Watch Your Tone: Social Conditions Modulate Singing Strategies

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Abstract

Bird song is typically depicted as a male singing a long-distance signal to potentially unknown receivers to (1) deter males and (2) attract females. Nevertheless, many songbirds sing from close distances to a known receiver; males of these species may be under more intense selective pressure to modify their songs depending on the sex of the receiver in order to convey different motivational states (aggression versus courtship) to the different sexes. In a laboratory setting, we examined how receiver sex affected within-song variation of the close-range singing behavior in the brown-headed cowbird (*Molothrus ater*). Although we know that cowbird song is influenced by flock composition, it is still unclear as to how the cowbird modifies his song based on social context. Using a cross-correlation analysis of each male's different song types, we found that pairs of songs were significantly more dissimilar if they were directed to females compared with songs directed to males. We subsequently tested whether there were any consistent spectral or temporal patterns in the songs males gave to females versus to males. Our results lend support for the Motivational Structural Rules Hypothesis as songs directed toward males had higher entropy (i.e., harshness) than the same song type directed toward females. Our results suggest that cowbirds may have evolved the ability to alter multiple dimensions of their singing behavior based on receiver sex.

Introduction

Studies of communication have often examined male songbirds that sing to multiple potential receivers: rival males that may encroach on their territory and females that may become a mating partner (Catchpole & Slater 2008). This conceptual model commonly assumes that communication is typically done over long-distances, in which the signal is designed to have a large active space (i.e., the distance from the sound source in which the signal is still detectable; Brenowitz 1982; Wiley & Richards 1982; Naguib & Wiley 2001). Additionally, during long-distance communication, the sender may not be aware of the presence or absence of a particular receiver.

Recent work, however, has demonstrated that many bird species sing at close distances to a known receiver (Titus 1998; Anderson et al. 2008; Catchpole & Slater 2008; Reichard et al. 2013). Within a close-distance communication framework, the sender is expected to modify his song to convey specific information or motivations (Morton 1977). Additionally, within this close-range context, the fine structure of the vocalization is not necessarily under selective pressure to propagate over far distances (Wiley & Richards 1978; Richards & Wiley 1980) and is therefore expected to show higher structural variability than long-range vocalizations (Marler 1967; Morton 1982; Fernandez-Juricic & Martella 2000). Compared with their long-range counterparts, close-range vocalizations may be under less selective pressure to have a large active space and may therefore be of lower amplitude than songs or calls that are broadcast over a large area.

Recent work on the close-range, low-amplitude songs (i.e., 'soft song', 'quiet song', 'twitter song', or 'whisper song') noted in over 24 species of North American passerines (Morton 2000) have largely focused on the role of such songs in aggressive contexts (Anderson et al. 2008, 2012; Searcy & Beecher

2009; Akcay et al. 2011). Nevertheless, there are some reports that low-amplitude songs may serve a dual function in both aggressive and courtship interactions (Dabelsteen et al. 1998; Balsby 2000; Reichard et al. 2013). Additionally, many group-living species often have close-distance, low-amplitude contact calls that potentially serve as a means of maintaining group cohesion (Fernandez-Juricic & Martella 2000).

Hypotheses related to how close-range songs should be modified depending on the social context are limited. The Motivational Structural Rules Hypothesis is, to the best of our knowledge, the only hypothesis that predicts context-induced structural changes in vocal communication made primarily for close-distance, directed signals (Morton 1977). The Motivational Structural Rules Hypothesis suggests that the physical structure of sound should be related to the motivation behind a signals use. For example, intrasexual signals used to convey aggression may be lower in frequency and harsher (i.e., less pure tones) than sounds used in an intersexual context. A vocalization's 'harshness' can be measured by its entropy, which is the amount of randomness in a sound, with harsher vocalizations having higher entropy values (Ho et al. 1998; Tchernichovski et al. 2000). Morton (1977) also suggested that harsh, low-frequency songs are a direct indication of body size, and therefore, an honest signal of the probability of winning an aggressive, intrasexual contest.

The goal of this study was to examine the effect of receiver sex on the spectral and temporal structure in the songs used primarily for close-distance communica-

tion of a group-living songbird: the brown-headed cowbird (*Molothrus ater*). As obligate brood parasites, cowbirds acquire their song skillfully in interactions with conspecifics upon joining a flock; their song is therefore thought to represent proximate quality and a male's ability to attend to social cues from flock mates (King & West 1983; Freeberg et al. 1995; Dohme et al. 2015). Investment in singing toward other singing males (i.e., counter-singing) and toward females has been shown to correlate with male mating success (White et al. 2010; Kohn et al. 2013).

