

**Understanding the variation in the adult male zebra
finch song and behaviour, during courtship, with
distance of communication**



A thesis submitted towards partial fulfilment of
BS-MS Dual Degree Programme

by

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For the study conducted under the guidance of

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at the

Department of Biology,

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Certificate

This is to certify that this dissertation entitled “**Understanding the variation in zebra finch song and behaviour during courtship, with distance**” towards the partial fulfilment of the BS-MS dual degree programme at the Indian Institute of Science Education and Research, Pune represents study/work carried out by **S Harini**, at **IISER, Pune** under the supervision of **Dr. Raghav Rajan, Department of Biology, IISER, Pune** during the academic year **2016-17**.



Signature of Student



Signature of Supervisor

Date: 20/03/2017

Declaration

I hereby declare that the matter embodied in the report entitled “**Understanding the variation in zebra finch song and behaviour during courtship, with distance**” are the results of the work carried out by me at the **Department of Biology, Indian Institute of Science Education and Research, Pune** under the supervision of **Dr. Raghav Rajan**, and the same has not been submitted elsewhere for any other degree.



Signature of Student



Signature of Supervisor

Date: 20/03/2017

CONTENTS

Abstract	05
List of Figures	06
List of Tables	07
Acknowledgements	08
Introduction	09
Methods	14
Results	24
Discussion	44
References	46

ABSTRACT

Communication in animals depends on factors that enhance or attenuate transmission of acoustic signals and which, in turn, are necessary for development of strategies for effective communication. One such prominent factor that affects signal transmission is the communication distance between the sender and the receiver. It has been shown that animals strategically change the intensity of their vocalisations to compensate for the losses incurred during signal transmission over long distances. However, the influence of this communication distance on acoustic features other than intensity has not been investigated. The song of the zebra finch birdsong model system provides us with an array of acoustic features which are distinct in different social contexts and hence, making these birds suitable candidates for examining the influence of communication distance on the acoustic properties of their song. In order to do this, we recorded and analysed the courtship song features at five different distances and found that, with increase in distance from the female zebra finch, the male bird song features become less rigorous. At longer distances, the courtship songs resemble the songs sung by these birds in isolation. On the other hand, when we tried to validate the variation in song amplitude with distance, we, surprisingly, found no change. Together, our findings suggest that communication distance is one of the crucial factors influencing acoustic properties essential for effective communication.

LIST OF FIGURES

Figure No.	Caption	Page No.
FIG 1	Reported difference in song features	11
FIG 2	Male zebra finch dance movements	12
FIG 3	Experimental Setup	16
FIG 4	Types of Microphone used	18
FIG 5	Representative Song Spectrogram	19
FIG 6	Visual cues - male and female bird	21
FIG 7	Matching scores for video scoring scheme	22
FIG 8	Schematic representation of distances used	24
FIG 9	Spectrographic representation of song features	25
FIG 10	Variation in number of INs with distance	26
FIG 11	Variation in number of motifs with distance	27
FIG 12	Variation in bout length with distance	28
FIG 13	Variation in first motif duration with distance	29
FIG 14	Variation in fundamental frequency with distance	31
FIG 15	Proposed models for changes in song features	33
FIG 16	Proportion of 'Directed' songs with distance	34
FIG 17	Changes in song features for 'directed' song bouts	36
FIG 18	Song amplitude variation for songs recorded with mixer microphone	38-39
FIG 19	Heat map representing changes in amplitude for different positions inside the cage	40
FIG 20	Song amplitude variation for songs recorded with backpack microphone	41
FIG 21	Mean change in amplitude with change in position of microphone from source	42
FIG 22	Song amplitude variation for songs recorded with head fixed microphone	42-43

LIST OF TABLES

Table No.	Caption	Page No.
1	Reported differences in song features for directed and undirected songs	25
2	Pearson's correlation coefficients and p values for the four song features	30
3	Summary of variations in song features for all song bouts	32
4	Number of bouts considered for analysis after categorisation into D, and UN	35
5	Summary of variations in song features for directed song bouts	37

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INTRODUCTION

Communication in animals can serve a multitude of functions like courting a mate, to provide warning signals, to hunt prey etc. For effective communication to take place, the signal produced by the sender must be received and discriminated by the receiver. This clearly implies that the animal must account for losses imposed by environmental constraints on signal transmission and detection (Wiley and Richards, 1978). Several studies have estimated the optimal communication distance for various birds and animals and their importance in effective communication, while others focus on how receivers assess the distance of the sender using properties of signal transmission (Brenowitz, 1986; Coen et al., 2016; Mouterde et al., 2014; Ornis et al., 1990). For instance, a particular study on male blackbirds emphasised the importance of directionality of vocalisations (Ornis et al., 1990). The study showed that while high frequency vocalisations are directed to a target individual, low frequency ones are meant for individuals with unknown locations. This could potentially support the argument that these birds can gauge the location of their targets before producing vocalisations. Another study proposed a model, using the example of Northern spring peeper (frog), to investigate the effect of habitat, caller position, and chorus noise on the effective distance of the call at a defined frequency. It was seen that the peeper will take up positions in the landscape which will increase the effective distance of call transmission, suggesting that these amphibians adapt to conditions in their landscape which will support maximisation of signal transmission (Parris, 2002). Other frogs and birds also tend to position themselves in habitats such that the losses incurred during signal transmission are reduced (Dabelsteen et al., 1993; Mouterde et al., 2014). Collectively, these studies suggest that animals tend to adopt strategies to maximise their acoustic signals for communication and a factor that contributes largely to the development of these strategies is the distance between the sender and the receiver (target). This is generally termed as distance of communication or communication distance.

Communication distance has been analysed as a contributing factor for effective communication in a number of animal systems. However, most of these studies focus on its effects on the strategies adopted by animals by varying the intensity of vocalisations to compensate for any loss in acoustic signal transmission due to

atmospheric absorption, scattering of signal, and ground attenuation (Brenowitz, 1986; Mouterde et al., 2014). In songbirds, it has been shown that the song's amplitude increases with increase in distance of communication to compensate for losses due to signal attenuation (Brumm and Slater, 2006). A study showed that *Drosophila* males modulate the amplitude of their courtship song to compensate for female distance and it was highly dependent on complex visual stimulus cues and stimulus history (Coen et al., 2016). Such behaviours are also observed in human beings for effective communication when the speaker tends to speak loudly if the receiver is far away and it was shown that this modulation in intensity of vocalisations followed the inverse square law of distance (Michael et al., 1995). Accounting for all these studies, it can be said that the distance of communication is crucial to modulating animal social behaviour, along with its implications in signal transmission.

Although adjustment of vocalisation intensity with distance has been established to be an important aspect of communication, it is imperative that we explore the possibility of other factors that could also be modified when the distance between the sender and receiver is varied. We investigated the influence of distance of communication on the adult male zebra finch song by quantifying various song features and its implications on the courtship behaviour in these birds.

In the zebra finch model system, the song is produced by the male bird to attract mate. These birds produce highly stereotyped and relatively simple songs which are amenable to quantitative analysis. The song is learnt by the juveniles from the tutor and provides little room for variability after song crystallisation (Zann et al., 1996).

The song of an adult male zebra finch consists of a sequence of song elements, beginning with a series of soft, short vocalisations known as introductory notes (INs), which are variable in number. INs are generally followed by a stereotyped sequence of vocalisations repeated either in a continuous manner or intermittently separated by calls or introductory notes. These sequences are called motifs. This structure of INs followed by motifs is generally referred to as a song bout.

Male zebra finches sing in two types of song based on social context - song produced during courtship, directed towards a female bird and is usually accompanied by a courtship dance, and song which is produced when alone, generally self-initiated (Sossinka and Bohner, 1980). The former is called Directed song and the latter is called undirected song. Since we are trying to understand the effects of distance of communication on song features, directed songs serve as suitable models. Zebra finches also provide us with an assortment of song features which are significantly different for directed and undirected songs and are convenient for use in this study. Following are some of the acoustic features that have been quantified for both song types in zebra finches (Sossinka and Bohner, 1980)-

- Number of introductory notes per song bout (FIG 1a)-
 - On an average, the directed songs are preceded by more number of INs than undirected songs.
- Number of motifs per song bout (FIG 1b)-
 - The directed songs have more number of motifs per bout than undirected songs.
- Song tempo -
 - To characterise song tempo, the durations of complete motifs produced by birds in both social contexts are measured. Motif durations for directed songs are less than undirected songs i.e. directed songs are faster.

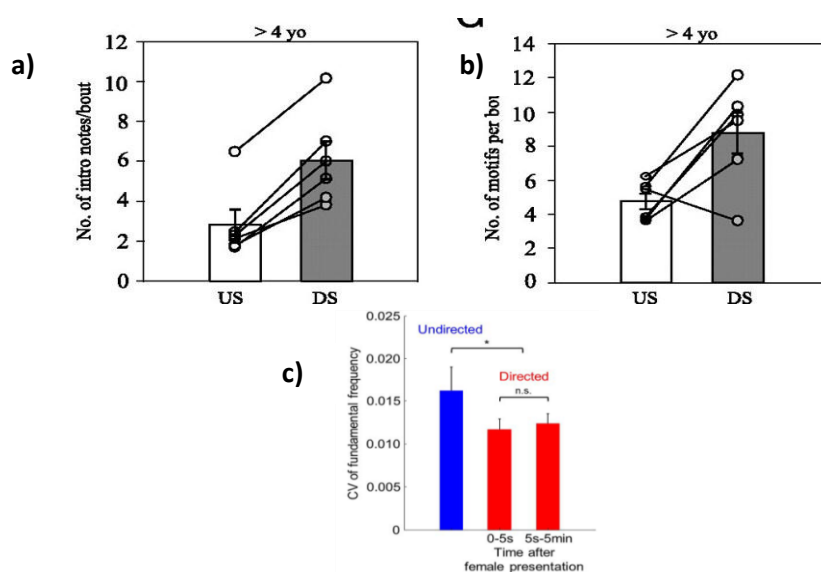


FIG 1. Reported difference in song features for the two types of song – directed and undirected US – Undirected song; DS – Directed Song.

a) Shows that the number of introductory notes is higher for DS than US. Data is significant. (Kao and Brainard, 2006); b) Shows that the number of motifs is higher for DS than US. Data is significant. (Kao and Brainard, 2006); c) Shows that the CV of fundamental frequency is higher for US than DS. (Aronov and Fee, 2012)

- Fundamental Frequency (FF) (FIG 1c) -
 - As a measure of changes in song structural features, the variability of fundamental frequency is estimated. It is seen that the variability in FF is less for directed songs than undirected songs.

The song in these birds is accompanied by visual cues which are different for both types of song. Birds singing directed song adopt postures which are suitable to attract females and these postures are called intense or high postures whereas the postures adopted during undirected song are referred as weak. An elaborate courtship ritual in zebra finches involves production of directed song along with the performance of the courtship dance (FIG 2) (Morris, 1953; Williams, 2001). The courtship dance constitutes a number of displays including beak wipes, turn-arounds, and hops which are unique to the bird as its song (FIG 6) (Ullrich et al., 2016). During this courtship display performed by the male bird, the female bird generally responds by producing a tail quiver or by hopping and calling. It is reported that the whole courtship display in these birds is a form of multimodal signalling to maximise information exchange.

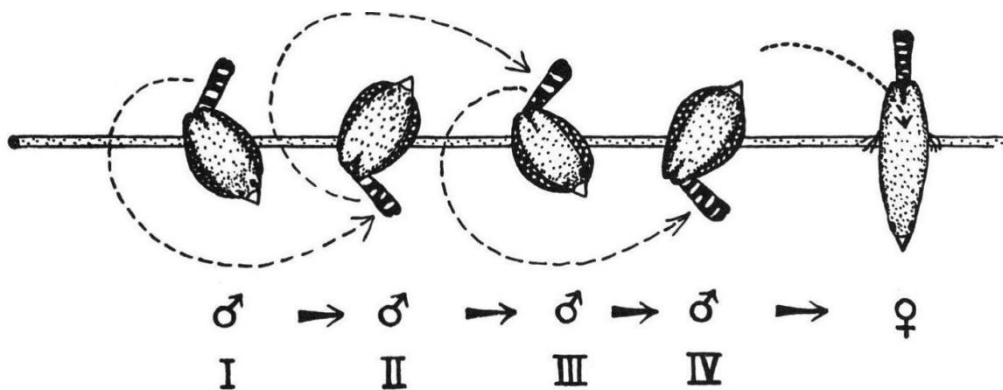


FIG 2. Male zebra finch dance movements during courtship. Each part of the figure depicts different stages of the display. (I-IV) The various positions that the male takes up while performing its courtship display. (Morris, 1953)

According to the results published by Sossinka and Bohner, 1980, the two types of songs – directed and undirected, show a clear bimodal distribution and are distinct. However, they do suggest the possibility of existence of differences in intensity within courtship song due to different reasons. A comparatively recent study (Iwasaki et al., 2013) showed that depending on the kind of females (paired, non-paired, or dummy), the male bird's courtship behaviour would be different. Their results, however,

showed such higher intensity courtship song for only one feature which is a measure of song tempo. Although this study doesn't concretely show the different intensities of courtship behaviour in zebra finches, it does point towards the possibility of variation in the degree of courtship intensity and implies that directed singing does not follow a homogenous pattern. There could be a spectrum of courtship songs which can vary from highly intense courtship displays on one end to weak postures (as seen during undirected songs) on the other end and hence providing us with a range of directed songs and displays to compare between.

Apart from this potential range of courtship behaviour in these birds, Brumm and Slater, 2006 have already reported that the song produced by the male zebra finch in presence of a female bird, increased in amplitude with increase in communication distance to compensate for losses incurred due to attenuation of signal with distance. However, the study didn't rigorously include the differences in directed and undirected song features and account for errors in song amplitude measurements due to changes in position of the bird within the cage with respect to the fixed position of the microphone on the cage.

Accounting for the aforementioned differences in social-context dependent song features and variations in courtship intensities, the zebra finch song bird model system makes a good candidate for investigating the influence of distance of communication* on courtship song features along with replication of the experiments to study its effects on song amplitude.

Specific aims and Hypothesis

The aim is to examine the influence of distance of the male bird from the female bird, on courtship and its intensity, by quantifying the changes in courtship song features - number of introductory notes preceding the bout, first motif duration, number of motifs per bout, amplitude and variability in fundamental frequency. We speculate that the changes in these song features with distance, could follow one of the following trends –

**Distance of communication is synonymously referred to as communication distance, distance, and distance from female throughout this report.*

- No change in courtship song features and intensity – the bird maintains these properties irrespective distance, implying that distance from female has no effect on them.
- An increase in courtship intensity with distance with most of the song features following the directed song properties at farther distances - This means that with increase in distance from the female bird, the male will always try to produce less variable and robust directed song to the female, compensating for losses incurred due to distance. In order to do so, the directed song should increase its number of INs, number of motifs and bout length, and decrease its first motif duration and variability in fundamental frequency, with increase in distance of communication.
- A decrease in courtship intensity with increase in distance and song features become more undirected-like. With increase in distance, number of INs, number of motifs, and bout length should decrease, and first motif duration, and variability in fundamental frequency should show an increase. Such a scenario might be occur if the male bird loses interest in attracting the female bird because he cannot see her or sense her presence, or doesn't want to get her attention as she is far away and production of courtship song might be an energy expensive process.

