



The function of song types and song components in Grasshopper Sparrows (*Ammodramus savannarum*)

Bernard Lohr^{a,*}, Sarah Ashby^a and Sarah M. Wakamiya^b

^a Department of Biological Sciences, University of Maryland,
Baltimore County, Baltimore, MD 21250, USA

^b Northern Great Plains Inventory and Monitoring Program,
National Park Service, Rapid City, SD 57701, USA

* Corresponding author's e-mail address: blohr@umbc.edu

Accepted 6 May 2013

Abstract

Song in birds is traditionally associated with two major functions: mate attraction and territory establishment/defence. In some birds, these functions are divided between different types or categories of signals, while in others the song repertoire is functionally interchangeable. Grasshopper Sparrows, *Ammodramus savannarum*, sing two distinct songs. Buzz song is prominent early in each breeding cycle, while warble song is typically more prominent later in the breeding cycle, and is only sung by paired males. To investigate the functions of these two song categories we did population-level song surveys, and performed a song playback experiment using buzz songs and warble songs as stimuli. Males singing buzz song responded to playbacks of both song categories almost exclusively with buzz song. Responses by birds singing warble or combined (buzz-warble) song were mixed, but these males responded to both song categories with buzz song at levels greater than chance. Our results suggest that buzz song is the principal category of song used for both the traditional intra- and inter-sexual functions associated with song. The function of the warble song class remains unclear. It may have predominantly inter-sexual functions such as social cohesion, reproductive synchrony within the pair, or extra-pair mate attraction. In a second playback experiment we examined song function further by testing the different components of buzz song. Results indicate that as in some other species, the initial segment of the Grasshopper Sparrow buzz song may act as an alerting component that enhances signal detection for long distance communication.

Keywords

birdsong, song function, song type, playback, Grasshopper Sparrow, *Ammodramus savannarum*.

1. Introduction

Acoustic signals such as birdsong can be used to convey multiple messages, though generally song is thought to have two major functions: mate attraction and male–male aggression, particularly in the context of territory establishment and defence (Collins, 2004; Nowicki & Searcy, 2004; Catchpole & Slater, 2008). Birds with repertoires of multiple song types often use these songs in a functionally interchangeable way, cycling through their repertoire either with immediate or eventual variety in similar contexts (Kroodsma, 1982). Many birds, however, use different song categories or song elements to transmit non-redundant information. That is, an increase in song complexity, either through an increased song repertoire, or through an increase in diversity of elements within a song, may be used to convey messages specific to a song category or song element (Nelson & Croner, 1991; Nelson & Poesel, 2007).

Grasshopper Sparrows (*Ammodramus savannarum*), like some other emberizids, produce two distinct categories of song. The ‘primary song’ (Vickery, 1996; Proppe & Ritchison, 2008), also known as the ‘grasshopper song’ (Smith, 1959) or ‘buzz song’ (Soha et al., 2009) consists of 3 or 4 brief, pure tone introductory notes followed by a longer duration, rapidly modulated, high-pitched trill. While the general form of this song is stereotyped across populations and subspecies, birds within populations produce individually distinctive songs that can be easily discriminated based on spectrograms, and with training, by ear (Figure 1). The second song category, the ‘sustained song’ (Smith, 1959; Vickery, 1996) or ‘warble song’ (Soha et al., 2009), consists of a series of short, frequency-modulated notes of variable structure, that are typically repeated and combined into a series of phrases. This song is also individually distinctive. The entire sequence of this latter song may be repeated two or more times. Hereafter we use the terms buzz and warble song, as these terms best describe the acoustic character of the signals (Soha et al., 2009). Birds also often produce a ‘combined’ song (buzz song immediately followed by a warble song, never the reverse) that is often sung interchangeably with warble song, and less commonly with buzz song.

Each male Grasshopper Sparrow has a repertoire of one buzz and one warble song. However, only paired males have been reported to sing warble or combined song, unpaired males only sing buzz song (Vickery, 1996; B.L., pers. obs.). Paired males change the rate and relative proportion of the song type they sing during the course of a breeding cycle (Proppe & Ritchison,

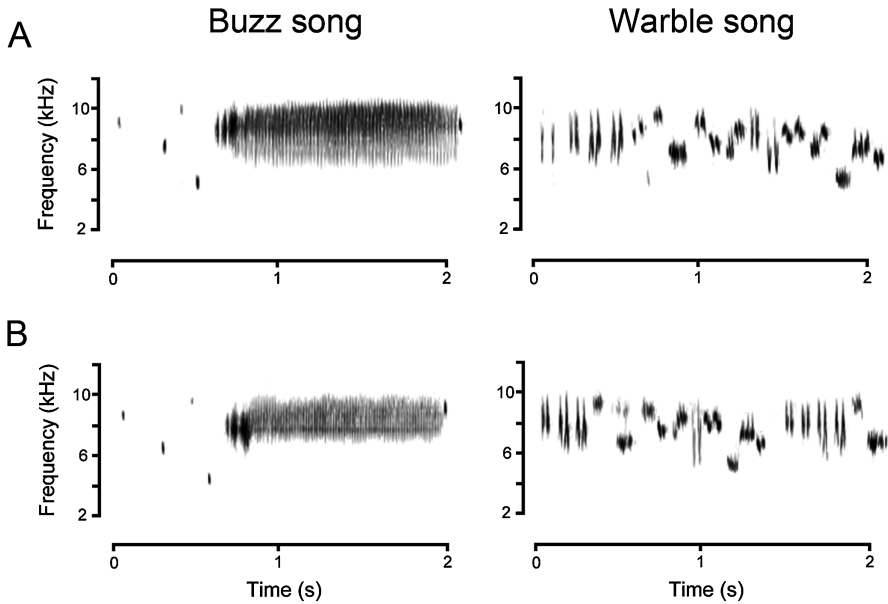


Figure 1. Spectrograms of buzz and warble songs of two male Grasshopper Sparrows (A and B) at the Chester River Field Research Station. The acoustic structure of the two song categories differs substantially, though both are in a high frequency range for songbirds (6–10 kHz). Spectrograms were generated using the SIGNAL/RTSD software (256 pt FFT, 195 Hz frequency resolution, 5 ms temporal resolution).

