

Male mate preferences in mutual mate choice: finches modulate their songs across and within male–female interactions



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Male songbirds use song to advertise their attractiveness as potential mates, and the properties of those songs have a powerful influence on female mate preferences. One idea is that males may exert themselves maximally in each song performance, consistent with female evaluation and formation of mate preferences being the primary contributors to mate choice. Alternatively, males may modulate their song behaviour to different degrees in the presence of different females, consistent with both male and female mate preferences contributing to mutual mate choice. Here we consider whether male Bengalese finches, *Lonchura striata domestica*, express mate preferences at the level of individual females, and whether those preferences are manifest as changes in song behaviour that are sufficient to influence female mate choice. We tested this idea by recording songs performed by individual unmated males during a series of 1 h interactions with each of many unmated females. Across recording sessions, males systematically varied both the quantity and the quality of the songs that they performed to different females. Males also varied their song properties throughout the course of each interaction, and behavioural tests using female birds revealed that songs performed at the onset of each interaction were significantly more attractive than songs performed by the same male later during the same interaction. This demonstration of context-specific variation in the properties of male reproductive signals and a role for that variation in shaping female mate preference reveals that male mate preferences play an important role in mutual mate choice in this species. Because these birds thrive so well in the laboratory and are so amenable to observation and experimentation across generations, these results yield a new model system that may prove especially advantageous in disentangling the role of male and female mate preferences in shaping mutual mate choice and its long-term benefits or consequences.

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Male song is a primary reproductive signal in songbird mate choice. Many studies have documented the importance of male song in female mate choice (Collins, 2004; MacDougall-Shackleton, 1997; Riebel, 2009; Searcy & Yasukawa, 1996; Tomaszycski & Adkins-Regan, 2005). In some cases, song can have such a profound influence that females will solicit copulation in response to song alone, even if no male is present (Dunning, Pant, Bass, Coburn, & Prather, 2014; Nagle, Kreutzer, & Vallet, 2002; Searcy, 1992; West & King, 1988b). These data have led to the idea that males use song to advertise their attractiveness as potential mates, and females evaluate those songs and use that information to choose their mate. In that light, it is tempting to conclude that the decision

of mate choice is made by the female, but it is not only female preferences that contribute to mate choice. Although the importance of female mate preferences has long been appreciated (Darwin, 1871), more recently we have also come to appreciate the importance of male mate preferences in mutual mate choice (reviewed in: Bonduriansky, 2009; Clutton-Brock, 2009; Edward & Chapman, 2011).

Theorists have posited that male mate preferences, evident as systematic differences in the quantity or quality of courtship behaviour that a male uses to court some females more than others, should be most evident in species in which (1) males encounter females simultaneously rather than sequentially, (2) males invest considerable resources in their displays to increase the number of females available for mating, (3) there is variation in female quality and the benefits of mate choice (male mate choice also occurs in species in which males do not make a significant contribution to offspring care; Amundsen, 2000; Amundsen & Forsgren, 2001; Bonduriansky, 2001) and (4) there is variation in the strength and

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direction of an individual male's mating preferences (reviewed in [Edward & Chapman, 2011](#)). Songbirds are excellent subjects for studies of male mate preferences because many species, including the Bengalese finches, *Lonchura striata domestica*, studied here, are highly social and live together in dense colonies. Therefore, males commonly encounter many females simultaneously. Males of those species also sing frequently throughout the day and across many different social contexts, and song is a primary means through which females select their mates ([Collins, Hubbard, & Houtman, 1994](#); [Dunning et al., 2014](#); [Woolley & Doupe, 2008](#)). Thus, males invest considerable time and energy in their displays to increase the number of females available for mating. In addition, there can be stark differences in the number of offspring produced by different mated pairs ([Godfray, Partridge, & Harvey, 1991](#); [Martin, 1987](#); [Winkler & Walters, 1983](#)), consistent with variation in female quality and the benefits of mate choice. What remains unknown, however, is the degree to which individual males vary in their mate preferences, whether those preferences are of sufficiently high resolution that males express preferences for individual females and whether the behavioural manifestations of such preferences achieve functional significance by influencing female mate choice.

Previous studies of male songbirds reveal at least some contribution of male mate preferences in courtship behaviour. In studies of zebra finches, *Taeniopygia guttata*, another highly social species that lives together in dense colonies, males prefer females that are in especially good condition, reflecting the benefits obtained from a richly supplemented diet and predictive of greater fecundity ([Jones, Monaghan, & Nager, 2001](#); [Monaghan, Metcalfe, & Houston, 1996](#); [Wynn & Price, 1993](#)). The male's preference is evident in a greater amount of courtship behaviour directed towards females that are in good condition (e.g. approaching and lingering near the preferred female, etc.; either song was not considered apart from other courtship behaviours ([Monaghan et al., 1996](#)), or there was no difference in the amount of song performed to the female of either condition ([Wynn & Price, 1993](#))). These data reveal that males can express a preference between categories of female quality, but they leave open the question of whether males modulate their song behaviour accordingly and whether they express that modulation as a function of individual female identity. In support of the possibility that males may modulate their songs to different degrees in the presence of different females, males of some species, including Bengalese finches, modulate their song properties when they sing directly to a female ('directed song') as opposed to when they sing alone ('undirected song') ([Sakata, Hampton, & Brainard, 2008](#); [Sossinka & Bohner, 1980](#)). Females can detect those subtle differences and prefer directed songs more than the undirected songs performed by the same male ([Dunning et al., 2014](#); [Woolley & Doupe, 2008](#)). Those data reveal at least some role for male song variation in shaping mate choice, but they do not address the resolution at which males express their mate preferences. It is known that males can modulate their behaviour based on female identity, as males perform greater amounts of song and express less aggression towards mates than they do towards nonmates ([Caryl, 1976](#); [Silcox & Evans, 1982](#)) and females prefer their mate's songs ([Miller, 1979](#); [O'Loughlin & Beecher, 1996](#)). However, those data do not address the important question of whether male song modulation is also evident at the level of individual females in the pre-mated condition and whether that modulation is sufficient to bias the mate preferences of unmated females. Detection of such changes in male behaviour and a role for those changes in affecting female mate preference would reveal that male mate preferences also greatly influence mutual decisions of mate choice.

Our goals in this study were to determine whether unmated adult male Bengalese finches modulate the quantity and the quality

of their songs to different degrees in different pairings with unmated adult females, and to determine whether those song changes achieve functional significance by affecting female mate preferences. To address our hypothesis that males vary their reproductive signalling when singing to each of many different females, we recorded males singing either alone or with each of many different females. We quantified the number of songs performed in each context, and we analysed the properties of those songs by computing their note transition entropy, a measure of note sequence complexity that has been shown previously to change between the directed and undirected conditions ([Sakata et al., 2008](#)) and that has been implicated as a possible contributor to female evaluation of song quality ([Dunning et al., 2014](#)). Differences in these parameters among songs performed by an individual male in different male–female contexts would support the idea that male Bengalese finches express mate preferences at the level of individual females and manifest those preferences as systematic variation in not only the quantity but also the quality of their reproductive signalling. To test our hypothesis that differences between songs recorded in different contexts are sufficient to affect female mate preferences, we used the songs performed by an individual male in different male–female contexts to test whether females could distinguish between those songs and whether they expressed a systematic preference for one or the other song variant. Detection of a systematic preference would reveal an important role for male mate preferences for individual females as a contributor to mutual mate choice and the control of vocal behaviour.

METHODS

Care and Handling of Experimental Subjects

We performed all experiments using adult (age > 120 days posthatch) male and female Bengalese finches obtained from a commercial breeder or from our breeding colony. Prior to experimentation, we identified males by their song performance and females by the presence of calls but the absence of song over 3 or more days of continuous recording. We housed birds in an aviary colony setting prior to experimentation (15:9 h light:dark photoperiod). During recording, we housed birds in a wire mesh cage (41 × 31 × 24 cm) inside a sound attenuation chamber (Industrial Acoustics Co., Bronx, NY, U.S.A., model MAC-1).

