Asymmetric behavioural responses to divergent vocal signals in allopatric Neotropical sparrows

Ronald A. Fernández-Gómez [a, *], Jorge E. Morales-Mávil [a], Laura T. Hernández-Salazar [a], J. Roberto Sosa-López [b, c]

[a] Instituto de Neuroetología, Universidad Veracruzana, Xalapa, Veracruz, Mexico
[b] Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional Unidad Oaxaca (CIIDIR), Instituto Politécnico Nacional, Oaxaca, Mexico
[c] Dirección de Cátedras, Consejo Nacional de Ciencia y Tecnología (CONACYT), Ciudad de México, Mexico

ARTICLE INFO

Birdsongs are signals that encode information for multiple behaviours such as species recognition. Species recognition is influenced by both geographical variation in song and birds' discrimination abilities, forming the basis for evolutionary processes driving reproductive isolation and speciation. Here, we assessed the behavioural responses to geographical variation in songs in olive sparrows, *Arremonops rufivirgatus*. We conducted a series of playback experiments in two allopatric populations (Pacific, Peninsula) to assess whether olive sparrows responded differentially to geographical variants of their song and whether such responses could be predicted by the acoustic similarity between subject and stimulus songs. Our results indicate an asymmetrical behavioural response, with the Pacific population responding strongly to both local and allopatric stimuli and weakly to the control, and the Peninsula population responding strongly to local stimuli and weakly to both allopatric stimuli and the control. Furthermore, despite the birds' lack of previous experience with the playback stimulus songs, we found that response intensity was predicted by the acoustic similarity in both populations, with stronger responses to more similar songs, suggesting that males from different population may use a similar mechanism to recognize signals, despite whether the signal comes from conspecifics or heterospecifics. Our findings support the hypothesis that song divergence could act as a premating barrier for at least one of the studies populations, and that birds' responses are dictated by the structural similarity between senders' and receivers' signals.

© 2021 The Association for the Study of Animal Behaviour. Published by Elsevier Ltd. All rights reserved.

Animals use a variety of signals in multiple contexts to defend resources from sexual and/or ecological competitors (Bradbury & Vehrencamp, 2011). Such signals must encode the minimum of information that allows species recognition, while avoiding the high cost of responding to heterospecific signals (Searcy & Nowicki, 2010). Therefore, signallers and receivers can distinguish between competitors and noncompetitors, an important component of species discrimination (i.e. an animal's ability to differentiate conspecific individuals from heterospecific individuals; Bradbury & Vehrencamp, 2011). Species discrimination might evolve through coevolution between receivers and signallers, with fundamental implications to the evolution of animal communication and speciation (Wiley, 2006). Thus, to understand species recognition

in animals, it is imperative to know how receivers perceive variation in signals.

Bird acoustic signals usually vary in time and space as result of phylogenetic constraints, ecological factors, sexual selection and cultural processes (Podos & Warren, 2007; Wilkins, Seddon, & Safran, 2013). It is known that among species exhibiting vocal learning, copy errors or accidental additions introduce novel song variation into bird populations (Catchpole & Slater, 2008). This process could produce constant cultural signal variation over time, resulting in marked phenotypic differences among isolated populations (e.g. *Campylorhynchus rufinucha*, Sosa-López et al., 2013; *Troglodytes* wrens, Sosa-López et al., 2016; *Melozone leucotis*, Sandoval, Bitton, Demko, Doucet, & Mennill, 2017). These vocal differences among populations (referred to as geographical variation) may, therefore, provide insight into the evolution of behavioural discrimination.

The olive sparrow species complex (*Arremonops rufivirgatus*) includes five allopatric populations ranging from the southern

---

* Corresponding author.
  E-mail address: ronfernandez@uv.mx (R. A. Fernández-Gómez).

United States to northern Costa Rica (Brush, 2020). Taxonomically, three groups have been recognized (Brush, 2020; Navarro-Sigüenza & Peterson, 2004): the *rufivirgatus* group (subspecies: *rufivirgatus*, *ridgwayi* and *crassirostris*), which is distributed from southern Texas to southern Veracruz; the *superciliosus* group (subspecies: *sinaloae*, *sumichrasti*, *chiapensis* and *superciliosus*), which is distributed from Sinaloa, Mexico to the Pacific coast of Costa Rica; and the *verticalis* group (subspecies: *verticalis* and *rhyptothorax*), which is restricted to the Yucatan Peninsula. Our previous study in this species complex revealed marked geographical variation in vocal traits between isolated populations (Fernández-Gómez, Morales-Mávil, & Sosa-López, 2020); however, whether olive sparrows discriminate among isolated populations or closely related taxonomic groups is still unknown.

Here, we examined the vocal and physical responses to acoustic signals from local and allopatric populations of olive sparrows and tested whether similarity between the songs of focal birds and stimuli explains variation in responses. We conducted a series of playback experiments in two allopatric olive sparrow populations using local and foreign songs (from different allopatric populations) to simulate intruders within the individual territories. First, we asked whether a differential behavioural response could be elicited by acoustically different olive sparrows' songs. We predicted that males would exhibit the strongest response intensity towards songs from local populations, an intermediate response intensity towards songs of foreign populations and the weakest response intensity towards the control stimulus (e.g. Demko, Sosa-López & Mennill, 2019; Derryberry, 2011; Hick, Doucet, & Mennill, 2016; Sosa-López et al., 2016). Second, we asked whether individual olive sparrow's behavioural response to playback could be explained by the resemblance between the stimulus song and the song of the focal bird, in the absence of previous experience with the allopatric populations' stimuli. If birds use the similarity between senders' and receivers' signals as a mechanism to respond to potential competitors, then receivers should vary the intensity of their responses according to the acoustic distance between the stimulus song and the song of the focal bird, regardless of whether stimuli come from local, allopatric or heterospecifics males. We predicted that individuals would show the strongest responses to more acoustically similar signals (e.g. Amézquita, Flechas, Lima, Gasser, & Hödl, 2011; Freeman & Montgomery, 2017; Sosa-López et al., 2016). This study increases our understanding of the mechanisms that receivers use to respond to sexual and territorial signals and the role of acoustic divergence in the evolution of reproductive isolation.

METHODS

Study Sites

We conducted a series of playback experiments in two allopatric populations: (1) the Pacific population (belonging to the *superciliosus* group) at Chamela Biological Station, San Patricio, Jalisco, Mexico (19.4991°N, 105.0422°W) from 17 April to 30 April 2018 and (2) the Peninsula population (belonging to the *verticalis* group) on private land at Merida City, Yucatan, Mexico (20.8928°N, 89.6318°W) from 17 May to 25 May 2018. We located olive sparrow territories by detecting singing males (typically during the dawn chorus), but occasionally broadcasted local songs to detect additional territories. We used mist nets to capture and uniquely colourband individuals to facilitate individual identification. The study included a total of 23 territorial males, of which 16 males were colour-banded and seven were unbanded. Olive sparrows are highly territorial during the breeding season (Brush, 2020; Fernández-Gómez et al., 2020), allowing identification of their territories by using males' dawn singing trees and the position of other neighbouring territories.

Playback Design

We used playback to simulate the presence of intruder birds into olive sparrows' territories. Males in both populations (Pacific and Peninsula) received six trials. At each trial, we played back one of the following treatments: a local song from either the Pacific population or the Peninsula population; foreign songs from four allopatric populations (i.e. Gulf, Chiapas, Central America and Peninsula/Pacific); a heterospecific control stimulus (Appendix, Fig. A1). The control stimulus was a congeneric species, the blackstriped sparrow, *Arremonops conirostris*, a phylogenetically close species to the olive sparrow (Klicka et al., 2014). Olive sparrows have no previous experience with black-striped sparrow songs, as they live in allopatry (Brush, 2020), and their songs are acoustically different (Fernández-Gómez et al., 2020).

Each playback trial had a duration of 10 min with three phases (following Sosa-López et al., 2017): phase 1 was a 5 min silent period, allowing us to confirm that there was not current vocal activity or territorial interaction with neighbouring males; phase 2 involved 2 min of acoustic playback, where one treatment was presented; and phase 3 was a 3 min silent period, in which we recorded all movements and vocalizations of the birds. We assessed behavioural parameters during the 5 min of phases 2 and 3. Each focal subject received all six treatments on different days, at approximately the same time of day. We counterbalanced the sequence of presentation with stimulus identity in a Latin square design (Quinn & Keough, 2002). All experiments were conducted between 0700 and 1200 hours. We avoided testing adjacent males on the same day.