2008), and may cease singing entirely toward the end of a breeding cycle (Smith, 1959; B.L., pers. obs.). At the beginning of the breeding season, and once young are independent and a new breeding cycle has begun, males typically sing buzz song at high rates. They then transition to combined song, and often eventually to warble song exclusively. During the middle of a breeding cycle males can sing all three types of song within a singing bout, though combinations of combined and warble song are most common (B.L., pers. obs.). Anecdotal evidence also suggests that males will switch within a day from singing predominantly warble or combined song to singing buzz song exclusively if their pair-mate has died or disappeared (B.L., pers. obs.). Given the somewhat unusual pattern of singing exhibited by this species, it is unclear how information is divided between the two song categories, and whether these song categories serve as redundant signals, or convey multiple, distinctive messages. Furthermore, the acoustically distinct components of

buzz song itself may convey different messages and may, therefore, serve different functions for intended receivers.

As an initial assessment of song use, we measured population-level song output by point counts along a transect through our study site. From these data we were able to determine the proportions of song category use throughout the season. In order to examine potential intra-sexual functions of the two song categories, we performed a song playback experiment with targeted males known to be singing either buzz song, or the warble/combined song. These experiments provided both quantitative and qualitative evaluations of the importance of these two song categories to territorial males. We also performed a second playback experiment in which we tested the separate components of buzz song to determine whether they were capable of eliciting equally strong and equally rapid responses from territorial males. Similar tests using subcomponents of White-crowned Sparrow (*Zonotrichia leucophrys*) song have shown differences in strength and type of response, suggesting that these components of song were involved in transmitting different messages (Soha & Whaling, 2002; Nelson & Poesel, 2007). In particular, we were interested in whether the introductory segment of the buzz song of Grasshopper Sparrows might act to 'alert' receivers to the message contained in the subsequent, rapidly-trilled segment of song. In long distance acoustic signals, an alerting mechanism might serve to direct the attention of the receiver, so that additional information in the signal is more likely to be detected (Richards, 1981; Wiley, 2006).

2. Material and methods

2.1. Study site

Experiments took place at the Chester River Field Research Station, a 92.4-ha restored grassland near Chestertown, MD, USA (39°13'N, 76°00'W). All adult Grasshopper Sparrows at this site have been individually colour-banded since its establishment in 1999. The breeding population has stabilized to about 70–80 breeding pairs (Gill et al., 2006). The territories of each male were mapped in detail using hand-held GPS units prior to experiments taking place in a given year. In 2003, the number of males singing each Grasshopper Sparrow song type was recorded using point counts along a regular transect of the separate subfields at the site. These data were collected as a baseline measure of singing activity across the season. During a 10-min period at each of 9 points along this transect, separated by a distance

of 3–4 territories to avoid re-counting previously sampled birds, we made a simple count of the number of males singing, and the song type being sung.

2.2. Audio recordings and playback stimuli

Audiotape recordings of Grasshopper Sparrow song were made using Sony TC-D5M professional analog tape decks and Sennheiser ME67 shotgun microphones. Songs were recorded at the Chester River Field Research Station in the same population, but were made several years prior to testing in experiment 1, and were arranged to ensure that any males that might have been present at the same time as recorded individuals were at least 3 territories away from these individuals in any year of overlap.

Recordings were digitised at a sampling rate of 50 kHz and 16 bits per sample, and songs were analysed and prepared for playback using the SIGNAL/RTSD sound analysis software (Beeman, 2004). Population measurements of song were used to generate a synthetic model song. The frequency, duration and relative amplitude was measured for each note or sub-component of each song segment for 5 songs each recorded from $N = 25$ birds in 2001 in the same population. An average value was determined for each note or subcomponent and a synthetic song matching these parameters was generated with the voltage-controlled oscillator function in SIGNAL/RTSD. This synthetic song (Figure 2) was used as a stimulus in experiment 2.

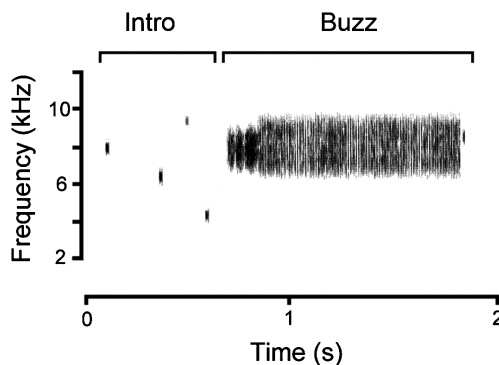


Figure 2. Spectrogram of the synthetic Grasshopper Sparrow song used as a stimulus in experiment 2. Song playback treatment 1 consisted of the Intro segment of the song only (comprising the four introductory notes), treatment 2 consisted of the Buzz segment of song only and treatment 3 consisted of the entire song. The spectrogram was generated using the SIGNAL/RTSD software (256 pt FFT, 195 Hz frequency resolution, 5 ms temporal resolution).

2.3. *Song playback experiments*

Song playback tests were conducted between 07:00 and 11:00 h EDT, and took place from May–July 2010 (experiment 1) and June–August 2004 (experiment 2). Songs were broadcast 1–4 m inside the edge of a bird's territory using a Sony TCM-5000EV connected via cable to a Radio-Shack 40-1219 horn tweeter mounted in custom-designed box with extended foam edges (to help directionalise sound propagation at the target bird). The speaker was situated 1 m above the ground. Grasshopper Sparrows at this site sing at heights of 1.1 ± 0.04 m (mean \pm SE). Song amplitude during playback was normalized to 90 dB SPL (RMS) at 1 m. We used a one-speaker, multiple-trial test procedure for our experiments. Songs were played at a rate of 10 songs per min (an average singing rate for this species). Males were visually sighted, perched within their territory prior to the initiation of playback tests. We observed focal birds for 1 min to measure baseline behaviour in that individual. Repeated tests with the same individual took place no sooner than 48 h since a previous test (to avoid habituation) and no later than 1 week since the first test (to ensure that birds were singing the same song type for all tests).

If birds produced either no response or a very weak response to a playback of buzz or warble song or the control song in experiment 2, if birds interacted with a neighbouring male before the end of the playback test, or if birds were singing different song types between the initial playback test and subsequent playback tests (experiment 1), data were excluded from these experiments. In experiment 1, 25% of playback attempts, and in experiment 2, 38% of playback attempts, were either halted early or excluded from analysis for these reasons. Subsequent reports of sample size include only the data that were not excluded for the reasons stated.