METHODS

All procedures and experiments were approved by the Institutional Animal Ethical Committee (IAEC), IISER Pune and performed in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi.

Twelve adult male and six adult female zebra finches (>120 days post hatch) were used in this study. These zebra finches were either reared in our colony or were bought from local vendor. These birds were provided with ad-libitum food and water along with regular food supplements of cuttlebone, sprouts, and boiled eggs. They were maintained in a bird colony where they are exposed to light from 6:00 AM to 8:00 PM (14 hours a day).

All male birds used in this study were separated into individual cages and isolated in sound-attenuating boxes prior to recording, for a period of 3-7 days. These birds had no contact with other males or females during the first 3-4 days of isolation. Female birds were introduced into the sound attenuating boxes for a period of 1-2 hours two days prior to the start of experiment.

Song and Video Recordings

During the last two days of isolation, directed and undirected songs were recorded. Songs were recorded using a microphone placed at a fixed position on top of the male bird's cage. The microphone used, was controlled using a mixer and customised software on a computer.

Signals from the microphone were filtered between 300 and 8000 kHz. Data was digitised and recorded at a sampling rate of 44100 kHz using custom software. The data was stored in 30 second long wav files which were used for further analysis(Rajan and Doupe, 2013a).

The position of the microphone and the settings used were optimised and kept a constant for each bird recorded throughout the two days of recording inside the sound-attenuating box. Undirected songs were recorded continuously from the time lights were turned on at 6:00 AM in the morning till 8:00 PM in the evening for about 10 hours a day. Directed songs were recorded for a period of 1-2 hours during these two days, by introducing a female bird to the male bird inside the sound-attenuating box. This female bird was isolated from the colony in an individual cage, at least half an hour before introduction to the male bird. These songs were used to quantify the amount of singing for each bird and identify the various song elements.

On the day of recording, each male bird was taken out of the sound-attenuating box and placed in the room used to conduct the experiment. The male bird was left to acclimate to its surroundings for an hour before the start of recording. A microphone was placed at a fixed position on top of the male bird's cage and the microphone controls were optimised for each bird in this room. A camera was attached to a stand and the male bird's movements were recorded (top view) in a video. The videos were recorded using ffmpeg, a software customised to record and edit videos and images on a computer and were later used to monitor the bird's behaviours.

To understand the influence of distance on the courtship behaviour of male zebra finches, the female birds were introduced to the male bird at five different distances (FIG 3). The location of the male bird's cage was fixed and female bird was introduced at a different distance, chosen randomly, during each recording session. Each recording session was 45 minutes long with 20 minutes intervals between each recording session. During each recording session, the female bird's behaviour was also noted either manually (n= 8 birds) and/or was video recorded using another camera (n =2 birds) (front view).

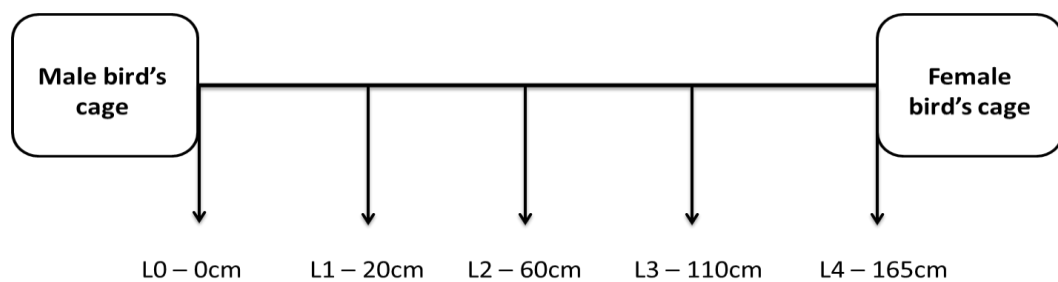


FIG 3. Distances at which different song properties were estimated (Top); Experimental Setup (Bottom)

Apart from this, undirected songs were also recorded as a control by placing an empty cage at L1, for each bird. For a subset of birds (n=5), L0 songs were recorded at a different time than the songs for other distances. This shouldn't affect the measurement of most of the song features (Unpublished data from the lab).

Microphones used in this study to record songs

Three types of microphones were used for song recording during this study (FIG 4). It was ensured that usage and attachment of any of the microphones didn't cause

any stress to the bird and was given enough time to acclimate before the start of the experiment.

1. Mixer microphone (MM) - This microphone is controlled via a mixer and is placed at a fixed position on the male bird's cage throughout the recording session. However, this microphone yielded us variable song amplitude (a measure of loudness of any vocalisation) for different positions of the bird within the cage (20cm x 20cm x 20cm). This was confirmed by playing back a monotone of fixed amplitude through a speaker at fixed volume from different positions within an empty cage and recorded using the mixer microphone.
2. Backpack Microphone (BP Mic) - As a solution to the variation in song amplitude with position of the bird within the cage, a microphone was attached to the back of the bird using a velcro strap for support. This microphone setup was customised into two parts - one part of the velcro was attached to the bird's back with the help of thread and the other part was attached to a 0.5g sensitive microphone wired to a computer. The first part of the setup was put on the bird initially and the bird was allowed to acclimate to it for a day or two. The second part with the microphone was attached to the bird about an hour prior to the start of recording. This setup was modified eventually due to the variability caused by changes in position of the microphone while attaching it to velcro on the bird's back every recording day. The modified setup had the microphone attached to the velcro strap which was fixed on the bird's back and the microphone was wired only during the recordings.
3. Head Fixed Microphone (HF Mic) - This setup had a microphone with a connector piece (weighing less than 0.5g) attached to the bird's head. It was held in place with the help of dental cement on the skull. This gave no room for any change in position of the microphone and reduced the variability in song amplitude and hence was used for further song recordings.

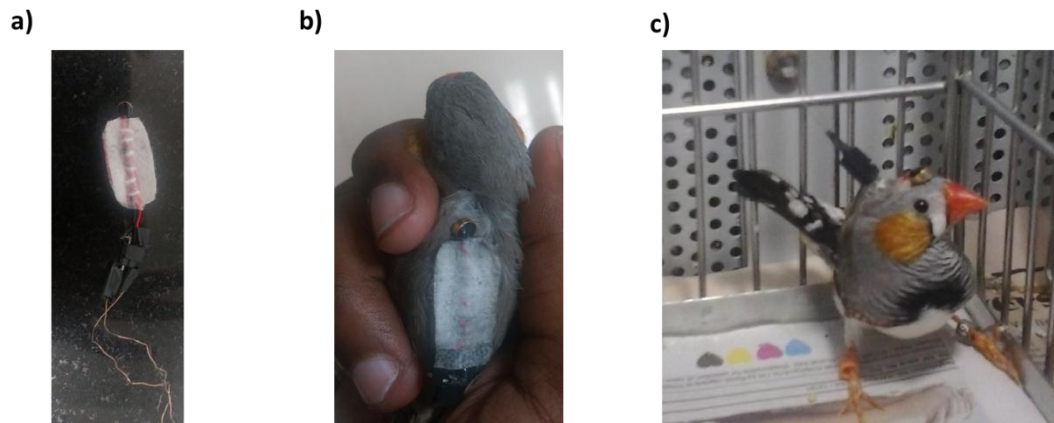


FIG 4. Types of microphone used in this study

a) Backpack Mic b) Backpack Mic on the bird's back. c) Head-fixed microphone on the bird.

Data Analysis

All analysis was done using custom-written scripts in MATLAB.

Song Analysis

To identify various song elements and look at the song features, song bouts were defined to be any period of vocalisations with 2s silence before and after. Only audio files with song bouts were considered for further analysis. These song files were segmented into vocalisations and gaps by using threshold log amplitude and represented as spectrograms. Spectrograms are frequency vs time plots, using a colour scale to depict the intensity of the vocalisations at different frequencies. Any vocalisation that lasted more than 10ms and had at least 5 ms silence on both ends was considered a syllable. The onset and offset of each syllable was determined by using amplitude thresholds and was optimised according to background noise for each song file. The syllables were labelled according to their similarity in amplitude profiles and visual spectral similarity, in a semi-automated manner. The syllable boundaries were adjusted using amplitude thresholds calculated for each syllable as half the maximum amplitude, in order to maintain the consistency of onset and offset times of the syllables.

Three song elements were considered in this study - motif, calls, and introductory notes (INs) (FIG 5).

A stereotyped sequence of syllables repeated either in a continuous manner or intermittently separated by calls or introductory notes, was considered to be a motif. Introductory notes were defined as soft vocalisations which were shorter in duration compared to motif syllables, and observed in variable numbers preceding the motif. Solitary vocalisations with variable duration were considered to be calls.

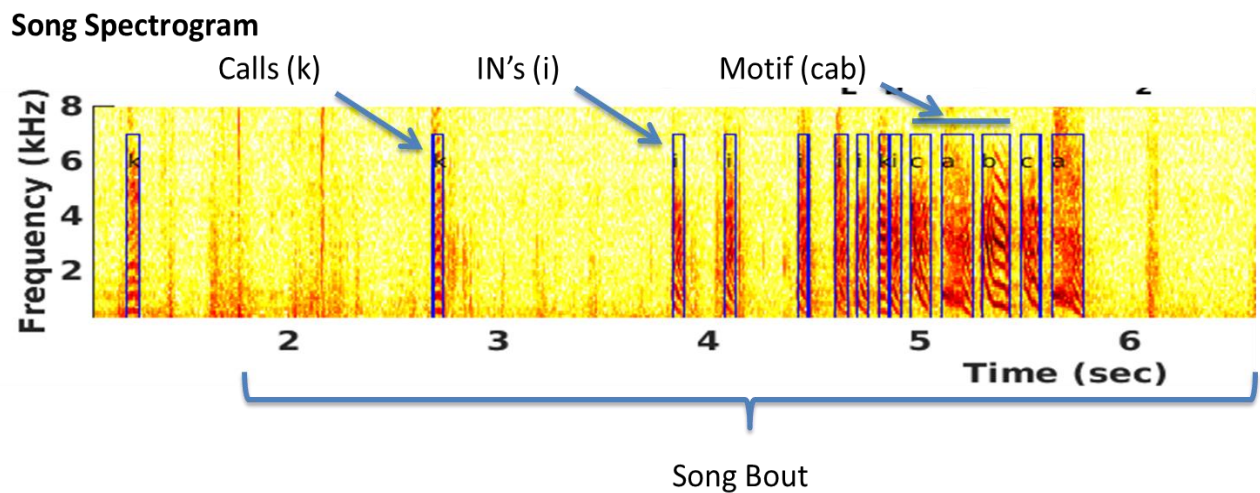


FIG 5. Representative spectrogram showing different song components – INs, motifs, and calls

Various syllable acoustic properties were analysed in this study. These acoustic properties were calculated using the descriptions of these properties in the Sound Analysis Pro (SAP) software. The features quantified in this study were measured as follows -

1. Log Amplitude (dB) - It is a measure of loudness of the song. Absolute deviation of the sound waveform, estimated by calculating the RMS of the acoustic signal, is taken as amplitude and an arbitrary baseline is set to 70 dB for the purpose of calculations as we do not measure background noise separately. Log Amplitude for song syllables was calculated according to -

$$\text{Log Amplitude(dB)} = 10 \log_{10} \sum_f P_f - \text{Baseline}$$

2. Fundamental Frequency (Hz) - It is an estimate of periodicity of sound and used in this study to assess the variability in song syllable structure across

distances. The fundamental frequency is calculated based on an algorithm called YIN (De Cheveigné and Kawahara, 2002) which incorporates autocorrelation and a smoothing algorithm to increase the accuracy of measurement.

3. Motif Duration - It is a measure of song tempo or rate and was calculated as -

Motif Duration

= Offset of the last motif syllable – Onset of the first motif syllable

We considered the motif duration of the first motif of each song bout for our analysis as the number of motifs varies from one bout to another.

4. Number of Motifs and Bout Length - Both these properties estimate the length of a song bout which is preceded and succeeded by 2s silence. Bout length was measured as the difference between the offset of the last bout syllable and the onset of the first bout syllable.
5. Number of Introductory Notes (INs) - A count of consecutively occurring INs at the beginning of the bout which does not have intervals greater than 500ms.

Video Analysis

Two different sets of videos were obtained for each recording session - one of the male and the other of the female. The videos were scored for different movements during the song bouts and each of these song bouts were classified into categories based on the criteria set. Each male bird was scored for visual cues that included puffing up, hopping, turn-arounds, beak-wipes, and looking in the direction of the female. If the male bird performed at least two of these movements, apart from the fixed criterion that the male should look at the female while singing, the songs were classified as directed (D) or else as undirected (UN).

The female bird's behaviour was also scored and the visual cues included puffing up, looking towards the male, proximity to the male in her own cage (near or far), hopping, and calling. The female bird's responses were classified as responding (R) for each corresponding song bout, if any of these two cues were performed by the female bird.

These classifications were incorporated into our analysis for different acoustic properties of song to understand the influence of social context on them for different distances between male and female bird.

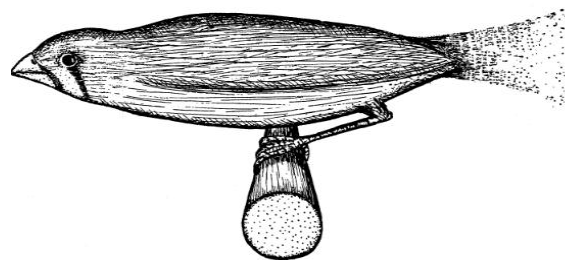
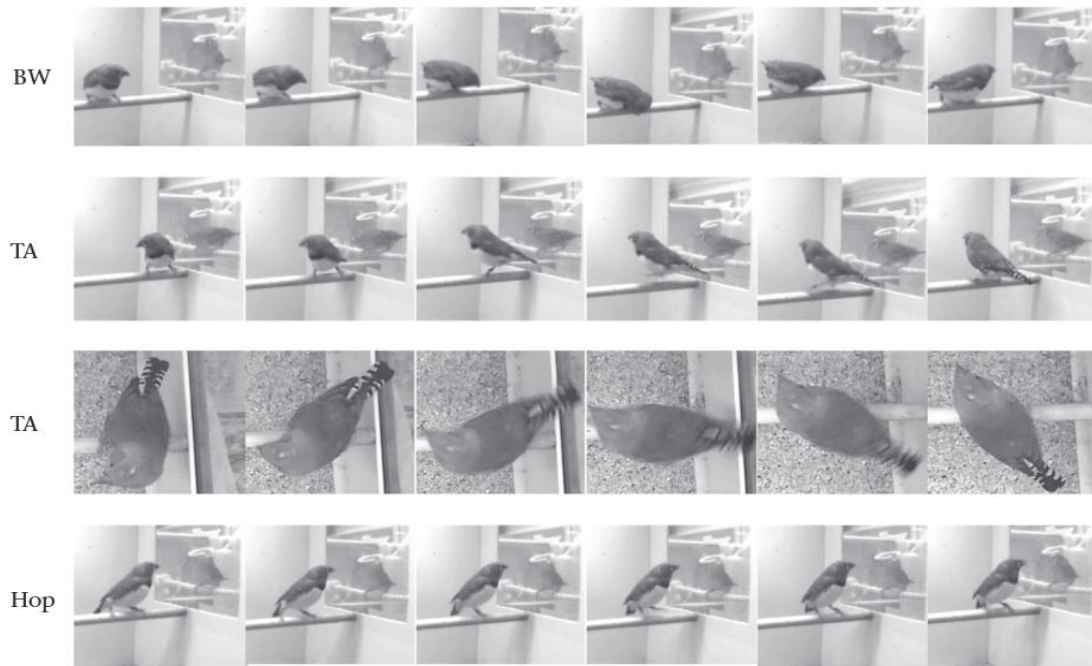


Fig. 8. The tail-quivering display of a responsive female.