Playback Stimuli

We created playback stimuli using recordings obtained directly in the field and from public and private sound libraries, including The Macaulay Library (www.macaulaylibrary.org), Xeno-canto (www.xeno-canto.org), Biblioteca de Sonidos Aves de México BISAM (www1.inecol.edu.mx/sonidos/menu.htm) and the private collection Sounds of Nature SONAT (http://www.antoniocelis.net/publications.html). For each stimulus, we used a single song exemplar that was repeated during 2 min with 4 s silent intervals, which corresponds to natural vocal behaviour in this species based on our field observations. We selected recordings with a high signal-to-noise ratio from different individuals and avoided the use of neighbouring males' songs to maximize the independence of replicates (McGregor, 2000). All stimuli were prepared in Audacity v.2.3.3 (Audacity Team, https://audacityteam.org/), high-pass filtered at 1 kHz and low-pass filtered at 12 kHz, to eliminate background noise, and normalized to -3 dB to ensure a constant amplitude across all treatments and trials. In total, we used 80 songs belonging to 70 different birds to create all stimuli (see Supplementary Table S1 for detailed information on recording localities and library sources).

Playback Procedure

We identified each territorial subject and positioned a loudspeaker (model: Firefox, FOXPRO Inc., Lewistown, PA, U.S.A.) near the centre of the territory, at a height of 1 m. We estimated the territory centre based on prior observations of the birds' singing posts during the dawn chorus. Before the experiments, the maximum amplitude levels of stimuli were standardized at -90 dB at 1 m from the speaker, using a sound pressure level meter

(model: 33—2055 Radio Shack Inc., Fort Worth, TX, U.S.A.; C-weighting; fast response). We marked the vegetation to estimate the focal male distance to the speaker using flagging tape at a distance of 1, 3, 5, 10 and 15 m in all four cardinal directions from the speaker (following Sosa-López et al., 2016).

We recorded the behavioural responses of each tested male using a digital recorder (models: Tascam DR40, TEAC America, Inc., Santa Fe Springs, CA, U.S.A. or a Marantz PMD661, Marantz America, Inc., Mahwah, NJ, U.S.A.; settings: WAV format, 44.1 kHz sampling frequency, 16-bit resolution) with two shotgun microphones (models: AT897, Audio-Technica U.S., Inc., Stow, OH, U.S.A., and a Sennheiser ME67/K6, Electronic GmbH & Co. KG, Hüfingen, Germany), each connected to a different channel. We recorded focal males' vocalizations using one microphone, while the second microphone was used by an observer to dictate the focal males' behavioural movements. The observer sat 15—25 m from the speaker and was blind to the order of stimuli presentation.

We quantified behavioural responses in terms of both vocal and physical activity. To describe vocal responses, we assessed: (1) song latency: the time (in seconds) between the start of playback and the first song response of the focal male; (2) singing duration: the duration (in seconds) of total vocal activity from the first song to the end of the last song recorded during the trial; (3) number of songs: the total number of songs recorded during the trial; and (4) song rate: the number of songs produced/s by the focal male. To describe physical responses, we also assessed the following measures: (5) approach latency: the time (in seconds) between the start of playback and the first movement of approach to speaker; (6) number of flyovers: the total number of times that subjects flew over the speaker; and (7) closest approach distance: the minimum distance of approach of the focal male to the speaker.

We assessed the behavioural responses to local and foreign songs in olive sparrows. For the analysis, we reduced the number of behavioural variables by conducting a single principal component analysis (PCA) for both populations. We eliminated superfluous variables from the analysis by removing variables with $KMO \leq 0.6$, suggested as a minimum requirement for sampling adequacy (Budaev, 2010). We eliminated one variable at a time until we achieved a general $KMO \geq 0.7$ (i.e. calculated for all variables combined). Using this procedure, we eliminated one variable (song rate) from the original seven behavioural measurements. After this procedure, we achieved acceptable sampling adequacy (KMO test = 0.76). We extracted components with an eigenvalue greater than 1. The PCA produced two principal components that together explained 84.21% of the total variation in the original six variables. The first component ($PC1_{vocal}$) explained 67.42% of the total variation and was strongly associated with mainly vocal variables: song latency, singing duration, number of songs and approach latency; and the second component ($PC2_{physical}$) explained 16.79% of the total variation and was strongly associated with physical variables: number of flyovers and closest approach distance (Appendix, Table A1 for PCA details).

Ethical Note

Our research was carried out under ethical guidelines and approved permits by the Dirección General de Vida Silvestre de México (SEMARNAT; Permits: SGPA/DGVS/01319/16; SGPA/DGVS/00853/17; SGPA/DGVS/002783/18; SGPA/DGVS/003508/18). Prior to playback experiments, we captured focal birds using mist nets and playback. We held each captured bird as briefly as possible to minimize stress and quickly released each bird after processing. To reduce birds' disturbance as much as possible, we performed one playback trial at day. Following playback experiments, all birds

showed normal behaviour within 5—10 min. No adverse effects on behaviours were noted during subsequent observations.

Acoustic Similarity Analysis

We calculated the acoustic distance between the song of the experimental subject and the stimulus song to assess whether acoustic similarity dictates the intensity of the response. For this analysis, we organized the treatments into three categories: local songs (Pacific or Peninsula), allopatric songs (Gulf, Chiapas, Central America and Peninsula or Pacific) and heterospecific songs (control treatment, black-striped sparrow). We included a total of 105 songs (23 subject songs and 82 stimulus songs). We measured 12 fine structural characteristics of all songs, following the methods in our previous research with olive sparrows (Fernández-Gómez et al., 2020). The acoustic measurement variables were: (1) mean frequency, (2) standard deviation of the mean frequency, (3) first quartile frequency, (4) third quartile frequency, (5) song duration, (6) number of elements in the song, (7) element rate, (8) minimum frequency, (9) maximum frequency, (10) bandwidth, (11) peak frequency and (12) entropy (see Fernández-Gómez et al., 2020, for variable definitions). Measurements were obtained in R (R Core Team, 2013) with the package 'warbleR' (Araya-Salas & Smith-Vidaurre, 2017), which uses functions from 'tuneR' (Ligges, Krey, Mersmann, & Schnackenberg, 2016), 'seewave' (Sueur, Aubin, & Simonis, 2008) and 'monitoR' (Katz, Hafner, & Donovan, 2016).

Following the procedure outlined above, we performed a PCA using the correlation matrix with varimax rotation, extracted components with eigenvalues greater than 1 and eliminated superfluous variables. We eliminated one variable (song duration) from the original 12 acoustic measurements. After this procedure, we achieved acceptable sampling adequacy (KMO test = 0.7). The PCA produced three principal components that together explained 88.2% of the total variation for 11 acoustic variables. The first component ($PC1_{acoustic}$) explained 62.2% of the total variation and was strongly associated with frequency variables (mean frequency, first and third quartile frequency, minimum and maximum frequency, peak frequency); the second component ($PC2_{acoustic}$) explained 16.9% of the total variation and was strongly associated with variables explaining frequency variation within songs (SD mean frequency, bandwidth, entropy); and the third component ($PC3_{acoustic}$) explained 9.1% of the total variation and was strongly associated with time variables (number of elements in the song, element rate; Appendix, Table A2). We used the component scores resulting from these PCAs to calculate the acoustic similarity as the Euclidean distance between the focal bird song and the stimulus song used in each trial.

Statistical Analysis

We tested for differences in olive sparrows' responses to local and foreign songs using linear mixed models (LMM). We included the behavioural components extracted ($PC1_{vocal}$ and $PC2_{physical}$) as the response variables, focal population (i.e. Pacific and Peninsula) and playback treatment (six levels: one local stimulus, four allopatric stimuli and one heterospecific control stimulus) as fixed effects and bird identity as a subject variable with random effect. We included in the model the interaction among focal population and playback treatment. The time of the day at which playbacks were performed and the order of treatment presentation had no significant effects and were dropped in the final models (time: $PC1_{vocal}$: $F_{1,23.2} = 0.0005$, $P = 0.9$; $PC2_{physical}$: $F_{1,21.4} = 0.27$, $P = 0.6$; order: $PC1_{vocal}$: $F_{5,110.3} = 0.68$, $P = 0.6$; $PC2_{physical}$: $F_{5,108.6} = 1.29$, $P = 0.2$).

