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Deciphering Information Encoded in Birdsong: Male Songbirds with Fertile Mates Respond Most Strongly to Complex, Low-Amplitude Songs Used in Courtship

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ABSTRACT: Research on the function of acoustic signals has focused on high-amplitude long-range songs (LRS) and largely ignored low-amplitude songs produced by many species during close-proximity, conspecific interactions. Low-amplitude songs can be structurally identical to LRS (soft LRS), or they can be widely divergent, sharing few spectral and temporal attributes with LRS (short-range song [SRS]). SRS is often more complex than LRS and is frequently sung by males during courtship. To assess function, we performed two playback experiments on males of a socially monogamous songbird. We compared responses of males whose mates were fertile or non-fertile with differences in song structure (SRS vs. LRS and soft LRS), amplitude (SRS and soft LRS vs. LRS), and tempo (slow SRS vs. fast SRS). Males responded more strongly to SRS than to LRS or soft LRS, indicating that song structure had a greater effect on response than song amplitude. SRS tempo did not detectably affect male response. Importantly, males responded more strongly to SRS when their mates were fertile, presumably because hearing SRS can indicate that a male's mate is being courted by an intruding male and a strong response can deter extrapair competitors. We conclude that low-amplitude songs can function in both inter- and intrasexual communication and should receive greater attention in future studies of mate choice and male-male competition.

Keywords: soft song, song function, territoriality, sexual selection, dark-eyed junco.

Introduction

Studies of the structure and function of acoustic signals have predominantly focused on conspicuous high-amplitude signals, or long-range songs (LRS), which, due to their broad transmission to receivers of both sexes, commonly serve as important dual-function signals in both territoriality and mate attraction (Searcy and Andersson

1986; McGregor 2005; Searcy and Nowicki 2005). In addition to LRS, many species sing a separate class of low-amplitude songs that are inconspicuous and often directed toward close-proximity conspecifics during interactions associated with courtship or elevated aggression (Dabelsteen et al. 1998; Robinson and Hall 2002). Low-amplitude songs can be divided into two distinct classes based on structure: (1) soft long-range song (soft LRS), which differs from LRS only in its reduced amplitude, and (2) short-range song (SRS; after Titus 1998), which typically shares few spectral and temporal characteristics with LRS. SRS is often substantially more complex than LRS in terms of the number and diversity of song elements and often covers a greater range of frequencies (e.g., Titus 1998; Anderson et al. 2008; Zuk et al. 2008).

The study of low-amplitude songs has been hindered by difficulties in observing and recording these inconspicuous signals produced infrequently by males, often in close proximity to a female or, less commonly, another male. Currently, low-amplitude songs have been identified in both vertebrates and invertebrates that rely on acoustic signals for intra- and intersexual communication; however, the terminology identifying low-amplitude songs has been inconsistent among taxa, making its overall prevalence difficult to assess (Dabelsteen et al. 1998; Robinson and Hall 2002). In taxa that have been studied, including crickets (Zuk and Simmons 1997; Robinson and Hall 2002), songbirds (Dabelsteen et al. 1998; Searcy and Beecher 2009), and at least one species of bat (Behr and von Helversen 2004), investigations have been predominantly observational and lacked rigorous tests of the function of low-amplitude song.

Song function is typically inferred by two main methods: (1) relating song production to context (i.e., the circumstances in which songs are produced) and (2) measuring receiver response. Although inferring function based solely

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on context is the dominant practice, it is at best an imprecise method for determining song function because it fails to reveal the song's impact on all potential receivers (Beebe 2004; Searcy and Beecher 2009). The production of a song can, for example, be related to a singer's social environment and reproductive status (e.g., mated or unmated), but even in cases where an intended receiver is apparent and its response can be measured, songs can be intercepted by unintended receivers, or eavesdroppers, whose responses may also affect the song's characteristics and function (Dabelsteen 2005).

Low-amplitude songs are typically directed to a receiver of a specific sex in close proximity, providing a strong contextual argument for both an intended receiver and a specific function, for example, courtship or aggression (Dabelsteen et al. 1998; Robinson and Hall 2002). However, even low-amplitude songs can be affected by eavesdroppers. For example, if the function of female-directed SRS is in courtship (e.g., increasing the likelihood of a copulation), then males that overhear SRS should respond strongly if they are to interrupt and potentially gain a copulation for themselves (Balsby and Dabelsteen 2003, 2005). Similarly, in pair-bonding species, a strong response to SRS overheard from an intruding male could deter a potential extrapair copulation and avoid a loss of paternity. In both cases, the singer will benefit from singing at low amplitude due to the retaliatory cost from eavesdroppers of advertising a courtship song. Thus, only through measuring the response of both the intended receiver and eavesdroppers can a complete understanding of a song's function and characteristics be identified.

