

Does audience affect the structure of warble song in budgerigars (*Melopsittacus undulatus*)?



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ABSTRACT

In many bird species, male song functions both to defend a territory against other males and to attract a female mate. Male budgerigars (*Melopsittacus undulatus*) produce a song-like vocal signal, the warble, that can be directed at either females or other males. Warble is a long, complex, low amplitude, and variable vocalization composed of different element types. While there is some evidence that warble can induce reproduction, the function of this signal is largely uncertain and it is unclear whether male- and female-directed warble differ in either function or structure. We recorded male budgerigars in the presence of either their mate or a familiar male to identify whether the warbles produced with different audiences differed in structure. We dissected each warble into specific element units, classified units into a limited number of types by rule-based visual classification and calculated the proportion of each element type, element diversity, and total duration for the male- and female-directed warbles of each male. We also examined the sequential organization of warble element types (syntax) using time-window lagged sequential analysis. We found no differences in the proportions of different elements used, element diversity or duration of warbles between male- and female-directed warble. The syntax of warbles is similar when directed at males or females. However, we found greater between-individual similarity in the sequential organization of warbles directed towards females than in those directed towards males. The greater syntactical consistency in female-directed warble suggests that females may prefer either specific types of element sequences, or consistency itself, and thus shape the organization of warbles.

1. Introduction

Acoustic signals are widely used by animals to mediate social interactions in multiple contexts. In many bird species, males produce songs in both aggressive and mating interactions. In some species, males produce songs to defend their territory from other males (Alatalo et al., 1990; Eens et al., 1993; Falls, 1988; Krebs et al., 1978; Nowicki et al., 1998; Radesäter et al., 1987; Smith, 1979), while in others, song is used to attract females for mating (Eriksson and Wallin, 1986; Gentner and Hulse, 1998; McDonald, 1989; Vallet and Kreutzer, 1995). In some species, males use song in both contexts (Catchpole and Slater, 2008; McDonald, 1989; Westcott, 1992).

Songs with different acoustic structure are often associated with unique functions, depending on the identity of the receiver(s). Some birds use syntax as the key acoustic structures to differentiate between audiences (Vallet and Kreutzer, 1995), while other birds rely on compositional structures to differentiate intended receivers (Kroodsmma et al., 1989; Lattin and Ritchison, 2009). For instance, blue grosbeak (*Passerina caerulea*) songs directed at males are longer in duration, have

more elements, and elicit more aggressive responses from other males than those songs directed to females (Lattin and Ritchison, 2009). Male chestnut-sided warblers (*Setophaga pensylvanica*) have two different types of song, with unaccented-ending songs used in exchanges with other males and accented-ending songs used to attract mates (Kroodsmma et al., 1989). Zebra finches (*Taeniopygia guttata*) use a directed courtship song and undirected song. Structurally, directed courtship songs have more introductory elements, more frequently repeated motifs, faster performance rate, and more rigid sequence of elements than undirected songs (Ölveczky et al., 2005; Sossinka and Bohner, 1980). Structural differences relating to audience effects also have been found in the vocal signals of non-avian species, including humans (Biersack et al., 2005; Dickson and Turner, 2015) and non-human primates (Cheney and Seyfarth, 1985). These examples indicate that specific acoustic structure of bird song and other signals can be associated with specific behavioral functions. However, it is not known to what extent specific structural differences, such as differences in song syntax, are associated with different intended receivers in other avian species.

Budgerigars (*Melopsittacus undulatus*) are highly gregarious small

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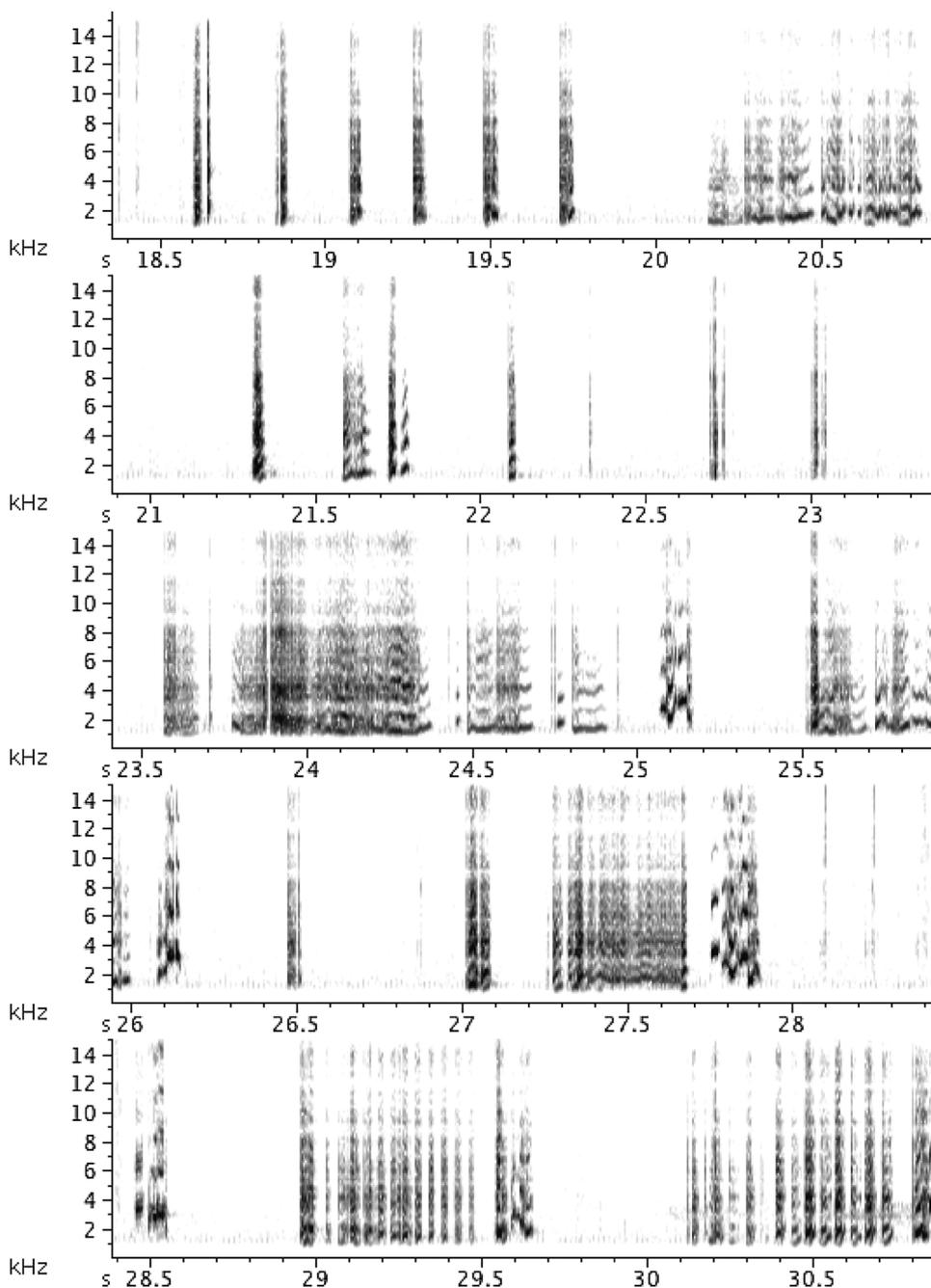