Then, we assessed whether behavioural responses to playback are explained by the acoustic similarity between the song of the

stimulus and the experimental subject using LMMs. We performed two sets of analyses, one for each PC behaviour (PC1$_{vocal}$ and PC2$_{physical}$). For each set, we first tested an overall relationship between playback responses and acoustic similarity by population. We included the principal components summarizing the behavioural response (PC1$_{vocal}$ or PC2$_{physical}$) as dependent variable, the Euclidian acoustic distances (PC1$_{acoustic}$, PC2$_{acoustic}$ and PC3$_{acoustic}$) and focal populations (Pacific and Peninsula) as fixed effects and bird identity as a random effect. We generated multiple models and chose the most parsimonious model by calculating the Akaike information criterion (AIC, Burnham & Anderson, 2002). We kept the principal components summarizing the behavioural response constant (PC1$_{vocal}$ or PC2$_{physical}$) and tested each interaction separately, including all their possible combinations. Second, we tested whether the relationship between playback responses and acoustic similarity by population could be explained by treatment. We included the principal components summarizing the behavioural response (PC1$_{vocal}$ or PC2$_{physical}$) as dependent variable, the Euclidian acoustic distances (PC1$_{acoustic}$, PC2$_{acoustic}$ and PC3$_{acoustic}$) and treatments (local, allopatric, heterospecific) as fixed effects and bird identity as a random effect. We tested the interactions between the Euclidian acoustic distances and treatments. For this

analysis, we chose the principal components with the Euclidian acoustic distances that had significant interactions with focal populations in the previous analysis.

We conducted PCAs using SPSS v.25 (IBM Corp., Armonk, NY, U.S.A.) and LMM using R v.4.0.0 (R Foundation for Statistical Computing, Vienna, Austria) with the package 'lme4' (Dray & Dufour, 2007) for fitting the models and the package 'multcomp' (Hothorn, Bretz, & Westfall, 2008) for estimated marginal means and Tukey's pairwise independent contrasts. All tests were two tailed with a significance threshold of 0.05. All assumptions for the LMM analyses such as normality and homogeneity were met (Zuur, Ieno, Walker, Saveliev, & Smith, 2009).

RESULTS

Do Olive Sparrows Respond Differently to Acoustic Signals from Allopatric Populations?

Populations showed significant differences in both vocal and physical responses across playback treatments (PC1$_{vocal}$: $F_{11,94.9} = 3.34$, $P < 0.001$; PC2$_{physical}$: $F_{11,97.2} = 11.03$, $P < 0.001$). For the Pacific population, a PC1$_{vocal}$ post hoc test showed no significant
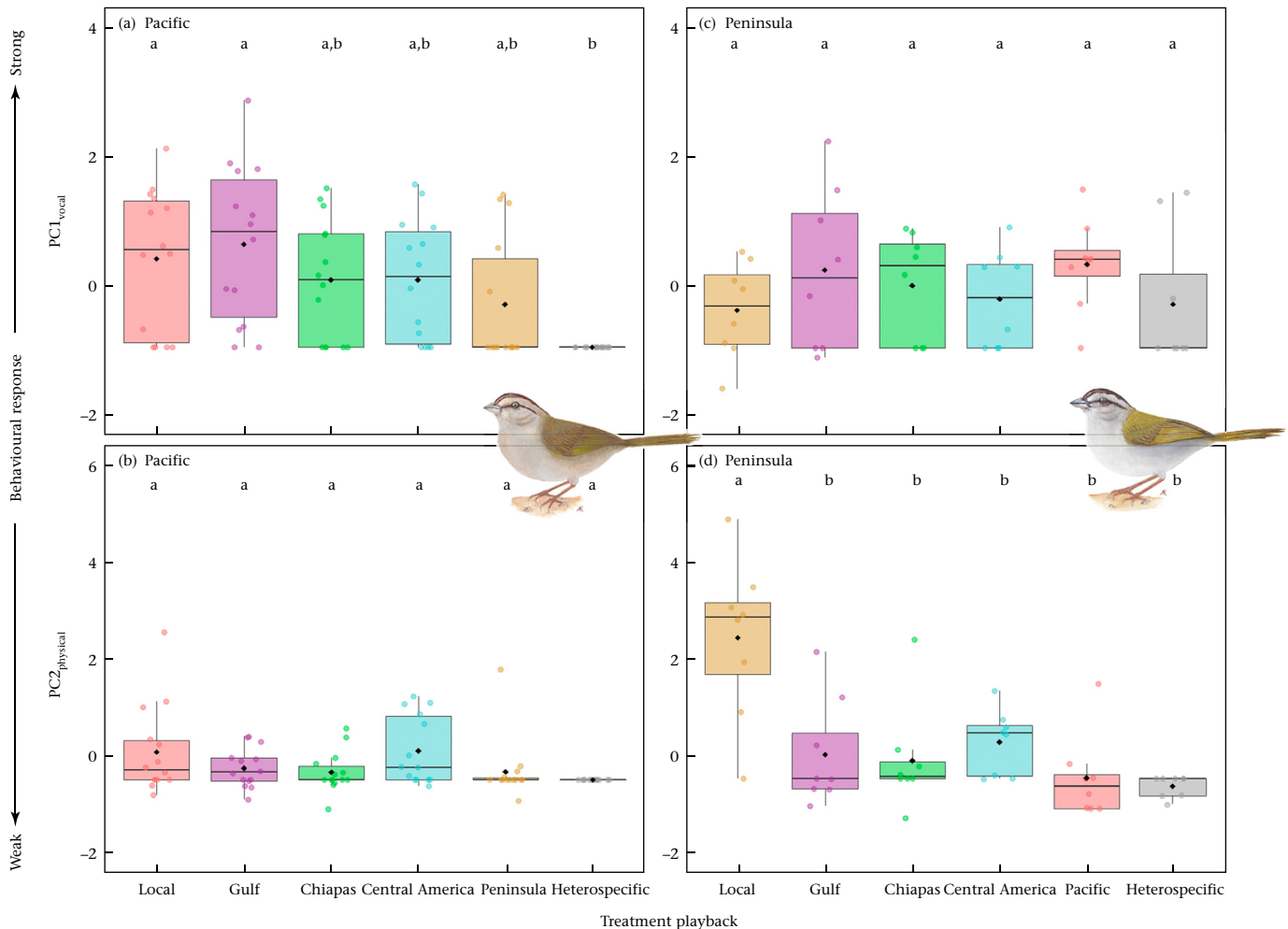
Figure 1. Behavioural response of olive sparrow males from (a, b) Pacific and (c, d) Peninsula populations to local, allopatric and heterospecific songs. Box plots show 25% and 75% quartiles, lines within the boxes show medians, and whiskers show outermost values within the range of 1.5 times the respective quartiles. Estimated marginal means and raw observations are shown as black dots and coloured dots, respectively. Letters indicate the results of the post hoc test, where treatments with the same letters indicate nonsignificant differences. Illustrations for the species in both populations are shown.

differences in the responses to local and allopatric treatments but a significantly different response to heterospecific stimuli (Fig. 1a); while a PC2$_{physical}$ post hoc test showed no significant differences between treatments (Fig. 1b). For the Peninsula population, a PC1$_{vocal}$ post hoc test showed no significant differences between treatments (Fig. 1c); while a PC2$_{physical}$ post hoc test showed a significant difference between local treatments and all other treatments (Fig. 1d, Supplementary Table S2, for detailed pairwise comparisons).

Are Playback Responses Driven by Acoustic Similarity?

The best models showed a significant effect of population on the association between acoustic distance with vocal responses (PC1$_{vocal}$ ~ PC1$_{acoustic}$*population: $F_{2,111.2} = 5.7$, $P = 0.004$; PC1$_{vocal}$ ~ PC2$_{acoustic}$*population: $F_{2,115.7} = 4.4$, $P = 0.01$; Fig. 2a and b, Table 1) and physical responses (PC2$_{physical}$ ~ PC2$_{acoustic}$*population: $F_{2,127} = 3.8$, $P = 0.02$; Fig. 2c, Table 1), suggesting that olive sparrow's behavioural responses are explained by the resemblance between the stimulus song and the song of the focal bird.

Furthermore, we found that the significant relationship between playback responses and acoustic similarity held for some treatment types. In particular, Pacific population males responded with higher vocal and physical intensity to heterospecific and local stimuli, respectively, that were more similar to their own songs (Fig. 3a and b, Table 2). In contrast, Peninsula population males responded with higher vocal and physical intensity to local and allopatric stimuli that were more similar to their own songs (Fig. 3c and d, Table 2).

