

Effect of spatial and temporal urban isolation on the genetic diversity, acoustic variation, and morphological characteristics of an urban survivor bird species

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Abstract

Urbanization alters ecosystems, fragmenting natural habitats, and hence, increasing isolation between populations. Therefore, a reduction in gene flow among isolated populations is expected with greater distance and time since fragmentation. Changes in the structure, density, or community composition in the remaining habitats often result in species' modifications of vocalizations and morphological traits. However, the relationship between genetic, vocalizations and morphological divergence in urban areas over time remains poorly understood. We analyzed ten years of genetic, acoustic, and morphological data from isolated populations of the white-eared ground-sparrow. We used seven microsatellites (SSRs), recorded, and measured five acoustic traits, and measured six morphological traits, and compared them over a period of ten years. We found an increase in inbreeding, increase in song duration, number of elements, and frequency of maximum amplitude, but a reduction in female body size and changes in male beak. However, we only identified a significant correlation between genetic diversity and the acoustic characteristics of song.

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Abstract

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increase in inbreeding, increase in song duration, number of elements, and frequency of maximum amplitude, but a reduction in female body size and changes in male beak. However, we only identified a significant correlation between genetic diversity and the acoustic characteristics of song.

Keywords: *Melospiza leucotis*, Costa Rica, genetic structure, Bioacoustic

Introduction

Urban development results in rapid environmental changes (Grimm et al., 2008; Johnson & Munshi-South, 2017) which transform natural habitats into small patches of vegetation surrounded by a matrix of man built constructions such as buildings and roads. These fragments with depauperate resources are also frequently highly contaminated by noise, lights, or solid debris (Fahrig & Rytwinski, 2009; Biamonte et al., 2011). This reduction in natural habitats isolates populations, as urban development restricts or eliminates the movement of individuals across populations (Lynch & Baker, 1994; Crooks et al., 2004), lowering gene flow and, consequently, the genetic diversity of populations (Lynch & Baker, 1994; Johnson & Munshi-South, 2017). In birds, changes in behavior (e.g., vocalizations, nest construction, predator responses) and morphology (e.g., bill and body size) have also frequently been reported as a consequence of different selective pressures acting on isolated populations (e.g., sexual selection, predation, environment) (Slabbekoorn & Smith, 2002; Brumm, 2004; Warren et al., 2006; Foster et al., 2008; Laiolo & Arroyo, 2011; Luther & Derryberry, 2012; Sandoval et al., 2014; Geffroy et al., 2020; Corrales-Moya et al., 2021; Méndez et al., 2021)

The increase in distance between populations and the effect of drift and inbreeding in populations of small size are the primary causes of the loss of genetic diversity within populations and the increase in spatial genetic structure in urban isolated bird populations (Keller & Waller, 2002; Whitlock, 2004; Johnson & Munshi-South, 2017; Miles et al., 2019). In wren-tits *Chamaea fasciata*, significant genetic structure was found when populations were separated by a highway and a strip of residential development, in southern California (Delaney et al., 2010b). In the song sparrow *Melospiza melodia*, researchers found lower genetic diversity in populations where urban development had been extensive and had occurred over long periods of time (Unfried et al., 2013). These results indicate that urbanization has the potential to lower genetic diversity within fragmented populations inside urbanized matrices and increase the geographic genetic structure of bird populations.

The observed patterns of genetic structure caused by urbanization are analogous to differences in songs reported for several bird species: white-bellied short wing *Brachypteryx major*, rufous-and-white wrens *Thryophilus rufalbus*, and wedge-tailed sabrewing *Campylopterus pampa*. (Shaw & Lesnick, 2009; Gonzalez et al., 2011; Purushotham & Robin, 2016; Graham et al., 2017). Variation or polymorphism in the acoustic traits of songs among populations occurs in response to different habitat characteristics (e.g., vegetation density or noise levels), and they are aimed at improving sound transmission and communication across different environments (Morton, 1975; Boncoraglio & Saino, 2007; Ey & Fischer, 2009; Hardt & Benedict, 2021). Acoustic variation may also occur via female selection, differences in female choice among populations may lead to selective changes in song characteristics by males (Searcy & Andersson, 1986; Laiolo & Arroyo, 2011). Acoustic variations may also be the result of acoustic drift, which occurs when a small group of birds randomly develops distinct repertoires. (Baker & Cunningham, 1985; Wilkins et al., 2013). For example, in bird species that inhabit both urban and rural environments, songs from urban populations had higher frequencies than populations from birds inhabiting rural environments to cope with anthropogenic noise (Hu & Cardoso, 2009; Luther & Derryberry, 2012; Méndez et al., 2021). Differences in song traits may have evolved through acoustic drift as in populations of Darwin's finches (B. R. Grant & Grant, 1996). These changes in songs can be maintained or increase over time, because songs are either learned or transmitted over generations (Boyd & Richerson, 1985; Warren et al., 2006). Hence, the correlation between genetic and vocal polymorphism may arise due to the relationship between song pattern diversity and the survival and reproductive success of individuals, as well as the presence of distinct genotypes within each population (Danielson-François et al., 2006; Irwin et al., 2008).