Studies to date investigating the function of low-amplitude songs have varied greatly in their use of contextual and receiver response data. In multiple species of crickets, for example, males that are approached by a female transition from LRS to SRS, and both contextual and receiver response data indicate that SRS serves an important function in courtship and mate choice (e.g., Wagner and Reiser 2000; Rantala and Kortet 2003; Tregenza et al. 2006; Rebar et al. 2009). Crickets do not sing soft LRS or produce any low-amplitude songs during aggressive interactions between males; they do, however, produce high-amplitude "aggressive songs" during agonistic interactions (Zuk and Simmons 1997).

Songbirds are known to produce low-amplitude songs during both male-male and male-female interactions. Strong contextual evidence from a number of songbird species suggests that, as in crickets, SRS functions primarily in courtship (e.g., Titus 1998; Balsby 2000; Balsby and Dabelsteen 2002, 2003; Collins et al. 2009). However, the only studies of songbirds thus far to include both contextual and receiver response data indicate that SRS functions only as an elevated aggressive signal (Dabelsteen and

Pedersen 1990; Searcy and Beecher 2009) and identified soft LRS as an accurate predictor of physical attack (Searcy et al. 2006; Ballentine et al. 2008; Hof and Hazlett 2010). Thus, the relative importance of low-amplitude songs in male-male and male-female interactions in songbirds remains unclear.

In this study, we tested the receiver response of males of a socially monogamous songbird, the dark-eyed junco (*Junco hyemalis*), in which prior contextual data have suggested that SRS functions predominantly in courtship (Titus 1998). We presented male juncos with territorial intrusions of LRS, soft LRS, and SRS to determine how male response is affected by differences in song structure (SRS vs. LRS and soft LRS), amplitude (SRS and soft LRS vs. LRS), and tempo (slow SRS vs. fast SRS). In contrast to all previous studies of low-amplitude song, we also compared male responses between two different contexts in the reproductive cycle when the male's mate was fertile and nonfertile. During their mate's fertile period, males are at their highest risk for losing paternity via extrapair copulation to an intruding male, which predicts that males should elevate their aggressive response to all intrusions or respond particularly strongly to vocalizations that signal a courtship event. Thus, in addition to measuring male response, we employed a contextual comparison between the fertile and nonfertile periods to provide an indirect test of the function of these low-amplitude songs (soft LRS, SRS) as courtship signals.

Methods

Study System

Male dark-eyed juncos sing LRS and soft LRS throughout the breeding season. LRS is produced most often by unmated males, suggesting a dual function in territorial maintenance and mate attraction (Ketterson et al. 1992; Nolan et al. 2002). Junco SRS is substantially more complex than LRS (fig. 1). SRS production peaks during courtship and the female fertile period, suggesting that SRS is important in courtship or female stimulation (Titus 1998). When singing SRS directed at a female during courtship, a male typically sings at a slow tempo (slow SRS: mean \pm SD = 0.84 \pm 0.27 s between syllables; fig. 1; D. G. Reichard and E. D. Ketterson, unpublished data); however, when singing while perched or foraging or during elevated aggressive interactions between males, SRS is sung at a much faster tempo (fast SRS: 0.12 \pm 0.14 s between syllables; fig. 1; D. G. Reichard, D. A. Enstrom, and E. D. Ketterson, unpublished data). Slow SRS and fast SRS differ significantly in the mean interval between syllables (independent samples *t*-test, $t_{1,398} = -31.69$, $P < .001$), but current data are insufficient to determine whether the syl-