Cowbirds have two types of vocalizations: a flight whistle which is a long-distance signal given most often during flight, and the perched song which is given most often during directed displays to both sexes at close distances (<1 m) but can also be sung in non-directed, long-distance displays (Rothstein et al. 1988). The perched song (Fig. 1) typically has three elements: (1) a series of low-frequency, complex glugs that are each comprised of frequency 'steps' formed from alternating sides of the syrinx during singing (phrase 1, or P1) (Allan & Suthers 1994), (2) the interphrase unit (IPU), a brief, 50 ms, high-frequency burst of energy, and (3) the second phrase (P2), a complex series of high-frequency, frequency-modulated tones (West et al. 1979). The first phrases of the cowbird song have been shown to be critical to inducing the female copulatory position, and the later portion of the song has been hypothesized to contain information regarding individual identity, which may be more important in male interactions (West et al. 1979).
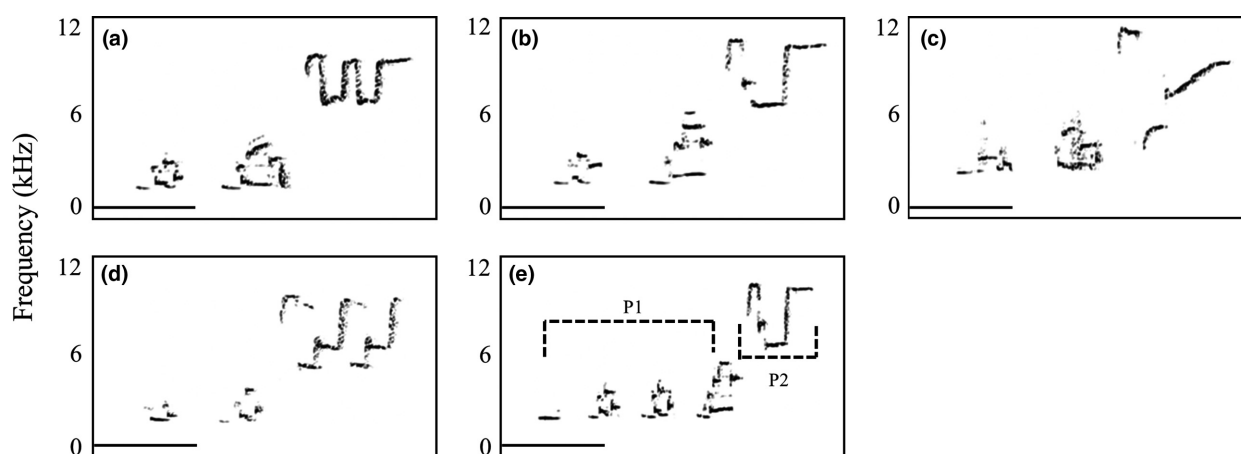

Fig. 1: Spectrograms of a focal male's full song repertoire (a–e). Cowbird perched songs are characterized by a series of low-frequency 'glugs' (Glug 1, Glug 2, etc.) which combined make up the first phase of the song (Phase 1; P1 hereafter). The second and final phase (Phase 2; P2 hereafter) is composed of high-frequency sweeps. Cowbird songs were characterized first by the overall shape of the P2, and then by the number of elements in P1. Both 'b-' and 'e-'type songs for this male have the same P2, but the number of elements in P1 differs (E has 3 glugs, and an introductory low-frequency 'whoo,' named Glug 0, while B has the more typical 2 glugs).

The cowbird perched-song repertoire usually ranges from 2 to 8 different songs types (Dufty 1986). Males will cycle quickly through their entire repertoire during interactions with both sexes (King & West 1983). Cowbirds often pair their perched songs with a visual bow and wing-spread display, and this entire visual display varies (depth of the bow, extent of wing extension) depending on the sex of the receiver (O'Loghlen & Rothstein 2010). Nevertheless, songs sung without a visual display are capable of generating normal reproductive responses such as copulatory solicitation displays (O'Loghlen & Rothstein 2010) or wing strokes (West & King 1988) from females and counter-singing responses from males.

Many studies have demonstrated that male cowbirds can modify their perched songs based on social information from conspecifics (reviewed in West et al. 2011). Changes in flock composition (e.g., the presence or absence of adults/juveniles or males/females) have been shown to influence song potency (West & King 1980) and reproductive success (White et al. 2002; Gersick et al. 2012; Kohn et al. 2013). For example, King & West (1977) showed that cowbird males reared in isolation develop very effective courtship songs. However, when these isolate males are reintroduced into a flock, their potent songs elicited aggressive attacks from the resident males. In response, the introduced males quickly learned to reduce their song potency to avoid subsequent attacks (West & King 1980). Moreover, there is considerable evidence that female cowbirds affect song learning by providing visual feedback in the form of wing strokes to indicate their preference for particular song elements (West & King 1988; West et al. 2011).

Although there is substantial evidence that cowbird song is influenced by the flock composition, we know relatively little about how a male cowbird modifies his song based on social context. Following the Motivational Structural Rules Hypothesis, we predicted that songs within a given song type directed to males would be lower in frequency and more entropic (i.e., harsher) with higher frequency-modulation rates than those same song types sung to females. We also predicted from the Motivational Structural Rules Hypothesis that there should be a negative correlation between frequency and body mass, and a positive correlation between entropy and body mass, as larger birds are able to produce lower frequency, higher entropy sounds (Greenewalt 1968; Morton 1977). We also measured the duration of the song elements but made no *a priori* predictions about song length because the evidence is mixed as to whether song duration is an aggressive or appeasing signal in passerines (Poesel et al. 2001; Nelson & Poesel 2011).

