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Low-amplitude songs produced by male dark-eyed juncos (*Junco hyemalis*) differ when sung during intra- and inter-sexual interactions

Dustin G. Reichard *, **Rebecca J. Rice**, **Elizabeth M. Schultz** ** and **Sara E. Schrock**

Department of Biology and the Center for the Integrative Study of Animal Behavior,
Indiana University, 1001 E. Third Street, Bloomington, IN 47405, USA
*Corresponding author's e-mail address: dgreicha@indiana.edu
**Current address: Animal Behavior Graduate Group and Department of Neurobiology,
Physiology and Behavior, One Shields Avenue, University of California Davis,
Davis, CA 95616, USA

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Abstract

The function and relative importance of low-amplitude (quiet) acoustic signals remains a poorly understood aspect of animal communication. Research in songbirds has predominantly focused on the role of low-amplitude songs as reliable predictors of attack and signals of aggressive intent. However, many species of songbirds produce low-amplitude song in the presence of both male and female conspecifics, suggesting a broader range of functions beyond aggressive behavior. Male dark-eyed juncos (*Junco hyemalis*) produce two distinct types of low-amplitude song: (1) soft long-range song (LRS), which is a lower amplitude version of junco LRS, and (2) short-range song (SRS), which is substantially more complex and structurally distinct from LRS. SRS is also sung at two distinct tempos (slow and fast) and has been observed in both male- and female-directed contexts. In this study, we presented free-living male juncos with a caged male or female conspecific accompanied by playback of LRS, a female precopulatory trill, or slow SRS and quantified the songs elicited by each social condition. Males produced slow SRS only in response to a female conspecific paired with a precopulatory trill or slow SRS, never to a male, even when males were paired with slow SRS. Together these observations indicate that slow SRS is a female-directed song. In contrast, males produced both LRS and soft LRS in the presence of conspecifics of both sexes, which suggests that LRS may serve a similar function with respect to male and female receivers regardless of amplitude. These results highlight the importance of investigating the production of low-amplitude songs in both intra- and inter-sexual contexts and provide further evidence that in juncos, slow SRS functions in courtship.

Keywords
short-range song, courtship, territoriality, song function, soft song.

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1. Introduction

The production of low-amplitude songs is common in both avian (Dabelsteen et al., 1998; Searcy & Beecher, 2009) and non-avian taxa (Robinson & Hall, 2002; Behr & von Helversen, 2004; Simoes et al., 2008; Zuk et al., 2008; Nakano et al., 2009; Osiejuk, 2011). Yet our understanding of the function and importance of low-amplitude songs relative to their high-amplitude, or long-range song (LRS), counterparts remains limited, particularly in birds (Reichard et al., 2011). In songbirds, the terminology identifying low-amplitude songs, which are predominantly sung by males, has been inconsistent. Low-amplitude songs have been referred to as 'soft song' (Nice, 1943; Anderson et al., 2007), 'quiet song' (Dabelsteen et al., 1998), 'whisper song' (Johnson & Kermott, 1991), 'strangled song' (Dabelsteen & Pedersen, 1990) and 'short-range song' (Titus, 1998). In general, all low-amplitude songs can be divided into two distinct classes based on differences in structure: (1) soft long-range songs (soft LRS), which are structurally identical to a species' LRS but are sung at reduced amplitude, and (2) short-range songs (SRS), which are structurally distinct from LRS and often diverge substantially from LRS both in frequency bandwidth and modulation and the number of distinct syllables appearing in each song (e.g., Anderson et al., 2008; Hof & Hazlett, 2010; Reichard et al., 2011; Figure 1).

Male songbirds rely on song to communicate multiple messages that are often related to male–male or male–female interactions, such as aggression or mate attraction and courtship. Because of the multiple functions of song, two evolutionary outcomes are likely: (1) a single class of song may evolve to serve multiple functions and convey information to many types of receivers, or (2) multiple, divergent classes of song may evolve with each song serving a unique function (Collins, 2004; Collins et al., 2009). In many species, a single class of LRS is thought to serve a 'dual function' both by attracting females as potential mates and repelling males as potential competitors (Searcy & Andersson, 1986; McGregor, 2005; Searcy & Nowicki, 2005); however, some species, such as the New World warblers, sing multiple classes of LRS with one class predominantly functioning in male–female interactions, while the other class functions in territorial behavior (Nolan, 1978; Kroodsma et al., 1989; Byers, 1996; Catchpole, 2000; Catchpole & Slater, 2008; but see Beebee, 2004).