2.3.1. *Experiment 1*

In experiment 1, we contrasted responses to song based both on the type of song being sung by the territorial male, and the type of song used to challenge the territorial male. Prior to playback birds were categorized as singing either buzz song exclusively (often characteristic of the beginning of a breeding cycle), or the warble or combined song (the latter two often sung interchangeably later in a breeding cycle). Each bird then received two playback tests, one of each song type. In total, 12 birds singing buzz songs were tested with song playbacks of a buzz song and warble song, and an additional 12 birds singing combined or warble songs were tested

with the same set of buzz and warble songs (for a total of $N = 24$ birds). Each of the 12 naturally recorded buzz and warble songs used in this experiment came from different individuals to minimize the potential for differential responsiveness due to undetected song idiosyncrasies (Kroodsma, 1989).

We ran each test trial for 10 min, consisting of a 1-min broadcast period (playback period) followed by 9 min of silence (post-playback period). Prior to the playback test, distance markers (flags) were placed perpendicular to the observer at 4 m intervals (distances from the speaker were estimated to within 2 m). During the playback we noted, or later calculated, several standard response measures of the territorial male: (i) number of songs, (ii) latency to sing, (iii) number of flights, (iv) average time spent within 12 m of the speaker, (v) mean approach distance to the speaker and (vi) closest approach to the speaker. Vocal responses by the subject, and all dictated observations by the investigator, were recorded into a Sennheiser ME67 shotgun microphone connected to a Sony TC-D5M cassette recorder.

2.3.2. *Experiment 2*

Experiment 2 involved a test of the distinct acoustic components within buzz song. A synthetic ‘mean’ song was used to standardize stimuli of each type. Playback tests involved three different song treatments: one containing only the four notes making up the initial half of the song (the ‘introductory’ segment of this song type), one containing only the rapidly amplitude- and frequency-modulated second half of the song (the ‘buzz’ segment), and one containing the full song (Figure 2). Each playback test began with an experimental treatment song. If the birds produced no response, or a very weak response, this was followed by the presentation of a normal (control) song. The presentation of song treatments was randomized and counterbalanced across test subjects so that equal numbers of birds received each treatment song first. In experiment 2, 9 birds were tested with all three treatments of song. We began testing with a 10th bird that became unavailable for subsequent tests; thus, our N for experiment 2 is 10 for the introductory portion of song, and 9 for the other two treatments.

We ran each playback trial for 12 min composed of a 1-min broadcast period (playback period) followed by 11 min of silence (post-playback period). During the playback we measured the same responses of male territory

holders as in experiment 1, as well as (vii) latency to orient toward the speaker and (viii) flight latency (latency to the first flight within 12 m of the speaker). We defined orientation toward the speaker as the bird facing within a 90 degree arc toward the speaker (i.e., the direction in which the beak was pointing was within 45 degrees either direction of the speaker). In practice, as birds were sighted prior to playback and almost never facing toward the speaker initially, orientation toward the speaker was normally very obvious and distinct, and usually happened quickly after the initiation of the playback.

2.4. Statistical analyses

We used the Sigmastat version 4.0 statistical software (Systat Software, Chicago, IL, USA) to evaluate responses to playback. Unless otherwise specified, reported statistical values are for the overall test rather than for post-hoc comparisons. In order to test differences in strength of response to playbacks in experiment 1, we used a two-way mixed model analysis of variance (ANOVA), with one across-subjects factor (the type of song the bird was currently singing prior to playback), and one within-subjects factor (the type of song treatment used to test birds during playback), with Bonferroni post-hoc tests to identify specific differences between treatments.

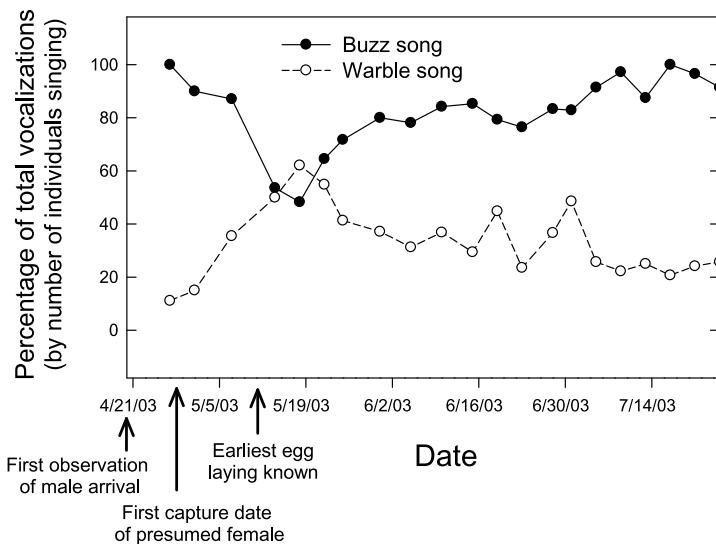
We used a one-way repeated measures ANOVA with Bonferroni post-hoc tests to evaluate playback responses in experiment 2. Because experiment 1 involved multiple natural exemplars of song as test stimuli, we used independent *t*-tests to examine whether responses to natural buzz songs differed from the responses to the synthetic song used in experiment 2. Behavioural response measures that could not be transformed to meet the normality assumptions of parametric tests were evaluated with nonparametric equivalents. Specifically, we used the Friedman test with Tukey post-hoc tests to examine song latency, flight latency, and orientation latency in experiment 2, and a Mann–Whitney *U*-test to examine closest approach to the speaker and amount of time spent within 12 m of the speaker in comparisons between natural and synthetic songs for experiments 1 and 2.

In order to assess responses to playback with a reduced number of composite measures, we used a principal components factor analysis with varimax rotation to aggregate the individual behavioural response measures into factors for further analysis (Yasukawa, 1978). We first describe the factor loadings and then report identical statistical tests on the factor scores.

3. Results

3.1. Singing activity

The number of songs of each type being sung by territorial males in the population as a whole changed throughout the course of the breeding season (Figure 3). During the initial transect, 100% of the males sang either the buzz or combined song (in this case approx. 10% were singing combined song, 90% sang buzz song exclusively). As the first breeding cycle progressed, eventually more birds sang warble song than sang buzz song. After a short period toward the middle to end of the initial breeding cycle (around May 15–May 21) this trend reversed again, and afterwards buzz song was heard more frequently than warble song for the remainder of the breeding season.



