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SISTEMA DE ESTUDIOS DE POSGRADO

EFECTO DEL AISLAMIENTO URBANO ESPACIAL Y TEMPORAL EN LA DIVERSIDAD GENÉTICA, CARACTERÍSTICAS ACÚSTICAS Y MORFOLÓGICAS DE *MELOZONE LEUCOTIS* (AVES: PASSERELLIDAE).

Tesis sometida a la consideración de la Comisión del Programa de Estudios de Posgrado en Biología para optar al grado y título de Maestría Académica en Biología

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Dedicatoria

Este trabajo lo dedico a mis padres por todo el apoyo y amor que me han brindado, por todo el esfuerzo realizado para formarme como persona y profesional, además a mi hermana uno de los más grandes amores de mi vida.

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Tabla de contenido

Resumen

La urbanización genera alteraciones en los ecosistemas, incluyendo la fragmentación de los hábitats naturales, lo que produce el aislamiento de poblaciones de una misma especie. Como consecuencia, se espera que el flujo de genes entre poblaciones aisladas se reduzca asociado con la distancia y el tiempo. Mientras tanto, las vocalizaciones y las características morfológicas pueden cambiar si los hábitats restantes cambian en estructura, densidad o composición de la comunidad. Sin embargo, la relación entre genética, vocalizaciones y divergencia morfológica en áreas urbanas a lo largo del tiempo sigue siendo poco conocida. En este estudio se evaluó, el efecto del tiempo sobre la diversidad genética, las características morfológicos y acústicas en una especie evitadora urbana, el gorrión orejiblanco (Melozone leucotis), además se evaluó si los cantos comunes en cada hábitat se transmiten mejor en su hábitat respectivo. Compararé la estructura genética, las características del canto masculino y la morfología en cuatro poblaciones con diferente desarrollo urbano durante un período de 10 años en Costa Rica. Realizaré experimentos de transmisión de sonido en cada población con los cantos comunes de las poblaciones. Encontramos que únicamente existe una relación positiva entre los cambios en la diversidad genética y las características acústicas de canto y que los cantos comunes que son emitidos en cada población no se transmiten mejor en su respectiva población.

Lista de tablas

Capítulo 1

Capítulo 2

Table 2. Main effects for the linear mixed models analyzing comparisons of background noise during the transmission experiments.

Lista de figuras

Capítulo 1

lack of letters on top of the whiskers mean that no significant differences were found in the post hoc tests.

Figure 4. Correlates 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

Capítulo 2

Figure 1. Spectrograms of type of solo songs of White-eared Ground-sparrows used in the transmission experiments.

Figure 2. Variation in four measurements of sound degradation according to playback site, source population, distance and microphone heights used in the transmission experiments. Error bars are standard errors of the mean, and bars with different letters indicate the values are significantly different from each other in post hoc tests.

Figure 3. Second order interactions between playback site and source population for the most common solo songs of White-eared ground-sparrow. The letters below the bars refer to the playback site and the letters below the set of playback sites refer to the source population. Error bars are standard errors of the mean, and bars with different letters indicate the values are significantly different from each other in post hoc tests.

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Introducción

Los seres humanos hemos colonizado casi todos los ecosistemas de la tierra en un periodo muy corto de tiempo. En 1900 solo un 10% de la población mundial vivía en ambientes urbanos (Grimm et al., 2008), pero desde el 2018 el 55% de la población vive en áreas urbanizadas (ONU, 2018). Esto ha generado alteraciones en los ciclos de los nutrientes, el clima, los hidrosistemas y la biodiversidad en hábitats naturales (Grimm et al., 2008). Lo que conlleva a un recambio de especies, de tal manera que especies generalistas incrementan sus poblaciones dentro de áreas urbanas, ya que son individuos que pueden adaptarse a una gran cantidad de hábitats, ingerir casi cualquier tipo de alimento y tolerar muy bien la urbanización (Leveau & Zuria, 2017; Meineke et al., 2013). Caso contrario, especies especialistas disminuyen o desaparecen de las zonas urbanas ya que necesitan de un alimento o hábitat específico para subsistir (Leveau & Zuria, 2017; Meineke et al., 2013).

La combinación de barreras, impuestas por la expansión urbana y la pérdida de bosques, producen la fragmentación de hábitats naturales (Jirón & Mansilla, 2014), promoviendo la división y aislamiento de poblaciones (Fahrig & Rytwinski, 2009). Como resultado se reduce el movimiento de individuos y disminuye el intercambio de genes (Lynch & Baker, 1994), que podría traer consecuencias desfavorables en la supervivencia y adaptación de la especie al medio (Ralls et al., 2018). En un estudio en el sur de California donde analizaron el efecto de la fragmentación por el rápido avance de la urbanización en cuatro especies comunes del lugar, tres lagartijas y un ave, encontraron diferencias genéticas significativas en las poblaciones de las cuatro especies, asociadas al tiempo de aislamiento del parche, así como a la presencia de carreteras y autopistas (Delaney et al., 2010).

En poblaciones aisladas, también se ha encontrado cambios en el comportamiento (ej.: vocalizaciones) y morfología (ej.: tamaño de picos y masa) entre poblaciones, por presiones selectivas locales como el tipo de hábitat, tipos de alimento, o selección sexual (Laiolo & Arroyo, 2011). En especies donde el canto es aprendido de otros individuos de la misma población, se puede encontrar diferencias en la frecuencia, duración y estructura de los cantos entre poblaciones (Sandoval & Mennill, 2014), que se dan debido a los procesos relacionados al aprendizaje (Lynch & Baker, 1994) o innovaciones en las características del canto (Slater, 1986). Por otro lado, un buen ejemplo en la variación de la morfología del pico debido al tipo de recurso alimenticio se puede ver en los pinzones de Darwin (Grant & Grant, 2006; Reaney et al., 2020) donde existen desde picos muy pequeños como la del pinzón cantor (*Certhidea olivácea*) cazador de insectos, hasta picos grandes y robustos como el pinzón terrero grande (*Geospiza magnirostris*) adaptado para consumir semillas (Sakamoto et al., 2019; Tebbich et al., 2004).

Variación genética

Los efectos del aislamiento urbano sobre la perdida de diversidad genética se dan por factores espaciales, debido a que la distancia entre poblaciones juega un papel fundamental en el movimiento de genes (Miles et al., 2019; Whitlock, 2004) y temporales, considerado como el tiempo (en años) de aislamiento de las poblaciones donde se ha ido perdiendo diversidad genética a lo largo de las generaciones (Keller & Waller, 2002). Por ende las especies que habitan dentro de los parches de vegetación natural, remanente en ambientes urbanos pueden presentar cambios en las frecuencias alélicas (Delaney et al., 2010). Por ejemplo, El tuatara (*Sphenodon punctatus)* en isla Stephens Nueva Zelanda mostró diferencia genética en poblaciones ubicadas a 500 metros una de otra (Moore, 2008). En mamíferos como linces (*Lynx rufus)* en California EEUU se ha reportado divergencia genética en gradiente urbano (Smith et al., 2020). Cabe recalcar que la distancia entre poblaciones puede ser irrelevante cuando una barrera es muy eficiente en el aislamiento de poblaciones (Marsh et al., 2008). De esta manera se ha reportado dos grupos genéticos de ~~s~~alamandras (*Plethodon cinereus*) en Virginia EEUU, a cada lado de una carretera de 104 metros de ancho, construida hace aproximadamente 35 años (Marsh et al., 2008). En este contexto queda claro que los cambios en el paisaje debido a la urbanización tienen efectos sobre los patrones de flujo y diferenciación genética de las especies (Moore et al., 2008; Smith et al., 2020).

Las aves aunque son un grupo que tiene facilidad de movilización por su capacidad de volar, y pueden disminuir el efecto de la fragmentación, hay especies que se mueven distancias cortas, tienen territorios pequeños o son dependientes de hábitat específicos (Delaney et al., 2010), por lo que son más susceptibles al efecto del aislamiento por urbanización (Whitlock, 2004). Por ejemplo, poblaciones del Carbonero común (*Parus major*) en parques ubicados a distancias entre 100 m y 5500 m, en Barcelona España presentan diferencias genéticas entre parques (Björklund et al., 2010). En el Herrerillo chochín (*Chamaea fasciata)* se encontró alta divergencia génica entre poblaciones separadas por una carretera y una franja de desarrollo residencial en el sur de California (Delaney et al., 2010). Lo que deriva en la consecuente pérdida de la diversidad genética y la adaptabilidad de las especies aisladas (Ralls et al., 2018).

Los efectos genéticos del aislamiento a nivel temporal en aves pueden comenzar a manifestarse en periodos de 40 años o menos (Delaney et al., 2010). En *Chamanea fasciata* se encontró estructura genética entre poblaciones ubicadas en parches aislados de 13 a 43 años (Delaney et al., 2010). En Washington, EEUU se encontró relación en la diferencia de la diversidad genética del Gorrión melódico (*Melospiza melodia*) con el inicio de la expansión metropolitana en los años 60 (Unfried et al., 2013). Proporcionando evidencia de que el tiempo de desarrollo en el paisaje urbano está relacionado con los cambios genéticos del gorrión melódico, debido al tiempo en ausencia de migración (Unfried et al., 2013). Los casos expuestos sugieren que la variación genética en ambientes urbanos es común tanto a nivel espacial como temporal (Björklund et al., 2010). Sin embargo, esta clase de estudios genéticos son escasos en zonas tropicales, a pesar de ser lugares que incluyen una alta biodiversidad y un rápido crecimiento urbano (Miles et al., 2019; Ortega-Álvarez & MacGregor-Fors, 2011)

Variación acústica y morfológica

La variación acústica entre poblaciones se da como respuesta a las diferentes características acústicas de cada hábitat y al aumento del ruido antropogénico (Luther & Derryberry, 2012; Slabbekoorn & Smith, 2002), ya que la comunicación se ve limitada por factores bióticos y abióticos propios de cada sitio y de las propiedades de transmisión del sonido relacionadas con el clima y la vegetación (Longman & Jenik, 1992). En ambientes urbanos la principal fuente de ruido es producida por los altos niveles de tráfico, la presencia constante de personas, casas y edificios (Croci et al., 2008). Este ruido generalmente es de baja frecuencia (<3 kHz), y puede enmascarar las señales acústicas de la fauna que vive en estos entornos (Brumm, 2004; Warren et al., 2006), lo que potencialmente dificulta que los animales defiendan territorios, atraigan a las hembras, o que las señales de alarma y auxilio sean correctamente percibidas (Sun & Narins, 2005).

Para solventar el enmascaramiento las aves que habitan ambientes urbanos adaptan sus señales acústicas para transmitirlas mejor en su medio (Boncoraglio & Saino, 2007). Por ello, entre poblaciones se puede encontrar variación en la amplitud, frecuencia o duración de los cantos o llamadas (Brumm, 2004; Warren et al., 2006). Cada uno de estos cambios en las señales acústica pueden suceder por respuestas fenotípicas por medio de variaciones acústicas aprendidas y transmitidas entre generaciones, o por respuestas evolutivas dadas por selección natural (Boyd & Richerson, 1985; Warren et al., 2006), por lo que mientras más tiempo las poblaciones están aisladas entre sí, más tienden a divergir (Lynch & Baker, 1994).

Por ejemplo, en poblaciones urbanas de la rana *Hylarana taipehensis,* en Tailandia, se reportó que para evitar ser solapadas por el ruido antropogénico, producen frecuencias más altas en las notas y aumentan la tasa de repetición de las llamadas (Sun & Narins, 2005). En el Pinzón orejiblanco (*Melozone leucotis*) en Costa Rica se ha visto un aumento en la frecuencia mínima en sus cantos, una disminución de la frecuencia máxima en los duetos, y aumento en la duración de sus cantos cuando aumenta el ruido antropogénico (Méndez et al., 2019). Con poblaciones del gorrión de corona blanca (*Zonotrichia leucophrys*) en California, donde se evaluaron cantos de 1969 y 2005 (Luther & Derryberry, 2012), se encontró un aumento en las frecuencias mínimas y máximas del canto. Además, diferencias en las respuestas de los individuos de la población a los cantos actuales vs los cantos antiguos, produciendo más cantos y acercándose más al lugar de donde se reproducían los cantos contemporáneos (Luther & Derryberry, 2012). Finalmente, en un estudio se analizaron grabaciones archivadas de 529 especies de aves de varios países, y encontraron que los individuos que residen en ambientes urbanos usan frecuencias más altas que las especies que no habitan la urbe (Hu & Cardoso, 2009).