Ethical Note

We monitored the birds daily and provided seed and water ad libitum. We also minimized the number of times that birds were moved between cages and the duration of handling in each case. These measures ensured that the birds were treated as well as possible given the constraints of the experimental design. Consistent with that goal, all birds remained healthy and robust throughout the study. All procedures were approved by the University of Wyoming Animal Care and Use Committee (protocol number 20140506JP00106-02), were consistent with the ABS Guidelines for the Use of Animals in Research, and were in compliance with all state and federal regulations governing the housing and use of songbirds.

Recording Male Songs Alone or in the Presence of Different Females

Procedures were similar to those reported previously ([Dunning et al., 2014](#)). Briefly, we placed a microphone inside the sound-attenuating chamber and recorded continuously ([Tchernichovski, Nottebohm, Ho, Pesaran, & Mitra, 2000](#)). We band-pass filtered

all recordings (300–10 000 Hz) and identified song notes (note durations typically 20–100 ms; e.g. Fig. 1a–c). A song comprised a series of notes in which no internote interval exceeded 500 ms (Sakata et al., 2008). We screened all recordings offline to ensure they were not corrupted by the presence of female calls or sounds associated with movements such as hops or wing flaps (custom software, MatLab, J. F. Prather).

We introduced female birds one at a time into the recording chamber. A wire-mesh cage divider bisected the cage, ensuring that the male and female remained in separate halves (seed and water were available ad libitum in each half). We placed a camera (General Electric model 45231) in the chamber to record male behaviours associated with singing. The experimenter could see the male bird and the female bird, when she was present, at all locations within the recording chamber, enabling reliable identification of songs directed towards the female receiver ('directed song') and songs performed with no apparent relation to the female receiver or when the female was absent ('undirected song'). Directed songs were accompanied by the male pointing its beak at the female, leaning forward and singing directly at the female (compare with Williams, 2001). All songs classified as undirected were recorded when the male was alone.

Experimental Design: Quantifying Male Song Performances

We moved the male from the colony to the recording chamber, allowed it to acclimate for 1 day, then recorded undirected song for 1 day ($N = 20$ birds; Fig. 1d). We then tested the male in a series of three trials in which we recorded directed songs while the male was paired with a series of females. Between each day of directed signing, we allowed the male a full day of residing in the recording chamber alone (Fig. 1d). In the three trials of female-directed singing, each trial consisted of six contiguous 1 h interactions with each of six different female Bengalese finches, after

which the male was housed in the recording chamber alone for the remainder of the day. The first female was introduced 30 min following lights on, and testing lasted until 6 h and 30 min after lights on (six 1 h interactions). We used the same set of six female birds in each of the three trials for each male bird, and we used different females in tests of different males (a total of 11 female birds, with each female presented to a mean \pm SE of 3.27 ± 0.65 male subjects, range 1–8 males per female). We pseudorandomized the sequence in which the females were introduced during each day of directed singing, with the only requirement being that no female could be presented first or last in more than one trial with the same male. This was done to minimize the effects of circadian factors on song properties, behavioural motivation (Deregnacourt, Mitra, Feher, Pytte, & Tchernichovski, 2005; Wang, Harpole, Trivedi, & Cassone, 2012; Wood, Osseward, Roseberry, & Perkel, 2013) or possible effects of recent experience with a specific female (e.g. as could happen if female B always followed female A). Thus, we recorded each male bird over a span of 15 h in the undirected condition (15 h of lights on during 24 h of recording the bird in the recording chamber alone) and 18 h spanning three 1 h interactions with each of six different female birds ($N = 20$ male birds).

Males commonly sang many songs to some females but very few songs to others. To determine the influence of each female on each male's song performance and to permit reliable quantification of song features, we enforced the criterion that each male must sing at least 15 undirected songs and eight directed songs in each interaction with at least one female bird to be included in this analysis ($N = 15$ of 20 male birds and 39 male–female pairings were valid for further analysis). To discern the degree to which male Bengalese finches modulate their songs in the presence of different females, we counted the number of songs performed by each male either alone or to each of many different female receivers and quantified the note transition entropy of each song.

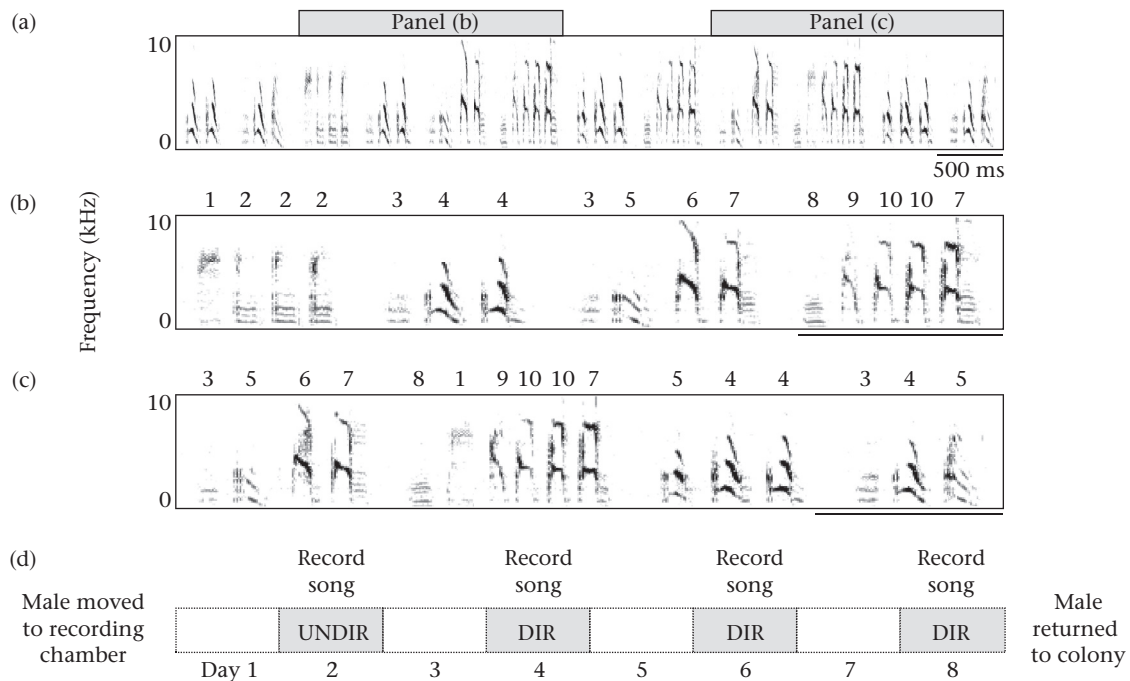
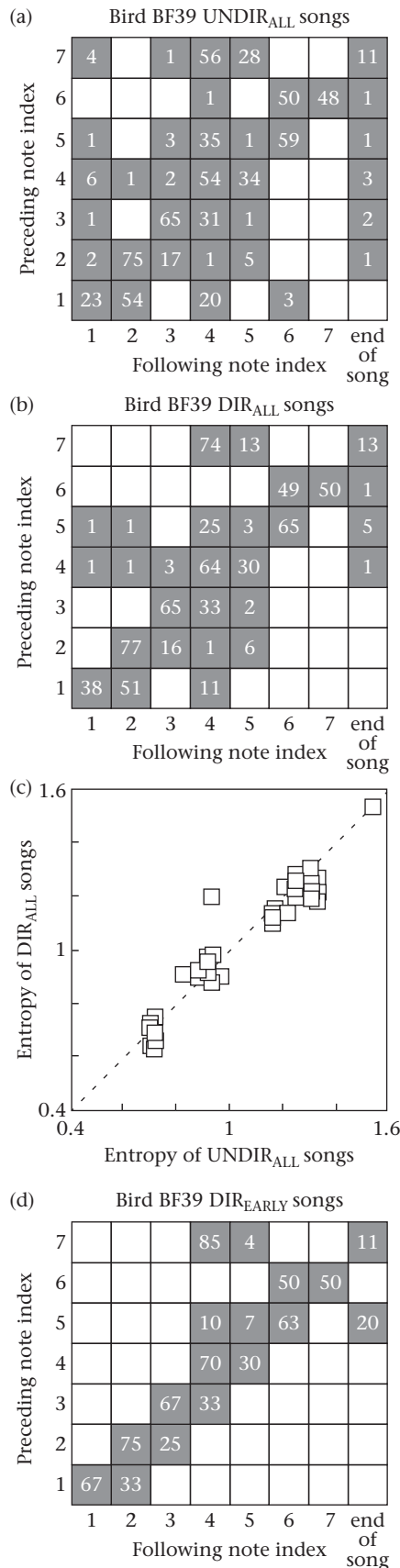