Fig. 1. Spectrogram of a 12.5 s portion of a 37 s long female-directed warble recorded from a male budgerigar. Frequency in kHz is shown on the y-axis and time in seconds on the x-axis.

parrots, endemic to Australia. They live in large nomadic flocks that breed opportunistically in the unpredictable wet season of Australia and do not defend territories (Brockway, 1964a,b). They have a large repertoire of vocal signals, the most complex of which is a song-like vocalization called ‘warble’ (Brockway, 1964a,b; Fujiwara and Okumura, 1992; Wyndham, 1980a). This warble is a long and variable vocalization composed of different element types (Fig. 1; Farabaugh et al., 1992) that is produced at low amplitude relative to other calls in the repertoire. It has been shown via operant conditioning experiments that budgerigars perceive these element types as distinct acoustic categories (Tu et al., 2011). The warble song is produced almost exclusively by males and can be directed at individuals of either sex in a variety of contexts (Brockway, 1964a,b; Farabaugh et al., 1992; Fujiwara and Okumura, 1992; Konishi, 1985; Trillmich, 1976; Wyndham, 1980a). Despite the fact that warble song is a common and frequently used acoustic signal among this species, its function is still uncertain. Previous research has suggested that males use warble song to coordinate

reproductive behavior, pair bond maintenance, and nest box activities, and to stimulate ovarian development in females (Brockway, 1962, 1964b,c, 1965). There is also some evidence that the presence of warble stimulates gonadal development and reproductive behavior in males (Brockway, 1964b,c). Wyndham (1980a) suggested that the warble might function for individual identification, however there is no evidence to date that this is the case.

A first step to understanding the function of a complex vocalization like the budgerigar warble is to examine its structure and whether it varies among different contexts (Bradbury and Vehrencamp, 2011; Dahlin and Benedict, 2013). Here we tested the hypothesis that the acoustic structure of the warble is different when directed at males versus females. We recorded warbles from male budgerigars in the presence of their mate or in the presence of another, familiar male. We compared the structure of warbles produced under these two conditions in relation to the relative frequency of different elements (composition) and the ordering of these elements (syntax). We predicted that males

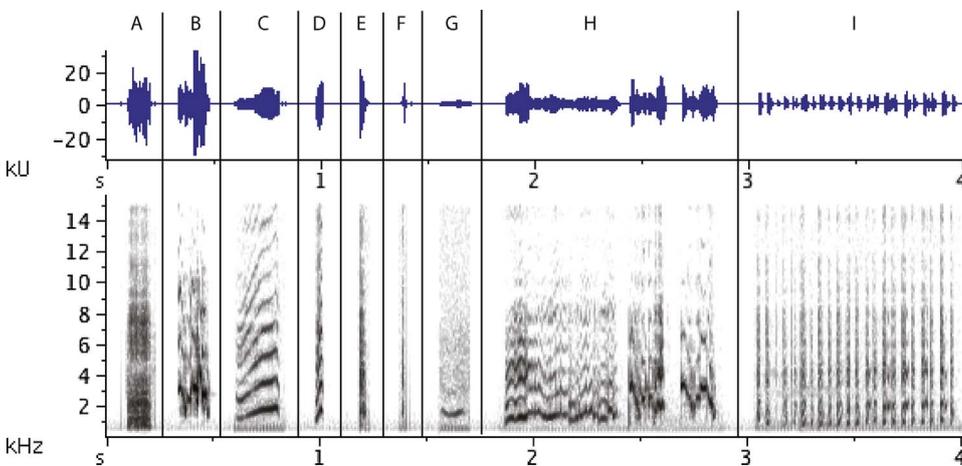


Fig. 2. Spectrogram examples of nine element categories (A-I): alarm call-like (A) elements, contact call-like (B) elements, long harmonic calls (C), short harmonic calls (D), noisy calls (E), clicks (F), pure tone-like (G) elements, and compounds (H) following Tu et al. (2011), and the “I” element which is composed of strings of short harmonic calls (D), noisy calls (E), and clicks (F). Frequency in kHz is shown on the y-axis and time in seconds on the x-axis.

Table 1
Means of measurements of warble parameters and results of nested ANOVAs for differences between male- and female-directed warbles.