3.2. Experiment 1

3.2.1. Analysis of individual response measures

Grasshopper Sparrows showed typical territorial reactions to song playbacks; focal males tested with playbacks responded by singing and approaching the speaker. Birds singing buzz song produced 43.4 ± 2.5 (mean \pm SE) songs in response to buzz song playbacks and 37.5 ± 3.6 songs in response to warble or combined song playbacks, though this difference was not significant ($F_{1,22} = 1.14$, $p = 0.30$). Latency to sing in response to playback differed for birds singing the two song types, however ($F_{1,22} = 6.64$, $p < 0.05$). Birds singing buzz song responded in 1.02 ± 0.21 min, while birds singing warble song or combined song responded in 1.92 ± 0.27 min. There were no significant differences in response to song between birds singing buzz songs and those singing warble or combined songs for any of the other individual playback response measures (number of flights, average distance from speaker, amount of time spent < 12 m from speaker, and closest approach to speaker) ($F_{1,22}$ values < 0.97 , p values > 0.34).

Birds showed no difference in strength of response to the different playback treatments they received, i.e., there were no significant differences for any individual response measures based on type of song (buzz versus warble) ($F_{1,22}$ values < 1.72 , p values > 0.20). There were no interaction effects in strength of response between the type of song a bird was singing, and the type of playback the bird received ($F_{1,22}$ values < 0.91 , p values > 0.35).

3.2.2. Aggregation into factor scores

A principal components factor analysis with varimax rotation aggregated individual response measures into three factors with eigenvalues greater than 1 (Table 1). Together these three factors accounted for 80% of the total variance. We labelled these 'approach', 'song', and 'flight' factors, as approach responses (mean approach distance, time spent within 12 m of the speaker) loaded most heavily on the first factor, song-related responses (number of songs and song latency) loaded most heavily on the second factor, and number of flights loaded most heavily on the third factor. Closest approach was split about evenly between the first and third factors. Responses with loadings greater than 0.60 (in bold in Table 1), were considered salient to that factor.

Table 1.

Loadings for principal components factor analysis of the response measures in experiment 1.

Response measure	Component		
	1: Approach	2: Song	3: Flight
Number of songs	0.280	0.756	0.312
Latency to sing	0.013	-0.904	0.201
Number of flights	-0.022	-0.078	0.931
Time spent within 12 m	0.901	0.101	0.008
Mean approach	-0.963	-0.071	-0.080
Closest approach	-0.506	-0.268	-0.532
Total variance explained	41.8%	21.1%	17.1%

The loadings shown in bold constitute the factors.

3.2.3. Analysis of factor scores

Focal males did not differ in strength of approach (PC1) based on either the song type they were played, or the song type they were singing. There were no significant differences in approach factor scores (PC1) across treatment types (Figure 4A, $F_{1,22}$ values < 0.13 , p values > 0.72). However, males producing buzz song prior to testing had stronger song responses to playback than males producing the warble or combined song prior to testing. Song factor scores (PC2) for birds singing buzz song (regardless of playback type) were significantly greater than for birds singing the warble or combined song (Figure 4B, $F_{1,22} = 5.16$, $p < 0.05$). Flight factor scores (PC3) did not differ significantly for either song type or playback type (Figure 4C, $F_{1,22}$ values < 1.46 , p values > 0.24).

In terms of the type of response rather than the strength of response, birds sang predominantly buzz song to song playbacks, though responses differed for territorial males singing the two song types (Figure 4D). Focal birds producing buzz songs sang buzz song almost exclusively in response to playback of either song type (99.2% of the time for birds receiving playbacks of buzz song, 100% of the time for birds receiving playbacks of warble song).

Focal birds producing warble or combined songs typically responded to playback with a mixture of song types. Only 4 birds in this category sang exclusively 1 song type in response (3 birds sang only buzz songs, 1 bird sang only warble songs). Of the total songs sung in response to playback by birds producing warble or combined songs, $68.5 \pm 7.3\%$ of these were buzz songs (by itself) for birds receiving buzz song playbacks, and $69.9 \pm$

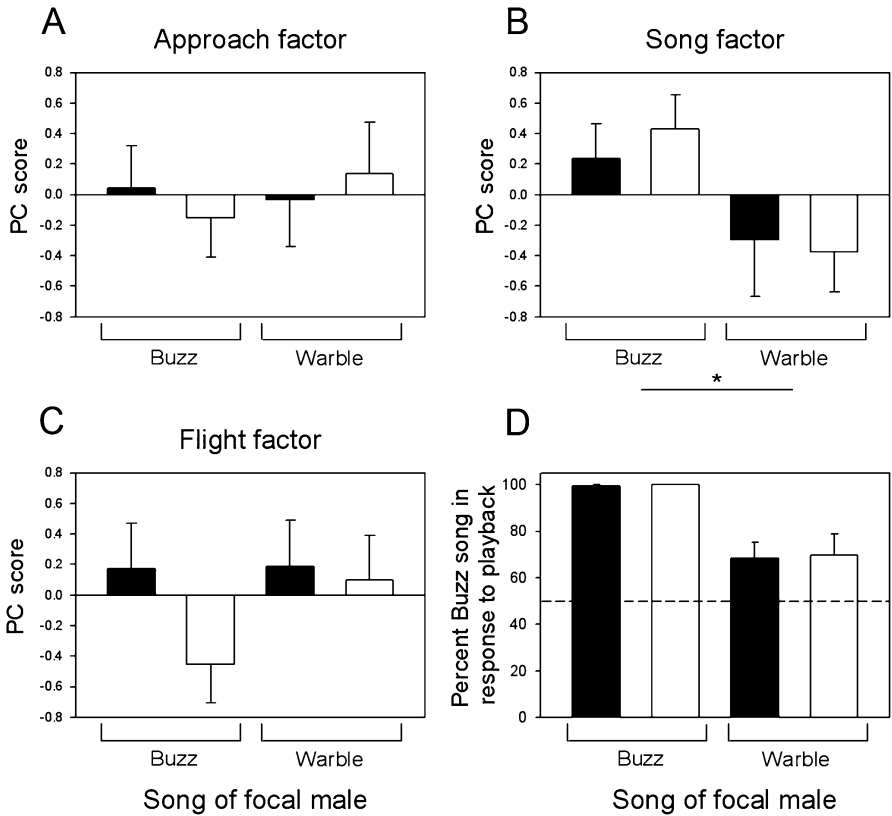


Figure 4. Strength of response to playback (mean + SE) for (A) approach factor scores, (B) song factor scores and (C) flight factor scores. Bars show responses for birds receiving buzz song playback (black) and warble song playback (white). Song being produced prior to testing by focal male targeted with playback is indicated by bars on left (buzz song) and bars on right (warble or combined song) for each set of factor scores. Approach to playback and flights did not differ significantly between buzz song and warble song playback treatments, nor between males singing the different song types. Song scores for birds producing buzz song were higher than those for birds producing the warble or combined song. (D) Type of song sung in response to playback. Birds producing buzz song prior to playback responded with buzz song nearly 100% of the time. Birds producing warble or combined song prior to playback responded with buzz song (only) in greater than 50% of cases.