Figure 1. (a) Bengalese finch song consists of a series of notes, shown as a spectrogram: amplitude is reflected in the darkness of each note; grey boxes indicate song portions shown in (b) and (c). (b, c) The sequence in which notes are sung can be stereotyped in some portions and variable in other portions. For example, note 6 is always followed by note 7 in this and every song that we recorded from this bird, whereas note 3 can be followed by either note 4 or note 5. (d) We quantified the variability of note sequence in songs performed by each male throughout a series of recordings when the male was singing alone (undirected song, UNDIR) and when the male was singing to each of six different females (directed song, DIR).



Quantifying Song Properties: Rationale for Emphasizing Note Transition Entropy

In our analysis of the properties of songs performed in the undirected or directed context, we focused on note sequencing, and we quantified changes in note sequencing by computing the note transition entropy for songs performed in each context (Sakata et al., 2008; Scharff & Nottebohm, 1991). The rationale for this emphasis on transition entropy is that a previous study of changes in Bengalese finch song between the directed and undirected contexts revealed that changes were much larger in the temporal domain, as measured using note transition entropy, than in the spectral domain, as measured using features such as mean frequency of individual notes (Sakata et al., 2008). Looking beyond that especially relevant study, other data from our group suggest that temporal features of Bengalese finch song may be important in female evaluation of song quality (Dunning et al., 2014), and other groups have also suggested that sequence complexity may influence female evaluation of song quality of male Bengalese finches (Morisaka, Katahira, & Okanoya, 2008). Therefore, we chose to focus our analysis on changes in temporal properties, specifically on the change in note transition entropy.

Quantifying Song Properties: Computing Note Transition Entropy

Note types performed by an individual male Bengalese finch are conserved within and across songs (Okanoya, 2004). We identified note types using visual inspection of song spectrograms. We indexed each note using a number code (e.g. Fig. 1b, c), and notes of the same type received the same code (Hampton, Sakata, & Brainard, 2009; Okanoya, 2004; Sakata et al., 2008). We quantified song note sequences using a method used previously to quantify behavioural sequencing in Bengalese finch song (Hampton et al., 2009; Sakata et al., 2008; Yamashita et al., 2008). This analysis can reveal stereotyped song portions in which certain note types occur in fixed sequences (e.g. notes always occur in the sequence 1 → 2 → 3) and other portions of the song in which transitions following a specific note can proceed to one of many possible notes ('branch points'). In Fig. 2, such variable transitions appear as many shaded boxes in each row of the grid, with the number of boxes in each row indicating the number of different transitions following a specific note type. Using those summaries of note transitions, we computed note transition entropy. This index of song variability ranges from 0, indicating a syllable sequence that is completely fixed, to progressively larger positive values, indicating progressively more variable note sequences (Sakata et al., 2008; Scharff & Nottebohm, 1991). We computed entropy values for each song recorded when the male was singing alone and when he was singing to each of the females for which that male performed at least eight songs. We computed entropy for each song as follows.

$$\text{Transition entropy} = \sum p_i \times \log_2(p_i)$$

where the sum is over all note types present in the song, and p_i is the probability of the i th transition following that specific note (Sakata et al., 2008). Following previous methods of computing the

song (all note transitions, including stereotyped sequences, were included in this analysis). (c) Comparison of note transition entropy of songs performed in the undirected and directed contexts for all males and all male–female interactions. (d) Songs performed at the onset of each male–female interaction (DIR_{EARLY}) were typically quite different from those performed in the undirected context. This panel depicts a DIR_{EARLY} song performed by the same male depicted in (a) and (b). As elaborated in the text, note transitions present in DIR_{EARLY} songs were more stereotyped than those in UNDIR_{ALL} and DIR_{ALL} songs (this bird is one of 15 males quantified in (c)).

Figure 2. Summary of note transitions performed by male BF39 during a 1 h recording in the (a) undirected state (UNDIR_{ALL}) and (b) directed state (DIR_{ALL}, one interaction between this male and one female bird). Grey boxes indicate transitions in this male's

entropy of Bengalese finch songs, we considered cases in which one transition constituted more than 95% of all outcomes following a particular note type to be stereotyped sequences, and we excluded stereotyped sequences in our computations of entropy (Sakata et al., 2008). We did this to ensure that our measurements were directly comparable to previous data, and we found that this had no significant effect on the entropy values that we observed. For example, only 12% of the notes in the songs we quantified ($N = 13$ notes) were associated with stereotyped note transitions. The remaining 88% ($N = 95$ notes) were branch points (syllables that were followed by variable note transitions such as note 3 in Fig. 1b and c, following the definition used by Sakata et al. (2008), $N = 108$ notes across 15 males in the primary data set described in Results). Furthermore, the entropy of undirected songs was similar regardless of whether we did or did not exclude stereotyped cases (Mann–Whitney U test: $U = 0.33$, $N_1 = N_2 = 15$ birds, $P = 0.74$; UNDIR_{ALL} data in the primary data set described in the Results). For simplicity, all entropy values reported here were computed with stereotyped cases excluded as in Sakata et al. (2008).

Making a Distinction between Early and Late Song Performances

As detailed in the Results, our initial analyses revealed that entropy values of songs recorded in the directed condition were indistinguishable from entropy values of songs recorded in the undirected condition. This differs from the distinction between undirected and directed Bengalese finch songs reported previously (Sakata et al., 2008), but there were important methodological differences between that study and ours, most notably that Sakata et al. presented females by placing each female into a cage next to the male bird's cage for no more than 2 min. They recorded all songs that the male performed during those 2 min and then removed the female. After approximately 5 min, the same female was presented again, or another female was presented in the same way (Sakata et al., 2008). Thus, each male–female interaction was brief and was repeatedly renewed. In contrast, interactions in our study lasted for 1 h. To investigate the possibility that changes in directed song are expressed only during the early portion of each male–female interaction, we identified the first three directed songs that the male performed in each interaction (DIR_{EARLY}). We also computed the entropy of all directed performed during the remainder of each 1 h interaction (DIR_{LATE}) and the entropy of the complete set of all directed songs (DIR_{ALL}) in each interaction (DIR_{ALL} comprises both DIR_{EARLY} and DIR_{LATE}). To determine whether possible differences between DIR_{EARLY} and DIR_{LATE} song properties were due to normal fluctuation in song properties, we also computed the entropy of the first three undirected songs for each male (UNDIR_{EARLY}), the entropy of all undirected songs performed during the remainder of the undirected recording session (UNDIR_{LATE}) and the entropy of all songs recorded throughout the undirected recording session (UNDIR_{ALL}; UNDIR_{ALL} comprises both UNDIR_{EARLY} and UNDIR_{LATE}). Thus, for each bird we computed three entropy values for songs collected in the undirected condition (UNDIR_{EARLY}, UNDIR_{LATE}, UNDIR_{ALL}), and we computed another three entropy values for directed songs in each valid male–female interaction (DIR_{EARLY}, DIR_{LATE}, DIR_{ALL}). We quantified the behaviour of each male in the presence of each female using the means of those values compiled from the three interactions with each female bird.