Measures	Female Mean \pm SE	Male Mean \pm SE	DF	F-Ratio	P-value
Duration (sec)	3.19 \pm 0.58	3.00 \pm 0.57	1,11.9	0.05	0.83
# of Total Elements	26.16 \pm 3.61	20.06 \pm 3.55	1,11.4	1.45	0.25
Diversity Index	1.44 \pm 0.05	1.45 \pm 0.05	1,10.9	0.00	0.98
Prop. of Alarm Call-like Elements (A)	0.02 \pm 0.01	0.02 \pm 0.00	1,11.6	0.08	0.78
Prop. of Contact Call-like Elements (B)	0.13 \pm 0.03	0.23 \pm 0.03	1,12.3	4.77	0.05
Prop. of Long Harmonic Call Elements (C)	0.11 \pm 0.02	0.11 \pm 0.02	1,12.0	0.02	0.89
Prop. of Short Harmonic Calls (D)	0.54 \pm 0.03	0.49 \pm 0.02	1,10.3	2.59	0.14
Prop. of Noisy Calls (E)	0.13 \pm 0.02	0.10 \pm 0.02	1,12.0	0.64	0.44
Prop. of Clicks (F)	0.06 \pm 0.01	0.05 \pm 0.01	1,10.2	0.52	0.49
Prop. of Pure Tone-like (G)	0.00 \pm 0.00	0.00 \pm 0.00	1,9.8	0.39	0.55
Prop. of Total Compounds (H)	0.13 \pm 0.02	0.12 \pm 0.02	1,11.8	0.47	0.51
Prop. of Total I Elements (I)	0.06 \pm 0.00	0.06 \pm 0.00	1,7.5	0.55	0.48

would change both the composition and syntax of their warble according to the sex of their audience.

2. Materials and methods

2.1. Study subjects

The subjects were captive-raised budgerigars acquired from local pet stores or commercial breeders. All animals were sexually mature at the time of the study and were housed with their mate in cages kept in a single room the New Mexico State University (NMSU) Animal Care Facility. They were kept on a 12:12 light: dark cycle under standard fluorescent lighting at a room temperature of $24 \pm 2^\circ\text{C}$ and fed an *ad libitum* diet of commercial bird seed and water. Research was conducted under approval of the NMSU Institutional Animal Care and Use Committee (IACUC protocol 2013-30).

2.2. Preliminary behavioral observations on audience composition

To confirm that budgerigars warble to individuals of either sex, we conducted behavioral observations employing scan sampling in an aviary (3 m wide, 7.5 m deep and 2.1 m high) that housed approximately the same amount of birds of each sex. Birds were observed twice a day in the mid-morning and mid-afternoon for one week. We conducted focal observations of 3 min length of randomly-selected individuals and recorded whether that individual produced a warble song, along with the sex and number of the intended receivers (i.e. birds that were in direct proximity of the bird producing these low-amplitude vocalizations).

2.3. Recording and segmenting warble bouts

Warbles were recorded from seven adult male budgerigars between April 2013 and July 2014. The birds were recorded within 49.2 l Igloo® coolers, lined with acoustic foam to limit echoes and external noise. A piece of transparent Plexiglas was placed over the opening of the coolers so that the target bird could view its audience while limiting interference in the recording. Warbles were recorded with using Audio-Technica Pro 37 microphones (frequency responses = 30–15,000 Hz). All recordings were processed with a Saffire Pro digitizer and directly saved to a Dell DHMPC computer using the sound program Sound Analysis Pro 2011 (Tchernichovski et al., 2000), with a sampling rate of 22,050 Hz. During recording sessions, each bird had *ad libitum* access to water and food.

Males were recorded under two treatments. In one treatment, the bird was recorded with its mate (female stimulus). In the other treatment, the bird was recorded with a familiar conspecific (male stimulus). The male stimuli birds were in the same age group and lived in the same colony as the experimental birds. Males were recorded in multiple sessions under each treatment to obtain enough warble samples with the recording sessions of each treatment interspersed to avoid order effects. Each recording session started at 5:30 P.M. and ended at 11:30 A.M. the following day because the birds tended to warble most frequently in the morning (C. Tobin and T. Wright, pers obs). This recording method allowed us to enhance our likelihood of recording the largest amount of warbles, with minimal handling and stress for the birds. Only vocalizations recorded between 6:00 A.M. to 11:00 A.M. were retained for analysis. After recording, warble bouts were identified using spectrograms generated with Raven Pro 1.5 (Cornell Lab of Ornithology). A warble bout was defined as a series of three or more elements belonging to three or more categories defined by Tu et al.

