect of urbanization was in a consistent direction for all similar traits. Urbanization was consistently associated with reduced expression of carotenoid-based plumage coloration in males, although in different patches for each species. For feather traits relevant to flight, both urbanization and time predicted changes in traits associated with increased maneuverability. We also observed that bills have become larger over time in males of both species.

The finding that male northern cardinal breast plumage was darker in the city of Tucson than in surrounding areas aligns with findings from Toledo, Ohio (Jones et al. 2010), but not with studies from Tampa, Florida (Leigh 2012) or Syracuse, New York (Baldassarre et al. 2022), neither of which found an association between breast brightness and urbanization. However, Baldassarre et al. (2022) found an inconsistent association between urbanization and breast hue across years. We also found that carotenoid-pigmented crests in male pyrrhuloxia were darker in the city, indicating that urban environments have similar effects on different carotenoid-pigmented plumage regions in males of the two species. Breast coloration of both sexes of northern cardinals is used in mate choice (Jawor et al. 2003), and nothing is known about the signals involved in mate choice for pyrrhuloxia, although given its conspicuousness and erection in social contexts, it would be surprising if the red crest of pyrrhuloxia served no social function (Tweit and Thompson 2020).

We also found a negative association between urbanization and saturation of melanin-based buffy-gray breast plumage in female pyrrhuloxia. Nothing is known about the role of plumage color variation in pyrrhuloxia, and it has been suggested that melanin patches across

species play more of a role in intrasexual than in intersexual communication (Badyaev and Hill 2000, Jawor et al. 2004); however, evidence from horned larks (*Eremophila alpestris*) shows a role for melanin patches in mate choice (de Zwaan et al. 2019). Other avian melanin-containing color patches are affected by urbanization, such as the black ties of male great tits (*Parus major*; Yeh 2004, Senar et al. 2014). Melanin-rich plumages can contain heavy metals (Isaksson et al. 2018), and heavy metal pollution is more prevalent in urban areas (Chatelain et al. 2021) and in association with mining (Rösner 1998), which could explain why female pyrrhuloxia breast plumage is more saturated in urban areas.

Year of collection was also associated with many color traits in both cardinal species, and much of this may be attributed to specimen degradation (Doucet and Hill 2009). In instances where temporal change was observed, older birds were less red and less saturated, but with some brighter features (male northern cardinal faces, male pyrrhuloxia breasts, female pyrrhuloxia faces and breasts) and some less bright features (male northern cardinal crests). It is likely that such changes are due to shifts in feather carotenoid content since brightness values are negatively associated with carotenoid content of mallard (*Anas platyrhynchos*) beaks (but are not associated with carotenoid content in house finch feathers; Butler et al. 2011). Urban birds also exhibited darker plumages in some carotenoid patches, so we demonstrate with both sets of results a general pattern of darker plumages over time and in urban areas for some patches in males of both species. Feathers were not washed prior to photographing due to preservation limitations, so these patterns could be driven by dirt accumulation in the city. Dirt would likely affect the entire bird and we did only see an effect on some patches, so this is an unlikely but still possible explanation.



348           The congruous effects of time on bill morphology in male northern cardinals and male  
349 pyrrhuloxia suggest that these species experience the same pressures on this trait. Urban male  
350 pyrrhuloxia also had wider bills. These trends could result from selection related to either  
351 foraging, as birds with larger beaks are faster at husking larger seeds (Nagy Koves Hrabar and  
352 Perrin 2002), or heat tolerance, as larger bills allow for greater heat dissipation without a  
353 corresponding increase in evaporative water loss (Greenberg et al. 2012, Danner et al. 2017,  
354 Tattersall et al. 2017). Other studies have found that male northern cardinals across their range  
355 have a larger bill size in association with urbanization, with heat tolerance proposed as a  
356 mechanism underlying an association between larger bill size (Miller et al. 2018). The Sonoran  
357 Desert has become hotter and drier over the last century (Weiss and Overpeck 2005, Zhao et al.  
358 2021), and urban areas are even hotter than the surrounding undeveloped areas (i.e. ‘heat  
359 island’ effect; Brazel et al. 2007), so heat tolerance is a likely explanation. Interestingly, studies  
360 of house finches in similar desert urban environments of Arizona found contrasting patterns, as  
361 urban finches have longer but narrower bills compared to rural birds (Badyaev et al. 2008,  
362 Giraudeau et al. 2014b), with the selective effects of consuming longer seeds at bird feeders  
363 listed as the probable factor. Both cardinal species frequent bird feeders around Tucson, often  
364 visiting the same feeders as house finches (pers. obs.). Other foraging factors could be affecting  
365 this trait, as both cardinal species consume many native seeds and, unlike house finches,  
366 commonly feed insects to their young (Halkin et al. 2021, Tweit and Thompson 2020), but we  
367 lack data on the foraging habits of these species in the Sonoran Desert, especially with respect  
368 to urbanization. It is also worth noting that an insectivorous bird species in New Zealand (New  
369 Zealand fantail, *Rhipidura fuliginosa*) had shorter and wider bills in association with long-term

370 urbanization, although the mechanisms underlying this pattern are not known (Amiot et al.  
371 2022). The effects of urbanization on insect prey species could also influence bill size, as  
372 urbanization favors small and medium beetles over larger beetles, although trends across all  
373 insects are undocumented (Diamond et al. 2015), and reduced access to water from foraging on  
374 larger insects could interact with urban heat stress to select for wider bills.