Studies investigating the function of low-amplitude songs have largely focused on the role of low-amplitude songs in mediating aggressive behavior between males (Searcy & Beecher, 2009; Akçay et al., 2011). Evidence from three species of songbirds (*Melospiza melodia*, Searcy et al., 2006; Templeton et al., 2012; *Melospiza georgiana*, Ballentine et al., 2008; *Dendroica caerulescens*, Hof & Hazlett, 2010) and one species of rail (*Crex crex*, Ręk & Osiejuk, 2011) has indicated that the production of low-amplitude songs, mainly soft LRS, is a strong predictor of physical attack and, thus, a reliable signal of aggressive intent. Male song sparrows (*Melospiza melodia*) will produce both a 'crystallized soft song' (soft LRS) and a 'warbled soft song' (SRS) during aggressive interactions, yet males presented with playbacks of both types of low-amplitude song and LRS respond most aggressively to the warbled soft song (Anderson et al., 2007, 2012). These data indicate that even low-amplitude songs thought to serve similar functions may still be distinct in terms of the information that they convey to the receiver.

In addition to aggressive behavior, many songbirds have been observed producing low-amplitude songs in the presence of both male and female conspecifics, indicating that low-amplitude songs may serve additional functions unrelated to aggression (Dabelsteen et al., 1998). Observations from a number of species suggest that low-amplitude songs, SRS in particular, may function specifically in courtship, or like most LRS, serve a dual function in both courtship and aggression (Dabelsteen et al., 1998; Balsby, 2000; Reichard et al., 2011). Some members of the genus *Turdus*, for example, are known to produce a complex, low-amplitude 'twitter' (SRS) that is directed towards both males and females during the breeding season, suggesting a possible dual function (Dabelsteen, 1988; Dabelsteen et al., 1998). However, in all songbirds, comparative data examining differences in the structure and production of low-amplitude songs and whether they are directed towards male or female conspecifics or both are rare (but see Balsby & Dabelsteen, 2003; Dabelsteen, 2005).

In this study, we focused on determining the social contexts in which male dark-eyed juncos produce low-amplitude songs, which is a crucial component of identifying all of the potential functions of an acoustic signal (Searcy & Beecher, 2009). Male juncos sing a distinct LRS and SRS and they differ substantially in structure and complexity (Figure 1). Junco LRS and soft LRS is sung as a discrete trill with a restricted frequency range (Titus, 1998). Males broadcast LRS throughout the breeding season, and unmated males sing more LRS than mated males, suggesting that LRS serves a dual function in territorial behavior and mate attraction (Nolan et al., 2002). Soft LRS,

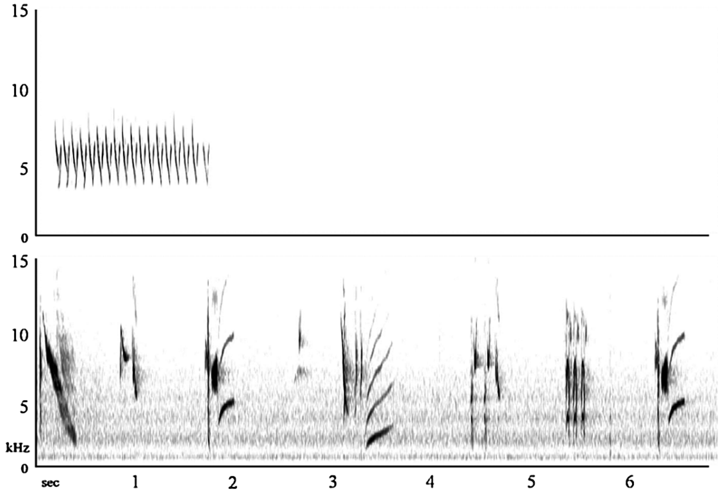

Figure 1. Representative sonograms of dark-eyed junco long-range song (LRS, top panel) and slow short-range song (SRS, bottom panel) recorded from different males. Relative darkness of the sonograms is not indicative of differences in amplitude between LRS and SRS.

which has received less attention, has been observed during close-proximity aggressive interactions between males and also when males are near their mate during nest building (Nolan et al., 2002). These non-quantitative observations would also suggest a dual function for soft LRS, but whether males differ in their use of soft LRS during male–male or male–female social contexts remains to be tested.

In contrast to soft LRS and LRS, junco SRS is a continuous, variable song containing more than 25 unique syllable types that often cover twice the frequency range of LRS (Figure 1; Titus, 1998; D.G. Reichard, unpubl. data), and SRS is sung at two distinct tempos: slow and fast (Reichard et al., 2011). Male juncos produce significantly more SRS during courtship and their mate's fertile period than during the nonfertile period in the field (Titus, 1998). In captivity, males in a Y-maze mate choice study produced more SRS than LRS only when a female was present in the maze (Enstrom et al., 1997). Collectively, these observations suggest that SRS may function predominantly in courtship, but both of these studies were completed before the discovery of two distinct classes of SRS (slow and fast; Reichard et al., 2011).