Variation in song acoustic characteristics (e.g., frequency and duration), have also been related with the

body and beak size of individuals (Podos, 2001, 2010), because larger birds and larger beaks tend to produce lower frequency songs (Wallschläger, 1980; Bradbury & Vehrencamp, 1998; Fletcher & Tarnopolsky, 1999; Slabbekoorn & Smith, 2002a; Brumm, 2009). For example, in purple-crowned fairy-wrens *Malurus coronatus*, barn swallows *Hirundo rustica*, and black swans *Cygnus atratus*; individuals with large body sizes had lower song frequencies (Galeotti et al., 1997; Patel et al., 2010; Hall et al., 2013). In House finches *Haemorhous mexicanus* from Arizona, urban individuals have longer and deeper beaks than rural birds and emit songs with slower trills and wider frequency range (Badyaev et al., 2008).

White-eared ground-sparrows, *Melospiza leucotis*, inhabit the central valley of Costa Rica in isolated populations embedded inside the greater urban area (Sandoval et al., 2015; Juárez et al., 2020; Rodríguez-Bardía et al., 2022). Previous work on this species reported that urbanization limits the local movement of individuals and gene flow between populations, which resulted in significant genetic structuring among Costa Rican populations (Rodríguez-Bardía et al., 2022). Previous research has also identified changes in song dialects among different populations (Sandoval et al., 2014, 2016; Bonilla-Badilla 2021). Additionally, it has been observed that differences in the frequency and duration of songs among populations are associated with levels of anthropogenic noise. Specifically, males in populations exposed to higher levels of noise tend to exhibit an increase in both the minimum frequency and duration of their songs. (Sandoval et al., 2014, 2015, 2016; Juárez et al., 2020; Méndez et al., 2021; Rodríguez-Bardía et al., 2022). These studies suggest that genetic and cultural traits are under intense selective pressures imposed by urbanized environments. However, it is still unknown how these traits change over time and how they track population dynamics.

The main objective of this study is to describe the changes in genetic diversity, vocalization, morphology, and their relationship, over a 10-year period in populations of white-eared ground-sparrow isolated by urban development in Costa Rica. We hypothesize that differences in genetic diversity, song acoustics, and morphology will correlate with the length of time communities have been separated, as population divergence increases with isolation.

Material and Methods

We conducted this study in four populations of white-eared ground-sparrows in Costa Rica: (1) Estación Biológica Monteverde (MTV), Puntarenas province (10° 18' N, 84° 48' W, 1600 m), characterized by coffee plantations and large forest patches that connect with more extensive mature forests (Rodríguez-Bardía et al., 2022) without an urban isolation effect yet. (2) Getsemani (HDA), Heredia province (10° 01' N, 84° 05' W, 1350 m); it is a non-urbanized site, with secondary forest patches and abandoned coffee plantations, and dense thickets with little human presence (Juárez et al. 2020). Urbanization started to expand from the edges of the study area since 2000. (3) Jardín Botánico Lankester (JBL), Cartago province (9° 50' N, 83° 53' W, 1370 m); it includes secondary forests, gardens, and buildings, with little disturbance and human presence, but immersed in a urban matrix that had rapidly extended since 1990 (Juárez et al. 2020). (4) The Universidad de Costa Rica (UCR), San José province (09° 56' N, 84° 05' W, 1200 m); is a highly urbanized site, with a secondary forest reserve surrounded by buildings, open areas and gardens, and it is exposed to intense human disturbance (Juárez et al., 2020). Urban development began before the 1900's but most fragmentation occurred after the 1970's (Biamonte et al., 2011).

Historical and current sampling

Historical acoustic and morphological data was collected in 2011 and 2012 and was deposited in the Laboratorio de Ecología Urbana y Comunicación Animal (LEUCA), Universidad de Costa Rica. These data are part of a long-term research focus on analyzing the effect of urbanization on behavior, morphology, and genetic diversity of bird populations. To obtain historic genetic diversity estimates, we used blood samples collected in 2011 – 2012. We will refer for the historical 2011 – 2012-year sampling as “period 1”.