9.4% of these were buzz songs (by itself) for birds receiving warble song playbacks. Chance level was set to 50% as a conservative estimate for these birds. All birds in this group were singing either warble or combined song (or both), but not buzz song by itself. Thus, the highest proportion of buzz song (as part of combined song) that could have been produced is 50% if

the focal birds in the latter treatment sang combined song exclusively. Focal birds responding to playback still produced more buzz song than would be expected by chance at this level, if they were either matching the song playback type, or responding at random with songs in their repertoire (χ^2 values > 7.05 , $df = 1$, p values < 0.01).

3.3. Experiment 2

3.3.1. Analysis of individual response measures

Grasshopper Sparrows showed typical territorial reactions to synthetic song, as they did to natural song playbacks in experiment 1; focal males responded by singing and approaching the speaker. Birds showed significantly stronger responses to the song as a whole and the second component of song (or buzz segment) than they did the introductory segment of song in the context of several approach-related measures: mean approach distance, closest approach to the speaker, number of flights ($F_{2,16}$ values > 8.00 , p values < 0.01). In addition, flight latency was significantly shorter in response to the whole song and buzz segment of song, than to the introductory segment of song ($\chi^2 = 15.93$, $df = 2$, $p < 0.001$). One approach-related measure, the amount of time spent less than 12 m from the speaker, did not differ significantly across the three treatment types ($F_{2,16} = 2.70$, $p = 0.10$).

The latency to orient toward the speaker did not differ significantly among the three treatment songs. Birds responded equally quickly to the introductory segment of song as they did to the buzz segment, or to the entire song ($\chi^2 = 3.5$, $df = 2$, $p = 0.57$). Song-related response measures also did not differ significantly between the three playback treatment types. Song latency was not significantly shorter ($F_{2,16}$ values < 0.94 , p values > 0.41), and number of songs did not differ between treatments ($\chi^2 = 1.82$, $df = 2$, $p = 0.40$).

3.3.2. Aggregation into factor scores

A principal components factor analysis with varimax rotation aggregated individual response measures into two factors with eigenvalues greater than 1 (Table 2). Together these two factors accounted for 59.4% of the total variance. We labelled these ‘approach’ and ‘song’ factors, as approach responses (closest approach, mean approach distance, time spent within 12 m of the speaker, as well as flight latency and number of flights) loaded most heavily on the first factor, and song-related responses (number of songs and song latency) loaded most heavily on the second factor. Responses with loadings

Table 2.

Loadings for principal components factor analysis of the response measures in experiment 2.

Response measure	Component	
	1: Approach	2: Song
Number of songs	0.136	0.832
Latency to sing	0.148	-0.801
Latency to orient	-0.533	0.145
Number of flights	0.666	0.086
Flight latency	-0.705	0.109
Time spent within 12 m	0.726	0.033
Mean approach	-0.868	-0.030
Closest approach	-0.907	-0.070
Total variance explained	42.1%	17.3%

The loadings shown in bold constitute the factors.

greater than 0.60 (in bold in Table 2), were considered salient to that factor. By that measure, latency to orient did not load heavily on either of these two principal components, though it was more strongly associated with the first of these two factors (Table 2).

3.3.3. Analysis of factor scores

As measured by approach factor scores (PC1), focal males responded less strongly to the introductory segment of song than to the entire song or just the buzz segment of song (Figure 5A) ($F_{2,16} = 24.53$, $p < 0.01$). Responses to song factor scores (PC2) did not differ among the three treatment types (Figure 5B; $F_{2,16} = 0.68$, $p = 0.52$).

3.3.4. Responses to synthetic song compared with natural song

Birds in the two experiments that received buzz song playbacks showed no difference in strength of response to behavioural measures based on the type of stimulus (synthetic versus natural). The number of songs, song latency, mean distance from the speaker, and number of flights did not differ significantly between the two stimulus types (t_{31} values < 1.36 , p values > 0.18), nor did the closest approach to the speaker or amount of time spent within 12 m of the speaker during the response period (U values > 76 , $N_1 = 24$, $N_2 = 9$, p values > 0.19). As responses were collected over different time periods in the two experiments (10 min versus 12 min), comparisons were made only over the first 10 min of response to playback in experiment 2.

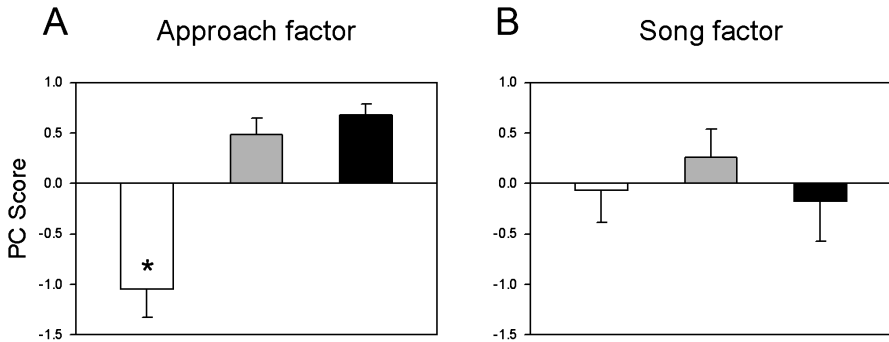


Figure 5. Strength of response to playback (mean + SE) for treatments 1–3 in experiment 2. (A) Approach factor scores, (B) song factor scores. White bars indicate factor scores for responses to playback of the Intro segment of song by itself (introductory notes only), grey bars to playback of the Buzz segment of song by itself, and black bars to playback of the entire song. Approach to song was significantly weaker to playbacks of the Intro segment of song than to either the Buzz segment of song or to the entire song. Song scores did not differ among treatments—song responses were equally strong to playbacks of the introductory notes, playbacks of the Buzz segment of song, and playbacks of the entire song.