375         The observed temporal increase in bill length of male northern cardinals, along with  
376 previous evidence that urbanization is associated with bill length increases across their range  
377 (Miller et al. 2018), suggests that both climate change and urbanization are acting in synchrony  
378 on this trait. This supports the idea that selection for improved heat tolerance is driving bill size  
379 increases. Yet, interestingly, we did not see an increased length in male pyrrhuloxia bills over  
380 time, although we did observe wider bills over time. This could result from a similar selective  
381 pressure for larger bills on both species due to climate change but a contrasting pressure on bill  
382 length in pyrrhuloxia. Bill length in pyrrhuloxia could be limited by their unique, more decurved  
383 bill shape (Tweit and Thompson 2020), with selection favoring stouter bills to allow for a  
384 stronger bite (van der Meij et al. 2008), or by foraging niche partitioning, if their bill shape  
385 allows them to access resources that are unavailable to northern cardinals or other  
386 competitors. Regardless of the mechanisms underlying this difference, this finding  
387 demonstrates that slight differences between species can affect their ability to respond to rapid  
388 anthropogenic change. Though broad, multi-taxa studies have offered important insights into  
389 general trends of avian trait evolution in response to climate change (Weeks et al. 2019), our  
390 findings show that taxon-specific analyses can highlight traits that might limit a species' ability  
391 to adapt.

Tail length increased in urban regions over time for male northern cardinals, and over time regardless of urban category for male pyrrhuloxia. Natural selection generally favors shorter tails in open environments, but favors longer tails in dense landscapes that require deft maneuverability (i.e. steering with a rudder; Thomas and Balmford 1995), such as urban environments. This may be species-specific, however, as great tits (*Parus major*) in the city have shorter tails than their rural conspecifics (Caizergues et al. 2021). An urban environment may represent a more open habitat to forest-adapted species like great tits, but a relatively denser environment to these desert-adapted cardinals. Urban-rural comparisons of this trait in other northern cardinal populations that inhabit forested landscapes (i.e. across much of the eastern USA) will hopefully provide important context for the effects of built structures in cities on this trait. It is also intriguing that tails of males of both species elongated over time and that wings of female pyrrhuloxia shortened over time, as both traits are expected to allow for improved maneuverability (Swaddle and Lockwood 2003). There is no obvious trend of undeveloped areas of Arizona becoming more vegetatively dense in a relevant context for cardinals, though no areas are truly undisturbed and undeveloped in our study area. The increased heat and reduced water of the region due to recent climate change could be driving species to utilize more resource-rich and interannually stable habitats along riparian and human-developed areas (e.g. farms, yards) even in more rural environments.

The sex-specific effects of morphological traits could indicate sex-specific responses to urbanization, especially given the unique effect of urbanization on female pyrrhuloxia wing length. Interestingly, urbanization also had a negative association with wing length in female, but not male, barn swallows (*Hirundo rustica*; Zhao et al. 2020). Although both sexes of

414 northern cardinals participate in territory defense (DeVries et al. 2020), males exert much more  
415 effort in territory establishment, intrasexual conflicts, and song performance (Gould 1961,  
416 Lemon 1968, Wilke 1995), and will feed females, while females dominantly nest build and raise  
417 young. It is unknown how these sex-specific behaviors interact with heat stress and why we  
418 might not have observed similar bill effects of time and urbanization on bill length and width in  
419 females. It is also possible that these patterns may result from differences in sample sizes  
420 between males and females. The response of female pyrrhuloxia wing length to urbanization  
421 could suggest that selection for increased maneuverability acts on wing length for female  
422 cardinals but on tail length for male cardinals. We had a very small sample size of female  
423 northern cardinals, and it is possible that this prevented us from detecting a similar pattern for  
424 them as well.

425         Our findings demonstrate impacts of temporal and urban pressures on a pair of related  
426 avian species and highlight the need for comprehensive studies that evaluate multiple social  
427 signals and morphological traits in the context of natural and urban ecologies. We show that  
428 two congeneric species with similar ecological niches can experience different phenotypic  
429 changes in urban landscapes, but that several traits in both species also respond similarly to  
430 urbanization. We also identify a variety of traits that differ in the city that likely have social  
431 functions, such that social selection may be driving these differences in coloration. We also  
432 show that northern cardinal color signals are affected by urbanization in similar ways in a desert  
433 city as they are in the temperate deciduous forest cities in the eastern United States, and we  
434 contribute novel findings of urban effects on bill length, bill width, and tail length that we hope  
435 will be examined in other cities, geographic regions, and species. Our findings fit into the

436 broader landscape of exciting new urban ecological literature, and we look forward to new  
437 advances at the intersection of urban ecology, physiology, animal behavior, and evolutionary  
438 biology.

439

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441 (removed for double-blind review)

442

#### 443 **Data and Code Accessibility Statement**

444 All photographs and raw data tables are available on Dryad ([reviewer sharing link](#)) and the code  
445 used to analyze these data can be found on GitHub ([link removed for double-blind review]).