Subsequent observations reported that slow SRS was produced most often during male–female interactions, while fast SRS was infrequently observed during both male–male and male–female interactions (Reichard et al., 2011; D.G. Reichard, D.A. Enstrom and E.D. Ketterson, unpubl. data). In a series of playback experiments, male juncos responded more aggressively to slow and fast SRS than to soft or loud LRS, and male response to slow SRS was significantly more aggressive when a male's mate was fertile, suggesting that slow SRS is interpreted by males as a courtship signal (Reichard et al., 2011). Male response to fast SRS was measured only during the nonfertile period making inferences about its function in courtship or aggression challenging (Reichard et al., 2011). However, males did not respond to any of the playbacks by singing slow or fast SRS with one exception (see Discussion). Thus, all assumptions about the functions of slow and fast SRS are currently based on non-quantitative observations of the social context accompanying their production or measures of male response to song playback alone, which warrants a more rigorous analysis of how males vary their use of low-amplitude song across a variety of social contexts.

To investigate how social context affects male production of low-amplitude songs (soft LRS, slow and fast SRS), we conducted two separate experiments each consisting of two simulated interactions. In Experiment 1, we asked whether male vocal behavior (LRS and SRS only) differed when presented with a live, male or female conspecific. To attract free-living males to the stimulus, we paired a captive male with playback of LRS, a common method for assaying aggressive behavior in songbirds (Searcy & Beecher, 2009). In contrast, females were paired with a precopulatory trill playback, a female vocalization often produced during a copulation solicitation display, to simulate a receptive, fertile female rather than an aggressive intruder. We predicted that songs functioning in courtship or female stimulation would be produced only to a female conspecific, aggressive songs would be produced only to a male conspecific, and dual function songs would be produced in both contexts.

In Experiment 2, we asked if playback of slow SRS accompanied by a male or female conspecific affected male vocal behavior (LRS, soft LRS, SRS). The pairing of slow SRS with a male conspecific was intended to mimic a male conspecific singing SRS on the focal male's territory, while the female conspecific paired with slow SRS was intended to mimic an interaction between the female conspecific and an unseen male singer (female juncos do not sing SRS) on the focal male's territory. This design was chosen to avoid any potential confounds of adding a second live conspecific, which likely would have resulted in variable levels of interaction between the two caged individuals. We predicted that songs that signal aggression or serve a dual function would be produced in both contexts owing to the persistent presence of a visible or nonvisible male singer, but songs that function in courtship would be produced only in the context of a female conspecific. In both experiments we also noted the presence or absence of the focal male's social mate to identify all of the potential receivers of each vocalization, and to determine whether males were producing certain types of song only when their social mate was present.

2. Materials and methods

2.1. Study site

Our study was performed at Mountain Lake Biological Station and Mountain Lake Hotel in Pembroke, VA, USA (37°22′N, 80°32′W). All dark-eyed juncos on our study site are captured and banded with a unique combination of colored leg bands at the beginning of each breeding season, which facilitated the identification of all focal individuals and their social mates during our experiments.

2.2. Experiment 1

Between 28 May and 10 July 2007 we conducted simulated social interactions with 24 free-living male juncos between 0800 and 1000 EST. Trials occurred near the estimated center of the focal male's territory on consecutive days in a counterbalanced order and consisted of a live, caged male or female conspecific paired with a 15-min playback. To serve as conspecific stimuli in both experiments, we captured male and female juncos at the beginning of each breeding season and housed them in small, same-sex flocks for the duration of the study. Captive juncos are generally docile when caged, and in both of our experiments the male conspecifics never sang LRS or SRS and the female conspecifics did not trill. Additionally, neither sex performed any noticeable visual displays (e.g., tail spreading or copulation solicitations) during our trials. Thus, any effect of the behavior of the caged conspecific on our results is likely minimal.

Male conspecifics were paired with a loud LRS playback tape previously created and used by McGlothlin et al. (2007) consisting of five unique LRS types recorded $\geqslant 10$ years prior to this study in our study population (for recording methods see Titus, 1998). Although all subjects heard the same vocal stimulus, the playback tape sampled a variety of male LRS types from our study population to limit any effects of pseudo-replication. Each LRS type was played nine times consecutively before transitioning to the next type at a rate of six songs per min. Female conspecifics were paired with playback of a precopulatory trill. We were unable to include recordings of trills from multiple individuals and, thus, this stimulus was pseudo-replicated (see justification below). To mimic the structure of the LRS playback tape, the female trill was repeated at a rate of six songs per min.

Playbacks were broadcast through a speaker (Radio Shack 40-1441) attached to a portable compact disc player (Duraband CD-855) placed directly beside the caged conspecific. The amplitude of each playback was standardized to 92 dB SPL measured at 1 m using a Radio Shack digital sound level meter (Model No. 33-2055), which is comparable to the natural amplitude of junco LRS (Nolan et al., 2002). Conspecifics were chosen randomly each day from the captive flock of four individuals of each sex to limit pseudo-replication.

