

Red-winged blackbirds *Agelaius phoeniceus* respond differently to song types with different performance levels

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Physical constraints on animal signals can have important consequences for communication. For bird songs that include a trill, performance is physically constrained by a trade-off between the rate at which notes are repeated in the trill and the sound frequency range covered, such that a trill cannot exceed a certain frequency range for a given note repetition rate. Producing trills that are closer to this performance limit is presumably more challenging for birds than producing trills that are farther from it. Male red-winged blackbirds *Agelaius phoeniceus* have repertoires of two to eight song types containing trills that span a range of performance levels. We determined the approximate trill performance maximum for a population of red-winged blackbirds, then conducted playback experiments to measure the responses of territorial males to song types with either high or low performance levels relative to this limit. Males responded significantly more strongly to songs containing low performance trills. Our results show that male red-winged blackbirds can discriminate between different song performance levels, suggesting that vocal proficiency plays a role in male-male interactions.

Studies of bird song have demonstrated that components of these complex vocalizations are limited by proximate constraints on performance (Gil and Gahr 2002, Podos et al. 2004). For example, the songs of many songbirds include a trill, in which a singer rapidly repeats a pattern of notes. Laboratory (Podos 1996) and comparative (Podos 1997, 2001) studies of species with trilled vocalizations have demonstrated that mechanical limitations on movements of the vocal tract impose a trade-off between the speed with which a singer can repeat notes (trill rate) and the range of sound frequencies these notes can cover (frequency bandwidth). To produce different frequencies a bird must change the configuration of its vocal tract, in part by opening and closing its beak (Suthers and Goller 1997, Hoese et al. 2000), and larger movements that produce broader frequency bandwidths require more time. A singer therefore cannot produce a very rapid trill while simultaneously covering a very wide frequency bandwidth (Podos 1997). Songs that cover the widest possible frequency bandwidth given their trill rate are considered high performance songs, and those that

cover less than the maximum frequency bandwidth are considered lower performance songs.

Trill performance levels appear to influence females' choice of mates in canaries *Serinus canaria* (Vallet and Kreutzer 1995, Vallet et al. 1998, Drăgănoiu et al. 2002) and swamp sparrows *Melospiza georgiana* (Ballentine et al. 2004). Females in these species are more sexually stimulated by higher performance songs. Less is known, however, about how trill performance characteristics influence communication among males. In our study, we investigated the responses of territorial male red-winged blackbirds *Agelaius phoeniceus* (tribe Icterini) to high and low performance song types produced by other males. Red-winged blackbirds have repertoires of two to eight song types per male, and males in an area typically do not share song types (Searcy and Yasukawa 1995). Songs consist of introductory notes followed by a trill, and different song types can have dramatically different trill rates (from <10 to >100 syllables/s; Searcy 1990, Kroodsm and James 1994). Our study focused on a red-winged blackbird population that sang mostly slow trills

(<25 syllables/s), which appeared to be constrained by a performance trade-off between trill rate and frequency bandwidth similar to that documented for species in the closely related tribe Emberizini (Podos 1997, 2001, Ballentine et al. 2004). We used differences in the performance characteristics of different song types to investigate the possibility that vocal proficiency plays a role in communication among territorial males.

Methods

Song recording and measurements

Our study was conducted from June-July 2003 and May-June 2004 on 25 territorial male red-winged blackbirds at two locations in St. Mary's County, Maryland, USA: 20 males on the campus of St. Mary's College of Maryland (SMCM; 76°25'W, 38°11'N) and 5 on St. George Island (SGI; 76°28'W, 38°6'N), a site about 10 km away. We captured most of the males at these locations, including 23 of the males used in this study, and banded each bird with a unique combination of colored celluloid leg bands. The two unbanded male subjects were easily recognized during the study by acoustic features of their songs and by the locations of their preferred song perches.

We tape-recorded the song repertoires of all 25 identified territorial males using a Sennheiser ME-67 shotgun microphone and either a Marantz PMD 222 audio cassette tape recorder or, in a few cases, a Sony MZ-N10 portable minidisc recorder. We digitized recordings using SoundEdit16 (version 2, Macromedia, San Francisco, CA; sampling rate 22.05 kHz and sample size 16 bits) and used on-screen spectrograms to sort songs into song types, which were stereotyped and easily categorized. We chose the best example of each song type (i.e., the least degraded recording with the least background noise) to measure in our study.