446

## References

- Amiot, C., Harmange, C., & Ji, W. (2022). Morphological differences along a chronological gradient of urbanisation in an endemic insectivorous bird of New Zealand. *Urban Ecosystems*, 25(2), 465-475.
- Armenta, J. K., Dunn, P. O., & Whittingham, L. A. (2008). Effects of specimen age on plumage color. *The Auk*, 125(4), 803-808.
- Asamoah, E. F., Di Marco, M., Watson, J. E., Beaumont, L. J., Venter, O., & Maina, J. M. (2022). Land-use and climate risk assessment for Earth's remaining wilderness. *Current Biology*, 32(22), 4890-4899.
- Baldassarre, D. T., Bedell, H. S., Drzewiecki, K. M., Goodman, B. D., Mills, M. L., & Ramos, D. A. (2022). Multiple carotenoid-based signals are enhanced despite poor body condition in urban male and female Northern Cardinals (*Cardinalis cardinalis*). *The Wilson Journal of Ornithology*, 134(4), 575-586.
- Badyaev, A. V., & Hill, G. E. (2000). Evolution of sexual dichromatism: contribution of carotenoid-versus melanin-based coloration. *Biological journal of the Linnean Society*, 69(2), 153-172.
- Badyaev, A. V., Young, R. L., Oh, K. P., & Addison, C. (2008). Evolution on a local scale: developmental, functional, and genetic bases of divergence in bill form and associated changes in song structure between adjacent habitats. *Evolution*, 62(8), 1951-1964.
- Bates D, Mächler M, Bolker B, Walker S (2015). "Fitting Linear Mixed-Effects Models Using lme4." *Journal of Statistical Software*, 67(1), 1–48. doi:10.18637/jss.v067.i01.

468 Beygelzimer, A., Kakadet, S., Langford, J., Arya, S., Mount, D., Li, S., & Li, M. S. (2015). Package  
469 'fnn'.

470 Bivand, R., Keitt, T., Rowlingson, B., Pebesma, E., Sumner, M., Hijmans, R., ... & Bivand, M. R.  
471 (2015). Package 'rgdal'. *Bindings for the Geospatial Data Abstraction Library*. Available  
472 online: <https://cran.r-project.org/web/packages/rgdal/index.html> (accessed on 15  
473 October 2017), 172.

474 Bonier, F., Martin, P. R., & Wingfield, J. C. (2007). Urban birds have broader environmental  
475 tolerance. *Biology Letters*, 3(6), 670-673.

476 Brazel, A., Gober, P., Lee, S. J., Grossman-Clarke, S., Zehnder, J., Hedquist, B., & Comparri, E.  
477 (2007). Determinants of changes in the regional urban heat island in metropolitan  
478 Phoenix (Arizona, USA) between 1990 and 2004. *Climate Research*, 33(2), 171-182.

479 Brown, C. R., & Brown, M. B. (2013). Where has all the road kill gone?. *Current Biology*, 23(6),  
480 R233-R234.

481 Brown, J. A., Lerman, S. B., Basile, A. J., Bateman, H. L., Deviche, P., Warren, P. S., & Sweazea, K.  
482 L. (2022). No fry zones: How restaurant distribution and abundance influence avian  
483 communities in the Phoenix, AZ metropolitan area. *PLoS One*, 17(10), e0269334.

484 Burns, K. J., McGraw, K. J., Shultz, A. J., Stoddard, M. C., Thomas, D. B., & Webster, M. W.  
485 (2017). Advanced methods for studying pigments and coloration using avian specimens.  
486 *Studies in Avian Biology*, 50, 23-55.

487 Butler, M. W., Toomey, M. B., & McGraw, K. J. (2011). How many color metrics do we need?  
488 Evaluating how different color-scoring procedures explain carotenoid pigment content

489 in avian bare-part and plumage ornaments. *Behavioral Ecology and Sociobiology*, 65,  
 490 401-413.

491 Caizergues, A. E., Charmantier, A., Lambrechts, M. M., Perret, S., Demeyrier, V., Lucas, A., &  
 492 Grégoire, A. (2021). An avian urban morphotype: how the city environment shapes  
 493 great tit morphology at different life stages. *Urban Ecosystems*, 24(5), 929-941.

494 Chatelain, Da Silva, A., Celej, M., Kurek, E., Bulska, E., Corsini, M., & Szulkin, M. (2021).  
 495 Replicated, urban-driven exposure to metallic trace elements in two passerines (vol 11,  
 496 19662, 2021). *Scientific Reports*, 11(1). <https://doi.org/10.1038/s41598-021-99964-9>

497 Constable, H., Guralnick, R., Wieczorek, J., Spencer, C., Peterson, A. T., & VertNet Steering  
 498 Committee. (2010). VertNet: a new model for biodiversity data sharing. *PLoS biology*,  
 499 8(2), e1000309.

500 Danner, R. M., Gulson-Castillo, E. R., James, H. F., Dzielski, S. A., Frank III, D. C., Sibbald, E. T., &  
 501 Winkler, D. W. (2017). Habitat-specific divergence of air conditioning structures in bird  
 502 bills. *The Auk: Ornithological Advances*, 134(1), 65-75.

503 de Zwaan, D. R., Barnes, S., & Martin, K. (2019). Plumage melanism is linked to male quality,  
 504 female parental investment and assortative mating in an alpine songbird. *Animal*  
 505 *Behaviour*, 156, 41-49.

506 DeVries, M. S., Winters, C. P., & Jawor, J. M. (2020). Similarities in expression of territorial  
 507 aggression in breeding pairs of northern cardinals, *Cardinalis cardinalis*. *Journal of*  
 508 *Ethology*, 38(3), 377-382.



509 Diamond, S. E., Dunn, R. R., Frank, S. D., Haddad, N. M., & Martin, R. A. (2015). Shared and  
 510 unique responses of insects to the interaction of urbanization and background  
 511 climate. *Current Opinion in Insect Science*, 11, 71-77.

512 Doucet, S. M., & Hill, G. E. (2009). Do museum specimens accurately represent wild birds? A  
 513 case study of carotenoid, melanin, and structural colours in long-tailed manakins  
 514 *Chiroxiphia linearis*. *Journal of Avian Biology*, 40(2), 146-156.

515 Everitt, B. S., Hothorn, T., Hothorn, M. T., & Hothorn, C. (2017). Package 'HSAUR2'. URL  
 516 <https://CRAN.R-project.org/package=HSAUR2>.