Por su parte el tamaño corporal y tamaño del pico, son variables que se encuentran significativamente relacionadas con la variación de las características acústicas de los cantos (ej.: frecuencia y duración), ya que si estas variables físicas empiezan a cambiar en un individuo la capacidad del ave para modular las configuraciones del tracto vocal durante el canto podría limitar la producción de algunas frecuencias (Foster et al., 2008; Podos, 2001). Esto se puede ver reflejado en poblaciones del Pinzón de pico mediano (*Geospiza fortis*) en Galápagos, donde existen poblaciones con dos morfotipos de pico (pequeño y grande) en los que también difieren las frecuencias de los cantos según el tamaño del pico (Hendry et al., 2009).

Al igual que la morfología, la genética de un individuo está estrechamente relacionada a las señales de comunicación acústica (Podos, 2001; Shaw & Lesnick, 2009; Shipilina et al., 2017), ya que las poblaciones que se encuentran geográficamente aisladas tienden a diferir en el canto debido a que las hembras usualmente eligen parejas en función del canto propio de su población (Freeman & Montgomery, 2017), lo que conlleva a que los cambios genéticos ocurran en conjunto con las señales acústicas a medida que las poblaciones divergen (Shaw & Lesnick, 2009). Por lo que es importante tomar en cuenta estas variables (genética, morfología y vocalizaciones) en conjunto para entender los efectos que produce el aislamiento de una especie a través del tiempo.

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Capítulo 1

Introduction

Fast urban development results in environmental changes (Grimm et al., 2008; Johnson & Munshi-South, 2017), such as the transformation of natural habitats into small patches of vegetation surrounded by buildings, road construction, and pollution (e.g., noise, light or solid) within cities (Fahrig & Rytwinski, 2009; Biamonte et al., 2011). This reduction of natural habitats generates isolated populations, because urban development is a barrier for the dispersion and movement of individuals between populations (Lynch & Baker, 1994; Crooks et al., 2004). As a result, the genetic diversity of populations is affected by reduced gene flow among remnant populations (Lynch & Baker, 1994; Johnson & Munshi-South, 2017). Changes in behavior have also been reported as a product of population isolation (e.g., vocalizations, nests, predator responses) as well as morphological changes (e.g., bill size and mass) due to local selective pressures (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; Méndez et al., 2021; Geffroy et al., 2020; Corrales-Moya et al., 2021)

The loss of genetic diversity as a result of urban isolation is mainly caused by the increase in distance between populations and the effect of drift over time (Whitlock, 2004; Miles et al., 2019). The more time has elapsed since fragmentation, a greater effect of drift is expected on changes in allele frequencies, the likelihood of allele fixation, and the magnitude of inbreeding withing populations (Keller & Waller, 2002; Johnson & Munshi-South, 2017). In wrentits *Chamaea fasciata*, significant genetic structure was found when compared populations separated by a highway and a strip of residential development in southern California (Delaney et al., 2010). In the song sparrow *Melospiza melodia,* researchers found higher differences in genetic diversity between populations that existed prior to the metropolitan expansion in 1960s compared to sparrow populations inhabiting recently developed urban sites in Washington (Unfried et al., 2013). These results show that urbanization has the potential to reduce genetic diversity and increase genetic structure in bird populations.

The patterns of the genetic structure generated by isolation are similar to those observed in patterns of acoustic variation in multiple bird species, as has been shown in white-bellied shortwing *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). Acoustic variations or polymorphism among populations occurs in response to different habitat characteristics (e.g., vegetation density or noise levels) to improve sound transmission and communication in different environments (Morton, 1975; Boncoraglio & Saino, 2007; Ey & Fischer, 2009; Hardt & Benedict, 2021). Acoustic variation may also occur via female selection where differences in female choice among populations may lead to differences in song characteristics (Searcy & Andersson, 1986; Laiolo & Arroyo, 2011). Finally, acoustic variations may be the result of acoustic drift, where a small group of individuals may randomly develop different repertories (Baker & Cunningham, 1985; Wilkins et al., 2013). For example, for several bird species that inhabit both urban and rural environments, songs from urban populations had higher frequencies than populations from birds inhabiting rural environments (Hu & Cardoso, 2009; Luther & Derryberry, 2012; Méndez et al., 2021). Finally, in populations of Darwin’s finches showed acoustic drift, because inaccurate imitation triggered geographic variation among islands (Grant & Grant, 1996). These changes in songs can be maintained or increase over the time, because songs are either learned and/or transmitted over generations (Boyd & Richerson, 1985; Warren et al., 2006). Therefore. the concordance between genetic and vocal variation might result when these changes in the song help the individuals to survive and breed, favoring a specific genotype inside each population (Danielson-François et al., 2006; Irwin et al., 2008).

Variation of the song acoustic characteristics (i.e., frequency and duration), also has been related with individuals' body and beak size (Podos, 2001, 2010), because bigger birds and longer beaks tend to produce songs with lower frequencies (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); but in greenish warblers *Phylloscopus trochiloides*, dark eyed juncos *Junco hyemalis*, and in black-bellied wrens *Pheugopedius fasciatoventris* the opposite pattern was found (Logue et al., 2007; Cardoso et al., 2008; Irwin et al., 2008). In the medium-billed finch *Geospiza fortis* in Galapagos, individuals of the large beak morphotype had songs with lower frequencies and lower trill rates, meanwhile individuals of small beak morphotype had songs with higher frequencies and faster trill rates (Podos, 2001; Huber & Podos, 2006).

In Costa Rica, the White-eared ground-sparrow *Melozone leucotis,* inhabits 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 thus gene flow between populations, which resulted in significant genetic structuring among Costa Rican populations (Rodríguez-Bardía et al., 2022). Studies also have found the occurrence of differences in song dialects between populations (Sandoval et al., 2014, 2016; Bonilla-Badilla 2021), besides differences in the frequency and duration of songs between populations was linked to anthropogenic noise levels, where males increased the minimum frequency and the duration of their song in populations with higher noise levels

(Sandoval et al., 2014, 2015, 2016; Méndez et al., 2021; Juárez et al., 2020; 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 whether these traits change in time with population dynamics.

The main objective of this study is to describe the changes in genetic diversity, vocalization, morphology and their relationship over the time in populations of white-eared ground-sparrow isolated by urban development. We expect that the genetic diversity, the acoustic characteristics of the song and the morphology will be correlated with the time in isolation of the population, because the most remote population will have less genetic diversity. so that, the longer the isolation time, the greater difference will be found between the populations.

Material and Methods

We conducted this study in four populations of White-eared Ground-sparrows in Costa Rica: (1) Estación Biológica Monteverde, Puntarenas province (10° 18′ N, 84° 48′ W, 1600 m.a.s.l), 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, Heredia province (10 ° 01’N, 84 ° 05’W, 1350 m.a.s.l); it is a non-urbanized site, with secondary forests and abandoned coffee plantations, and dense thickets with little human presence (Juárez et al. 2020). Urbanization started to occur in the edges of the study area since 2000. (3) The Universidad de Costa Rica, San José province (09 ° 56'N, 84 ° 05'W, 1200 m.a.s.l); is a highly urbanized site, with secondary forest reserve surrounded by buildings, open areas and gardens, and it is exposed to human disturbance (Juárez et al., 2020). Urban development ocurred before 1900’s but most fragmentation occurring after the 1970´s (Biamonte et al., 2012). (4) Jardín Botánico Lankester, Cartago province (9 ° 50'N, 83 ° 53'W, 1370 m.a.s.l); it is a sparsely urbanized area, with a mix of secondary forests, gardens, and buildings, with little disturbance and human presences (Juárez et al. 2020). This site, urbanization occurred after 1990.

Historical vs current sampling

We used acoustic and morphological data collected in 2011 and 2012 that was deposited in databases from the Laboratorio de Ecología Urbana y Comunicación Animal (LEUCA), of the Universidad de Costa Rica, as part of a long-term study of the effect of urbanizationon bird behavior divergence. For genetic data, we used blood samples collected in 2011 – 2012. White-eared ground-sparrows captures were done from 2018 – 2022 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. We collected 10 µL of blood sample using brachial vein method and stored it in 95% ethanol or Lysis buffer for molecular analyses (Seutin et al., 1991; Rodríguez-Bardía et al., 2022). Procedures were conducted in accordance with the current laws in Costa Rica. Research permits and animal handling protocols were approved by the Research Committee of Biology School and by the animal Care Committee (ACC) of Universidad de Costa Rica to LS how is the principal investigator.

We conducted focal recordings from 2018 – 2022 of each banded pair using a Marantz PMD661 digital recorder and a Sennheiser ME66/K6 unidirectional microphone. The recordings were done 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).

DNA extraction and SSR amplification

We extracted DNA from blood using the DNeasy blood and tissue Kit (Qiagen Inc., Valencia, CA, USA) following the manufacturer's protocol. We used seven primers: Mme2, Mme7, Mme8, Asµ15, Escµ6, Gf01 y Gf05 (Petren, 1998; Jeffery et al., 2001; Bulgin et al., 2003) which have been shown to be polymorphic and amplify well for this species (Rodríguez-Bardía et al., 2022). To amplify the described SSRs we used the procedure detailed in the Qiagen multiplex master kit (Qiagen Inc., Valencia, CA). All markers were amplified through Polymerase Chain Reactions (PCR) using 10 µL reaction, containing 2 µL of 0.4 µM primer mix, 1.5 µL of 20 ng of template DNA, 5 µL of Multiplex Master Kit (Qiagen) for mixed primers and Top Taq Master Kit (Qiagen) for Gf01 and 1.5 µL of water nuclease free (Qiagen). We follow 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 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 (Méndez et al., 2020; Sandoval et al., 2014, 2015, 2016). 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. The used the threshold method, where different windows will be analyzed such as: the spectrogram, which was used to identify and classify sounds, the power spectrum, which served to measure frequency characteristics with a threshold of -30dB related to vocalization, finally the oscillogram to measure the temporal characteristics of sounds (Podos, 1997). Spectrograms were constructed using a Hann window with window size of 512 samples, 3 dB bandwidth of 124 Hz, Time grid: Overlap of 50%, hop size of 5.80 ms. frequency grid spacing: 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 sole of foot), 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

Mme7 is a sex-linked marker and it deviated from Hardy Weinberg Equilibrium (HWE) (Rodríguez-Bardía et al., 2022). For that reason, to use in further analysis we coded as missing allele as missing for females and juveniles (Rasner et al., 2004; Rodríguez-Bardía et al., 2022). To estimate genetic variation during the time we genotyped 76 individuals, 53 males and 23 females. We calculated expected heterozygosity (H_e), observed heterozygosity (H_o), allelic richness (A_r), and inbreeding coefficients (F_{IS}) per population in a decade (e.g., MTV 2011-2012 and MTV 2018-2022). Confidence intervals for FIS values were estimated using 9999 bootstraps as implemented in the “hierfstat” package in R (R Core Team, 2020). To assess differences in heterozygosity between populations in a decade we performed Wilcoxon signed rank test with z-scores using the “coin” package in R (R Core Team, 2020). To estimate genetic structure among populations in a decade, we calculated Nei's coefficient (G_{st}) using 9999 bootstrap values to estimate confidence intervals. Also, we tested for differences in genetic diversity between 2011 – 2012 and 2018 – 2022 with an analysis of molecular variance (AMOVA) with 9999 permutations using the “poppr” package in R (R Core Team, 2020). Populations were clustered in each time periods using the Bayesian clustering algorithms implemented in the STRUCTURE V.2.3.4 program (Pritchard et al., 2000). Correlated allele frequencies and the admixture model was used 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 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 a proper mixture of the chains (Rodríguez-Bardía et al., 2022).