Methods

Overview

We examined the effect of receiver sex on spectral and temporal differences within a given song type. We recorded male song presented during trials to both males (N = 25) and females (N = 10) and categorized each song into particular song types for each individual male by examining the number of glug elements in P1, and the shape of the final P2 element (see Fig. 1). We selected up to five exemplars (see Table S1) of each male's song types and ran two analyses on the assemblage of songs: (1) A cross-correlation analysis to determine whether males adjusted their songs depending on receiver sex, and (2) a spectral and temporal analysis to examine whether any differences found in the cross-correlation analysis could be explained by differences in frequency or entropy, as predicted by the Motivational Structural Rules Hypothesis.

Animal capture and housing

All animal care and experimental procedures were approved by Purdue Universities Animal Care and Use Committee (PACUC) Protocol # 1111000151. Between May 2, 2011 and Apr. 26, 2012, 40 adult male and 10 adult female cowbirds were wild-caught in decoy traps in collaboration with the USDA APHIS (Sandusky, OH). Specifically, 30 adult males were caught between May–Jun. 2011 and 10 adult males and 10 adult females were caught in Apr. 2012. We did not include juvenile males in this study. Adult and juvenile males were differentiated based on plumage patterns characteristic to the different age classes (e.g., only adult males have completed their molt into their black iridescent plumage). Birds were housed at Purdue University in individual enclosures (size equal to 0.5 m^3) in single-sex rooms and provided mixed seed, grit, and water *ad libitum*. Birds' water was treated with a 9.6% oral solution of Amprolium (1:1000) (to prevent Coccidial infection) for five consecutive days after being brought into the laboratory. Every other day, birds were given two mealworms and their water was supplemented with vitamins (Premium Multi-Drops Vitamins). The lighting schedule was adjusted weekly to follow the natural lighting conditions of West Lafayette, IN (ranging

from 14:10 Light: Dark in the summer to 10:14 during the winter).

During the 2011 molting season (Aug.–Nov.), 30 males were included in a food-deprivation experiment to examine the effects of stress on plumage reflectance (15 birds were randomly assigned to the food-deprived condition and 15 birds were assigned to the non-food-deprived condition). While current condition (manipulated via food deprivation) has been shown to decrease singing rate in some species (e.g., Ritschard & Brumm 2012), there is no evidence suggesting that past food deprivation (over 6 mo prior) has any impact on current singing behavior. Moreover, our non-food-deprived birds and food-deprived birds did not vary in body condition (i.e., there was no significant effect on PCA scores that combined body mass and tarsus length) at the beginning of this experiment ($F_{1,10} = 1.33$, p = 0.28). When we included deprivation treatment in our analyses, we did not find a significant effect on any measured parameter (all $F_{1,45} \leq 2.67$, p $\geq$ 0.11); thus, we removed this covariate from our statistical models.

Between May 2 and Jul. 2, 2012, all males were implanted with testosterone in an attempt to reduce any hormonal profile differences between individuals captured in 2011 and 2012 and to increase display motivation. Testosterone is known to increase singing motivation, but has not been shown to influence song syntax in this species (O'Loghlen & Rothstein 1993, 2002; O'Loghlen et al. 2013). Additionally, all female cowbirds were implanted with estrogen in order to induce breeding season behavior (e.g., copulatory solicitation displays) for an ongoing study (results not presented here). Following previous studies (O'Loghlen & Rothstein 1993, 2002; O'Loghlen et al. 2013), testosterone and estrogen implants were made by packing either 10 mm crystalline testosterone or estrogen (Sigma Chemical Co., St. Louis, MO) into Silastic tubing (outer diameter 1.96 mm) and sealed with Silastic adhesive. This amount of hormone is a long-lasting, physiological dose that is typically within the natural bounds for songbirds in the breeding season (Hunt & Wingfield 2004). Such implants will keep the hormone levels stable until they are removed. All birds were sedated with a combination of ketamine (40–60 mg/kg) and midazolam (6–8 mg/kg) injected into the breast muscle so that birds could be implanted subcutaneously in the chest. Birds were placed on a heating pad and allowed to recover in their individual home enclosures after implantation; they were allowed 3 wk of rest prior to being a part of song recording trials.

Song recordings

Trials were conducted in the late breeding season of late Jul.–Aug. 2012. A summary table including each individuals' trial days, the total number of songs collected, and the number of songs included in the final analyses is provided (see Table S1). A single trial consisted of a male being taken from his home enclosure and placed in a 0.5-m³ wire mesh experimental enclosure with a single perch. One side of the experimental enclosure contained a small Plexiglas window (30 cm × 15 cm) which was adjacent to another identical cage enclosure containing an unfamiliar male or female. This arena setup allowed for bidirectional communication between the two individuals. A camera (HD Everio GZ-E10) was placed adjacent to this window to record male visual displays. Although Plexiglas generally does not allow the transfer of light in the UV spectrum, cowbird feathers have not been shown to reflect in the UV (McGraw et al. 2002), and thus, we assumed the lack of this signal would have no effect on the receiver responses. Additionally, we assumed that the small size of the window would not significantly affect the transmission of visual or acoustic stimuli. Both experimental enclosures were situated on a table within a 3 × 3 × 4 m indoor room lined with acoustic tiles and acoustic foam (Foam Factory, Clinton Twp., MI). No other birds were within earshot of this experimental setup. A Sennheiser ME66 short directional microphone powered by a K6 powering unit was placed above the arena, equidistant (1 m) from the two perches. All audio recordings were sampled at a rate of 44.1 kHz on a Marantz PDM-690 professional solid-state recorder and saved as.wav files. A second camera (Samsung SMX-F40BN) was positioned to view the entire arena so that the identity of a singing male during male–male trials could be confirmed. Trials were run between 0600 and 1600 h and lasted approx. 30 min. Males were exposed to a bird of a randomly chosen sex within a given day, but two males were never included in a trial together if they were neighbors in their home enclosures. This reduced the potential effects of familiarity in differences in song production. No bird completed more than four trials in a given day, and birds were not run on consecutive days. Each bird was weighed and returned to their home enclosures for 2 h between subsequent trials. Of the 25 males tested, 18 males vocalized during at least one of their trials, and seven birds sang to both a male and a female stimulus.