Assessing Functional Significance of Differences between DIR_{EARLY} and DIR_{LATE} Songs

To investigate the functional significance of the song changes that we noted in DIR_{EARLY} songs, we used the directed songs

performed by an individual male in an individual male–female interaction as stimuli in behavioural tests of female song preference. We selected from our colony a group of adult female Bengalese finches ($N = 10$ birds) that did not include any of the females used to evoke directed song in the previous portion of this study. Therefore, there was no possibility that the female was responding to the features of a song that was directed towards her and thus could have been influenced by her preference or her behavioural responses.

Female Bengalese finches typically express a strong preference for the song of one individual when tested using an array of songs from many Bengalese finch males (Dunning et al., 2014). To account for this preference in these experiments, females were first tested to identify each female's most preferred song from among the 15 male birds quantified in the first part of this study (following the methods of Dunning et al., 2014). In tests of the functional significance of song changes that we noted in DIR_{EARLY} songs, we used stimuli consisting of (1) the three DIR_{EARLY} songs performed by each female's most preferred male in a randomly selected male–female pairing (i.e. randomly selected from among the set of all females that were effective in evoking song from that male) and (2) three directed songs recorded later during the same male–female interaction. To prevent confusion regarding this set of three songs versus the larger set of DIR_{LATE} songs from that interaction, we will refer to these songs as DIR_{LATE_3}. We selected the DIR_{LATE_3} songs pseudorandomly, screening only by duration so that the duration of DIR_{LATE_3} songs was closely matched to the duration of the corresponding DIR_{EARLY} songs. None of these songs had introductory notes, as introductory notes either were absent in the original song performance, or we excluded them when songs were assembled into the aggregate song stimulus. We created the aggregate DIR_{EARLY} and DIR_{LATE_3} stimuli by combining the three songs into one file (700 ms of silence inserted between each song and normalized by amplitude as in Dunning et al., 2014). The duration of those aggregate DIR_{EARLY} and DIR_{LATE_3} stimuli were very similar within each set of stimuli (ratio of DIR_{EARLY} to DIR_{LATE_3} duration: 0.99 ± 0.04 , $N = 7$ sets of stimuli; durations were indistinguishable in each condition: paired t test: $t_6 = 0.20$, $P = 0.85$) although durations varied among different sets of stimuli (mean \pm SE: 31.00 ± 3.05 s, range 14.5–54 s, $N = 7$ sets of stimuli).

In each test, we presented each female with three repetitions of the DIR_{EARLY} and DIR_{LATE_3} songs of her most preferred male and recorded the number of calls that the female produced during each song stimulus (20–25 s of silence between each stimulus, randomly specified by computer). Calls are a sensitive measure of female Bengalese finch mate preference (Dunning et al., 2014), and we compared the number of calls produced in response to each stimulus to investigate each female's possible preference for one of those song variants. We tested each female's preference across five tests of DIR_{EARLY} versus DIR_{LATE_3} songs of her most preferred male, and we quantified the female's preference by computing the difference between the percentage of responses evoked by DIR_{EARLY} songs versus the percentage of responses evoked by DIR_{LATE_3} songs. Using this metric, a positive significant value indicates a preference for DIR_{EARLY}, and a negative significant value indicates a preference for DIR_{LATE_3}.

Quantifying Song Properties for Stimuli Used in Tests of Female Song Preference

To investigate the degree to which differences not only in note transition entropy but also in other song properties are associated with differences in the attractiveness of male songs, we quantified an array of properties for each of the DIR_{EARLY} and DIR_{LATE_3} songs that we used in tests of female preference. Those parameters were

measured as described in Dunning et al. (2014) (summarized in Table 1), and that set of parameters is quite similar to those that we and others have used previously to quantify songs of Bengalese finch and other species (e.g. Dunning et al., 2014; Sakata et al., 2008; Sossinka & Bohner, 1980). Together with measures of note transition entropy, these metrics enable us to describe the songs used in tests of female preference comprehensively.

Statistical Analyses

In our analyses of male song performances, we used two-way ANOVAs to compare the entropy values of songs performed by each male in the undirected and directed states. We computed Pearson rank correlation coefficients to investigate the strength of correlation among entropy values recorded in specific conditions. In our tests of the number of calls that female birds produced in response to DIR_{EARLY} versus DIR_{LATE_3} songs, we used paired *t* tests to investigate whether the female expressed a significant preference for either set of stimuli. We computed Pearson rank correlation coefficients to investigate the degree to which female song preference may be related to each of 11 properties of the DIR_{EARLY} and DIR_{LATE} song stimuli. In all tests, significance was assessed at $\alpha = 0.05$, and results are reported as means \pm SE.

RESULTS

Males Vary the Amount of Directed Songs They Perform to Different Females

We compared the numbers of songs produced by each male in the presence of six different females ($N = 20$ males). Males typically

sang at least one song to each female (males performed at least one song to 5.47 ± 0.21 females, $N = 20$ males), revealing that males engaged in at least some courtship behaviour in nearly all interactions (90%, 324 of 360 interactions, $N = 20$ birds). Among the six females to which each male sang, the number of song performances varied dramatically (range 0–70 songs/1 h interaction, $N = 20$ birds). Males typically performed far more songs to one female than to any of the others. Specifically, the female that received the most songs from each male evoked $44 \pm 3\%$ ($N = 20$ birds) of all song performances recorded from that male, and the identity of that especially provocative female varied across male singers (11 different females were identified as the most preferred female, $N = 20$ males). In addition, approximately one quarter of all interactions consisted of less than two song performances during the entire hour (28%, 101 of 360 interactions, $N = 20$ birds). These performances commonly occurred soon after the female was introduced, but they could occur at any point throughout the 1 h interaction. Together, these data reveal that the song performance of an individual male varied across different male–female pairings and that the effects of those contexts could be quite dramatic.

To ensure that subsequent analyses of song properties were based on sufficient data to permit reliable quantification of those properties, we enforced the criterion that an individual male must sing at least 15 undirected songs and eight directed songs in each interaction with at least one female bird in order to be included in the data set. With this criterion, 15 male birds were valid for further analysis, and the characteristics of those birds were broadly similar to the full set of 20 birds. Specifically, these 15 males sang to 5.60 ± 0.19 of the six females with which they were paired, performing at least one song in nearly all interactions (94%, 254 of 270 interactions). Within interactions that passed our criterion of eight

Table 1
Properties of songs performed by male Bengalese finches in the undirected context (UNDIR_{ALL}), during the early portion of a male–female interaction (DIR_{EARLY}) and later during the same interaction (DIR_{LATE_3})

