



Different vocal signals, but not prior experience, influence heterospecific from conspecific discrimination

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ARTICLE INFO

Article history:

Received 1 October 2012
Initial acceptance 31 October 2012
Final acceptance 23 January 2013
Available online 25 March 2013
MS. number: A12-00758R

Keywords:

allopatric population
conspecific recognition
innate discrimination
learned discrimination
Melospiza leucotis
sparrow
sympatric population
vocal duet

Efficient communication between animals requires specificity to ensure that animals distinguish relevant signals from background noise. Research on discrimination between the acoustic signals of heterospecific versus conspecific animals, especially in birds, has focused on the songs produced by breeding males, in spite of the fact that animals produce other types of acoustic signals such as calls and duets. We used acoustic playback experiments to evaluate whether tropical white-eared ground-sparrows, *Melospiza leucotis*, use calls, male solo songs and duets to discriminate conspecific from heterospecific competitors. We also evaluated whether prior experience influences competitors' discrimination by comparing responses among populations of white-eared ground-sparrows that are allopatric and sympatric with a congeneric competitor species (Prevost's ground-sparrows, *Melospiza biarcuatum*). White-eared ground-sparrows displayed more intense responses to conspecific vocalizations than they did to congeneric vocalizations. The duets produced in response to conspecific playback exhibited higher bandwidth and maximum frequency, lower minimum frequency and longer duration than duets produced in response to heterospecific playback. These results suggest that white-eared ground-sparrows use information encoded in vocalizations to discriminate competitors from noncompetitor species. The observed responses were not influenced by previous experience; white-eared ground-sparrows displayed similar responses whether they lived in sympatry or allopatry with the congener simulated through playback. Our results expand our understanding of how animals use different types of vocalizations to discriminate conspecific from heterospecific signals.

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Species specificity of animal signals is important for efficient communication (Bradbury & Vehrencamp 2011). Species-specific components of signals ensure that animals do not attend to signals that are not beneficial to their own interests, such as defending territories against heterospecifics that are not true competitors (Ryan & Rand 1993; Grether et al. 2009; Ord et al. 2011). The signals used by animals to distinguish their own species from potential competitors vary across taxa (Matyjasiak 2005; Bradbury & Vehrencamp 2011; Grether 2011), and are related to the modality of communication (Anderson & Grether 2010; Bradbury & Vehrencamp 2011) and also to individual discrimination (Marler 1960; Nelson 1989; Tibbetts 2002; Tibbetts & Dale 2004).

Acoustic signals have been particularly well studied as a species recognition signal, yet research on species discrimination via acoustic signals has focused almost exclusively on the songs

produced by breeding males (Grether et al. 2009; Ord & Stamps 2009; Grether 2011; Ord et al. 2011). The primary functions of breeding males' acoustic signals are mate attraction and resource defence (Andersson 1994; Catchpole & Slater 2008). Species discrimination appears to be particularly important for male breeding signals because this reduces the chance of misidentification of relevant territorial competitors or prospective mates during the reproductive season (Murray 1981; Ptacek 2000; Ord & Stamps 2009; Grether 2011). Yet many animals, including birds, produce other types of acoustic signals beyond male breeding signals, such as calls and duets (Langmore 1998; Matrosova et al. 2011; Geissmann 2002; Marler 2004; Catchpole & Slater 2008; Furrer & Manser 2009; Bradbury & Vehrencamp 2011); these other types of signals may also include species-specific elements. Therefore, to understand the role of these other acoustic signals in conspecific and heterospecific discrimination, it is worthwhile to conduct comparative studies between different categories of acoustic signals, rather than focusing on a single signal type.

Given the complexity and diversity of their vocalizations (Catchpole & Slater 2008), birds provide an excellent model for studying conspecific and heterospecific discrimination. The most

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biodiverse order of birds is Passeriformes, which is subdivided into oscine birds (suborder: Passeres) where birds learn songs from tutors, and suboscine birds (suborder: Tyranni) where birds inherit songs without learning (Kroodsma 2004). Although the mode of development of songs varies between these groups, calls appear to be nonlearned vocalizations for both groups (Marler 2004). Whether female songs and male–female duets are learned or innate is poorly understood; however, there are many species where duets comprise the same vocalizations as solo songs, suggesting that duets are probably learned in the same manner as male solo songs (e.g. Mennill & Rogers 2006). Based on the assumption that the songs and duets of oscine songbirds are learned, these vocalizations are more likely to show differences between species than are calls. These differences arise because songs and duets evolve under strong social evolutionary pressures (e.g. sexual preferences of the opposite sex for specific acoustic features, aggressive responses of same-sex animals to specific acoustic features, and the influence of neighbours vocalizations during periods of song learning) that are understood to lead to faster changes in culturally transmitted traits compared to genetically transmitted traits (Andersson 1994; Price 2007). Conversely, calls are more likely to show similarity between species than are songs or duets (e.g. Klump & Shalter 1984; Marler 2004; Templeton & Greene 2007). This similarity may arise because calls are used in inter-specific communication, as is the case for mobbing calls, alarm calls or food calls (Marler 2004; Radford & Ridley 2007; Templeton & Greene 2007; Bradbury & Vehrencamp 2011).

In this study our objective was to conduct a comparative playback experiment to explore the role of different vocalization types (calls, male solo songs and male–female duets) for differentiating between heterospecific and conspecific competitors in Neotropical white-eared ground-sparrows, *Melospiza leucotis*. This songbird specializes in thicket habitats in Central America (Stiles & Skutch 1989; Howell & Webb 1995; Sandoval & Mennill 2012), which are characterized by dense vegetation where visual signals do not propagate well; vocal signals are therefore expected to be the dominant forms of communication and interaction between competitors in this habitat (Sandoval & Barrantes 2012). At different locations in Costa Rica, white-eared ground-sparrows live in sympatry or allopatry with respect to their closest relative, Prevost's ground-sparrows, *Melospiza biarcuatum* (Stiles & Skutch 1989; DaCosta et al. 2009). Duets and calls of these two ground-sparrows are superficially similar (Fig. 1), so that there is ample opportunity for competitor misidentification when both species are present in the same area. As in *Hypocnemis* antbirds (Tobias & Seddon 2009; Seddon & Tobias 2010), and *Ficedula* flycatchers (Qvarnström et al. 2006), the vocal similarities between these two species, as well as their reliance on common resources, give rise to direct interactions between these two ground-sparrow species. Therefore, comparison of the sympatric and allopatric populations allowed us to evaluate the influence of vocal familiarity on the discrimination of heterospecific competitors.