FIG 6. Different visual cues produced by the male and female birds during courtship (Morris, 1953; Ullrich et al., 2016)

Top panel: Three different movements performed by the male bird during courtship – Beak Wipe (BW), Turn-arounds (TA), and Hops.

DATA CONSIDERED FOR ANALYSIS:

Song bouts recorded at each distance across different days were put together for analysis. For 2 out of the 10 birds, only the first 50 bouts of song at each distance was considered for analysis, while all the song data was pooled in for the rest of the 8 birds. Some song bouts occurring at the end of each audio file were lost due to errors in recordings and were not included. While measuring fundamental frequency, amplitude, and motif duration, song syllables that were above three times the

interquartile range from the 75th percentile of the data or below three times the interquartile range from the 25th percentile of the data were considered outliers and eliminated,. Amongst these selected song bouts, scoring for male and female movements were done for only those for which videos were available (some videos were lost due to problems with the video recording software) and of credible quality (audio and video is synced and movements are clearly visible).

CROSS CHECKING THE VIDEO SCORING SCHEME FOR COURTSHIP BEHAVIOUR:

In order to validate the categorisation of song bouts as directed and undirected through video scoring, and to eliminate any biases developed by the experimenter, two observers familiar with the zebra finch model system and blind to the experimental conditions and experimenter's scoring were asked to judge the male and female birds' behaviours during courtship in a subset of videos corresponding to 90 song bouts at different distances. They were asked to assess the male behaviour based on whether he is puffing up, looking towards the female, hopping, dancing, doing turn-arounds, beak-wipes, and the female behaviours based on her movements (hopping), calls, puffing up, orientation with respect to the male bird, and copulation solicitation displays (including tail quivers, tail wags). Similar to the scoring scheme followed by the experimenter, they were asked to score the male or female movement as D or R respectively when any two of the criteria were satisfied. Scores of each of the two observers were compared against the scores of the

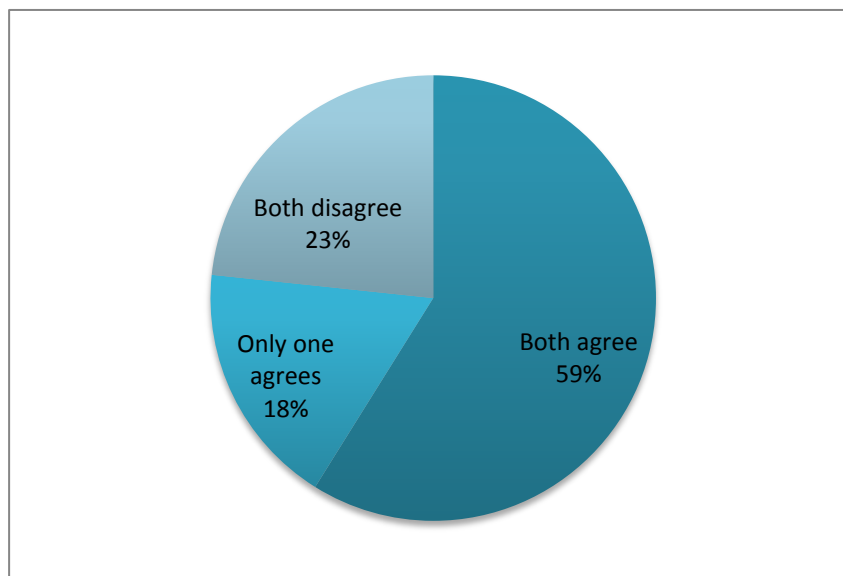


FIG 7. Pie chart showing the degree of matching of male bird's movements during courtship when scored by two observers other than the experimenter. Both observers agreed with the experimenter 59% of the times; only one of them agreed 18% of the times and both disagreed with the experimenter 23% of the times.

experimenter. It was seen that for 59% of the cases both of them agreed with the experimenter's scoring of the videos and for 77% of the cases at least one of the two observers agreed with the experimenter. Hence, the video scoring done by the experimenter to classify song bouts was considered adequate and used for further analysis.

Statistical Analysis

All comparisons across various groups (distances in this study) were quantified by Kruskal-wallis ANOVA with the application of a post hoc Tukey-Kramer criterion. For all the trends for different song feature values, correlations were calculated using Pearson's correlation coefficient and significance was checked for an alpha value = 0.05. All correlations were computed using Spearman's correlation coefficient as well and since the data didn't show much of a difference, we decided to use Pearson's correlation coefficient to estimate trends in the data.

RESULTS

Distance of communication has been shown to be an important factor influencing the acoustic transmission of signals. To understand the effect of distance of communication on different song features of the adult male zebra finch, we recorded and analysed songs and visual displays of the male and female zebra finches during courtship across five distances (FIG 8) (See Methods).

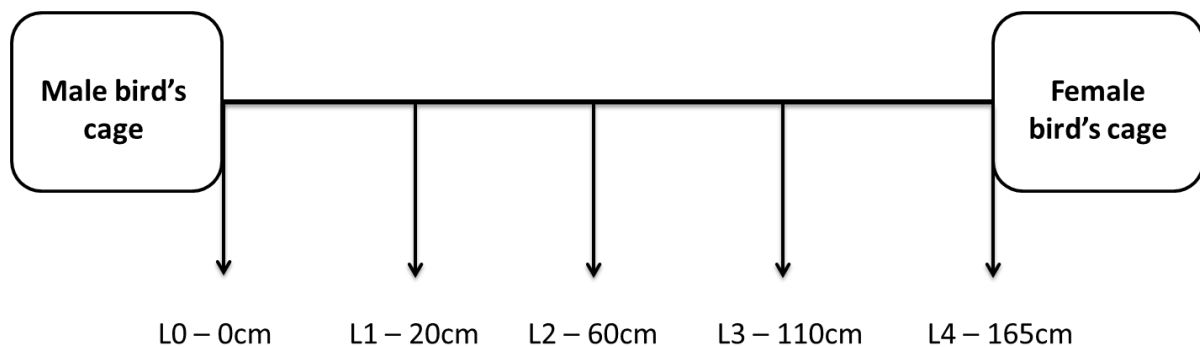


FIG. 8: Schematic representation of various distances at which the song features were estimated during the experiment.

Previous studies have shown context dependent differences in song features. Songs sung in presence of female (Directed songs) are preceded by a higher number of introductory notes per bout, have higher number of motifs and hence longer bouts, show less variability in syllable structure, and are faster in tempo than the songs sung by the male bird when alone (Undirected songs) (FIG 9, Table 1). Using these observations as basis, we quantified the aforementioned song features to understand influence of distance of communication on them.

1 a) Courtship song features change with distance from female, for all song bouts independent of visual cues

Changes in number of INs, number of motifs, motif duration, and variability in fundamental frequency were assessed with distance and the following trends were observed. It was verified that these features were not influenced by the changes in amplitude and changes in position of the microphone.

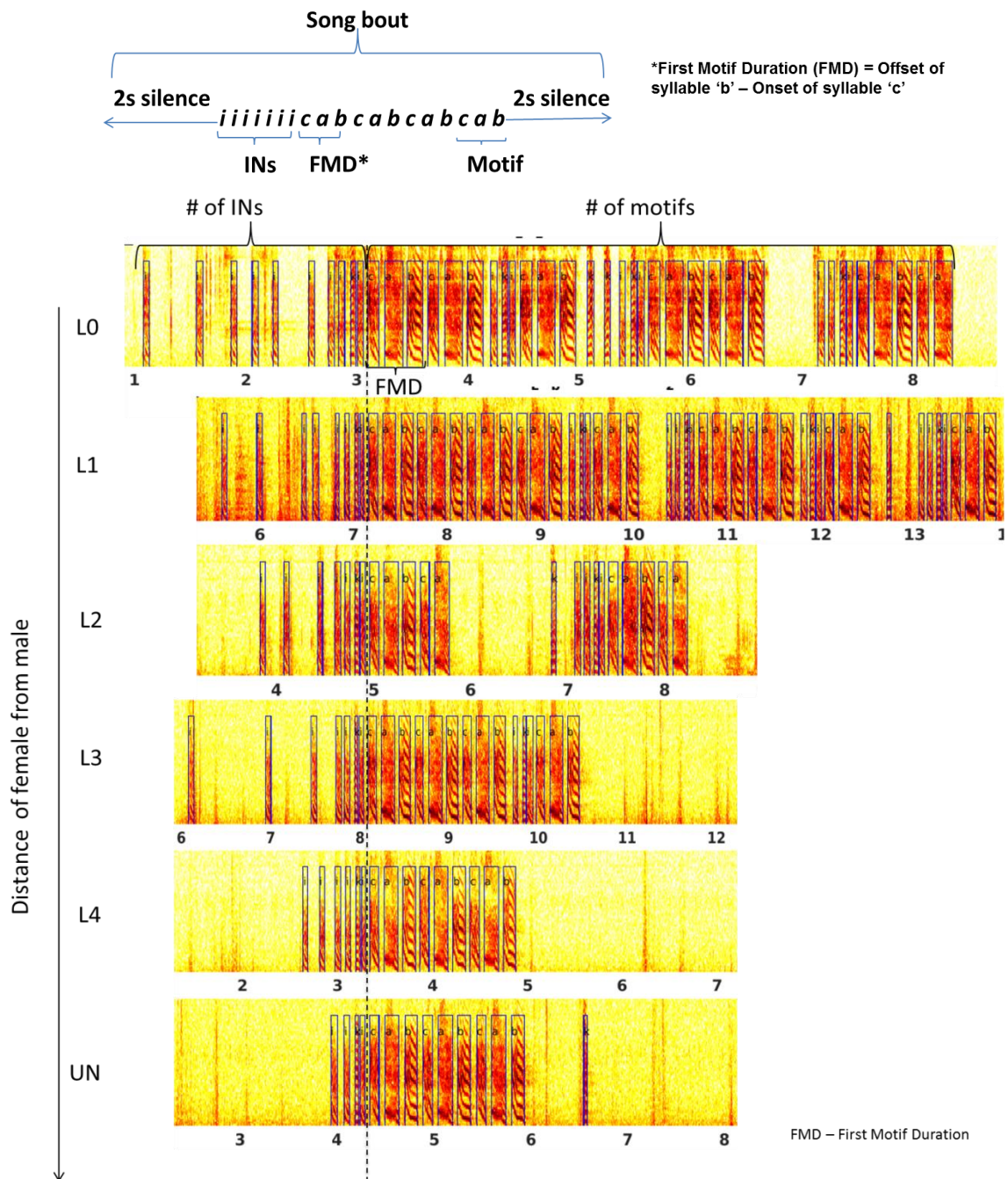


FIG. 9: (Top) Components of a song bout. (Bottom) Representation of changes in different song features through song spectrograms (frequency vs time) across distances from the songs recorded from one representative bird. *i* – INs. *cab* – motif. FMD – First motif duration.

Table 1 – Reported differences in song features for directed and undirected songs (Aronov and Fee, 2012; Kao and Brainard, 2006; Kao et al., 2005; Sossinka and Bohner, 1980)

Song feature	Directed Songs	Undirected Songs
Number of INs	More in number	Less in number
Number of motifs and bout length	More in number or longer songs	Less in number or shorter songs
First motif duration ~ song tempo	Faster songs or shorter in duration	Slow songs or longer in duration
Fundamental Frequency	Less variable	Highly variable

We characterised the change in the number of introductory notes (INs) preceding bouts and observed that the number of INs, in fact, decreased with increase in distance for 8 out of 10 birds individually ($p < 0.05$; median $r = -0.24$; range = -0.7 to 0.07) (FIG.10a,b). When the mean number of INs for directed songs at each distance were compared against the undirected song bouts (UN), we see a significant difference between the undirected songs and directed songs at L0