Current acoustic and morphological data was collected from 2018 to 2022. We recorded each banded pair using a Marantz PMD661 digital recorder and a Sennheiser ME66/K6 unidirectional microphone. The recordings were stored in WAV format with a sample rate of 44.1 kHz and a precision of 24 bits. All recording were conducted between 4:55 - 6:00 h when this species is most vocally active (Sandoval et al., 2016). We captured

individuals using mist nets (12 x 2.5m, 15 mm mesh eye) inside each pair territory. We provided each captured individual with a unique numbered metal ring and a color combination of plastic rings. From each bird, we obtained 10 μ L of blood from the brachial vein and stored it in 95% ethanol or Lysis buffer for molecular analyses (Seutin et al., 1991; Rodríguez-Bardía et al., 2022). We will refer for the period 2018 – 2022-year sampling as “period 2”.

All procedures were conducted in accordance with Costa Rican law. The Research Committee of the Biology School and the Animal Care Committee (ACC) of the University of Costa Rica granted to LS, the principal investigator, research permits and animal care protocols.

DNA extraction and SSR amplification

We extracted DNA from blood samples using the DNeasy blood and tissue Kit (Qiagen Inc., Valencia, CA, USA) following the manufacturer’s protocol. We used seven primers: Mme2, Mme7, Mme8, As μ 15, Esc μ 6, Gf01 y Gf05 (Petren, 1998; Jeffery et al., 2001; Bulgin et al., 2003) which have been previously shown to be polymorphic and amplified well for this species (Rodríguez-Bardía et al., 2022). We followed the Qiagen Multiplex master kit ((Qiagen Inc., Valencia, CA) procedure to amplify the aforementioned SSRs. All markers were amplified through Polymerase Chain Reactions (PCR) using 10 μ L reaction, containing 2 μ L of 0.4 μ M primer mix, 1.5 μ L of 20 ng of template DNA, 5 μ L of Multiplex Master Kit (Qiagen) for mixed primers and Top Taq Master Kit (Qiagen) for Gf01 and 1.5 μ L of water nuclease free (Qiagen). We followed the PCR thermal profiles and mixes described in Rodriguez et al. (2022). The PCR was performed in a Veriti thermocycler (Applied Biosystems, Foster City, CA, USA). Capillary electrophoresis was performed on a 3500 genetic analyzer (Applied Biosystems) using Hi-Di formamide and GeneScan 500 LIZ dye size standard (Applied Biosystems). Multilocus genotypes were scored using GeneMarker 1.91 (SoftGenetics, State College, PA, USA).

Acoustic analyses

We analyzed all songs using Raven pro 1.6 software (Cornell Lab of Ornithology, Ithaca, NY, USA). We classified each song according to its structure in different types following the classification of the sound library for white-eared ground-sparrow available in LEUCA and used in previous studies (Sandoval et al., 2014, 2015, 2016; Méndez et al., 2020). In addition, we measured the following acoustic characteristics: duration (s), minimum frequency (kHz), maximum frequency (kHz), frequency of maximum amplitude (kHz), and the number of elements. We used different windows to obtain the measurements: the spectrogram window was used to identify and classify sounds, the power spectrum was used to measure frequency characteristics with a threshold of -30dB relative to vocalization peak, and the oscillogram to measure the temporal characteristics of sounds. Spectrograms were constructed using a Hann window with a size of 512 samples, 3 dB bandwidth of 124 Hz; a Time grid with an overlap of 50% and hop size of 5.80 ms; and a frequency grid spacing with DFT size of 512 samples and grip spacing of 86.1 Hz.

Morphometric measurements

We measured six morphological traits: tarsus length (from the intertarsal joint to the middle of the foot sole), tail length, wing chord length (unflattened), exposed culmen length (from the tip of the beak to base of skull), beak width (at the beak gape), and beak depth (measured at right angles to the point on the lower mandible where the feathers end) following the protocol described in Sandoval & Mennill, (2013). We used a dial caliper (model: SPI Plastic Caliper 150mm, AVINET, NY, USA) to get the bill and tarsus measurements, a metal wing ruler (model: WING15ECON, AVINET, NY, USA) to measure wing chord and tail length.

Statistical analyses

Genetic analyses

Mme7 is a sex-linked marker and it deviated from Hardy Weinberg Equilibrium (HWE), therefore, we coded it as a missing allele for females and juveniles in further analyses (Rasner et al., 2004; Rodríguez-