Song similarity and spectral/temporal analyses

Song selection and noise reduction

Songs for each male were classified into different song types using COOL EDIT PRO (version 2). We selected a maximum of five exemplars of each male's different song types from both a male-directed and female-directed trial in order to determine the influence of receiver sex on song spectral and temporal parameters (see Table S1). Exemplars were always selected from the beginning of each trial, until five high-quality exemplars (songs in which no other bird was singing and/or creating noise by moving inside the enclosure) were reached. Most birds sang the majority of their song types to both a male and female receiver within a 2-wk time-frame; thus, we chose exemplars from trials that were conducted as close in date as possible but never more than 2 wk apart in order to have the most balanced dataset possible and also to be conservative with respect to any seasonal changes that may occur within a male's song type. This slightly decreased our overall sample size of songs as some birds did not sing their full repertoires during this 2-wk cutoff period to both males and females. Moreover, in some cases (four individuals), this also resulted in all of a male's female-directed songs to be collected from one sampling session, and all of his male-directed songs to be collected from another. Therefore, we included date as a covariate in our original spectral analyses to tease apart the potential confounding effects of receiver sex and trial date. We reduced the background noise from each recorded song with the noise reduction function in CoolEdit Pro. We then normalized the amplitude of all the songs in the wav file to 80%. We chose not to analyze the Glug 0, Glug 3, or IPU (see Fig. 1) elements because these song components could not be found across all male song types.

Song similarity statistics: cross-correlations

We examined the effect of receiver sex on the fine spectral and temporal differences within a given song type with a cross-correlation analysis. Cross-correlation can be used to measure the similarity between two waveforms as a function of a time-lag applied to one of the waveforms. We used normalized cross-correlation values; normalization results in identical waveforms having a cross-correlation coefficient of 1 and a waveform cross-correlated with white noise has a cross-correlation coefficient of 0 (Boersma & Weenink 2009). We limited the cross-correlation analyses to the exemplars of each song type sung by

each male irrespective of the sex of the receiver (e.g., all of a male's 'A' song types were cross-correlated with each other, but these 'A' types were not correlated with his 'B' song types). Cross-correlation analyses were generated using a Praat script ("cross-correlate" in Boersma & Weenink 2009; version 5.1.32). We also repeated this procedure separately for several components of the song (e.g., first glug, P1, and P2).

We used multidimensional scaling (MDS; Proc MDS, SAS Institute., v 9.3) to reduce the dimensionality of the cross-correlation matrix. Each song type and song component for each male was analyzed separately. MDS estimates the relative position of a set of objects (e.g., male- and female-directed waveforms of a particular song type from a single male) in a space with a user-specified number of dimensions. We fit the MDS model with 3 dimensions, as the estimated R value for 3 dimensions was > 0.95 for all MDS models [R values were calculated from the MDS generated badness-of-fit statistic: badness of fit = $\sqrt{(1-R^*)}$]. Our MDS analyses used absolute values of dissimilarity (calculated as 1 minus the cross-correlation coefficient), which yields approximate values of dissimilarity between the different exemplars of a male's song type across MDS space.

We calculated the distance in MDS space for all pairs of songs of a given type directed to males, or songs directed to females, and for pairs songs sung to receivers of different sexes. The same was done for each song component (first glug, P1, and P2) within a particular song type. Thus, for each male, we had the mean distance and variation within all his male-directed songs, female-directed songs, and also the distance between pairs of songs directed to receivers of different sexes songs within a particular song type. We then used repeated-measures ANOVAs with Proc MIXED in SAS to model the main effects of sex, year of capture, mass of the singer, and their interactions on the MDS distance (measured as dissimilarity). We specified a variance component covariance structure and the Kenward–Roger method to calculate the degrees of freedom.

Spectral and temporal measurements

We used Sound Analysis Pro (version 2011.104) to measure different spectral components of the song exemplars from each male. We measured the frequency (fundamental, mean, and peak), frequency modulation (FM), entropy, and duration for glug 1, glug 2, and P2 for each song. Sound Analysis Pro calculates the Weiner entropy value, a pure number (i.e., unitless) measured on a logarithmic scale from 0

(e.g., white noise) to minus infinity (e.g., complete order, or a pure tone) (Tchernichovski et al. 2000). Thus, this scale provides an index of the harshness of a sound, where harsher sounds are more entropic and closer to a score of 0. In this analysis, we chose not to analyze amplitude or amplitude modulation (AM) because birds did not always vocalize from the same distance or orientation to the microphone.