DISCUSSION

We assessed behavioural responses in two allopatric olive sparrow populations to songs with marked geographical variation and explored whether acoustic similarity drives response strength. Both populations responded strongly to local songs and weakly to heterospecific songs; however, while the Pacific population showed no differences between local songs and all four allopatric songs, the Peninsula population responses were significantly stronger for local songs than for allopatric songs. Furthermore, our analyses

revealed that the strength of response was predicted by the acoustic similarity between the subject's song and the stimulus song. Together, these results suggest an asymmetrical pattern of response, with independent evolutionary lineages having divergent discrimination patterns, and that birds use similarity between their own songs and stimulus songs to recognize potential competitors.

Asymmetric discrimination, which has been reported in multiple studies (Uy, Irwin, & Webster, 2018), is thought to occur as a result of learning, sexual selection and social selection (Burbidge, Parson, Caycedo-Rosales, Cadena, & Slabbekoorn, 2015; Colbeck, Sillett, & Webster, 2010; Demko et al., 2019; Dingle, Poelstra, Halfwerk, Brinkhuizen, & Slabbekoorn, 2010; Greig & Webster, 2013; Hamao, 2016; Hick et al., 2016; Pearson & Rohwer, 2000). Most studies, however, have reported asymmetrical responses in species with sympatric and parapatric populations and have rarely reported this pattern in allopatric populations as in wood-wrens (Dingle et al., 2010). We suggest two nonmutually exclusive hypotheses that could explain the asymmetric response pattern in olive sparrows.

Under the first scenario, isolation between populations with only cultural drift acting on songs could promote song divergence with consequences in perception across the populations (Wilkins et al., 2013). Learning is related to an auditory template that could be modified through social interactions, driving differences in both song structure and recognition among populations (Irwin, Thimgan, & Irwin, 2008; Lachlan & Servedio, 2004; Lynch, 1996; Ord, King, & Young, 2011; Sosa-López et al., 2013), as has been proposed to occur in greenish warblers, *Phylloscopus trochiloides* (Irwin et al., 2008). In line with this hypothesis, and supported by the results of our previous study, where we showed a relationship between acoustic divergence and geographical distance (Fernández-Gómez et al., 2020), a possible explanation for variation in olive sparrows' perception is that random changes in vocal traits accrued linearly with distance as result of learning processes shaping males' auditory templates (Koetz, Westcott, & Congdon, 2007; Lipshutz, Overcast, Hickerson, Brumfield, & Derryberry, 2017).

Under the second scenario, ecological and sexual selection could act on song divergence, with little or no role of isolation on song variation. In line with the sensory drive hypothesis (Endler, 1992), adaptation of each olive sparrow population to a divergent habitat
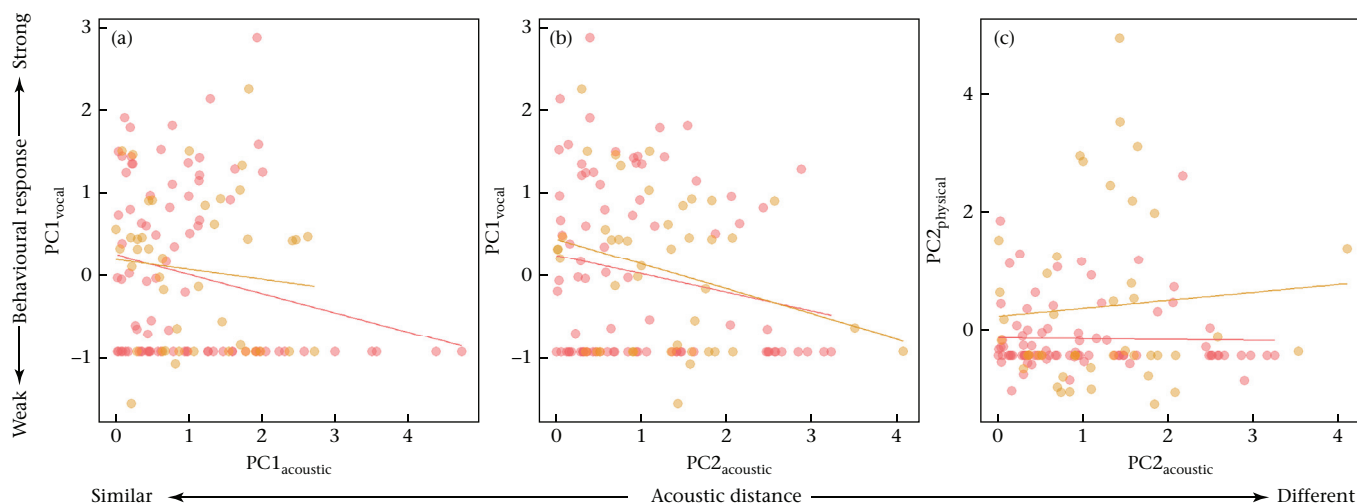
Figure 2. Association between the strength of olive sparrows' behavioural responses and the acoustic song similarity between subject and stimulus songs. Graphs depict the relationships between (a) PC1$_{vocal}$ responses and PC1$_{acoustic}$ responses, (b) PC1$_{vocal}$ responses and PC2$_{acoustic}$ responses and (c) PC2$_{physical}$ responses and PC2$_{acoustic}$ responses. Light red and yellow dots represent Pacific and Peninsula populations, respectively. Only significant associations are shown.

Table 1
Linear mixed models to assess the effects of population on the association between acoustic distance and acoustic similarity ($PC1_{vocal}$ and $PC2_{physical}$)

Dependent variable	Fixed effect	No. of parameters	AIC
$PC1_{vocal}$	$PC1_{acoustic}*population+PC2_{acoustic}*population$	7	358.11
	$PC1_{acoustic}*population+PC2_{acoustic}*population+PC3_{acoustic}*population$	9	358.02
$PC2_{physical}$	$PC2_{acoustic}*population$	5	365.40
	$PC1_{acoustic}*population$	5	367.77
	$PC3_{acoustic}*population$	5	369.90
	$PC1_{acoustic}*population+PC2_{acoustic}*population$	7	366.98
	$PC2_{acoustic}*population+PC3_{acoustic}*population$	7	368.07
	$PC1_{acoustic}*population+PC2_{acoustic}*population + PC3_{acoustic}*population$	9	369.89
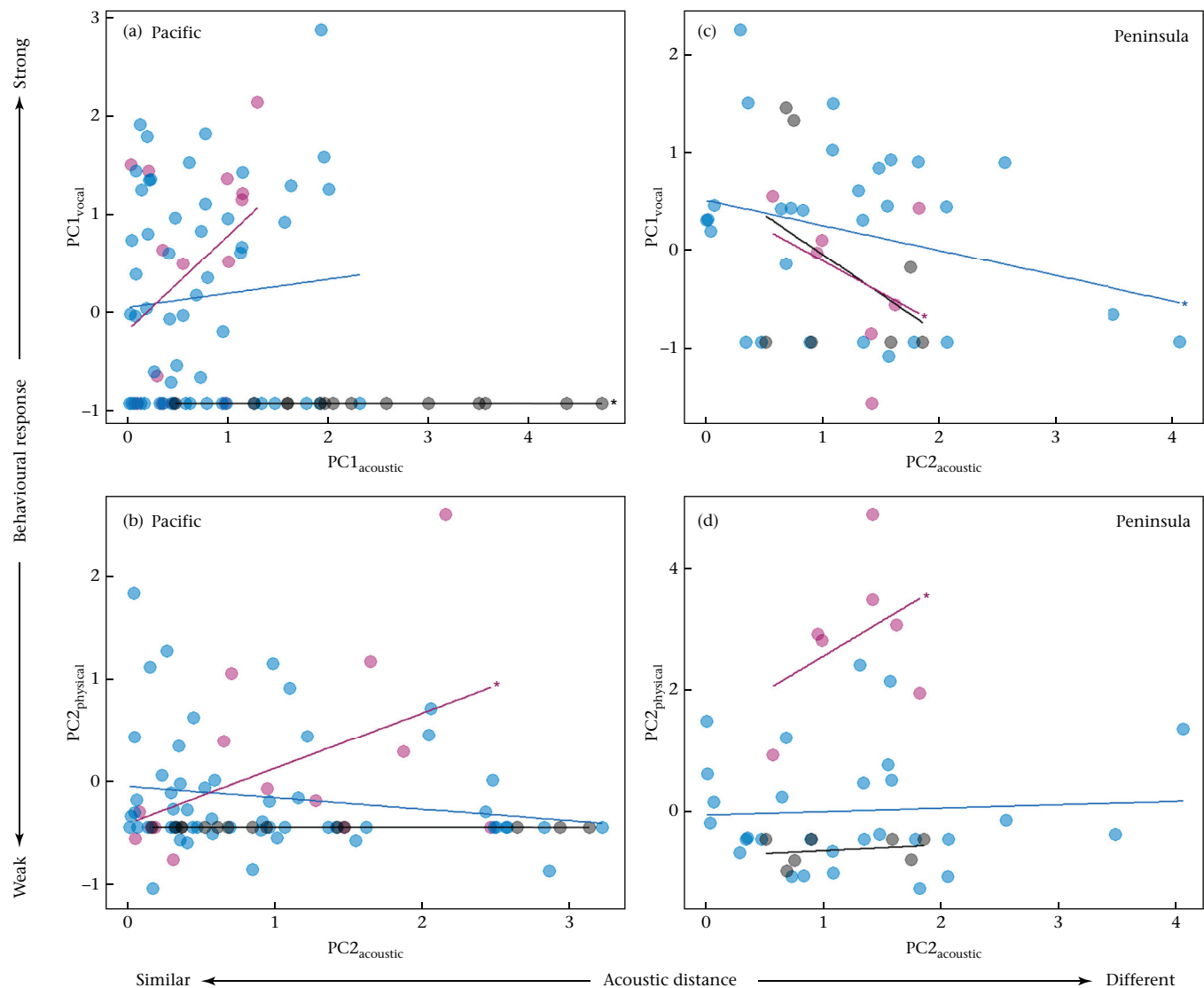
Figure 3. Graphics depicting treatment slopes for the association between the strength of olive sparrows' behavioural responses and the acoustic song similarity between subject and stimulus songs. (a, c) Association between $PC1_{vocal}$ responses and $PC1_{acoustic}$ responses for Pacific and Peninsula populations, respectively. (b, d) Association between $PC2_{physical}$ responses and $PC2_{acoustic}$ responses for Pacific and Peninsula populations, respectively. Purple, blue and black points represent local, allopatric and heterospecific treatments, respectively. Asterisks denote significant relationships.