517 Fidino, M., & Magle, S. B. (2017). Trends in long-term urban bird research. *Ecology and*  
 518 *conservation of birds in urban environments*, 161-184.

519 Fox, J., Weisberg S (2019). An R Companion to Applied Regression, 3rd edition. Sage, Thousand  
 520 Oaks CA. <https://socialsciences.mcmaster.ca/jfox/Books/Companion/index.html>.

521 Fox, J., Weisberg S (2018). "Visualizing Fit and Lack of Fit in Complex Regression Models with  
 522 Predictor Effect Plots and Partial Residuals." *Journal of Statistical Software*, 87(9), 1–27.  
 523 doi:10.18637/jss.v087.i09.

524 Fox, J., Weisberg, S., Adler, D., Bates, D., Baud-Bovy, G., Ellison, S., ... & Monette, G. (2012).  
 525 Package 'car'. *Vienna: R Foundation for Statistical Computing*, 16.

526 Giraudeau, M., Mousel, M., Earl, S., & McGraw, K. (2014). Parasites in the city: degree of  
 527 urbanization predicts poxvirus and coccidian infections in house finches (*Haemorhous*  
 528 *mexicanus*). *PloS One*, 9(2), e86747.

529 Giraudeau, M., Nolan, P. M., Black, C. E., Earl, S. R., Hasegawa, M., & McGraw, K. J. (2014). Song  
 530 characteristics track bill morphology along a gradient of urbanization in house finches  
 531 (*Haemorhous mexicanus*). *Frontiers in Zoology*, 11(1), 1-8.

532 Giraudeau, M., Toomey, M. B., & McGraw, K. J. (2012). Can house finches (*Carpodacus*  
 533 *mexicanus*) use non-visual cues to discriminate the carotenoid content of foods?.  
 534 *Journal of Ornithology*, 153, 1017-1023.

535 Gould, P. J. (1961). Territorial relationships between Cardinals and Pyrrhuloxias. *The Condor*,  
 536 63(3), 246-256.

537 Green, D. M., & Baker, M. G. (2003). Urbanization impacts on habitat and bird communities in a  
 538 Sonoran desert ecosystem. *Landscape and urban planning*, 63(4), 225-239.

539 Greenberg, R., Cadena, V., Danner, R. M., & Tattersall, G. (2012). Heat loss may explain bill size  
 540 differences between birds occupying different habitats. *PloS one*, 7(7), e40933.

541 Harrell Jr, F. E., & Harrell Jr, M. F. E. (2019). Package 'hmisc'. CRAN2018, 2019, 235-236.

542 Halkin, S. L., D. P. Shustack, M. S. DeVries, J. M. Jawor, and S. U. Linville (2021). Northern  
 543 Cardinal (*Cardinalis cardinalis*), version 2.0. *Birds of the World* (P. G. Rodewald and B. K.  
 544 Keeney, Editors). Cornell Lab of Ornithology, Ithaca, NY, USA.  
 545 <https://doi.org/10.2173/bow.norcar.02>

546 Heinen-Kay, J. L., Kay, A. D., & Zuk, M. (2021). How urbanization affects sexual communication.  
 547 *Ecology and Evolution*, 11(24), 17625-17650.

548 Hensley, C. B., Trisos, C. H., Warren, P. S., MacFarland, J., Blumenshine, S., Reece, J., & Katti, M.  
 549 (2019). Effects of urbanization on native bird species in three southwestern US Cities.  
 550 *Frontiers in Ecology and Evolution*, 7, 71.

551 Hijmans, R. J., Bivand, R., Forner, K., Ooms, J., Pebesma, E., & Sumner, M. D. (2022). *Package*  
 552 *'terra'*. Maintainer: Vienna, Austria.

553 Hijmans, R. J., Van Etten, J., Cheng, J., Mattiuzzi, M., Sumner, M., Greenberg, J. A., ... & Hijmans,  
 554 M. R. J. (2015). Package *'raster'*. *R package*, 734.

555 Hostetler, M., & Knowles-Yanez, K. (2003). Land use, scale, and bird distributions in the Phoenix  
 556 metropolitan area. *Landscape and Urban Planning*, 62(2), 55-68.

557 Hutton, P., & McGraw, K. J. (2016). Urban–rural differences in eye, bill, and skull allometry in  
 558 house finches (*Haemorhous mexicanus*). *Integrative and comparative biology*, 56(6),  
 559 1215-1224.

560 Isaksson, C. (2018). Impact of urbanization on birds. *Bird species*, 235, 257.

561 Isaksson, C., & Andersson, S. (2007). Carotenoid diet and nestling provisioning in urban and  
 562 rural great tits *Parus major*. *Journal of Avian Biology*, 38(5), 564-572.

563 Isaksson, C., Örnborg, J., Stephensen, E., & Andersson, S. (2005). Plasma glutathione and  
 564 carotenoid coloration as potential biomarkers of environmental stress in great  
 565 tits. *EcoHealth*, 2, 138-146.

566 Jawor, J. M., & Breitwisch, R. (2004). Multiple ornaments in male northern cardinals, *Cardinalis*  
 567 *cardinalis*, as indicators of condition. *Ethology*, 110(2), 113-126.

568 Jawor, J. M., & Breitwisch, R. (2006). Is mate provisioning predicted by ornamentation? A test  
 569 with northern cardinals (*Cardinalis cardinalis*). *Ethology*, 112(9), 888-895.