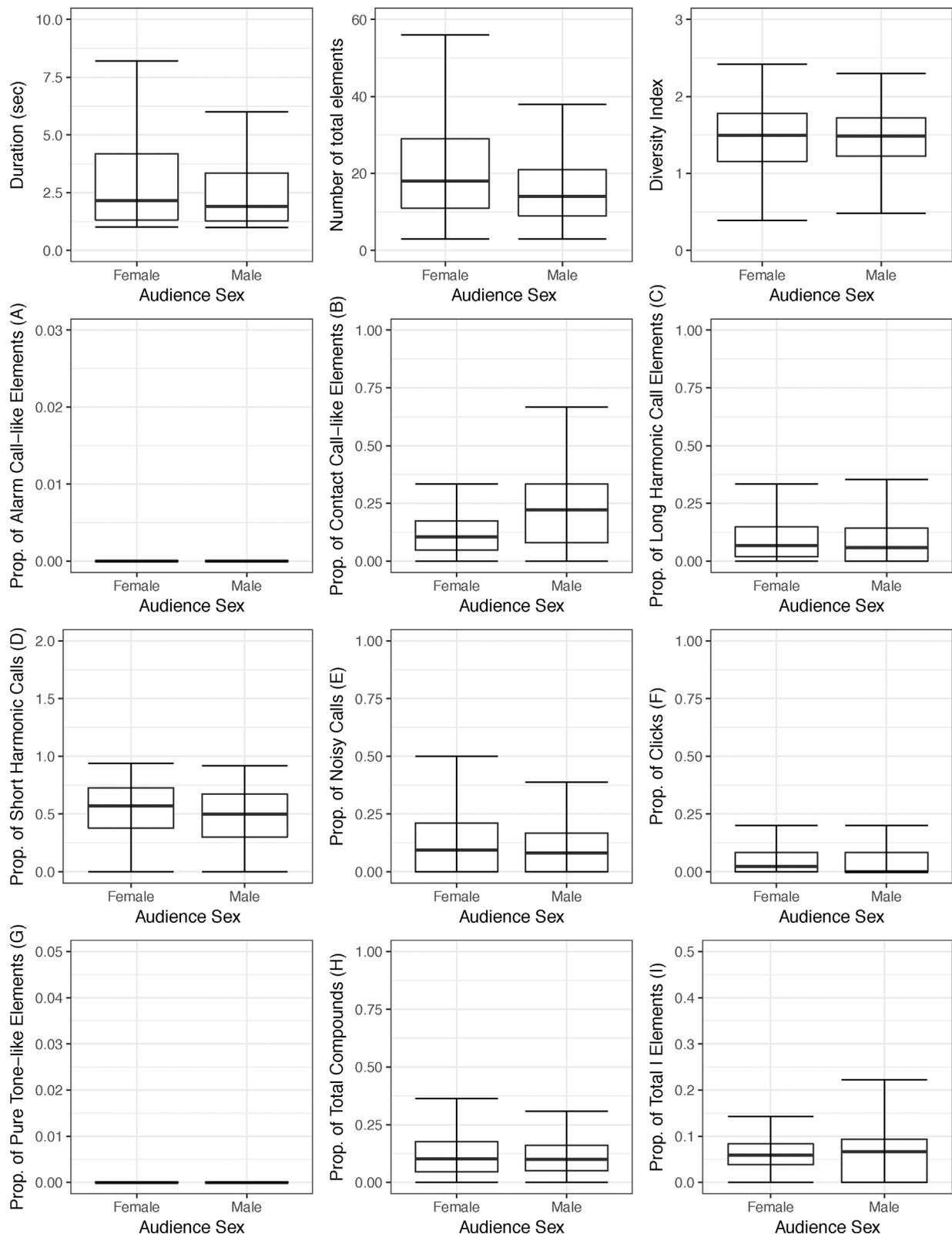


Fig. 3. Comparison of all warble parameters between female-directed and male-directed warbles. A total of 193 female-directed warbles and 236 male-directed warbles were measured from seven birds. Data are represented as outlier box plots showing medians as the thick lines, first and third quartiles as boxes, and 1.5 times the interquartile range as whiskers. There was a significantly higher proportion of ‘B’ elements in male-directed than in female-directed warbles, but there were no significant differences in any of the other 11 measured parameters.

(2011), with a duration of at least one second. Warble elements separated by less than one second of silence were considered to be part of the same warble bout. A total of 193 female-directed and 236 male-

directed warble bouts were obtained from seven males over 853 h 51 min of recording for a total of 429 bouts. This resulted in an average of 27.6 female-directed and 33.7 male-directed bouts per individual.

Table 2
Factor loading scores for each warble measure resulting from the Principal Component Analysis. Variable loadings > |0.3| (absolute value) are bolded.

Measures	PC1	PC2	PC3
Duration (sec)	-0.390	0.135	-0.162
# of Total Elements	-0.407	-0.193	-0.058
Diversity Index	-0.057	0.644	0.360
Total Alarm Call-like Elements (A)	-0.163	0.368	-0.212
Total Contact Call-like Elements (B)	-0.242	0.348	-0.260
Total Long Harmonic Call Elements (C)	-0.277	0.159	-0.075
Total Short Harmonic Calls (D)	-0.357	-0.371	-0.119
Total Noisy Calls (E)	-0.310	-0.038	0.122
Total Clicks (F)	-0.290	-0.007	0.449
Total Pure Tone-like (G)	-0.105	-0.066	0.691
Total Compounds (H)	-0.253	0.194	-0.111
Total I Elements (I)	-0.367	-0.259	0.041
Standard Deviation	2.345	1.251	1.090
Proportion of Variance	0.458	0.131	0.099
Cumulative Proportion	0.458	0.589	0.688

Table 3
Results from the nested ANOVA performed on factor scores resulting from the Principal Component Analysis.

PCA	Female Mean ± SE	Male Mean ± SE	DF	F-Ratio	P-value
PCA 1	-0.327 ± 0.394	0.173 ± 0.388	1,11.5	0.818	0.384
PCA 2	-0.180 ± 0.168	0.067 ± 0.164	1,11.3	1.105	0.315
PCA 3	0.205 ± 0.177	-0.101 ± 0.174	1,11.1	1.526	0.242

2.4. Classification of warble elements by visual inspection

We classified the different warble elements using visual inspection of spectrograms and the element categories defined by Tu et al. (2011) and Farabaugh et al. (1992). The warble elements (A–G) (Fig. 2) were categorized following Tu et al. (2011) and compounds or H elements following Farabaugh et al. (1992). We also identified a new warble element category (“I”) (Fig. 2). This type of element is a quick repetition of primarily “D” elements and occasionally “E” and “F” elements that together make a trill-like sound. We recorded the following parameters for each warble: total duration (seconds), total number of elements, the Shannon-Weiner Diversity Index (a measurement of the diversity of elements within a given warble that incorporates both richness of elements in a song and the evenness of their representation– the higher the value of this index, the more diverse the song), and proportion of Alarm Call-like (A) Elements, Contact Call-like (B) Elements, Long Harmonic Calls (C), Short Harmonic Calls (D), Noisy Calls (E), Clicks (F), Pure Tone-like (G) Elements, compounds (H), and “I” Elements.