To analyze the effect of time on the acoustic measurements, we analyzed 10,707 solo songs. We used 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) reflects duration and number of elements, the second principal component (PC2) reflects minimum and maximum frequency, and the third principal component (PC3) reflects the frequency of maximum amplitude (Table 1). We performed linear mixed models (LMMs) using the “lme4” package (Bates et al., 2015). There were two independent variables: Populations (three levels: Monteverde, Heredia UCR, and Lankester), time periods (two levels: historic and current), and the interactions between both variables. The response variables were the three principal song components (PC1, PC2, and PC3). As a random effect we included the territory inside each population in which each individual was recorded. Each response variable was analyzed in a separate LMM. We carry out *post hoc* tests on all pairwise comparisons between the main effects and the two-factor interactions

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, that together explained 60% of total variance. The first principal component (PC1) reflected tarsus length, tail length, wing chord length, and beak depth, the second principal component (PC2) reflected exposed culmen length and beak width (Table 2). We performed a generalized linear mixed model (GLMMs) with a gamma distribution, using “lme4”. There were two independent variables: Populations (Monteverde, Heredia, UCR, and Lankester), time periods (historic and current) and the interactions between both variables. The response variables were the two principal components (PC1 and PC2). As a random effect we included the territory inside each population in which each individual was captured. Each principal component was analyzed in a separate GLMM. We also performed models for females and males because males are larger than females (Sandoval & Mennill, 2013) We carried out post hoc tests on all pairwise comparisons between the main effects and the two-factor interactions

Finally, to evaluate if divergence between type of traits (genetic, acoustic, and morphology) are related, we conducted a partial Mantel test to assess the correlation between variables using 10,000 permutations to determine significance as implemented in the “Vegan” package in R (Oksanen et al., 2012). Females in this analysis were not taken into account 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. After that with the PCAs we performed 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, the observed heterozygosity (H_o) was higher in 2011 – 2012 than in 2018 – 2022 (z = 1.95, p = 0.05), while the other populations did not show differences in genetic diversity in the decade of comparison (all comparisons: z < 1.014, p > 0.05) (Table 3) In Heredia the expected heterozygosity (H_e) was lower in 2011 – 2012 compared to 2018 – 2022 (z = -2.366, p < 0.05). In contrast, heterozygosity was lower in 2011 – 2012 for the Lankaster population than in 2018 – 2022 (z = -2.366, p < 0.05), the other populations did not present differences (all comparisons: z < 0.676, p > 0.05) (Table 3). Three populations (Heredia 2018 – 2022, Lankester 2018 – 2022, and UCR 2018 – 2022) showed a significant deficit of heterozygotes and inbreeding in the 2018-2022 decade (Table 3).

Genetic diversity was significantly structured in the metapopulation and it is similar between 2011 – 2012 (G_{st} = 0.0753, 95% CI = 0.056 and 0.094) and 2018 – 2022 (G_{st} = 0.0735, 95% CI = 0.056 and 0.091). Our AMOVA results showed that there were significant differences in allele frequencies in a decade (i.e., 2011 – 2012 vs. 2018 – 2022; F_{ct} = 0.120, p < 0.05), as well as among populations within time periods (F_{sc} = 0.154, p < 0.001). The Evanno method suggested that populations may be grouped into two clusters (i.e., K=2) in both decades. In 2011 all Monteverde individuals were grouped in cluster 2, whereas individuals in Heredia, UCR, and Lankester populations were grouped mostly in cluster 1 (Figure 1). In 2018-2022 the Evanno method also grouped individuals into K = 2 clusters. All Monteverde individuals were grouped in cluster 2, whereas individuals in all other populations included individuals from both clusters 1 and 2 (Figure 1).

Acoustic characteristics of songs

Our model showed that the duration and number of elements (PC1) were affected by the time and by the interaction population × time period (Table 4). In such away, the songs increased the duration and number of elements in 2018 – 2022 (Figure 2). In the interaction population × time period, Monteverde was the population that increase the duration and number of elements in 2018 – 2022. The minimum and maximum frequency (PC2) were not affected by the time, but they showed significant differences among populations (Table 4). UCR had higher minimum frequency and lower maximum frequency than Monteverde and Heredia. Lankester had lower minimum frequency and higher maximum frequency than the other populations (Figure 2). The frequency of maximum amplitude (PC3) showed significant differences in the interaction population × time period (Table 4). The songs increased the frequency of maximum amplitude in Heredia 2018 – 2022 than 2011 – 2012 and decreased in UCR and Lankester 2018 – 2022 regarding 2011 – 2012.

Morphology

Our model showed that tarsus length, tail length, wing chord length, and beak depth (PC1) were affected by the interaction population × time period on females. (Table 5). Tarsus length, tail length, wing chord length, and beak depth decreased in Monteverde and Lankester 2018 – 2022 regarding 2011 – 2012. (Figure 3). The exposed culmen length and beak width (PC2) did not show significant differences (Table 5) among years or sites. For males our model showed that tarsus length, tail length, wing chord length, and beak depth (PC1) were affected by the interaction population × time period (Table 5). Tarsus length, tail length, wing chord length, and beak depth were smaller in Lankester 2018 – 2022 compared to 2011 - 2012 (Figure 3). The exposed culmen length and beak width (PC2) were affected by the interaction population × time period (Table 5). Monteverde 2018 – 2022 had smaller exposed culmen length and bigger beak width than 2011 – 2012 and Lankester 2018 – 2022 had bigger exposed culmen length and smaller beak width than 2011 – 2012 (Figure 3).

Relationship between variables

Partial Mantel test carried out for correlation between genetic distances and morphology distances, accounting for acoustic, result in a not significant correlation r= – 0.06, (p = 0.40) (Figure 4). We found a correlation between genetic distances and acoustic distances, accounting for body size, r = 0.49, p < 0.05 (Figure 4). Finally, the partial mantel test between morphology distances and acoustic distances, accounting for genetic distances, result in a correlation coefficient of r = 0.24, which was not significant (p = 0.10) (Figure 4).

Discussion

In terms of genetic diversity, our results showed that the genetic diversity was more affected by distance than by time. This pattern is common in species with low mobility and specific habitat requirements such as the white-eared ground sparrow (Soulé et al., 1988; Delaney et al., 2010; Rodríguez-Bardía et al., 2022), which inhabit dense and early successional vegetation (e.g., riparian forests, forest edges, thickets, or shade coffee plantations; Sandoval and Mennill, 2012; Sandoval et al., 2016)within urban areas that result in isolation (Juárez et al., 2020; Rodríguez-Bardía et al., 2022). The pattern we found is worrying from a conservation perspective because we found a loss of genetic diversity and increased inbreeding associated with urban development (Delaney et al., 2010; Johnson & Munshi-South, 2017; Sattler et al., 2017; Miles et al., 2019). The increase in inbreeding in the two populations with middle urban development (Heredia and Lankester 2018 – 2022), and the higher inbreeding and loss of genetic diversity observed in the population within the most urbanized site (UCR population; Stiles, 1990), suggest that urbanization is a proximal factor determining inbreeding in bird populations. Similar pattern have been found red foxes *Vulpes vulpes* (Wandeler et al., 2003), in the European treefrog *Hyla arborea* (Andersen et al., 2004), in the blackbird *Turdus merula* (Evans et al., 2009), wrentit *Chamaea fasciata* (Delaney et al., 2010), and the side-blotched lizard *Uta stansburiana*, western skink *Plestiodon skiltonianus*, and the western fence lizard

Sceloporus occidentalis (Delaney et al., 2010). In each of these examples, genetic diversity decreased significantly when urban development was oldest and most extensive; hence, genetic diversity was greater in rural areas than in urban ones.

Differences in solo songs of white-eared ground-sparrows were to be expected because previous studies reported dialects among populations and acoustic differences such as adaptation to urban noise (Sandoval & Mennill, 2014; Sandoval et al., 2016; Méndez et al., 2021; Bonilla-Badilla 2021). However, we discovered a correlation between acoustic and genetic distances, implying that the variation in ground-sparrow songs reported here and in previous studies is not evolving in populations through morphological changes or acoustic adaptation to the environment. This type of relationship has been observed in birds such as the greenish warbler Phylloscopus trochiloides (Irwin et al., 2008), mammals such as the short-tailed singing mouse Scotinomys teguina (Campbell et al., 2010), and frogs such as the ornate pigmy frog Microhyla fissipes (Lee et al., 2010). Another explanation for the observed relationship between acoustic and genetic distances is the role of cultural drift or sexual selection guiding the evolution of songs within populations (West-Eberhard, 1983; Irwin et al., 2008; Camacho-Alpízar et al., 2018). This could apply for white-eared ground-sparrows, because solo songs are used by males only for female attraction (Sandoval et al., 2016), new males imitate the solo songs of males in the populations to which they arrive and this produces microgeographic song variation among populations that endure over time (Sandoval et al., 2014, 2016; Bonilla-Badilla 2021). If we take account that younger males may imitate dominant or older individuals inside their populations or that females may prefer specific songs, then a specific phenotype would be favored (West-Eberhard, 1983). Therefore, inside populations of white-eared ground-sparrow some kind of preference may guide song structure and in consequence shape the distribution of genetic diversity., Because after learning the songs males are not available to learn new songs over time (Bonilla-Badilla 2021).

Females of Monteverde and Lankester 2018 – 2022 were smaller than the same populations in the previous years. Males of the middle-urbanized population of 2018- 2022 (Lankester) were smaller than populations in 2011 – 2012. Also, beak measurements vary in males from Monteverde 2018 – 2022 than the previous years, which is the population inside the most natural habitat. our results show that changes in morphology vary among populations or time periods. Studies have reported several reasons for the reduction of individuals such as: quality and availability of food (Yom-Tov & Geffen, 2011; Goodman et al., 2012; Salewski et al., 2014), predation risk (Mcleod et al 2005), and an increase in contamination (Roux & Marra, 2007). In white-eared ground-sparrows smaller individuals may reflect the low-quality of food found in urbanized areas (Mennechez & Clergeau, 2006). In urbanized areas this species may also increases their territory to find more food sources (Juárez et al., 2020). The variation in morphology along the time has been reported in insects, fish, amphibians, reptiles, birds and mammals (Gardner et al., 2011; Yom-Tov & Geffen, 2011; Gotanda et al., 2015; Siepielski et al., 2019). This variation may be the result of different pressures and triggers on phenotypic or genetic responses (Yom-Tov & Geffen, 2011; Gotanda et al., 2015; Siepielski et al., 2019). At the same time, different phenotypic traits (morphological and acoustic characters) may evolve under different selective pressures (Sathyan et al., 2017). Also, morphological traits evolve at slow temporal rates (Carnicer et al., 2009)..

In conclusion, comparing four populations of white-eared ground-sparrow over the years showed changes in genetic diversity, spectrotemporal song characteristics, and morphology. Interestingly, we only found a positive relationship between the changes in genetic diversity and songs. We suggest that this correlation may be explained by cultural drift or sexual selection because if preferences act a constant rate in a population, it is possible to observed changes in acoustic signals related with genetic divergence (Irwin et al., 2008; Sathyan et al., 2017). Also the lack of relationship between morphological and the other two characteristics studied may suggest that morphology has little influence on sexual signaling (Wilkins et al., 2013; Sathyan et al., 2017). The processes of divergence and relationship between genetic and phenotypic traits are slow and complex to see, for the different forces acting at the same time (Irwin et al., 2008; Carnicer et al., 2009; Sathyan et al., 2017), in consequence, it is important to continue long-term studies in urban environments to understand how urban populations are changing and adapting at the increase of the fragmentation and consequently isolation by urbanization. Since, the species with different natural history may response and adapt in different ways to habitat change (Rodríguez-Bardía et al., 2022).

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Table 1. Results of principal components analysis of solo songs of white-eared ground-sparrow *Melozone leucotis*. The asterisk indicates the variables of major contribution in each component.

Song measurements	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*
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 of major contribution in each component.

Body measurements	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 ± 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. G*iven in parenthesis the number of females in each population.

Population	Time period	n	Ho		He		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. Effects and two-factor interactions in linear mixed-effects models comparing acoustic characteristics of solo songs in two periods of time (2011 – 2012, 2018 – 2022) of populations of white-eared ground-sparrow *Melozone leucotis*.

	PC1			PC2			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 × time period	3	2.73	< 0.05	3	1.04	0.38	3	3.65	< 0.05

PC1 is the duration and number of elements, PC2 is the minimum and maximum frequency, and PC3 is the frequency of maximum amplitude.

Table 5. Effects and two-factor interactions in generalized linear mixed models comparing body measurements in two periods of time (2011 – 2012 vs 2018 – 2022) of populations of white-eared ground-sparrows *Melozone leucotis*.

	Females						Males					
	PC1			PC2			PC1			PC2		
	X2	DF	P	X2	DF	P	X2	DF	P	X2	DF	P
Population	0.75	3	0.86	1.72	3	0.63	2.28	3	0.52	0.62	3	0.89
Time period	0.24	1	0.63	1.25	1	0.26	0.001	1	0.97	0.005	1	0.94
Population × time period	31.73	3	< 0.001	2.68	3	0.44	27.14	3	< 0.001	10.15	3	< 0.05

PC1 is tarsus length, tail length, wing chord length, and beak depth, PC2 is exposed culmen length and beak width.