During each simulated interaction an observer (E.M.S.) and a recorder located 10–15 m from the stimulus quantified the number of LRS produced. Additionally, we recorded the presence and type of SRS (slow or fast) produced. Previous studies in other songbirds have shown that low-amplitude songs can be reliably heard, identified, and differentiated from LRS by human observers during interactions similar to those presented here (Searcy et al., 2006; Anderson et al., 2008; Hof & Hazlett, 2010). One difference between this study and previous experiments is that we did not record the vocalizations of our subjects simultaneously with our observations. While it is possible that we failed to hear some low-amplitude vocalizations, we think this is unlikely because two human observers were present for each trial and junco soft LRS and SRS are regularly heard at distances greater than 15 m from the singer (D.G. Reichard, pers. obs.). We also noted the appearance of other conspecifics during each trial, including the focal male's mate. A Wilcoxon signed-ranks test was used to compare the amount of LRS produced towards each sex.

2.3. *Justification for pseudo-replication in Experiment 1*

By presenting only a single female trill playback to all of our subjects, our results may suffer from the risks of pseudo-replication (Kroodsma, 1990;

Kroodsma et al., 2001; Wiley, 2003). At the time of this study, our only high-quality recording of a female precopulatory trill was a single trill recorded previously from a captive female junco. In subsequent breeding seasons, we were able to record additional precopulatory trills from six females in the field, and we analyzed those trills to determine whether the trill playback used in this study was representative of the standing variation in the population. The trills recorded from free-living females contained the identical note type (i.e., a high-frequency chip) present in the trill playback, and the trill length (1.58 s), number of notes within each trill (35) and trill rate (22.1 notes/s) of the trill playback were nearly identical to the means observed from the sample of six trills ($\bar{x}$ (range); trill length 1.62 (0.86–2.37) s; no. of notes: 35 (20–45); trill rate 22.1 (18.9–24.4)), although the average minimum (5777 Hz) and maximum (8607 Hz) note frequencies of the playback trill were lower than the grand averages of the notes in all six trills (Min: 6435 Hz, Max: 10 756 Hz).

Thus, based on the apparent lack of divergence in the note and temporal structure of the trill playback and the substantial overlap in frequency despite a decreased bandwidth, we think it unlikely that the results we describe here are due to a pseudo-replicated vocal stimulus. Note also that four different females chosen at random served as visual stimuli, thus ensuring variation among visual stimuli. Finally, we followed the practice of Poole (1999) and the suggestions of Kroodsma et al. (2001), and used minimal statistics in our analysis of these data.

2.4. Experiment 2

Between 18 June and 13 July 2009, we conducted simulated social interactions containing slow SRS playbacks paired with a live, caged male or female conspecific instead of LRS or a precopulatory trill. Playback tapes were created using recordings and methods previously reported by Reichard et al. (2011). For this study playback tapes consisted of one 30-s segment of slow SRS repeated continuously for 5 min. We did not alter the tempo of the initial slow SRS recordings. We created enough tapes that each male heard a unique recording for each trial. Playbacks were broadcast through a Pignose amplified speaker (Model No. 7-100) covered in camouflage cloth that was placed on the ground directly beside the caged conspecific. The SRS playback amplitude was standardized to 65–70 dB SPL measured at 1 m using the same Radio Shack digital sound level meter to mimic the estimated natural amplitude of SRS (Anderson et al., 2008).

The playback and live male or female conspecific were presented to 17 male juncos on consecutive days in a counterbalanced order between 1300 and 1700 EST. None of the male subjects were previously sampled in Experiment 1. Conspecifics were chosen randomly each day from the captive colony of five juncos of each sex to limit pseudo-replication. To ensure that the low-amplitude playback was heard, each trial began with the conspecific and speaker placed in the center of the focal male's territory and a playback of high-amplitude LRS of approximately 85–90 dB SPL. Once the focal male approached to within 10 m of the cage, the LRS playback was stopped, and we quantified the male's behavior for 1-min of silence to account for any response elicited by the LRS. If the male ranged beyond 10 m during the 1 min of silence (rare due to the presence of a visual conspecific), we restarted the attraction playback and repeated the minute of silence. After 1 min of silence, we played the 5-min slow SRS playback and continued to quantify the male's behavior for the duration of the playback and an additional 5 min of post-playback silence. The SRS playback tape and speaker location were changed slightly between days (approx. 1–3 m away) to limit habituation. To avoid 'dear enemy' effects (Temeles, 1994) we chose playback recordings collected a minimum of 500 m away from each subject's territory.