We made a priori predictions about the responses of white-eared ground-sparrows pairs to playback simulating calls, solo songs and duets of conspecific and congeneric animals. For responses to playback of male solo songs (which are known to be important in territory defence in this species, Sandoval & Mennill 2012, and in birds generally, Catchpole & Slater 2008), we predicted that both male and female white-eared ground-sparrows would show the highest intensity of response to conspecific signals versus congeneric signals (i.e. strong discrimination). We made this prediction for two reasons. First, the songs of the two congeners show substantial spectrotemporal differences, more so than the other two types of vocalizations (Fig. 1). Second, males and females of the two congeneric species do not compete for breeding partners

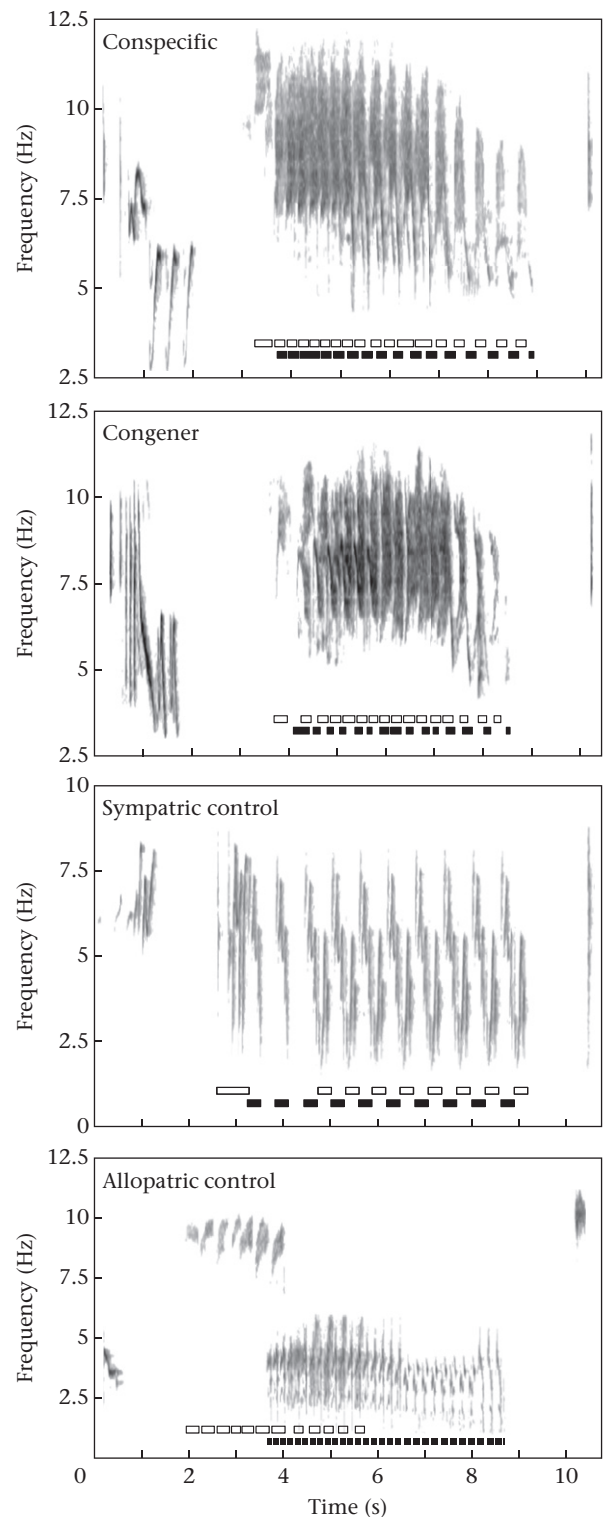


Figure 1. Sound spectrograms of three types of vocalizations used in the playback experiment to study species recognition in white-eared ground-sparrow. In each spectrogram, a male solo song is shown at the far left, a male–female duet is shown in the centre, and a call is shown at the far right. Conspecific stimuli were white-eared ground-sparrows; congeneric stimuli were Prevost's ground-sparrows; sympatric control stimuli were plain wrens; and allopatric control stimuli were large-footed finches. White and black bars underscore the contribution of each individual to the duets.

(there is no evidence of hybridization between white-eared and Prevost's ground-sparrows), so that white-eared ground-sparrows should show the highest intensity of response to conspecific signals. For responses to playback of vocal duets (which appear to be important in territory defence in white-eared ground-sparrows; Sandoval & Mennill 2012) and calls (which appear to serve as signals of alarm and contact signals in this species), we predicted that both male and female white-eared ground-sparrows would be less discriminating in their responses to conspecific versus congeneric competitors. We made this prediction for two reasons. First, unlike their songs, both the calls and the duets of these two species are very similar in fine structural features (Fig. 1). Second, the cost of responding to the wrong species may be lower for duets and calls than for solo songs; given the function of these three types of signals (calls for alarm or contact, duets for territory defence, and solo songs for mate attraction), the cost of mistaking a congener for a conspecific should be highest for solo songs. If all vocalizations produced by white-eared ground-sparrows encode species information, we predicted a less aggressive response to all hetero-specific vocalizations than to conspecific ones (Grether 2011). If species identity is not encoded in all vocalization types, we predicted the same intensity of response to conspecific and hetero-specific vocalizations for those types of signals.