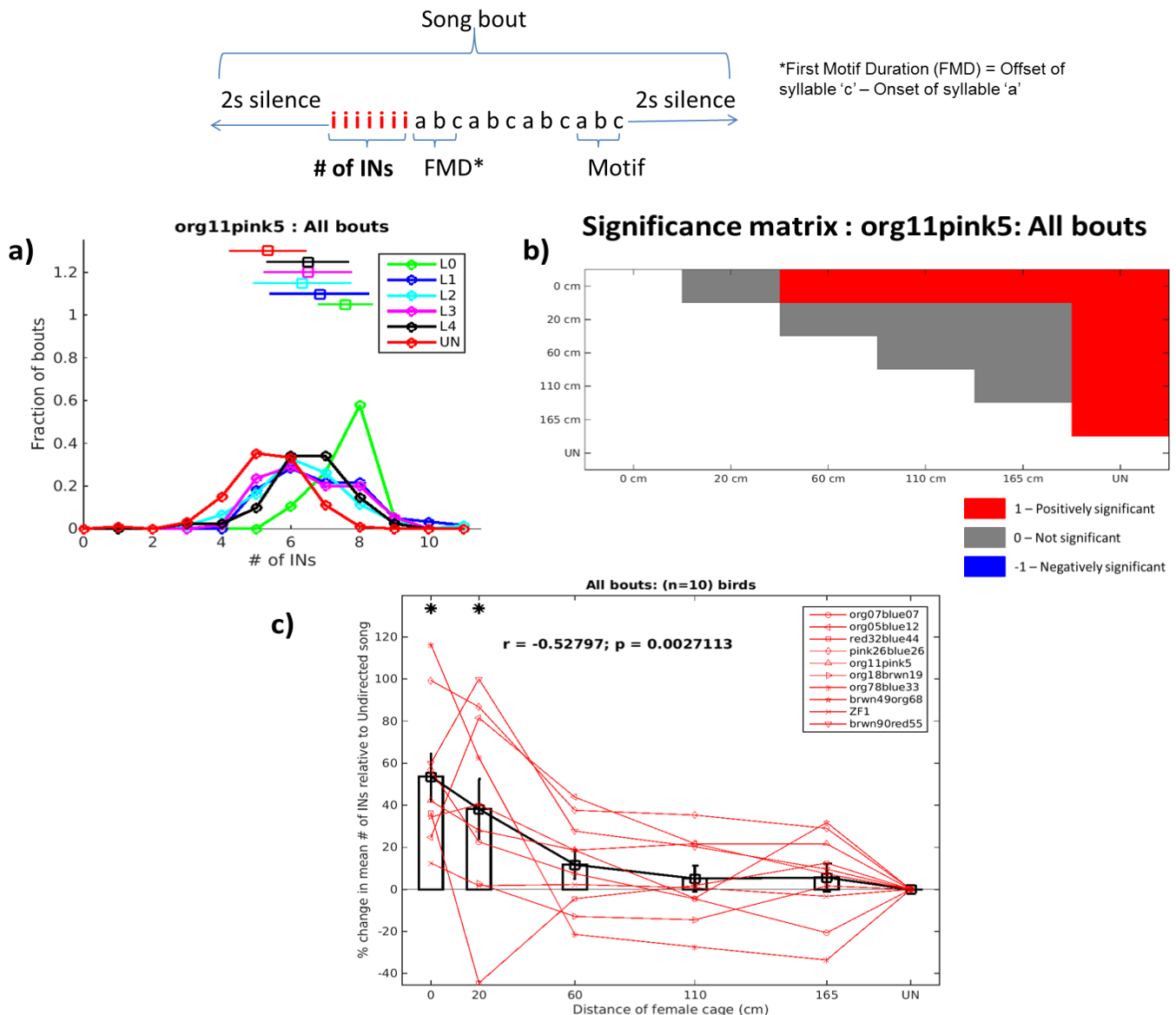


FIG 10. Change in mean number of introductory notes with increase in distance of communication

a) Variation in mean number of introductory notes (INs) for a representative bird (org11pink5) across distances (L0, L1, L2, L3, L4) and UN. The lines on top represent the average number of INs at each distance. (Horizontal error bars - SD)

b) A representation of test for significance of difference in mean number of INs against each distance for one bird (Kruskal-Wallis ANOVA, post-hoc Tukey-Kramer criterion) Significant differences ($p < 0.05$) are expressed as -1 and 1 whereas no significance is given by 0. Red (1) represents changes which are positively significant (eg. (Value at L0 - Value at L2) > 0); Grey represents no significance

c) Plot of percentage change in mean number of INs relative to undirected songs across distance for 10 birds. Each red line with a different symbol represents a different bird. The bars show the mean percentage change for all birds together at each location relative to the mean number of INs of undirected song. The error bar represents standard error. The black line joining the means shows the negative correlation of number of INs with distance (Pearson's correlation: $r = -0.527$; $p = 0.0027$) for all birds.

(Distance = 0 cm) and L1 (Distance = 20 cm), for 6 out of 8 birds and this significance was maintained across all birds for all distances (FIG 10c). This is consistent with the results reported by Rajan and Doupe, 2013 and Sossinka and Bohner, 1980 stating that directed songs have higher number of INs preceding them and suggesting that more preparation is needed before the start of a directed song. It was also seen that the percentage change in the mean number of INs for directed songs at each distance relative to UN songs showed a significant negative correlation with distance, when the data from all birds was pooled in ($r = -0.527$; $p = 0.0027$). Together, these results show that the male bird produces higher number of INs at shorter distances.

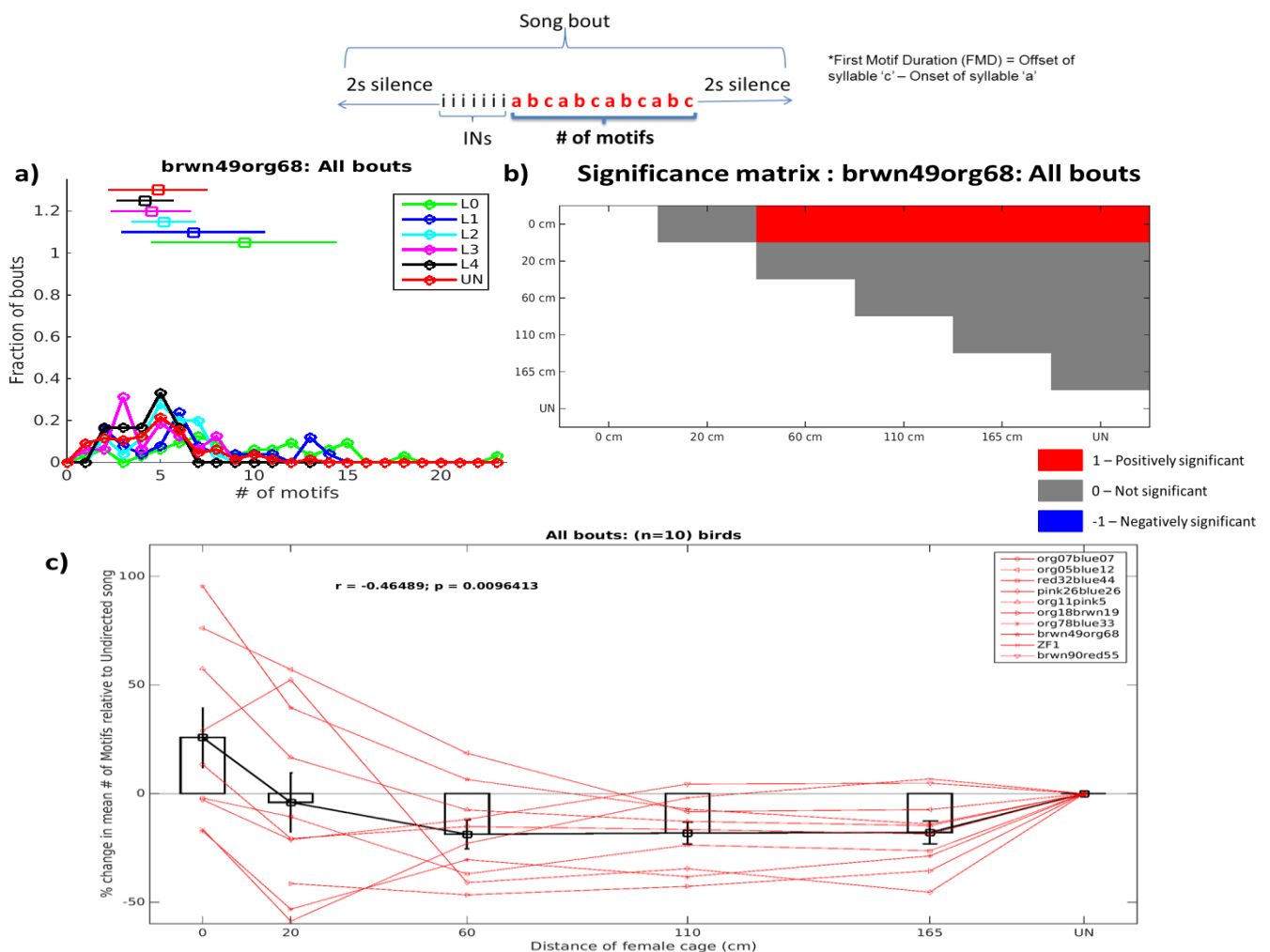


FIG 11. Change in mean number of motifs with increase in distance of communication

a) Variation in mean number of motifs for a representative bird (brwn90red55) across distances (L0, L1, L2, L3, L4) and in comparison with undirected song (UN). The lines on top represent the average number of motifs at each distance. (Horizontal error bars - SD)

b) A representation of test for significance of difference in mean number of motifs against each distance for one bird (Kruskal-Wallis ANOVA, post-hoc Tukey-Kramer criterion). Significant differences ($p < 0.05$) are expressed as -1 and 1 whereas no significance is given by 0. Red (1) represents changes which are positively significant (eg. (Value at L0 – Value at L2) > 0); Grey represents no significance

c) Plot of percentage change in mean number of motifs relative to undirected songs across distance for 10 birds. Each red line with a different symbol represents a different bird. The bars show the mean percentage change for all birds together at each location relative to the mean number of motifs of undirected song. The error bar represents standard error. The black line joining the means shows the negative correlation of number of motifs with distance (Pearson's correlation: $r = -0.46489$; $p = 0.0096$) for all birds.

Next, we quantified the changes in length of each song bout with distance through two parameters - Number of motifs and bout length (in milliseconds). Both these features decreased with increase in distance of communication. However, only number of motifs showed a significant decrease for 6 out of 10 birds individually, (FIG.11a,b) ($p < 0.05$; median $r = -0.127$, range = -0.471 to 0.217) while bout length decreases for 5 out of 10 birds significantly ($p = 0.2$; median $r = -0.144$, range = -0.46 to 0.082) (FIG. 12a,b).

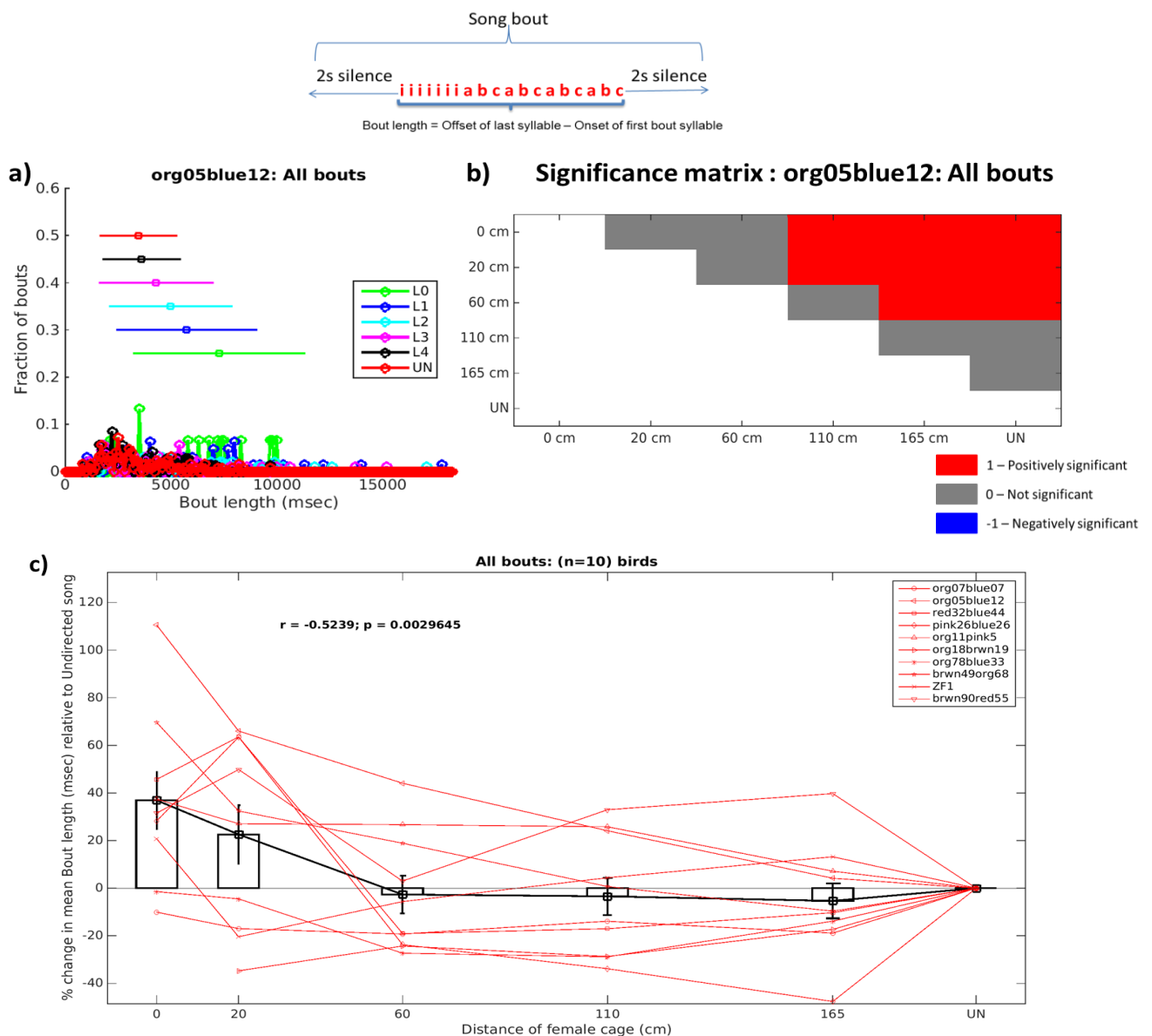


FIG 12. Change in mean bout length with increase in distance of communication

a) Variation in mean bout length (ms) for a representative bird (org05blue12) across distances (L0, L1, L2, L3, L4) and undirected song (UN). The lines on top represent the average bout length at each distance. (Horizontal error bars - SD)

b) A representation of test for significance of difference in mean bout length against each distance for one bird (Kruskal-Wallis ANOVA, post-hoc Tukey-Kramer criterion). Significant differences ($p < 0.05$) are expressed as -1 and 1 whereas no significance is given by 0. Red (1) represents changes which are positively significant (eg. (Value at L0 – Value at L2) > 0); Grey represents no significance.

c) Plot of percentage change in mean bout length relative to undirected songs across distance for 10 birds. Each red line with a different symbol represents a different bird. The bars show the mean percentage change for all birds together at each location relative to the mean bout length of undirected song. The error bar represents standard error. The black line joining the means shows the negative correlation of bout length with distance (Pearson's correlation: $r = -0.5239$; $p = 0.0029$) for all birds.

Comparison of percentage change in mean number of motifs and bout length with respect to undirected song (UN) yielded a significant negative correlation with distance for 5 out of 10 birds for motif number, and 4 out of 10 birds for bout length, and was significant across all birds, for both these features (motif number: $-r = -0.46$; $p = 0.09$) (FIG. 11c); (bout length: $r = -0.5239$, $p = 0.002$) (FIG. 12c). Trends in both of these features together showed that song bouts become shorter with increase in distance.