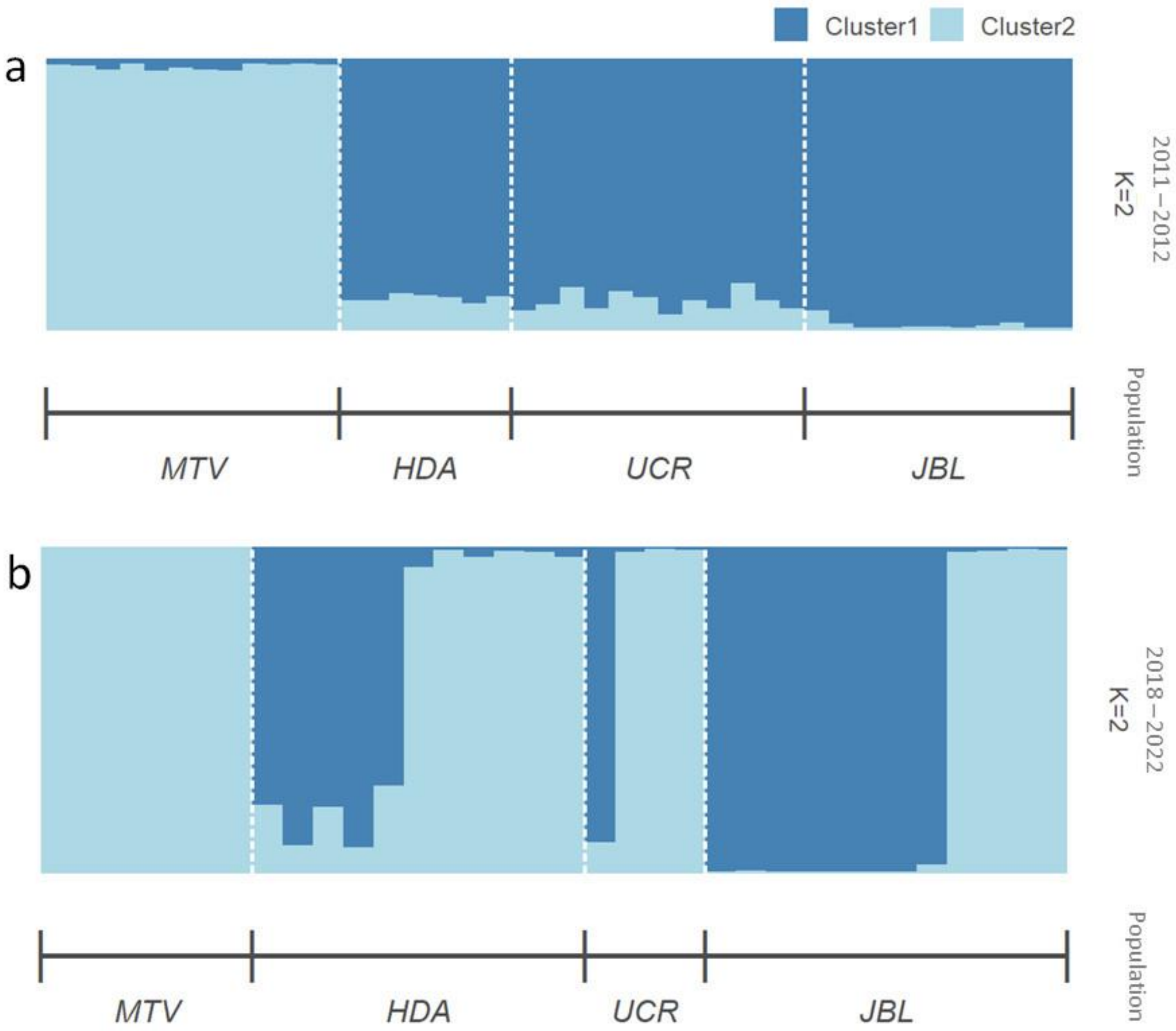

Figure 1. STRUCTURE barplots for (a) 2011 – 2012 populations of white-eared ground-sparrow *Melozone leucotis* into K = 2, and (b) 2018 – 2022 populations of white-eared ground-sparrow *Melozone leucotis* into K = 2. Plots are sorted by sampling location, each bar represents one individual, each color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

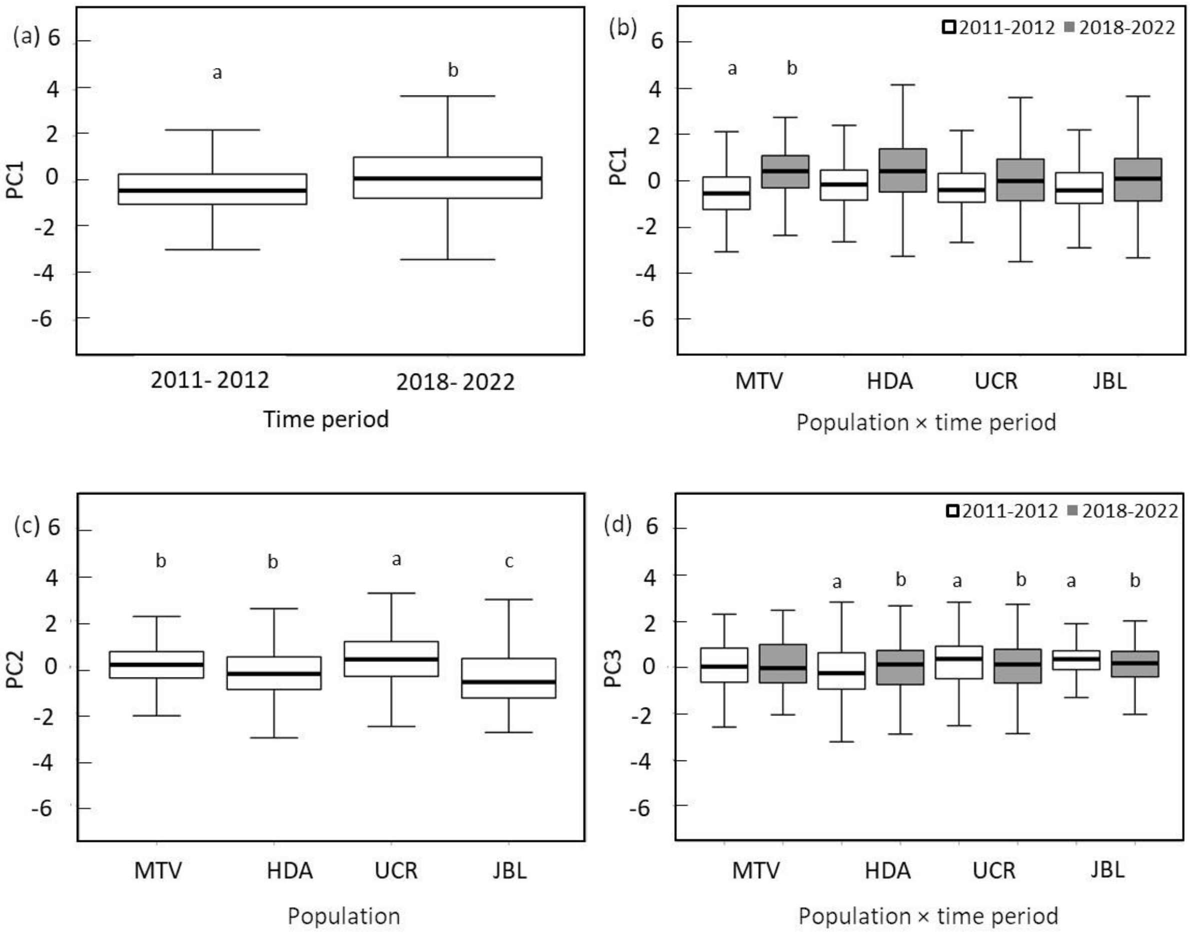

Figure 2. Box-plots of the variation in acoustic characteristics of white-eared ground-sparrow *Melozone leucotis*, according linear mixed-effects models done. The responses are measured as principal components scores summarizing (a), (b) the duration and number of elements (PC1), (c) The minimum and maximum frequency (PC2), and (d) The frequency of maximum amplitude (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 on top of the whiskers mean that no significant differences were found in the post hoc tests.

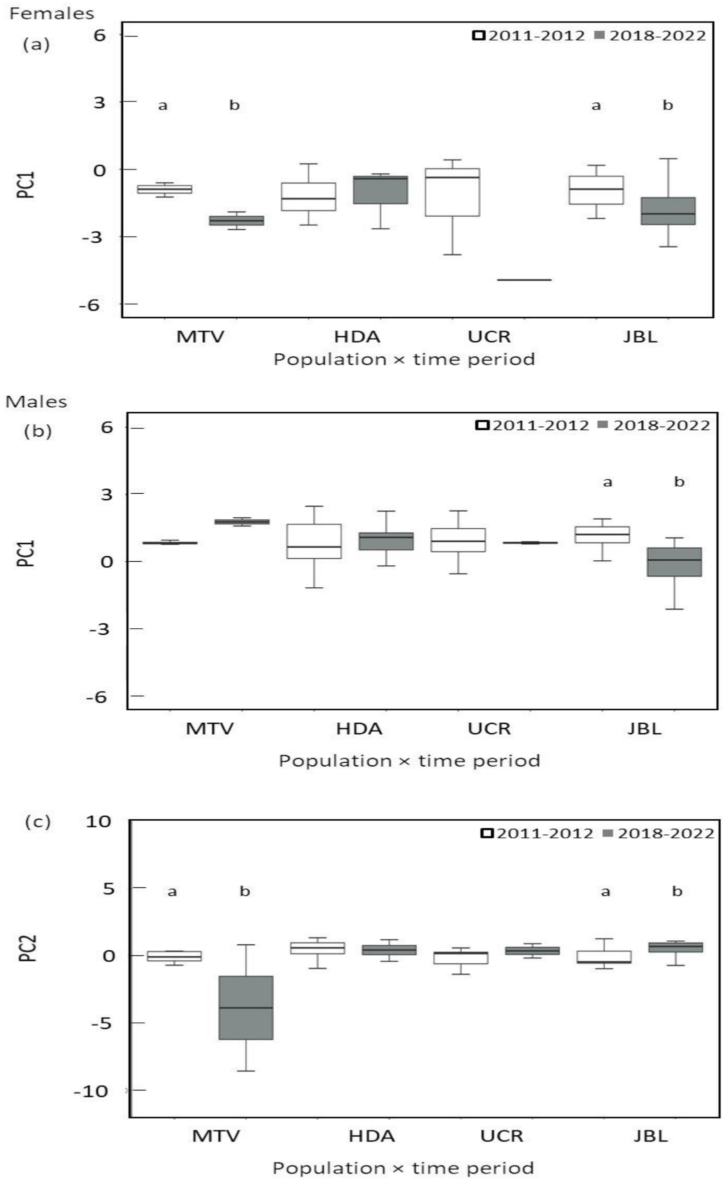

Figure 3. Box-plot of variation in morphology of white-eared ground-sparrow *Melozone leucotis*, according generalized linear mixed models done. The responses are measured as principal components scores summarizing (a) tarsus length, tail length, wing chord length, and beak depth (PC1) of females, (b) tarsus length, tail length, wing chord length, and beak depth (PC1) of males, (c) the exposed culmen length and beak width (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 on top of the whiskers mean that no significant differences were found in the post hoc tests.