Bardía et al., 2022). We genotyped 76 individuals, 28 males from ‘period 1’, 25 males from ‘period 2’, 14 females from ‘period 1’, and 9 females from ‘period 2’. We calculated expected heterozygosity (H_e), observed heterozygosity (H_o), allelic richness (A_r), and inbreeding coefficients (F_{IS}) for each population and time period (e.g., MTV period 1 and MTV period 2). We estimated confidence intervals for F_{IS} values using 9999 bootstraps as implemented in the “hierfstat” package in R (R Core Team, 2020). To compare differences in heterozygosity for the same populations between time periods we used z-scores from a Wilcoxon signed rank test as implemented in the “coin” package in R (R Core Team, 2020). To estimate changes in genetic structure among populations over a decade, we calculated Nei’s coefficient (G_{st}) among populations within time periods, using 9999 bootstrap values to estimate confidence intervals. We assumed that nonoverlapping confidence intervals indicated significant differences in genetic structure between periods. We also tested for differences in genetic diversity between time periods using an analysis of molecular variance (AMOVA) with 9999 permutations using the “poppr” package in R (R Core Team, 2020). We clustered individuals in each time period into the most likely configuration of clusters based on allele frequency similarity using the Bayesian clustering algorithms implemented in the program STRUCTURE V.2.3.4 (Pritchard et al., 2000). To determine the most probable number K, we utilized a method implemented in a study that used data from some of the same populations (Rodríguez-Bardía et al., 2022). Thus, we used correlated allele frequencies and the admixture model with 300 000 Markov chains, and a burning of 30 000 chains. We grouped individuals in 1 to 6 clusters with 20 repetitions for each cluster (Rodríguez-Bardía et al., 2022). To determine the most likely number of K clusters we used Structure Harvester 0.6.94 (Evanno et al., 2005). After determining the most likely number of K cluster, we conducted another structure analysis with 1 000 000 Markov chains and a burn-in of 100 000 chains to assure proper chain mixture (Rodríguez-Bardía et al., 2022).

Acoustic analyses

To analyze the effect of time on acoustic measurements, we analyzed 10,707 solo songs. We used a principal component analysis (PCA) using the “FactoMineR” R package (Lê et al., 2008) to condense the five song measurements into three variables with eigenvalues > 1 , that explained 76% of total variance. The first principal component (PC1) correlated with duration and number of elements, the second principal component (PC2) correlated with minimum and maximum frequency, and the third principal component (PC3) correlated with the frequency of maximum amplitude (Table 1). We performed linear mixed models (GLMMs) using the “lme4” package (Bates et al., 2015) to determine the effect of populations and time periods on the variation of acoustic traits. Specifically, we included in the model two independent variables: Populations (four levels: MTV, HDA, UCR, and JBL), time periods (two levels: period 1 and period 2), and the interactions between both variables. Each of the three principal components (PC1, PC2, and PC3) of the song was included as a response variable in the model, and the territory inside each population as random factor. We additionally carry out *post hoc* tests when the model showed us significant differences on pairwise comparisons between the main effects and the two-factor interactions.

Morphological analyses

To analyze the effect of time on body measurements, we measured 87 individuals, 55 males and 32 females. We used principal component analysis (PCA) using “FactoMineR” to condense the six body measurements into two variables with eigenvalues > 1 , which together explained 60% of the total variance. The first principal component (PC1) correlated with tarsus length, tail length, wing chord length, and beak depth; the second principal component (PC2) correlated with exposed culmen length and beak width (Table 2). We performed a generalized linear mixed models (GLMMs) with a gamma error distribution, using “lme4”. In these analyses we included populations (MTV, HDA, UCR, and JBL), time periods (period 1 and period 2) and the interactions between both variables, as independent variables; each of the two principal components (PC1 and PC2) as the response variable, and the territory inside each population in which individuals were captured as random factor. We also performed models for females and males because males are larger than females (Sandoval & Mennill, 2013). We carried out post hoc tests when the model showed significant differences on pairwise comparisons between the main effects and the two-factor interactions.

Finally, to evaluate if divergence between different traits (i.e., genetic, acoustic, and morphology) is related,

we conducted a partial Mantel test to determine the strength of the correlation between variables using 10,000 permutations implemented in the “vegan” package in R (Oksanen et al., 2012). Females in this analysis were not considered because they do not sing (Sandoval and Mennill 2014; Sandoval et al. 2016). We used 46 males with data of the three variables. First, we performed a PCA to condense the six body measurements and another PCA to condense the five song measurements of the males. With the PCAs we then obtained acoustic and morphological distance matrices created from Euclidean distances of averaged population traits in time periods, and the genetic distance matrix from Gst values.

Results

Genetic diversity

At UCR, observed heterozygosity (H_o) was greater in ‘period 1’ than in ‘period 2’ ($z = 1.95$, $p = 0.05$), whereas the genetic diversity of the other populations did not differ between time periods (all comparisons: $z = 1.01$, $p > 0.05$) (Table 3). In Heredia, period 1 had a lower expected heterozygosity (H_e) than period 2 ($z = -2.37$, $p < 0.05$). In contrast, heterozygosity was lower in ‘period 1’ for the JBL population than in ‘period 2’ ($z = -2.37$, $p < 0.05$), whereas the other populations did not differ (all comparisons: $z = 0.68$, $p > 0.05$) (Table 3). HDA, JBL, and UCR showed significant inbreeding in ‘period 2’ (Table 3).