Throughout each trial we quantified the number of LRS and soft LRS produced and the duration and type of SRS produced. Distinguishing between soft and loud LRS was determined by both the observer (R.J.R.) and a recorder who sat at the same location, 10–15 m from the caged conspecific and speaker. We conservatively identified LRS as soft only when both the observer and recorder were in agreement. All other LRS were lumped into the LRS category for analysis. We also noted the presence of other conspecifics.

All statistics were performed with IBM SPSS Statistics version 20.0 (IBM, Armonk, NY, USA). We used Wilcoxon signed ranks tests to compare the amount of LRS and soft LRS produced towards each sex. Additionally, we used two repeated measures general linear models to compare the number of LRS and soft LRS produced during the slow SRS playback and post-playback silent periods (within-subjects factors) in response to both conspecific sexes (between-subjects factor).

3. Results

3.1. Experiment 1

Nine out of 17 males that interacted with both a male and female conspecific sang SRS, but in all cases, SRS was slow SRS and was produced during trials with a female conspecific, never a male conspecific (Figure 2). All males produced LRS with 13 of 17 males producing LRS in response to both sexes while 3 males produced LRS only to males and 1 male produced LRS only to a female (Figure 2). We did not differentiate between soft and loud LRS in this experiment, so the LRS data is a combination of both. Males did not detectably differ in the amount of LRS produced to each sex (Figure 3; $Z = -0.758$, $p = 0.448$).

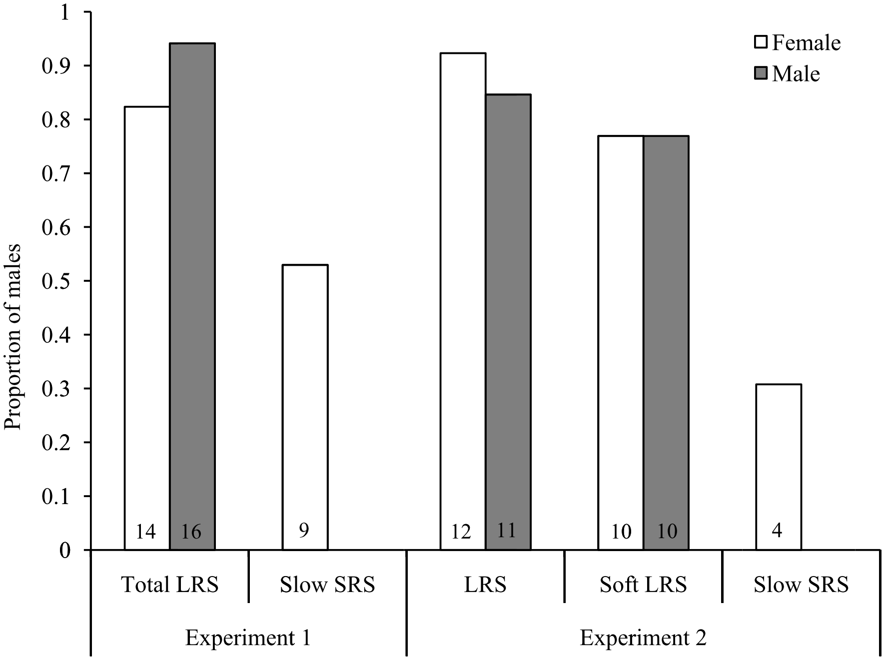

Figure 2. Proportion of males that produced slow SRS, soft LRS, or LRS during an interaction with a female or male conspecific. In Experiment 1, female conspecifics were paired with playback of a female precopulatory trill and male conspecifics were paired with LRS playback ($N = 17$). In Experiment 2, conspecifics of both sexes were paired with slow SRS playback ($N = 13$). Numbers at the base of each bar represent the number of males that sang each song type.