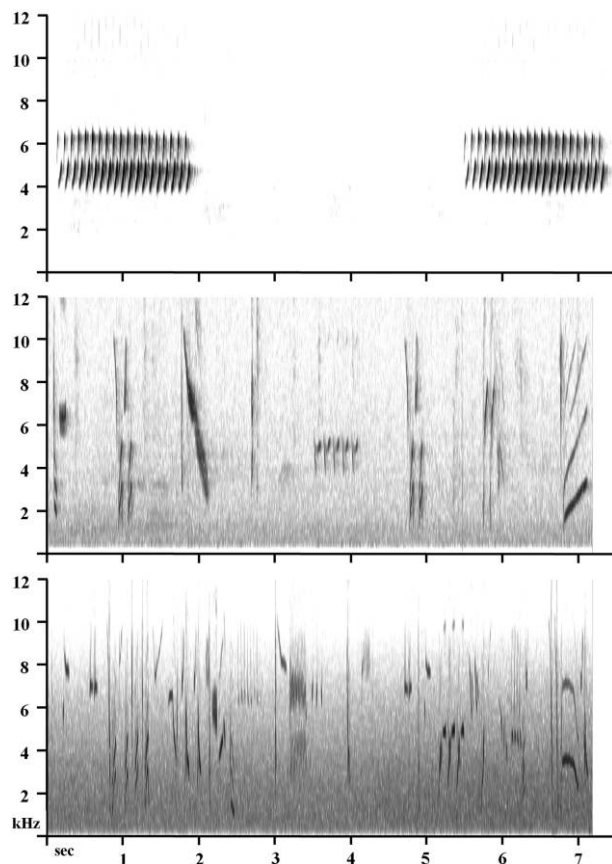


Figure 1: Sonograms of long-range song (LRS; top), slow short-range song (SRS; middle), and fast SRS (bottom) plotted on the same frequency and timescales. The darkness of the sonograms does not represent their relative amplitudes at recording or playback. All sonograms are from different individuals and thus are not indicative of syllable sharing. Audio files corresponding to each sonogram (LRS [audio file 1], slow SRS [audio file 2], fast SRS [audio file 3]) can be found in the online edition of the *American Naturalist*.

lable types that appear in slow SRS differ from those that appear in fast SRS.

Song Recordings and Playback Tapes

In the summers of 2008 and 2009, we recorded LRS and SRS from breeding juncos at Mountain Lake Biological Station and the adjoining grounds of Mountain Lake Hotel in Pembroke, Virginia (37°22'N, 80°32'W), using an Audio-Technica shotgun microphone (model AT835b) and a Marantz digital recorder (model PMD660). LRS was recorded opportunistically from spontaneously singing males or elicited by a brief playback of LRS. SRS was elicited and recorded from territorial males with an intrusion of a caged female conspecific in breeding condition

and playback of a female precopulatory trill. All SRS recordings were of slow SRS. To prepare playback tapes, we generated spectrograms (digitized at 44.1 kHz, 32 bits) of both song classes, using Adobe Audition 1.5 (Adobe Systems, San Jose, CA) and chose the highest-quality recordings as determined by a high signal-to-noise ratio.

All playback tapes consisted of an initial 1-min silent period followed by 3 min of playback stimulus. Slow SRS tapes contained a 30-s segment of slow SRS repeated continuously for 3 min to mimic the structure of natural SRS (fig. 1). SRS bouts can vary substantially in length from less than 30 s to more than 30 min of continuous song (D. G. Reichard, personal observation); therefore, our choice of a 3-min playback is well within the range of natural variation. For the study of the fertile period, the interval between song elements was not altered from the initial recording. In the study of the nonfertile period, the same playback tapes were used again, with the interval between elements standardized to 0.8 s for the slow SRS stimuli or 0.05 s for the fast SRS stimuli. These intervals are consistent with natural variation in the length of intervals in slow female-directed SRS and fast male-directed SRS (see "Study System"), while keeping the phonology of the syllables identical. LRS tapes were used for two playback treatments (high-amplitude LRS and low-amplitude soft LRS) and contained a single trill repeated every 10 s to mimic the structure of normal LRS and soft LRS (fig. 1; Titus 1998). All tapes were normalized to 90% of the peak amplitude. LRS tapes were processed using a high-pass equalizer to remove low-frequency background noise below 250 Hz. SRS tapes contained more background noise, owing to the high gain settings necessary to record low-amplitude song, and were processed using a high-pass equalizer to remove all noise below 1,000 Hz.

To minimize pseudoreplication, we created enough playback tapes so that each subject heard a unique playback for all treatments in each study, with two exceptions. For treatments that differed only in amplitude (LRS, soft LRS) and tempo (slow SRS, fast SRS), we used the same files for both treatments but never played two songs with the same phonology (e.g., slow and fast versions of the same SRS) to the same subject. This method eliminated the potential for other structural variation between the playback types to cause any differences in male response. To avoid "dear enemy" effects (Temeles 1994), we chose playback recordings collected a minimum of one-half kilometer away from each subject's territory.

Simulated Territorial Intrusions (STIs) during the Fertile Period

Between May 28 and July 10, 2009, we conducted three separate STIs, each consisting solely of a playback of slow

SRS, LRS, or soft LRS, between 0700 and 1200 hours on the territories of 20 male dark-eyed juncos known to have fertile mates. Nests and territories were monitored throughout the summer to determine the fertility status of the female paired with each male. If a female was known to be building a nest or laying eggs or if her nest had recently failed (<5 days before the experiment), she was identified as fertile (see Birkhead and Møller 1992). Additionally, one of our subjects was of unknown nesting status, but the male was seen courting his mate by frequent feather displays (e.g., tail spreading and erecting body feathers) and SRS within a day before the experiment, so the female was considered fertile. Courtship displays and SRS production in juncos are known to peak during the fertile period in the field (Titus 1998) and in the presence of a fertile female in captivity (Enstrom et al. 1997). We played songs through an Apple iPod (Apple, Cupertino, CA) connected to a Pignose amplified speaker (model 7-100) covered in camouflage cloth and placed flat on the ground with its cone facing up.