2.5. Statistical analyses

A Principal Component Analysis (PCA) was conducted to identify correlations among measures of song structure. The PCA was conducted in R version 3.2.1 (R Core Team, 2013, <http://www.r-project.org>). For this analysis, each warble bout was included along with its corresponding individual ID and audience sex. Since the resulting first three components explained 68.83% of the variance, they were retained for further analysis to compare female-directed versus male-directed warbles.

A nested ANOVA was conducted to determine if there were significant differences in warble characteristics between female-directed and male-directed warbles. Individual ID was entered as a random factor nested within the sex of the audience, which was entered as a fixed factor. An ANOVA was performed with each one of the structural measures described above as the dependent variable. We also ran nested ANOVAs on the first three principal components from the PCA on measures of song structure, entering these principal components as

the dependent variables. To identify specific differences *p*-values smaller than 0.05 were considered significant. These analyses were conducted with JMP statistical software (JMP®, Version 13. SAS Institute Inc., Cary, NC, 1989–2007).

We used time-window lagged sequential analysis to examine the sequential organization of warbles and whether it differed when delivered to different audiences (Bakeman and Quera, 2011). Our analysis focused on identifying *diagram sequences* where the occurrence of a specific element (at lag 0) was directly followed by another element (at lag 1). For example, a warble stream for one warble might consist of two types of elements (A and B) looking like this, “A B B B A A.” In this example, the diagram sequential pattern A to B, and B to A, occurred only once, while the diagram sequential pattern B to B and A to A each occurred two times. A *transition matrix* can then be constructed that captures the entire diagram sequences observed in each warble. This analysis was conducted in R using the package *markovchain* (Spedicato et al., 2016), *statnet* (Handcock et al., 2008), and custom designed functions.

The first step of this analysis was to see if budgerigar warbles exhibited significant temporal organization. To do this we converted each warble sequence into a transition matrix that contained all transitions between all elements in the warble. For each individual, we summed the transition matrices for all their warbles, and then divided each cell of the matrix by the sum of the row in order to get a *transition probability matrix*. The transition probability matrix contains the probability of observing each diagram sequence. Likelihood-ratio tests were then used to assess if the observed transition probability matrices were different from those expected if the elements were evenly distributed (Bakeman and Quera, 2011; Cornwell, 2015; Gottman and Roy, 1990).

The next step in this analysis was to find which dyadic sequences are significantly different from expected sequences. Here we summed the transition matrices for the warbles directed at males or females for all individuals. An expected distribution was created that assumed independence between all warble elements (Bakeman and Quera, 2011). To test for the difference between the observed and expected sequences we calculated a *z* score for each dyadic sequence in the transition matrix using Eq. (1).

$$Z_{[i,j]} = \frac{X_{i,j} - E_{i,j}}{\sqrt{E_{[i,j]}(1 - P_{row[i,j]})(1 - P_{col[i,j]})}} \tag{1}$$

Where $X_{[i,j]}$ is the observed dyadic sequences between warble elements, $E_{[i,j]}$ is the expected dyadic sequences, $P_{row[i,j]}$ is the adjusted residual for the row of the matrix, and $P_{col[i,j]}$ is the adjusted residual for the column of the matrix. These *z* scores were then translated into *p* values with a Bonferroni correction for multiple comparisons. This analysis identifies which dyadic sequences occurred above or below chance level (Bakeman and Quera, 2011).

To investigate the similarity in the sequential patterns across individuals we used structural graph correlations. This procedure uses resampling techniques to create a row-wise Pearson correlation between two different matrices, with higher correlation coefficients indicating that individuals exhibited similar sequential organization in their warbles. Structural graph correlations were used to compare the transition probability matrices across all individuals. Conditional uniform graph tests (*cugtest* in *statnet*) were used to assess the significance of the structural correlations (Butts and Carley, 2001; Handcock et al., 2008). Permutation based Wilcoxon-signed rank tests were then used to compare the correlation coefficients for female directed warbles with male directed warbles. Significant differences would indicate that all individuals exhibited greater similarity in the sequential organization of warbles when they were directed at a particular sex. For the sequential analysis, only six birds were included in the sample because element sequence data was not available for the seventh bird.