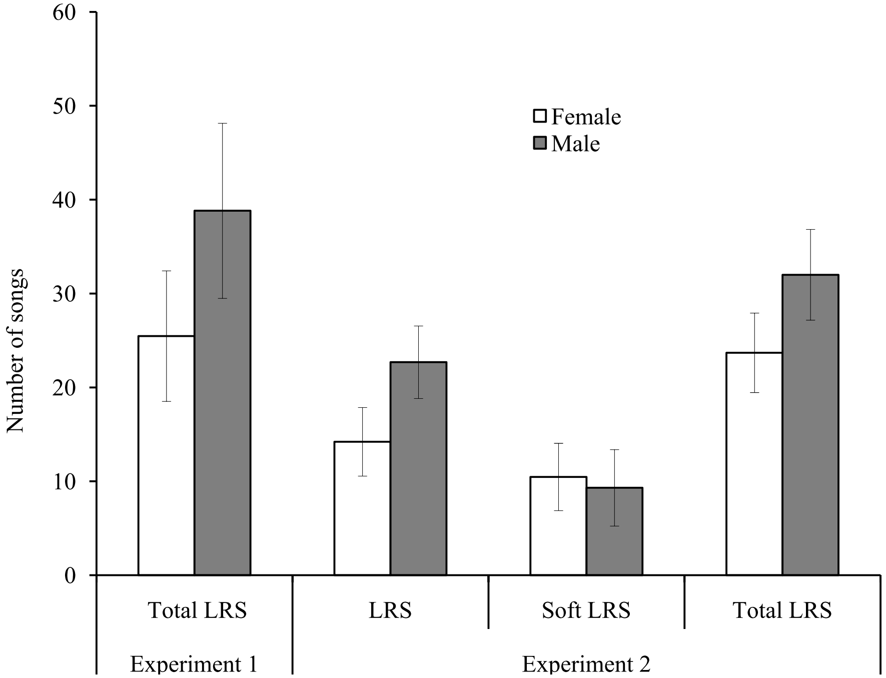

Figure 3. Mean number of long-range songs (LRS), soft LRS, and total LRS (LRS + soft LRS) produced in response to a male or female conspecific in two experimental conditions. In Experiment 1, male conspecifics were paired with a LRS playback and female conspecifics were paired with a playback of a female precopulatory trill; trials were 15 min ($N = 17$). In Experiment 2, both conspecifics were paired with a slow SRS playback; trials were 5-min ($N = 13$). No statistical differences between the conspecific stimuli were found for any song class. Error bars represent ± 1 SEM.

The focal male's mate was present for 12 of 17 trials involving a female conspecific and 9 of 17 trials involving a male conspecific. Focusing only on trials in which focal males sang slow SRS, the focal male's mate was present in 7 of 9 trials. A second male appeared for one of our trials and was quickly chased away by the focal male without any SRS production by either male.

We were able to collect response data for only one sex from seven males with two males receiving only a female conspecific and five receiving only a male conspecific. All seven males produced LRS in the presence of their conspecific stimuli and both males that received a female conspecific produced slow SRS.

3.2. Experiment 2

Four out of 13 males that received conspecifics of both sexes sang SRS during the simulated interactions, and identically to Experiment 1, in all cases SRS was slow SRS and was produced only in response to a female conspecific (Figure 2). Two of the male subjects began producing slow SRS during the pre-playback silent period, but of these two only one male persisted in singing throughout the slow SRS playback. The other two males began singing slow SRS during the post-playback silent period after the SRS playback had ended.

Twelve of 13 males sang at least one soft LRS and 8 of 13 produced soft LRS in response to both a male and female conspecific. Of the other 4 males that sang soft LRS, half produced soft LRS only in response to a female and the other half only in response to a male. Similarly, 11 of 13 males sang LRS to both a male and female conspecific and 1 male sang LRS only to a female (Figure 2). An additional four males received only a female conspecific and of those males, three sang LRS, one sang soft LRS, and one sang SRS. During our trials, the focal male's mate was present in 5 of 13 female trials and 2 of 13 male trials. There were no instances of neighboring males responding to our trials. In trials where focal males sang slow SRS, the focal male's mate was present in 2 out of 4 trials.

Males did not detectably differ in the number of LRS (Figure 3; $Z = -1.691$, $p = 0.091$), soft LRS ($Z = -0.578$, $p = 0.563$), or total number of LRS ($Z = -1.452$, $p = 0.147$) produced in response to a female or male conspecific during the slow SRS playback (Figure 3). Additionally, males did not differ in the number of LRS or soft LRS produced during the pre-playback (LRS: $Z = -0.275$, $p = 0.783$; soft LRS: -1.069, $p = 0.285$) or post-playback (LRS: $Z = -1.255$, $p = 0.209$; soft LRS: $Z = -0.677$, $p = 0.498$) silent periods.

However, males produced significantly less soft LRS during the post-playback silent period than during the slow SRS playback ($F_{1,24} = 6.170$, $p = 0.020$). But, there was no detectable effect of the sex of the conspecific intruder ($F_{1,24} = 0.002$, $p = 0.962$) on soft LRS production during the playback and post-playback periods, and no significant interaction between playback period and conspecific sex ($F_{1,24} = 0.403$, $p = 0.532$). In contrast, males did not differ detectably in the amount of LRS produced during the slow SRS playback and post-playback silent periods ($F_{1,24} = 0.277$, $p = 0.603$). There was also no significant effect of conspecific sex on LRS production during the playback and post-playback periods ($F_{1,24} = 1.871$, $p = 0.184$), and no significant interaction between playback period and conspecific sex ($F_{1,24} = 0.002$, $p = 0.962$).