Before each STI, a LRS recording was played to attract the focal male and ensure that the low-amplitude playbacks were heard. The initial LRS playback was changed for each treatment and played for a maximum of 10 min, or until the focal male was within 10 m of the speaker. If a male did not respond after 10 min, the trial was aborted and reattempted the next day. If a male approached within 10 m of the speaker, we stopped the playback and began a 1-min silent observation period to control for any initial behavioral response elicited by the initial LRS playback. Next, the experimental playback (slow SRS, LRS, or soft LRS) was played for 3 min and was followed by a second silent observation period of 3 min. The average amplitude of each treatment mimicked the estimated natural amplitude of each song class; thus, SRS and soft LRS were played at 65 dB sound pressure level (SPL), and LRS was played at 85 dB SPL (Anderson et al. 2007). We standardized amplitude before each playback by using a Radio Shack digital sound level meter (model 33-2055) held 1 m from the speaker. We did not perform STIs on days with excessive wind or anthropogenic (e.g., construction) noise that may have hindered the male's ability to hear the low-amplitude playbacks.

During each playback, we noted LRS, flights longer than 1 m, time spent within 1 and 5 m of the speaker, latency to approach within 1 and 5 m of the speaker, latency to first LRS, and closest approach to the speaker by the focal male. Four of the response measures (LRS, flights, time within 1 m, time within 5 m) were also quantified during the pre- and postplayback silent observation periods. Males frequently produced LRS and occasionally produced soft LRS during playback (14 out of 60 trials). We were not confident in our ability to discriminate LRS from soft

LRS; therefore, we included all songs into a single LRS category for analysis. In addition, only one male produced SRS during a trial, so SRS was not considered in our statistical analysis. Each playback treatment was separated by a 45-min silent period to limit habituation and fatigue. During the time between treatments, we relocated the speaker to a different site equidistant from the estimated center of the male's territory. We randomized and counterbalanced treatment order to control for order effects.

STIs during the Nonfertile Period

Between May 15 and July 15, 2010, we again conducted three separate STIs, each consisting solely of a playback of soft LRS, slow SRS, or fast SRS, between 0700 and 1200 hours on the territories of 14 male dark-eyed juncos with nonfertile mates that had been incubating eggs for a minimum of 3 days. The fertile period of female songbirds extends from the initiation of nest building until the laying of the penultimate egg; thus, any lingering behavioral effects from that period had likely passed (Birkhead and Møller 1992). Only two males from the fertile period study were repeated in the nonfertile period study. As all the playbacks were low-amplitude songs, we standardized the playback volume to 65 dB SPL by using the same method as above. All other methods relating to the behaviors quantified, playback order, and speaker relocation were also identical.

Statistical Analysis

We performed two separate principal component analyses (PCAs) for the fertile and nonfertile periods by using all eight behavioral measures to generate composite response scores for each individual and each treatment. We then used a linear mixed model on the PC scores for each period, with subject as a random effect to account for the repeated sampling of individuals; order, playback treatment, and their interaction as fixed factors; and Julian date as a covariate. A Šidák correction for multiple comparisons was used to evaluate differences between playback treatments and orders. Additionally, we used linear mixed models with the same factors and covariates as above to examine the between-treatment differences in each behavior measured during the pre- and postplayback silences for both the fertile and nonfertile periods.

To test for differences in male response between the fertile and nonfertile periods, we combined the results for male response to slow SRS and soft LRS from both studies into a third PCA. We then used a linear mixed model to analyze PC scores, with subject as a random effect to account for the repeated sampling of individuals; order, playback treatment, fertility status, and their interactions as

Table 1: Measures (loadings) of behavioral responses to playback of long-range song (LRS), soft LRS, and slow short-range song (SRS) during the fertile period; soft LRS, slow SRS, and fast SRS during the nonfertile period; and soft LRS and slow SRS in both the fertile and nonfertile periods

Component (% of total variance)	Fertile		Nonfertile		Comparison	
	PC1 (51.4)	PC2 (17.3)	PC1 (44.5)	PC2 (25.1)	PC1 (49.0)	PC2 (20.6)
Latency to 1 m	-.804	-.224	-.770	.271	-.834	.205
Latency to 5 m	-.689	-.177	-.781	-.040	-.757	.059
Closest approach	-.915	-.138	-.844	.134	-.870	.115
Latency to song	.511	-.754	.405	.872	.397	.855
Flights	.612	-.110	.642	-.261	.635	.019
LRS	-.536	.753	-.326	-.879	-.407	-.819
Time within 5 m	.841	.204	.787	.167	.844	-.099
Time within 1 m	.805	.317	.585	-.537	.674	-.424

Note: In all cases, only the first and second principal components (PC1 and PC2, respectively) had eigenvalues greater than 1.0 and were included in the mixed-model analyses.

fixed factors; and Julian date as a covariate. We used SPSS for Windows 11.5 (SPSS, Chicago) for all statistical tests.