Our methods for measuring trill characteristics were similar to those used by Podos (1997) and Ballentine et al. (2004). We measured song features using Canary (version 1.2.4, Cornell Laboratory of Ornithology, Ithaca, New York; frequency resolution 349.7 Hz, temporal resolution 11.61 ms). Trill rate was measured from spectrograms as the number of syllables (i.e., patterns of repeated notes) in the trill per second (Fig. 1a). Frequency bandwidth was measured from a power spectrum that averaged power spectra from each sampling frame over the entire trill portion of the song. We calculated frequency bandwidth as the difference between the highest and lowest frequencies with amplitudes above -24 dB relative to the loudest sound in the trill (Fig. 1b). This threshold excluded most background noise while including most of the bird's signal (Podos 1997).

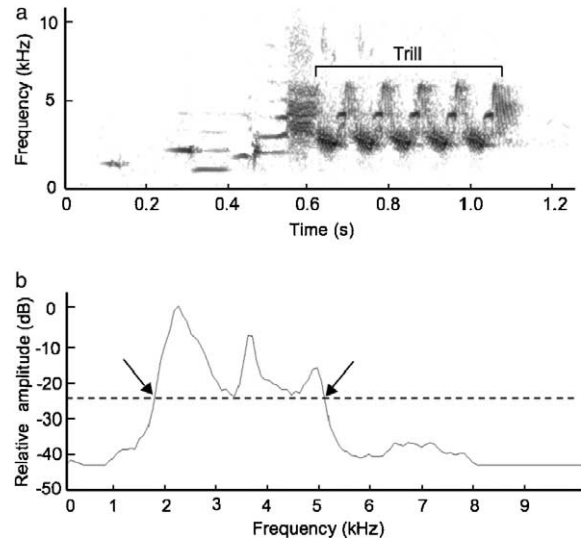


Fig. 1. (a) Song spectrogram showing frequency (kHz) as a function of time (s) and (b) power spectrum showing relative amplitude (dB) as a function of frequency (kHz) for the trill portion of the song. The number of trill syllables (five in this example) was divided by trill duration to find trill rate (syllables/s). We calculated frequency bandwidth as the difference between the highest and lowest frequencies (arrows) with amplitudes above -24 dB (dashed line) relative to the loudest sound in the trill.

After we were confident that we had recorded the song type repertoires of all territorial male red-winged blackbirds in our study area (126 song types by 25 males; more than 5,600 songs recorded in total), we sorted trill exemplars into bins by their trill rate and selected the trill with the broadest frequency bandwidth in each bin for linear regression (Blackburn et al. 1992, Podos 1997). The resulting regression line estimated the performance limit for trill production (Podos 1997). We used a bin size of 3 syllables/s, which maximized the number of song types included in the regression while minimizing the inclusion of lower performance songs. We then determined the relative performance level of each song by measuring its "vocal deviation," or the orthogonal distance from the song's trill measurements to the regression line (Podos 2001, Ballentine et al. 2004). High performance songs were close to the performance limit and so had low vocal deviation measurements, whereas low performance songs were farther from the performance limit and had higher vocal deviations.

For seven song types we could not obtain clear enough recordings to measure frequency bandwidth, so these song types were not included in the analyses. Of the 119 measured songs, only songs with trill rates <36 syllables/s (112 out of 119 song types) were included in our measurements of vocal performance

levels, based on evidence that trills with rates faster than about 36–39 syllables/s are not subject to the same constraints on performance as slower trills because they do not involve the same cyclical movements of the vocal tract (Podos 1997).

After measuring the trill rates and frequency bandwidths of all 112 song types in the 25 males' repertoires, we selected the song type with the highest and that with the lowest trill performance level from each male to use in making our playback tapes. We recorded the songs to be used for playback onto cassette tapes using a Marantz PMD 222 recorder. Each tape consisted of one song repeated at a rate of 4 songs/minute for three minutes (12 repetitions of the song). This song rate is close to the average natural song rate of the species (Yasukawa 1981) and has been used in previous playback studies of red-winged blackbirds (e.g., Searcy 1990).