570 Jawor, J. M., Gray, N., Beall, S. M., & Breitwisch, R. (2004). Multiple ornaments correlate with  
 571 aspects of condition and behaviour in female northern cardinals, *Cardinalis cardinalis*.  
 572 *Animal Behaviour*, 67(5), 875-882.

573 Jawor, J. M., Linville, S. U., Beall, S. M., & Breitwisch, R. (2003). Assortative mating by multiple  
 574 ornaments in northern cardinals (*Cardinalis cardinalis*). *Behavioral Ecology*, 14(4), 515-  
 575 520.

576 Jiménez-Peñuela, J., Ferraguti, M., Martínez-de la Puente, J., Soriguer, R., & Figuerola, J. (2019).  
 577 Urbanization and blood parasite infections affect the body condition of wild  
 578 birds. *Science of the Total Environment*, 651, 3015-3022.

579 Jones, K. R., Klein, C. J., Halpern, B. S., Venter, O., Grantham, H., Kuempel, C. D., ... & Watson, J.  
 580 E. (2018). The location and protection status of Earth's diminishing marine wilderness.  
 581 *Current Biology*, 28(15), 2506-2512.

582 Jones, T. M., Rodewald, A. D., & Shustack, D. P. (2010). Variation in plumage coloration of  
 583 northern cardinals in urbanizing landscapes. *The Wilson Journal of Ornithology*, 122(2),  
 584 326-333.

585 Kern, E. M., & Langerhans, R. B. (2018). Urbanization drives contemporary evolution in stream  
 586 fish. *Global Change Biology*, 24(8), 3791-3803.

587 Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest package: tests in linear  
 588 mixed effects models. *Journal of statistical software*, 82(13).

589 Leigh, B. (2012). Carotenoid-based plumage and its effects on the immune system of Northern  
 590 Cardinals (*Cardinalis cardinalis*) within an Urban-rural gradient [honors thesis. Tampa  
 591 (FL): University of South Florida

592 Lemon, R. E. (1968). The displays and call notes of cardinals. *Canadian Journal of Zoology*, 46(2),  
 593 141-151.

594 Lendvai, Á. Z., Giraudeau, M., Németh, J., Bakó, V., & McGraw, K. J. (2013). Carotenoid-based  
 595 plumage coloration reflects feather corticosterone levels in male house finches  
 596 (*Haemorrhous mexicanus*). *Behavioral Ecology and Sociobiology*, 67, 1817-1824.

597 Lenth, R., Singmann, H., Love, J., Buerkner, P., & Herve, M. (2018). Emmeans: Estimated  
 598 marginal means, aka least-squares means. *R package version*, 1(1), 3.

599 Li, X., Zhou, Y., Hejazi, M., Wise, M., Vernon, C., Iyer, G., & Chen, W. (2021). Global urban  
 600 growth between 1870 and 2100 from integrated high resolution mapped data and  
 601 urban dynamic modeling. *Communications Earth & Environment*, 2(1), 1-10.

602 Litteral, J., & Wu, J. (2012). Urban landscape matrix affects avian diversity in remnant  
 603 vegetation fragments: evidence from the Phoenix metropolitan region, USA. *Urban*  
 604 *Ecosystems*, 15, 939-959.

605 Long, J. A., & Long, M. J. A. (2017). *Package 'jtools'*.

606 Long, J., (2019). interactions: Comprehensive, User-Friendly Toolkit for Probing Interactions. *R*  
 607 *package version 1.1.0*, <https://cran.r-project.org/package=interactions>.

608 Luke, S. G. (2017). Evaluating significance in linear mixed-effects models in R. *Behavior Research*  
 609 *Methods*, 49(4), 1494-1502.

610 McAtee, W. L. (1908). *Food habits of the grosbeaks* (No. 32). US Department of Agriculture,  
 611 Biological Survey.

612 McGraw, K. J., Mackillop, E. A., Dale, J., & Hauber, M. E. (2002). Different colors reveal different  
 613 information: how nutritional stress affects the expression of melanin-and structurally  
 614 based ornamental plumage. *Journal of Experimental Biology*, 205(23), 3747-3755.

615 McGraw, K. J., Safran, R. J., & Wakamatsu, K. (2005). How feather colour reflects its melanin  
616 content. *Functional Ecology*, 19(5), 816-821.

617 McNew, S. M., Beck, D., Sadler-Riggleman, I., Knutie, S. A., Koop, J. A., Clayton, D. H., & Skinner,  
618 M. K. (2017). Epigenetic variation between urban and rural populations of Darwin's  
619 finches. *BMC Evolutionary Biology*, 17, 1-14.

620 Miller, C. R., Latimer, C. E., & Zuckerberg, B. (2018). Bill size variation in northern cardinals  
621 associated with anthropogenic drivers across North America. *Ecology and*  
622 *Evolution*, 8(10), 4841-4851.

623 Nagy Koves Hrabar, H. D., & Perrin, M. (2002). The effect of bill structure on seed selection by  
624 granivorous birds. *African Zoology*, 37(1), 67-80.

625 Narango, D. L., & Rodewald, A. D. (2016). Urban-associated drivers of song variation along a  
626 rural–urban gradient. *Behavioral Ecology*, 27(2), 608-616.

627 Palacio, F. X. (2020). Urban exploiters have broader dietary niches than urban avoiders. *Ibis*,  
628 162(1), 42-49.

629 Quesada, J., & Senar, J. C. (2006). Comparing plumage colour measurements obtained directly  
630 from live birds and from collected feathers: the case of the great tit *Parus major*. *Journal*  
631 *of Avian Biology*, 37(6), 609-616.

632 R Core Team (2021). R: A language and environment for statistical computing. R Foundation for  
633 Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.