4. Discussion

Song in Grasshopper Sparrows clearly serves the two main functions typically associated with song: mate attraction and territory defence (Collins, 2004; Catchpole & Slater, 2008). As buzz song is far more prevalent than warble song at the start of the breeding season, when birds initially return to the breeding grounds, it most likely serves as the initial song for mate attraction and pairing (Figure 3; Proppe & Ritchison, 2008). It is also the only song sung by males known to be unpaired, and without a nest (Vickery, 1996; B.L., S.A. and S.M.W., pers. obs.). For most of the season, buzz song is heard more commonly than warble song, though for at least some time later in the initial breeding cycle, warble song was more commonly heard than buzz song in the population as a whole (Figure 3). During the remainder of the breeding season, buzz song predominated in the population, though this was likely at least in part a consequence of the less synchronized nature of nesting cycles later in the season. The first nesting cycle in late April/early May begins relatively synchronously, but as the season progresses and nests become depredated, subsequent nesting cycles grow increasingly out of phase as birds re-nest immediately following nest loss. Buzz song, thus, seems to serve the primary inter-sexual function of song, mate attraction, especially at the start of the breeding season.

We provide evidence that buzz song also seems to serve as the major acoustic signal involved in the principal intra-sexual function of song, territory defence. In experiment 1 we tested the response of birds to both types of song in two different groups of males. One group comprised males that sang buzz song exclusively, the second group sang either warble song or combined (buzz-warble) song, corresponding typically to birds farther along in their breeding cycle. Both song types elicited a similarly strong response in playback experiments with regard to number of flights or general approach measures, regardless of the type of song a focal male was singing. However, focal birds singing buzz song produced more songs in response to playback, and sang those songs with shorter latency following the initiation of a playback test than birds singing the warble or combined song (Figure 4B). This might be, at least in part, a consequence of generally greater song output when birds are producing buzz song compared with when they are producing warble or combined song (Proppe & Ritchison, 2008; B.L., S.A. and S.M.W., pers. obs.). However, as singing often precedes and follows territorial encounters (Smith, 1959; Vickery, 1996), this result seems to suggest a more active, vigorous response to playback by birds producing buzz song.

In terms of the type of response given to playback, there were four possible outcomes: (1) the focal bird would respond with buzz song, (2) the focal bird would respond with a warble or combined song, (3) the focal bird would respond with the song that it was currently singing, or (4) the focal bird would song match to the playback and respond with the song it heard. A focal bird could also respond with some combination of these possibilities. Results suggest that Grasshopper Sparrows respond primarily with buzz song to playback (outcome 1 above), with some tendency to respond with the song that the bird was already singing (outcome 3 above). Birds that were singing buzz song when tested responded exclusively with that song type, while birds that were singing combined or warble song responded at greater than chance levels with the buzz song as well (Figure 4D). This result elaborates upon earlier, anecdotal reports that buzz song and some calls, though not warble song, are produced in response to playback by Florida Grasshopper Sparrows (see Vickery, 1996). While both song types were used when males were paired and patrolling the borders of their territory during normal daily activity, our results from these playback experiments support earlier suggestions that buzz song is the predominant song used in encounters with intruding males.

Given that buzz song seems to serve as the major song both in initial mate attraction and territory defence in Grasshopper Sparrows, what is the principal function of the warble (or combined) song? There are several other functions that have been suggested or proposed for multiple song types or categories when songs are not functionally interchangeable (i.e., when they carry different ‘messages’). Some songbirds subdivide their repertoires into categories that may differ in temporal patterning (for example, sung at different times of day), and may be either male-directed or female-directed, parsing the dual traditional functions outlined above (Catchpole & Slater, 2008). Examples of this type of categorization are numerous, and occur notably in the paruline warblers (Lein, 1978; Kroodsma et al., 1989; Staicer, 1989, 1996; Byers & Kroodsma, 1992; Spector, 1992; Beebee, 2004a, b). In other species, both contextual and experimental evidence has demonstrated that while one song category may serve the dual intra- and inter-sexual functions of song, other song categories may be associated with additional information, including motivational tendencies (Schroeder & Wiley, 1983; Nelson & Croner, 1991), location within the territory (Schroeder & Wiley, 1983; Weary et al., 1994), stages of the breeding cycle (Schroeder & Wiley, 1983; Weary et al., 1994; Wiebe & Lein, 1999), etc. Grasshopper Sparrows seem to fall in the latter category, as the single buzz song in a typical male’s repertoire clearly functions in both the traditional male- and female-directed contexts, while the warble song is associated with later stages of the breeding cycle (Smith, 1959; Proppe & Ritchison, 2008), and occurs only if the male is already paired. It should be emphasized that in these latter cases in particular, functional distinctions between song categories may not be mutually exclusive.

Several potential uses not associated with the traditional dual functions of song have been proposed for warble song in Grasshopper Sparrows. One possibility, suggested by Smith (1959), is that this song functions to strengthen the pair bond between a male and female, as it is produced only after pairing. Another possibility is that song acts either to advance the reproductive physiology of the female, or synchronize the female’s reproductive physiology with the behaviour of the male (Lehrman, 1958, 1964; Hinde & Steele, 1976; Wright & Cuthill, 1992; Nowicki & Searcy, 2004). In this case warble or combined song might somehow serve this function either exclusively or more effectively than buzz song. More recently, it also has been suggested that warble song may serve in part as an alarm, or anti-predator, signal to

females of the pair (Proppe & Ritchison, 2008). Perhaps most intriguingly, it is also possible that warble song has evolved as a specialized extra-pair mate attractant, advertising the availability of paired males as extra-pair mates to neighbouring females, though the specifics of extra-pair mating awaits detailed further study in Grasshopper Sparrows. Grasshopper Sparrows are socially monogamous, though rare cases of polygyny have been reported (Vickery, 1996; Small et al., 2009). It is certainly possible that warble song, and especially combined song, may serve more than one of these additional functions. More contextual and experimental evidence is needed to narrow the possible roles of this song type.