Playback methods

Playback experiments were performed on 15 territorial males (10 at SMCM and 5 at SGI) from 19 May to 18 June, 2004. Each male subject received two playback treatments: one using a high performance song type and another using a low performance song type. High and low performance song types presented to each subject were recorded from the repertoire of the same male to control for possible familiarity to the playback songs. We attempted to present each male with the songs of a non-neighboring, presumably unfamiliar male. To avoid pseudoreplication (Wiley 2003), each song type was used for only one playback trial, and each subject heard a pair of songs from the repertoire of a different male. Treatments to the same subject were conducted in random order and on different days. To minimize the impact of the first playback on the second, playbacks to a male and his immediate neighbors were separated by at least two days (Brenowitz 1981, Wiley 2003); however, treatments to the same subject were also presented less than four days apart for all but one individual to minimize the effect of changing levels of aggression over the breeding season (Peek 1971). To control for the effect of time of day on subjects' behavior, the start times of playbacks to the same subject were within 45 min of each other on the different days. To minimize observer bias, the researchers conducting the trials did not know at the time of playback the performance level of the song being played.

Playback songs were broadcast using a Marantz PMD 222 recorder, an Amplivox portable stereo amplifier (model S-805A), and a RadioShack 50-W Powerhorn speaker (model 40-1440) mounted on a metal fence post. We set up the speaker several meters inside each subject's territory and used this same

speaker location for both playback treatments to that male. We standardized the playback volume as much as possible using a Realistic digital sound level meter (fast response, C weighting).

Each playback trial included observations made for five minutes before playback, three minutes during playback, and three minutes after playback. During each trial, we used a shotgun microphone and an additional cassette-tape or minidisk recorder to record the vocalizations of the subject and spoken behavioral observations by the experimenter. Behaviors we noted were the number of songs given by the subject per min, the number of flights per minute, and the amount of time the male spent within 5 m of the speaker. To control for variation in male behaviors prior to playback, we subtracted the behavioral measurements made before playback from the combined measurements made during and after playback. Reported values for song rate, flight rate, and time near the speaker therefore represent the change in behavior from the period before playback to the period after playback began. We also analyzed tape-recordings of subjects made during the trials to see whether males tended to switch to higher or lower performance song types in response to playback.

To compare the overall response of each male to the two experimental treatments, we performed a principal components analysis (PCA) using as variables the increase in song rates, flight rates, and time within 5 m of the speaker. We compared the PCA scores as well as the separate response values for the two treatments using Wilcoxon matched-pairs signed-rank tests, two-tailed. To see if subjects consistently switched to a higher or lower performance song type in response to either playback treatment, we used a two-tailed Sign test to compare the direction of change in the performance level of the song type the male was singing before playback to that of the first different song type he used after playback began. We also compared the performance levels of each subject's first new song type between treatments using a matched-pairs *t*-test. Only trials that had a first new song type with a trill rate < 36 syllables/s were used in these comparisons (12 out of 15 high performance treatments and 13 out of 15 low performance treatments).

Results

For the 112 song types included in the study, trill performance appeared to be limited by a trade-off between frequency bandwidth and trill rate similar to that found in other songbirds with trilled vocalizations (Fig. 2; Podos 1997, Ballentine et al. 2004, Beebe 2004). The negative upper-bound regression line estimating the performance limit for red-winged

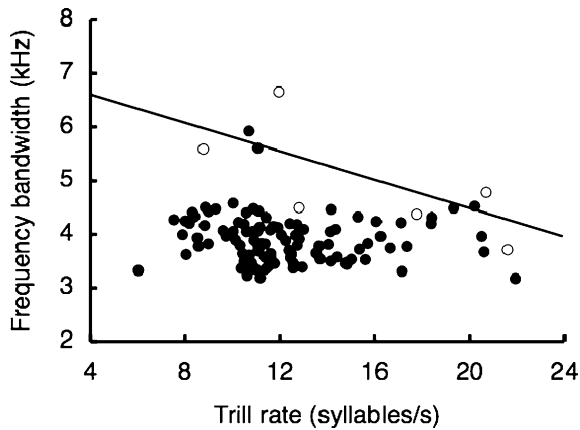


Fig. 2. Frequency bandwidth as a function of trill rate for 112 song types recorded from 25 male red-winged blackbirds. Open circles are songs used to calculate the upper-bound regression line estimating the performance maximum for trill production. Songs with trill rates > 36 Hz were not included based on evidence that these fast trills are not subject to this performance trade-off (Podos 1997).