	Tempo	Mean frequency	<i>N</i> different note types	Spectral entropy	Sequence				<i>N</i> epochs of note repetition	<i>N</i> repeated notes per epoch	% Song duration comprising note repetition
					Entropy	Linearity	Consistency	Stereotypy			
Male 1											
UNDIR _{ALL}	12.14	3973	7	−3.66	1.25	0.27	0.50	0.38	18	1.57	34
DIR _{EARLY}	13.24	3906	7	−3.68	0.97	0.31	0.57	0.44	15	1.82	38
DIR _{LATE_3}	13.35	3064	7	−1.88	1.10	0.29	0.52	0.40	15	1.63	32
Male 2											
UNDIR _{ALL}	12.66	3630	6	−2.14	1.33	0.33	0.58	0.45	18	1.89	44
DIR _{EARLY}	14.89	3863	6	−1.91	0.93	0.43	0.63	0.53	12	1.87	51
DIR _{LATE_3}	13.17	3799	6	−1.97	0.92	0.41	0.64	0.54	14	2.04	51
Male 3											
UNDIR _{ALL}	9.37	3221	6	−1.58	1.21	0.45	0.63	0.54	9	1.13	18
DIR _{EARLY}	8.41	3000	6	−1.57	1.17	0.49	0.58	0.54	3	1.00	15
DIR _{LATE_3}	8.93	3211	6	−1.48	1.31	0.45	0.63	0.54	7	1.07	15
Male 4											
UNDIR _{ALL}	10.65	3567	8	−2.29	0.72	0.42	0.65	0.53	14	2.07	40
DIR _{EARLY}	10.64	3500	8	−2.15	0.57	0.43	0.64	0.53	11	1.73	29
DIR _{LATE_3}	10.23	3220	8	−2.25	0.71	0.43	0.63	0.53	11	1.81	32
Male 5											
UNDIR _{ALL}	8.34	3116	6	−2.46	0.94	0.34	0.54	0.44	10	1.16	19
DIR _{EARLY}	10.10	3439	6	−2.51	0.94	0.36	0.57	0.47	5	1.30	14
DIR _{LATE_3}	7.84	3520	6	−2.48	0.92	0.36	0.56	0.46	7	1.00	18
Male 6											
UNDIR _{ALL}	11.71	3485	4	−3.24	1.24	0.37	0.52	0.44	10	1.77	34
DIR _{EARLY}	11.24	3782	4	−3.18	1.14	0.41	0.59	0.50	7	1.89	36
DIR _{LATE_3}	11.51	3637	4	−3.16	1.26	0.38	0.61	0.49	12	1.90	41
Male 7											
UNDIR _{ALL}	11.96	3563	5	−2.09	0.92	0.40	0.60	0.50	20	1.43	36
DIR _{EARLY}	12.43	4950	5	−2.98	0.79	0.47	0.56	0.51	11	1.35	36
DIR _{LATE_3}	13.99	5116	5	−3.19	0.88	0.40	0.60	0.50	18	1.50	39

songs, the number of song performances varied widely (range 8–70 songs/1 h interaction). This subset of 15 males also tended to sing more to one female than to any of the others ($44 \pm 3\%$ of all songs to one female), and the identity of the most preferred female varied across singers (nine females were identified as the most preferred female). Each of these 15 males sang eight or more times in each interaction with 2.67 ± 0.25 different females (deemed valid male–female interactions, a total of 39 valid interactions across 15 male birds), and each female typically evoked 21.6 ± 3.9 songs/1 h interaction. We computed the remainder of these Results using this set of 15 males to investigate the effects of social context on the properties of male Bengalese finch song.

To begin to investigate possible differences between songs performed in the directed and undirected states, we compared the number of different note types (range 4–11) and the number of different types of note transitions (range 19–42) in the songs of each bird ($N = 15$ males). The number of notes and note transitions was not different across UNDIR_{ALL} and DIR_{ALL} contexts (Mann–Whitney U test: note types: $U = 0.02$, $N_1 = N_2 = 15$ males, $P = 0.98$; note transitions: $U = 0.17$, $N_1 = N_2 = 15$ males, $P = 0.86$). A repeated measures two-way ANOVA revealed no difference between the numbers of songs performed by each male to each female across the 3 days of testing ($F_{2,28} = 0.14$, $N = 15$ males, $P = 0.87$; [Supplementary Table S1](#)) and the identity of each male's most preferred female was consistent across trials (the most preferred female that received the greatest total number of songs for each male also received the greatest number of songs in 76% of all trials, $N = 15$ males). Consistent with reports from other species showing that song complexity is not systematically greater in longer songs ([Palmero, Espelosin, Laiolo, & Illera, 2014](#)), these data indicate that we adequately sampled the bird's repertoire in the songs that we recorded. Together with our randomization of the sequence in which females were presented, this absence of systematic changes across days indicates that changes in male song behaviour can be interpreted as indicative of mate preference and not a consequence of the order of testing. To investigate further the degree to which males may modulate their songs across these contexts, we quantified the nature of those note transitions and used those data to compute the entropy of undirected and directed songs.

Comparison with a Previous Study of Context-dependent Changes in Bengalese Finch Note Transition Entropy

Our initial comparisons of the note transitions in songs performed by one bird in the undirected state (e.g. [Fig. 2a](#)) versus the directed state (e.g. [Fig. 2b](#)) revealed very little difference, which was surprising in light of the differences reported by [Sakata et al. \(2008\)](#). To investigate that across our complete data set, we compared the note transition entropy of UNDIR_{ALL} songs versus DIR_{ALL} songs performed by each male ($N = 15$ birds). Consistent with our initial impressions, we found that entropy values were indistinguishable between DIR_{ALL} and UNDIR_{ALL} songs (two-way ANOVA: $F_{1,24} = 0.27$, $N = 39$ valid interactions across 15 male birds, $P = 0.61$; [Fig. 2c](#), [Supplementary Table S2](#)). We allowed our male–female interactions to persist for 1 h, whereas Sakata et al. allowed interactions to persist for at most 2 min. One possibility is that males may strongly modulate their song properties during the early portion of each interaction but not maintain that throughout the entire 1 h interaction. In support of that idea, the note transitions in the subset of directed songs performed early during a male–female interaction (DIR_{EARLY}; e.g. [Fig. 2d](#)) were less variable than in the complete set of directed songs recorded throughout that interaction (DIR_{ALL}; e.g. [Fig. 2b](#)) or in undirected songs (UNDIR_{ALL}; e.g. [Fig. 2a](#); statistical comparisons are reported below). In the

following sections, we investigate the possibility that male Bengalese finches modulate their directed songs, and therefore the attractiveness of those songs, during the course of a male–female interaction.

Males Modulate the Properties of Their Directed Songs within Male–Female Interactions

We computed the entropy of the first three songs recorded at the onset of each male–female interaction (DIR_{EARLY} songs from 39 male–female pairings across 15 male birds). Consistent with the idea that those songs represent the onset of an interaction, those songs occurred 6.95 ± 0.85 min following the introduction of the female (range 0.12–40.16 min; onset of each of the three DIR_{EARLY} songs in each interaction was considered separately). We compared the entropy of DIR_{EARLY} songs versus the entropy of songs performed by the same bird singing alone (UNDIR_{ALL}). In contrast to our findings of similarity between DIR_{ALL} and UNDIR_{ALL} songs, entropy values for DIR_{EARLY} songs were significantly lower than when the male was singing alone (two-way ANOVA: $F_{1,24} = 8.75$, $N = 39$ valid interactions across 15 male birds, $P < 0.01$; [Fig. 3a](#), [Supplementary Table S2](#)). Lower entropy values in the DIR_{EARLY} state reveal that Bengalese finch note sequences typically became less variable immediately following introduction of a female bird.