The ability to discriminate between acoustic signals may arise because the animals have an innate auditory template of the intra-specific signals or it may be learned (or modified) through experience (Ord et al. 2011). If discrimination is learned, then we predicted that birds living in sympatry would have frequent contact with the vocalizations of both congeneric and conspecific individuals during critical periods in their development, resulting in the ability to differentiate congeneric versus conspecific vocalizations (Catchpole 1978; Catchpole & Leister 1986). Conversely, birds living in allopatry would lack such experience, and should not show the ability to differentiate between the vocalizations that have high structural similarity (e.g. calls and duets). If species differentiation develops in the absence of learning through experience with the congener (i.e. if discrimination is a genetic trait), and the trait is shared across the allopatric and sympatric populations, then birds should respond more to conspecific than to congeneric vocalizations in both our allopatric and sympatric populations. It is possible that the genetic mechanism that facilitates conspecific from congeneric differentiation may have diverged between the sympatric and allopatric populations, in which case we predicted that birds living in sympatry would show the ability to differentiate congeneric versus conspecific vocalizations, whereas those living in allopatry would not.

METHODS

We studied four populations of white-eared ground-sparrows in Costa Rica from June to July 2011, during this species' breeding season (Sandoval & Mennill 2012). Two populations included white-eared ground-sparrows but no congeneric Prevost's ground sparrows: (1) Monteverde, Puntarenas Province (10°18'N, 84°48'W; altitude: 1600 m) and (2) Lankester Botanical Garden, Cartago Province (09°50'N, 83°53'W; altitude: 1400 m). We refer to these as 'allopatric populations' hereafter. The remaining two populations included coexisting white-eared ground-sparrows and Prevost's ground-sparrows: (3) North Heredia, Heredia Province (10°01'N, 84°05'W; elevation: 1200–1500 m) and (4) University of Costa Rica campus, San Jose Province (09°56'N, 84°05'W; elevation: 1200 m). We refer to these as 'sympatric populations' hereafter. In the sympatric populations, both species were found occupying the same type of habitat, and they typically showed overlapping territories. We have observed the two species interacting with each other in the field.

Playback Experiment

In all four study populations, we used playback to simulate the presence of four different species of birds inside the territories of white-eared ground-sparrows: (1) conspecific white-eared ground-sparrows; (2) congeneric Prevost's ground-sparrows; (3) a 'sympatric control', plain wrens, *Thryothorus modestus*; and (4) an 'allopatric control', large-footed finches, *Pezopetes capitalis*. We selected plain wrens as a sympatric control because they are common in the same habitat as white-eared ground-sparrows throughout their range in Costa Rica (Stiles & Skutch 1989), but they produce vocalizations that are highly different from ground-sparrows (Fig. 1), and they are not known to be ecological competitors with ground-sparrows, feeding on different resources at different strata in the same habitat. We selected large-footed finches as an allopatric control because they live in similar habitats to both *Melospiza* species, but have a completely nonoverlapping distribution with white-eared ground-sparrows (Stiles & Skutch 1989), and therefore they are not ecological competitors. Large-footed finch vocalizations are somewhat similar in structure to white-eared ground-sparrow vocalizations, although they contain ample spectrotemporal differences (Fig. 1). The two control species were also selected because they produce all three types of vocalizations (calls, solo songs and duets) of interest in our experiments (Fig. 1).

We generated playback stimuli by isolating recorded vocalizations with a high signal-to-noise ratio (assessed visually) from recordings we collected in Costa Rica. Recordings were gathered with a shotgun microphone (Sennheiser ME66/K6) and a solid-state digital recorder (Marantz PMD661; sampling rate: 44.1 kHz; accuracy: 16-bit; file format: WAVE). To minimize the effects of familiarity with vocalizations and any possible 'dear enemy effects' in our results (Temeles 1994; Catchpole & Slater 2008), we played back white-eared ground-sparrow vocalizations from the same geographical location but from the territory that was farthest from that of the playback subjects (minimum distance between the subject's territory and the stimulus bird's territory was two intervening territories). We used different stimuli for every pair. For the Prevost's ground-sparrow playback stimuli, we used vocalizations recorded from the two sympatric populations. For plain wren stimuli, we used recordings from the Central Valley. For large-footed finch stimuli, we used recordings from Cerro de la Muerte, Costa Rica.

We filtered out background noise outside of the range of the species' vocalizations using the Fast Fourier Transform filter function in Cool Edit 2000 (Syntrillium Software Co., Phoenix, AZ, U.S.A.). Each type of vocalization had different frequency characteristics, necessitating different types of filters. For white-eared and Prevost's ground-sparrows, we filtered all sounds below 3 kHz and all sounds above 12.5 kHz. For plain wrens, we filtered all sounds above 10 kHz; for calls and duets, we filtered all sounds below 2 kHz; and for songs, we filtered all sounds below 5 kHz. For large-footed finch, we filtered all sounds below 8 kHz and all sounds above 11 kHz for calls, all sounds below 2 kHz and above 6 kHz for songs, and all sounds below 1 kHz and above 10 kHz for duets (see Fig. 1). The resulting filtered stimuli included only the signal of interest, allowing us to rule out the influence of background noise on the responses of the focal pair. We normalized all the recordings to –1 dB using the amplify function of Cool Edit 2000. After filtering and normalizing sounds, we confirmed that the filtered stimuli sounded realistic based on acoustic comparison to live birds in the field. All playback tracks consisted of one stimulus vocalization repeated several times. Each vocalization type differs in length; rather than holding playback rate constant, we held duty cycle constant. Calls were broadcast at a rate of 12 calls/min, songs were broadcast at a rate of eight songs/min, and duets were broadcast at

a rate of four duets/min. These values also allowed us to broadcast stimuli at rates that corresponded with normal rates of delivery for these vocalizations based on our observations of wild birds, while still producing stimuli with an equivalent duty cycle between treatments.