could promote lineage diversification of sensory systems to local environments, affecting how songs are detected and perceived (Burbidge et al., 2015; Dingle et al., 2010; Endler, 1992; Kirschel et al., 2011; Seddon, 2005; Seddon & Tobias, 2010). Furthermore, additional selection forces could promote changes in phenotypic

traits functionally related to sound production such as body mass or beak morphology (Derryberry et al., 2012, 2018). For instance, a study in tropical Africa with little greenbuls, *Andropadus virens*, showed asymmetric discrimination between divergent songs by individuals from different habitats; songs from the same habitat

elicited similar response levels irrespective of the geographical origin of the songs, whereas songs from different habitats elicited different intensities of response (Kirschel et al., 2011). Thus, either the lack of habitat differentiation or no sexual selection acting on the Pacific populations could explain the low song divergence among allopatric populations (Fernández-Gómez et al., 2020) and similar responses between local populations and all four allopatric populations. On the other hand, olive sparrow habitat differs greatly between the Peninsula population and the Pacific population. Some evidence suggests that the climate in the Yucatan Peninsula changed during the Pleistocene, producing ecological divergence and promoting habitat and species differentiation (Cortés-Ramírez, Gordillo-Martínez, & Navarro-Sigüenza, 2012; Estrada-Loera, 1991), as is thought to have occurred in the wedge-tailed sabrewing, *Campylopterus curvipennis* (Cruz-Yepez, González, & Ornelas, 2020). Further phylogenetic analysis and playback experiments focusing on sexual and ecological selection would help to better understand how divergence in song recognition has evolved in olive sparrows.

Our results indicate that the responses of olive sparrows vary with the degree of similitude between the focal individual's song and the stimulus song, and that males likely use the same fine acoustic song structural characteristics to differentiate between songs despite whether the stimulus comes from local, allopatric or heterospecific birds. This finding supports the idea that a perceptual mechanism of acoustic signal recognition known as generalization could be acting in olive sparrows. Generalization occurs when subjects compare signals that they perceive with a signal template (i.e. a complex perceptual system of a neuronal substrate of memory for recognition; Bolhuis & Gahr, 2006). Under this scenario, the similitude between the perceived signal and the template should predict the strength of the response (Shepard, 1987). Many studies in frogs and songbirds support this hypothesis (Derryberry, 2011; Freeman & Montgomery, 2017; Ryan, Rand, Hurd, Phelps, & Rand, 2003; Sosa-López et al., 2016) and explain the differential responses towards conspecific and heterospecific songs. In the present study, the strength of the behavioural response by birds in both populations was associated with the similitude between the birds' own songs and the stimulus,

regardless of whether the stimulus came from an allopatric population or a heterospecific population. Pacific and Peninsula males appear to respond to allopatric divergent songs based on how much those songs differ from their own song template, suggesting that generalization could be a mechanism explaining such a response pattern. However, it seems to be that the Peninsula population uses a different strategy, showing a trade-off pattern between physical and vocal response. Indeed, the physical responses of Peninsula birds towards songs of their closest neighbours were stronger and decreased with the distance between neighbours; vocal responses, in contrast, showed an inverted pattern, with response intensity increasing with the distance (Fig. 4). Both patterns might be explained by the birds' familiarity with neighbouring songs. However, why Pacific and Peninsula populations showed different physical patterns in their responses is still unclear. Previous studies have shown inconsistent results regarding the sequential order of aggressive signals in birds. For instance, in some species such as swamp sparrows, *Melospiza georgiana*, physical behaviours (wing waving) predict attack (Ballentine, Searcy, & Nowicki, 2008), while in other species such as black-capped chickadees, *Poecile atricapillus*, attacks are predicted by vocal behaviours (Baker, Wilson, & Mennill, 2012).

In conclusion, our findings indicate an asymmetrical behavioural response to allopatric populations in olive sparrows. Despite this asymmetry, the responses in both populations were driven by the acoustic resemblance between birds' focal songs and stimulus songs. Moreover, despite the divergence in songs between allopatric populations reported in our previous study (Fernández-Gómez et al., 2020), our current playback experiments suggest that the Peninsula population does not recognize other allopatric populations as competitors. Thus, song could act as a behavioural premating barrier, providing support for considering the *verticalis* group as an independent evolutionary unit. Conversely, the Pacific population does recognize other allopatric populations as competitors, providing support for considering all allopatric populations within the *superciliosus* group as an evolutionary unit (Brush, 2020; Navarro-Sigüenza & Peterson, 2004). We propose that geographical isolation followed by cultural drift and ecological and sexual pressures, working alone or together, could explain the

Table 2
Results of linear mixed models explaining the effect of treatment on the relationship between olive sparrows' responses to playback and the acoustic similarity between the subject and the playback stimulus

Behavioural response	Population	Fixed effect	Estimate	SE	df	t	P
PC1$_{vocal}$	Pacific	(Intercept)	0.21	0.23	50.68	0.93	0.357
		PC1$_{acoustic}$*local	0.57	0.40	76.84	1.43	0.156
		PC1$_{acoustic}$*allopatric	0.15	0.21	80.48	0.71	0.482
		PC1$_{acoustic}$*heterospecific	-0.31	0.12	81.20	-2.68	**0.009**
		PC2$_{acoustic}$*local	-0.10	0.24	82.90	-0.41	0.682
		PC2$_{acoustic}$*allopatric	-0.20	0.14	83.87	-1.48	0.143
		PC2$_{acoustic}$*heterospecific	-0.26	0.20	80.84	-1.34	0.184
	Peninsula	(Intercept)	1.04	0.37	29.55	2.83	**0.008**
		PC1$_{acoustic}$*local	-0.32	0.54	42.20	-0.59	0.556
		PC1$_{acoustic}$*allopatric	-0.29	0.21	25.31	-1.35	0.191
		PC1$_{acoustic}$*heterospecific	-0.35	0.32	39.92	-1.12	0.272
		PC2$_{acoustic}$*local	-0.89	0.44	41.02	-2.02	**0.050**
		PC2$_{acoustic}$*allopatric	-0.41	0.15	41.96	-2.70	**0.010**
		PC2$_{acoustic}$*heterospecific	-0.68	0.43	42.57	-1.59	0.119
PC2$_{physical}$	Pacific	(Intercept)	-0.17	0.10	31.71	-1.64	0.112
		PC2$_{acoustic}$*local	0.37	0.13	81.47	2.88	**0.005**
		PC2$_{acoustic}$*allopatric	-0.04	0.08	83.59	-0.48	0.634
		PC2$_{acoustic}$*heterospecific	-0.15	0.11	81.71	-1.38	0.170
	Peninsula	(Intercept)	-0.09	0.27	27.29	-0.34	0.734
		PC2$_{acoustic}$*local	2.27	0.31	39.65	7.25	**<0.001**
		PC2$_{acoustic}$*allopatric	0.09	0.17	42.99	0.51	0.613
		PC2$_{acoustic}$*heterospecific	-0.38	0.32	39.39	-1.16	0.251