Results

Principal Component Analyses

In all three PCAs (fertile, nonfertile, comparison), all eight behaviors loaded strongly onto the first principal component (PC1), which typically explained around half of the variation (table 1). Only vocal behaviors loaded strongly onto the second component (PC2), which generally explained around 20% of the variation (table 1). To facilitate an intuitive interpretation of our data, we multiplied our loading and PC scores by (-1) so that greater PC1 scores were indicative of a shorter latency to approach, longer latency to song and lower LRS production, more flights, a closer approach to the speaker, and more time spent within 1 and 5 m of the speaker in each analysis (table 1). Thus, greater PC1 scores were indicative of a stronger territorial response.

STIs during the Fertile Period

We found a significant effect of playback treatment on male response (linear mixed model, $F_{2,34.18} = 37.336$, $P < .001$) during the fertile period. Males responded significantly more strongly to playback with SRS than with soft LRS (pairwise comparison with Šidák correction, $P < .001$) or LRS ($P < .001$; fig. 2). There was no detectable difference in response to soft LRS and LRS ($P = .322$). There was also no detectable effect of treatment when comparing PC2 scores (linear mixed model, $F_{2,34.44} = 0.373$, $P = .691$).

When we focused on PC1 scores, treatment order ($F_{2,34.18} = 1.221$, $P = .308$) and Julian date ($F_{2,16.74} =$

1.951, $P = .181$) did not significantly affect response; however, there was a significant treatment-by-order interaction ($F_{4,44.87} = 2.674$, $P = .044$). Males that received SRS as the second or third treatment responded more strongly than males that received SRS as their first treatment (fig. 3). Responses to soft LRS and LRS declined as order number increased (fig. 3).

Males spent significantly more time within 5 m of the speaker after playback with SRS than after playback of soft LRS (linear mixed model, pairwise comparison with Šidák correction, $P < .001$) or LRS ($P = .001$). There was no detectable difference in time spent within 5 m of the speaker after playback with soft LRS or LRS ($P = .403$).

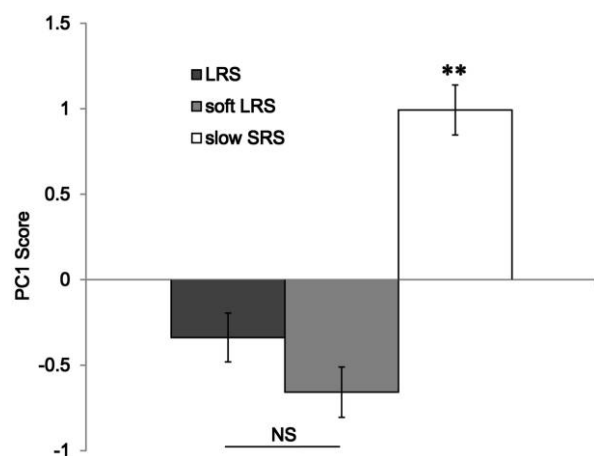


Figure 2: Estimated marginal mean (\pm SE) of principal component 1 (PC1) scores for response to playback of long-range song (LRS), soft LRS, and slow short-range song (SRS). Males responded significantly more strongly to playback of SRS than soft LRS ($P < .001$) or LRS ($P < .001$). There was no detectable difference in response to soft LRS and LRS ($P < .322$). For each treatment group, $n = 20$.

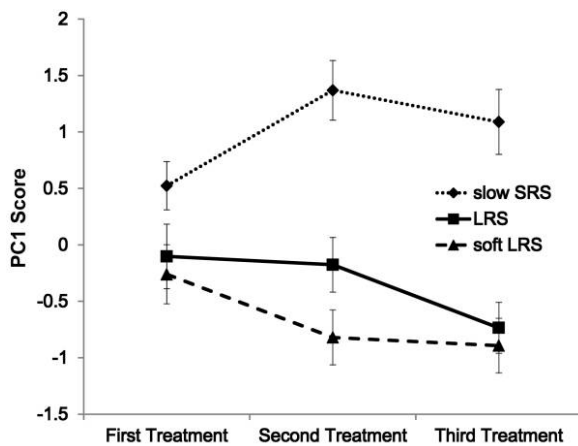


Figure 3: Estimated marginal mean (\pm SE) of principal component 1 (PC1) scores for male juncos when receiving soft long-range song (LRS), LRS, and slow short-range song (SRS) as the first, second, or third treatment. Treatment-by-order interaction was significant ($P = .044$). Males respond more strongly to SRS when presented as the second or third treatment. Response to soft LRS and LRS declines as treatment number increases.

We found no other significant differences in any of the postplayback behaviors (linear mixed models, flights: $F_{2,50} = 1.272$, $P = .289$; LRS: $F_{2,32.90} = 0.121$, $P = .887$; time within 1 m: $F_{2,33.30} = 1.943$, $P = .159$). Additionally, we found no significant differences in any of the preplayback behaviors (flights: $F_{2,34.99} = 0.276$, $P = .761$; LRS: $F_{2,32.18} = 1.236$, $P = .304$; time within 1 m: $F_{2,33.27} = 0.657$, $P = .525$; time within 5 m: $F_{2,50} = 2.018$, $P = .144$).