Each playback trial included presentations of the same type of vocalization (calls, solo songs or duets) from each of the four species. Playback involved 2 min of vocalizations followed by 5 min of silence (Fig. 2), with multiple trials in quick succession, similar to other experimental designs (e.g. Bolton 2007; Geberzahn et al. 2009; Ripmeester et al. 2010). We observed birds' response behaviour during playback and during the first 3 min of the silent period, and we treated the remaining 2 min of silence as a recovery period, allowing the focal pair to return to normal activities. Our field observations confirmed that birds consistently left the playback area by the end of the silent recovery periods. Within each trial we randomly selected the stimulus order (using the random number generator in Microsoft Excel), with the condition that we never presented vocalizations of two ground-sparrow species consecutively. Each focal pair received playback trials on 3 consecutive days (1 day receiving the four species' calls, 1 day receiving the four species' solo songs, 1 day receiving the four species' duets) where the order of trials followed a randomized design.

Playback of the four species were presented to 20 territorial white-eared ground-sparrows pairs in the allopatric populations (13 at Monteverde and 7 at Lankester Botanical Garden), and to 24 pairs in the sympatric populations (10 at Heredia and 14 at University of Costa Rica). Five pairs at each location had at least one individual banded, and our observations of these banded animals confirmed that they used the same territory during successive days and were not observed moving between territories throughout the breeding season. Therefore, we are confident that the unbanded pairs that received playback were unique pairs. Playback sessions were conducted between 0600 and 1000 hours, a time when all four species were vocally active.

Playback sounds were broadcast using an active loudspeaker (Anchor Audio; Minivox; frequency response: 100–12 000 Hz) and a portable audio player (Apple iPod classic). Loudspeakers were mounted at a height of 0.8–1.5 m, and were positioned inside the subjects' territory, 5–10 m from the edge of the territory. We hung flags at 3 m on either side of the loudspeaker to use as a reference during playback trials. Playback volume was held constant across all trials at 80 dB SPL, measured at 1 m from the speaker with a digital sound level meter (Radio Shack model 33-2055 using C weighting, slow response). We considered this to be similar to the amplitude of birds' voices based on our assessments in the field. Playback trials on different days were always broadcast from the same loudspeaker location, and the same observer was located at the same position, 8 m from the loudspeaker.

Response Measures

We quantified birds' reactions to each playback stimulus by measuring both their behavioural responses (i.e. their physical

reaction to playback) and the fine structural features of their vocal responses (i.e. their acoustic reaction to playback). We measured the following behavioural response variables: (1) the latency from the start of playback to the subjects' first vocalization, in seconds (if the pair did not vocalize we assigned a value of 300 s); (2) the latency to approach to within 3 m of the speaker, in seconds (if the pair did not approach we assigned a value of 300 s); (3) the time spent inside a 3 m radius from the speaker, in seconds (if the pair did not expend any time inside the 3 m radius we assigned a value of 0 s); and (4) the total number of vocalizations produced during the 5 min, from the start of the playback to 3 min after playback finished.

Previous research shows that males may vary the structure of their vocalizations in response to playback experiments (e.g. Slabbekoorn & ten Cate 1997; Mennill & Ratcliffe 2004; Sandoval 2011; Bartsch et al. 2012). To evaluate whether ground-sparrows show similar behaviour, we measured the spectrotemporal characteristics of vocalizations produced by the focal pair during the 3 min of silence after each playback stimulus. Vocalizations produced during the 2 min of playback were often overlapped by playback and were therefore difficult to analyse in detail based on the sound spectrograms. The birds' vocalizations were recorded with a directional microphone (Sennheiser ME66/K6) and a solid-state digital recorder (Marantz PMD660 or PMD661). Using Raven Pro 1.4 (Cornell Lab of Ornithology, Ithaca, NY, U.S.A.), for each recorded vocalization we measured: (1) the minimum frequency, in Hz; (2) the maximum frequency, in Hz; (3) the frequency bandwidth, in Hz; and (4) the duration, in seconds. We used Raven Pro 1.4 settings to achieve a temporal resolution of 5.8 ms and a frequency resolution of 188 Hz (settings: Hann window; 256 kHz sampling, and 50% overlap). The measurements were made through visual assessment of the spectrogram, wave and power spectrum windows in Raven Pro; the spectrogram window was used to identify the vocalization, and the wave and power spectrum windows were used to measure time and frequency limits, respectively. We calculated an average value when pairs produced more than one type of vocalization in response to playback.

Statistical Analysis

We conducted principal component analysis to combine the four behavioural responses into two multivariate response measures, using varimax rotation on the correlation matrix. The first two rotated components had eigenvalues greater than 1.0 and together explained 77.3% of the variance in the original four behavioural variables. The first rotated component explained 44.4% of the variation and showed a strong relationship with rapid approach to the loudspeaker ($r = 0.93$; we present correlation coefficients between factor 1 and the raw variables) and time within 3 m of the loudspeaker ($r = 0.93$), and a weak relationship with latency to first vocalization ($r = 0.23$) and the total number of vocalizations produced ($r = 0.04$). We call this first rotated component 'close approach', where pairs that received a high score approached rapidly and spent more time close to the speaker. The

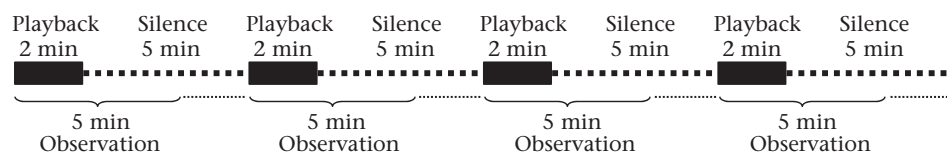


Figure 2. Schematic representation of the timing of playback trials delivered to white-eared ground-sparrows. During each trial, territorial pairs received four playback treatments (either calls, solos or duets of the four playback species); each pair received three trials on three subsequent days. Playback treatments are represented by black bars and the time between treatments is represented by a thick dotted line. The responses of the subjects were assessed for the first 5 min following the first playback stimulus, and the remaining 2 min were treated as a recovery period.

second rotated component explained 32.9% of the variation and showed a strong relationship with latency to first vocalization ($r = 0.76$) and the total number of vocalizations produced in response to the stimulus ($r = 0.85$), but a weak relationship with rapid approach to the loudspeaker ($r = 0.14$) and time within 3 m of the loudspeaker ($r = 0.60$). We therefore call this variable 'song output', where pairs that received a high score for this second principal component vocalized sooner and produced more vocalizations in response to the stimuli. The raw data for the behavioural measurements are presented in the [Supplementary Material \(Table S1\)](#).