blackbird trills was not statistically significant (ANOVA, $N=6$, $P=0.122$), but its equation was very similar to that calculated by Podos (1997) for a pooled sample of 34 species in the Emberizini ($y = -0.141x + 7.131$ and $y = -0.124x + 7.55$, respectively). Males had an average repertoire size of 5.04 ± 0.25 song types (Mean \pm SE, $N=25$ males), and each exhibited a range of trill performance levels in his repertoire (mean vocal deviation range was 10.14 ± 0.96).

Table 1. Song repertoire sizes of recorded males and performance levels^a of song types used in playback experiments.

Recorded male	Repertoire size	High performance playback songs	Low performance playback songs
BOBO	3	7.15	15.69
BORW	5	0.40	11.27
CCRR	6	10.19	22.10
CWCW	4	8.63	14.10
FCFU	5	-3.32	13.51
GGWW	4	-12.97	10.97
GOGO	4	-2.36	14.74
GWGW	7	4.49	11.12
GWOO	8	7.84	16.23
LBBW	5	11.51	22.21
LWLW	6	3.56	25.70
OLOL	4	3.88	14.22
ROWW	5	3.16	14.14
WOWO	6	3.69	14.35
WWOO	5	8.48	13.20

^aPerformance levels were measured as vocal deviation, or the orthogonal distance on a scatter plot of frequency bandwidth versus trill rate from the song to a line estimating the performance maximum. High vocal deviations indicate low performance levels and vice versa.

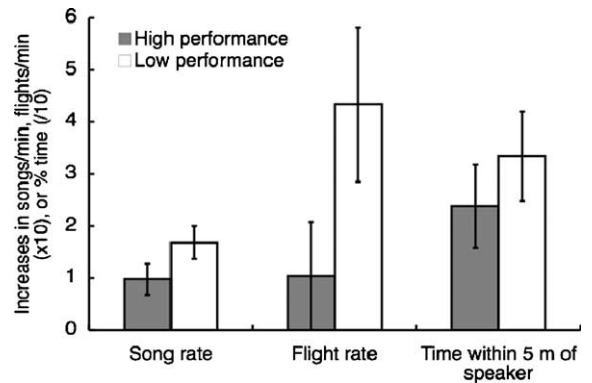


Fig. 3. Mean (\pm SE) responses to playbacks of high performance (grey bars) and low performance (white bars) songs. Values represent increases relative to the rates measured before playback.

For the songs used as stimuli in playback experiments (Table 1), high performance song types had both significantly faster trill rates (matched-pairs t -test: $t_{14} = 5.731$, $P < 0.001$) and significantly broader frequency bandwidths ($t_{14} = 3.622$, $P = 0.003$) than low performance playback songs. Trill durations did not differ consistently between treatments ($t_{14} = -0.008$, $P = 0.994$).

In response to playback, males typically increased their song rates and flight rates and spent more time near the speaker (Fig. 3). The increase in song rate was significantly greater for low performance playbacks than for high performance playbacks (Wilcoxon signed-ranks test, $N=15$: $Z = -2.385$, $P = 0.017$). Males also tended to increase their flight rate more and spend more time within 5 m of the speaker in response to low performance playbacks, though neither difference was statistically significant ($Z = -1.647$, $P = 0.100$ for flight rate and $Z = -0.866$, $P = 0.386$ for time within 5 m of the speaker). A PCA combining these behavioral measurements (component loadings shown in Table 2) showed that males responded significantly differently to high and low performance playbacks (Fig. 4; $Z = -2.897$, $P = 0.004$).

Although males responded differently to songs with different performance levels, they did not appear to preferentially use either high or low performance song types in response to playback. Subjects did not consistently switch to song types of either higher or lower performance levels in response to either treatment (Sign test: $N=10$, $P > 0.05$ for high performance playbacks and $N=12$, $P > 0.05$ for low performance playbacks). The performance level of the first new song type used in response to playback also did not differ between high and low performance playbacks ($t_9 = -0.901$, $P = 0.391$).