4. Discussion

The production of low-amplitude vocalizations is common in both vertebrates and invertebrates and is often observed in songbirds during close proximity, male–male and male–female interactions associated with aggression or courtship. Low-amplitude songs can be structurally identical to a species' LRS, but produced at a lower volume (soft LRS), or they can differ substantially in structure from a species' LRS (SRS). Few studies have examined how the use of low-amplitude songs differs when singing occurs in both male–male and male–female interactions, which is a critical component of identifying the function or functions of low-amplitude songs (Searcy & Beecher, 2009).

In this study, male dark-eyed juncos produced slow SRS only when presented with a female conspecific accompanied by playback of either a female precopulatory trill (Experiment 1) or slow SRS (Experiment 2). Males produced slow SRS both in the presence and absence of their own social mate, but never produced slow SRS in response to a male conspecific paired with either LRS or slow SRS. In contrast, males did not differ detectably in the production of LRS or soft LRS to both sexes. We did not observe any fast SRS during any of our trials. These results are consistent with previous data from captive juncos in which males placed in a Y-maze choice test produced more SRS than LRS after a female was introduced (Enstrom et al., 1997) and strengthen our interpretation of previous results of male responses to low-amplitude song playback.

In a previous study, male juncos in this study population responded significantly more aggressively to slow SRS when their mates were fertile, but did not differ in their response to soft LRS between the fertile and non-fertile periods (Reichard et al., 2011). The current finding that males sing slow SRS only in response to females supports the conclusion that males respond more strongly to slow SRS during the fertile period because they interpret slow SRS as an indication that extra-pair courtship is occurring on their territory. This conclusion is further supported by the observation that some males in the current study produced slow SRS in response to a female conspecific when their social mate was absent, suggesting a function for slow SRS in soliciting extra-pair copulations, which are common in our study population (Gerlach et al., 2011). Furthermore, the result that soft LRS was produced at similar levels in response to both male and female conspecifics is consistent with the idea that soft LRS is not a reliable indicator of whether an interaction involves two males or a male and a female, which may explain why males did not elevate their aggressive response to soft LRS during their mate's fertile period.

4.1. SRS and aggressive signaling

A common form of aggressive signaling in songbirds involves countersinging with LRS and soft LRS during agonistic interactions between males (Searcy & Beecher, 2009). Here, we show that in the presence of a male conspecific paired with playback of slow SRS, male juncos do not respond with their own slow SRS or fast SRS, but instead produce only LRS or soft LRS. The absence of SRS production in response to slow SRS playback and a male conspecific parallels the lack of slow SRS production observed in a previous study of male response to SRS, soft LRS, and LRS playbacks alone (Reichard et al., 2011) and further supports the lack of an aggressive function for slow SRS in juncos. The only condition in which males did produce slow SRS in response to slow SRS playback occurred when slow SRS was paired with a female conspecific. Additionally, only one of the four males that sang slow SRS overlapped their song with the slow SRS playback, and in that single case the male began singing slow SRS during the pre-playback silent period rather than starting after the playback started.

The aforementioned results, when considered with our observation from Experiment 1 that males produce slow SRS in response to a female conspecific paired with a precopulatory trill, strongly suggest that males were producing slow SRS to court or stimulate the visible female rather than to signal an aggressive response to the nonvisible male singer. We also noted that the proportion of males that sang slow SRS was lower when the female conspecific was paired with slow SRS rather than the precopulatory trill. This decrease in SRS production in response to both a female and a perceived male may have been a result of males dividing their vocal response between courtship of the visible female and aggression towards the male singer. Given that males do not produce slow SRS in response to SRS playback alone (Reichard et al., 2011), it seems unlikely that males were using their slow SRS in this context as a dual function signal to both stimulate the female and repel the unseen male singer.

It also remains plausible that, in some cases, male songbirds may direct complex, low-amplitude songs such as SRS towards females as an aggressive signal. In Experiment 1, we paired each female conspecific with a precopulatory trill playback to simulate a non-threatening social interaction that would provide insight into the vocal signals used during courtship or female stimulation rather than aggression. As juncos are socially monogamous and regularly engage in extra-pair behavior (Gerlach et al., 2011), it seems unlikely that males would respond aggressively to a potential extra-pair mate during the breeding season. One context in which a male might be expected to exhibit aggressive behavior towards a female conspecific is in the presence of his social mate (e.g., Ung et al., 2011). Contrary to this prediction, males produced slow SRS towards a female conspecific in both the presence and absence of their social mate in each of our experiments.