We analysed variation in these two response variables using a linear mixed-effects model. We included the following four fixed factors: (1) the species that produced the stimulus (white-eared ground-sparrow, Prevost's ground-sparrow, plain wren, large-footed finch); (2) the type of vocalization (call, solo song, duet); (3) whether the subjects lived in allopatry with Prevost's ground-sparrows (allopatric or sympatric); and (4) the order of the playback stimulus presentation (first, second, third or fourth stimulus of the day). We also included all second-order interactions between these four factors. To account for the fact that each pair was sampled repeatedly, we included subject identity as a random effect. The interaction between order of playback and species that produced the stimulus allowed us to evaluate whether responses varied with particular species being presented at particular positions within the stimulus set, and thereby assess position effects of playback order. We used the restricted maximum likelihood method for estimating fixed effects. For all factors or second-order interactions that explained significant variation in subjects' playback responses, we performed post hoc tests where we conducted all pairwise comparisons within each stimulus and vocalization type, followed by Bonferroni correction to account for multiple comparisons.

Focal pairs only produced calls and duets in response to playback; we never detected a solo song in response to playback. We analysed the structural features of subjects' calls and duets separately, because these vocalizations are structurally different ([Fig. 1](#)) and presumed to be functionally distinct. We conducted principal component analysis to combine the four acoustic responses into one multivariate response measure for calls and one multivariate response measure for duets. For the analysis of calls, the first component had an eigenvalue greater than 1.0 and explained 54.6% of the variance in the original four variables. The first component showed a strong relationship with the frequency bandwidth ($r = 0.97$), maximum frequency ($r = 0.71$), minimum frequency ($r = 0.63$) and duration ($r = 0.51$). For the analysis of duets, the first component had an eigenvalue greater than 1.0 and explained 53.9% of the variance in the original four variables. The first component showed a strong relationship with the frequency bandwidth ($r = 0.97$), minimum frequency ($r = 0.76$), maximum frequency ($r = 0.60$) and duration ($r = 0.52$). Therefore, for both calls and duets, responses with a high principal component score had longer duration, broader bandwidth, higher maximum frequency and lower minimum frequency. The raw data for the acoustics measurements are presented in the [Supplementary Material \(Tables S2, S3\)](#).

We conducted two linear mixed-effects model (one for calls, one for duets) to evaluate whether the characteristics of vocalizations produced in response to playback of the four species varied according to the type of vocalization and whether subjects lived in allopatry or sympatry with Prevost's ground-sparrows. We followed the exact same approach as in the first linear mixed-effects model for behavioural responses (above).

We used a significance threshold of $\alpha = 0.025$ to reject the null hypothesis for these linear mixed-effects models, due to the fact

that we conducted two comparisons of behavioural responses and vocal responses, instead of just one. All tests were two tailed. All values are reported as means $\pm$ SE. All statistical analyses were conducted in JMP (version 10.0; SAS Institute, Cary, NC, U.S.A.) and SYSTAT (version 11.00.01; SYSTAT Software, Chicago, IL, U.S.A.).

Ethical Note

In this study we presented acoustic stimuli that produced aggressive responses by territorial white-eared ground-sparrows. The aggression levels observed during playback trials were similar to the natural interactions we have observed between the focal species with other individuals of their own species and/or other species. We also observed the subjects for several minutes after conclusion of each experiment and confirmed that the focal pair resumed normal activities, similar to the behaviour they displayed prior to the experiment. We conducted this study following the regulations of the Animal Care Committee of the University of Windsor (AUPP: 09-06) and the Government of Costa Rica (071-2011-SINAC).

RESULTS

White-eared ground-sparrows responded strongly in many playback trials, often approaching the loudspeaker and producing calls and duets near the playback-simulated intruders. The approach responses of white-eared ground-sparrows, summarized by the first principal component (PC1), varied according to the species of intruder simulated through playback and the type of vocalization, as well as the interaction between these two factors ([Fig. 3a](#); linear mixed-effects model of variation in PC1; effect of playback species: $F_{3,466} = 8.4$, $P < 0.0001$; effect of playback vocalization type: $F_{2,447} = 9.2$, $P = 0.0001$; interaction of playback species and playback vocalization type: $F_{6,447} = 6.6$, $P < 0.0001$). Close approach responses did not vary between populations that were sympatric versus allopatric with respect to Prevost's ground-sparrows ($F_{1,267} = 0.3$, $P = 0.58$), nor did they vary with presentation order ($F_{3,447} = 2.4$, $P = 0.07$), or any of the remaining interaction terms (all $F < 2.5$, $P > 0.06$) including the interaction of presentation order and stimulus type ($F_{9,473} = 0.9$, $P = 0.55$). Post hoc analysis of the species simulated through playback revealed that white-eared ground-sparrows showed closer approach responses to all conspecific and congeneric vocalizations than to the two control species ([Fig. 3a](#)). Post hoc analysis of stimulus type revealed that white-eared ground-sparrows showed a closer and faster approach to the duets than to songs and calls ([Fig. 4a](#)). Post hoc analysis of the interaction between species and stimulus type revealed that white-eared ground-sparrows showed a closer and faster approach response to the duets of conspecific and congeneric playbacks than to solo songs and calls, whereas they showed no differences in response to the calls, solo songs and duets of the two control species (ANOVA: white-eared ground-sparrow: $F_{2,129} = 13.7$, $P < 0.001$; Prevost's ground-sparrow: $F_{2,129} = 7.0$, $P = 0.001$; plain wren: $F_{2,129} = 1.2$, $P = 0.29$; large-footed finch: $F_{2,129} = 1.9$, $P = 0.16$; [Fig. 3a](#)).