Genetic diversity was significantly structured in the metapopulation; however, structure did not differ between time periods: period 1 ($G_{st} = 0.07$, 95% CI = 0.06 and 0.09) and period 2 ($G_{st} = 0.07$, 95% CI = 0.06 and 0.09). AMOVA results showed that 12% of total genetic variation was partition among individuals in different time periods. There were significant differences in allele frequencies between periods (i.e., period 1 vs. period 2; $F_{ct} = 0.120$, $p < 0.05$), as well as among populations within time periods ($F_{sc} = 0.15$, $p < 0.001$). In both time periods, the Evanno method suggested that populations were likely grouped into two clusters (i.e., $K=2$). In ‘period 1’, all MTV individuals were assigned to cluster 1, while nearly all HDA, UCR, and JBL individuals were assigned to cluster 2 (Figure 1). In ‘period 2’ individuals were also grouped into $K = 2$ clusters, with individuals from MTV grouped in cluster 1, and the individuals from the other populations were almost equally distributed into clusters 1 and 2 (Figure 1).

Acoustic characteristics of songs

We found that the duration and number of elements (PC1) were affected by the time period and by the interaction between population and time period (Table 4). In MTV, songs increased in duration and number of elements in ‘period 2’ (Figure 2). The minimum and maximum frequency (PC2) were not affected by time, but it differed significantly among populations (Table 4). UCR had higher minimum frequency and lower maximum frequency than MTV and HDA; while JBL had lower minimum frequency and higher maximum frequency compared to other populations (Figure 2). The frequency of maximum amplitude (PC3) showed significant differences in the interaction population \times time period (Table 4). The songs increased the frequency of maximum amplitude in HDA period 2, relative to ‘period 1’, whereas UCR and JBL decreased in ‘period 2’ compared to ‘period 1’.

Morphology

We found a significant interaction between population and time period in females for tarsus length, tail length, wing chord length, and beak depth (PC1) (Table 5). These morphological variables represented by the PC1 decreased between periods 1 and 2 for MTV and JBL (Figure 3). The exposed culmen length and beak width (PC2) did not show significant differences (Table 5) among sites or time periods. For males we also found a significant interaction between population and time period for PC1 (Table 5). Tarsus length, tail length, wing chord length, and beak depth were smaller in JBL period 2 compared to period 1 (Figure 3). The exposed culmen length and beak width (PC2) were affected by the interaction between population and time period (Table 5). Birds in MTV period 2 had shorter exposed culmen and bigger beak width than in period 1, and birds in JBL period 2 had bigger exposed culmen length and smaller beak width than in period 1 (Figure 3).

Relationship between variables

When acoustic differences were controlled, the partial Mantel test revealed a non-significant association between genetic and morphological distances ($r = -0.06$, $p = 0.40$; Figure 4). Similarly, we found no significant correlation between morphology and acoustic distances, while accounting for genetic differences ($r = 0.24$, $p = 0.10$; Figure 4). However, we found a significant correlation between genetic distances and acoustic distances when morphological distances were accounted for ($r = 0.49$, $p < 0.05$; Figure 4).

Discussion

Our findings showed an increase in inbreeding, as well as changes in acoustic and morphological traits over a ten-year period, however only genetic distances and song divergence correlated. These results are likely explained by the interaction between changes in habitat quality and the barriers to gene flow produced by rapid urban development in the sampling area (Biamonte et al., 2011; Rodríguez-Bardía et al., 2022). Particularly considering the low mobility, isolation and specific habitat requirements of white-eared ground-sparrow (Delaney et al., 2010a; Rodríguez-Bardía et al., 2022; Soulé et al., 1988).

Genetics

We found an increase in inbreeding in three populations exposed to rapid urbanization in the Costa Rican Central Valley (HDA, JBL, and UCR). Inbreeding is caused by consanguineous matings, and its extent is proportional to a reduction in effective population sizes, or a reduction in the dispersal of individuals among populations (Frankham et al., 2002). Therefore, the increase in inbreeding could be explained by urban expansion which rapidly transforms natural ecosystems into homogenized environment that limits resources to small suboptimal patches of vegetation (Biamonte et al., 2011; Fahrig & Rytwinski, 2009; Miles et al., 2019). These drastic changes brought about by urbanization restrict the movement of birds between populations (Rodríguez-Bardía et al., 2022). Consequently, small and isolated populations increase the likelihood of mating with relatives (Wright et al., 2008). A similar increase in inbreeding associated with isolation and urban development was reported for the european treefrogs *Hyla arborea* (Andersen et al., 2004) and was also found in birds, the taita thrush *Turdus helleri* (Lens et al., 2000).

Our analysis found that spatial genetic structure is more affected by distance than by time. This result is consistent with the isolation by distance and the urban resistance to gene flow previously reported for this species (Rodríguez-Bardía et al., 2022). Additionally, this condition is typical for species with low mobility and specific habitat requirements, such as the white-eared ground-sparrow (Delaney et al., 2010b; Rodríguez-Bardía et al., 2022; Soulé et al., 1988). However, the Bayesian clustering suggests that some movement of individuals may still occur among populations. Therefore, our results suggest that we are witnessing the progressive decline of gen flow and genetic diversity over time.