We also tested whether different components of song, in this case buzz song, might serve to convey different types of information to receivers. In experiment 2, we examined responses to the introductory notes of song, as well as the subsequent rapidly amplitude-modulated segment, compared with the song as a whole. In this experiment, we used a synthetic song that was based on population mean values for all measurable song parameters as a way of standardising the stimulus across trials. Responses to this song did not differ statistically from responses to the natural playback songs in experiment 1; it was equally potent in eliciting territorial responses from males in the field. The introductory notes of Grasshopper Sparrow buzz song clearly elicited a weaker response than the buzz segment, paralleling similar results in other species having songs with pure tone introductory notes followed by rapidly-modulated notes or song segments (Richards, 1981; Soha & Whaling, 2002). When exposed to the introductory notes, subjects took more time to fly in towards the speaker, took fewer flights within the first minute, and remained farther from the speaker. The buzz segment, on the other hand, induced strong reactions similar to those of the full song. Overall, while song-related measures were generally similar across all three stimulus types (Figure 5B), approach responses as a whole were significantly weaker to the introductory notes alone than to the buzz segment or full song, which did not differ significantly (Figure 5A). These results suggest that the complex, rapidly amplitude- and frequency-modulated buzz segment conveys the information necessary for song recognition by Grasshopper Sparrows, at least to other males.

One possibility for the function of the introductory notes in buzz song is suggested by the latency of birds to orient toward the stimulus. Despite the weaker approach response to introductory notes by themselves, there was no

difference in the latency to orient to the playback presentation between the three song treatments. This result is consistent with the idea that the introductory segment of song may be used as an alerting signal preceding the message conveyed by the buzz segment. Grasshopper Sparrows recognize and orient towards the speaker when the introductory notes are played, but usually only fly in once they hear the rapidly-modulated buzz segment. The introductory notes may, thus, act as a ‘pointer’ or ‘attractive modifier’ (Hasson, 1997; Gualla et al., 2008), with the buzz segment serving the function of an ‘activator’ (Hasson, 1997), though in this case for males as receivers rather than females. Alerting components, by serving to direct attention at the signaller (thus preparing the receiver for subsequent information), likely enhance signal detection and recognition in vocal and visual signals, especially in noisy environments (Richards, 1981; Ord & Stamps, 2008). Grasshopper Sparrow songs are rapidly-modulated, very high frequency signals for birds (Figure 1) and, therefore, susceptible to distortion in the form of both attenuation and degradation, even in acoustically open habitats (Wiley & Richards, 1982; Romer & Lewald, 1992; Lohr et al., 2003; Naguib, 2003). An alerting and messaging mechanism would maximize the detectability of these long-range signals by using a highly degradation-resistant component, and then relay information about the signaller’s identity and quality through a complex and more feature-rich song segment (Richards, 1981; Hasson, 1997). The use of such alerting signals has now been identified in a variety of taxa (Gerhardt, 1976; Richards, 1981; Brenowitz, 1982; Mitchell et al., 2006; Bloomfield et al., 2008; Ord & Stamps, 2008; Greig & Pruett-Jones, 2010).

While male Grasshopper Sparrows have a repertoire of only two songs, our results suggest a diversity of functions for the two song categories and different components of the principal advertisement song. We confirm earlier suggestions that buzz song functions as both the predominant intra-sexual signal in territorial interactions between males, as well as the advertisement signal to females for pairing. This song consists of a series of pure tone notes that precede a rapid trill. While the trill seems to be necessary and sufficient to relay the messages associated with this song type, the introductory notes may function as an ‘alerting’ signal, directing a receiver’s attention to the impending information, thus increasing the detection and reliability of the signal. The potential messages conveyed by warble song remain enigmatic, and more detailed studies are needed to explore fully its possible function given the associations that have been made with its prevalence in certain contexts.

Acknowledgements

We thank Douglas E. Gill for his generous support and advice, and for guiding the establishment of the Chester River Field Research Station. Dan Small, Maren Gimpel, Jason Guerard and Jared Parks provided invaluable assistance in the field. We also thank Henry F. Sears for creating the Chester River Field Research Station, and for allowing us access to the site for these experiments. Financial support for these studies was provided by the University of Maryland Baltimore County (UMBC) and the Center for Environment and Society at Washington College. Experiments reported here conform to the guidelines for studies in animal behaviour established by the Animal Behaviour Society, and were approved by the University of Maryland Baltimore County Institutional Animal Care and Use Committee.

References

- Beebee, M.D. (2004a). The functions of multiple singing modes: experimental tests in yellow warblers, *Dendroica petechia*. — *Anim. Behav.* 67: 1089-1097.
- Beebee, M.D. (2004b). Variation in vocal performance in the songs of a wood-warbler: evidence for the function of distinct singing modes. — *Ethology* 110: 531-542.
- Beeman, K. (2004). SIGNAL/RTSD user's guide. — Engineering Design, Belmont, MA.
- Bloomfield, L.L., Farrell, T.M. & Sturdy, C.B. (2008). All "chick-a-dee" calls are not created equally Part II. Mechanisms for discrimination by sympatric and allopatric chickadees. — *Behav. Proc.* 77: 87-99.
- Brenowitz, E.A. (1982). Long-range communication of species identity by song in the red-winged blackbird. — *Behav. Ecol. Sociobiol.* 10: 29-38.
- Byers, B.E. & Kroodsma, D.E. (1992). Development of two song categories by chestnut-sided warblers. — *Anim. Behav.* 44: 799-810.
- Catchpole, C.K. & Slater, P.J.B. (2008). Bird song: biological themes and variations. — Cambridge University Press, Cambridge.
- Collins, S. (2004). Vocal fighting and flirting: the functions of birdsong. — In: Nature's music: the science of birdsong (Marler, P. & Slabbekoom, H., eds). Elsevier Academic Press, San Diego, CA, p. 39-79.
- Gerhardt, H.C. (1976). Significance of two frequency bands in long distance vocal communication in the green treefrog. — *Nature* 261: 692-694.
- Gill, D.E., Blank, P., Parks, J., Guerard, J.B., Lohr, B., Schwartzman, E., Gruber, J.G., Dodge, G., Rewa, C.A. & Sears, H.F. (2006). Plants and birds on a CRP restored grasslands in Maryland. — *Wild. Soc. Bull.* 34: 944-956.
- Greig, E.I. & Pruett-Jones, S. (2010). Danger may enhance communication: predator calls alert females to male displays. — *Behav. Ecol.* 21: 1360-1366.
- Guala, F., Cermelli, P. & Castellano, S. (2008). Is there a role for amplifiers in sexual selection? — *J. Theor. Biol.* 252: 255-271.