Song output, summarized by PC2, varied according to the species of intruder simulated through playback (linear mixed-effects model of variation in PC1, effect of playback species: $F_{3,454} = 3.7$, $P = 0.01$; [Fig. 3b](#)). Song output did not vary between populations that were sympatric versus allopatric with respect to Prevost's ground-sparrows ($F_{1,109} = 0.1$, $P = 0.80$), type of vocalization ($F_{2,447} = 3.0$, $P = 0.05$) or presentation order ($F_{3,447} = 1.5$, $P = 0.21$), or any interaction terms (all $F < 1.5$, $P > 0.05$), including the interaction of presentation order and stimulus type ($F_{9,458} = 1.2$, $P = 0.29$). Post hoc analysis revealed that the species simulated

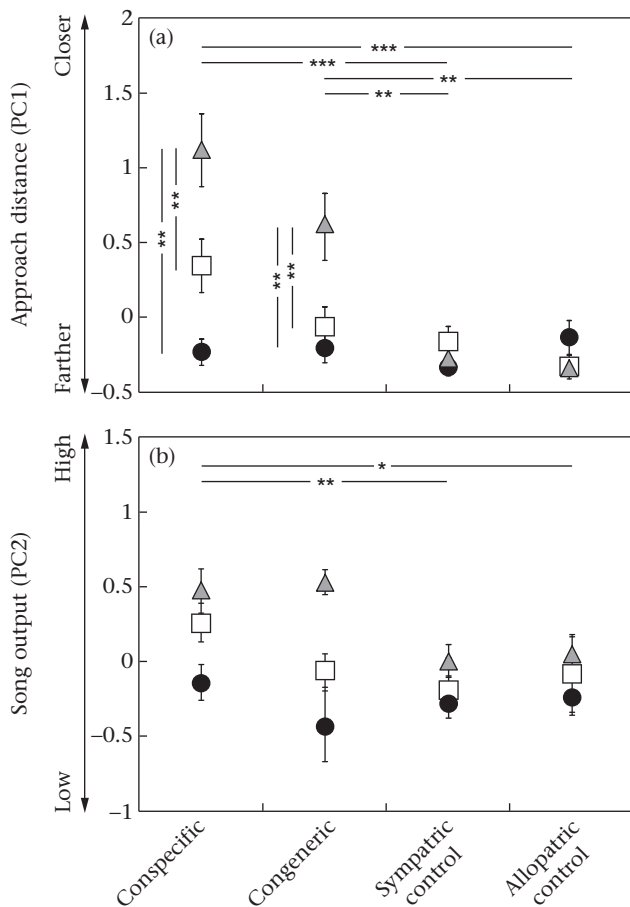


Figure 3. Comparison of responses displayed by white-eared ground-sparrow pairs to playback of three types of vocalizations (circles: calls; squares: solo songs; triangles: duets) from four species (conspecific: white-eared ground-sparrow; congeneric: Prevost's ground-sparrow, sympatric control: plain wren; allopatric control: large-footed finch). The responses are measured as principal components scores summarizing (a) variation in approach distance (PC1) and (b) variation in song output (PC2; see text for details). Post hoc statistical differences in response to the four species are represented by horizontal lines; post hoc statistical differences in response to the three types of vocalizations are represented by vertical lines (* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$).

through playback showed a significant effect; white-eared ground-sparrows showed higher song output in response to the conspecific and congeneric playbacks than they did in response to the two control species (Fig. 3b).

In response to playback, white-eared ground-sparrows produced calls and duets, but never solo songs. Analysis of the fine structure of subjects' calls revealed that duration and frequency measurements did not differ significantly with simulated species ($F_{3,347} = 0.5$, $P = 0.72$; Fig. 5a), vocalization type ($F_{2,349} = 0.6$, $P = 0.05$), sympatric versus allopatric population with respect to Prevost's ground-sparrows ($F_{1,33} = 2.4$, $P = 0.13$), presentation order ($F_{3,349} = 0.5$, $P = 0.72$) or any interaction terms (all $F < 2.4$, $P > 0.06$).

The fine structure of duets produced in response to playback varied according to the species of intruder simulated ($F_{2,414} = 13.9$, $P < 0.001$; Fig. 5b) and the type of vocalization played ($F_{2,414} = 16.9$, $P < 0.001$; Fig. 5c), but did not vary between populations that were sympatric versus allopatric with respect to Prevost's ground-sparrows ($F_{1,39} = 5.3$, $P = 0.026$), or with presentation order ($F_{3,414} = 2.3$, $P = 0.074$) or any interaction term ($F < 1.5$, $P > 0.18$). Based on post hoc analysis, the species simulated through playback showed a significant effect; white-eared ground-sparrows