We used repeated-measures ANOVAs with Proc MIXED in SAS to analyze FM rates, frequency (fundamental, mean, and peak), entropy, and duration separately for all song components (in this analysis: glug1, glug2, P2). We split the P1 into separate glugs in order to have a finer analysis of the spectral and temporal profiles of this part of the song. Fundamental and peak frequency of the glugs and P2 song elements were log-transformed to meet the normality assumption. In several cases, a single outlier was removed to meet the normality assumption; we verified that the outlier removed had a residual value of at least 4 standard deviations from the mean. We specified a variance component covariance structure and the Kenward–Roger method to calculate the degrees of freedom. Our independent factors included the effect of song type (nested within singer), receiver sex, mass of the singer, recording date, stimulus identity nested within sex, and the interaction between song type and receiver sex.

Results

We collected a total of 630 songs from the seven birds that sang their repertoires to both sexes: 346 were directed toward females and 284 were directed toward males. We found that pairs of songs were significantly more dissimilar if they were directed to different sexes compared with songs directed to the same sex (Fig. 2). This effect was found for all components of the songs examined: the first glug, the entire P1, the P2, and the whole song (Table 1). This is evidence that males alter the structure of a given song type based on the intended receiver. Capture date and singer mass significantly affected dissimilarity of the P2 and the P1 components, respectively. To examine the
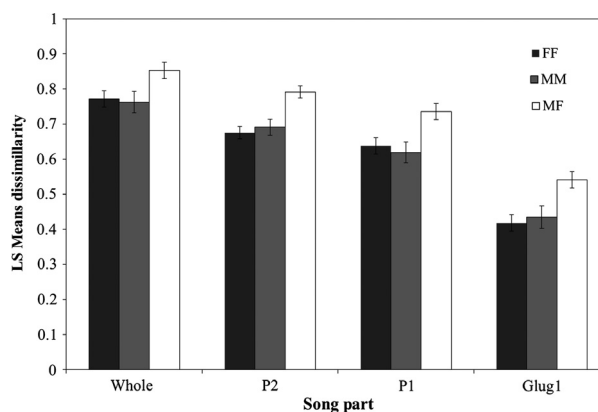

Fig. 2: Dissimilarity (derived from multidimensional scaling analysis) of cross correlations between all pairs of any specific song given by each male. Songs sung within a sex (females: FF, dark gray bars) and (males: MM, light gray bars) are more similar that songs sung to the opposite sex (MF, white bars) across all song types and components: the whole song, P2, P1, and Glug 1.

relationship between singer mass and the difference in P1 components, we examined β, the slope of the line predicted by SAS describing the relationship between continuous independent and dependent factors. Larger birds tended to have more dissimilar P1 components than smaller males ($β = 0.011 \pm 0.004$). Additionally, birds caught in 2012 had more dissimilar P2 components (0.73 ± 0.01) than those caught in 2011 (0.68 ± 0.02). Nevertheless, the interaction between capture year and receiver sex was never significant (Table 1), so the general pattern of males singing more dissimilar songs to females was consistent across all birds.

Spectral and temporal differences

We examined whether there were any consistent spectral or temporal patterns in the songs males sang to females versus to males that might result in the significant differences we observed in the cross-correlation analyses. Not surprisingly, because song types were visually categorized by the spectral properties of the song, the main effect of song type was significant for all variables for every song component (see

Table 1: Dissimiliarity between pairs of songs directed to different sexes; bold values indicate statistical significance

Song component	Receiver sex	Capture date	Capture date*Receiver sex	Singer mass
Whole Song	$\boldsymbol{F_{2,46} = 4.80, p = 0.01}$	$F_{1,46} = 1.64, p = 0.21$	$F_{2,46} = 0.85, p = 0.43$	$F_{1,46} = 0.43, p = 0.51$
First Glug	$\boldsymbol{F_{2,43} = 8.04, p = 0.001}$	$F_{1,43} = 0.93, p = 0.34$	$F_{2,43} = 2.02, p = 0.12$	$F_{1,43} = 0.58, p = 0.45$
P1	$\boldsymbol{F_{2,45} = 6.68, p = 0.003}$	$F_{1,46} = 1.74, p = 0.19$	$F_{2,46} = 1.185, p = 0.32$	$\boldsymbol{F_{1,46} = 8.89, p = 0.005}$
P2	$\boldsymbol{F_{2,46} = 13.6, p < 0.001}$	$\boldsymbol{F_{1,46} = 4.28, p = 0.04}$	$F_{2,46} = 0.68, p = 0.51$	$F_{1,46} < 0.001, p = 0.97$

Table S2). Several of our covariates were also significantly related to the measured song properties. For example, we discovered that stimulus identity (nested within sex) was significant across multiple spectral and temporal properties (see Table 2). In particular, all measured parameters (e.g., duration, frequency, entropy, and frequency modulation) of the P2 song component were significantly affected by the stimulus identity (all $F_{10,119} \geq 1.92$, $p \leq 0.05$), and the entropy and fundamental frequency of all song components were altered by the identity of the receiver (all $F_{10,119} \geq 1.95$, $p \leq 0.05$) (see Table 2). Moreover, we observed that recording date significantly affected the frequency parameters of several different song components. The fundamental frequency ($F_{1,118} = 15.78$, $p < 0.001$) and mean frequency ($F_{1,118} = 6.20$, $p = 0.01$) of the P2 were negatively associated ($\beta = -0.90 \pm 0.23$; $\beta = -28.25 \pm 11.35$, respectively) with recording date, while the P2 peak frequency was positively associated with date ($F_{1,118} = 6.89$, $p = 0.01$; $\beta = 31.73 \pm 12.09$). Additionally, recording date was also negatively related to both the fundamental frequency of glug 1 ($F_{1,119} = 5.85$, $p = 0.02$; $\beta = -0.03 \pm 0.01$) and the peak frequency of glug 2 ($F_{1,119} = 8.28$, $p = 0.005$; $\beta = 22.03 \pm 7.66$).