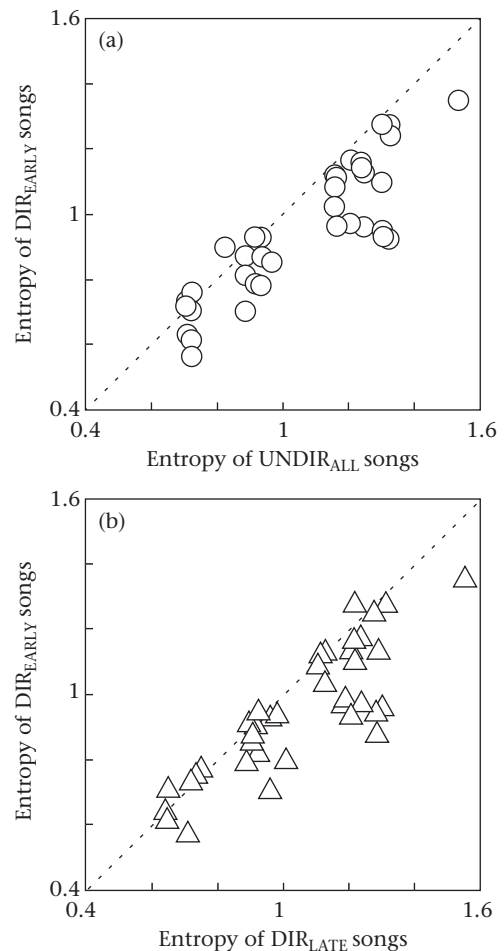


Figure 3. Comparison of note transition entropy for songs performed at the onset of each male–female interaction (DIR_{EARLY}) and (a) throughout the undirected context (UNDIR_{ALL}) and (b) by the same birds later during each 1 h male–female interaction (DIR_{LATE}). Dashed line indicates the line of identity in each panel.

Next we asked whether the changes detected in DIR_{EARLY} song performances were preserved in the DIR_{LATE} songs recorded throughout the remainder of the 1 h interaction. In contrast to the DIR_{EARLY} songs recorded shortly after introduction of the female, these DIR_{LATE} songs occurred after 31.68 ± 0.90 min (range 0.95–59.42 min). The entropy of DIR_{EARLY} songs was less than the entropy of DIR_{LATE} songs performed by the same male later during each male–female interaction (two-way ANOVA: $F_{1,48} = 19.24$, $N = 39$ valid interactions across 15 male birds, $P < 0.001$; Fig. 3b, Supplementary Table S2). There was also a trend towards fewer branch points in DIR_{EARLY} songs (range 17–36) than in DIR_{LATE} songs (range 17–44; two-way ANOVA: $F_{1,48} = 6.79$, $N = 39$ valid interactions across 15 male birds, $P = 0.01$; Supplementary Table S3). These differences in the DIR_{EARLY} state were not simply due to normal fluctuations in Bengalese finch song properties or the smaller number of songs in the early data set, because there was no difference in the entropy or the number of different note transitions in $UNDIR_{EARLY}$ songs versus $UNDIR_{LATE}$ songs (Mann–Whitney U test: entropy: $U = 1.37$, $N_1 = N_2 = 15$ birds, $P = 0.17$; note transitions: $U = 0.20$, $N_1 = N_2 = 15$ birds, $P = 0.83$). In fact, the mean entropy of $UNDIR_{EARLY}$ songs (1.11) was slightly greater than that of DIR_{LATE} songs (1.08; difference was not significant). That trend runs counter to the decreases that we observed in the DIR_{EARLY} state, further indicating that the changes we detected were not the result of chance or differences in group size. Therefore, the presence of a female has a powerful influence on the structure of Bengalese finch song, but that influence is not constant throughout prolonged interactions.

Males Modulate the Properties of DIR_{EARLY} Song to Different Degrees in the Presence of Different Females

Having determined that the presence of a female can influence DIR_{EARLY} song performances, we asked whether those changes reflect the presence of a female receiver regardless of the female's identity, or whether changes in male behaviour differ across different females. To determine the possible influence of different female receivers on songs performed by each male, we expanded our earlier criterion (each male performed at least eight songs in each interaction with at least one female, $N = 15$ birds) to now require that each male performed at least eight songs in each interaction with at least two female birds ($N = 12$ birds). Each male sang to 3.00 ± 0.17 different valid females (range 2–4), and each valid female typically evoked 20.00 ± 2.25 songs/h (range 8–70). Using this data set, we compared entropy values for one and the same male singing many times in each of at least two different male–female pairings.

We computed the entropy of songs that each bird performed when singing alone ($UNDIR_{ALL}$) and used that value to normalize the entropy of the DIR_{EARLY} and DIR_{LATE} songs performed by the same male. We observed wide ranges of normalized entropy values for DIR_{EARLY} songs performed by each male to different females (Fig. 4a), indicating that male Bengalese finches modulate the properties of their reproductive signals to different degrees in the presence of different females. In light of our previous findings that song changes evident in DIR_{EARLY} songs are not preserved throughout the remainder of that interaction, we also tested whether the degree to which a male modulates his songs in the presence of different females is also not preserved throughout the course of each interaction (Fig. 4b). The coefficient of variation for DIR_{EARLY} songs (0.09 ± 0.01); the coefficient of variation among the points in each column of Fig. 4a) was significantly greater than that for DIR_{LATE} songs (0.04 ± 0.01 ; paired t test: $t_{11} = 3.53$, $N = 12$ males, $P < 0.01$; Fig. 4b). Therefore, the female-specific changes in DIR_{EARLY} songs became less stark over the course of a prolonged

interaction. Consistent with both the quality and the quantity of song serving as an indication of male mate preference, the degree of song modulation was correlated with the number of songs that the male performed to each female (Pearson rank correlation: $r_{34} = 0.41$, $N = 36$ valid interactions across 12 birds, $P = 0.01$; Fig. 4c). Finally, normalized entropy values of DIR_{EARLY} songs were significantly less than those for DIR_{LATE} songs, indicating that the less variable note sequence that characterizes DIR_{EARLY} songs did not persist over the course of a prolonged interaction (two-way ANOVA: $F_{1,48} = 28.79$, $N = 36$ valid interactions across 12 male birds, $P < 0.001$; Fig. 4d, Supplementary Table S2). Together with the results described above, these data reveal not only that male Bengalese finches modulate the properties of their reproductive signalling throughout the course of each male–female interaction, but also that males modulate the properties of DIR_{EARLY} songs to different degrees in the presence of different female receivers.

Socially Induced Changes in Directed Song Are Functionally Relevant

To test the degree to which the changes evident in DIR_{EARLY} songs may influence female mate choice, we used DIR_{EARLY} songs as stimuli in behavioural tests of female song preference (stimuli consisted of three DIR_{EARLY} and three DIR_{LATE_3} songs from the female's most preferred male). Using calls to quantify each female's song preference (Dunning et al., 2014), 9 of 10 birds responded more strongly to DIR_{EARLY} songs than to DIR_{LATE_3} songs (Fig. 5). Among the six birds that expressed a significant preference (paired t test: $P < 0.05$; filled symbols in Fig. 5), all of those females preferred DIR_{EARLY} more than DIR_{LATE_3} . Therefore, female Bengalese finches can distinguish DIR_{EARLY} from DIR_{LATE_3} songs, and the modulated properties that characterize DIR_{EARLY} songs positively influence a female's evaluation of that song and the associated singer.

To begin to explore what song properties might be most influential in biasing female evaluation, we quantified 11 parameters of DIR_{EARLY} and DIR_{LATE_3} stimuli. The values we measured here are like those reported previously for Bengalese finches (e.g. Dunning et al., 2014). We compared each female's degree of preference (distance from zero along the Y axis of Fig. 5) against the difference between those songs in each parameter ($N = 7$ sets of DIR_{EARLY} and DIR_{LATE_3} stimuli recorded from seven male birds singing to one of seven different females; Table 1). In no case was preference significantly correlated with any individual song parameter (Spearman rank correlations: $P \geq 0.14$; Supplementary Table S4). However, it is intriguing that an earlier study from our group suggested that female Bengalese finch song preference may be associated with note sequence complexity, song tempo, the extent of note repetition, or some combination of those properties (Dunning et al., 2014), and each of those parameters is arranged in a similar way in five of the seven stimuli (i.e. more consistent note sequence, faster tempo, fewer repeated notes in DIR_{EARLY} songs than in DIR_{LATE_3} songs).