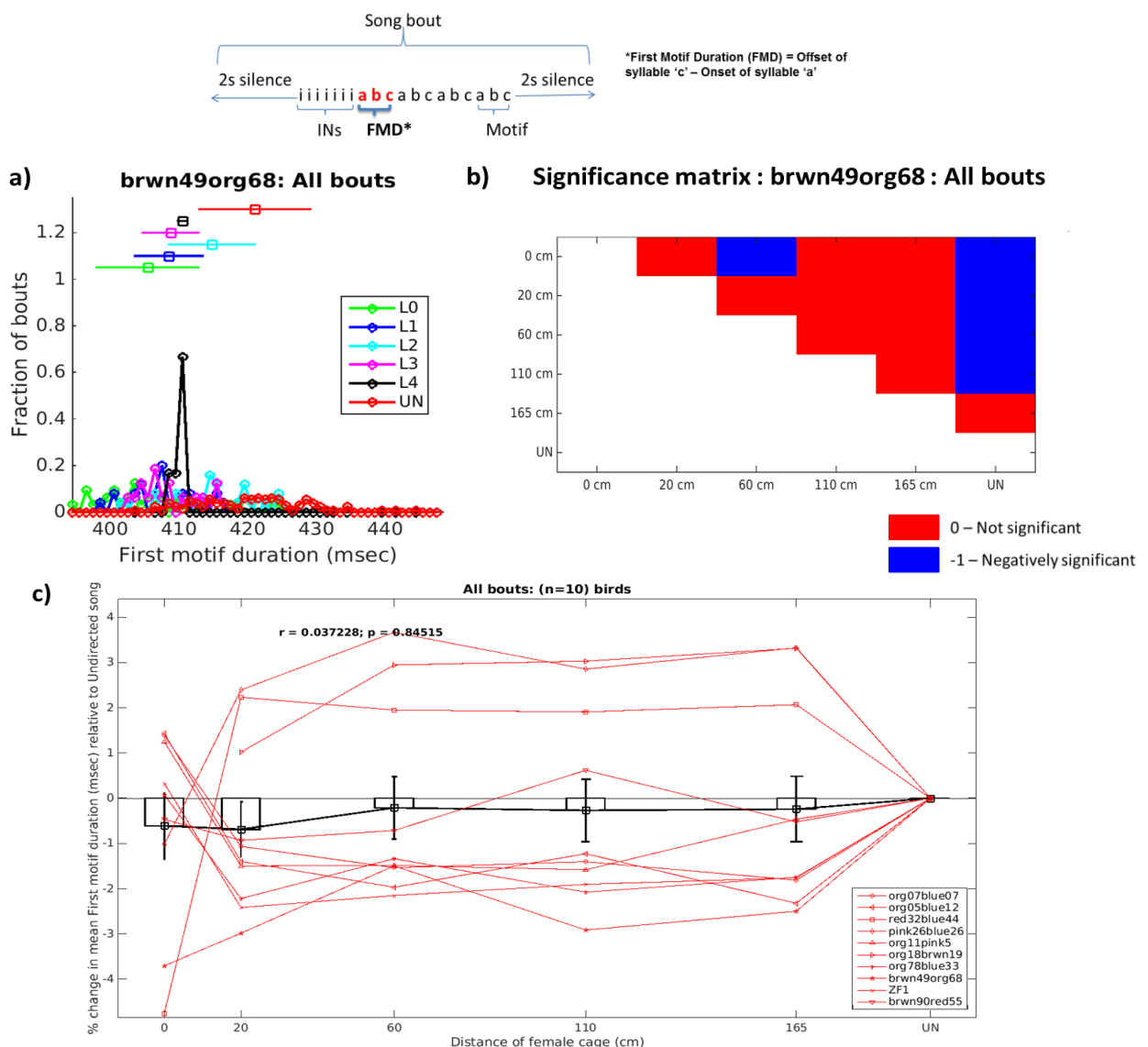


FIG 13. Change in mean first motif duration with increase in distance of communication
a) Variation in mean first motif duration (ms) for a representative bird (brwn49org68) across distances (L0, L1, L2, L3, L4) and in comparison with undirected song (UN). The lines on top represent the average first motif duration (ms) at each distance. (Horizontal error bars - SD)
b) A representation of test for significance of difference in mean first motif duration against each distance for one bird (Kruskal-Wallis ANOVA, post-hoc Tukey-Kramer criterion). Significant differences ($p < 0.05$) are expressed as -1 and 1 whereas no significance is given by 0. Blue (-1) represents changes which are negatively significant (eg. (Value at L0 – Value at L2) < 0); Red (0) represents no significance.
c) Plot of percentage change in mean first motif duration (FMD) relative to undirected songs across distance for 10 birds. Each red line with a different symbol represents a different bird. The bars show the mean percentage change for all birds together at each location relative to the mean FMD of undirected song. The error bar represents standard error. The black line joining the means shows the positive correlation of FMD with distance (Pearson's correlation: $r = 0.037$; $p = 0.84515$) for all birds.

In order to assess the differences in song tempo with distance, the motif duration of song bouts were quantified. Since each song bout has variable number of motifs, we calculated only the first motif duration (FMD). It is known that the difference in first motif duration between directed and undirected song is about 5% (Aronov and Fee, 2012). Hence, we hypothesised that the first motif duration could increase with increase in distance from female. We saw that the FMD doesn't change significantly with distance, when data for all the birds was grouped and showed a maximum change of 3-4% with respect to the FMD of undirected song. However, when we inspected the data individually, the FMD for 7 out of 10 birds, showed an increase for distances L1 to L2-L3 (20 cm to 110 cm). This was attributed to the time gap (~3-4 months) between L0 (0 cm) recordings and other distance recordings as it has been shown that song tempo could change with age (Pytte et al., 2007).

Table 2 – Pearson's correlation coefficients and p values for four song features; The ones highlighted in yellow show a significant negative correlation whereas the ones highlighted in green show a significant positive correlation

Bird Name	Number of INs		Number of Motifs		Bout Length		First Motif Duration	
	r	p	r	p	r	p	r	p
org07blue07	-0.227	0	-0.054	0.274	0.012	0.808	-0.209	0
org05blue12	-0.199	0.001	-0.355	0	-0.248	0	-0.172	0.005
red32blue44	-0.062	0.477	-0.193	0.026	-0.46	0	0.649	0
pink26blue26	-0.546	0	-0.433	0	-0.403	0	0.666	0
org11pink5	-0.216	0.002	-0.352	0	-0.043	0.554	-0.263	0
org18brwn19	-0.261	0.057	-0.018	0.9	0.082	0.556	0.369	0.006
org78blue33	-0.695	0	-0.062	0.371	-0.245	0	-0.264	0
brwn49org68	-0.294	0.003	-0.471	0	-0.355	0	0.335	0.001
ZF1	-0.126	0.132	0.217	0.009	-0.021	0.803	-0.24	0.004
brwn90red55	-0.253	0	0.073	0.275	-0.036	0.589	0.127	0.058
Median	-0.24	0.0015	-0.1275	0.0175	-0.144	0.277	-0.0225	0.0005
Min	-0.695	0	-0.471	0	-0.46	0	-0.264	0
Max	-0.062	0.477	0.217	0.9	0.082	0.808	0.666	0.058

Lastly, we evaluated the variability in song structure by measuring the fundamental frequency (FF) of parts of the syllable containing harmonic stacks (FIG 14). Since FF is known to be a precisely learned component of song, the changes in FF directly correlate with the changes in song syllable structure (Kao and Brainard, 2006; Kao et al., 2005). We estimated the FF for 20 motif syllables across 10 birds and observed that though the average FF significantly decreased for 10/20 syllables and increased for 5/20 syllables, the overall average FF for all 20 syllables together doesn't change

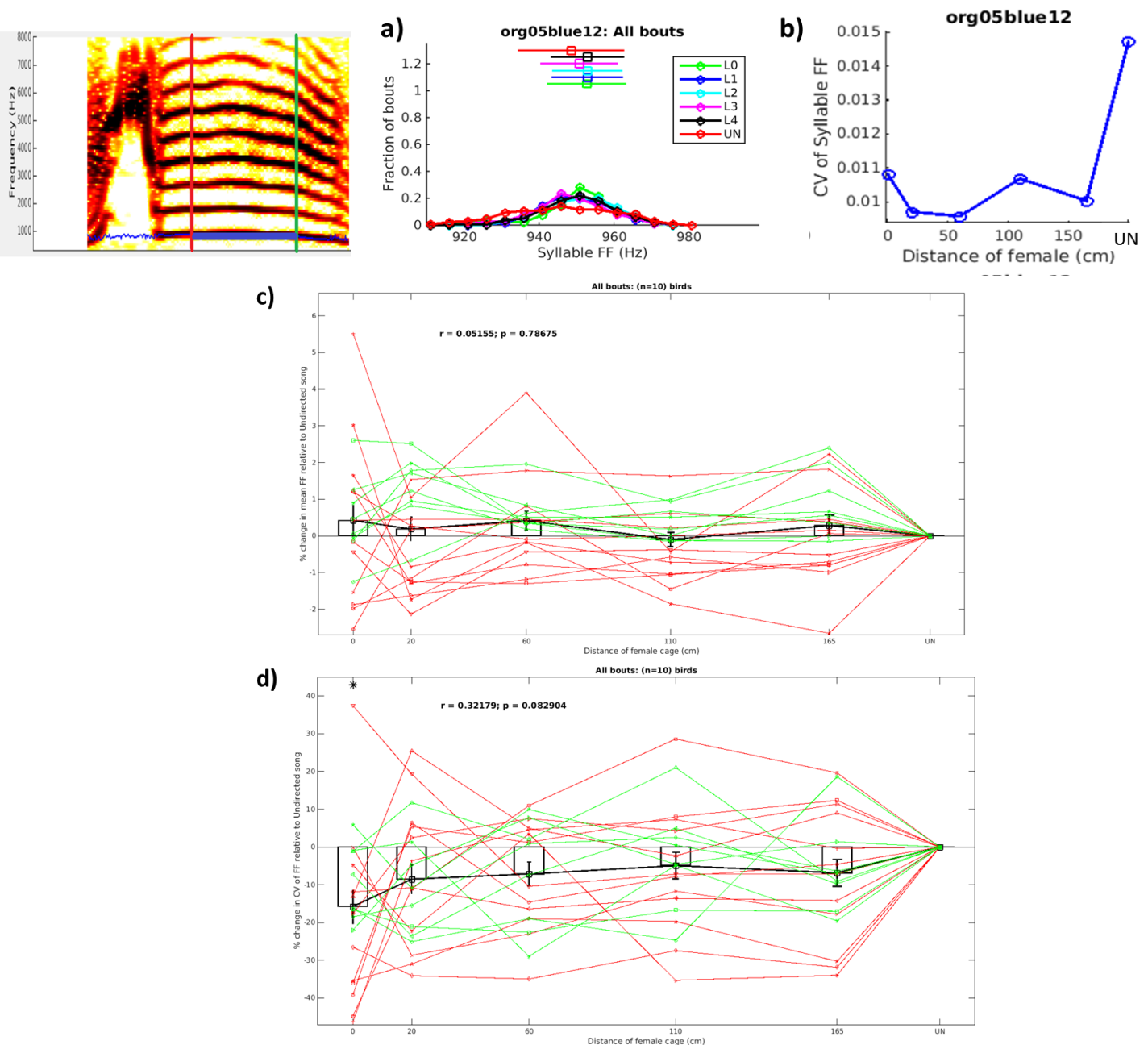


FIG 14. Changes in mean fundamental frequency and variability in FF with distance from female

(First panel) Spectrographic representation of a syllable with the red and green lines marking the boundaries demarcating the region chosen to calculate FF for. The blue line represents the FF for the syllable.

a) Distribution of fundamental frequency (FF) (Hz) for one syllable for a representative bird (org05blue12) for different distances. The mean FF has been shown on the top of the plot with different colours for each distance. (Horizontal error bars - SD).

b) Variability in FF plotted as average of CV (std/mean) for a representative bird for all bouts across distances. CV of FF is less than UN at all distances.

c) Plot of percentage change in mean FF relative to undirected songs across distances for 20 syllables. Each line with a different symbol and colour represents a different syllable. The bars show the mean percentage change for all birds together at each location relative to the mean FF of undirected song. The error bar represents standard error. The black line joining the means shows the correlation of mean FF with distance ($r = 0.05$; $p = 0.78$).

d) Plot of percentage change in mean CV of FF relative to undirected songs across distances for 20 syllables. Each line represents a different syllable. The bars show the mean percentage change for all birds together at each location relative to the mean CV of FF of undirected song. The error bar represents standard error. The black line joining the means shows the correlation of CV of FF with distance ($r = 0.32$; $p = 0.08$). The star notifies the significance of CV at L0 with respect to UN.

significantly with distance from female (FIG 14a,c). The significant changes in mean FF at the level of individual syllables could be potentially explained by looking at the range of FF values at each distance relative to the range of FF values for the

undirected song bouts. The range of variation seemed to be similar for all distances and undirected songs in 12/15 syllables that showed significant changes in mean FF across distance.

The variability in FF was determined by calculating the coefficient of variation (CV = standard deviation/mean). It is known that CV of song syllables is more for undirected songs in comparison to directed songs (Kao and Brainard, 2006; Kao et al., 2005). We saw that the CV for syllables at the shortest distance (L0 = 0cm) were significantly lower than undirected song syllables for all the 20 syllables (FIG 14b, d). However, the change in CV with distance was not significant for the 20 syllables (FIG 14b, d). This suggests that the variability in song syllable structure may or may not increase with distance.

Table 3 – Summarised trends in song features with distance for each bird (n =10) for all song bouts

Bird Name	Number of INs	Number of motifs	Bout length	First Motif Duration	Mean FF	CV of FF
org07blue07	Black	Black	Black	Blue	Black	Red
org05blue12	Blue	Blue	Blue	Blue	Blue	Red
red32blue44	Black	Blue	Blue	Red	Blue	Black
pink26blue26	Blue	Blue	Blue	Red	Red	Black
org11pink5	Blue	Blue	Black	Blue	Blue	Red
org18brwn19	Blue	Black	Black	Red	Black	Black
org78blue33	Blue	Black	Blue	Blue	Blue	Red
brwn49org68	Blue	Blue	Blue	Red	Blue	Black
ZF1	Black	Blue	Black	Blue	Blue	Black
brwn90red55	Blue	Black	Black	Black	Black	Black
*Birds which show significant changes in at least three song features as per expected trends are in bold						
Black	No significant change					
Blue	Significant decrease with distance					
Red	Significant increase with distance					

Together, these results indicate that six out of the ten birds showed a significant change in at least 3 of the song features (TABLE 2 and 3) and these changes followed trends which would suggest that song bouts become more undirected-like with increase in distance from female.

I b) Songs scored as ‘Directed’ based on visual cues suggest that directed songs can vary in intensity with distance from female

According to the results reported by Sossinka and Bohner, (1980), the two types of song classified on the basis of social context - directed and undirected, are distinct and show a bimodal distribution. In order to compare the changes in song features for these two types of song over distance, songs sung by male birds were scored as Directed (D) or Undirected (UN) based on the visual displays like hopping, beak-wipes, turn-arounds etc (See Methods). We speculate that the changes in song features with distance for these song types would follow either one of the following two propositions (FIG 15) –

- a) Songs categorised as Directed and Undirected would be distinct at each distance and the change could be attributed to differences in number of bouts for each song type at each distance as we saw that song features become more undirected-like with distance.
- b) Song bouts classified as directed would show a decrease in intensity with distance i.e. the four song features quantified would increase or decrease in such a manner that they become similar to undirected song features at longer distances. This trend in directed song bouts would be the same as that observed when all song bout types are clubbed together (results for which are shown previously).