- Hasson, O. (1997). Towards a general theory of biological signaling. — J. Theor. Biol. 185: 139-156.
- Hinde, R.A. & Steele, E. (1976). The effect of male song on an oestrogen dependant behaviour in the female canary *Serinus canaries*. — Horm. Behav. 7: 293-304.
- Kroodsma, D.E. (1982). Song repertoires: problems in their definition and use. — In: Acoustic communication in birds (Kroodsma, D.E. & Miller, E.H., eds). Academic Press, New York, NY, p. 125-146.
- Kroodsma, D.E. (1989). Suggested experimental designs for song playbacks. — Anim. Behav. 37: 600-609.
- Kroodsma, D.E., Bereson, R.C., Byers, B.E. & Minear, E. (1989). Use of song types of the chestnut-sided warbler: evidence for both intra-sexual and inter-sexual functions. — Can. J. Zool. 67: 447-456.
- Lehrman, D.S. (1958). Effect of female sex hormones on incubation behavior in the ring dove (*Streptopelia risoria*). — J. Comp. Physiol. Psychol. 51: 142-145.
- Lehrman, D.S. (1964). Control of behavior cycles in reproduction. — In: Social behavior and organization among vertebrates (Etkin, W., ed.). University of Chicago Press, Chicago, IL, p. 143-166.
- Lein, M.R. (1978). Song variation in a population of chestnut-sided warblers (*Dendroica pensylvanica*): its nature and suggested significance. — Can. J. Zool. 56: 1266-1283.
- Lohr, B., Wright, T.F. & Dooling, R.J. (2003). Detection and discrimination of natural calls in masking noise by birds: estimating the active space of a signal. — Anim. Behav. 65: 763-777.
- Mitchell, B.R., Makagon, M.M., Jaeger, M.M. & Barrett, R.H. (2006). Information content of coyote barks and howls. — Bioacoustics 15: 289-314.
- Naguib, M. (2003). Reverberation of rapid and slow trills: implications for signal adaptations to long-range communication. — J. Acoust. Soc. Am. 113: 1749-1756.
- Nelson, D.A. & Croner, L.J. (1991). Song categories and their functions in the field sparrow (*Spizella pusilla*). — Auk 108: 42-52.
- Nelson, D.A. & Poesel, A. (2007). Segregation of information in a complex acoustic signal: individual and dialect identity in white-crowned sparrows song. — Anim. Behav. 74: 1073-1084.
- Nowicki, S. & Searcy, W.A. (2004). Song function and the evolution of female preferences: why birds sing, why brains matter. — Ann. N.Y. Acad. Sci. 1016: 704-723.
- Ord, T.J. & Stamps, J.A. (2008). Alert signals enhance animal communication in “noisy” environments. — Proc. Natl. Acad. Sci. USA 105: 18830-18835.
- Proppe, D.S. & Ritchison, G. (2008). Use and possible functions of the primary and sustained songs of male grasshopper sparrows. — Am. Midl. Nat. 160: 1-6.
- Richards, D.G. (1981). Alerting and message components in songs of rufous-sided towhees. — Behaviour 76: 223-249.
- Romer, H. & Lewald, J. (1992). High-frequency sound transmission in natural habitats: implications for the evolution of insect acoustic communication. — Behav. Ecol. Sociobiol. 29: 437-444.

- Schroeder, D.J. & Wiley, R.H. (1983). Communication with repertoires of song themes in tufted titmice. — *Anim. Behav.* 31: 1128-1138.
- Small, D.M., Gimpel, M.E., Parks, J., Guerard, J.B. & Gill, D.E. (2009). First documented cases of polygyny in the grasshopper sparrow. — *Wilson J. Ornithol.* 121: 822-825.
- Smith, R.L. (1959). The songs of the grasshopper sparrow. — *Wilson Bull.* 71: 141-151.
- Soha, J.A., Lohr, B. & Gill, D.E. (2009). Song development in the grasshopper sparrow, *Ammodramus savannarum*. — *Anim. Behav.* 77: 1479-1489.
- Soha, J.A. & Whaling, C. (2002). Responses of adult white-crowned sparrows to playbacks of song phrases: implications for the ontogeny of song recognition. — *Condor* 104: 848-854.
- Spector, D.A. (1992). Wood-warbler song systems. A review of paruline singing behaviors. — *Curr. Ornithol.* 9: 199-238.
- Staicer, C.A. (1989). Characteristics, use, and significance of two singing behaviors in Grace's warbler (*Dendroica graciae*). — *Auk* 106: 49-63.
- Staicer, C.A. (1996). Acoustical features of song categories of the Adelaide's warbler (*Dendroica adalaidae*). — *Auk* 113: 771-783.
- Vickery, P.D. (1996). Grasshopper Sparrow (*Ammadramus savannarum*). — In: *The birds of North America*, Vol. 239 (Poole, A. & Gill, F., eds). The Academy of Natural Sciences, Philadelphia, PA and the American Ornithologists' Union, Washington, DC.
- Weary, D.M., Lemon, R.E. & Perreault, S. (1994). Male yellow warblers vary use of song types depending on pairing status and distance from nest. — *Auk* 111: 727-729.
- Wiebe, M.O. & Lein, M.R. (1999). Use of song types by mountain chickadees (*Poecile gambeli*). — *Wilson Bull.* 111: 368-375.
- Wiley, R.H. (2006). Signal detection and animal communication. — *Adv. Stud. Behav.* 6: 217-247.
- Wiley, R.H. & Richards, D.G. (1982). Adaptations for acoustic communication in birds: sound transmission and signal detection. — In: *Acoustic communication in birds*, Vol. 1 (Kroodsma, D.E. & Miller, E.H., eds). Academic Press, New York, NY, p. 131-181.
- Wright, J. & Cuthill, I. (1992). Monogamy in the European starling. — *Behaviour* 120: 262-285.
- Yasukawa, K. (1978). Aggressive tendencies and levels of a graded display factor analysis of response to song playback in the redwinged blackbird (*Agelaius phoeniceus*). — *Behav. Biol.* 23: 446-459.