Significant values are shown in bold.
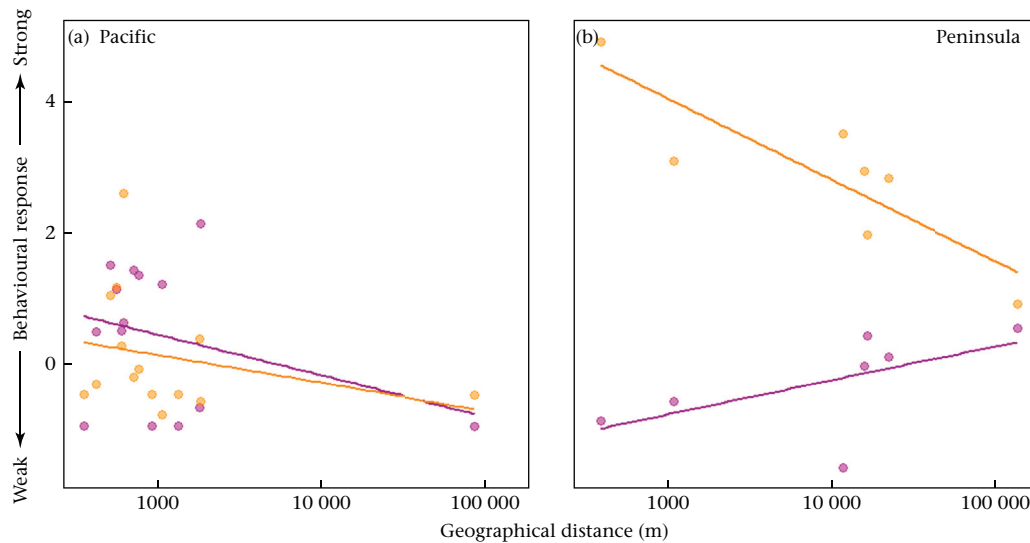
Figure 4. Relationship between the strength of olive sparrows' behavioural responses (PC1$_{vocal}$ and PC2$_{physical}$) and the geographical distance (in metres) between the territory of the focal bird and the territory of the bird whose song was used as stimulus for the local treatment in the playback experiment. (a) Pacific population (Pearson correlation: PC1$_{vocal}$: $r_{12} = -0.35$, $P = 0.21$; PC2$_{physical}$: $r_{12} = -0.18$, $P = 0.52$). (b) Peninsula population (PC1$_{vocal}$: $r_5 = 0.55$, $P = 0.19$; PC2$_{physical}$: $r_5 = -0.77$, $P = 0.04$). Light magenta points indicate vocal responses (PC1$_{vocal}$) and light orange points represent physical responses (PC2$_{physical}$).

asymmetrical response pattern, and that males use fine structural characteristics of the songs to recognize potential competitors.

Author Contributions

R.A.F.G. and J.R.S.L. conceived the idea. R.A.F.G. carried out the field work, collected the data and performed the analyses. J.R.S.L. and J.E.M.M. coordinated the study, provided funding, participated in the design of the study and provided guidance for all analyses. R.A.F.G., J.E.M.M., L.T.H.S. and J.R.S.L. wrote the manuscript.

Funding

Declaration of Interests

We declare we have no competing interests.

Acknowledgments

Supplementary Material

Supplementary material associated with this article is available, in the online version, at https://doi.org/10.1016/j.anbehav.2021.01.018.

References

Amézquita, A., Flechas, S. V., Lima, A. P., Gasser, H., & Hodl, W. (2011). Acoustic interference and recognition space within a complex assemblage of dendrobatid frogs. *Proceedings of the National Academy of Sciences of the United States of America, 108*(41), 17058–17063. https://doi.org/10.1073/pnas.1104773108

Araya-Salas, M., & Smith-Vidaurre, G. (2017). warbleR: An R package to streamline analysis of animal acoustic signals. *Methods in Ecology and Evolution, 8*(2), 184–191. https://doi.org/10.1111/2041-210X.12624

Baker, T. M., Wilson, D. R., & Mennill, D. J. (2012). Vocal signals predict attack during aggressive interactions in black-capped chickadees. *Animal Behaviour, 84*(4), 965–974. https://doi.org/10.1016/j.anbehav.2012.07.022

Ballentine, B., Searcy, W. A., & Nowicki, S. (2008). Reliable aggressive signalling in swamp sparrows. *Animal Behaviour, 75*(2), 693–703. https://doi.org/10.1016/j.anbehav.2007.07.025

Bolhuis, J. J., & Gahr, M. (2006). Neural mechanisms of birdsong memory. *Nature Reviews Neuroscience, 7*(5), 347–357. https://doi.org/10.1038/nrn1904

Bradbury, J. W., & Vehrencamp, S. L. (2011). *Principles of animal communication*. Sunderland, MA: Sinauer.

Brush, T. (2020). Olive sparrow (*Arremonops rufivirgatus*). In A. F. Poole (Ed.), *Birds of the world*. Ithaca, NY: Cornell Lab of Ornithology. https://doi.org/10.2173/bow.olispa.01.

Budaev, S. V. (2010). Using principal components and factor analysis in animal behaviour research: Caveats and guidelines. *Ethology, 116*(5), 472–480. https://doi.org/10.1111/j.1439-0310.2010.01758.x

Burbidge, T., Parson, T., Caycedo-Rosales, P. C., Cadena, C. D., & Slabbekoorn, H. (2015). Playbacks revisited: Asymmetry in behavioural response across an acoustic boundary between two parapatric bird species. *Behaviour, 152*(14), 1933–1951. https://doi.org/10.1163/1568539X-00003309

Burnham, K. P., & Anderson, D. R. (2002). Model selection and multimodel inference: A practical information-theoretic approach. In K. P. Burnham, & D. R. Anderson (Eds.), *Model selection and multimodel inference*. New York, NY: Springer. https://doi.org/10.1007/b97636.

Catchpole, C. K., & Slater, P. J. B. (2008). *Bird song: Biological themes and variations* (2nd ed.). Cambridge, U.K: Cambridge University Press. https://doi.org/10.1017/CBO9780511754791

Colbeck, G. J., Sillett, T. S., & Webster, M. S. (2010). Asymmetric discrimination of geographical variation in song in a migratory passerine. *Animal Behaviour, 80*(2), 311–318. https://doi.org/10.1016/j.anbehav.2010.05.013

Cortés-Ramírez, G., Gordillo-Martínez, A., & Navarro-Sigüenza, A. G. (2012). Biogeographical patterns of the Yucatán Peninsula birds. *Revista Mexicana de Biodiversidad, 83*(2), 530–542. https://doi.org/10.7550/rmb.32326

Cruz-Yepez, N., González, C., & Ornelas, J. F. (2020). Vocal recognition suggests premating isolation between lineages of a lekking hummingbird. *Behavioral Ecology, 31*(4), 1046–1053. https://doi.org/10.1093/beheco/araa050

Demko, A. D., Sosa-López, J. R., & Mennill, D. J. (2019). Subspecies discrimination on the basis of acoustic signals: A playback experiment in a Neotropical songbird. *Animal Behaviour, 157*, 77–85. https://doi.org/10.1016/j.anbehav.2019.08.021

Derryberry, E. P. (2011). Male response to historical and geographical variation in bird song. *Biology Letters, 7*(1), 57–59. https://doi.org/10.1098/rsbl.2010.0519

Derryberry, E. P., Seddon, N., Claramunt, S., Tobias, J. A., Baker, A., Aleixo, A., et al. (2012). Correlated evolution of beak morphology and song in the neotropical woodcreeper radiation. *Evolution, 66*(9), 2784–2797. https://doi.org/10.1111/j.1558-5646.2012.01642.x