DISCUSSION

Male Bengalese finches express mate preferences, and those preferences are evident as variation of male reproductive signalling within and across different male–female contexts. Males vary not only the number of songs that they perform to each female, with some females evoking robust singing and others evoking little or no song, but also the properties of those songs, with less variable note sequences when singing to a female than when singing alone. Furthermore, the degree to which males modulate their female-directed song also varies within a prolonged male–female interaction. Song modulation is typically most clearly evident

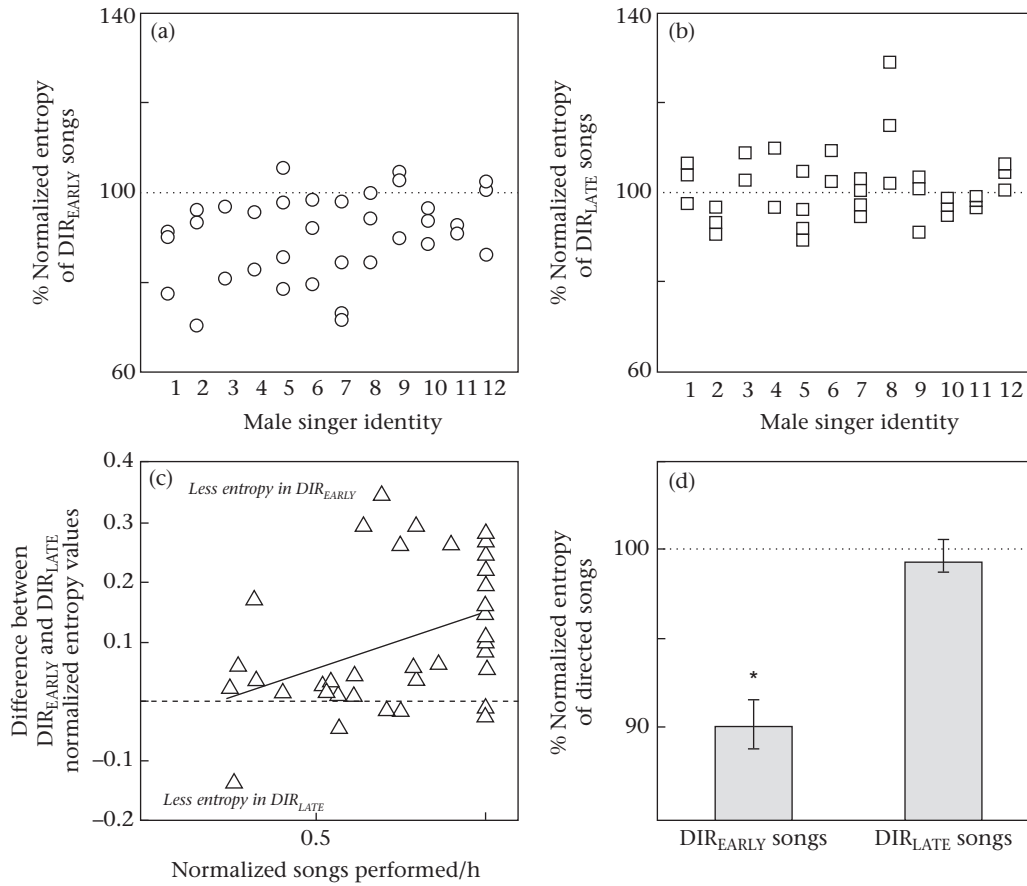


Figure 4. Entropy values across (a) DIR_{EARLY} and (b) DIR_{LATE} songs performed by each male to each of several different females (all females that passed the criterion for inclusion in our data set are shown for each male). Dotted line indicates no change from the entropy detected in undirected songs; each point represents one male–female interaction. (c) Difference in entropy values of DIR_{EARLY} and DIR_{LATE} songs performed to each female during each 1 h interaction (each point represents one male–female interaction; X axis is normalized by the maximum number of songs that each male performed in any male–female interaction; solid line represents linear regression). (d) Mean \pm SE percentage of normalized entropy of directed songs during the early and late portion of each male–female interaction. Dotted line indicates no change from the entropy detected in undirected songs. * $P < 0.05$. Results for the difference in entropy values between the DIR_{LATE} and $UNDIR_{ALL}$ conditions are given in [Supplementary Table S2](#).

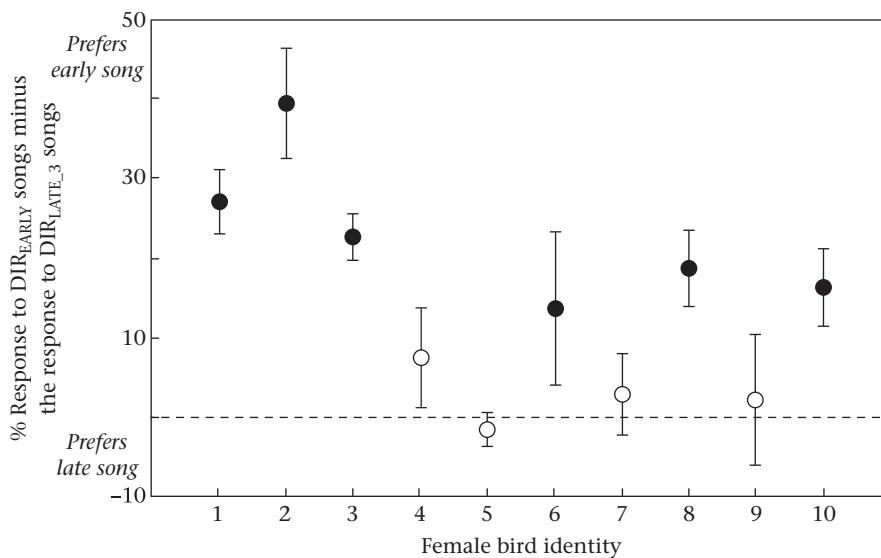


Figure 5. Mean \pm SE differences in the degree of song modulation between DIR_{EARLY} and DIR_{LATE_3} stimuli. Filled symbols: females that expressed a significant preference; open symbols: females that expressed no significant preference.

immediately following introduction of the female, and those changes subside over the course of a 1 h interaction. Therefore, directed song is not a monolithic entity. Instead, it varies in both quantity and quality within and across interactions with different females, indicating that male mate preferences and the associated song modulation are evident at the level of individual females. Those differences in male song behaviour are functionally significant because they are effective in modulating female evaluation of song quality. Therefore, males not only express preferences for individual females, but also express those preferences in a way that actively influences female preference.

Considered more broadly, these data make clear the importance of studying both participants if we are to understand the respective contributions of male and female mate preferences in the mutual decision of mate choice. In this study, we defined male Bengalese finch mate preferences with high resolution, and in a recent study by our group we also documented female mate preferences in this population with similarly high resolution (Dunning et al., 2014). Our data reveal that male mate preferences are evident in modulation of the stereotypy of note sequence, suggesting that stereotypy is important in female evaluation of song quality. Others have shown that the complexity of song syntax can also influence female Bengalese finch song preference (Morisaka et al., 2008; Okanoya, 2004). An important goal of future studies will be to use synthetic stimuli in which individual song parameters are altered in order to define the importance of these and other song parameters in female song evaluation and mate choice. Together with the ease of breeding these birds and observing them across generations in the laboratory, our present findings and the results of those future studies will provide an especially tractable context in which to begin to disentangle the contributions of male and female mate preferences to the outcomes of mutual mate choice and its long-term benefits or consequences.

Female Characteristics That May Influence Male Mate Preference

Individual males expressed their preferences very clearly and very early during each interaction (see also Sakata et al., 2008), leading us to suspect that male mate preference is based on some overt feature(s) of the female bird and that males do not require an abundance of data to form their assessment. In considering possible aspects of a female Bengalese finch's condition that may distinguish an individual bird and thereby influence a male Bengalese finch's preference for her among many other females, our data suggest that visual cues may be important (e.g. size or feather condition; Bonduriansky, 2001, 2009; Takahasi, Ikebuchi, & Okanoya, 2005; Takahasi & Okanoya, 2013). Males commonly sang as the female was being brought close to the cage where they would reside throughout testing. Such a clear response so quickly after the female coming into view suggests that males are basing their evaluation at least partially on some facet of the female's appearance.