The indirect evidence of limited gene flow found in our study, could be also a result of the interaction between philopatry, territoriality and the lack of suitable habitat for populations of ground-sparrows in an urban setting. Philopatry and territoriality keep individuals within a population, thereby decreasing gene flow and increasing genetic differentiation between populations (Bounas et al., 2018; Rodríguez-Bardía et al., 2022). In urbanized sites ground-sparrow males may increase their territories to search for additional food sources (Juárez et al., 2020). This foraging behavior could obligate males to migrate when resources are scarce. Thus, this behavior could explain to the movement of individuals showed in our Bayesian clustering.

On the contrary, individuals inhabiting isolated populations might develop local adaptations, such as different dialects and variations in the frequency and duration of songs, as shown for the white-eared ground-sparrow (Sandoval et al., 2014, 2015, 2016; Juárez et al., 2020; Méndez et al., 2021). These adaptations could further reduce individuals dispersal between populations, thus contributing to increase genetic differences among remaining populations (Morhina et al., 2017). For example, in white-crowned sparrows *Zonotrichia leucophrys*, the genetic structure was correlated with song dialects, because song dialects act as barriers for migrant males, increasing genetic structure of populations (MacDougall-Shackleton & MacDougall-Shackleton, 2001).

We only observed decline in genetic diversity in the population at the most urbanized location (H_o UCR period 1: 0.62 vs H_o UCR period 2: 0.32). This result is consistent with previous research indicating that

urbanization reduces genetic diversity in wren-tits *Chamaea fasciata*, side-blotched lizards *Uta stansburiana*, western skinks *Plestiodon skiltonianus*, and western fence lizards *Sceloporus occidentalis* inhabiting more isolated habitat patches (Delaney et al., 2010b). Therefore, cities and human-made structures are clearly acting as barriers to gene flow (Rodríguez-Bardía et al., 2022), that limits the movement of individuals among vegetation remnants within cities. So, in the span of a decade we were able to document a decrease in genetic diversity of this bird species in the most urbanized populations. Due to the small number of individuals captured at UCR during ‘period 2’ our findings must be interpreted with caution.

Acoustics

Differences in solo songs of white-eared ground-sparrows are concordant with previous studies which reported acoustic differences as a possible adaptation to urban noise (Méndez et al., 2020; Sandoval et al., 2016; Sandoval & Mennill, 2014; Bonilla-Badilla 2021). However, we additionally discovered a correlation between acoustic and genetic distances while controlling for morphology. These results reaffirmed the urban development as an important barrier among populations, that isolate and limit the movements of individuals (Rodríguez-Bardía et al., 2022; Sosa-López et al., 2013). The same relationship between genetic and acoustic distances has been reported in populations of rufous-naped wren *Campylorhynchus rufinucha* which lost connectivity by an historically isolation due to a marine barrier during the formation of the Isthmus of Tehuantepec in the late Pleistocene (Vázquez-Miranda et al., 2009). The correlation between genetic and acoustic distances, and the pattern of isolation by distance previously reported in the white-eared ground-sparrow (Rodríguez-Bardía et al., 2022), suggest an important role of cultural drift or sexual selection (Camacho-Alpízar et al., 2018; Irwin et al., 2008; West-Eberhard, 1983). White-eared ground-sparrow uses solo songs for female attraction, and males, after learning songs are not able to learn new songs (Sandoval et al., 2016; Bonilla-Badilla 2021). Thus, if females are choosing specific acoustic traits, specific song types or young males are imitating specific songs in each population, a specific phenotype would be favored over generations (West-Eberhard, 1983). For example, female great tits, *Parus major*, prefer males that emit song types with higher frequencies in noisy environments (Halfwerk et al., 2011). In this context the higher frequencies in urbanized populations and the different dialects among populations of white-eared ground-sparrows (Méndez et al., 2020; Sandoval et al., 2016; Sandoval & Mennill, 2014; Bonilla-Badilla 2021), could be reflecting sexual selection acting over populations.

Morphology

The female body size of white-eared ground-sparrow decreases in MTV and JBL as well as males of JBL population along the sampling periods. Body size often decreases in response to a decrease in food availability and quality (Goodman et al., 2012; Salewski et al., 2014; Yom-Tov & Geffen, 2011). Introduced species such as rats and mice may compete for food resources of our study species. A similar reduction in body size was observed in the lesser sheathbills *Chionis minor*, in Marion Island, due to the introduction of rats and cats that eat terrestrial macro-invertebrates, the sheathbills primary food resource (Huyser et al., 2000). In white-eared ground-sparrows reduction in body size may also be caused by lower-quality food as a result of urbanization (Mennechez & Clergeau, 2006). Higher predation risks could also play an important role in size reduction, because smaller individuals may be better able to avoid cats and other predators (Seress et al., 2011). Given that sparrows are a common prey of cats (Seress et al., 2011; Yom-Tov & Geffen, 2011), this would be an important adaptation (or selection process) for ground-sparrows facing urbanization.