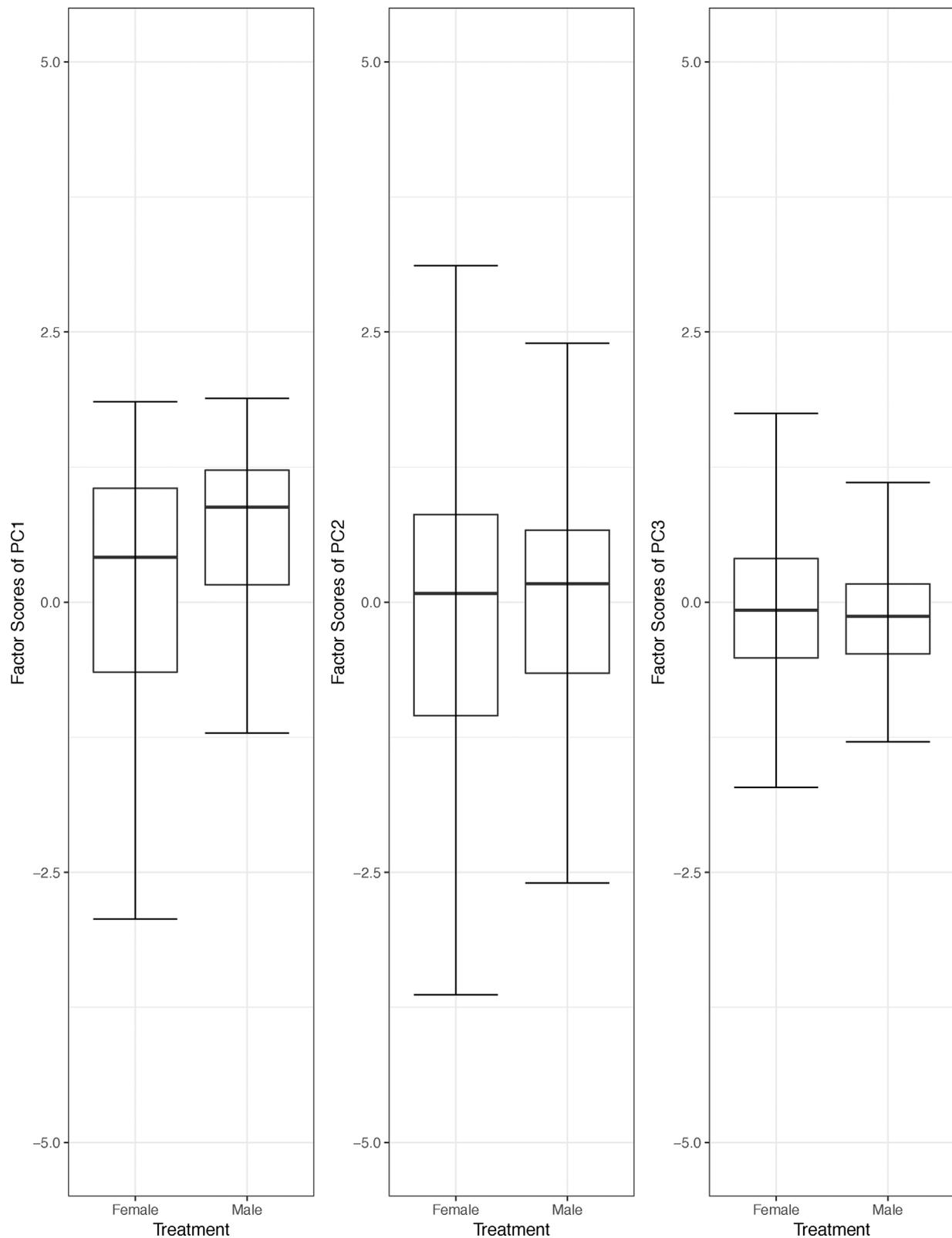


Fig. 4. Comparison of factor scores from Principal Component Analysis on warble parameters measured on 193 female-directed warbles and 236 male-directed warbles recorded from seven birds. Data are represented as outlier box plots showing medians as the thick lines, first and third quartiles as boxes, and 1.5 times the interquartile range as whiskers. There were no significant differences between female-and male-directed warbles in the first three PCA factors.

3. Results

3.1. Warble rates and audience composition

Spontaneous warbling was common in a mixed-group aviary setting.

Over 32 h of observation, warbles were produced by males when in close proximity to other females at a rate of 17.3 warbles/h when in proximity to other males at 12.8 warbles/h, when multiple birds were present at a rate of 3.8 warbles/h and when no other birds were in close proximity at a rate of 21.7 warbles/h.

Table 4

Results of Transition-Probability matrices for each individual male, showing that sequential organization significantly differs from random expectations. * Indicates significant transitions at alpha 0.05.

Likelihood-ratio	Individual	G	df	p
	Big John	228.72	36	< 0.0001*
	Doc	180.45	36	< 0.0001*
	Gilbert	73.736	36	0.0002
	Mar	272	36	< 0.0001*
	Neptune	311.14	36	< 0.0001*
	Pavarati	110.97	36	< 0.0001*

3.2. Compositional differences between audiences

The ANOVAs examining compositional differences between male- and female-directed warble revealed only one marginally significant difference: warbles directed at males contained a higher proportion of contact call-like (B) elements than did warbles directed at females ($F_{(1,12.3)} = 4.772$, $P = 0.049$). There was no significant differences between female- and male-directed warbles in duration, number of total elements, the Shannon-Weiner index, proportion of alarm call-like (A) elements, proportion of long harmonic calls (C), proportion of short harmonic calls (D), proportion of noisy calls (E), proportion of clicks (F), proportion of pure tone-like (G), proportion of total compounds (H), and proportion of total I elements (Table 1 and Fig. 3).

The first three principal components explained 68.8% of the variance (Table 2). We did not find significant differences between female-directed and male-directed warbles with any of the nested ANOVAs performed on the first three principal components (Table 3 and Fig. 4).

3.3. Syntactical differences between audiences

For all six individuals examined, we found significant organization in the transition-probability matrices for both female- and male-directed warbles (Table 4), indicating that the overall sequential organization of warbles was significantly different from random expectations.

The majority of dyadic sequences observed were also significantly different from expected. (Table 5), indicating that most transitions between different warble elements showed significant organization. This organization was similar across male- and female-directed warble, with only two dyadic sequences (c → d, f → e) reaching significance in female-directed warble that did not also reach significance in male-directed warble (Fig. 5).

Males were more similar to each other in the syntax of their warbles when they were directed at females than when they were directed at males. We found significant between-individual correlations in the transition-probability matrices for both warbles directed towards females (Table 5a, c) and warbles directed towards males (Table 5b, d), indicating that the sequential organization of warbles was similar across individuals. Nonetheless, individuals exhibited greater between-individual structural correlation coefficients when warbles were directed towards females than males, indicating a greater amount of between-individual similarity in female directed warbles than male directed warbles (Median female-directed correlation coefficient = 0.71, Median male-directed correlation coefficient = 0.55, Wilcoxon-signed rank test: $Z = 2.52$, $p = 0.01$, Fig. 6).