It is possible that such a rapidly induced modulation of male courtship behaviour could also be evoked by some vocal cue that the female expressed as she first saw the male and then was placed into the cage; however, our data suggest that is not the case. Although female birds commonly called throughout the course of each male–female interaction, males very commonly sang their DIR_{EARLY} songs prior to any vocalization from the female bird. Therefore, female-specific patterns of male courtship modulation are apparently not evoked by auditory signals. Immediate modulation of male behaviour also suggests that taste, olfaction and somatosensation play little or no role in shaping the male's initial assessment, but the absence of somatosensory input could at least partially explain the transition from modulated DIR_{EARLY} songs to unmodulated DIR_{LATE} songs. Consistent with our findings of

strongly modulated DIR_{EARLY} songs, experiments performed using zebra finches reveal that the first stages of mate choice (e.g. direction of courtship towards the eventual mate) are evident within minutes of introducing the female bird (Silcox & Evans, 1982). Interestingly, those experiments also indicate that pair bonds are cemented through interactions that continue for days following introduction (e.g. allopreening and maintained proximity), and a pair bond typically fails to emerge if the male and female are kept separated and therefore denied a chance to interact, even if they are separated by only a transparent screen (Silcox & Evans, 1982). It is an interesting idea that different cues may have different degrees of importance throughout the course of courtship and mate choice. For example, perhaps somatosensory input is not necessary for males to form their initial assessment of female attractiveness, but physical contact is necessary later during the formation of a bond (Nilsen & Vrana, 1998; Uvnas-Moberg, 1999). That idea is consistent with our observation that the most effective courtship signals are performed early during a male–female interaction, with males performing less attractive songs when they are kept separated from the female by a wire mesh divider. It will be interesting to explore the degree to which the patterns we observed here might vary when males and females have no separation between them and when song performance of an individual male in the presence of the same female is tracked throughout the entire process of courtship and pair bond formation.

In addition to the female's appearance, or possibly as an alternative female characteristic, male mate choice may also be influenced by some aspect of the female's nonvocal behaviour. In support of that idea, studies of brown-headed cowbirds, *Molothrus ater*, have revealed a female response to male song that, when detected by the male, causes the male to modulate his song behaviour (West & King, 1988a). Furthermore, subtle behaviours expressed by female satin bowerbirds, *Ptilonorhynchus violaceus*, also influence how males perform their reproductive displays (Patricelli, Uy, & Borgia, 2004). If female Bengalese finches also express some form of behavioural feedback very early during an interaction, then that response could be the basis on which males decide to invest their effort in robust singing of modulated songs. If males base their mate preferences on multiple features, perhaps including aspects of both appearance and behaviour, as in the case of female mate preferences (Collins et al., 1994; Miller, 1979; Williams, Kilander, & Sotanski, 1993), then the male would have a range of relevant information on which to base his assessment. Discovery of an influence of female behaviour on male modulation of reproductive signalling in a species that is as experimentally tractable as the Bengalese finch would open the door to detailed studies of both adult and juvenile birds to define the degree to which males may learn their mate preferences through early life experience just as females learn their mate preferences (Holveck & Riebel, 2014; Kato, Hasegawa, & Okanoya, 2010; Riebel, 2009; Verzijden et al., 2012).

Implications for Understanding the Evolution of the Population

We have developed a new animal model in which we can assess the mate preferences of individual males for each of many different females. This model provides a new tool to enable us and others to investigate the degree to which male mate preferences are translated into fitness benefits through resources provided to the female and offspring. For example, males may provide greater levels of nest care or defence when mated with females that they find especially attractive (Matessi, Carmagnani, Griggio, & Pilastro, 2009). In addition, male mate preferences may translate into modulation of male reproductive physiology. Models of the consequences of male mate preference predict differences in male

allocation of ejaculates, manifest as possible increases in sperm production or seminal fluid production when males are courting females that they find especially attractive (reviewed in [Wigby & Chapman, 2004](#)). Those models also predict greater allocation of sperm to females that the male perceives as higher quality ([Galvani & Johnstone, 1998](#); [Parker & Pizzari, 2010](#); [Reinhold, Kurtz, & Engqvist, 2002](#)). The Bengalese finch model developed here provides an excellent new context in which to address long-standing questions of whether dynamics of male mate preferences that are evident as differences in precopulatory behaviour translate into equivalent dynamics in sperm competition and other aspects of reproductive physiology ([Edward & Chapman, 2011](#)). Because Bengalese finches can be relatively easily housed, studied and bred over multiple generations in the carefully controlled conditions of the laboratory, these animals afford an opportunity to document, and perhaps even modulate, the life history of experimental subjects. The ability to perform detailed observations on each of many individuals and to manipulate their interactions quickly and easily will vastly facilitate future efforts to understand the relation between mate preferences and the evolution of a population.

Implications for Understanding the Neural Basis of Vocal Performance

Our results reveal that directed song is not a monolithic entity in which males exert themselves maximally in each song performance. Instead, males exert themselves to different degrees in different contexts, evident as nuanced modulation of their directed song performances (compare with [Patricelli, Uy, Walsh, & Borgia, 2002](#)). Directed song was originally defined by social context (singing in the presence of one or more females) and features of the male's behavioural display (orienting his beak and body directly towards a female receiver) ([Morris, 1954](#); [Sossinka & Bohner, 1980](#); [Williams, 2001](#)). Using that definition, valuable insight into the neurophysiology of male song control has come from studying song performances in the directed versus undirected contexts (e.g. [Jarvis, Scharff, Grossman, Ramos, & Nottebohm, 1998](#); [Kao & Brainard, 2006](#); [Woolley, Rajan, Joshua, & Doupe, 2014](#)). However, we now realize that directed song is not a categorical distinction, as the degree to which a male modulates his song varies not only across different male–female interactions but also across different times within each interaction. Prior to this realization, such variation may have confounded efforts to understand the relation between note sequencing and the function of the underlying neural circuits. Specific neural circuits, emerging from a premotor cortical site and projecting either to the vocal motor cortex or to the basal ganglia, have each been implicated in the sequencing of individual notes into the complex sequences that characterize song ([Ali et al., 2013](#); [Fujimoto, Hasegawa, & Watanabe, 2011](#); [Hampton et al., 2009](#); [Kobayashi, Uno, & Okanoya, 2001](#)), and the pathway projecting into the basal ganglia has also been implicated in auditory perception ([Prather, Nowicki, Anderson, Peters, & Mooney, 2009](#)). In this study, we reveal that song sequence is modulated in a highly context-specific manner. Although the context-specific song modulation that we describe is subtle, those changes are functionally significant. Our results are consistent with previous findings that birds are exquisitely sensitive to small changes in song structure ([Cynx, Williams, & Nottebohm, 1990](#); [Vernaleo & Dooling, 2011](#)), and neurons in the pathways implicated in auditory perception and vocal sequencing are similarly sensitive ([Lewicki, 1996](#); [Margoliash, 1986](#); [Theunissen et al., 2004](#)), with very precise relations between the occurrence of specific song features and the timing of auditory action potentials in awake birds (reviewed in [Prather, 2013](#)). It will be useful to include a consideration of these context-specific changes in future efforts to understand how auditory perception

emerges in the brain and exerts its influence on the network of brain sites that govern vocal sequencing.

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Supplementary Material

Supplementary material for this article is available, in the online version, at <http://dx.doi.org/10.1016/j.anbehav.2014.08.016>.

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