Our inability to elicit fast SRS with any of our stimuli failed to provide insight into the social contexts associated with fast SRS production. One potential explanation for this result may be the fact that we did not use fast SRS with a male or female conspecific as a stimulus. However, in two previous studies of male response to both high- and low-amplitude songs (Reichard et al., 2011; Rosvall et al., 2012), only one male (of 74 total males) sang fast SRS, and the fast SRS was produced in response to multiple song stimuli including slow SRS (D.G. Reichard, C.C. Vanderbilt and E.D. Ketterson, unpubl. data). Observations of fast SRS from free-living juncos are infrequent, but fast SRS is often followed or accompanied by intense physical interactions between the male singer and a rival male or his social mate (D.G. Reichard, pers. obs.). It is important to note that in the observations of male–male interactions involving fast SRS in the field and the male that responded to playback by singing fast SRS, a female was often nearby limiting the ability to identify an intended receiver and assign a clear function to the fast SRS. Therefore, whether fast SRS is predominantly a female-directed signal like slow SRS, or whether it is serving an aggressive or dual function remains poorly understood.

4.2. *Soft LRS and aggressive signaling*

The production of soft LRS has repeatedly been identified as a signal of aggressive intent and strong predictor of physical attack in a number of avian species (Searcy & Beecher, 2009; Hof & Hazlett, 2010; Ręk & Osiejuk, 2011). Consistent with this hypothesis, male dark-eyed juncos produce soft LRS during aggressive interactions, but whether soft LRS production predicts the likelihood of attack remains to be tested. In this study, we noted soft LRS produced in the presence of both male and female conspecifics, which may suggest a dual function for soft LRS. However, in Experiment 1 we did not differentiate between loud and soft LRS, and in Experiment 2 the combination of a visible female with a male song makes it difficult to distinguish whether the intended receiver of the soft LRS was the visible female or the unseen male. Thus, we cannot definitively say whether soft LRS was produced near the female as a signal of aggression, courtship, or both.

During Experiment 2, males experienced either a visible or nonvisible, vocalizing male intruder followed by a silent period where only a male or female conspecific was present. In response to both conditions, we noted that male LRS production did not detectably change between the playback and post-playback periods, but male soft LRS production was significantly higher during playback. If both soft and loud LRS function as aggressive signals, then the fact that soft LRS is produced more often in response to a persistently signaling intruder may suggest that soft LRS is a signal of elevated aggression. However, a stronger test of this idea would involve first establishing a baseline of soft and loud LRS production in the absence of any male intruder and then comparing the production of both song classes before, during, and after the intrusion.

In support of a non-aggressive function for soft LRS, male juncos are known to produce soft LRS near their mates during nest building (Nolan et al., 2002), a behavior that also occurs in white-crowned sparrows (*Zonotrichia leucophrys*) more frequently during the fertile period and also near the nest during incubation (Nelson & Poesel, 2011). Nelson & Poesel (2011) have hypothesized that this soft LRS may function in courtship and female stimulation or as an ‘all-clear’ signal to incubating females, similar to the role of male LRS in the reed bunting (*Emberiza schoeniclus*, Wingelmaier et al., 2007). In summary, identifying soft LRS as an aggressive signal appears accurate in many species, including juncos, but soft LRS likely serves additional functions in male–female communication beyond aggression.

4.3. Conclusions

Based on the observations of slow SRS and social context in this study and previous work on male responses to various types of song playback (Reichard et al., 2011), it is apparent that slow SRS functions in courtship in dark-eyed juncos. However, the strongest test of a signal's courtship function involves measuring female response. Females juncos were previously shown to prefer males that were more active, performed more visual displays, and sang more SRS in a Y-maze choice test (Enstrom et al., 1997), but direct tests of female preferences for male songs are currently lacking. Beyond juncos, tests of female preferences for low-amplitude songs in other species have yielded mixed results. Song sparrows, for example, do not produce SRS during courtship, and as predicted, females presented with SRS, LRS and soft LRS produced the most copulation solicitation displays (a measure of preference and willingness to mate) in response to LRS not SRS (Anderson et al., 2007). On the other hand, Eurasian blackbirds (*Turdus merula*) produce SRS during courtship, and females perform a similar amount of copulation solicitation displays in response to SRS and LRS (Dabelsteen, 1988). Considered as a whole, these data suggest that SRS, and low-amplitude songs in general, likely serve functions that vary depending on the species being sampled.

The functions of junco soft LRS and fast SRS are less clear, but our results are consistent with previous observations of soft LRS production during both male–male and male–female interactions, suggesting that junco soft LRS is a dual function signal. However, due to a methodological confound, we cannot definitely say whether males in our study were using soft LRS as an aggressive or dual function signal. The lack of fast SRS production in response to any of our stimuli raises important topics for future research such as determining the types of social conditions necessary to elicit fast SRS and the functional importance of a signal that elicits an intense aggressive reaction from males (Reichard et al., 2011) but is rarely observed. More broadly, the results of this study highlight that a thorough and rigorous sampling of the importance of low-amplitude songs to both sexes across many species is essential for gaining a more complete understanding of the complexity of song types and functions specific to low-amplitude songs.

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