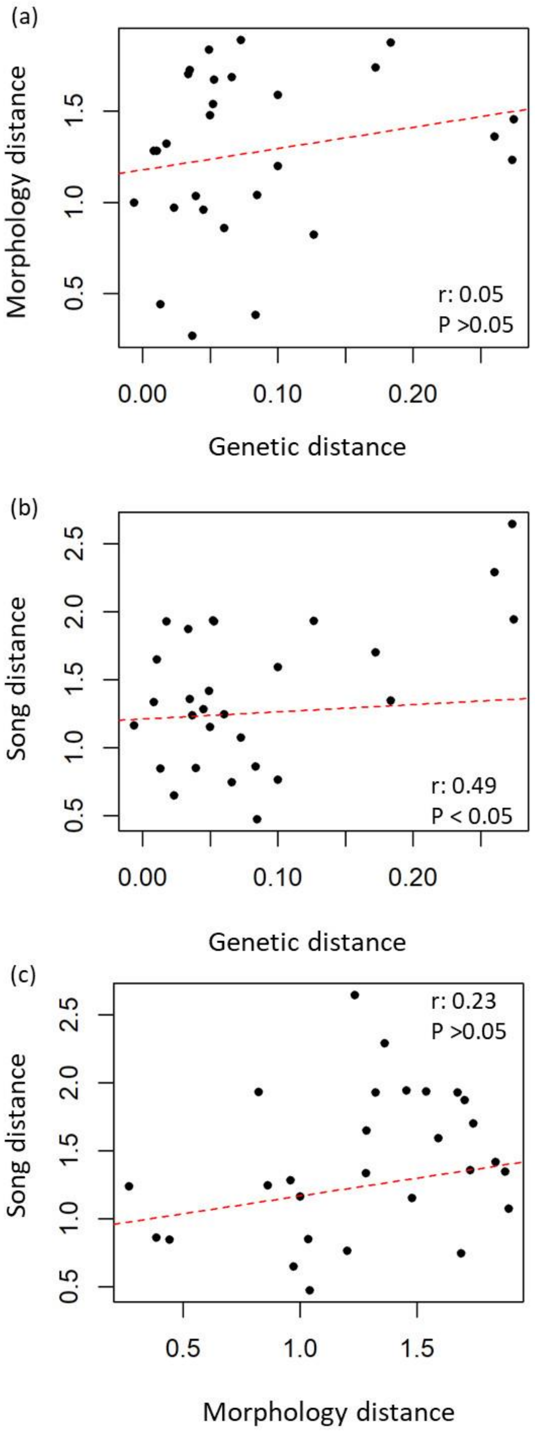

Figure 4. Correlates in white-eared ground-sparrow *Melozone leucotis.* (a) song and morphology distances, (b) morphology and genetic distances, and (c) song and genetic

distances. Linear regressions are shown, but statistical significance was evaluated using Mantel tests

Capitulo 2

The Acoustic Adaptation Hypothesis does not support the occurrence of common songs in White-eared Ground-sparrow an urban bird species

Introduction:

Songs are acoustic signals that have two main functions in birds: mate attraction and territory establishment and defense (Bradbury and Vehrencamp 1998; Collins 2004). The acoustic characteristics of songs (i.e., frequency and duration) may vary according to habitat characteristics (Longman and Jenik 1992; Slabbekoorn and Smith 2002), female selection (Dingle et al. 2008), and/or acoustic drift (Wilkins et al. 2013). These forces produce a great variety of acoustic signals between populations (Baker and Cunningham 1985; Lynch and Baker 1994; Ellers and Slabbekoorn 2003).

According to the Acoustic Adaptation Hypothesis (AAH) habitats have a very strong influence on the frequency and duration traits of acoustic signals, as they must be audible and discernible by receivers (Patricelli and Blickley 2006). Particularly, the AAH proposes that acoustic signals evolve to optimize their transmission and detectability in the habitat where they are more commonly produced (Morton 1975). This pattern, has been shown in approximately 26 investigations (Boncoraglio and Saino 2007) where the main result supports the existence of songs with convergent characteristics for similar habitats (lower frequencies and longer duration in close habitats, and higher frequency and shorter duration in open habitats), because these characteristics enhance the distance at which signals can be heard and interpreted reliably by a receiver in a specific habitat (Morton

1975, 1986; Boncoraglio and Saino 2007; Ey and Fischer 2009; Piza and Sandoval 2016; Graham et al. 2017; Hardt and Benedict 2021).

When the AAH does not explain differences in the acoustic characteristics of songs between populations, investigators propose that female preferences are the main force underlying the observed differences (Date and Lemon 1993; Fotheringham et al. 1997; Slabbekoorn 2004a; Mathevon et al. 2005; Penna et al. 2006; Llusia et al. 2013; Penna and Moreno-Gómez 2015; Graham et al. 2017; Velásquez et al. 2018). Under sexual selection, female preferences shape song differences between populations (Searcy and Andersson 1986; Gerhardt 1995; Grant and Grant 1996; Collins 2004). For example, in Rufous-collared Sparrow *Zonotrichia capensis* in Ecuador, females in the Papallacta population prefer songs with two or three modulated notes, but in Pintag population females prefer songs with one or two modulated notes in the songs. Females preferred the autochthonous dialect to their population (Moore et al. 2005; Danner et al. 2011). In White-crowned Sparrow *Zonotrichia leucophrys nuttalli* in USA there are two populations separated by 10 km in Bass lake where males produced songs that differed from each other by an internal buzz, with females belonging to population without buzzy note preferring males that sang songs without buzzy (Baker 1975, 1983). Both studies support the hypothesis that female preference is a major factor shaping the differences in male solos among populations.

White-eared Ground-sparrows, *Melozone leucotis* have 33 song types that may share between individuals and between populations, each male sings an average of 3.5 song types, (Sandoval et al., 2014, 2016). Inside each population the song variation is given by the introduction of new songs by replacement of males, hence, the common songs are songs that permanence in the population into consecutive years (Bonilla-Badilla 2021). In such a way, among populations have a micrographic song variation (dialects), where each population has a different repertoire of common songs (Sandoval et al. 2014, 2016; Bonilla-

Badilla 2021). Songs are produced only by males and are used to attract females to form a pair or have extra-copulations during breeding seasons (Sandoval and Mennill 2014; Sandoval et al. 2016). Ground-sparrows inhabit isolated populations inside urban, suburban and rural areas, where habitats vary in vegetation structure (e.g., density and strata number) and degree of openness (Sandoval et al. 2015; Juárez et al. 2020; Rodríguez-Bardía et al. 2022). Hence, this is a suitable model species to provide information for the variation in song repertoire between populations like a consequence of signals adaptation to maximize sound transmission as proposed by the AAH.

Studies of sound transmission have focused on convergent acoustic characteristics of the vocalizations to different environments (Boncoraglio & Saino 2007; Ey & Fischer 2009; Hardt & Benedict 2021). But we did not find studies that test if the common songs that occur in different populations of the same species inside an urban gradient occur guided by AAH. Our main objective in this study is to test the AAH by comparing the transmission properties of the common songs of White-eared Ground-sparrows within the population where they are produced more commonly. Under this hypothesis, we expect that the common songs are adapted to transmit better within the habitats in which they are commonly emitted, showing minor degradation and attenuation compared to when they are played in other habitats. Also we expect to find more degradation and attenuation in urbanized habitats because human constructions generate an excess of echoes (Warren et al. 2006). Finally we expect to see degradation and attenuation of the signals at greater distances and near to the ground (Graham et al. 2017).

Material and Methods

Study sites

We conducted this study in four populations of White-eared Ground-sparrows previously characterized by Juárez et al (2020), located in the Central Valley of Costa Rica: (1) The Universidad de Costa Rica main campus, San José province (09 ° 56'N, 84 ° 05'W, 1200 m); it is a highly urbanized site, which includes a secondary forest reserve surrounded by buildings and gardens, and exposed to human disturbance (Juárez et al., 2020). (2) Instalaciones Deportivas, Universidad de Costa Rica, San José province (09 ° 56'N, 84 ° 02'W, 1200 m); it is a site with patches of secondary forest and abundant human disturbance, surrounded by gardens, open fields, and buildings (Juárez et al. 2020). (3) Jardín Botánico Lankester, Cartago province (9 ° 50'N, 83 ° 53'W, 1370 m); it is an area considered sparsely urbanized, which includes a mix of secondary forest, gardens, and buildings, with little disturbance and human influx (Juárez et al. 2020). (4) Getsemani, Heredia province (10 ° 01’N, 84 ° 05’W, 1350 m); it is a non-urbanized site, which it has secondary forest and abandoned coffee plantations, in addition to dense thickets with little human presence (Juárez et al. 2020). The study was conducted in 2021 on August 7 - 27 during the last part of White-eared Ground-sparrow breeding season (Sandoval & Mennill 2012), from 6:00 – 9:00 h, because male solo songs are produced during these hours (Sandoval et al. 2016), in three different territories inside each population of White-eared Ground-sparrow.

Playback stimuli

The songs used to generate the stimuli were recorded during previous studies and are deposited in Laboratorio de Ecología Urbana y Comunicación Animal (LEUCA) of the Universidad de Costa Rica. The recordings were collected using a Marantz PMD661 digital recorder (sampling rate: 44.1 kHz; accuracy: 16-bit and file format: WAV) and a Sennheiser ME66/K6 directional microphone. We selected the three common songs per population following the reports in Sandoval et al. (2014) and Bonilla-Badilla (2021). The three common songs per population were from three males (one per male), to take into account the variation inside the populations due to male differences. We selected recordings with songs without overlapping background sounds and with the highest signal-to-noise ratio (Figure 1).

The transmission stimuli were created with a sequence of five repetitions of the three common song types per population of White-eared Ground-sparrows. Each repetition was separated by 1 sec of silence, and before the first stimulus a silence of 3 sec was settled. We filtered the background noise using the Fast Fourier transform Filter (FFT) in Audition 1.0 (Adobe Systems, San José, CA, USA). Since FFT is used to remove frequencies that do not correspond to the signal, with the aim to improve the signal-to-noise ratio and delete background sound according to the recordings (López et al., 2008). The filters used per population and song type were: UCR population: type songs T18: 3.2 – 11.5 kHz; T14: 3 -11.5 kHz; T16: 4 – 11.5 kHz. Deportivas population: Type songs T13: 2.7 – 11 kHz; T35: 4 – 12 kHz; T40: 2 – 12 kHz. Lankester population: Type songs T44: 2 -11.2 kHz; T42: 2.2 – 12 kHz; T21: 2 -10 kHz. Heredia population: Type songs T23: 3.5 – 12 kHz; T2: 2.8 – 12 kHz; T3: 3.9 – 12 kHz. We standardize the stimuli to -1 dB using the normalize function from Audition 1.0. The stimuli were stored in an iPod Touch Nano portable player (Apple, Cupertino, CA) for playback in the field.

Transmission experiment

We broadcasted the stimuli from an iPod Touch nano connected to an active loudspeaker (Anchor Audio; Minivox; frequency response: 0.1–20 kHz) constantly at 80 dB, measured at 1 m from the speaker using a digital sound level meter (Sper Scientific NIB-850014 Scottsdale, AZ; using A weighting and fast response). The broadcasted amplitude match how loud the White-eared Ground-sparrow solo songs are in the field (Sandoval et al. 2015). We re-recorded the stimuli using an omnidirectional microphone (Sennheiser ME62 / K6) and a solid-state digital recorder (Marantz PMD661; sampling rate: 44.1 kHz; accuracy: 16-bit; file format: WAVE), connected via a microphone preamplifier (Sound Device MP-1; frequency response: 0.02–22 kHz). As the distance between the loudspeaker and the microphone increased, we adjusted the level of the preamplifier to be able to record songs (Sandoval et al. 2015). Then, we set up the pre-amplifier at 0 dB gain at 4, 8 and 16 m, and 18 dB gain at 32 m of distance between the loudspeaker and microphone.

We played sound stimuli at four different horizontal distances (4, 8, 16, and 32 m between the loudspeaker and microphone), because these represent the distances between the pair members, males and females (the two shorter distances) and between neighboring pairs (the two longer distances), at two microphone heights (0.4 and 2.2 m), that represent the common perch height of potential receivers, and at one speaker height (2.2 m) that represent the common height for males to sing (Sandoval et al. 2015). The horizontal distances in each territory were distributed according to the cardinal points (north, south, east and west) but started at the same point, in order to obtain a more representative sampling of the effect of habitat on sound transmission (Sandoval et al. 2015).

Sound analysis

To analyze re-recorded stimuli we used SigPro 3.25 software (Pedersen 1998). To avoid changes in the sound that may have arisen because of the playback equipment (Sandoval et al. 2015; Graham et al. 2017), we compared the re-recorded sounds at the four distances of our experiment (4, 8, 16 and 32 m) against re-recorded sounds at a distance of 1.0 m and at 2.2 m height, on an area with no vegetation in Jadín Botánico Lankester.

We measured background noise variation using the same FFT filter settings we used to isolate each common song. In addition, we measured noise levels before the start of the playback stimuli that we analyzed in the 3 sec. of silence before the first stimulus (As described in Debelsteen, 1993). We assumed that the background noise before each stimulus was the same as the noise that overlapped the common songs during the experiment as in other transmission studies (Debelsteen, 1993; Graham et al., 2017; Piza & Sandoval, 2016; Sandoval et al., 2015). We measured four variables after applying the FFT filter settings to remove the background noise outside the range of the common songs: the signal-to-noise ratio (the comparison between the amount of energy in the observed sound versus energy in the background noise immediately before the sound of interest), the tail-to-signal ratio (the amount of energy in the reverberant tail compared with the energy in the observed sound), the blur ratio (the distortion of the signal’s frequency and amplitude pattern over time), and the excess attenuation (attenuation beyond the spherical spreading of 6 dB per doubling of the distance) (Sandoval et al. 2015; Piza and Sandoval 2016). The formulas and details used to obtain these four measurements in SigPro are presented in (Debelsteen, 1993; Holland et al., 1998; Lampe et al., 2007).