634 Rodewald, A. D., Shustack, D. P., & Jones, T. M. (2011). Dynamic selective environments and  
635 evolutionary traps in human-dominated landscapes. *Ecology*, 92(9), 1781-1788.

636 Rösner, U. (1998). Effects of historical mining activities on surface water and groundwater-an  
637 example from northwest Arizona. *Environmental Geology*, 33, 224-230.

638 Senar, J. C., Conroy, M. J., Quesada, J., & Mateos-Gonzalez, F. (2014). Selection based on the  
639 size of the black tie of the great tit may be reversed in urban habitats. *Ecology and*  
640 *Evolution*, 4(13), 2625-2632.

641 Sepp, T., McGraw, K. J., & Giraudeau, M. (2020). Urban sexual selection. *Urban Evolutionary*  
642 *Biology*, 234-252.

643 Shultz, A. J., Adams, B. J., Bell, K. C., Ludt, W. B., Pauly, G. B., & Vendetti, J. E. (2021). Natural  
644 history collections are critical resources for contemporary and future studies of urban  
645 evolution. *Evolutionary Applications*, 14(1), 233-247.

646 Singmann, H., Bolker, B., Westfall, J., Aust, F., & Ben-Shachar, M. S. (2015). afex: Analysis of  
647 factorial experiments. *R package version 0.13–145*.

648 Smith, B. T., Escalante, P., Hernández Baños, B. E., Navarro-Sigüenza, A. G., Rohwer, S., & Klicka,  
649 J. (2011). The role of historical and contemporary processes on phylogeographic  
650 structure and genetic diversity in the Northern Cardinal, *Cardinalis cardinalis*. *BMC*  
651 *Evolutionary Biology*, 11(1), 1-12.

652 Snell-Rood, E. C., & Wick, N. (2013). Anthropogenic environments exert variable selection on  
653 cranial capacity in mammals. *Proceedings of the Royal Society B: Biological*  
654 *Sciences*, 280(1769), 20131384.

655 Swaddle, J. P., & Lockwood, R. (2003). Wingtip shape and flight performance in the European  
656 Starling *Sturnus vulgaris*. *Ibis*, 145(3), 457-464.

657 Tattersall, G. J., Arnaout, B., & Symonds, M. R. (2017). The evolution of the avian bill as a  
 658 thermoregulatory organ. *Biological Reviews*, 92(3), 1630-1656.

659 Thomas, A. L., & Balmford, A. (1995). How natural selection shapes birds' tails. *The American*  
 660 *Naturalist*, 146(6), 848-868.

661 Tryjanowski, P., Morelli, F., & Møller, A. P. (2020). Urban birds: urban avoiders, urban adapters,  
 662 and urban exploiters. *The Routledge Handbook of Urban Ecology* (pp. 399-411).  
 663 Routledge.

664 Tweit, R. C. and C. W. Thompson (2020). Pyrrhuloxia (*Cardinalis sinuatus*), version 1.0. In *Birds*  
 665 *of the World* (A. F. Poole and F. B. Gill, Editors). Cornell Lab of Ornithology, Ithaca, NY,  
 666 USA. <https://doi.org/10.2173/bow.pyrrhu.01>

667 van der Meij, M. A., & Bout, R. G. (2008). The relationship between shape of the skull and bite  
 668 force in finches. *Journal of Experimental Biology*, 211(10), 1668-1680.

669 vanRossum, G. (1995). Python reference manual. *Department of Computer Science [CS]*, (R  
 670 9525).

671 Venables WN, Ripley BD (2002). *Modern Applied Statistics with S*, Fourth edition. Springer, New  
 672 York. ISBN 0-387-95457-0, <https://www.stats.ox.ac.uk/pub/MASS4/>.

673 Vu, V., VQV/ggbiplot: A biplot based on Ggplot2. *GitHub*. Retrieved January 20, 2023, from  
 674 <https://github.com/vqv/ggbiplot>

675 Watson, J. E., Shanahan, D. F., Di Marco, M., Allan, J., Laurance, W. F., Sanderson, E. W., ... &  
 676 Venter, O. (2016). Catastrophic declines in wilderness areas undermine global  
 677 environment targets. *Current Biology*, 26(21), 2929-2934.



678 Weeks, B. C., Willard, D. E., Zimova, M., Ellis, A. A., Witynski, M. L., Hennen, M., & Winger, B. M.  
679 (2019). Shared morphological consequences of global warming in North American  
680 migratory birds. *Ecology Letters*, 23(2), 316-325.

681 Weiss, J. L., & Overpeck, J. T. (2005). Is the Sonoran Desert losing its cool? *Global Change*  
682 *Biology*, 11(12), 2065-2077.

683 Wickham, H., Chang, W., & Wickham, M. H. (2016). Package 'ggplot2'. *Create elegant data*  
684 *visualisations using the grammar of graphics. Version, 2(1)*, 1-189.

685 Wickham, H., & Wickham, M. H. (2017). Package 'tidyr'. *Easily Tidy Data with 'spread' and*  
686 *'gather ()' Functions*.

687 Wilke, R. R. (1995). *The territorial behavior of Pyrrhuloxia (Cardinalis sinuatus) in west-central*  
688 *Texas with observations of breeding biology* (Doctoral dissertation).

689 Winchell, K. M., Carlen, E. J., Puente-Rolón, A. R., & Revell, L. J. (2018). Divergent habitat use of  
690 two urban lizard species. *Ecology and Evolution*, 8(1), 25-35.

691 Winters, C. P., & Jawor, J. M. (2017). Melanin ornament brightness and aggression at the nest in  
692 female Northern Cardinals (*Cardinalis cardinalis*). *The Auk: Ornithological Advances*,  
693 134(1), 128-136.