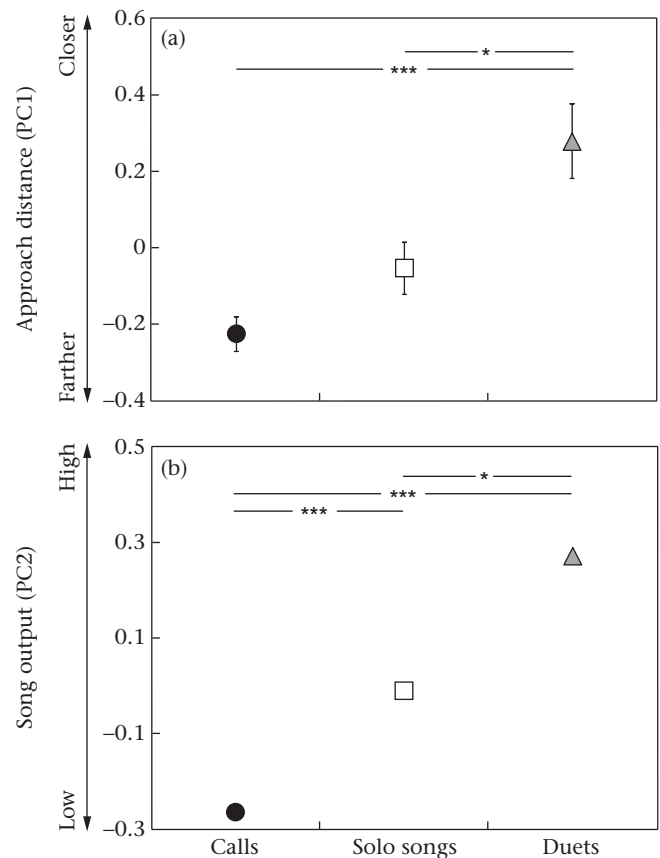


Figure 4. Comparison of responses displayed by white-eared ground-sparrow pairs to playback of three types of vocalizations (circles: calls; squares: solo songs; triangles: duets) averaged across the four species and the two populations. The responses are measured as principal components scores summarizing (a) variation in approach distance (PC1) and (b) variation in song output (PC2; see text for details). Post hoc statistical differences are represented by horizontal lines (* $P \leq 0.05$; *** $P \leq 0.001$).

produced duets with higher PC1 scores (i.e. higher maximum frequencies and bandwidths, lower minimum frequencies and longer durations) than to congeneric duets and duets of the two control species (Fig. 5c).

DISCUSSION

Pairs of white-eared ground-sparrows displayed stronger responses to playback of conspecific calls, solo songs and duets compared to the same types of vocalizations from congeneric species and two unrelated control species. Duet playback incited the strongest responses in comparisons to calls and solo songs. Although subjects' responses varied according to the species and the type of vocalization simulated, responses were unrelated to previous experience; there were no differences in response to conspecific and congeneric playback between allopatric and sympatric populations.

Territorial pairs of white-eared ground-sparrows responded to playback of conspecific vocalizations by producing duets with longer duration, broader bandwidth, higher maximum frequency and lower minimum frequency (as summarized with a principal component score) in comparison to vocalizations they produced in response to the other three species. This result supports our prediction that white-eared ground-sparrow vocalizations encode species information, and that white-eared ground-sparrows distinguish conspecific from congeneric vocalizations. Therefore,

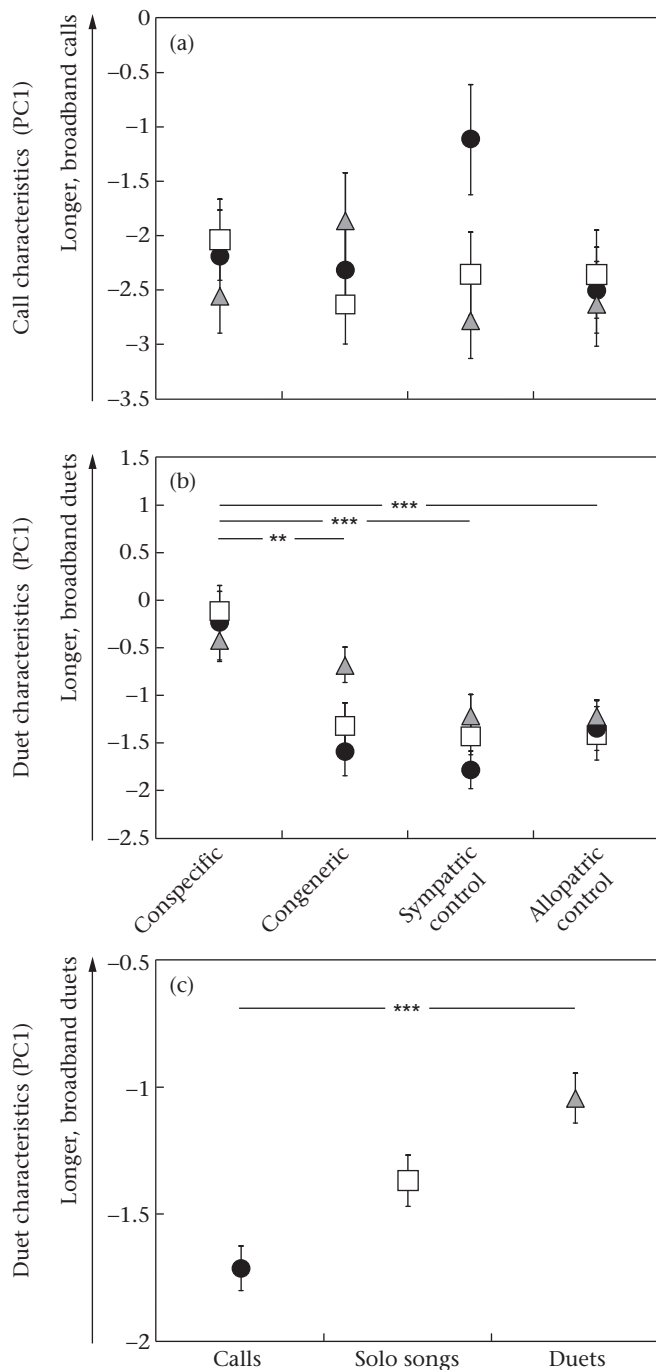


Figure 5. Vocal responses of white-eared ground-sparrow pairs to playback of three types of vocalizations (circles: calls; squares: solo songs; triangles: duets) from four species (conspecific: white-eared ground-sparrow; congeneric: Prevost's ground-sparrow; sympatric control: plain wren; allopatric control: large-footed finch). Responses were measured as principal components scores summarizing variation in (a) call and (b, c) duet characteristics (PC1; see text for details). Post hoc statistical differences are represented by horizontal lines (** $P \leq 0.01$; *** $P \leq 0.001$).

the lack of difference in behavioural responses (approach behaviour and song output, explored below) towards Prevost's ground-sparrow duets and songs did not arise due to a lack of differentiation between their vocalizations. Instead, we think that the statistically similar behaviours shown towards the conspecific and congeneric playback arose because the subjects recognized both species as ecological competitors (Grether 2011; Ord et al. 2011).