We found significant main effects of sex across the different spectral and temporal measurements of several song components (Table 2). The only consistent result across all components of the song was a significant main effect of sex on a song's entropy (all $F_{1,118} > 4.19$, $p < 0.04$). Indeed, entropy was consistently higher for songs sung to males than those sung to females for glug 1, glug 2, and P2 (see Fig. 3). In addition, we also found a significant song type by receiver–sex interaction across multiple different song components and measured variables (see Table 2). Again, here the only consistent result across all components of the song was a significant interaction between sex and a singer's song type on a song's entropy (all $F_{11,118} > 2.01$, $p < 0.03$). We investigated this interaction by plotting sex and song type for all the males and verified that the patterns for entropy were consistent across males' different song types (i.e., the majority of the male song types followed the pattern of the main effect; see Fig. S1–S3). Singer mass was significantly related to the entropy ($F_{1,119} = 13.69$, $p < 0.001$) and duration ($F_{1,117} = 14.71$, $p < 0.001$) of the P2 component of the songs analyzed. Both entropy and duration were positively related to the P2 ($\beta = 0.100 \pm 0.03$; $\beta = 8.68 \pm 2.26$, respectively), and no other spectral or temporal measurements (mean frequency, peak

Table 2: Statistics associated with the effect of stimulus identity, stimulus sex, and the interaction between stimulus sex and song type on measured song parameters; bold values indicate statistical significance

| Independent Factor	Song Part	Fundamental Frequency	Mean Frequency	Peak Frequency	Frequency Modulation	Entropy	Duration
Stimulus Identity (Stimulus Sex)	Glug1	**$F_{10,119} = 4.66$, p < 0.001**	$F_{10,119} = 1.00$, p = 0.45	$F_{10,118} = 1.8$, p = 0.07	$F_{10,119} = 0.59$, p = 0.82	**$F_{10,119} = 7.97$, p < 0.001**	**$F_{11,119} = 2.24$, p = 0.02**
	Glug2	**$F_{10,119} = 1.95$, p = 0.05**	$F_{10,119} = 1.39$, p = 0.19	$F_{10,119} = 1.66$, p = 0.10	$F_{10,119} = 1.07$, p = 0.39	**$F_{10,118} = 4.91$, p < 0.001**	$F_{11,119} = 1.51$, p = 0.14
	P2	**$F_{10,118} = 15.78$, p < 0.001**	**$F_{10,118} = 2.75$, p = 0.005**	**$F_{10,118} = 2.87$, p = 0.003**	**$F_{10,119} = 1.92$, p = 0.05**	**$F_{10,119} = 2.86$, p = 0.003**	**$F_{11,117} = 3.96$, p < 0.001**
Stimulus Sex	Glug1	$F_{1,119} = 0.12$, p = 0.73	$F_{1,119} = 0.34$, p = 0.54	$F_{1,118} = 0.04$, p = 0.85	$F_{1,119} = 0.42$, p = 0.52	**$F_{1,119} = 6.58$, p = 0.01**	$F_{1,119} = 0.49$, p = 0.48
	Glug2	$F_{1,119} = 0.94$, p = 0.34	$F_{1,119} = 2.33$, p = 0.13	$F_{1,119} = 1.11$, p = 0.30	$F_{1,119} = 0.25$, p = 0.62	**$F_{1,118} = 4.19$, p = 0.04**	**$F_{1,119} = 4.37$, p = 0.04**
	P2	**$F_{1,118} = 11.38$, p = 0.001**	$F_{1,118} = 2.31$, p = 0.15	$F_{1,118} = 1.55$, p = 0.22	$F_{1,119} = 0.01$, p = 0.76	**$F_{1,119} = 16.4$, p = 0.001**	**$F_{1,117} = 24.4$, p < 0.001**
Stimulus Sex * Song Type	Glug1	**$F_{11,119} = 2.29$, p = 0.01**	**$F_{11,119} = 3.31$, p = 0.001**	**$F_{11,118} = 2.19$, p = 0.02**	$F_{11,119} = 1.16$, p = 0.32	**$F_{11,118} = 2.01$, p = 0.03**	**$F_{11,119} = 2.24$, p = 0.02**
	Glug2	$F_{11,119} = 1.66$, p = 0.09	**$F_{11,119} = 4.27$, p < 0.001**	**$F_{11,119} = 4.20$, p < 0.001**	**$F_{11,119} = 3.53$, p < 0.001**	**$F_{11,118} = 2.24$, p = 0.02**	$F_{11,119} = 1.51$, p = 0.14
	P2	$F_{11,118} = 2.62$, p = 0.11	$F_{11,118} = 1.5$, p = 0.14	$F_{11,118} = 1.63$, p = 0.10	$F_{11,119} = 1.12$, p = 0.35	**$F_{11,119} = 4.44$, p < 0.001**	**$F_{11,117} = 3.96$, p < 0.001**
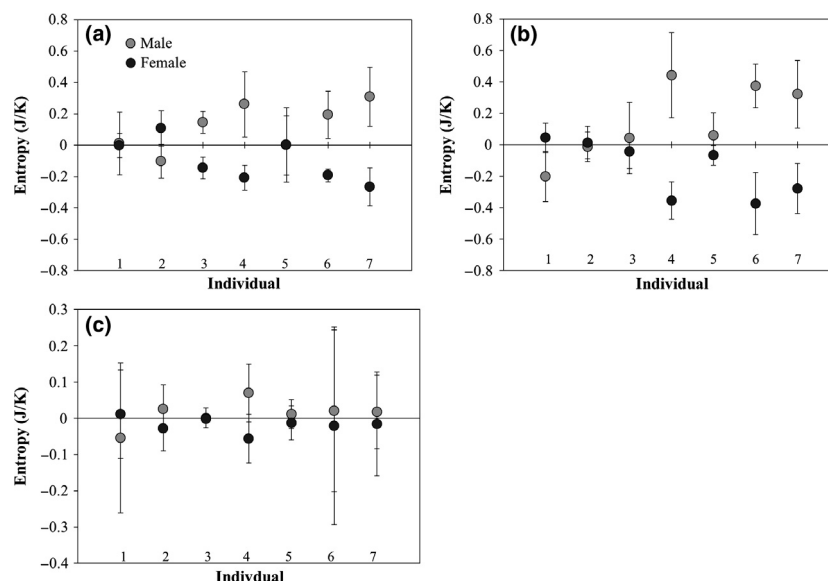
Fig. 3: Main effect of entropy across the different song components: Glug 1 (a), Glug 2 (b), and P2 (c) for each individual singer. Entropy values were standardized based on the deviation from the mean for each singer. Entropy is consistently lower in songs sung to females (dark gray circles) than those songs sung to males (light gray circles).