Statistical analysis

To analyze the effect of the sound transmission experiments on signal degradation we performed linear mixed-effects models (LMMs). There were four independent variables: playback site (four levels that correspond to the four populations where we conducted the playbacks), source population (four levels that correspond to the four populations where birds were recorded), distance (four levels: 4, 8, 16, 32 m), microphone height (two levels: 0.4m and 2.2 m), and the second order interaction between independent variables. The response variables were the four sound degradation measurements (signal-to-noise ratio, tail-to-signal ratio, blur ratio and excess attenuation) and each variable was analyzed in an individual LMM. As a random effect we included, the territory where each experiment was conducted and song type. Finally, we carry out post hoc tests on all pairwise comparisons between the main effects and the two-factor interactions using Bonferroni corrections. To analyze background noise (dB), we conducted LMM on measurements collected in the 3 sec. of silence before the first stimulus and after applied the filters previously mentioned. We had four independent variables (playback site, distance, and microphone height) and the response variable was the background noise level measurement. We examined only main effects such as Graham et al. (2017). We report all values as mean ± SE. Statistical analyses were conducted in R with lmer library (R Core Team 2020).

Results

Our transmission experiments showed that playback site and source population had different effects on the degradation and attenuation of common songs of White-eared Ground-sparrows. The playback site had a significant effect on the transmission properties of the songs, but the source population did not show a significant effect on the transmission properties. Here, we present a detailed analysis of the main effects and interactions.

Signal-to-noise ratio was higher and tail-to-signal ratio lower at less urbanized sites and with more vegetation (Heredia and Lankester; Table 1. Figure 2). Blur ratio was higher at higher urbanized sites (Deportivas and UCR; Figure 2). The excess attenuation was greater at Heredia (Figure 2). None of the four measurements were affected by source population (Table 1). Degradation (tail-to signal and blur ratio) and attenuation (signal-to-noise ratio and excess of attenuation) increased with distance and close to the ground

(Figure 2), showed the higher values at the two larger distances (16 and 32 m; Figure 2) and when the receiver was closer to the ground (microphone at 0.4 m; Figure 2).

Two measurements; the signal-to-noise ratio and tail-to-signal ratio, showed significant interactions between playback site × source population and source population × distance (Table 1). Playback site × source population did not show less degradation at the sites where they were recorded and played (i.e., the degradation of songs recorded in Deportivas population was not lower than songs recorded in Heredia population when played at Deportivas site, Figure 3). All four songs had higher signal-to-noise ratio at Heredia and Lankester sites (Figure 3). Tail-to-signal ratio was higher at all four songs played in Heredia and Lankester sites (Figure 3). Source population × distance show that degradation increased as distance increased, because signal-to-noise ratio and tail-to-signal ratio decreased as the distance increased (Supplementary Figure S1).

All four sound degradation measurements presented significant interaction effects between playback site × distance, playback site × microphone height, and microphone height × distance (Table 1). Signal-to-noise ratio of common songs for playback site × distance was significantly higher at shorter distances (4 and 8 m) and lowest at the furthest distances (16 and 32m). Tail-to-signal ratio was significantly higher at shorter distances (4 and 8 m) and lowest at furthest distances (16 and 32 m) in three sites, in Lankester it was significantly higher at the 4 m distance than the other three distances (Supplementary Figure S2). Blur ratio was significantly higher at the furthest distances (16 and 32 m) and lowest at shorter distances (4 and 8 m). Excess attenuation was greater at 16 m and smaller at 4 and 8 m, at 32 m it presented a reduction in all four sites (Supplementary Figure S2).

For the interaction between playback site × microphone height; signal-to-noise ratio of common solo songs was significantly higher at 2.2 m only in Lankester site. Tail-to-signal ratio was significantly higher at 2.2 m in Heredia and Lankester sites. Blur ratio was significantly higher at 0.4 m in Heredia, Lankester and UCR sites, and in Deportivas site it was significantly higher at 2.2m. Excess attenuation was significantly higher at 0.4 m in Heredia and Lankester sites (Supplementary Figure S3). For the interaction between microphone height × distance; signal-to-noise ratio increased at shorter distance at both heights. Tail-to-signal ratio presented the same pattern observed for signal-to-noise ratio. Blur ratio increased at furthest distance at both heights. Excess attenuation presented the same pattern observed for blur ratio except for 32 m, where the excess attenuation decreased at both heights (Supplementary Figure S3). Only a single interaction (tail-to-signal ratio) was significant for the interaction source population × microphone height where the songs had significantly higher tail-to-signal ratio at 2.2 m, except in Lankester site where heights were not significantly different (Supplementary Figure S3).

Background noise variation

The background noise in our transmission experiments varied between playback sites (Table 2). Background noise at the Deportivas and UCR sites (more urbanized sites) was higher than at the Lankester and Heredia sites (sites with more vegetation) (Table 2). Background noise at 32 m was higher than 16m, 8m and 4m. Microphone height did not show a significant effect on background noise (Table 2).

Discussion

We found that playback site (population where the experiments were conducted) and source population (the location where songs were recorded) affect song transmission, but not as predicted according to the Acoustic Adaptation Hypothesis. The interaction between ‘playback site × source population’ did not show that common songs were locally adapted, because songs did not experience less degradation at their respective habitats. Previously, it was reported that White-eared Ground-sparrows songs are not well adapted to transmit in dense habitats because they degrade very fast with distance (Sandoval et al. 2015). Our data revealed that common songs within a population are not specifically adapted to transmit better in their habitats. Therefore, considering that song in White-eared Ground-sparrows are used for female attraction and extra-pair copulation (Sandoval et al. 2016), common songs are probably maintained in each population over the years, because they are the most attractive songs for females.

We found that playback site had a significant effect in song degradation in all four studied populations. Our four populations differ in vegetation structure and ambient noise (Sandoval et al. 2015; Juárez et al. 2020), which may be the main factor that affects song transmission (Brumm and Naguib 2009). We observed lower signal-to-noise ratio, lower tail-to-signal ratio, and higher blur ratio in Deportivas and UCR , the two most urbanized populations (territories surrounded by buildings, streets, and sidewalks) and most exposed to anthropogenic noise (Juárez et al. 2020). These patterns were expected in these two populations, because the land cover of open areas, gardens, and buildings generate an excess of echoes as results of reflectance and rapid bouncing of sound waves between walls (Warren et al. 2006). Also, the greater amplitude of urban noise scatter, and overshadow sounds producing fast degradation and attenuation at Deportivas and UCR (Brumm 2004; Warren et al. 2006; Slabbekoorn et al. 2007). Excess attenuation was higher in the Heredia population, where habitat was dominated by a mix of secondary forest, coffee plantations, and dense thickets (Sandoval and Mennill 2012; Sandoval et al. 2015; Juárez et al. 2020). Dense habitats produce more attenuation in sounds because sounds have to go through many obstacles like leaves, branches and tree trunks, which cause the signal to lose energy rapidly (Dingle et al. 2008; Brumm and Naguib 2009; Tobias et al. 2010). Our results were consistent with this pattern.

All common songs tested of White-eared Ground-sparrow had higher signal-to-noise ratio when they were played in Heredia and Lankester (habitats with more vegetation) and they had lower signal-to-noise ratio when they were played in Deportivas and UCR (urbanized habitats). This pattern may be caused because songs are still adapted to transmit in the natural habitats of this sparrow (thickets or habitats with dense vegetation; Sandoval and Mennill 2012; Sandoval et al. 2015). Since degradation is generally minimized in the native environments of each species (Boncoraglio and Saino 2007; Hardt and Benedict 2021).

The tail-to-signal ratios were lower when the songs were played in Deportivas and UCR than in the other two populations, these patterns were expected because these habitats have a lot of reflective surfaces from human constructions that increase reverberation and the energy of the tail (Naguib 2003; Phillips et al. 2020). On the other hand, the different pattern of tail-to-signal ratio of Heredia and Lankester songs was expected because Deportivas and UCR songs have higher minimum frequency than the other ones (Méndez et al. 2019), and songs with higher frequencies produce more reverberation (Mockford et al. 2011; Phillips et al. 2020). In consequence urbanized places and songs with higher minimum frequencies produce high rates of reverberation.

Songs degraded and attenuated less when they were transmitted at the same height between signals and receiver (2.2 m speaker and microphone). This pattern was consistent with previous studies when the height of signaler and receiver were similar and at higher perches attenuation and degradation of vocalizations was lower (Debelsteen 1993; Mathevon et al. 1996; Holland et al. 1998; Mathevon et al. 2005;Barker et al. 2009; Sandoval et al. 2015). Microphones placed at 2.2 m in height songs presented higher signal-to-noise ratios, higher tail-to-signal ratios, lower blur ratios, and lower excess attenuations than songs recorded in microphones placed at 0.4m in height. Most degradation occurs at

0.4 m, because the signal must travel diagonally and cross more vegetation and gradually lose energy (Barker et al. 2009). This means that the receiver perch height influences song reception (Debelsteen 1993; Balsby et al. 2003; Barker et al. 2009), because songs may be less recognizable for the receiver close to the ground because vocalizations interacts with strata nearer to the ground increasing the tail and the distortion (Slabbekoorn 2004b; Barker et al. 2009; Sandoval et al. 2015).

The distance between the speaker and microphone also affected the four variables of degradation and attenuation that we measured as it was expected. Signal-to-noise ratio and tail-to-signal ratio decreased and the blur ratio and excess attenuation increased with the distance. These findings are according to previous studies where the atmospheric absorption, geometrical spreading, and ground effect increase the degradation and attenuation with the distance (Piercy et al. 1977; Holland et al. 1998; Sandoval et al. 2015). The excess attenuation was highest at 16 m and not at 32 m, we think that this probably happened because the majority of the experiments at 32m were with less obstacles (e.g., vegetation) than the other distances. Also, we found that the background noise increased with the distance between the loudspeaker and the microphone. In consequence background noise was higher at 32 m than the other distances as has been reported in other studies (Sandoval et al., 2015; Piza & Sandoval, 2016; Graham et al., 2017). The perception of noise increase with the distance, since the microphone may record sounds out of the stimuli with major intensity and more sources of noise (Matsinos et al., 2008; Gill et al., 2015; Grabarczyk & Gill, 2020).

In conclusion, we did not find evidence that the maintenance of common songs in populations of the White-eared Ground-sparrow were guided by the Acoustic Adaptation Hypothesis. Given that the interaction ‘playback site and source population’ did not show that songs are better transmitted in their habitat. Therefore, variation in common songs between populations is probably maintained through sexual selection by females as has been reported in White-crowned Sparrows, *Zonotrichia leucophrys* (Baker 1983), yellowhammers *Emberiza citronella* (Baker et al. 1987), Swamp sparrows *Melospiza georgina* (Balaban 1988), Song Sparrows *Melospiza melodia* (Searcy et al. 1997), and Rufous-collared Sparrows *Zonotrichia capensis* (Danner et al. 2011). However, experiments that test female preferences by particular song types are necessary to further support our conclusions.

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Tables

Table 2. Effects and two-factor interactions in linear mixed-effects models comparing the most common solo songs of four populations of White-eared Ground-sparrows for each of four measures of degradation and attenuation.

	Signal to noise ratio			Tail to signal ratio			Blur-ratio			Excess attenuation		
	Df	F	P	Df	F	P	DF	F	P	DF	F	P
Playback site	3	9.1531	<0.005	3	2.1933	<0.005	3	2.2819	<0.001	3	26.966	<0.001
Source population	3	2.4566	0.13255	3	2.7787	0.108	3	0.043	0.9874	3	0.3278	0.8053
Distance	1	990.99	<0.001	1	1491.035	<0.001	1	1481.35	<0.001	1	64.784	<0.001
Microphone height	1	238.07	<0.001	1	148.2323	<0.001	1	538.33	<0.001	1	51.271	<0.001
Playback site × Source population	9	6.3841	<0.001	9	4.4476	<0.001	9	0.5834	0.8119	9	0.0633	0.9999
Playback site × Distance	3	35.076	<0.001	3	85.4485	<0.001	3	73.6515	<0.001	3	138.39	<0.001
Playback site × Microphone height	3	26.388	<0.001	3	26.8268	<0.001	3	129.475	<0.001	3	7.108	<0.001
Source population × Distance	3	4.4033	<0.005	3	8.3441	<0.001	3	2.0085	0.1105	3	0.0666	0.9776
Source population × microphone height	3	0.3217	0.80971	3	2.7477	<0.005	3	0.1486	0.9306	3	0.0532	0.9838
Microphone height × Distance	1	239.04	<0.001	1	79.2619	<0.001	1	254.099	<0.001	1	58.861	<0.001

Table 2. Main effects for the linear mixed models analyzing comparisons of background noise during the transmission experiments.