STIs during the Nonfertile Period

We again found a significant effect of playback treatment on male response (linear mixed model, $F_{2,20.07} = 5.709$, $P < .011$) during the nonfertile period. Males responded significantly more strongly to playback of fast SRS than soft LRS (pairwise comparison with Šidák correction, $P < .010$) but did not detectably differ in their responses to slow SRS and soft LRS ($P = .109$; fig. 4). There also was no detectable difference in response to slow SRS and fast SRS ($P = .632$; fig. 4). Treatment order (linear mixed model, $F_{2,20.07} = 0.054$, $P = .948$) and Julian date ($F_{1,9.85} = 0.516$, $P = .489$) did not significantly affect response, and there was no significant treatment-by-order interaction ($F_{4,31.95} = 0.285$, $P = .886$). There was no detectable effect of treatment when comparing PC2 scores ($F_{2,18.79} = 2.781$, $P = .088$).

We found no detectable differences in any of the postplayback behaviors (linear mixed models, flights:

$F_{2,20.33} = 0.672$, $P = .522$; LRS: $F_{2,16.04} = 1.021$, $P = .383$; time within 1 m: $F_{2,32} = 0.848$, $P = .438$; time within 5 m: $F_{2,32} = 2.356$, $P = .111$). Additionally, we found no significant differences in any of the preplayback behaviors (flights: $F_{2,21.51} = 0.710$, $P = .503$; LRS: $F_{2,19.12} = 0.499$, $P = .615$; time within 1 m: $F_{2,32} = 1.419$, $P = .257$; time within 5 m: $F_{2,32} = 0.588$, $P = .561$).

Comparison of Male Response during the Fertile and Nonfertile Periods

As above, there was a significant effect of treatment on PC1 scores (linear mixed model, $F_{1,27.86} = 44.305$, $P < .001$), with males responding significantly more strongly to slow SRS than to soft LRS. No detectable effects were found for any of the other model factors, but there was a significant interaction between treatment and fertility status ($F_{1,27.92} = 6.933$, $P = .014$). More specifically, male response to soft LRS did not differ detectably between the fertile and nonfertile periods (independent samples t -test, $t_{1,32} = 0.805$, $P = .427$), but males responded significantly more strongly to slow SRS when their mates were fertile ($t_{1,32} = 2.772$, $P = .009$; fig. 5).

There was no detectable effect of treatment on PC2 scores; however, there was a significant effect of fertility status (linear mixed model, $F_{1,45.37} = 6.434$, $P = .015$). Males had significantly higher PC2 scores during the nonfertile period, which is indicative of a longer latency to

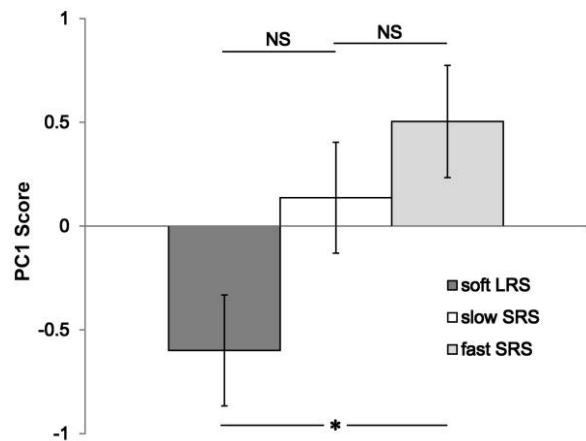


Figure 4: Estimated marginal mean (\pm SE) of principal component 1 (PC1) scores for response to playback of soft long-range song (LRS), slow short-range song (SRS), and fast SRS. Males responded significantly more strongly to playback of fast SRS than soft LRS ($P = .01$). There was no detectable difference in response to slow SRS and soft LRS ($P = .109$) or to slow SRS and fast SRS ($P = .632$). For each treatment group, $n = 14$.

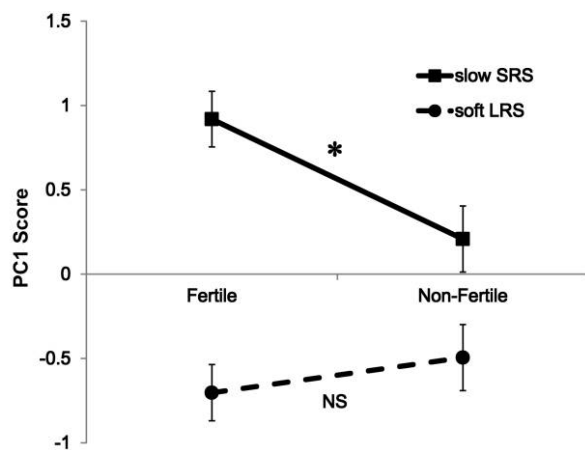


Figure 5: Estimated marginal mean (\pm SE) of principal component 1 (PC1) scores for male response to soft long-range song (LRS) and slow short-range song (SRS) during the fertile and nonfertile periods. Males do not differ detectably in response to soft LRS ($P = .43$) but respond more strongly to slow SRS during the fertile period ($P < .01$).

song and less LRS produced. No other factors in the model differed detectably.