However, we do not rule out the possibility of the causality for the changes observed in these song features across distances, for the two song types, to be a combination

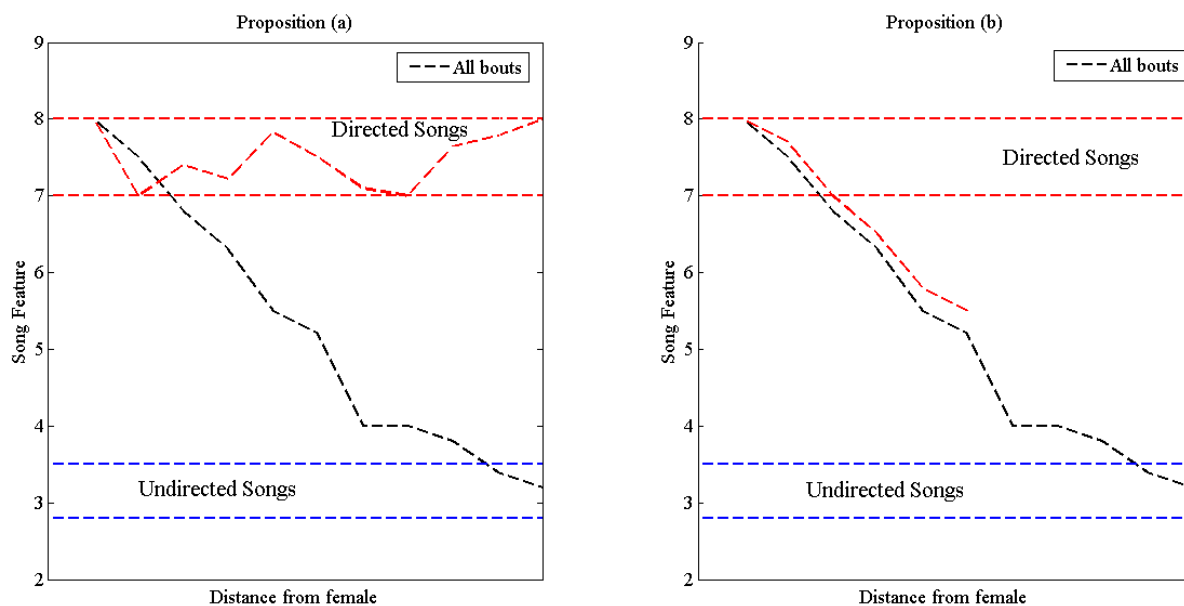


FIG 15. Schematic representation of the two propositions to explain changes in song features with distance for different song types
 Red lines parallel to the axis – demarcates the directed song feature values; Blue lines parallel to the axis – demarcates the undirected song feature values; Red dotted line – shows the trend of directed song bouts for the song feature; Black dotted line – shows the data for all song bouts clubbed together

of both the propositions stated above.

To assess if the changes in song features followed either or both the propositions, the five song features were computed for the classified song bouts and compared against all song bouts. Song bouts were considered for analysis only if they satisfied two conditions i.e. Number of bouts for each category > 5 and at least 3 distances must have song bouts of each category (TABLE 4). After elimination of the outliers (See Methods), we calculated the percentage of song bouts categorised as Directed at each distance and observed that the number decreased with distance, as expected (FIG 16).

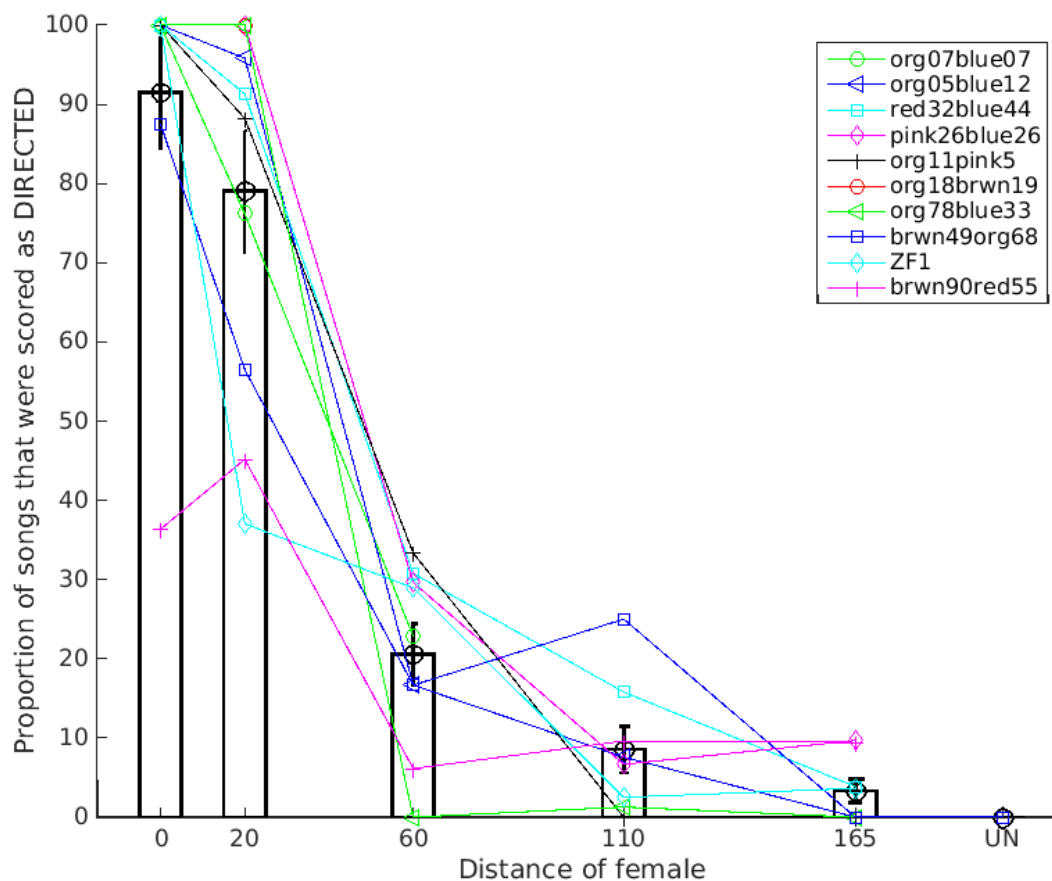


FIG 16. Percentage of songs that were scored as Directed at each distance. Different lines represent different birds (n =10)

We, then, calculated the variation in number of INs, number of motifs, bout length, variation in fundamental frequency and first motif duration for each of the classified song bouts across distance.

Table 4 – Number of bouts considered for analysis after categorisation into D, and UN.

Bird Name	Condition	Total # of bouts	# of bouts with video scoring	# of Directed bouts	# of Undirected bouts
org07blue07	L0	18	18	18	0
	L1	75	63	48	9
	L2	169	92	21	43
	L3	148	0	0	0
	L4	168	0	0	0
	UN	148	148	148	148
org05blue12	L0	15	15	15	0
	L1	63	24	23	1
	L2	94	60	10	27
	L3	88	66	5	47
	L4	70	45	0	38
	UN	123	123	123	123
red32blue44	L0	23	16	16	0
	L1	23	23	21	0
	L2	26	13	4	7
	L3	61	38	6	27
	L4	43	26	1	23
	UN	68	68	68	68
pink26blue26	L0	29	3	3	0
	L1	14	14	14	0
	L2	28	27	8	2
	L3	30	30	2	10
	L4	32	31	3	26
	UN	75	75	75	75
org11pink5	L0	19	19	19	0
	L1	60	17	15	0
	L2	61	24	8	0
	L3	55	14	0	0
	L4	41	22	0	21
	UN	99	99	99	99
org18brwn19	L1	22	22	22	0
	L2	14	0	0	0
	L3	18	0	0	0
	L4	12	0	0	0
	UN	24	24	24	24
	org78blue33	L0	24	23	23
L1		36	22	22	0
L2		71	25	0	25
L3		80	78	1	75
L4		70	11	0	11
UN		81	81	81	81
brwn49org68	L0	32	8	7	1
	L1	25	23	13	6
	L2	25	24	4	17
	L3	16	16	4	8
	L4	6	6	0	6
	UN	130	130	130	130
ZF1	L0	24	24	24	0
	L1	28	27	10	14
	L2	38	38	11	16
	L3	53	40	1	29
	L4	58	55	2	51
	UN	65	65	65	65
brwn90red55	L0	65	55	20	25
	L1	38	31	14	15
	L2	52	49	3	46
	L3	69	52	5	38
	L4	55	53	5	45
	UN	90	90	90	90

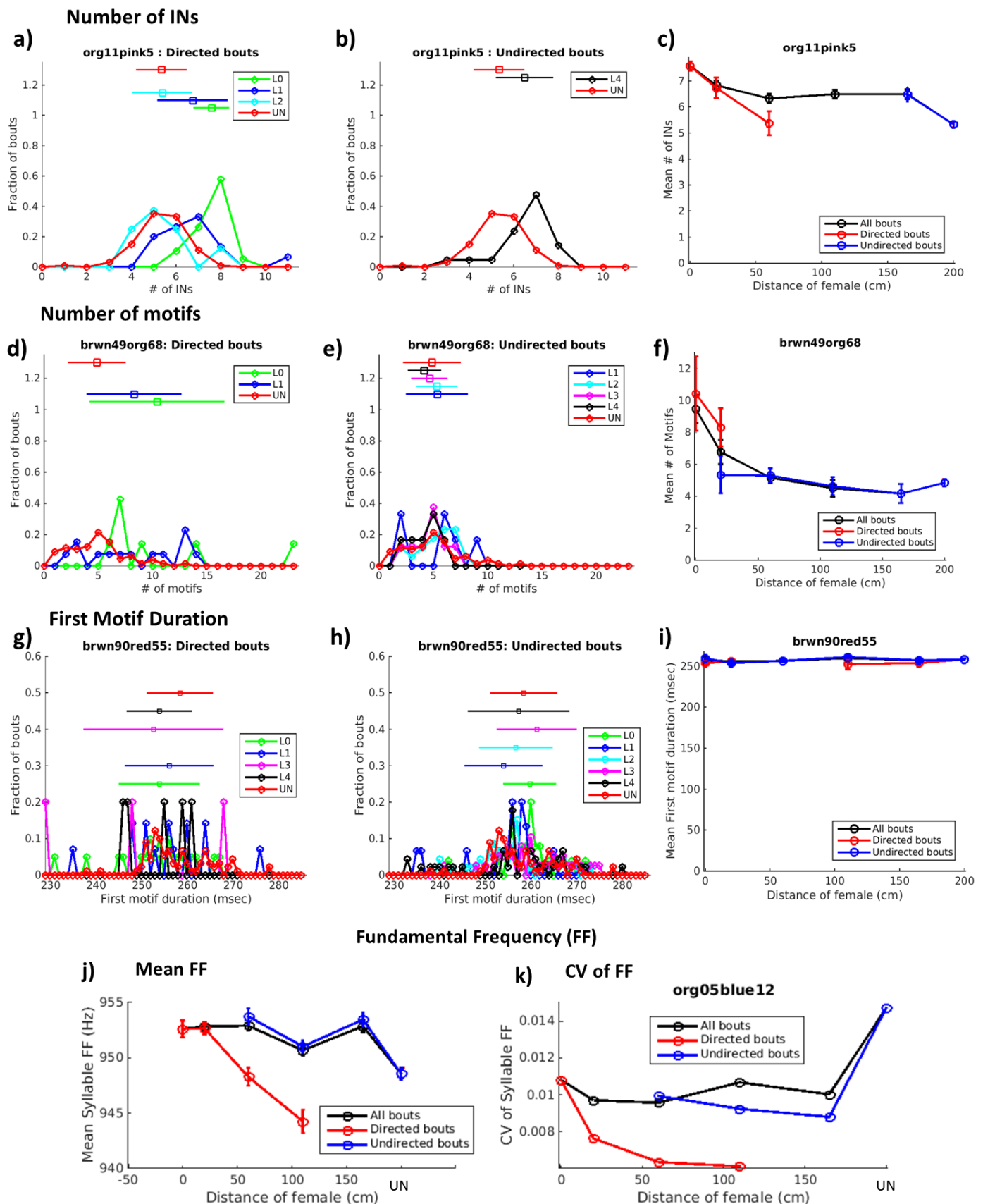


FIG 17. Examples of changes in each song feature for song bouts categorised as Directed and Undirected in comparison to changes in all bouts

(a,b,d,e,g,h) – Solid lines on top represent means and errors bars represent SD; (c,f,i,j) Error bars – SE
 (a-i) Using representative bird for each song feature, mean variation with distance is shown for directed and undirected song bouts separately and then is compared to variation in all song bouts.

(j-k) Variation in mean FF and CV of FF is shown for directed, undirected, and all song bouts.

Mean number of INs, and motifs for song bouts classified as directed showed a negative correlation with distance (TABLE 5) but the change wasn't significant due to small number of directed bouts at longer distances (TABLE 4). Directed songs at shorter distances do show a gradual fall in mean number of INs and motifs whereas undirected songs are largely confined to longer distances and are generally lower in number than directed song bouts at shorter distances (FIG 17 a-c, d-f; TABLE 5). As observed previously, first motif duration doesn't essentially change with distance even after categorisation of the songs as directed and undirected at each distance (FIG 17 g-i; TABLE 5). Fundamental frequency for song bouts scored as 'directed' showed no change in mean FF with distance (n = 20 syllables, 10 birds) while the CV of FF increased with distance but the change was not significant (FIG 17 j,k; TABLE 5).