	Df	F	P
Playback site	3	185.377	<0.001
Distance	1	504.514642	<0.001
Microphone height	3	0.67278	0.41226189

Figures

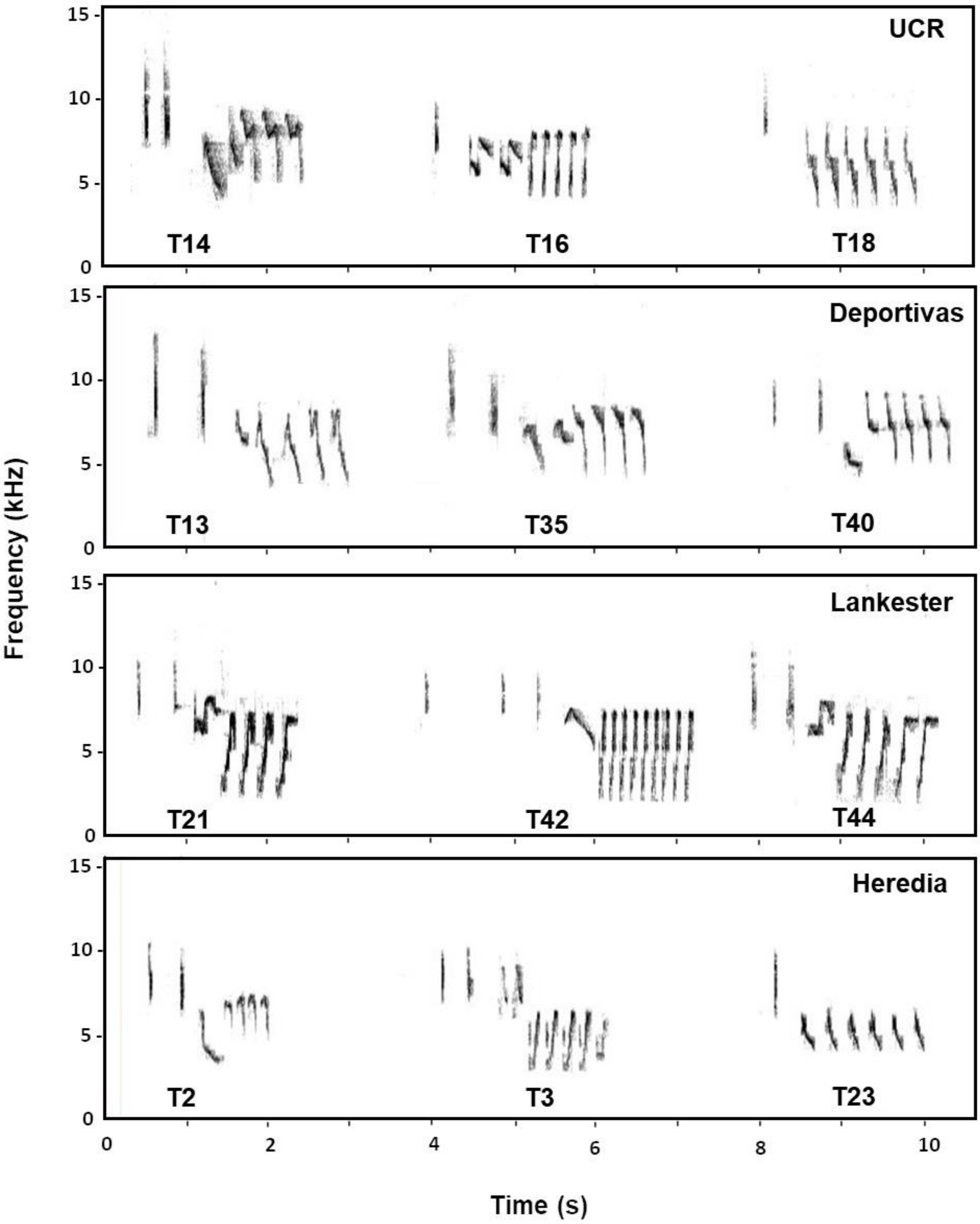


Figure 1. Spectrograms of type of solo songs of White-eared Ground-sparrows used in the transmission experiments.

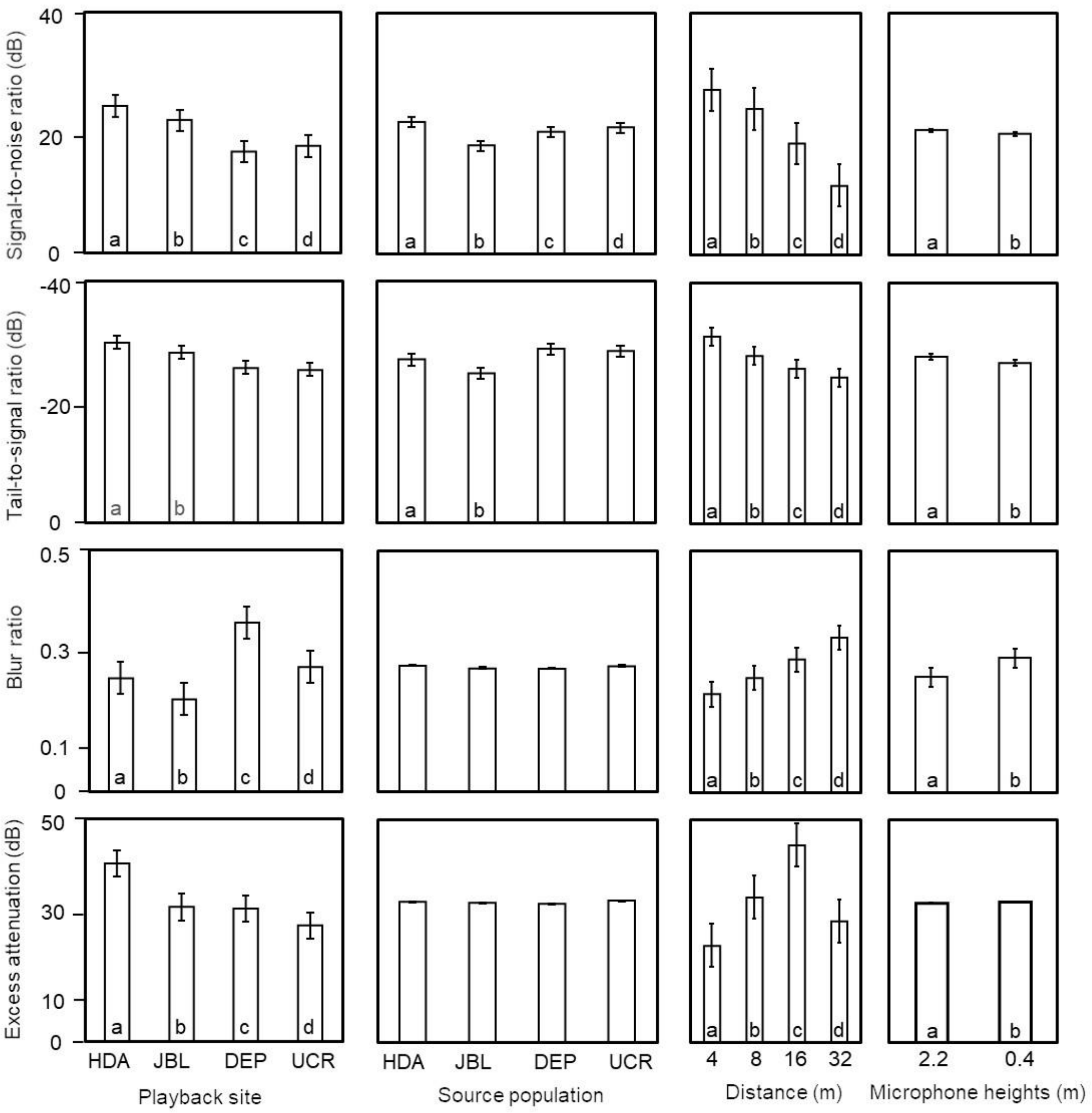


Figure 2. Variation in four measurements of sound degradation according to playback site, source population, distance and microphone heights used in the transmission expriments. Error bars are standard errors of the mean, and bars with different letters indicate the values are significantly different from each other in post hoc tests.