Discussion

Low-amplitude songs are produced by many species of vertebrates and invertebrates during intra- and intersexual interactions. These low-amplitude songs can be structurally identical to a species' high-amplitude LRS but sung at low amplitude (soft LRS), or these songs can be structurally distinct and quite complex (SRS). The function of low-amplitude songs is poorly understood with respect to mate choice and their role in mediating aggressive interactions. Our results in dark-eyed juncos are consistent with previous data in suggesting that slow SRS serves a function in courtship. The importance of changes in SRS tempo remains unclear, and more work is needed to determine whether increased tempo is signaling increased courtship effort or aggressive intent. We found no evidence that soft LRS functions in courtship, but we did find that soft LRS performs a function similar to that of high-amplitude LRS in territoriality. The response of female juncos to low-amplitude songs remains to be tested.

On the basis of previous contextual evidence in juncos, SRS is sung by males and most often directed to females during courtship (Titus 1998). Thus, male eavesdroppers that overhear SRS may interpret SRS as an active courtship event and respond strongly in an attempt to interrupt an extrapair copulation. In this study, we found that responses to SRS were characterized by rapid, close approaches to

the speaker, which are typical aggressive responses of juncos (e.g., Newman et al. 2006; McGlothlin et al. 2007). Responses to SRS also showed an increase in activity as measured by flights but a decline in vocal behavior.

When comparing the receiver response of male juncos to both slow SRS and soft LRS between two distinct reproductive contexts, we found that males responded significantly more strongly to slow SRS during their mate's fertile period than during their mate's nonfertile period. This greater response to slow SRS during the fertile period, when males are at their highest risk for losing paternity to an intruding male, is consistent with SRS functioning in and signaling courtship. Male responses to soft LRS, in contrast, were weaker than responses to slow SRS and did not differ detectably between the fertile and nonfertile periods. The lack of difference between reproductive contexts in response to soft LRS suggests that soft LRS may serve a similar function regardless of fertility status and that males are not simply elevating their response to all songs when their mates are fertile.

Independent of reproductive context, we found that SRS generally incites a stronger aggressive response from male juncos than LRS or soft LRS, which suggests that male response is dependent not on amplitude but rather on song structure. Males did not differ detectably in their responses to slow SRS and fast SRS. However, during the nonfertile period, male response to fast SRS but not to slow SRS was significantly stronger than male response to soft LRS. These results are somewhat difficult to interpret but may suggest that the tempo of SRS indicates elevated motivation or intent from the signaler, which could be related to courtship effort or aggression. The finding that SRS incites a more aggressive response than LRS or soft LRS provides one explanation for why SRS is produced at low amplitude, as males that are overheard singing SRS, such as during an attempted extrapair copulation, may experience a strong retaliatory cost from eavesdropping males.

Males did not differ detectably in their response to LRS and soft LRS, suggesting that the function of LRS in male-male territorial interactions remains constant regardless of changes in amplitude. During the fertile period, male response to LRS and soft LRS declined to a similar degree when either treatment was presented as the second or third treatment, possibly as a result of habituation to the stimulus. Male response to slow SRS, in contrast, increased when slow SRS was presented as the second or third treatment. This similarity in response to LRS and soft LRS further implies similar functions. The elevated response to slow SRS may also indicate a lower perceived risk from the LRS stimuli in comparison with slow SRS, which may signal a courtship event.

Why Sing Softly?

Low-amplitude songs appear to function in both male-male and male-female close-proximity interactions, but these functions differ across taxa, raising the question of what conditions are necessary for the evolution of low-amplitude signals. Dabelsteen (2005) hypothesized that cryptic signals, such as low-amplitude songs, are favored in situations where the production of long-range signals would incur a cost from eavesdroppers, namely, hetero-specific predators and conspecific competitors. Broadly transmitted signals, such as LRS, can advertise location and potentially decreased vigilance to predators, which can decrease survival (Zuk and Kolluru 1998). In the case of conspecific eavesdroppers, neighboring or rival males can use information from acoustic signals that function in courtship to interrupt copulations (Balsby and Dabelsteen 2003, 2005) and information relating to agonistic interactions to judge aspects of the signaler's quality for use in future interactions (Naguib and Todt 1997; Mennill and Ratcliffe 2004; Peake et al. 2005) or to intrude onto the signaler's territory when the signaler is distracted (Naguib et al. 2004). The selective pressure from predation on acoustic signals will vary greatly among taxa, depending on predator abundance and reliance on acoustic cues, which is difficult to quantify. However, the selective pressure exerted by conspecific eavesdroppers will likely vary predictably with population density and mating system.