4. Discussion

In this study, we compared the structure of the budgerigar warble

Table 5

Transition Matrix (a and b) and Z-score Matrix for Dyadic Sequences (c and d) for female- and male-directed warbles. Values represent totals for all males combined.

Note Types	A	B	C	D	E	F	G
a. Transition Matrix for Female-Directed Warble							
A	4	13	11	16	9	2	0
B	14	118	42	126	99	17	0
C	2	56	64	128	31	17	1
D	6	163	123	2174	232	137	6
E	19	76	30	253	234	66	2
F	1	33	16	131	56	56	2
G	0	0	1	7	0	3	0
b. Transition Matrix for Male-Directed Warble							
A	7	12	1	17	7	5	0
B	18	300	96	177	89	25	1
C	7	58	60	134	20	2	1
D	9	221	92	1687	175	107	2
E	4	89	14	190	114	31	0
F	1	42	6	90	25	48	1
G	0	1	1	2	1	0	1
c. Z Score Matrix for Dyadic Sequences for Female-Directed Warble							
A	6.84*	6.36*	-2.92*	-27.06*	-1.16	2.48	-0.05
B	13.63*	309.64*	82.17*	-194.86*	27.49*	-3.27*	0.40
C	3.95	34.79*	46.41*	-93.9*	-29.06*	-21.93*	0.18
D	-40.53*	-113.46*	-166.7*	-236.58*	-421.86*	-126.73*	-7.99
E	-4.67*	27.6*	-36.27*	-396.26*	-2.82*	-16.38*	-1.37
F	-2.06	17.89*	-17.21*	-156.18*	-23.49*	26.21*	-0.42
G	-0.05	0.40	0.18	-8.00	-0.21	-1.55	0.94
d. Z Score Matrix for Dyadic Sequences for Male-Directed Warble							
A	6.88*	3.42*	-3.62*	-20.36*	2.21	2.69	-0.08
B	10.72*	155.55*	60.43*	-490.59*	6.57*	-15.8*	0.23
C	3.27*	12.77*	42.2*	-56.46*	-13.91*	-16.97*	0.59
D	-33.9*	-409.3*	-129.64*	945.41*	-139.34*	-30.98*	-2.54
E	-1.32	6.59*	-21.15*	-112.87*	78.67*	7.91*	-0.86
F	-1.87	5.41*	-12.25*	-60.49*	0.71	41.19*	0.70
G	-0.08	0.23	0.59	-2.55	0.32	-0.44	1.09

Note: * indicates significant z score with Bonferroni adjustment for multiple comparisons.

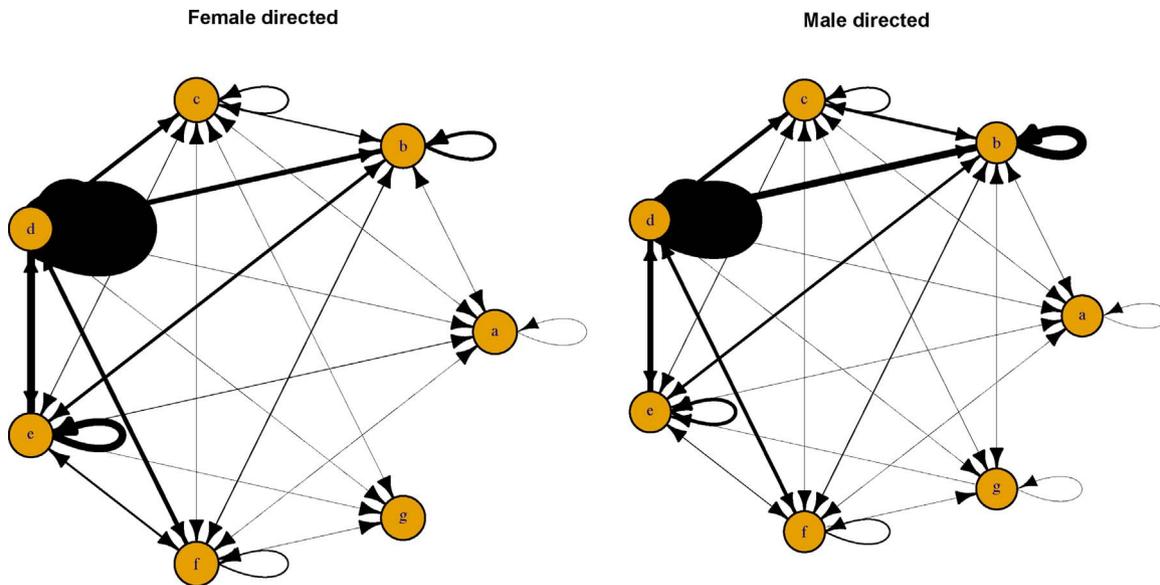


Fig. 5. Visualization of the dyadic sequences for female- and male-directed warbles for all males combined. Arrows represent transitions from one note type to another, with thicker arrows representing more frequent transitions. The particularly thick circular arrow next to d elements represents the high frequency in which d notes were followed by other d notes in warble sequences. While the syntactical patterns were generally similar in male- and female-directed warbles, two dyadic sequences, (c → d) and (f → e), were significantly more frequent than chance in female-directed warbles, but in not male-directed warbles.