694 Wolfenbarger, L. L. (1999). Female mate choice in Northern Cardinals: is there a preference for  
695 redder males?. *The Wilson Bulletin*, 76-83.

696 Yeh, P. J. (2004). Rapid evolution of a sexually selected trait following population establishment  
697 in a novel habitat. *Evolution*, 58(1), 166-174.

698 Zhao, Y., Liu, Y., Scordato, E. S., Lee, M. B., Xing, X., Pan, X., ... & Pagani-Núñez, E. (2020). The  
699 impact of urbanization on body size of Barn Swallows *Hirundo rustica gutturalis*. *Ecology*  
700 *and Evolution*, 11(1), 612-625.

701 Zhao, Y., Norouzi, H., Azarderakhsh, M., & AghaKouchak, A. (2021). Global Patterns of Hottest,  
702 Coldest, and Extreme Diurnal Variability on Earth. *Bulletin of the American*  
703 *Meteorological Society*, 102(9), E1672-E1681.

704

705

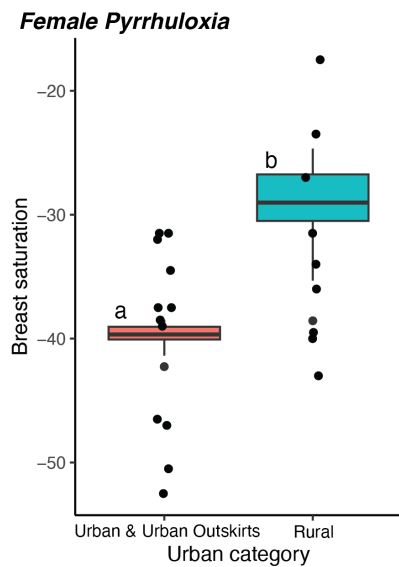
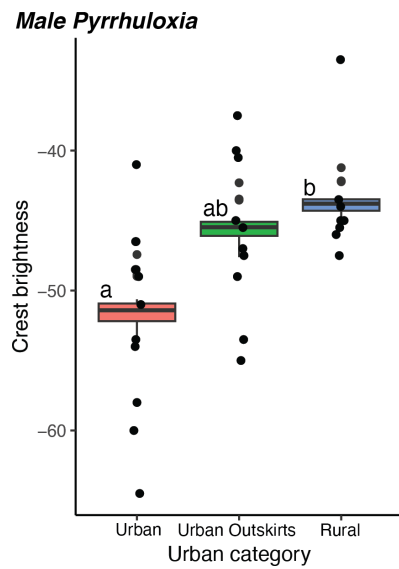
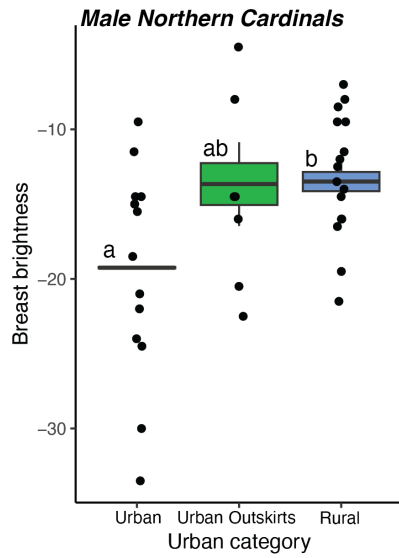
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707 **Figures**

708 **Figure 1.** Head and breast photographs of (from top to bottom) a male northern cardinal, a  
709 female northern cardinal, a male pyrrhuloxia, and a female pyrrhuloxia. Photographs on the left  
710 are from field caught birds, and photographs on the right are from University of Arizona  
711 museum specimens.

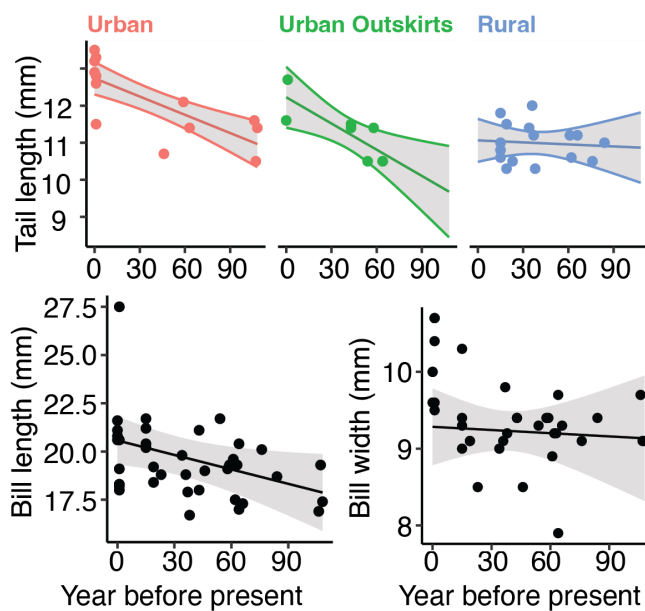


**Figure 2.** Plots of color trait values by urban category. We present only the four traits for which urbanization was significant and year was not, which were breast brightness for male northern cardinals, crest brightness for male pyrrhuloxia, and breast saturation for female pyrrhuloxia. Predicted median and interquartile range, based on the linear model of each color trait, are presented as lines, and actual trait measurements of the specimens are presented as points.