Table 5 – Summarised trends in song features with distance for all song bouts and song bouts categorised as Directed, for each bird (n =10)

Bird Name	Number of INs		Number of motifs		Bout length		First Motif Duration		Mean FF		CV of FF	
	A	D	A	D	A	D	A	D	A	D	A	D
org07blue07	Black	Black	Black	Black	Black	Black	Blue	Blue	Black	Black	Red	Black
org05blue12	Blue	Black	Blue	Blue	Blue	Black	Blue	Blue	Blue	Black	Red	Black
red32blue44	Black	Black	Blue	Black	Blue	Blue	Red	Red	Blue	Black	Black	Black
pink26blue26	Blue	Black	Blue	Black	Blue	Black	Red	Black	Red	Black	Black	Black
org11pink5	Blue	Blue	Blue	Black	Black	Black	Blue	Blue	Blue	Black	Red	Black
org18brwn19	Blue	Black	Black	Black	Black	Black	Red	Black	Black	Black	Black	Black
org78blue33	Blue	Black	Black	Black	Blue	Black	Blue	Black	Blue	Black	Red	Black
brwn49org68	Blue	Black	Blue	Black	Blue	Black	Red	Black	Blue	Black	Black	Black
ZF1	Black	Black	Blue	Black	Black	Black	Blue	Blue	Blue	Black	Black	Black
brwn90red55	Blue	Black	Black	Black	Black	Black	Black	Black	Black	Black	Black	Black

A -All song bouts; D - Directed song bouts

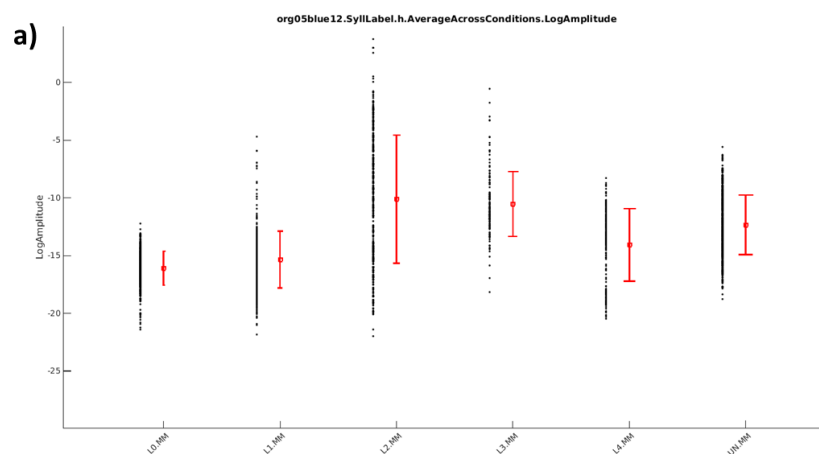
Black	No significant change
Blue	Significant decrease with distance
Red	Significant increase with distance

Summary

So far, the findings of this study has shown that number of INs, number of motifs, and bout length decrease with distance and first motif duration and variability in FF increase with increase in distance. Six out of the ten birds followed these trends significantly for at least three of the above mentioned song features, when all the song bouts were considered for analysis (TABLE 4). When the song bouts were classified as directed and undirected on the basis of visual cues, we observed that the percentage of songs classified as directed decreased with distance. We also showed that song bouts classified as directed followed changes in song features with distance, similar to the trends observed when all the song bouts were considered. Only two out of the ten birds demonstrated these changes in song features significantly for directed bouts (TABLE 5). Altogether, it was seen that song features became more undirected-like with distance and that changes in song features for directed songs were not significant due to a combination of two reasons – small number of directed songs at each distance, and directed song bouts themselves changed their song properties with distance.

Changes in song amplitude depends on a number of factors, including distance

Having characterised the changes in other song features with distance, we, next, quantified the changes in song amplitude with distance. According to the study conducted by Brumm and Slater (2006), amplitude of song motif syllables increased with distance of communication. We replicated their experiments over a smaller range of distances and observed the changes in song amplitude* with distance. Initially songs were recorded using the mixer microphone (as in (Brumm and Slater, 2006b)) and we observed that the song amplitude decreased with distance, though



the change was not significant (FIG 18) (n = 13 motif syllables, 6 birds).

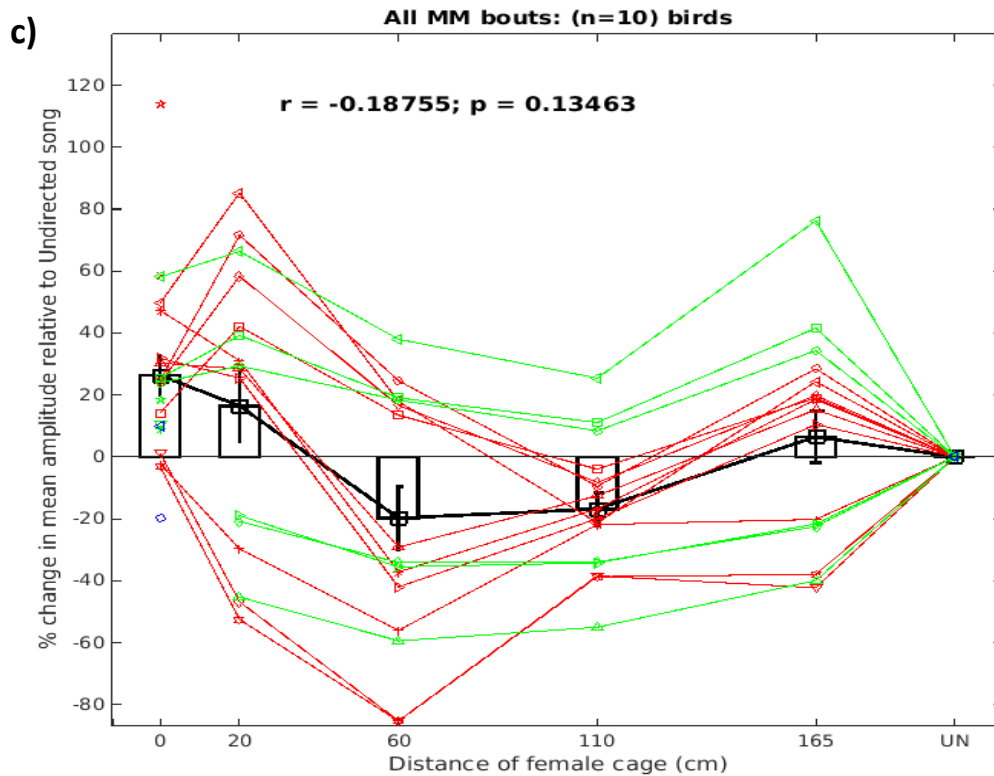
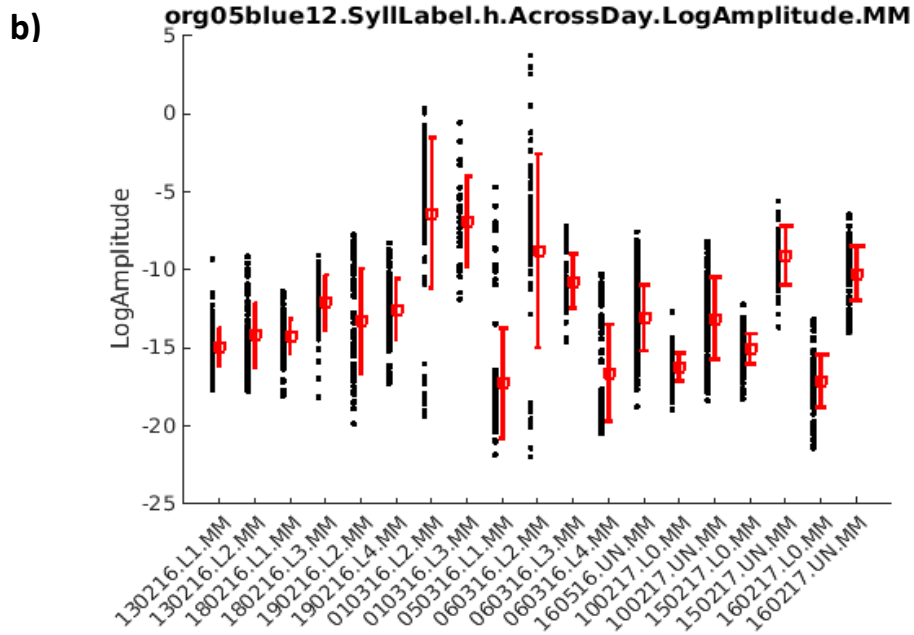


FIG 18. Change in log amplitude for a representative bird when recorded with *mixer microphone*

a,b – Black data points represent amplitude values for all individual syllable on the said day or distance. The red square marks the mean at each distance/day and the errors bars represent standard deviation.

a) Average change in amplitude across distances for a representative syllable. Data is not significant.

b) Day-to-day variability in amplitude for each distance (L0, L1, L2, L3, L4) for a representative bird.

c) Plot of percentage change in mean amplitude relative to undirected songs across distance for 13 syllables for 6 birds. Each line represents a different syllable (Irrespective of colour). The bars show the mean percentage change for all syllables together at each location relative to the mean amplitude of the same syllables occurring during undirected songs. The black line joining the means shows the negative correlation of amplitude with distance for mixer microphone recordings.

However, when we sought out to understand the reason behind this change, we realised that there was a variation in song amplitude for different positions of the bird inside the cage with respect to the fixed position of the microphone on top of the cage. When a monotone of fixed amplitude was played back through a speaker, we observed a variation of 4dB for prominently occupied positions of the male within the cage (FIG 19). The monotone amplitude ranged from 34 dB to 48 dB across all the 16 positions at which the monotone was played back to the microphone fixed at a position on the top of the cage.

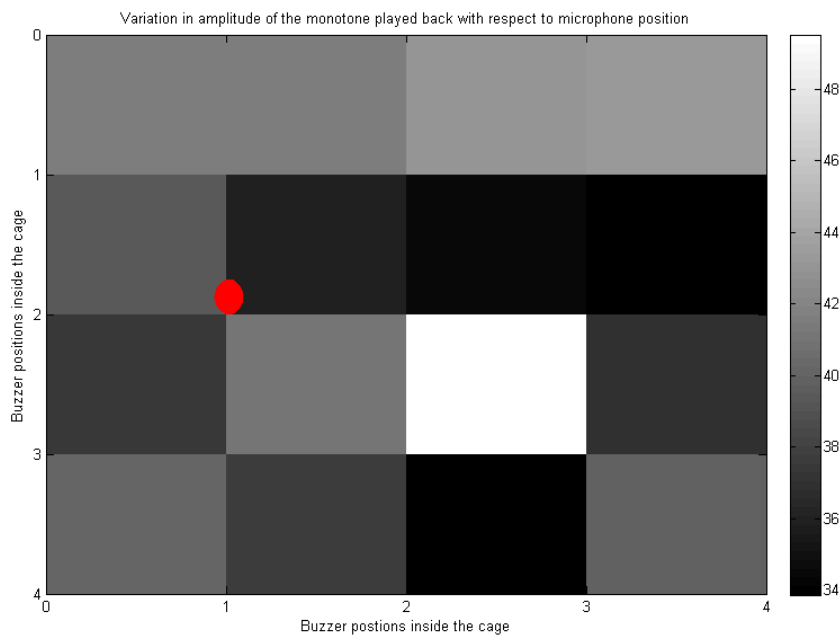


FIG 19. Variation in monotone amplitude played back at different positions within the cage
A monotone was played back at 16 positions within a cage used for recordings. Red circle represents microphone position.

To fix this issue, we used the backpack microphone (See Methods) to measure amplitude changes in song as this would be on the bird and moved with the bird around the cage. We observed that the song amplitude, in fact, showed no significant change with distance but the trend in amplitude variation with distance was seen (FIG 20). Amplitude decreased with increase in distance (n = 16 syllables, 7 birds).

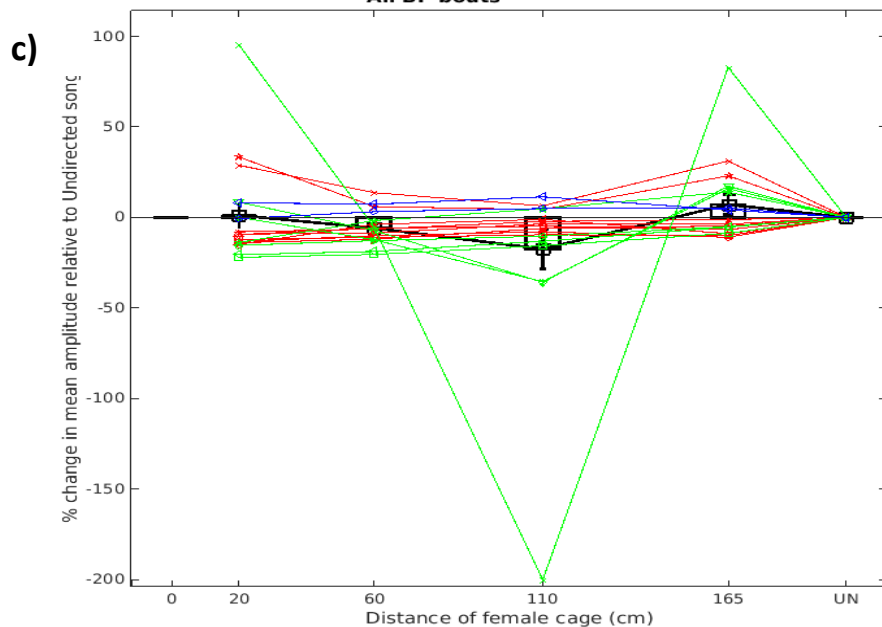
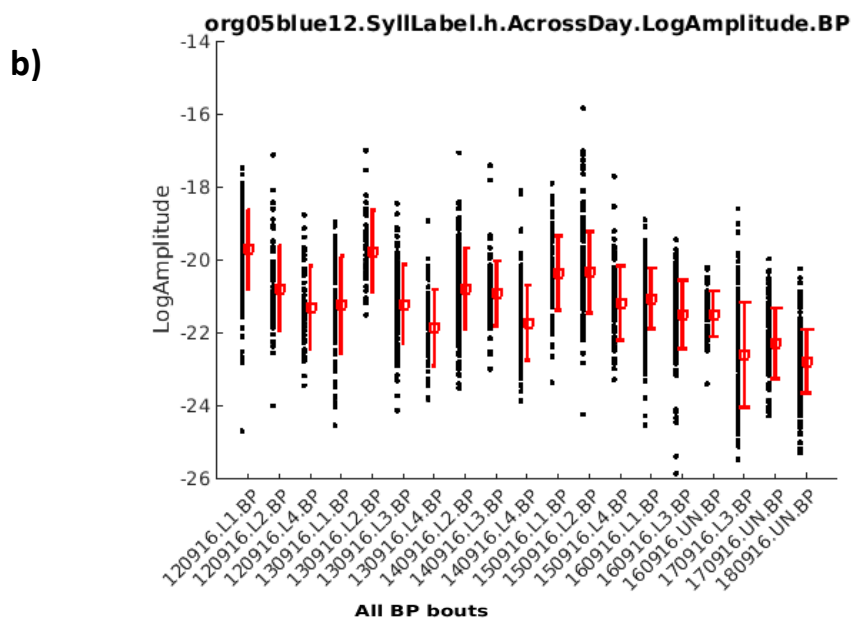
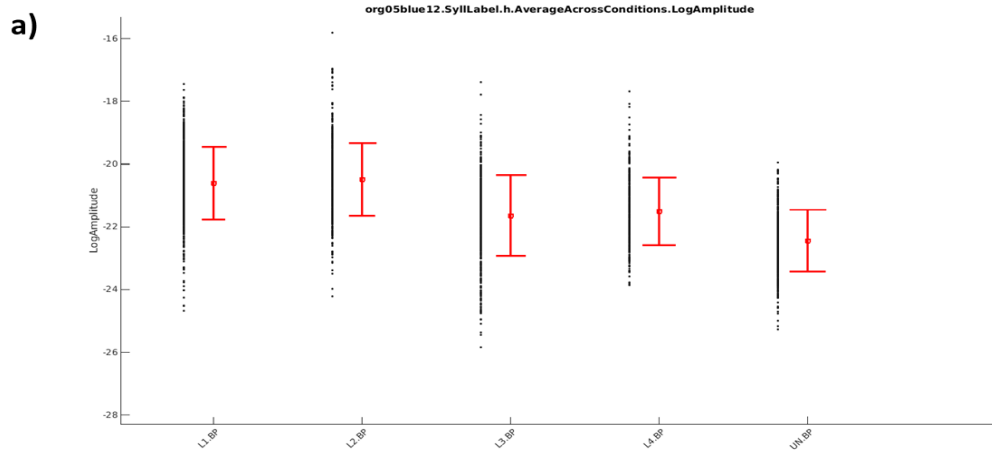


FIG 20. Change in log amplitude for a representative bird when recorded with *backpack microphone*

a,b – Black data points represent amplitude values for all individual syllable on the said day or distance. The red square marks the mean at each distance/day and the errors bars represent standard deviation.

a) Average change in amplitude across distances for a representative syllable. Data is not significant.

b) Day-to-day variability in amplitude for each distance (L0, L1, L2, L3, L4) for a representative bird.

c) Plot of percentage change in mean amplitude relative to undirected songs across distance for 16 syllables for 7 birds. Each line represents a different syllable. The bars show the mean percentage change for all syllables together at each location relative to the mean amplitude of the same syllables occurring during undirected songs. The black line joining the means shows the negative correlation of amplitude with distance for backpack microphone recordings.