A potential fitness cost exists for signalers that broadly advertise a courtship event, as it can alert rival males that a female is nearby and increase the likelihood that courtship is interrupted and the copulation is lost (e.g., Balsby and Dabelsteen 2005; Stoltz and Andrade 2010). Selection for inconspicuous courtship signals by eavesdroppers can occur in any mating system in which males compete for females or extrapair fertilizations occur, as long as the population density is high enough to allow eavesdropping. In corroboration with this hypothesis, low-amplitude songs that function in courtship have now been identified in crickets, songbirds, and one species of bat, all of which encompass both socially monogamous and polygynous mating systems.

The fitness costs associated with advertising an agonistic interaction may differ greatly depending on whether males actively defend females. In species in which males defend females, broadly advertising an agonistic interaction also transmits information that the signaler's territorial defense and mate guarding are potentially compromised. Broadcasting this decline in vigilance may increase the likelihood that an additional intrusion will result in an extrapair copulation and a fitness cost to the signaler. Species without mate guarding do not incur this potential cost of eavesdropping. Alternatively, males may also gain a fitness ben-

efit from broadly advertising their agonistic interactions if they are a dominant male and females eavesdrop on aggressive interactions, which has been shown in both socially monogamous (Otter et al. 1999; Mennill et al. 2002) and polygynous (Doutrelant and McGregor 2000; Aquiloni et al. 2008) species. Currently, low-amplitude songs that function in aggression have been identified only in pair-bonding songbirds (but also see Ręk and Osiejuk 2011), suggesting that there may be a cost to broadcasting aggressive interactions when pair bonding occurs. Crickets, in contrast, do not form lasting pair bonds and do not produce low-amplitude aggressive songs but instead produce high-amplitude aggressive songs during male-male interactions (Zuk and Simmons 1997).

In addition to mating system and population density, the likelihood that low-amplitude songs will evolve to serve a function in courtship or aggression also depends heavily on the sensory biases of the intended and unintended receivers and the relative importance of acoustic signals within the species. As an alternative to producing a low-amplitude song, species may rely on other short-range sensory modalities such as vision, touch, and olfaction in close proximities to counteract eavesdropping (Roemer et al. 2010). However, future studies investigating the presence or absence of low-amplitude song should consider both mating system and population density in determining the likelihood that low-amplitude songs are functioning in male-female or male-male interactions.

Implications for Mate Choice and Speciation

The finding that low-amplitude songs, such as SRS, can serve an important function in courtship may have broad implications across all taxa that rely on acoustic signals for mate choice. If females are using SRS as an important trait in mate choice, then studies investigating female preference that focus only on conspicuous high-amplitude LRS will miss an integral part of the male's courtship display. In variable field crickets (*Gryllus lineaticeps*), for example, females prefer males producing low-quality LRS paired with high-quality SRS over males producing high-quality LRS paired with low-quality SRS, indicating that females' mating decisions are based predominantly on SRS (Wagner and Reiser 2000). Songbird researchers, on the other hand, have struggled to find a consistent link between female preference and certain song characteristics such as repertoire size (Searcy and Nowicki 2005; Byers and Kroodsma 2009), but those studies have predominantly focused on LRS (but see Dabelsteen and Pederson 1988; Anderson et al. 2007), which is often less complex than SRS and potentially less important in courtship. The dark-eyed junco highlights this discrepancy, as male juncos sing only one to eight syllable types in their LRS but typically

sing more than 20 syllable types in their SRS (Titus 1998; Newman et al. 2008; Cardoso et al. 2009).

Additionally, due to the fact that SRS is transmitted to a receiver over short distances, SRS is less likely than LRS to be influenced by selection for effective sound transmission (Wiley and Richards 1982; Dabelsteen et al. 1993; Balsby et al. 2003). SRS may therefore be more labile in response to other selective pressures such as divergence in female preference. Consequently, divergence in SRS may be less constrained than divergence in LRS. Given the function of SRS in courtship in some species, divergence in SRS between populations has the potential to act as a reproductive isolating mechanism and contribute to speciation (Coyne and Orr 2004; Price 2008). Divergence in LRS and SRS has been tested between two species of field crickets (*Gryllus texensis* and *Gryllus rubens*), and, contrary to the above predictions, LRS had diverged more substantially than SRS (Fitzpatrick and Gray 2001). In addition, females of both species lacked strong preferences for conspecific SRS, indicating that SRS is not acting as an isolating mechanism (Gray 2005; but see Jang et al. 2007). However, such divergence remains to be tested in species such as oscine songbirds that learn their songs.

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