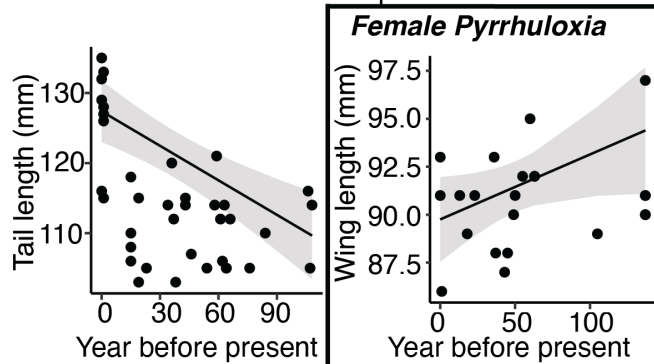
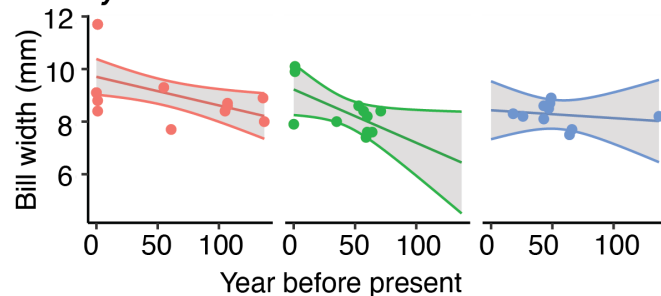


720 **Figure 3.** Plots of trait sizes by urban category. We present only the six traits for which  
721 urbanization, year, or the interaction between the two were significant, which were bill width,  
722 tail length, and bill length for male northern cardinals, bill width and tail length for male  
723 pyrrhuloxia, and wing length for female pyrrhuloxia. Interquartile ranges of each trait size are  
724 presented as lines, with actual trait measurements of the specimens plotted as points.  
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### Male Northern Cardinals



### Male Pyrrhuloxia



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728 **Tables**

Species and Sex	Trait of interest	Factor	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Northern cardinal males	Face saturation	Urban category	2	106.90	53.44	0.44	0.65
		Year	1	74.80	74.81	0.62	0.44
		Urban category x Year	2	1940.60	970.29	8.04	0.00
		Residuals	31	3741.10	120.68		
	Breast brightness	Urban category	2	291.56	145.78	4.06	0.03
		Year	1	0.00	0.00	0.00	1.00
		Urban category x Year	2	17.73	8.86	0.25	0.78
		Residuals	31	1114.24	35.94		
Pyrrhuloxia males	Crest brightness	Urban category	2	348.87	174.43	5.22	0.01
		Year	1	93.07	93.07	2.78	0.11
		Urban category x Year	2	2.36	1.18	0.04	0.97
		Residuals	24	802.05	33.42		
Pyrrhuloxia females	Face hue	Urban category	1	9.53	9.53	0.51	0.49
		Year	1	116.96	116.96	6.21	0.02
		Urban category x Year	1	90.28	90.28	4.79	0.04
		Residuals	17	320.23	18.84		
	Breast saturation	Urban category	1	283.95	283.95	5.44	0.03
		Year	1	232.01	232.01	4.45	0.05
		Urban category x Year	1	60.48	60.48	1.16	0.30
		Residuals	17	886.79	52.16		

729 **Table 1.** ANOVA tables showing the effects of urbanization, year, and/or the interaction on  
730 color traits. Here, we only present results with either significant effects of urban category or of  
731 the interaction between urban category and year. Results of every model can be found in the  
732 supplement (Tables S15-S18).

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737 **Table 2.** ANOVA tables showing the effects of urbanization, year, and their interaction on trait  
738 sizes. We only present results with either significant effects of urban category, year, or of the  
739 interaction between the two. All results can be found in the supplement (Tables S19-S22).

Species and Sex	Trait of interest	Factor	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Northern cardinal males	Bill length	Urban category	2	0.95	0.47	0.25	0.78
		Tarsus	1	4.18	4.18	2.19	0.15
		Year	1	10.87	10.87	5.71	0.02
		Urban category x Year	2	0.72	0.36	0.19	0.83
		Residuals	31	58.985	1.90		
	<u>Bill width</u>	Urban category	2	1.50	0.75	2.84	
		Tarsus	1	0.05	0.05	0.17	0.07
		Year	1	2.58	2.58	9.81	0.68
		Urban category x Year	2	0.88	0.44	1.67	<0.01
		Residuals	31	8.15	0.26		0.20
	<u>Tail length</u>	Urban category	2	936.68	468.34	13.44	
		Tarsus	1	0.14	0.14	0.00	
		Year	1	765.76	765.76	21.97	0.00
		Urban category x Year	2	201.16	100.58	2.89	0.95
		Residuals	30	1045.45	34.85		0.00
<u>Pyrrhuloxia males</u>	<u>Bill width</u>	Urban category	2	4.99	2.49	3.38	
		Tarsus	1	0.00	0.00	0.00	0.05
		Year	1	6.61	6.61	8.95	0.99
		Urban category x Year	2	1.29	0.64	0.87	0.01
		Residuals	27	19.95	0.74		0.43
	<u>Tail length</u>	Urban category	2	94.99	47.50	1.46	
		Tarsus	1	12.66	12.66	0.39	
		Year	1	226.39	226.39	6.98	0.25
		Urban category x Year	2	124.82	62.41	1.92	0.54
		Residuals	23	745.87	32.43		0.01
<u>Pyrrhuloxia females</u>	<u>Wing length</u>	Urban category	1	15.08	15.08	3.34	

Tarsus	1	13.86	13.86	3.07	0.09
Year	1	24.75	24.75	5.48	0.10
Urban category x Year	1	1.42	1.42	0.32	0.03
Residuals	17	76.76	4.52		0.58

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