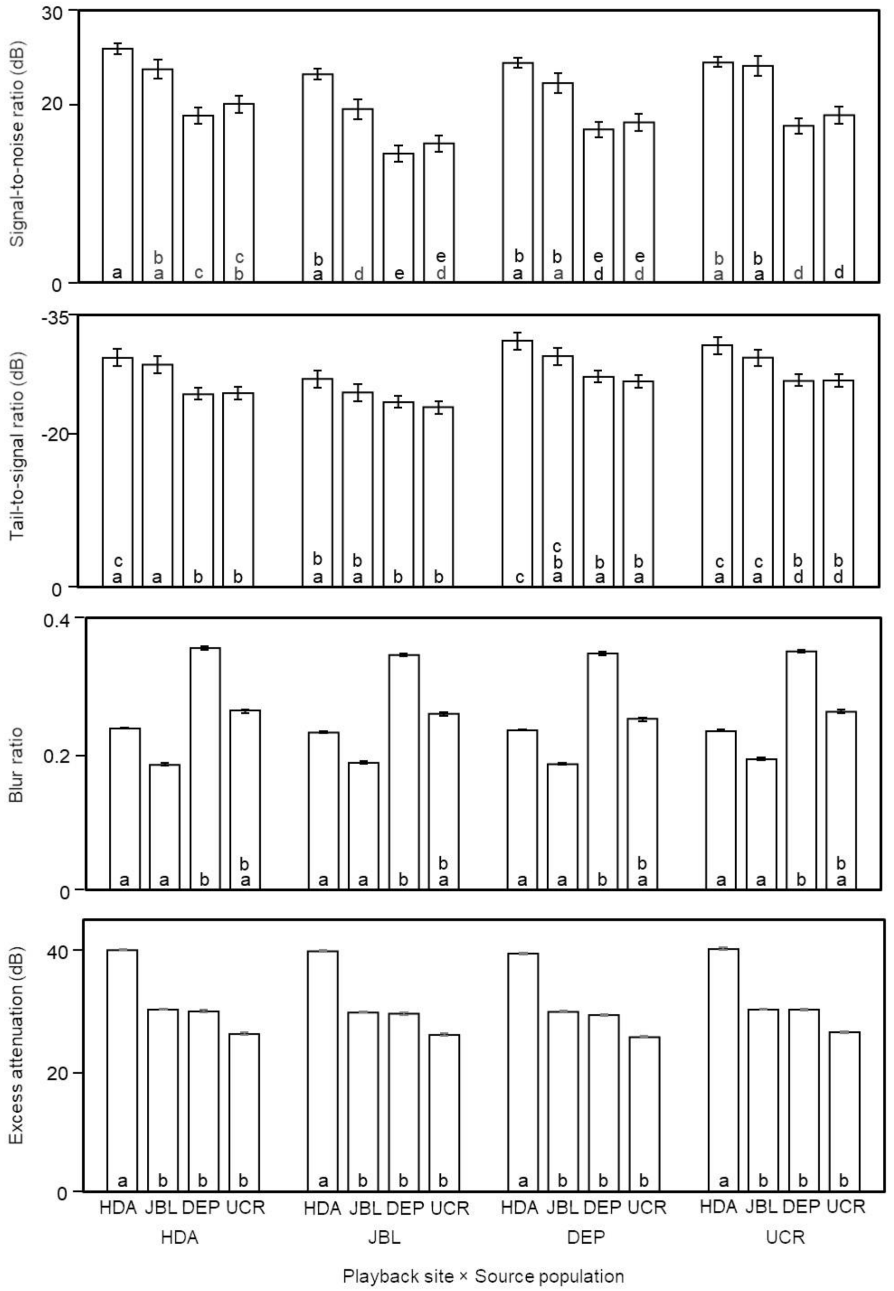
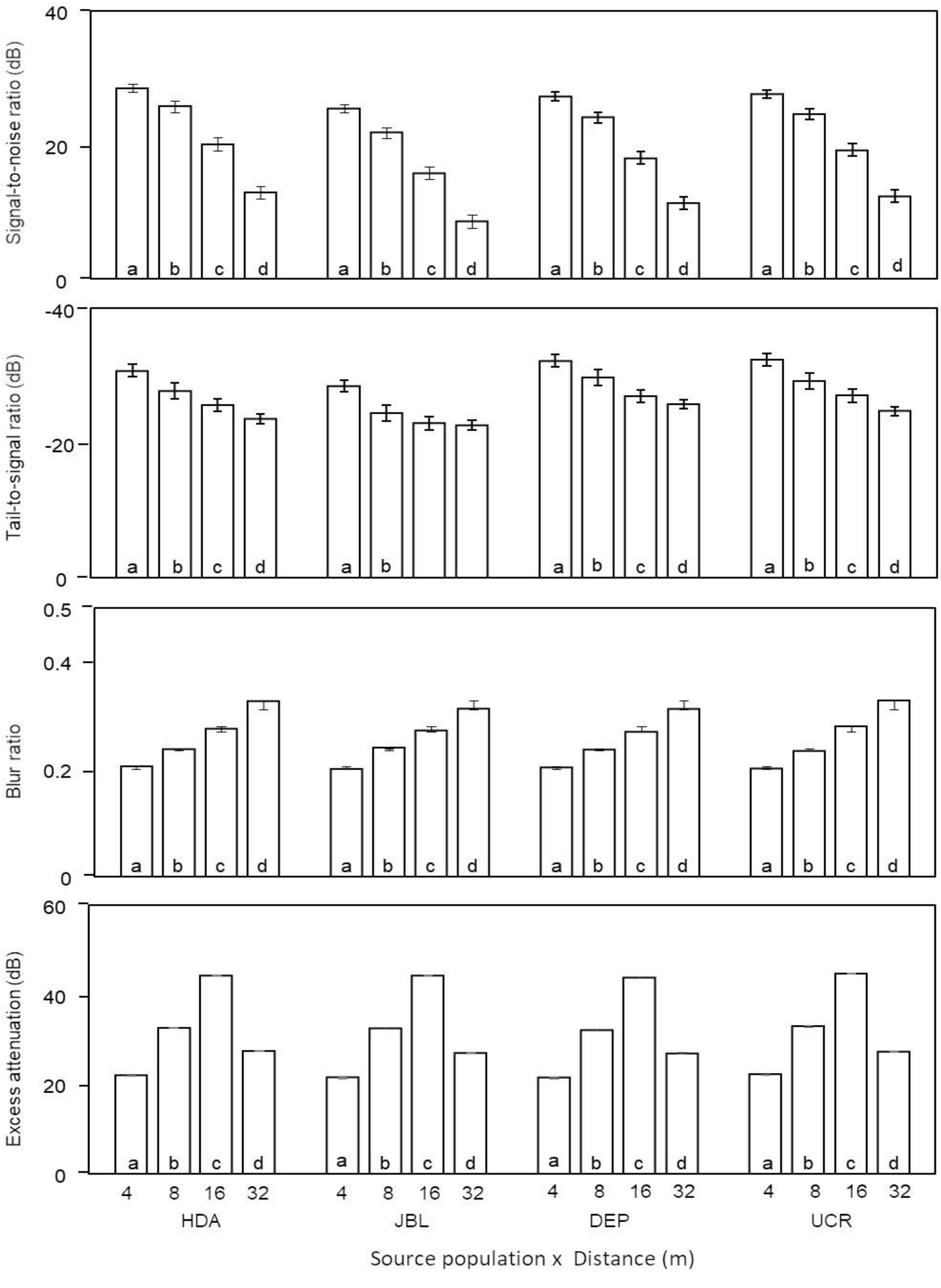
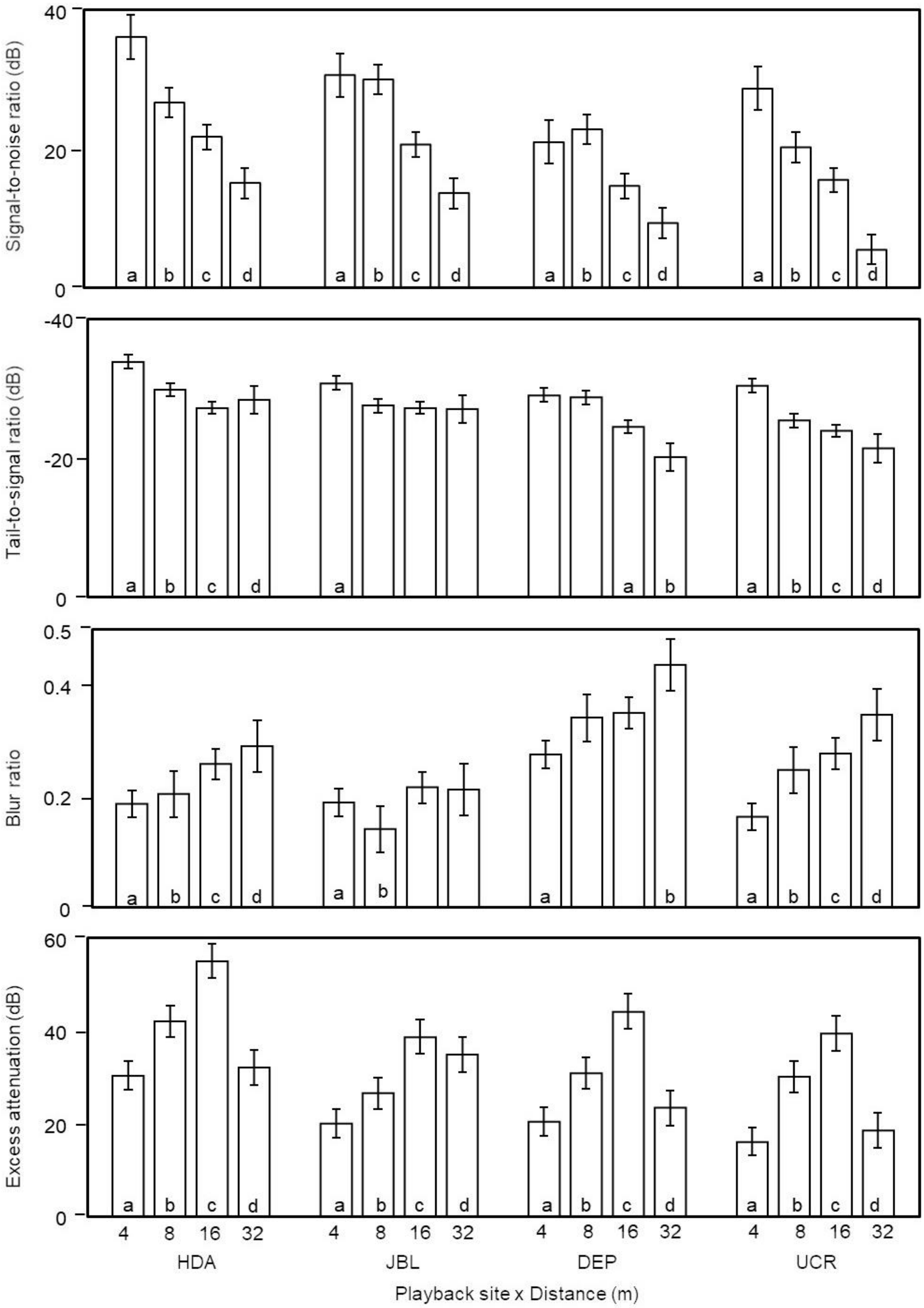


Figure 3. Second order interactions between playback site and source population for the most common solo songs of White-eared ground-sparrow. The letters below the bars refer to the playback site and the letters below the set of playback sites refer to the source population. Error bars are standard errors of the mean, and bars with different letters indicate the values are significantly different from each other in post hoc tests.

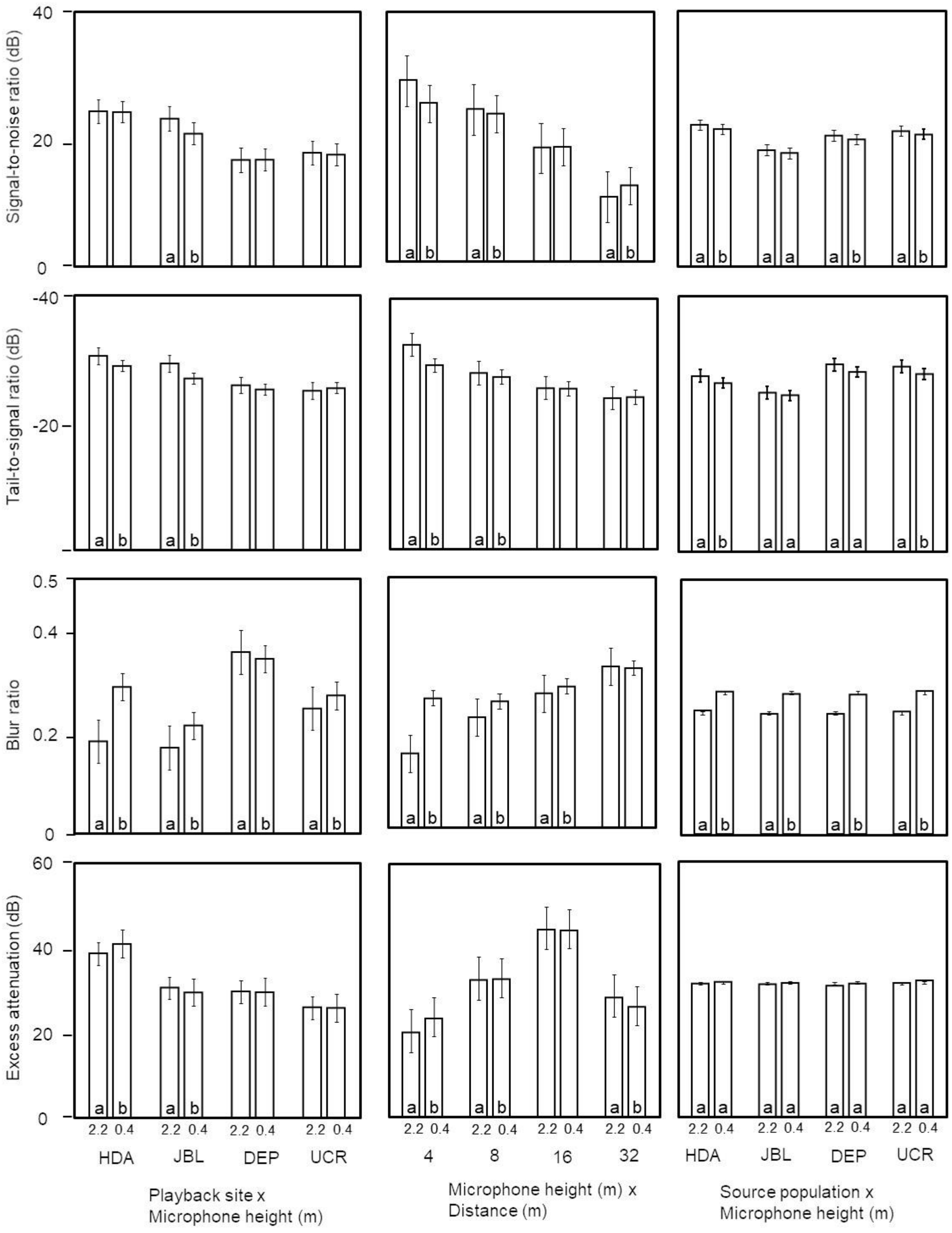
Supplementary Information



Supplementary Figure S1. Second order interactions between source population and distance for the most common solo songs of White-eared ground-sparrow. Error bars are standard errors of the mean, and bars with different letters indicate the values are significantly different from each other in post hoc tests.



Supplementary Figure S2. Second order interactions between playback site and distance for the most common solo songs of White-eared ground-sparrow. Error bars are standard errors of the mean, and bars with different letters indicate the values are significantly different from each other in post hoc tests.



Supplementary Figure S3. Second order interactions between playback site and microphone height, microphone heigth and distance and source population and microphone height for the most common solo songs of White-eared ground-sparrow. Error bars are standard errors of the mean, and bars with different letters indicate the values are significantly different from each other in post hoc tests.

Conclusiones

En la comparación de 10 años de las características genéticas, acústicas y morfológicas de las cuatro poblaciones del gorrión de cuatro ojos encontramos variación en cada una de las características mencionadas. Leve variación en la diversidad genética afectada principalmente por la distancia entre poblaciones, variación morfológica únicamente en dos poblaciones y variación acústica en todas las poblaciones y en los dos períodos de tiempo. A pesar del cambio registrado en las diferentes características estudiadas, solamente encontramos relación entre los cambios en la diversidad genética y los cantos. Creemos que esta única relación encontrada se debe a que la estructura del canto en cada población está siendo guiada por deriva cultural o selección sexual.

No encontramos evidencia alguna de que el mantenimiento de los cantos comunes dentro de cada población a lo largo del tiempo esté siendo guiado por la hipótesis de adaptación acústica. Debido a que la interacción entre la población donde se realizaron los experimentos (playback site) con la población donde el canto fue grabado (Source population) no mostró que los cantos comunes estén localmente adaptados, ya que los cantos no se transmitieron mejor en su propia población, por tanto, no presentaron menos degradación ni atenuación en sus hábitats respectivos como lo predice la hipótesis de adaptación acústica.