frequency, FM, or duration) were significantly influenced by singer mass (all $F_{1,119} \leq 2.73$, $p \geq 0.10$).

Discussion

The results of our cross-correlation analysis suggest that within a close-distance communication framework, cowbirds modify their perched songs depending on the sex of the receiver. An investigation of the spectral and temporal properties of songs given in different social contexts shows that males appear to modify the fine structure (e.g., entropy) depending on the sex of the receiver. Consequently, even subtle changes within a song type may be meaningful to the intended receiver. This is, to the best of our knowledge, the first evidence to report how male cowbirds modify the spectral properties of their song based on social context. Furthermore, males adjusted different song types within their perched song repertoire in different ways. We found significant interactions between the sex of receiver and song type for the variables we measured: frequency modulation, duration, entropy, and frequency (mean, peak, and fundamental), across all the different song components: glug1, glug2, and P2. These interactions suggest that cowbirds may modify each song type differently depending on the receiver. Although we found significant interactions between sex and song type, we only found a consistent main effect of sex for entropy across the different song components, which partly supports our predictions made from the Motivational Structural Rules Hypothesis (Morton 1977). Males sing the same song with higher entropies to other males, but use lower entropies when directing these songs to females. All additional variables measured (duration, fundamental frequency, peak frequency, mean frequency, FM) did not show consistent patterns across the different song parts. Thus, we did not find direct support for our predictions regarding frequency: males did not lower song frequency when singing to males versus singing to females.

Our findings at the song structure level are in agreement with previous research showing that male cowbirds modify their visual displays depending on the sex of the receiver (O'Loghlen & Rothstein 2010). Male cowbirds display more intensely (e.g., longer display duration, deeper bows, wider wing-spread) when displaying toward males than when displaying toward females. The multimodal combination of both the vocal and the visual displays may be important for mate choice in this species (O'Loghlen & Rothstein 2010), and it appears that females prefer female-directed, low-intensity wingspread displays (O'Loghlen & Rothstein 2012). Along these lines, female cowbirds may discriminate between male- and female-directed songs. In the present study, we did not examine visual display intensities; consequently, it is possible that motions involved in these intense displays affected the acoustic properties of the song (Cooper & Goller 2004). Future research should examine both properties of the multimodal signal simultaneously to determine whether differences in song are the by-product of extreme body movements.

Our predictions regarding body size were also somewhat supported as we predicted that there should be a negative correlation between frequency and body

mass, and a positive correlation between entropy and body mass. The Motivational Structural Rules Hypothesis proposes that lower frequency vocalizations may be an indication of larger body mass, and thus convey information regarding the potential to win an aggressive encounter (Morton 1977). In this study, we did find that singer mass was positively related to the entropy and duration of the P2, but we failed to detect a relationship between singer mass and frequency.

It is interesting to note that the effect of body mass on the entropy properties of perch song correlate with the predicted functions of the P2 components of the song. The P2 is predicted to signal individual identity or dominance status, potentially to males (West et al. 1979). Thus, if male body size is an indication of quality or fighting-ability, perhaps males are using highly entropic notes within the P2 component to signal to males. Several other bird species also seem to use different parts of the same song to perform different functions (Marler & Slabbekoorn 2004). In chaffinch (*Fringilla coelebs*) song, for example, the end flourish appears to be more important in mate choice, while the trill is important in interactions with other males (Leitao & Riebel 2003). Similar to cowbirds, in the barn swallow (*Hirundo rustica*), a series of complex notes at the beginning of the song appear to function in female choice (Møller et al. 1998), while the ending rattle was implicated in male–male competition (Galeotti et al. 1997).