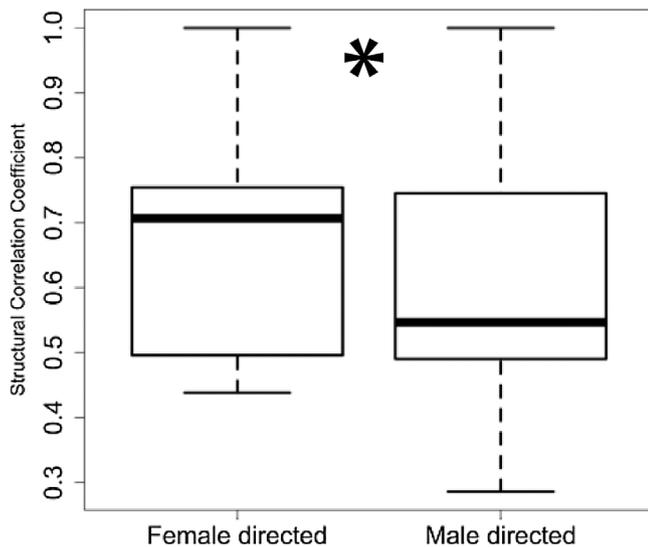


Fig. 6. Graphs of the structural correlation coefficient values that compare between-individual similarity in sequential organization for female-directed and male-directed warbles. For each class of warble there are 15 unique comparisons across all the individuals. Data are represented as outlier box plots showing medians as the thick lines, first and third quartiles as boxes, and 1.5 times the interquartile range as whiskers. Female-directed warbles are significantly higher in between-individual similarity than to male-directed warbles (Wilcoxon-singed rank test: $Z = 2.52$, $p = 0.01$) as indicated by asterisk above the contrast.

song when directed towards males versus females. We did not find significant differences when examining compositional measures such as total duration, proportion of element types, and element diversity, with the exception of one measure, the proportion of contact-call like ‘B’ elements. However, when examining the sequential organization of warble element types (syntax), we did find differences between female-directed and male-directed songs in their consistency across individuals. Below we discuss these results in more detail and their implications for the function of warble song in budgerigars.

We found strong audience effects on budgerigar warble only at the level of syntax. First, we found that the overall sequential organization of warble elements was significantly different than random, confirming

that these complex vocalizations do have a syntactical organization, a result previously suggested by perceptual studies using operant conditioning approaches (Tu and Dooling, 2012). We also found that the sequential organization of female- and male-directed warbles tends to be similar across individuals. Even though similarity in elemental ordering was exhibited by both female- and male-directed warbles, female-directed warbles tended to be less variable in elemental ordering as indicated by the higher structural correlation coefficient values. Specifically, female-directed warble bouts tend to have long harmonic call elements (C) followed by short harmonic calls (D) and clicks (F) followed by noisy calls (E). Overall, these results suggest that the audience towards which a warble is directed does affect the production of warbles, with less variation in warbles directed towards females than in warbles directed towards males.

The greater syntactical consistency in female-directed than in male-directed warble suggests that different intended receivers may exert different selective pressures on the form of warble. One possibility is that females potentially prefer specific types of element sequences, and thus shape the organization of warbles to greater extent than males. Future studies could test such female preferences with playback experiments that expose females to the specific elemental transitions that occur more often in female-directed warbles in playbacks of either of the same elements in different orders each time, or of consistent strings of different elements. Alternatively, females may have a preference for consistency itself. Differences in the relative consistency of song in different contexts are seen in zebra finches, *Taeniopygia guttata*, in which males singing in the presence of females sing highly stereotyped songs while those singing in isolation sing more plastic song (Ölveczky et al., 2005; Sossinka and Bohner, 1980). This undirected song by male zebra finches has been suggested to be a form of vocal practice that improves the ability of males to sing stereotyped song when directing it to females (Miller et al., 2010); male-directed warble could play a similar role in budgerigars.

This study did not directly examine warble function but we can suggest possible functions based our results and previous studies on budgerigar warble. Our finding that male budgerigars are more consistent in the sequential organization of warble element types when singing to females is consistent with the idea that females exert stronger directional selection of some form on warble organization, perhaps through a mating preference. Such a preference might exist if females

use warble consistency to assess the overall quality or cognitive abilities of a male (A. Medina-García and T. Wright, unpublished results). This hypothesis could be tested with playbacks as discussed above, or by examining mating success of males who produce warble with different levels of consistency. Studies of warble development would also be of value to examine whether females shape the development of warble structure in juvenile males as found in the brown-headed cowbird, *Molothrus ater* (West and King, 1988). Alternatively, or additionally, more consistent warble may serve as a better stimulus of females during budgerigar courtship; Brockway (1965) showed that warble stimulates female ovarian development and nesting behavior within budgerigars.

It is unclear whether warble structure is less consistent when directed at males because consistency is less important for communication among males, or because variable warbles are actually more salient signals to male receivers. There is some evidence that male-directed warble stimulates male reproductive behavior and physiology in group-living budgerigars, just as female-directed warble stimulates female reproduction (Brockway, 1964c, 1965). Budgerigars are nomadic and opportunistic breeders within their arid natural range in central Australia. Warble may be an aid in promoting coordinated breeding conditions among all members of the colony during a short window of opportunity (Brockway, 1964b, 1969; Wyndham, 1980a,b); such simultaneous breeding by multiple pairs within an ephemeral colony would be advantageous if it reduces predation risk during a compressed window of breeding opportunity. Further studies could test whether males exposed to different levels of variability in the sequential ordering of elements within warbles exhibited different effects on breeding physiology and behavior.

This study provides a foundation for future research examining audience effect in budgerigar warble song. Future research that aims to identify the function of warble song could incorporate consistency in the sequential organization of warbles, and potentially the presence or absence of key elemental sequences, as key variables when testing functional hypotheses. Studying budgerigar warble within more naturalistic social contexts, and employing technology such as wireless microphones (Couchoux et al., 2015; Ter Maat et al., 2014), as well as analytical approaches like social network analysis (Hobson et al., 2015; Kohn et al., 2015), would also be logical next steps in understanding its function. In sum, these results reinforce the idea that examining audience effects on signal variation is a key step in understanding the function of complex and variable animal signals.

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