Derryberry, E. P., Seddon, N., Derryberry, G. E., Claramunt, S., Seeholzer, G. F., Brumfield, R. T., et al. (2018). Ecological drivers of song evolution in birds: Disentangling the effects of habitat and morphology. *Ecology and Evolution, 8*(3), 1890–1905. https://doi.org/10.1002/ece3.3760

Dingle, C., Poelstra, J. W., Halfwerk, W., Brinkhuizen, D. M., & Slabbekoorn, H. (2010). Asymmetric response patterns to subspecies-specific song differences in allopatry and parapatry in the gray-breasted wood-wren. *Evolution, 64*(12), 3537–3548. https://doi.org/10.1111/j.1558-5646.2010.01089.x

Dray, S., & Dufour, A.-B. (2007). The ade4 Package: Implementing the duality diagram for ecologists. *Journal of Statistical Software, 22*(4). https://doi.org/10.18637/jss.v022.i04

Endler, J. A. (1992). Signals, signal conditions, and the direction of evolution. *American Naturalist, 139*(Suppl), S125–S153. https://doi.org/10.1086/285308

Estrada-Loera, E. (1991). Phytogeographic relationships of the Yucatan Peninsula. *Journal of Biogeography, 18*(6), 687–697. https://doi.org/10.2307/2845550

Fernández-Gómez, R. A., Morales-Mávil, J. E., & Sosa-López, J. R. (2020). Geographic variation and divergence of songs in the olive sparrow species complex. *Journal of Field Ornithology, 91*(1), 77–91. https://doi.org/10.1111/jofo.12320

Freeman, B. G., & Montgomery, G. A. (2017). Using song playback experiments to measure species recognition between geographically isolated populations: A comparison with acoustic trait analyses. *Auk: Ornithological Advances, 134*(4), 857–870. https://doi.org/10.1642/auk-17-63.1

Greig, E. I., & Webster, M. S. (2013). Spatial decoupling of song and plumage generates novel phenotypes between 2 avian subspecies. *Behavioral Ecology, 24*(4), 1004–1013. https://doi.org/10.1093/beheco/art005

Hamao, S. (2016). Asymmetric response to song dialects among bird populations: The effect of sympatric related species. *Animal Behaviour, 119*, 143–150. https://doi.org/10.1016/j.anbehav.2016.06.009

Hick, K. G., Doucet, S. M., & Mennill, D. J. (2016). Tropical wrens rely more on acoustic signals than visual signals for inter- and intraspecific discrimination. *Animal Behaviour, 118*, 153–163. https://doi.org/10.1016/j.anbehav.2016.05.024

Hothorn, T., Bretz, F., & Westfall, P. (2008). Simultaneous inference in general parametric models. *Biometrical Journal, 50*(3), 346–363. https://doi.org/10.1002/bimj.200810425

Irwin, D. E., Thimgan, M. P., & Irwin, J. H. (2008). Call divergence is correlated with geographic and genetic distance in greenish warblers (*Phylloscopus trochiloides*): A strong role for stochasticity in signal evolution? *Journal of Evolutionary Biology, 21*(2), 435–448. https://doi.org/10.1111/j.1420-9101.2007.01499.x

Kaiser, H. F. (1974). An index of factorial simplicity. *Psychometrika, 39*, 31–36. https://doi.org/10.1007/BF02291575

Katz, J., Hafner, S. D., & Donovan, T. (2016). Tools for automated acoustic monitoring within the R package monitoR. *Bioacoustics, 25*(2), 197–210. https://doi.org/10.1080/09524622.2016.1138415

Kirschel, A. N. G., Slabbekoorn, H., Blumstein, D. T., Cohen, R. E., de Kort, S. R., Buermann, W., et al. (2011). Testing alternative hypotheses for evolutionary diversification in an African songbird: Rainforest refugia versus ecological gradients. *Evolution, 65*(11), 3162–3174. https://doi.org/10.1111/j.1558-5646.2011.01386.x

Klicka, J., Keith Barker, F., Burns, K. J., Lanyon, S. M., Lovette, I. J., Chaves, J. A., et al. (2014). A comprehensive multilocus assessment of sparrow (Aves: Passerellidae) relationships. *Molecular Phylogenetics and Evolution, 77*(1), 177–182. https://doi.org/10.1016/j.ympev.2014.04.025

Koetz, A. H., Westcott, D. A., & Congdon, B. C. (2007). Geographical variation in song frequency and structure: The effects of vicariant isolation, habitat type and body size. *Animal Behaviour, 74*(5), 1573–1583. https://doi.org/10.1016/j.anbehav.2007.03.022

Lachlan, R. F., & Servedio, M. R. (2004). Song learning accelerates allopatric speciation. *Evolution, 58*(9), 2049–2063. https://doi.org/10.1111/j.0014-3820.2004.tb00489.x

Ligges, U., Krey, S., Mersmann, O., & Schnackenberg, S. (2016). *TuneR: Analysis of music* (Version 1.3.3). http://r-forge.r-project.org/projects/tuner.

Lipshutz, S. E., Overcast, I. A., Hickerson, M. J., Brumfield, R. T., & Derryberry, E. P. (2017). Behavioural response to song and genetic divergence in two subspecies of white-crowned sparrows (*Zonotrichia leucophrys*). *Molecular Ecology, 26*(11), 3011–3027. https://doi.org/10.1111/mec.14002

Lynch, A. (1996). The population memetics of birdsong. In D. Kroodsma, & E. H. Miller (Eds.), *Ecology and evolution of acoustic communication in birds* (pp. 181–197). Ithaca, NY: Cornell University Press.

McGregor, P. K. (2000). Playback experiments: Design and analysis. *Acta Ethologica, 3*(1), 3–8. https://doi.org/10.1007/s102110000023

Navarro-Sigüenza, A. G., & Peterson, A. T. (2004). An alternative species taxonomy of the birds of Mexico. *Biota Neotropica, 4*(2), 1–32. https://doi.org/10.1590/s1676-06032004000200013

Ord, T. J., King, L., & Young, A. R. (2011). Contrasting theory with the empirical data of species recognition. *Evolution, 65*(9), 2572–2591. https://doi.org/10.1111/j.1558-5646.2011.01319.x

Pearson, S. F., & Rohwer, S. (2000). Asymmetries in male aggression across an avian hybrid zone. *Behavioral Ecology, 11*(1), 93–101. https://doi.org/10.1093/beheco/11.1.93

Podos, J., & Warren, P. S. (2007). The evolution of geographic variation in birdsong. *Advances in the Study of Behavior, 37*(7), 403–458. https://doi.org/10.1016/S0065-3454(07)37009-5

Quinn, G. P., & Keough, M. J. (2002). In *Experimental design and data analysis for biologists* (Vol. 1). Cambridge, U.K: Cambridge University Press. https://doi.org/10.1017/CBO9780511806384.

Ryan, M. J., Rand, W., Hurd, P. L., Phelps, S. M., & Rand, A. S. (2003). Generalization in response to mate recognition signals. *American Naturalist, 161*(3), 380–394. https://doi.org/10.1086/367588

Sandoval, L., Bitton, P.-P., Demko, A. D., Doucet, S. M., & Mennill, D. J. (2017). Phenotypic variation and vocal divergence reveals a species complex in white-eared ground-sparrows (Cabanis) (Aves: Passerellidae). *Zootaxa, 4291*(1), 155–170. https://doi.org/10.11646/zootaxa.4291.1.9

Schulte, A., Sweet, S. A., & Grace-Martin, K. (2011). *Data analysis with SPSS: A first course in applied statistics* (4th ed.). Boston, MA: Pearson.

Schumacker, R. E. (2015). *Using R with multivariate statistics*. Thousand Oaks, CA: Sage.