In JBL males increased the exposed culmen length and decreased beak width between sampling periods. Due to the widespread use of bird feeders, the trend toward longer beaks has been linked to the consumption of larger prey or generalized feeding (Bosse et al., 2017; Hüppi & Geiger, 2022; Rolshausen et al., 2009). In great tits, *Parus major*, an increase in birds with longer beaks was associated with an increase in fitness, because birds were able to feed from bird feeders (Bosse et al., 2017). A decrease in beak width reflects a decrease in bite force (Badyaev et al., 2008). For example, the house finches *Haemorhous mexicanus* that inhabit urban populations in Arizona have a narrower beak, and less bite force, because they consume softer human food. (Badyaev et al., 2008; de León et al., 2011; Hüppi & Geiger, 2022). In contrast, in MTV males decrease their exposed culmen length and increase their beak width. The increase in beak width reflect that

birds are likely consuming larger and tougher food, which would require an increase in bite force (de León et al., 2011; Hüppi & Geiger, 2022). Differences in beak lengths between populations may also be the result of individuals consuming novel foods, regardless of the morphology of the species; indicating adaptation toward exploiting different food resources (de León et al., 2011; P. R. Grant & Grant, 2002).

Comparing four populations of white-eared ground-sparrows over a decade revealed changes in levels of inbreeding, spectrotemporal song characteristics, and morphological traits. Interestingly, we only found a positive relationship between differences in genetic divergence among populations and song divergence. We suggest that this correlation corroborates the role of urbanization as a barrier for white-eared ground-sparrows. The correlation may also be explained by cultural drift or sexual selection, as it is possible to observe changes in acoustic signals associated with genetic divergence if selection for song preferences acts continuously in populations (Irwin et al., 2008; Sathyan et al., 2017). The relationship between genetic and phenotypic traits is often slow and complex, due to the different forces acting at the same time (Irwin et al., 2008; Carnicer et al., 2009; Sathyan et al., 2017). Only long-term studies in urban environments shed light on the processes of adaptation of different species to the drastic changes imposed by urbanization, since species with different life history traits may response and adapt in different ways to the same environmental changes (Rodríguez-Bardía et al., 2022).

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Table 1. Results of principal components analysis of solo songs of the white-eared ground-sparrow *Melozona leucotis* . The asterisk indicates the variables that have a larger contribution in each component.

Song measurements	Principal component	Principal component	Principal component
	PC1	PC2	PC3
Duration (s)	0.82*	0.25	-0.09
Minimum frequency (kHz)	-0.26	0.78*	0.24
Maximum frequency (kHz)	0.55	-0.58*	0.30
Frequency of maximum amplitude (kHz)	0.11	0.09	0.93*

Song measurements	Principal component	Principal component	Principal component
Number of elements (n)	0.71*	0.43	-0.18

Table 2. Results of principal components analysis of body measurements of white-eared ground-sparrow *Melozone leucotis*. The asterisk indicates the variables that have a larger contribution in each component.

Body measurements	Principal component	Principal component
	PC1	PC2
Tarsus length (mm)	0.73*	0.28
Tail length (mm)	0.77*	0.13
Wing chord length (mm)	0.75*	-0.15
Exposed culmen length (mm)	0.02	0.83*
Beak width (mm)	0.38	-0.75*
Beak depth (mm)	0.65*	0.11

Table 3. Mean \pm SD of observed heterozygosity (H_o), expected heterozygosity (H_e), inbreeding coefficient (F_{is}), and allelic richness (A_r) for each population assessed in two periods of time (2011-2012 and 2018-2022) for white-eared ground-sparrow *Melozone leucotis*. In parentheses the number of females in each population.