Table 2. Loadings of individual response measures on the first principal component for playback of high and low performance songs.

Response measure	PC1
Song rate	0.572
Flight rate	0.668
Time within 5 m	0.707

Discussion

Our results show that male red-winged blackbirds can distinguish between songs based on trill performance levels. Subjects gave stronger responses to lower performance trills, which was surprising given the expectation that high performance songs should appear more threatening to territory residents: a variety of previous playback studies of territorial songbirds have documented stronger responses to signals that represent a greater potential threat (reviewed in Collins 2004). However, previous investigators have also found the opposite pattern, that high-threat signals can result in reduced aggression or even retreating responses by subjects (e.g., Dabelsteen and Pedersen 1990, Luschi and Del Seppia 1995, Langemann et al. 2000; reviewed in Vehrencamp 2001). Our playback treatments differed only in their trill performance levels, so the difference in our subjects' responses presumably reflected a difference in their perception of a potential intruder's abilities or motivation. Faced with an unfamiliar opponent, subjects might have been less hesitant to approach a poor-quality or poorly-motivated

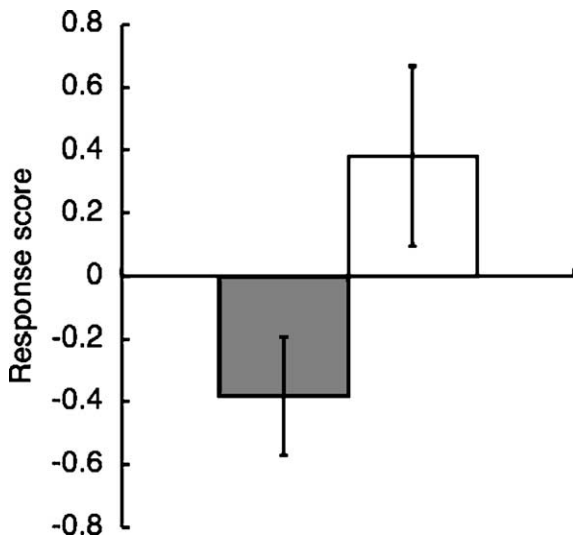


Fig. 4. Mean (\pm SE) scores from a principal components analysis combining increases in song rate, flight rate, and time within 5 m of the speaker in response to high performance (grey bars) and low performance (white bars) playback.

bird than one with the ability and motivation to sing high performance songs.

Previous studies of red-winged blackbird song have found little evidence that different song types are used in different contexts (Smith and Reid 1979) or that they elicit different responses from conspecifics (Yasukawa 1981, Searcy 1988). These studies may have failed to uncover such differences in part because they did not consider vocal performance levels in their comparisons. Moreover, most previous studies of red-winged blackbirds have focused on populations with primarily fast-trilled (>39 syllables/s) songs (Searcy and Yasukawa 1995), which are probably not subject to the trade-off measured in our study (Podos 1997). Our study population in southern Maryland used predominantly slow trills (94% of song types had trill rates of <25 syllables/s), which allowed us to quantitatively assess differences in vocal performance among songs. Songs with fast trills might be bound by other performance constraints, such as trill duration or song amplitude (Gil and Gahr 2002), and possibly males can distinguish between different performance levels in these songs using cues other than those measured here.

Given our finding that males discriminate between songs with different levels of vocal performance, it might be surprising that our male subjects did not appear to modify their performance levels in response to playback. Perhaps resident males have such an advantage over intruders that they do not need to emphasize their vocal abilities, or perhaps residents disguise their own performance limitations by not changing to song types with consistently higher or lower performance levels. It is also possible that subjects' song types varied in characteristics we did not measure, any of which could have reflected vocal proficiency. Furthermore, although we found little evidence that trill performance levels vary much within song types during normal singing, we did not investigate the possibility that males altered the performance levels of particular song types in response to playback. Male songbirds are known to vary certain acoustic features of their song types during social interactions (e.g., Searcy et al. 2000, Price et al. 2006). Whether such within-song type variation occurs in red-winged blackbirds, and whether these variants reflect vocal proficiency, remains to be tested.

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