Searcy, W. A., & Nowicki, S. (2010). *The evolution of animal communication: Reliability and deception in signaling systems*. Princeton, NJ: Princeton University Press. https://doi.org/10.1515/9781400835720

Seddon, N. (2005). Ecological adaptation and species recognition drives vocal evolution in neotropical suboscine birds. *Evolution, 59*(1), 200–215. https://doi.org/10.1111/j.0014-3820.2005.tb00906.x

Seddon, N., & Tobias, J. A. (2010). Character displacement from the receiver's perspective: Species and mate recognition despite convergent signals in suboscine birds. *Proceedings of the Royal Society B: Biological Sciences, 277*(1693), 2475–2483. https://doi.org/10.1098/rspb.2010.0210

Shepard, R. (1987). Toward a universal law of generalization for psychological science. *Science, 237*(4820), 1317–1323. https://doi.org/10.1126/science.3629243

Sosa-López, J. R., Martínez Gómez, J. E., & Mennill, D. J. (2016). Divergence in mating signals correlates with genetic distance and behavioural responses to playback. *Journal of Evolutionary Biology, 29*(2), 306–318. https://doi.org/10.1111/jeb.12782

Sosa-López, J. R., Mennill, D. J., & Navarro-Sigüenza, A. G. (2013). Geographic variation and the evolution of song in Mesoamerican rufous-naped wrens *Campylorhynchus rufinucha*. *Journal of Avian Biology, 44*(1), 27–38. https://doi.org/10.1111/j.1600-048X.2012.05651.x

Sosa-López, J. R., Mennill, D. J., & Renton, K. (2017). Sexual differentiation and seasonal variation in response to conspecific and heterospecific acoustic signals. *Ethology, 123*(6–7), 460–466. https://doi.org/10.1111/eth.12616

Sueur, J., Aubin, T., & Simonis, C. (2008). Seewave: A free modular tool for sound analysis and synthesis. *Bioacoustics, 18*(2), 213–226. https://doi.org/10.1080/09524622.2008.9753600

Uy, J. A. C., Irwin, D. E., & Webster, M. S. (2018). Behavioral isolation and incipient speciation in birds. *Annual Review of Ecology, Evolution, and Systematics, 49*(1), 1–24. https://doi.org/10.1146/annurev-ecolsys-110617-062646

Wiley, R. H. (2006). Signal detection and animal communication. *Advances in the Study of Behavior, 36*, 217–247. https://doi.org/10.1016/S0065-3454(06)36005-6

Wilkins, M. R., Seddon, N., & Safran, R. J. (2013). Evolutionary divergence in acoustic signals: Causes and consequences. *Trends in Ecology & Evolution, 28*(3), 156–166. https://doi.org/10.1016/j.tree.2012.10.002

Zuur, A. F., Ieno, E. N., Walker, N., Saveliev, A. A., & Smith, G. M. (2009). *Mixed effects models and extensions in ecology with R*. New York, NY: Springer. https://doi.org/10.1007/978-0-387-87458-6

Appendix

Table A1
Factor loadings of the extracted PCA components summarizing olive sparrow behavioural response variables

| Variables	$PC1_{vocal}$	$PC2_{physical}$
Eigenvalues	4.05	1.01
Variance explained (%)	67.42	16.79
Song latency	**-0.91**	-0.17
Singing duration	**0.92**	0.19
Number of songs	**0.88**	0.15
Song rate	Dropped	Dropped
Approach latency	**-0.72**	-0.53
Number of flyovers	0.05	**0.94**
Closest approach distance	-0.59	**-0.71**

The PCA combines behavioural responses of individuals from both Pacific and Peninsula populations. Eigenvalues and the percentage of variation explained are presented for each component. The removed superfluous variables are indicated as 'dropped'. Variables with factor loadings greater than 7 are shown in bold. PCA was based on the correlation matrix using varimax rotation. PC1 summarizes mainly vocal responses, while PC2 summarizes physical responses. The PCA assumptions were met (Budaev, 2010; Kaiser, 1974; Schulte, Sweet, & Grace-Martin, 2011; Schumacker, 2015), with a positive correlation determinant (0.006), acceptable sampling adequacy (KMO test = 0.76) and sphericity indicating correlations among variables (Bartlett's test: $\chi^2_{15} = 663.5$, $P < 0.001$).

Table A2
Factor loadings of the extracted PCA components summarizing fine structural variables of olive sparrow songs

	$PC1_{acoustic}$	$PC2_{acoustic}$	$PC3_{acoustic}$
Eigenvalues	6.84	1.87	1.00
Variance explained (%)	62.22	16.96	9.11
Mean frequency	**0.80**	0.50	0.30
SD of the mean frequency	-0.15	**0.93**	0.08
First quartile frequency	**0.90**	0.21	0.28
Third quartile frequency	**0.77**	0.53	0.31
Song duration	Dropped	Dropped	Dropped
Number of elements in the song	-0.27	-0.18	**-0.89**
Element rate	-0.25	-0.24	**-0.87**
Minimum frequency	**0.87**	-0.09	0.07
Maximum frequency	**0.85**	0.32	0.22
Bandwidth	0.36	**0.78**	0.40
Peak frequency	**0.80**	0.01	0.27
Entropy	0.37	**0.86**	0.20

Eigenvalues and the percentage of variation explained are presented for each component. Variables with factor loadings greater than 7 are shown in bold. PCA was based on the correlation matrix using varimax rotation. The PCA assumptions were met (Budaev, 2010; Kaiser, 1974; Schulte et al., 2011; Schumacker, 2015), with a positive correlation determinant (1.971E-8), acceptable sampling adequacy (KMO test = 0.77) and sphericity indicating strong correlations among variables (Bartlett's test: $\chi^2_{55} = 2528.23$, $P < 0.001$).
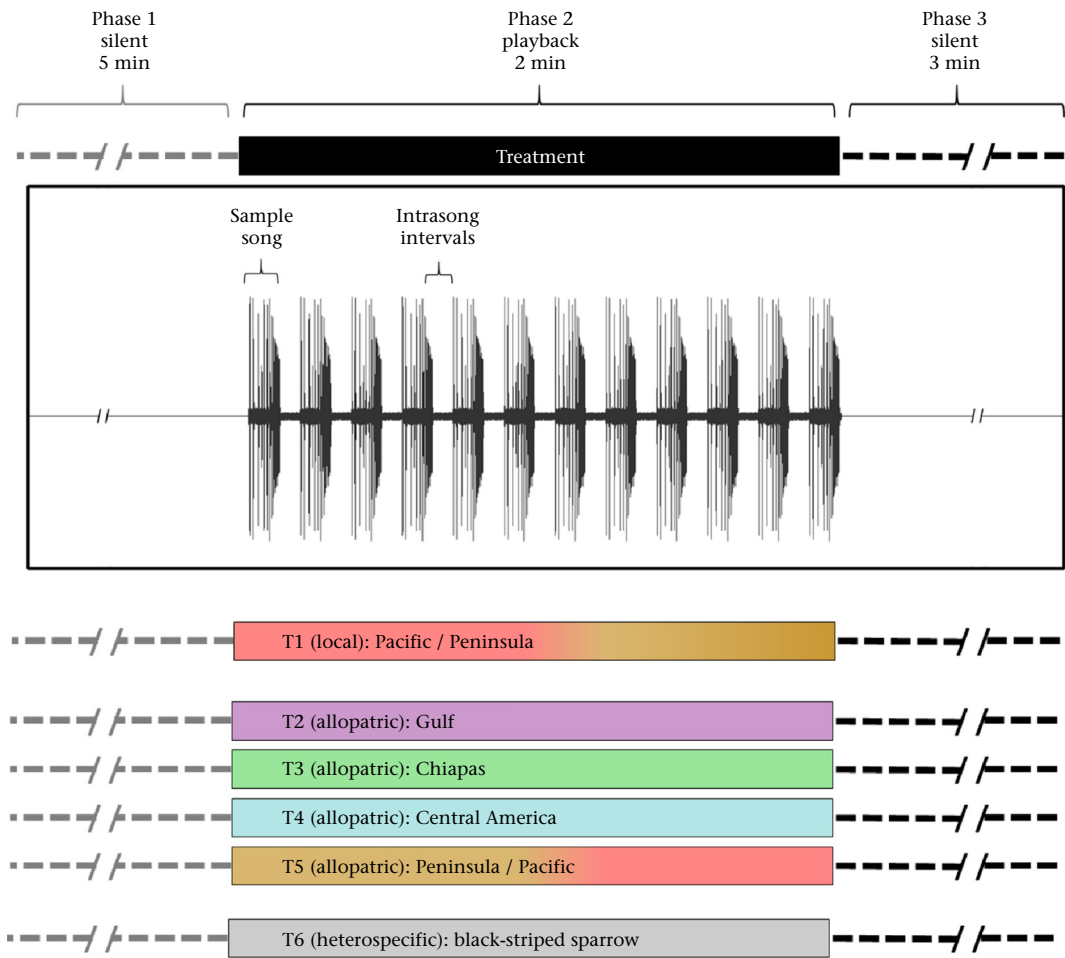
Figure A1. Diagram of the playback design showing 10 min trials with three phases. Each subject received six trials on subsequent days (T1–T6). We assessed behavioural responses during phases 2 and 3.