Population	Time period	n	Ho	Ho	He	He	Fis	Fis	Ar
			Mean	SD	Mean	SD	Mean	SD	
Monteverde	2011-2012	12 (4)	0.64	0.09	0.60	0.09	-0.09	0.05	3.16
Monteverde	2018-2022	7(2)	0.54	0.11	0.58	0.11	0.09	0.07	3.09
Heredia	2011-2012	7(3)	0.59	0.12	0.49	0.10	-0.20	0.05	2.72
Heredia	2018-2022	11(3)	0.59	0.10	0.71	0.05	0.18	0.12	3.59
Lankester	2011-2012	11(5)	0.55	0.08	0.52	0.08	-0.07	0.09	2.64
Lankester	2018-2022	12(4)	0.57	0.11	0.68	0.06	0.22	0.12	3.48
UCR	2011-2012	12(2)	0.62	0.13	0.50	0.11	-0.26	0.05	2.76
UCR	2018-2022	4	0.32	0.12	0.59	0.07	0.48	0.20	3.14

Table 4. Comparison of acoustic characteristics of solo songs between two periods of time (2011 – 2012, 2018 – 2022) in four populations, and their interaction of white-eared ground-sparrow *Melozone leucotis*, based on linear mixed-effects models. PC1 correlates with the duration and number of elements, PC2 correlates with the minimum and maximum frequency, and PC3 correlates with the frequency of maximum amplitude.

	PC1	PC1	PC1	PC2	PC2	PC2	PC3	PC3	PC3
	DF	F	P	DF	F	P	DF	F	P
Population	3	1.67	0.18	3	10.58	< 0.001	3	1.90	0.13
Time period	1	8.84	< 0.05	1	2.86	0.09	1	1.38	0.24
Population \times time period	3	2.73	< 0.05	3	1.04	0.38	3	3.65	< 0.05

Table 5. Comparison of body measurements in two periods of time (2011 – 2012 vs 2018 – 2022) in four populations, and their interaction of white-eared ground-sparrows *Melozone leucotis*, based on generalized linear mixed models. PC1 correlates with tarsus length, tail length, wing chord length, and beak depth, PC2 correlates with exposed culmen length and beak width.

	Females	Females	Females	Females	Females	Females	Males	Males	Males	Mal
	PC1	PC1	PC1	PC2	PC2	PC2	PC1	PC1	PC1	PC2
	X2	DF	P	X2	DF	P	X2	DF	P	X2
Population	0.75	3	0.86	1.72	3	0.63	2.28	3	0.52	0.62
Time period	0.24	1	0.63	1.25	1	0.26	0.001	1	0.97	0.00
Population \times time period	31.73	3	< 0.001	2.68	3	0.44	27.14	3	< 0.001	10.1

Figure legends

Figure 1. STRUCTURE barplots for (a) 2011 – 2012 populations of white-eared ground-sparrow *Melozone leucotis* into $K = 2$, and (b) 2018 – 2022 populations of white-eared ground-sparrow *Melozone leucotis* into $K = 2$. Plots are sorted by sampling location, each bar represents one individual, each color represent a cluster. MTV Monteverde, HDA Heredia, UCR Universidad de Costa Rica, JBL Jardín Botánico Lankester.

Figure 2. Box-plots of the variation in acoustic characteristics of white-eared ground-sparrow *Melozone leucotis*, based on linear mixed-effects models. The responses are measured as principal components scores. summarizing. (a -b) Duration and number of elements correlated with PC1, © The minimum and maximum frequency correlated with PC2, and (d) The frequency of maximum amplitude correlated with PC3. The box-plot shows the median (central horizontal line), 75th and 25th percentile (top and bottom of the box) and the maximum and minimum values (top and bottom whisker). Letters indicate the values are significantly different from each other in post hoc tests, and lack of letters mean that no significant differences were found in the post hoc tests.

Figure 3. Variation in morphology of white-eared ground-sparrow *Melozone leucotis*, based on generalized linear mixed models. The responses are measured as principal components scores; summarizing (a) tarsus length, tail length, wing chord length, and beak depth correlated with PC1 of females, (b) tarsus length, tail length, wing chord length, and beak depth correlated with PC1 of males, (c) the exposed culmen length and beak width correlated with PC2 of males. The box-plot shows the median (central horizontal line), 75th and 25th percentile (top and bottom of the box) and the maximum and minimum values (top and bottom whisker). Letters indicate the values are significantly different from each other in post hoc tests, and lack of letters mean that no significant differences were found in the post hoc tests.

Figure 4. Correlations between different sets of variables in white-eared ground-sparrow *Melozone leucotis*. (a) Song and morphology distances, (b) morphology and genetic distances, and (c) song and genetic distances. Linear regressions are shown, but statistical significance was evaluated using Mantel tests.

Data Accessibility

Cueva, L., Fuchs, E. J., Gilbert, B., Madrigal-Brenes, R., & Sandoval, L. (2024). Data from: Effect of spatial and temporal urban isolation on the genetic diversity, acoustic variation, and morphological characteristics of an urban survivor bird species. Zenodo. <https://doi.org/10.5281/zenodo.10472250>

Competing interests

The authors declare no competing interests.

Author Contributions

L.C., E.J.F., G.B., and L.S. designed the study. L.C. and L.S. collected the samples. L.C. and R.M.-B. performed lab work. L.C., E.J.F. and L.S. performed statistical and genetic analyses. L.C. drafted the manuscript. All authors contributed, completed, and approved the final manuscript.

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