We saw that there were significant variations in song amplitude at each distance across different days of recordings. This day-to-day variability in amplitude was attributed to the minute changes in position (in the scale of millimetres) of the microphone to the sides while it was on the back of the bird throughout the recording

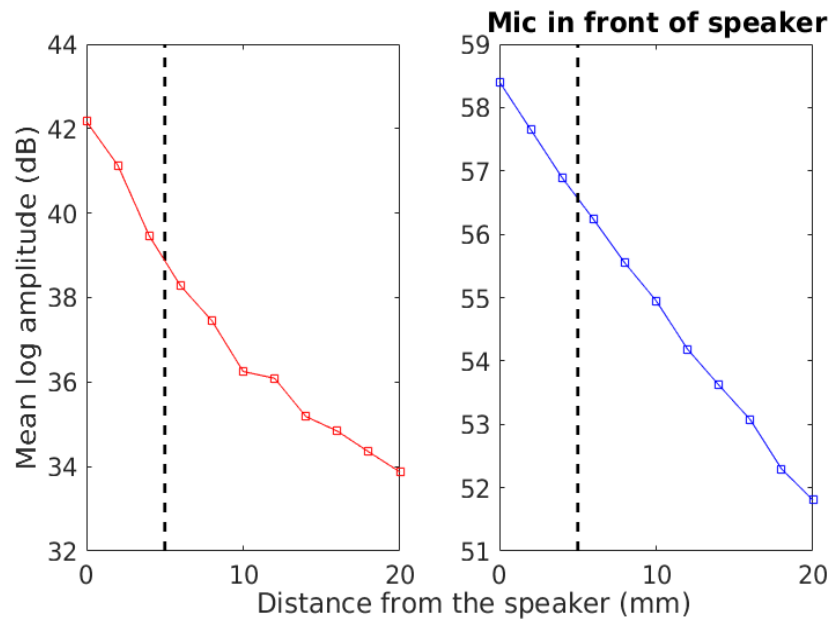
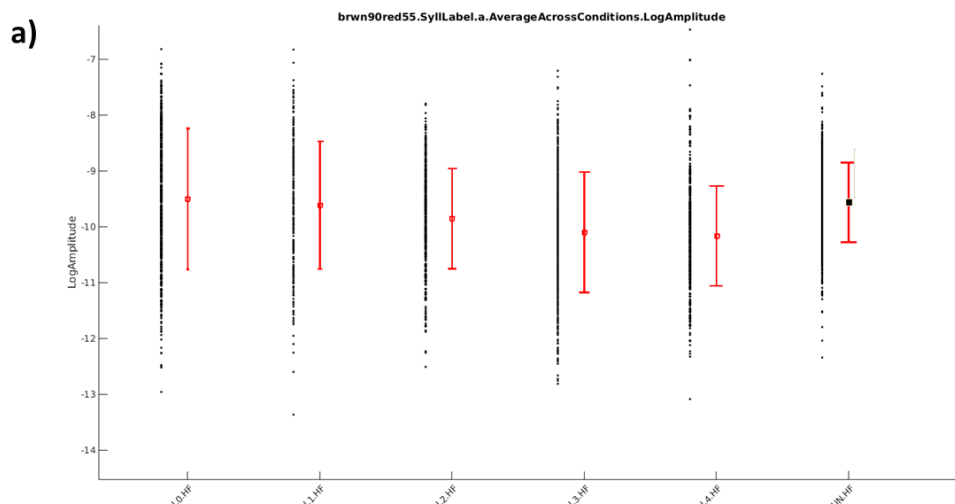


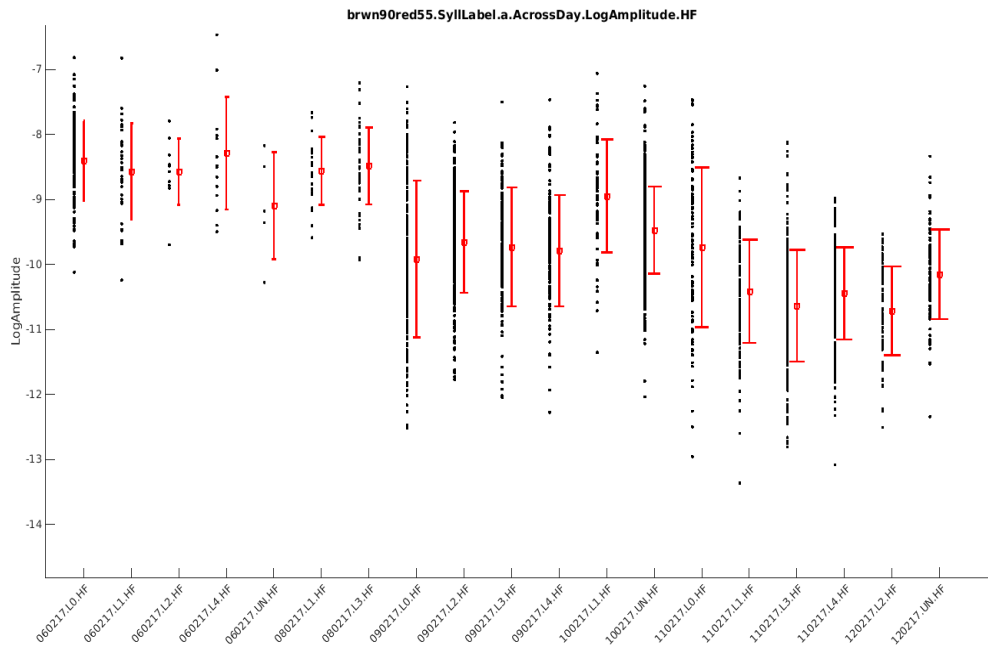
FIG 21. Mean Log amplitude change quantified with respect to the position of the microphone relative to source of sound (Distance from speaker)

Change in amplitude of a monotone of fixed amplitude played back via speaker at various distances from the microphone. Left panel – Microphone facing away from the speaker; Right panel – Microphone facing the speaker,

session. The change in amplitude with distance from the microphone was quantified using a fixed amplitude monotone played back via speakers. 2.5 - 3dB variability was observed for 5mm change in position of the microphone (FIG 21) and hence, a technique to attach the microphone at a stable position on the bird had to be devised.



b)



c)

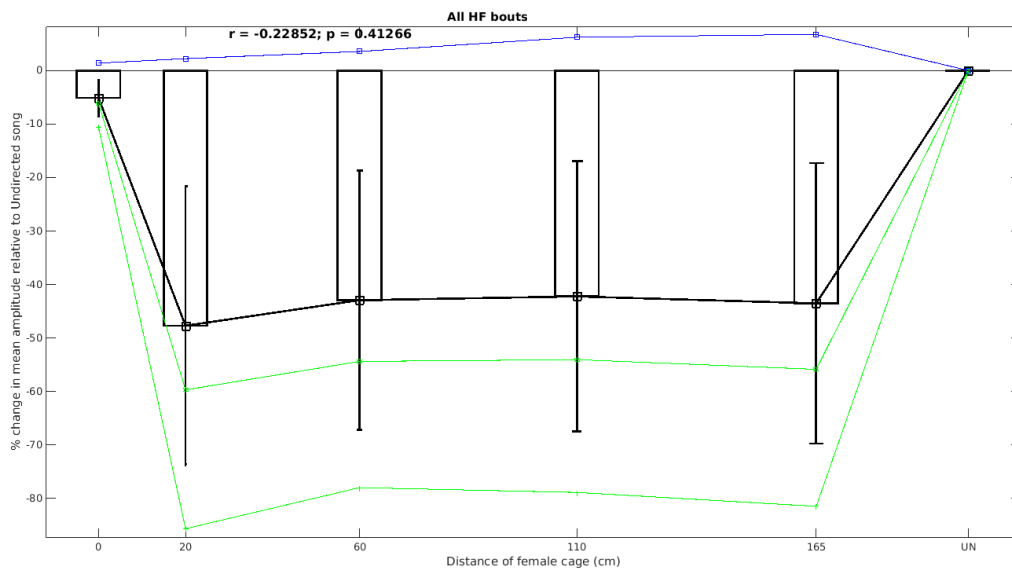


FIG 22. Change in log amplitude for a representative bird when recorded with head-fixed microphone

a) Average change in amplitude across distances for a representative bird. Data is not significant.
 b) Day-to-day variability in amplitude for each distance (L0, L1, L2, L3, L4) for a representative bird.
 c) Plot of percentage change in mean amplitude relative to undirected songs across distance for 3 syllables for 2 birds. Each line represents a different syllable. The bars show the mean percentage change for all birds together at each location relative to the mean amplitude of undirected song. The black line joining the means shows the negative correlation of amplitude with distance for head fixed microphone recordings.

As a result, we used the head fixed microphone (See Methods) to alleviate any errors in amplitude measurement due to changes in position of the microphone even by a small degree. The estimated amplitude with head-fixed microphone showed no significant change with distance (FIG 22) (n = 3 syllables, 2 birds).

On the whole, we observed no change in amplitude of motif syllables with distance even with the head fixed microphone recordings. In spite of these results, we believe that a number of factors could contribute to this lack of change in amplitude, with day-to-day variability in amplitude and time of interaction between male and female birds, being the most prominent ones.

DISCUSSION

By quantifying the changes in the song features of the adult male zebra finch song, we demonstrate the importance of distance of communication between the male and female birds, during courtship. It is known that the song and behaviour of adult male zebra finch is social context-dependent and to check for influence of distance on the male bird's song and behaviour in two different social contexts (directed and undirected), only features known to be different for both were chosen. We observed that the number of introductory notes, number of motifs, and bout length decreased with distance while the first motif duration didn't change significantly, showing maximum of 3-4% increase and the variability in the fundamental frequency of syllables also increased, with increase in distance between the male and female birds, during courtship. On comparison of these song features with the undirected song bouts (different social context and acted as control – when the bird was presented with an empty cage instead of a female), it was seen that number of introductory notes, and variability in fundamental frequency of syllables were the only two features which showed a significant difference from the undirected song features at the closest distances (0 cm and 20 cm). Consistent with one of our predicted hypothesis, these findings suggest that song features become more undirected-like with distance and are not random changes in song features elicited by female bird's presence or the male bird's motivation alone. Though female presence can trigger the production of song, it would imply that the song quality shouldn't change with distance and the male bird's behaviour should be synonymous to that of a vigorous courtship ritual, irrespective of the distance from the female bird. However, we do realise that indirectly we are hinting at the male bird's ability to clearly see the female at farther distances but even at shorter distances (up to 60 cm), we see significant differences in song properties, and hence strengthening our proposition.

When we inspected each of the features individually, we see that each of them corroborate and show a gradual change from being highly similar to directed songs to becoming more undirected-like songs with increase in distance. Higher number of INs and lower variability in fundamental frequency of syllables at shorter distances could imply that the bird wants to produce a less variable i.e. more stereotyped song to the female bird, and to do so he needs more preparation. This is in accordance with the preparation hypothesis for INs, stating the need for the bird to produce more INs to reach the pre-motif state due to sudden introduction of the female (Rajan and Doupe, 2013).

Although our result does not show a significant increase in motif duration with distance, we find that this could be due to the long time periods of data collection, spread over 6-7 months. Essentially this implies the influence of time of recording on the song tempo. In accordance with this, when we looked at songs recorded within a short range of time, we see that first motif duration is lower for shorter distances relative to undirected and hence, albeit the need for rigorous quantification, we could say that songs are faster at shorter distances. Production of longer and faster songs when the female is in close proximity could pinpoint the need for active female response to perform an intense courtship repertoire. As the female response can be seen and heard by the male bird clearly at shorter distances, the bird responds back by singing faster and longer bouts. When she is farther away, her lack of response due to distance or due to the lower resolution of the male bird's visual acuity, the male tends to slow down and sing shorter song bouts. However, we are yet to determine the effect of female response on the male bird's song and behaviour and how it influences the role of distance in causing these changes in song.

The categorisation of bouts as directed and undirected based on the behaviour of the male bird implies the existence of variation in directed song intensities across distances as proposed previously. This, along with the effect of female response, could influence the quality of stimulus that is being provided to the male to initiate and maintain robust song production at various distances. Besides, this also provides a much stringent measure to categorise songs based on social context in the zebra finch model system.

Though it has been already shown that song amplitude increases with distance, we do not see any changes in song amplitude with distance (Brumm and Slater, 2006a). However, we strongly believe that this might actually not be the case and a number of factors could cause the variation in song amplitude, apart from distance. While we have tried to the best of our abilities to alleviate the technical errors contributing to these variations, the role of time at various scales seems to be influencing it broadly, along with distance. The usage of head-fixed microphone might have reduced the variability due to change in position of the microphone, but hasn't completely eliminated the day-to-day variability in amplitude. Determining the changes in amplitude within a recording session, within the same day, and across days, could shed some light on why we observe these changes or lack of.

Although earlier studies hint at sharp distinction between directed and undirected song properties, our work shows that there is a possibility for a range of variation within the directed song bouts. We might not be seeing a binary distinction between the two as a number of other factors especially female response, might also be contributing to the male bird's song quality. We could classify songs on the basis of on song properties alone and validate if the categorisation of songs on the basis of visual cues is good enough or not. This could clear all our concerns regarding the kind of transition we see in song properties with distance.

Together, the results of this study hints at the influence of distance on acoustic features, other than intensity of vocalisations, and potentially tries to understand the roles they play in effective exchange of information during communication. Along with distance of communication, we would like suggest that other factors like sender motivation; receiver response etc. could also be attributed to the changes observed in various acoustic features. Lastly, we would like to put forth that all these factors collectively could contribute to the long term adaptations in signal transmission during communication, amongst various animal systems, over time.

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