Similar levels of aggressiveness are known, for example, in Virginia's warblers, *Oreothlypis virginiae*, and orange-crowned warblers, *Oreothlypis celata* (Martin & Martin 2001), and in collared flycatchers, *Ficedula albicollis*, and pied flycatchers, *Ficedula hypoleuca* (Qvarnström et al. 2006). In both of these examples, territorial birds responded similarly to signals of congenics and conspecifics.

The behavioural responses of white-eared ground sparrows to calls of the four simulated species did not differ significantly. The similar behavioural responses to calls may arise due to similarity in call function between species (i.e. to communicate alarm or as a contact signal). We cannot distinguish whether birds failed to distinguish which species was simulated by call playback, or whether the birds recognized the species but responded in similar fashion to calls of the four species (Klump & Shalter 1984; Radford & Ridley 2007; Templeton & Greene 2007; Sandoval & Wilson 2012). Our results contrast with those of previous studies showing stronger responses to conspecific calls than to other species' calls, as in satin bowerbirds, *Ptilonorhynchus violaceus*, where males show stronger responses to calls from their own population (Nicholls 2008). The calls of satin bowerbirds are much more complex than the simple calls of the four species that we simulated in the current experiment, which may account for the differences between these studies.

White-eared ground-sparrows did not respond differently to conspecific Prevost's ground-sparrows vocalizations whether they were in zones of sympatry or allopatry. Birds living in two of our study populations have historically lived in isolation of this congeneric species (Slud 1964; Stiles & Skutch 1989), and yet they still discriminated between the two species based on playback. This supports our prediction that the mechanism for conspecific discrimination is genetic and that the competitor recognition system has clearly not diverged between sympatric and allopatric populations; otherwise, we would have seen different responses in the sympatric versus allopatric populations. Previous investigations of two subspecies of *Sylvia* warblers (Brambilla et al. 2008) and populations of medium ground-finch (Podos 2007) showed that previous experience was not necessary to distinguish between competitors. For example, the two populations of medium ground-finch were separated by 11 km; males in each population responded more strongly to their own population's songs, even though the songs were not distinguishable by acoustic measurements (Podos 2007). In the case of *Sylvia* warblers, males of two subspecies show the same degree of reduced aggressiveness to the other subspecies song in allopatric and sympatric populations (Brambilla et al. 2008).

It is easy to imagine that white-eared ground-sparrows combine vocal signals (e.g. duets) with visual signals (e.g. plumage features) to distinguish conspecific from heterospecific competitors, as occurs in *Sylvia* warblers (Matyjasiak 2005). Our observations of birds' behaviour during playback support this idea; pairs rapidly approached playback of duets of both *Melospiza* species and they typically moved around the speaker, as if to search for the source of the sound (behaviours that were not observed during responses to the two control species). This behaviour is consistent with the idea that birds may have been searching for additional information, possibly in the form of plumage-based signals of species identity, although confirming this idea would require a complex experiment on the interplay of acoustic and visual signals in species discrimination.

By focusing on the responses of white-eared ground-sparrows to playback of their own species' calls, solo songs and duets, we can gain insight into the functions of these different signals. Interestingly, we found that territorial pairs showed their closest approaches and highest song output in response to duets, and less

intense responses to playback of solos and calls. If we interpret close approach and high vocal output as aggressive behaviours, these intense responses to duets compared to solo songs and calls offer strong support for the territory resource hypothesis for duet function in white-eared ground sparrows (Hall 2004). A similar pattern has been revealed previously in at least three other species of territorial duetting birds, although there are also duetting animals that respond with similar high intensity to solo songs and duets (reviewed in Hall 2009).

In conclusion, results of this playback study demonstrate that three different types of avian vocalizations may encode species information that facilitates discrimination between conspecific and congeneric competitors versus heterospecific noncompetitors (i.e. allopatric and sympatric controls). However, each type of vocalization elicits different intensities of response against conspecific and heterospecific rivals. To develop a better understanding of acoustic signals and their role in species discrimination (e.g. species recognition, competitor discrimination and mate selection), it is worthwhile to conduct comparative studies between all types of acoustic signals and avoid focusing on a single type of signal (e.g. solo songs). Our experiments using allopatric and sympatric population comparisons allow us to conclude that familiarity based on previous experiences and interactions between sympatric species are not a prerequisite for species-specific signal recognition, and our results suggest that this discrimination may be an innate process independent of experience with other species.

Acknowledgments

We thank the Reserva Biológica Monteverde, Lankester Botanical Garden and Bosquesito Leonel Oviedo from Universidad de Costa Rica for all of the support they provided to conduct research in their reserves. We thank O. Sandoval for help conducting field research and D. Wilson for input with our analyses. We also thank G. Grether, two anonymous referees, R. Sosa and B. Graham for comments that improved the manuscript. L.S. was supported by scholarships and grants from the Ministerio de Ciencia y Tecnología (MICIT) and the Consejo Nacional para Investigaciones Científicas y Tecnológicas (CONICIT) of Costa Rica, the Government of Ontario and the University of Windsor. Additional funding was provided by the Natural Sciences and Engineering Research Council of Canada (NSERC), the Canada Foundation for Innovation (CFI) and the Government of Ontario to D.J.M. This investigation was conducted under the permit 071-2011-SINAC of Ministerio de Ambiente Energía y Telecomunicaciones and the Sistema Nacional de Áreas de Conservación de Costa Rica. None of the authors have a conflict of interest to declare.

Supplementary Material

Supplementary material for this article is available, in the online version, at <http://dx.doi.org/10.1016/j.anbehav.2013.02.006>.

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