It is not altogether surprising that we also found a significant relationship between stimulus identity and several of our dependent variables; most notably we found that receiver identity significantly affected all measured spectral and temporal measures of the P2 song component in addition to the entropy and fundamental frequency of all song parts. This suggests that cowbirds may not only modify their song based on the sex of the receiver, but also tailor their songs to communicate with a specific individual. As mentioned previously, the P2 is the most variable portion of the cowbird song and has been hypothesized to function in individual identity (West et al. 1979); our results suggest that this portion of song may be the most malleable and thus contain information pertinent to specific receivers.

A critical next step is to examine whether there are fitness benefits to males that can modify their song to a greater extent depending on the sex of the receiver. The facultative modification of song is not particularly well described, especially for songbirds that sing the same repertoire to males and females (Leitao et al. 2006; Benedict et al. 2012). However, growing evidence suggests that male quality may be associated with the ability to signal appropriately in different social contexts (reviewed in Taborsky & Oliveira 2012; West et al. 2011).

In brown-headed cowbirds it has been hypothesized that male age and experience may play a significant role in signaling ability (O'Loghlen & Rothstein 1995, 2012). Male cowbirds have a delayed development of local, shared perched songs until after their second breeding season, and, as such, second-year males rarely obtain copulations even though they are fully sexually mature (Rothstein et al. 1986; Yokel et al. 1986; O'Loghlen & Rothstein 1993). Female cowbirds, indeed, tend to show a preference for the local perched songs over the non-shared perched songs sung by second-year males (O'Loghlen & Rothstein 2003). Similarly, a male's experience with fluctuations in group size and composition (such that would occur over a breeding season) have also been shown to influence the dominance relationships and singing behavior of cowbird individuals (White et al. 2010, 2012; Kohn et al. 2011, 2013; Gersick et al. 2012). Many of our males were housed in a same-sex, socially static environment since 2011 and it is likely that lack of a dynamic interaction with other individuals may have decreased the motivation to sing, resulting in a relatively low number of individuals that sang to both sexes. Indeed, the males that did sing to both males and females seemed to have more species-typical interactions with conspecifics than those males that only sang to one sex: male–male trials frequently involved counter-singing and male–female trials often had female chattering in response to male singing. Nevertheless, capture date never significantly interacted with sex of the receiver so males did not sing differently to males or females depending on their date of capture. Although we were unable to differentiate the age (i.e., experience) between the adult males (juvenile males were not included) in this study, it would be interesting to test the development of singing behavior and whether within-song tuning with changes in receiver sex is a learned phenomenon.

In addition to understanding the role of singer age and experience in the tuning of songs to different receivers, it may also be interesting to investigate whether differences in hormonal profiles underlie the ability to tune songs in different social contexts. In the current study, all males were implanted with testosterone and females with estrogen in order to encourage singing and typical receiver responses in a laboratory setting. The physiological dose used had previously been shown to be effective in multiple

studies of courtship behavior in this species (O'Loghlen & Rothstein 1993, 2002; O'Loghlen et al. 2013). Nevertheless, testosterone has been shown to increase aggressiveness in this species (Dufty 1986) and may also shape how cowbirds communicate with conspecifics. Therefore, it may be worthwhile to examine whether non-hormone implanted birds also vary the fine structure of their songs depending on the social context.

Overall, our findings suggest that communication is dependent on the social environment. Moreover, there are multiple levels of signal complexity that may be modified depending on social context: from overall alterations of singing performance or rate, to within-song variability in spectral and temporal measurements. Therefore, our interpretation of a signal's content must be done within the framework of the social scene in which the signal evolved. Future studies should investigate whether similar signal flexibility can be detected in larger groups of social animals (McGregor 2005; Bradbury & Vehrencamp 2014), where the presence of more than one receiver may affect the motivation of the sender, and the signal evaluation of the receiver. Indeed, the potential for eavesdropping by conspecifics may alter the costs and benefits between finding a mating partner and resisting attack from more dominant individuals in the group (Freed-Brown & White 2009, West & King 1980). Perhaps the ability to adjust a signal with the social context is an honest indication of the signaler's quality or condition. We urge that more research is necessary to determine the fitness payoffs of signal plasticity or adjustment at multiple levels of sociality: from pairs of individuals to larger groups. Moreover, in a world where animal communication is rarely only done in one signal modality, it is imperative that more studies incorporate how signalers use multiple sensory modalities within different social contexts.

Acknowledgements

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Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web-site:

Table S1: Data Description.

Table S2: Statistics associated with the main effect of song type on spectral and temporal song properties.

Fig S1-S3: Interaction between Song Type (Singer) and sex on entropy across the different song components: Glug 1, Glug 2, and P2 for 5 of the 7 birds examined. Entropy is consistently lower in songs sung to females (dark gray circles) than those songs sung to males (light gray circles). Least Squares Means were derived from a repeated measures analysis of variance analysis (see text). Note that these LS Means are estimated within songs sung by each male.